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Short Communication

Hair can be a good biomarker of occupational exposure to mercury vapor: Simulated experiments and field data analysis

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ABSTRACT

Generally, urine mercury (U-Hg) is widely used for assessment of inorganic mercury (I-Hg) exposure in humans. The reliability of scalp hair as an indicator of mercury vapor exposure is contentious. However, significant correlations were found between hair total mercury (T-Hg) and U-Hg and between hair I-Hg and total gaseous mercury (TGM) in ambient air in our previous studies. Simulated experiments were designed to assess the contribution of direct absorption/adsorption of mercury vapor in the hair. Results indicated that the increases of hair T-Hg concentrations were less than 1 μg/g, which was negligible compared with hair T-Hg concentrations in occupationally exposed workers. The β-mercaptoethanol washing can remove 30% of mercury (Hg) in the exposed hair samples. The inhaled Hg constituted the major fraction (97.4%) of I-Hg exposure for the artisanal Hg mining workers. From the simulated experiments and field data analysis, we can conclude that hair I-Hg can be a useful tool for monitoring occupational exposure to Hg vapor.

1. Introduction

Human hair and blood are used as biological indicators for estimating methylmercury (Me-Hg) exposure (USEPA, 1997; NRC, 2000). Compared with blood specimen, hair has several advantages: it can be obtained by non-invasive sampling, it is easy for storage, and it contains higher concentrations of elements. Generally, hair is 250 to 300 times more concentrated in mercury (Hg) than blood (WHO, 1990). Moreover, segmental analyses of hair Hg can trace dynamic change of exposure over hair growing period (Dolbec et al., 2001; Morrissette et al., 2004; Malm et al., 2010), since hair growth rate is generally estimated at 1 cm/month. Recent advances in single hair strand analysis at micron resolution (Legrand et al., 2004, 2005) should yield more detailed information on historical Hg exposure.

Mercury is incorporated into scalp hair at the hair follicle in proportion to its content in blood. Once incorporated into the hair, the Hg is stable and it gives a longitudinal history of blood Hg levels (WHO, 1990). Generally, hair Me-Hg constitutes from 80% to 98% of total Hg (T-Hg) in fish eating population (WHO, 1990; Dolbec et al., 2001), and the use of T-Hg as a surrogate for Me-Hg concentration should not lead to significant exposure misclassification. But with hair T-Hg analysis, it is difficult to distinguish between exogenous contaminations and deposited endogenously in occupational settings.

The reliability of human scalp hair as an indicator of Hg vapor exposure is contentious. The key points are quantification of the contributions from different exposure sources and elimination of the exogenous contaminations. For Hg vapor exposure, about 80% of inhaled is retained in the body (WHO, 1991). The absorbed Hg vapor is oxidized to divalent inorganic Hg in red blood cells and is carried to all tissues in the body. The kidney is the main depository. Excretion takes place via both urine and feces. Urinary Hg (U-Hg) originates mainly from kidney and it is the commonly used biomarker for Hg vapor exposure, as it reflects the cumulative dose in the kidney (WHO, 1991).

Li et al. (2008a) spiked human hair samples with Hg chloride solutions and washed with different detergents. Serious Hg pollution were found in the spiked hair samples and these could not be fully washed off even using reagents with high affinity to Hg. It suggested that hair was not a suitable biomarker for evaluation of T-Hg exposure especially for occupational population with serious external Hg exposure.

However, Wilhelm et al. (1996) found that hair was a useful tool for monitoring external exposure by observation of hair and blood Hg before and after dental amalgam. Significant correlation was observed between hair inorganic Hg (I-Hg) concentrations and total gaseous mercury (TGM) concentrations in ambient air in Wuchuan Hg mining areas, Guizhou, China (Li et al., 2008b). Significant correlations between hair T-Hg and U-Hg were also found in artisanal Hg mining workers in Wuchuan (Li et al., 2008c) and Tongren, China (Li et al., 2011). These indicated that hair might be a useful tool for monitoring exposure of Hg vapor in occupational workers.

This study was designed to: (1) quantify the contribution of direct absorption/adsorption of Hg vapor in the hair by simulated experiments and different washing styles; (2) evaluate the possibility of hair as a biomarker of exposure to Hg vapor for occupational population.

