Accred Qual Assur
DOI 10.1007/s00769-009-0506-y

PRACTITIONER'S REPORT

Instability of mercury in specimens of human urine for external quality assessment

Andrew Taylor · Robert L. Jones · Alain Leblanc · Olav Mazarrasa · Mi-Young Lee · Patrick J. Parsons · Marina Patriarca · Jean-Philippe Weber · Cas Weykamp

Received: 16 November 2008 / Accepted: 20 February 2009

Abstract An under-recovery of inorganic mercury added to urine and a wide range of results is observed in quality assessment schemes (EQAS) for trace elements. Furthermore, the under-recoveries are inconsistent suggesting features associated with the urine matrix may make the mercury unavailable for measurement. To investigate the instability of mercury in urine the following experiments were set up: (1) a sample of Hg^{2+} in water with various 'stabilizers' added was sent to UK external quality assessment scheme participants. (2) Urine was collected from volunteers who also completed a 3-day food diary. Hg, Ca, Mg, Se, uric acid, phosphate, creatinine, reducing substances and protein were measured. Inorganic mercury was spiked into the urine, stabilizers were added and the mercury determined following storage. The results confirmed under-recovery of mercury in association with the urine matrix. Further investigations of how urinary components affect the measurement of mercury are necessary.

Keywords Occupational and environmental laboratory medicine · External quality assessment · Mercury in urine

Introduction

Mercury is a metal with extensive industrial application. Among its many uses the metallic form is employed, for

Presented at the Eurachem PT Workshop, October 2008, Rome, Italy.

All the authors are members of the thematic network "Organisers of external quality assessment/proficiency testing schemes related to occupational and environmental medicine".

A. Taylor (✉)
Centre for Clinical Science and Measurement,
Faculty of Health and Medical Sciences,
University of Surrey, Guildford GU2 7XH, UK
e-mail: a.taylor@surrey.ac.uk

R. L. Jones
Nutritional Biochemistry, Division of Laboratory Sciences,
National Center for Environmental Health, CDC,
Atlanta, GA 30341-3724, USA

A. Leblanc · J.-P. Weber
Centre de Toxicologie,
Institut National de Santé Publique du Québec,
945 Wolfe Avenue, Saint-Foy,
QC G1V 5B3, Canada

O. Mazarrasa
Laboratorio de Higiene Industrial,
Centro de Seguridad y Salud en el Trabajo,
Gobierno de Cantabria,
39012 Santander, Spain

M.-Y. Lee
Occupational Safety and Health Research Institute,
#34-4 Gusan-Dong, Bupyong-gu, Incheon 403-711,
Republic of Korea

P. J. Parsons
New York State Department of Health,
Wadsworth Center, PO Box 509, Albany,
NY 12201-0509, USA

M. Patriarca
Department of Veterinary Public Health and Food Safety,
Istituto Superiore di Sanità, 00161 Rome, Italy

C. Weykamp
MCA Laboratory, Queen Beatrix Hospital,
7101 BN Winterswijk, The Netherlands

Published online: 18 March 2009

example, in the production of chlorine, in scientific instruments and in dental amalgam. Inorganic mercury compounds have been used in batteries and within agro-chemical industries while organo-chemical forms are also important industrially. The environmental relevance of mercury derives from the presence of methyl mercury in foods, especially shellfish and large predatory seafoods such as tuna and swordfish. Release of mercury from dental amalgam and the possible implications on the health of individuals with large numbers of restorations is also seen as a matter of concern [1, 2].

Harmful effects of mercury, in all its forms, are well recognised. Occupational exposure is controlled in many countries by general legislation [3] and by regulations specific to this metal [4, 5]. Despite the imposition of maximum allowable occupational and environmental exposures and of biological monitoring standards, incidents affecting individuals or whole communities leading to severe clinical problems are reported. Therefore, programmes of risk evaluation and reduction, involving measurements of exposure to and absorption of the metal, are of obvious importance.

