

## Comparison of hair, nails and urine for biological monitoring of low level inorganic mercury exposure in dental workers

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*Received 6 October 2003, revised form accepted 2 February 2004*

Creatinine-corrected urine mercury measurements in spot urine samples are routinely used in monitoring workers exposed to inorganic mercury. However, mercury measurement in other non-invasive biological material has been used in some epidemiological studies. Dentists and dental nurses remain a group of workers with potential exposure to inorganic mercury through their handling of mercury-containing amalgam, although changes in work practices have reduced the current, likely exposure to mercury. Therefore, dental workers remain an occupational cohort in whom the value of using different biological media to identify exposure to low level inorganic mercury can be investigated. Samples of head hair, pubic hair, fingernails, toenails and urine were analysed for mercury content from a cohort of UK dentists ( $n = 167$ ) and a socioeconomically similar reference population ( $n = 68$ ) in whom any mercury exposure was primarily through diet. The mercury content in all biological material was significantly higher in the dental workers than in the control population ( $p < 0.0001$ ). The geometric mean and 90th percentile mercury concentrations in the urine samples from dentists were  $1.7$  and  $7.3 \mu\text{mol mol}^{-1}$  creatinine, respectively, with only one sample having a value at around the UK's Health and Safety Executive biological monitoring health guidance level of  $20 \mu\text{mol mol}^{-1}$  creatinine. Receiver operator characteristic analyses suggested that the ability of the biological material to discriminate between dentists and referents were fingernails  $>$  urine  $\approx$  toenails  $>$  pubic hair  $\approx$  head hair. Further investigation is warranted as to why fingernails appear to be such a good discriminator, possibly reflecting some contribution of direct finger contact with amalgam or contaminated surfaces rather than systemic incorporation of mercury into growing nails. Good correlation between head hair and pubic hair mercury levels in all subjects was obtained ( $r = 0.832$ ), which was significantly improved when hair samples weighing  $< 10$  mg were excluded ( $r = 0.868$ ). Therefore, under these exposure conditions and using the described pre-analytical washing steps, there is little influence from atmospheric contamination on the level of mercury content of head hair. The choice of non-invasive biological materials for mercury analysis depends on a number of considerations. These include the toxicokinetics of urinary mercury excretion, the growth rates of hair and nail, the nature and time-frame of exposure, and the fact that urine mercury may not reflect the body burden level from dietary methyl mercury. However, the data from this study suggests that urine mercury remains the most practical and sensitive means of monitoring low level occupational exposure to inorganic mercury.

**Keywords:** mercury, hair, urine, occupational exposure.

### Introduction

Dentists and dental nurses, through their handling of mercury-containing amalgam, remain a group of workers with potential exposure to inorganic mercury,

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although changes in work practices have reduced the current, likely exposure to mercury. Symptoms of low level mercury poisoning are subtle (e.g. headaches, fatigue, nausea, personality changes) and the possibility of effects at low levels are still controversial, even though regulatory bodies such as the UK Health and Safety Executive (HSE) have established health-based atmospheric and biological exposure standards.

Exposure to mercury has been determined in different biological matrices, with the use of hair and nail samples gaining a significant amount of attention over the past three decades. Hair and nails have been used to investigate environmental and occupational exposure to mercury because of the ease of collection and storage of the samples and the fact that these media can reflect long-term exposure to mercury. It is known that hair mercury concentration reflects the blood mercury concentration at the time the hair was formed (Suzuki *et al.* 1989). The regular analysis of mercury in hair or urine has been recommended for monitoring mercury exposure in dental workers in the UK (McMullin *et al.* 1982, Pritchard *et al.* 1982).

However, hair and nail analysis can be problematic due to possible external contamination, and there are wide ranges of normal values that can make the interpretation of exposure data difficult (Morton *et al.* 2002a). Occupational exposure assessment is usually based on the concentration of mercury in urine and blood (Mason *et al.* 2001). Urinary mercury is known to reflect exposure over the previous 2–4 months, whilst blood mercury reflects more recent exposure and is used to establish acute exposure to mercury. In the UK the HSE have set a health guidance value for mercury exposure based on urinary measurements of  $20 \mu\text{mol mol}^{-1}$  creatinine (HSE 1996).

In 1978 a Glasgow study carried out and reviewed by Lenihan indicated that head and body hair and nails from dentists and dental assistants showed elevated mercury levels when compared with samples from subjects who were not exposed. In most cases, the levels were two or three times higher than in unexposed workers (Lenihan *et al.* 1978). Other studies have also documented elevated mercury levels in hair from dentists (Hefferen 1976, Lin *et al.* 1982), and it is accepted that occupational mercury exposure in dental workers can result in elevated hair and nail concentrations.

The aim of this study was to compare the mercury content in five different biological media (head hair, pubic hair, fingernails, toenails and urine) in occupationally exposed and control samples. Pubic hair and toenails were incorporated into the survey to investigate the possible influence of external contamination.