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2. Material and methods

2.1. Simulated experiments

The hair samples used for the simulated experiments were collected from a volunteer with stainless steel scissors. The average of hair T-Hg concentrations was 0.258 μg/g with a Standard Deviation (SD) of 0.011 μg/g ($n=5$), which indicated that hair T-Hg concentrations were stable in different scalp regions. Before the experiments of Hg vapor exposure, the hair samples were washed with detergent and distilled water, and then dried in an oven at 60 °C overnight.

The experimental facility was consisted of a Dynacal mercury vapor (Hg^0) permeation device (VICI Metronics), a packed-bed reactor, an on-line Hg analyzer, and a data acquisition system, as shown in Fig. 1 (revised from Wan et al., 2011). A flow (500 mL/min) of pure N_2 (99.999%) passed the permeation tube and yielded a stable concentration of Hg^0. The Hg^0 concentrations can be adjusted with the temperature controlled by a water bath. The reactor was a U-shaped quartz tube with a total length of 760 mm (outer diameter: 8.0 mm; inner diameter: 6.0 mm).

The hair samples were placed in the reactor with a length of 10 mm at room temperature. The Hg^0 concentrations in the flow were measured by a Lumex RA 915+ Hg analyzer, which is based on Zeeman atomic absorption spectrometry, and is capable of providing a real-time response every 1 s.

The Hg^0 concentration of 200 μg/m^3, which was at same level with Hg vapor exposure for the artisanal Hg mining workers, was used in the simulated experiments. The hair samples were exposed to Hg vapor at different time periods.

2.2. Hair washing methods

The exposed hair samples were washed with acetone and β-mercaptoethanol (ME). The acetone washing method was recommended by International Atomic Energy Agency (IAEA) and it was widely used in most studies (Batzevich, 1995; Feng et al., 1995) as a pretreatment step to remove external contamination. In this method, hair samples were sequentially washed with acetone once, three times with deionized water, and with acetone once more at room temperature.

The chelating stability constant for ME to Hg is 45.4, which indicates that it's a strong Hg chelator. It has been used in the reduction/elimination of memory effect of Hg analysis (Li et al., 2006). ME shows better washing efficiency than Ethylene Diamine Tetracetic Acid (EDTA) and cysteine in a previous study (Li et al., 2008a).

Hair samples were divided into three categories by different washing detergents: un-washed (UW), acetone (AC) and β-mercaptoethanol (ME) washed. Finally, the hair samples were left in an oven overnight to dry at 60 °C prior to Hg analysis.

2.3. Mercury determination and quality control

T-Hg concentrations in hair samples were analyzed by RA-915+ Hg analyzer coupled with PYRO-915+ attachment. It is based on atomization of Hg by thermal decomposition and subsequent measurement by Zeeman atomic absorption spectrometry. The detection limit was 0.020 μg/g on a basis of 10 mg hair sample.

As a part of a strict Quality Control program, method blanks, certified reference material (CRM) and blind duplicates were analyzed for the hair samples. The CRMs (NIES-13 and GBW09101b) were used to evaluate the accuracy of Hg analysis. The mean of T-Hg in NIES-13 was 4.30 ± 0.09 μg/g ($n=9$), which was consistent with the certified value of 4.4 ± 0.2 μg/g; the detected T-Hg concentration in GBW09101b was 1.09 ± 0.08 μg/g ($n=9$), in a good agreement with the certified value (1.06 ± 0.28 μg/g). The relative percent difference was lower than 5% in duplicate samples.

2.4. Calculation of contribution from different sources

In order to assess the contribution to I-Hg exposure from different routes, we estimated the daily intake via drinking water, diet (fish, rice, corn, vegetables, meat, and poultry), and respiration for the artisanal mining workers. For calculation, absorption efficiency of Hg species in diet by human body was considered as 8% for I-Hg (WHO, 1991). For inhalation of Hg vapor, approximately 80% is retained in the body (WHO, 1991). The diet data was adopted from the results obtained in Wanshan Hg mining area by Zhang et al. (2010a). The air