Procedures used to analyse biological specimens are often complex; nevertheless, accurate results are crucial. It is recognised that truly independent assessments of laboratory performance are provided by properly constructed and managed external quality assessment schemes (EQAS) and participation in EQAS (or proficiency testing schemes) is recommended as one of the methods to assure the quality of results.

Measurement of mercury, both in real samples and those prepared for EQA, is complicated by the volatility of this element and its potential loss from the test sample. This topic has been previously investigated with specimen types having minimal matrix, such as water and urine [6–8] and it is recommended that oxidising agents should be added to samples to ensure that the mercury is in the ionised form and is not reduced to the volatile, uncharged Hg^0 state. Oxidising agents that have been taken for this purpose include HNO_3, K_2CrO_7, KBr/BrO_3 and sulfamic acid.

The organisers of EQAS for trace elements have observed that when test samples are prepared by addition of known amounts of inorganic mercury to urine there is failure to recover all the added analyte even though stabilizing agents have been added (Fig. 1). The UK samples are prepared and stored at −20 °C for up to 3 months before being analysed by scheme participants. The level of under-recovery is seen to be associated with the particular urine pool used for preparation and not to the interval between manufacture and analysis. In addition, the range of results reported by participants is much wider than is typically seen with similar assays such as measuring cadmium in urine. Furthermore, the under-recovery is inconsistent

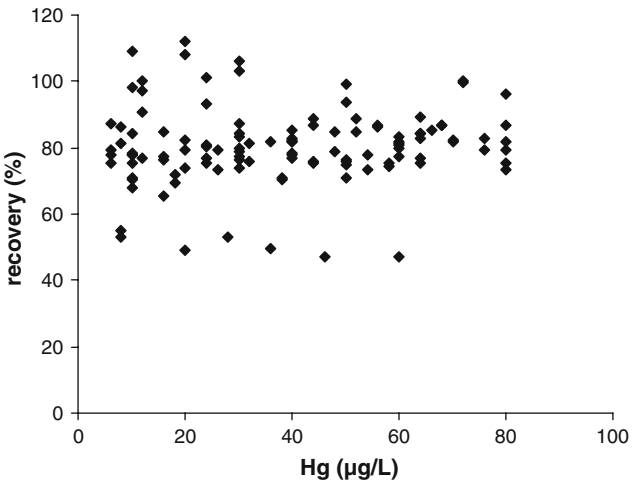

Fig. 1 Recovery (%) of mercury (as $HgCl_2$) added to urine. UK Scheme. Samples distributed between October 2004 and June 2008. The recovery was calculated using the arithmetic average of participant's results for each sample. The arithmetic mean and standard deviation for all data points is 80.5 ± 9.3%

with differences dependent on the original urine base material. This under-recovery and the wide range of results are not seen with blood samples.

Possible factors that may be relevant to the stability of mercury in urine and to the poor inter-laboratory agreement were considered. A lack of skill or expertise among the laboratories was discounted as many had considerable experience and report very good results when measuring other elements or with mercury in blood. Loss of mercury consequent upon reduction and volatilization or because of adsorption onto or diffusion through the walls of the containers was thought to be possible, though unlikely when stabilizing agents were added. Two further features that might be influencing results were suggested. These were a method-related problem and an undescribed interference from the urine matrix.

Experiments to investigate these factors were set up and the preliminary results reported here.

Experimental

1. Mercury, as $HgCl_2$ was added to four aqueous solutions to give a final concentration of 100 µg/L. The aqueous solutions had been prepared from purified water having a resistivity of greater than 18 MΩ cm, and each contained one of the following stabilizing agents; 10 mL/L HNO_3, 10 g/L sulfamic acid, 10 g/L bovine serum albumin. The fourth solution had no stabilizer added. The concentration of 100 µg/L was used as it is well above the detection limits of most methods and results should not be influenced by poor precision associated with lower levels. Such

concentrations may be seen in real samples from workers where there is poor occupational hygiene or in patients with mercury poisoning. Aliquots of these solutions were sent to participants of the UK EQAS for measurement of the mercury concentrations.