## Materials and methods

All hair and nail analyses were carried out at the Health and Safety Laboratory, Sheffield, UK, and urine mercury levels were determined by the Trace Element Unit, Glasgow Royal Infirmary, Glasgow, UK.

### Study population

In total, 180 dentists and 180 controls took part in the survey, providing biological samples and completing questionnaires about the use of mercury, the number of dental fillings, any symptoms and general health questions.

**Dentist.** The Dental Practice Division of the Common Services Agency provided a list of registered dentists practising in four health board areas in the West of Scotland. A total of 129 dentists (71.7%) were recruited by randomly sampling from the register (of about 900) and 51 dentists (28.3%) were self-selected volunteers (Ritchie *et al.* 2002).

**Controls.** Control subjects, matched to dentists by academic ability, were recruited from the staff of the University of Glasgow. Subjects in the control group were invited to participate via an article published in the university newsletter and through e-mail mailing lists of university employees and postgraduate students. E-mails were targeted to those staff likely to meet the requirements of having a first degree and not having been exposed to mercury on a regular basis.

#### Sample collection

Hair and nail samples were collected directly from the donors. Head hair samples were taken from the occipital region of the head and cut with scissors about 1 cm from the scalp. Pubic hair, urine, finger and toenail samples were collected by the donors themselves. Urine samples were collected as a spot sample. Plastic bags were provided for the hair and nail samples, and 25 ml universal containers were provided for the urine samples.

#### Hair and nail analysis

The hair and nail samples were weighed and then washed overnight with 5 ml of a solution of 0.1% v/v Triton X-100. Following drying and re-weighing, the samples were digested with concentrated nitric acid at room temperature overnight in sealed vessels. Then 10  $\mu\text{l}$  of gold standard ( $10\,000\text{ mg l}^{-1}$ ) was added to stabilize the mercury in the digest solution. The digests were then diluted with water and 10 ng  $\text{ml}^{-1}$  platinum was added as an internal standard. Analysis of the sample digests was carried out using an Elan 6100 ICP-MS (Perkin Elmer, Beaconsfield, UK). A digested certified reference material (CRM) of hair was analysed with every set of hair digests. The digestion method was validated by analysing a Chinese hair reference material (GBW09101) (LGC, Teddington, UK) with a reference range of  $2.16 \pm 0.21\text{ }\mu\text{g g}^{-1}$ , where a mean of  $2.22 \pm 0.30\text{ }\mu\text{g g}^{-1}$  ( $n = 12$ ) over nine sample batches was seen. Batch quality control was monitored using in-house reference urine and a reference hair sample (total weight 15 g). The reference hair sample had a nominal range of  $0.39 \pm 0.02\text{ }\mu\text{g g}^{-1}$ . The inter-batch range of this sample was  $0.42 \pm 0.08\text{ }\mu\text{g g}^{-1}$  ( $n = 19$ ), giving an inter-batch variation of 18% relative standard deviation.

#### Urine analysis

The urine samples were first acidified and gold added to stabilize the mercury in the sample, and then potassium dichromate solution was added to oxidize the mercury species. Following dilution, analysis of the urine was carried using cold-vapour atomic absorption spectroscopy at the Trace Element Unit, Glasgow Royal Infirmary (Ritchie *et al.* 2002). Results were expressed in relation to creatinine content, which was also measured at the Trace Element Unit.

## Results

The distribution of mercury concentration in the various biological materials was shown to be log-normal in both controls and dental workers. Therefore appropriate transformation of data was undertaken for statistical analyses. Geometric means and ranges for the concentrations of mercury measured in control and exposed populations are given in table 1; comparisons using unpaired *t*-tests are also shown. Mercury levels in urine, head hair, pubic hair, toenail and fingernail were significantly higher in the dental workers. Only one of the dental workers had a urinary mercury level ( $20.9\text{ }\mu\text{mol Hg mol}^{-1}$  creatinine) marginally greater than the Health Guidance Value for mercury exposure of  $20\text{ }\mu\text{mol Hg mol}^{-1}$  creatinine set by the HSE. Figure 1 shows the distribution of urinary mercury results in the dental workers.

Correlations between mercury levels in the various biological samples were also investigated after combining control and dental workers (table 2). During the

Table 1. Geometric means and ranges of mercury concentrations found in hair, nails and urine from controls and a potentially exposed group of dental workers. Appropriate log-transformation of the data was performed before comparison using the *t*-test.