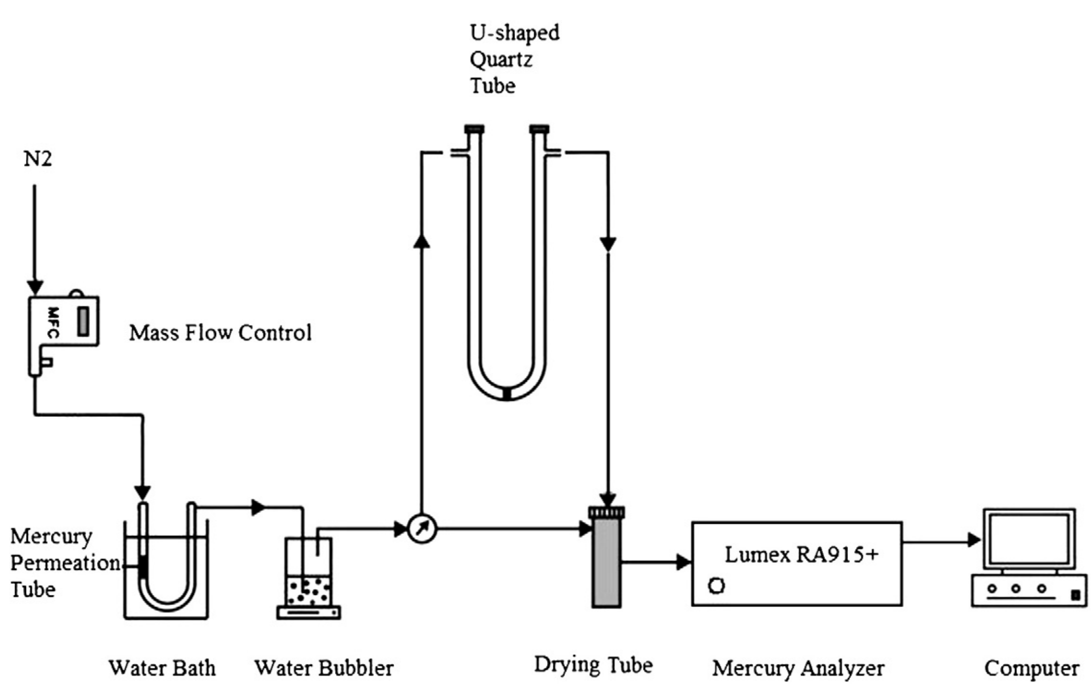

Fig. 1. Schematic diagram of experimental facility for hair exposure to Hg vapor.

Hg concentrations were assumed to be 50 μg/m^3 in the work place (Li et al., 2008a) and 93 ng/m^3 in the residence area (Zhang et al., 2010a).

2.5. Field data analysis

The data of hair and urine Hg for artisanal mining workers was adopted from Li et al. (2008c, 2011), which were investigated in Wuchuan and Tongren area. Artisanal mercury mining is periodically operated by local peasants seasonally or according to the market price development. It is known for low recovery efficiency (Li et al., 2009), high environmental costs, and poor health and safety measures (Li et al., 2008c, 2011). The local residents in Hg mining areas (Li et al., 2008b; Feng et al., 2008) and a control group (Li et al., 2008c) were also selected for comparison.

2.6. Statistical methods

Statistical analyses were performed using SPSS 11.5 for windows. The characteristics of Hg concentrations were described in Mean ± SD and examined using descriptive statistics. The correlation coefficients among U-Hg, hair T-Hg, and hair Me-Hg in each group were studied by the Pearson correlation analysis. The results of statistical tests were considered statistically significant if $p<0.05$.

3. Results

3.1. Absorption/adsorption of Hg vapor in hair

T-Hg concentrations in the exposed hair samples for different times are shown in Fig. 2. There was nearly no increase in the initial 12 h of exposure in UW samples. T-Hg concentrations increased about 15% in the 1st day to 3rd day of exposure and increased significantly 40–50% in the 4th day to 7th day of exposure in UW samples.

Hair Hg contaminations could not be removed by washing with acetone in the initial 3 days. But the T-Hg concentrations in AC samples were elevated about 50% in the 4th day to 7th day of exposure when compared with UW hair samples. It was difficult to explain and similar results were obtained in the duplicate experiments.

When the exposed hair samples were washed with ME, about 30% of Hg was washed off, which was similar to the results observed by Li et al. (2008a). Among EDTA, cysteine, and ME, ME showed the best washing efficiency to Hg (Li et al., 2008a).