2. Urine samples were collected from 15 human volunteers, living in Guildford, at the conclusion of a 3-day period during which they completed a food diary to record the details of their dietary intake. A portion from each urine sample was analysed to determine the concentrations of sodium, potassium, chloride, urea, glucose, calcium, phosphate, magnesium, uric acid, protein, creatinine using the methods established for use on the Siemans Advia 1800 clinical analyser. Mercury, as $HgCl_2$, was added to further portions of the urine specimens to increase the endogenous concentrations by 20 µg/L. These samples were stored at +4 °C for 7 days and the mercury concentrations of these and the unspiked samples were measured by inductively coupled plasma mass spectrometry (ICP-MS) at the Centre for Clinical Science and Measurement, University of Surrey.

All human specimens/data were collected and used in accordance with procedures approved by the Surrey Regional Ethics Committee.

Statistical methods

Results from participants in the EQAS for mercury in urine were evaluated by calculation of the arithmetic average, standard deviation and the coefficient of variation (%) following removal of outliers according to the method of Healy [9]. For the results reported from the aqueous samples, the same parameters were determined, without outlier removal, using Microsoft Excel. Student's *t* test was used to compare data obtained from different analytical techniques. A probability value (p) of <0.05 was taken to indicate statistical significance. Pearson's correlation coefficient was determined to test for possible association between components of urine samples and the recovery of mercury [10].

Results and discussion

The results reported for the four aqueous samples are summarised in Fig. 2. Consistent with many previous studies, at only 55% the recovery of mercury was very low for the solution with no stabilizer added. Following addition of nitric or sulfamic acids or bovine serum albumin, recovery was approximately 90%. However, when the

