



Occupational Exposure to Mercury: Air Exposure Assessment and Biological Monitoring based on Dispersive Ionic Liquid-Liquid Microextraction

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Abstract

Background: Exposure to mercury (Hg) as a heavy metal can cause health effects. The objective of this study was to assess occupational exposure to Hg in a chlor-alkali petrochemical industry in Iran by determining of Hg concentrations in air, blood and urine samples.

Methods: The study was performed on 50 exposed subjects and 50 unexposed controls. Air samples were collected in the breathing zone of exposed subjects, using hopcalite sorbents. Analysis was performed using a cold vapor atomic absorption spectrophotometer (CV-AAS) according to NIOSH analytical method 6009. For all participants, blood and urine samples were collected and then transferred into sterile glass tubes. After micro-extraction with ionic liquid and back extraction with nitric acid, Hg concentrations in blood and urine samples were determined by CV-AAS.

Results: The mean concentration of air Hg was 0.042 ± 0.003 mg/m³. The mean concentrations of Hg in blood and urine samples of exposed subjects were significantly higher than unexposed controls (22.41 ± 12.58 versus 1.19 ± 0.95 µg/l and 30.61 ± 10.86 versus 1.99 ± 1.34 µg/g creatinine, respectively). Correlation of air Hg with blood Hg, urine Hg and blood Hg-urine Hg ratio were significant statistically ($P < 0.05$).

Conclusions: The values of Hg in blood and urine samples of chlor-alkali workers were considerably high. Correlation coefficients showed that blood Hg and blood Hg-urine Hg ratio are better indicators than urine Hg for assessing occupationally exposed workers in terms of current exposure assessment.

Keywords: Blood, Blood Hg-urine Hg ratio, Mercury, Urine

Introduction

Occupational exposure to mercury (Hg) as a heavy metal can cause health effects especially on central nerves system (CNS) and kidneys (1). Moreover, inflammation of the gums and excessive salivation observed among chronically exposed workers (2). The health effects related to exposure to Hg on the CNS may include subjective symptoms such as nervousness, fatigue, and

depression and dysfunction on the CNS such as increased tremor, insomnia and memory impairments (3-8). However, nausea, vomiting, abdominal pain, bloody diarrhea, kidney damage, and death will occur in acute exposures (2). Elemental Hg is a silver-white and it is liquid at room temperature. The concentration of air Hg rapidly increases as the temperature increases and after

distribution in the environment easily enters to the human lungs (8). Exposure can occur through respiratory system and skin contact. Occupational exposure to Hg and its compounds may occur in various occupations such as amalgam makers, barometer makers, battery makers, chemical laboratory workers, chlor-alkali petrochemical workers, dentists, fluorescent lamp makers, gold and silver extractors, insecticide makers, Hg miner workers and thermometer makers (2, 9,10). It should be noted that exposure to Hg compounds may occur from different sources (11). Hg contaminated fish is a major source of methylHg for humans (6, 11-15). Environmental pollution due to methylHg contamination observed in the vicinity of some industries such as the Chisso Corporation chemical factory in Minamata Bay in kyushu, Japan or the Dryden chlor-alkali facility and paper mill in the English-Wabigon River, Ontario as well as three epidemic poisonings caused by consumption of methylHg-contaminated seeds have been reported in Iraq (5, 8). Exposure to Hg can also have potential risks for people who living near the chlor-alkali plants. Gibicar et al. (12) studied human exposure to Hg in the vicinity of chlor-alkali plant in Italia. They stated that “14% of emitted gaseous Hg from the Hg cell chlor-alkali plant is deposited within 5 km from the source”. However, they suggested that potential Hg risks are related to various sources and consumption of contaminated fish is one of the important cases. Chlor-alkali industry is one of the main sources of Hg pollution (14). Chlor-alkali workers are mostly exposed through breathing air polluted with Hg vapors released from chlor-alkali electrochemical reactor (ECR) or direct skin contact. Family members of these workers may also become exposed to Hg through personnel’s clothes contaminated with Hg in the workplaces. Hg compounds get distinguished from other toxic pollutants due to their non-biodegradability can accumulate in living tissues of human body. Exposure to Hg can cause physiological or neurological damages to the human body, so exposure assessment and precaution measures should be considered (16, 17). In the present study we assessed occupational exposure to mercury in a chlor-alkali petrochemi-

cal industry by determining of mercury concentrations in air, blood and urine samples. For evaluation of food and air effect on mercury concentration in human biological samples, speciation of mercury based on a new applied analytical method (IL-DLLME) was done.