Hac and Krechniak (1993) did a similar experiment on the Hg^0 concentration of 210 μg/m^3 with a longer time period and found that hair T-Hg level increased to 3 times during 28 days of exposure, when compared with the initial value of 0.38 μg/g. T-Hg levels in the exposed hair samples attained a steady state on the 21st day of exposure. The variation of T-Hg concentrations in the hair exposed to Hg vapor is shown in Fig. 3.

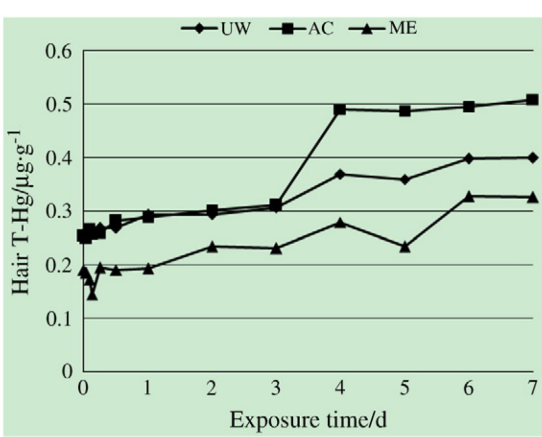

Fig. 2. Hair T-Hg concentrations with different washing procedures for different exposed times.

In the simulated experiments, the increases of hair T-Hg concentrations are less than 1 μg/g when the hair samples were directly exposed to Hg vapor of 200 μg/m^3. The comparisons of hair Hg concentrations between different groups are given in Table 1. Compared with hair T-Hg concentrations in artisanal mining workers, the observed absorption/adsorption of Hg vapor in the hair was negligible. The direct absorption/adsorption of Hg vapor in hair was not an important exposure route for the artisanal mining workers.

3.2. Identification of source of hair I-Hg

The daily intakes of I-Hg from different routes are listed in Table 2. The inhaled Hg represented the major fraction (97.4%) of I-Hg intake for the artisanal Hg mining workers and this was consistent with our previous study (Li et al., 2008b).

Hair T-Hg and U-Hg concentrations in the artisanal mining workers were highly elevated when compared with general population from Hg mining areas and the control group, which confirmed significant occupational exposures to Hg vapor (Table 1). Furthermore, the percentage of Hg as Me-Hg in hair was 12.4% on average in occupational workers and it showed significant negative correlation with hair T-Hg ($r=-0.47$, $p<0.01$; Fig. 4). This confirmed that Me-Hg and I-Hg had different exposure sources. The populations in Hg mining area are exposed to Hg vapor through inhalation and exposed to Me-Hg through intake of Me-Hg polluted rice (Feng et al., 2008; Zhang et al., 2010a). Recent studies highlighted that rice can accumulate relatively high levels of Me-Hg in Hg contaminated areas (Qiu et al., 2008; Zhang et al., 2010b).

4. Discussion

Generally, U-Hg measurements are widely used for assessment of I-Hg (mainly Hg vapor) exposure in humans, because U-Hg is thought to indicate most close Hg levels present in the kidneys (Barregard, 1993; Clarkson, 2002). Many studies have confirmed the strong correlation between U-Hg level and TGM in occupational settings. WHO (World Health Organization) (1991) recommended that the values of air concentration (in μg/m^3) were approximately the same as those of U-Hg concentration (expressed in μg/g Creatinine). By data fitting from various studies, a significant correlation was found between Hg in air versus urine ($U\text{-}Hg=3.24{*}TGM^{0.833}$) in the air Hg concentration range of 10 to 50 μg/m^3 (Tsuji et al., 2003).

A significant correlation ($p<0.05$) between hair I-Hg and U-Hg in the artisanal mining workers was also observed (Fig. 5). In cases of occupational exposure, hair could become a useful tool for monitoring exposures to Hg vapor as the widely used U-Hg, since there was nearly no direct absorption/adsorption in the hair.