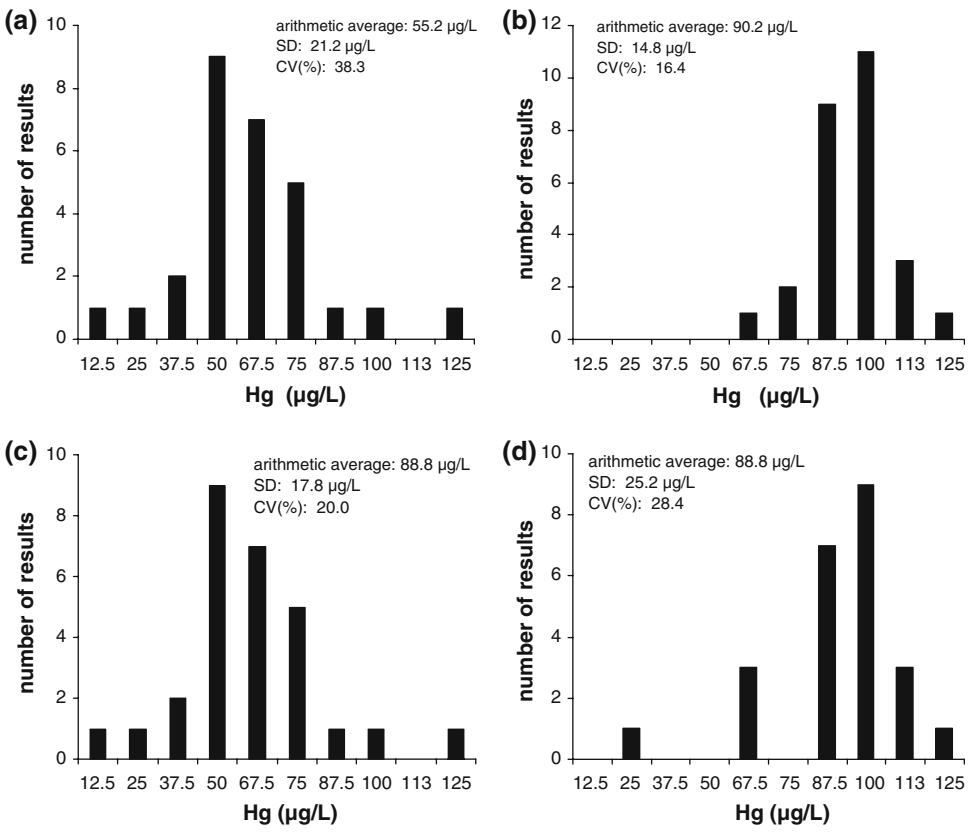

Fig. 2 Distribution of results reported by participating laboratories ($n = 28$) who measured the concentration of mercury in aqueous samples prepared to contain 100 µg/L Hg, with or without the addition of stabilizing agents. **a** No addition, **b** 10 mL/L nitric acid, **c** 10 g/L sulfamic acid, **d** 10 g/L bovine serum albumin. The arithmetic average (µg/L), standard deviation (SD) and coefficient of variation (CV) are shown for each sample

distribution of results is examined (Fig. 2), it is seen that, for the stabilized samples, many of the laboratories reported results close to the formulated concentration and the arithmetic average values were reduced due to the influence of a small number of low results. However, recalculation of the robust mean using Algorithm A from ISO 13528: 2005 failed to revise these values. These data were compared with those achieved for a urine specimen with a similar concentration of mercury and with 10 mL/L nitric acid, which was analysed by the participants at about the same time (Fig. 3). At 89% the recovery was higher than seen with many other samples but the spread of results as indicated by the coefficient of variation (%) and the distribution (Fig. 3) was about twice that of the simple aqueous solution.

The data were examined according to whether results were obtained by cold vapour atomic absorption spectrometry (CVAAS) or by ICP-MS. The results are summarised in Table 1. There was no significant difference ($p > 0.2$) between results for the two techniques with any of the samples.

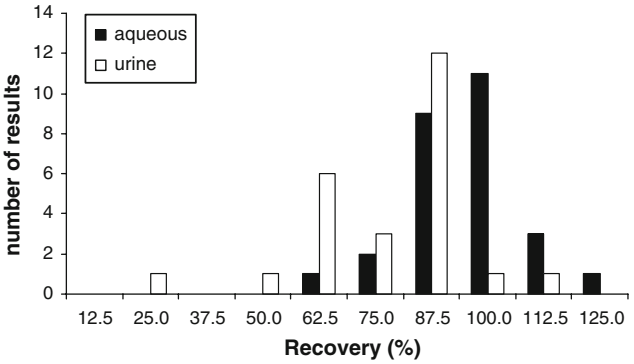

Fig. 3 Distribution of results reported by participating laboratories who measured the concentration of mercury in an aqueous sample prepared to contain 100 μg/L Hg and 10 mL/L HNO_3 ($n = 28$), in May 2008, and in a urine sample prepared to contain 80 μg/L Hg and 10 mL/LHNO_3 ($n = 24$), in February 2008. The arithmetic average (μg/L), standard deviation (μg/L) and coefficient of variation (%), respectively, were aqueous sample—90.2, 14.8 and 16.4; urine sample—71.2, 21.5 and 34.4. Results are expressed as percentage of recovery to allow direct comparison

The results confirm that recovery from unstabilized aqueous solutions is poor whereas those to which stabilizing agents had been added recoveries were closer to 100% suggesting that there is little or no actual loss of mercury from the solutions. Nitric and sulfamic acids were investigated as one or other of these are added to urine in several of the EQAS schemes. Bovine serum albumin was added as an attempt to mimic a blood sample. Similar results were obtained with CVAAS and ICP-MS. The range of results reported for the aqueous solutions is less than with urine samples. From these results it was concluded that the problems are associated with the matrix of the urine used to prepare the EQAS sample.