Materials and methods

Study groups

The population of this study consisted of two groups: chlor-alkali workers (subjects) who exposed to Hg (n=50) and office employees as unexposed controls (n=50). Control group was selected from matched people of the same age and sex (male) without diseases affecting from the same factory. First, participants were given information about the research and then individual informed consents were obtained from all volunteers. Most of subjects said that they often did not use respiratory protection devices, but they wear safety gloves during their works. The study was performed at a petrochemical industry in Abadan, Iran (Because of ethical considerations the name of industry is not mentioned).

Air sampling and analysis

Air samples were collected in the breathing zone of exposed workers by using hopcalite sorbents. The sampling duration was about 4 hours of the work shift. Samples were collected during three work days of a week with similar working conditions. All pumps were calibrated before and after use and air samples were collected at flow rate of 0.20 l/min. Analysis was performed using a cold vapor atomic absorption spectrophotometer (CV-AAS) (CV-AAS, GBC – 932, 3000, Australia) according to NIOSH analytical method 6009 (18).

Mercury concentrations in blood and urine samples

For all participants, blood and urine samples were collected at the end of shift and then transferred into sterile glass tubes. Samples were maintained at -20°C until analyzed. Ionic Liquid-liquid

extraction was combined with CV/HG-AAS to develop a new procedure for the determination and speciation of trace amount of Hg in human blood samples. Dispersive liquid-liquid micro-extraction has been developed as a new mode of liquid phase micro-extraction and attracted increasing attention for its simple operation high enrichment factor, rapidity and high extraction efficiency (19). In this work, 1 mL of 1% (w/v) APDC (Ammonium pyrrolidin-dithiocarbamat) solution was added to 10 mL of blood as well as urine samples and pH was adjusted to 7 with buffer solution in a centrifuge tube. Then, 0.2 g of IL (Ionic liquid) was added to the mixtures and they were shaken with a vortex apparatus for 2 min. Hg (Hg^{2+}) was complexed and pre-concentrated as Hg-APDC in IL. The phases were separated by centrifuging of turbid solution at 3 min with 3500 rpm. After micro-extraction with ionic liquid and back extraction with nitric acid, Hg concentrations in blood and urine samples were determined by CV-AAS. For inorganic Hg (Hg^{2+}) determination, 1.5 mL of 1% (w/v) NaDDC solution was added to 10 mL of blood samples at pH=6 and to be continued with the same above way by FI-CVAAS. Total Hg (organic and inorganic Hg) determined after 10 mL blood sample placed in microwave (210°C, HNO_3 Con., UV). Total organic Hg (R-Hg) is simply calculated by difference between concentration of total Hg and inorganic Hg in blood samples.

The instrumental and extraction conditions for Hg determination by CV-AAS are listed in Table 1. Working range was between 0.5- 27 $\mu\text{g/l}$ for samples at peak area. Method validation was performed using additional standard (Table 2 and 3) and standard reference material (NIST SRM 955c) with certified values for mercury speciation. The results are shown in Table 4.

The urine Hg concentration may be influenced by dilution of urine due to the intake of fluids, physical activity or temperature, so urine Hg concentrations were adjusted by urine creatinine concentrations.

Table1: Instrumental and extraction conditions for Hg determination by CV-AAS

Instrumental Parameters	Hg
Wavelength (nm)	253.7
Lamp current (mA)	3
Spectral bandwidth (nm)	0.5
LOD* ($\mu\text{g/l}$)	0.3
Working range ($\mu\text{g/l}$)	1-55
Extraction conditions	Hg
LOD ($\mu\text{g/l}$)	0.06
Working range ($\mu\text{g/l}$)	0.5-27
Enrichment Factor	5
Volume sample (ml)	10
Amount of IL** (g)	0.2
PH	7

*LOD: limit of detection. **IL: Ionic liquid

Table 2: Validation of proposed method for determining Hg in blood and urine samples ($\mu\text{g/l}$)

Sample	Added Hg	Found Hg ^a	Recovery (%)
Blood ₁	-	23.51 ± 0.32	-
	10	32.82 ± 1.11	98
	20	44.94 ± 2.82	103
Blood ₂	-	1.21 ± 0.02	-
	2	3.18 ± 0.10	98
	4	4.98 ± 2.83	103
Urine	-	11.79 ± 0.36	-
	2	13.42 ± 0.53	97
	4	16.02 ± 0.64	102

^a Mean ± SD of five determinations

Table 3: Speciation and determination of R-Hg and Hg (II,I) by proposed method in spiked real samples