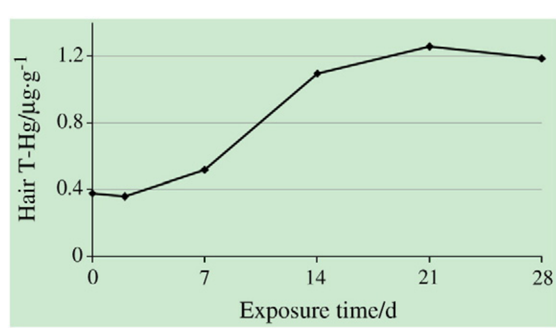

Fig. 3. T-Hg concentrations in hair samples exposed to Hg vapor (210 μg/m^3) for different exposed times.
Data from Hac and Krechniak, 1993.

Table 1
The comparisons of hair and urine Hg concentrations among different groups/Mean ± SD (Range).

	n	Hair T-Hg/μg·g^{-1}	Hair Me-Hg/μg·g^{-1}	Hair I-Hg/μg·g^{-1}	Hair Me-Hg ratio/%	U-Hg/μg·g^{-1} Creatinine	Reference
Occupational workers	79	43.5 ± 47.2 (6.28–123)	2.59 ± 2.21 (0.63–8.65)	41.0 ± 46.7 (4.27–117)	12.4 ± 15.5 (1.12–51.8)	698 ± 1140 (22.5–3190)	Li et al., 2008c, 2011
Local residents	218	3.59 ± 2.64 (1.19–8.72)	1.74 ± 1.28 (0.60–4.37)	1.85 ± 1.98 (0.069–5.92)	55.4 ± 23.8 (16.9–96.2)	36.9 ± 55.5 (1.50–166)	Li et al., 2008b; Feng et al., 2008
Control group	40	0.77 ± 0.28 (0.41–1.49)	0.65 ± 0.25 (0.33–1.23)	0.13 ± 0.12 (0.00–0.35)	83.1 ± 12.6 (62.5–99.4)	1.30 ± 0.39 (0.77–2.17)	Li et al., 2008c

Table 2
The estimated daily intake of I-Hg through different routes for artisanal mining workers.
Data from Zhang et al., 2010a.

Medium	Daily consumption	I-Hg	Daily intake/μg·d^{-1}	Ratio/%
Air (work time)	8 m^3	50000 ng/m^3	320	97.1
Air (rest time)	12 m^3	93 ng/m^3	0.89	0.27
Water	2 L	49.9 ng/L	0.008	0.002
Rice	600 g	68.7 ng/g dw	3.30	1.0
Corn	60 g	2.05 ng/g dw	0.0098	0.003
Vegetable	368 g	129.9 ng/g ww	3.82	1.16
Meat	79.3 g	219.1 ng/g ww	1.39	0.42
Fish	1.2 g	230 ng/g ww	0.022	0.007
Poultry	4.9 g	157.6 ng/g ww	0.062	0.018
Total			329.5	

dw, dry weight; ww, wet weight.

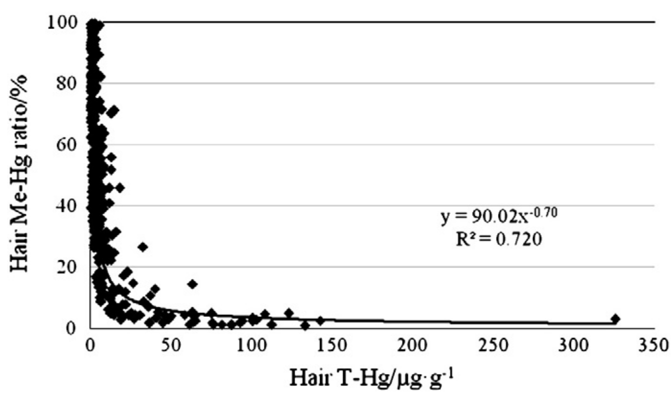

Fig. 4. The correlation between T-Hg and Me-Hg ratio in hair samples.
Data from Feng et al., 2008; Li et al., 2008b,c, 2011.