Results from the measurement of various components of urine are given in Table 2. The table also shows that recovery of mercury from these specimens was between 51 and 98%. This analysis provided a little insight as to what might be interacting with Hg to cause the metal to be unavailable for measurement. Four components showed a correlation coefficient greater than ±0.3, i.e., protein (Fig. 4), sodium, calcium and glucose. However, the associations were not straightforward as is seen for urinary protein. The highest recovery of mercury, 98%, was from the sample with the most protein, 0.28 g/L yet the sample with the next highest protein concentration had one of the lowest recoveries of mercury. It is likely, therefore, that interactions are rather more complex involving a number of components. No dietary items recorded in the diet diaries appeared to be associated with the recovery of mercury.

Conclusion

Previous investigations of mercury in dilute solutions have been directed at the use of stabilizing agents and container

Table 1 Arithmetic average (μg/L), standard deviation (μg/L) and coefficient of variation (%) of results for mercury in aqueous samples prepared to contain 100 μg/L Hg, with or without the addition of stabilizing agents, reported by participating laboratories who used cold vapour atomic absorption spectrometry ($n = 16$) or inductively coupled plasma mass spectrometry ($n = 12$)

	None		Nitric acid		Sulfamic acid		BSA	
	CV-AAS	ICP-MS	CV-AAS	ICP-MS	CV-AAS	ICP-MS	CV-AAS	ICP-MS
Arithmetic average	52.2	58.2	90.6	89.2	89.2	87.8	87.2	97.2
SD	21.0	22.4	12.2	18.8	18.4	18.2	17.8	23.8
CV (%)	40.2	38.5	13.4	21.2	20.7	20.8	20.3	24.4
p	>0.5		>0.8		>0.8		>0.2	

p statistical probability, *BSA* bovine serum albumin

Table 2 Concentrations of urinary components in 15 different urine specimens, the recovery of mercury added to the urine [*R* (%)] and the correlation coefficients (*r*) between recovery and concentrations of these components

R (%)	Na	K	Cl	Urea	Gluc	Ca	PO_4	Mg	Uric	Prot	Creat
76.1	66.0	52.6	74	229.8	0.1	4.19	20.33	3.37		0.08	8.34
74.4	71.7	53.8	78	231.9		2.87	20.98	3.19	0.872	0.07	7.60
76.1	71.4	52.7	270	189.4	0.1	3.23	15.80	2.50	0.058		6.48
86.5	44.8	23.5	402	99.8	0.1	1.16	9.54	0.92	0.035	0.01	3.15
98.0	64.8	29.6	531	121.7	1.5	0.95	11.27	1.69	0.037	0.28	4.16
78.2	68.4	33.3	529	173.5	0.3	1.59	14.51	1.70	0.030	0.01	5.21
84.0	63.4	41.9	552	182.0	6.5	2.91	17.34	2.48	0.405	0.02	6.28
83.0	52.5	33.4	489	21.8	0.5	1.96	17.87	2.10	0.024	0.03	6.95
79.0	51.9	24.5	482	103.8	0.1	1.79	8.23	2.12	0.034	0.02	3.46
83.3	65.1	23.7		134.7	11.3	1.39	13.81	1.65	0.038	0.03	4.79
71.4	50.3	32.5	483	111.0	0.2	1.70	10.10	1.50	0.023	0.04	3.68
81.2	83.8	45.3		171.0	0.2	1.95	14.38	1.45	0.018	0.03	5.74
78.2	52.0	38.0	499	142.0	0.2	2.01	12.50	2.25	0.018	0.04	4.28
96.9	78.7	36.2	551	193.0	2.2	1.43	14.07	2.41	0.078	0.06	5.90
51.0	49.4	33.6	559	119.0	0.2	1.92	10.20	1.67	0.028	0.01	4.07
r	0.39	−0.18	0.13	0.03	0.35	−0.36	0.10	0.02	−0.05	0.52	0.10