Sample	Added ($\mu\text{g/l}$) ^a		Found ($\mu\text{g/l}$) ^a		Total	Recovery (%)	
	R-Hg ^b	Hg (II)	R-Hg	Hg (II,I)		R-Hg	Hg (II)
WB ^c	-----	-----	3.084 \pm 0.097	2.181 \pm 0.107	5.265 \pm 0.204	-----	-----
	2	-----	4.926 \pm 0.175	2.253 \pm 0.131	7.179 \pm 0.306	97	103
	-----	2	3.119 \pm 0.124	3.989 \pm 0.216	6.617 \pm 0.338	101	95

^a Mean \pm SD of three determinations, ^b R-Hg: organic Hg, ^c WB: Whole blood

Table 4: Analytical results of mercury speciation and determination in standard reference material

Sample	Certified Hg(II,I)	Certified R-Hg	Found ^a Hg(II,I)	Found ^a R-Hg
SRM 955c	9.0 \pm 1.3	9.6 \pm 1.5	8.8 \pm 0.9	9.4 \pm 0.6

^aNIST SRM 955c, organic Hg speciation, pH 6.0, Microwave, -20°C, Mean \pm SD of three determinations

Statistical analysis

The statistical package for the social sciences software (SPSS version 17) was used for all statistical analyses. The One-Sample t-test was used to determine potential differences between means for some values of the two studied groups. Correlation of air Hg with blood Hg, urine Hg and blood Hg-urine Hg ratio were based on Pearson coefficients. Statistical significance was taken as $P < 0.05$.

Results

The average age values for subjects and controls were 31 \pm 5.52 and 32 \pm 6.28 years, respectively. There were no significant differences between exposed subjects and unexposed controls in terms of age ($P = 0.301$) and sex (all workers were male). The values of concentrations of Hg in the air, blood and urine samples are shown in Table 5. The mean concentration of air Hg was 0.042 \pm 0.003 mg/m³. This value is more than the threshold limit value-time weighted average (TLV-TWA: 0.025 mg/m³) recommended by American Conference of Governmental Industrial Hygienists (ACGIH) (20). Our data was normally distributed. Absorption and repeatability of the results were investigated for the determination

blood and urine Hg by CV-AAS (Table 2). The results showed that the percentage of recovery was 97 and more. Also, the values obtained for CRM analysis indicated 98% recovery. The results showed that the Hg concentrations in blood samples ranged from 2.95 to 45.21 $\mu\text{g/l}$ in the exposed subjects (Table 5). The mean concentration of blood Hg of exposed subjects (22.41 \pm 12.58 $\mu\text{g/l}$) was significantly higher than unexposed controls (1.19 \pm 0.95 $\mu\text{g/l}$) ($P < 0.001$). Also, this value is 1.5 times more than the biological exposure indices (BEIs) for blood samples (15 $\mu\text{g/l}$) recommended by ACGIH (8). Similarly, the mean concentration of urine Hg of exposed subjects (30.61 \pm 10.86 $\mu\text{g/g}$ creatinine) was significantly higher than unexposed controls (1.99 \pm 1.34 $\mu\text{g/g}$ creatinine) ($P < 0.001$), (Table 5). The relationship between blood Hg and urine Hg was significant statistically ($r = 0.739$ and $P < 0.001$), (Fig 1). Also, the correlation test showed significant relations of air Hg with blood Hg, urine Hg and blood Hg-urine Hg ratio in the subjects (Table 6).

By proposed method, organic Hg (R-CH₃) concentrations in blood samples were determined and R-CH₃ concentrations in exposed and unexposed subjects were similar (0.78 \pm 0.05 versus 0.61 \pm 0.03 $\mu\text{g/l}$, respectively).

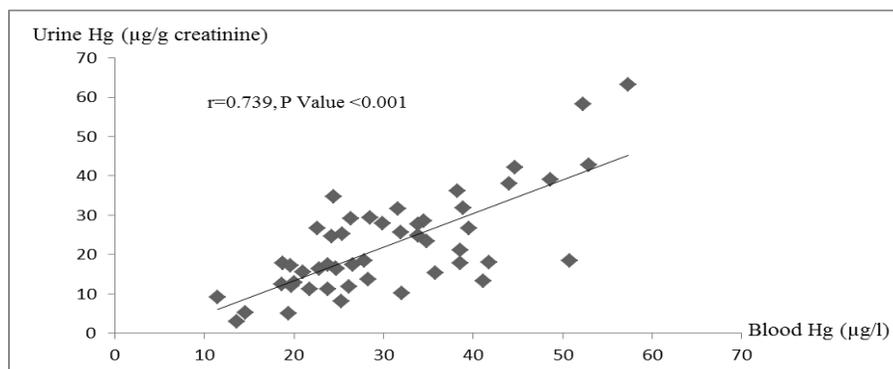


Fig. 1: The relationship between blood Hg and urine Hg in the subjects (n=50)