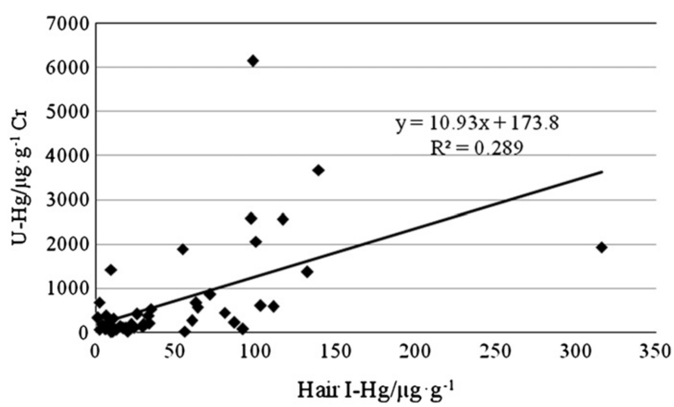

Fig. 5. The corelation between hair I-Hg and U-Hg in the artisanal mining workers.
Data from Li et al., 2008c, 2011.

Furthermore, a significant correlation ($r = 0.989$, $p < 0.01$) was observed between hair I-Hg and TGM in ambient air in Wuchuan Hg mining area (Fig. 6a, Li et al., 2008b). Moreover, a significant correlation ($r = 0.67$, $p < 0.05$) was still obtained when two extremely high values were excluded (Fig. 6b, Li et al., 2008b). Counter et al. (2005) also observed a significant association between U-Hg and hair T-Hg in children involved in artisanal gold mining in South America. These confirmed that hair might be a useful tool for monitoring occupational exposure to Hg vapor.

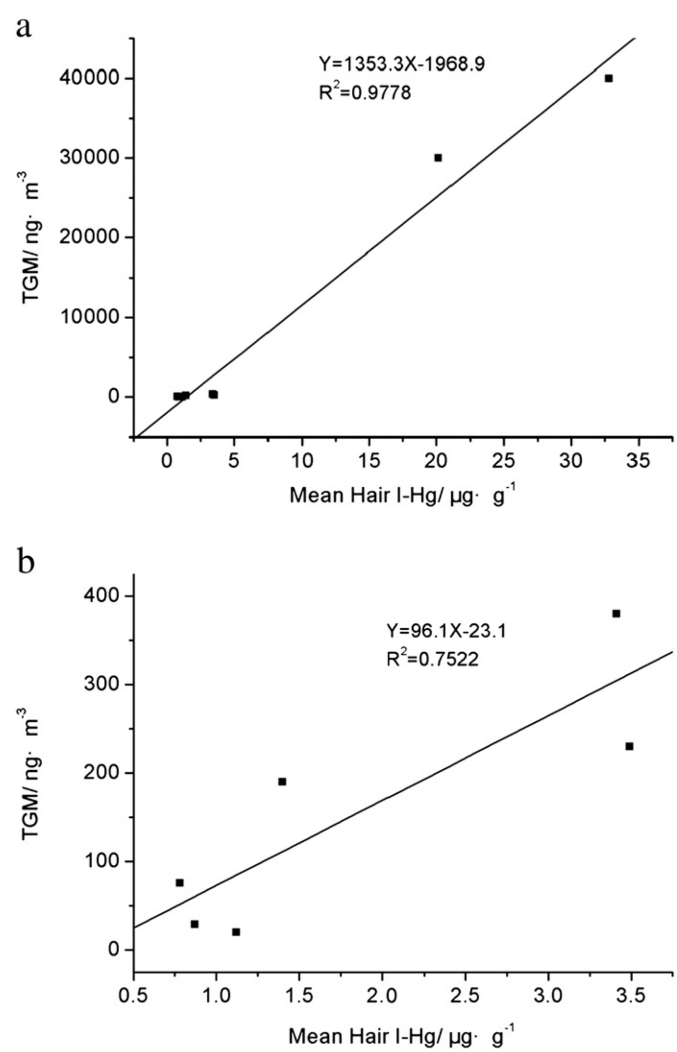

Fig. 6. The correlation between mean hair I-Hg and TGM concentrations in Wuchuan Hg mining area.
Data from Li et al., 2008b.

5. Conclusions

The results from the simulated experiments indicated that the direct absorption/adsorption of Hg vapor in hair was negligible compared with hair I-Hg in artisanal mining workers. But the washing styles have significant effects on hair Hg concentrations. Inhalation is the most important exposure route for the artisanal Hg mining workers. By field data analysis, we found that hair I-Hg level was significantly correlated with U-Hg in artisanal mining workers and with TGM concentrations in the atmosphere. These indicated that hair can be a good biomarker for estimating exposure to Hg vapor in occupational settings.

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