Abbreviations and concentration units are *Na* sodium (mmol/L), *K* potassium (mmol/L), *Cl* chloride (mmol/L), Urea (mmol/L), *Gluc* glucose (mmol/L), *Ca* calcium (mmol/L) PO_4 inorganic phosphate (mmol/L), *Mg* magnesium (mmol/L), *Uric* uric acid (mmol/L), *Prot* protein (g/L), *Creat* creatinine (mmol/L)

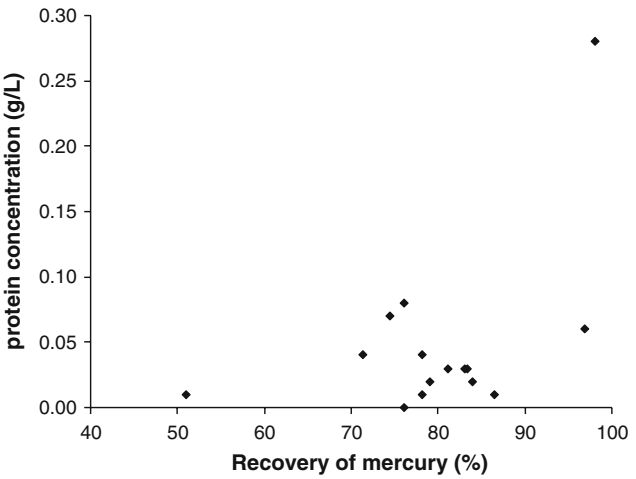

Fig. 4 Correlation between concentrations of protein in urine and the recovery of added mercury

materials to prevent losses by evaporation, diffusion and/or adsorption. Experience of the EQAS organisers suggests that other mechanisms are important with urine samples. From the work reported here it is seen that the under recovery of inorganic mercury added to urine and the poor interlaboratory agreement are seen to be associated with the matrix of the urine rather than loss of the metal. The presence of protein in urine may afford some stability but other factors are also involved. Further work to investigate this problem is necessary. Experiments using samples from occupationally exposed individuals, with naturally increased concentrations, are in place. It is also planned to assess whether lyophilisation will produce stable test items for use in EQA schemes. The issue of assessing accuracy of measurement will be addressed by distribution of a certified reference material on a single occasion.

References

1. World Health Organisation (1991) Environmental Health Criteria Series 118. Inorganic mercury. ILO/UNEP/WHO, Geneva
2. Commission on Life Sciences (2000) Toxicological effects of methylmercury. National Academy Press, Washington, DC
3. European Union (2005). Proposal for a Regulation of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency and amending Directive 1999/45/EC and Regulation (EC) [on Persistent Organic Pollutants] Proposal for a Directive of the European Parliament and of the Council amending Council Directive 67/548/EEC in order to adapt it to Regulation (EC) of the European Parliament and of the Council concerning the registration, evaluation, authorisation and restriction of chemicals. (COM 2003 0644 (03))
4. Health & Safety Executive (2005) EH40/2005 Workplace exposure limits. HSE Books, London
5. Health & Safety Executive (1997) Biological monitoring in the workplace. A guide to its practical application to chemicals. HSE Books, London

6. Rosain RM, Wai CM (1973) The rate of loss of mercury from aqueous solution when stored in various containers. Anal Chim Acta 65:279–284. doi:10.1016/S0003-2670(01)82493-4
7. Trujillo P, Stein P, Campbell E (1974) The preservation and storage of urine samples for the determination of mercury. Am Ind Hyg Assoc J 35:257–261
8. Lo LM, Wai CM (1975) Mercury loss from water during storage: mechanisms and prevention. Anal Chem 47:1869–1870. doi:10.1021/ac60361a003
9. Healy M (1982) Algorithm AS 180: a linear estimator of standard deviation in symmetrically trimmed normal samples. Appl Stat 31:174–175. doi:10.2307/2347985
10. Wessa P (2008), Pearson Correlation (v1.0.3) in Free Statistics Software (v1.1.23-r3), Office for Research Development and Education. http://www.wessa.net/rwasp_correlation.wasp/