Table 5: Concentrations of Hg in the air, blood and urine samples

Sample	Subjects (n=50)		Controls (n=50)		P value
	(mean± SD)	Range	(mean± SD)	Range	
Air Hg (mg/m ³)	0.042± 0.003	0.01- 0.06	-	-	-
Blood Hg (µg/l)	22.41± 12.58	2.95- 45.21	1.19± 0.95	0.66- 7.32	< 0.001
Urine Hg (µg/g creatinine)	30.61± 10.86	11.47- 57.32	1.99± 1.34	0.39-6.29	< 0.001

Table 6: Correlation* of air Hg with blood Hg, urine Hg and blood Hg-urine Hg ratio in the subjects (n=50)

	Blood Hg= 22.41± 12.58 (µg/l)		Urine Hg= 30.61± 10.86 (µg/g creatinine)		Blood Hg-urine Hg ratio	
	r	P value	r	P value	r	P value
Air Hg= 0.042± 0.003 (mg/m ³)	0.532	< 0.001	0.317	0.025	0.470	0.001

*Correlations are based on Pearson coefficients (r). Statistical significance will be observed if $P < 0.05$.

Discussion

The results of our study showed that chlor-alkali workers had significant exposure to Hg. Exposure to Hg, even at low levels, can cause adverse effects (1, 10). Mniszek (21) was investigated exposure to Hg in two chlor-alkali industries in Poland. In this study, Hg vapor concentrations were considerably high in both industries and values exceeded 0.025 mg/m³. So, these results confirmed that chlor-alkali workers are at the risk of developing adverse effects, because of considerable exposure to Hg.

Hg levels in blood and urine samples can be used for exposure assessment, which it determines identifying individuals subjected to higher levels

of exposure. "Biological monitoring can also be used for risk assessment if the relationships between the exposure parameters and the adverse effects are known" (11). In the present study, the mean concentration of blood and urine Hg of exposed subjects was significantly higher than unexposed controls. Similarly, in a study by Longworth et al. (11), the concentrations of Hg in the blood and urine samples were considerably higher in the chlor-alkali workers (exposed group) than in the control group. The reference values for blood and urine Hg are 1-8 and 4-5 µg/l, respectively. However, these values may differ according to seafood consumption (8). Significant correlation between blood Hg and hair Hg was found in a study by Akagi et al. (22). Satoh (8)

stated that blood and urine samples are good markers for occupational Hg exposure assessment, whereas scalp hair is good indicator for methylHg exposure especially for environmental exposure. Lately, Li et al. (23) noted that hair can be used as a good biomarker for monitoring occupational exposure to Hg vapor. We observed significant relations for blood and urine samples as biomarkers of Hg exposure. We also calculated blood Hg-urine Hg ratio as an indicator. Correlation test showed significant relation between blood Hg-urine Hg ratio and air Hg. However, correlation coefficients showed that blood Hg and blood Hg-urine Hg ratio are better indicators than urine Hg indicator for assessing occupationally exposed workers in terms of current exposure assessment. This finding is consistent with the findings of Barrewards' study (24).

Limitations

One of the limitations for our study is the lack of data related to Hg levels enters to the human body through non-occupational sources especially seafood, which can affect blood Hg concentration. The number of seafood meals per week was less than 2 times in the studied population, so this limitation is not very influential in our results.

Conclusion

Chlor-alkali workers are exposed to considerable concentrations of Hg. The Hg concentrations in blood and urine samples were significantly higher in the exposed subjects than in the unexposed controls. Correlation of air Hg with blood Hg, urine Hg and blood Hg-urine Hg ratio were significant statistically. However, correlation coefficients showed that blood Hg and blood Hg-urine Hg ratio are better indicators than urine Hg indicator for assessing occupationally exposed workers in terms of current exposure assessment. IL-DLLME can be considered as a new applied analytical method for determination and speciation of mercury in blood samples.

Ethical issues

Ethical issues have been conducted by the authors in accordance with the recommendations outlined in the Declaration of Helsinki. Individual informed consents were obtained from the participants. The study was approved by the ethical committee of Iranian Petroleum Industry Health Research Institute (IPIHRI).

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