	Controls	Dental workers	<i>p</i> value
Head hair – all samples			
No. of samples	161	161	
Mean ( $\mu\text{g g}^{-1}$ )	0.43	0.71	< 0.0001
Range ( $\mu\text{g g}^{-1}$ )	0.04–3.86	0.10–5.67	
Head hair – samples > 10 mg			
No. of samples	108	105	
Mean ( $\mu\text{g g}^{-1}$ )	0.40	0.68	< 0.0001
Range ( $\mu\text{g g}^{-1}$ )	0.06–2.46	0.10–3.19	
Pubic hair			
No. of samples	168	167	
Mean ( $\mu\text{g g}^{-1}$ )	0.35	0.69	< 0.0001
Range ( $\mu\text{g g}^{-1}$ )	0.03–2.54	0.09–11.71	
Fingernail			
No. of samples	155	164	
Mean ( $\mu\text{g g}^{-1}$ )	0.24	1.42	< 0.0001
Range ( $\mu\text{g g}^{-1}$ )	0.02–2.49	0.12–239.6	
Toenail			
No. of samples	155	163	
Mean ( $\mu\text{g g}^{-1}$ )	0.18	0.43	< 0.0001
Range ( $\mu\text{g g}^{-1}$ )	0.02–1.22	0.02–14.74	
Urinary $\mu\text{mol Hg mol}^{-1}$ creatinine			
No. of samples	163	162	
Mean	0.38	1.73	< 0.0001
Range	0.03–4.2	0.20–20.9	

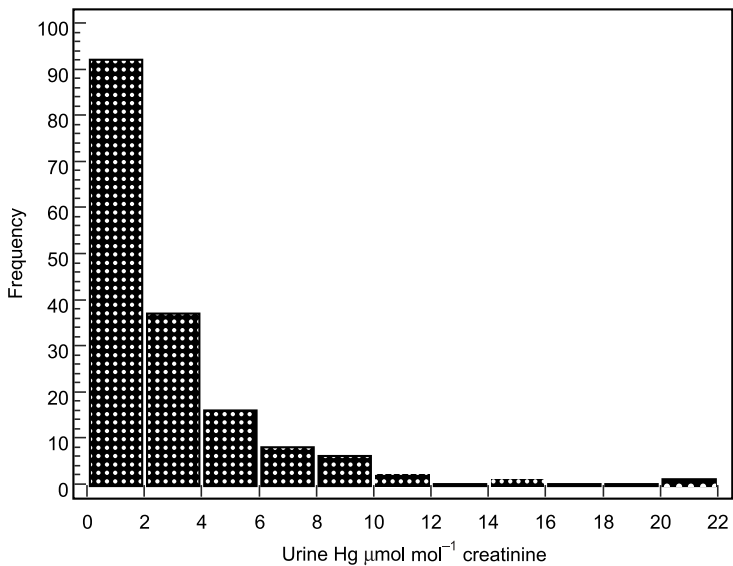


Figure 1. Histogram of urine mercury concentrations in dental workers (*n* = 167).

Table 2. Pearson correlation coefficients from analysis of log-transformed variables.

Correlations	r	95% confidence interval
Head hair samples > 10 mg versus pubic hair	0.868	0.832–0.898
Head hair (all samples) versus pubic hair	0.832	0.794–0.863
Fingernail versus toenail	0.707	0.647–0.759
Fingernail versus head hair samples > 10 mg	0.632	0.202–0.454
Fingernail versus head hair (all samples)	0.605	0.529–0.672
Urine versus fingernail	0.419	0.318–0.510
Urine versus toenail	0.315	0.206–0.416
Urine versus pubic hair	0.291	0.183–0.391
Urine versus head hair (all samples)	0.278	0.168–0.381
Urine versus head hair samples > 10 mg	0.295	0.069–0.332

course of the study, it became apparent that in head hair samples with a mass < 10 mg the method detection limit and the variance due to sample inhomogeneity was raised above acceptable levels. Once samples < 10 mg were excluded, the correlation between head and pubic hair showed no significant difference.

Using receiver operator characteristic (ROC) analysis, a comparison was made regarding the ability of the mercury analyses in varying biological media to discriminate between the control group, whose exposure to mercury was principally through diet, and the dental workers, where there is the additional potential source of occupational exposure to inorganic mercury through the handling of amalgam (table 3). Interestingly, ROC analyses suggested that fingernail mercury measurements were a significantly better discriminator between dental workers and controls than other measurements ( $p < 0.001$ ). Urine ( $p = 0.005$ ) and toenail ( $p = 0.002$ ) mercury levels were better discriminators than measurements in head hair. Whereas there was no significant difference in the discriminatory power of hair from different areas of the body, it was clear that discriminatory differences existed between nails obtained from fingers and those from toes. The order for the biological material in terms of discriminating between dentists and referents was fingernails > urine  $\approx$  toenails > pubic hair  $\approx$  head hair.

The criteria for fingernail, toenail, head hair, pubic hair and urine mercury concentrations that offer the best accuracy (lowest false-positive and false-negative results) in discriminating between controls and dentists were 0.51, 0.27, 0.63 and 0.49  $\mu\text{g g}^{-1}$  and 1.0  $\mu\text{mol mol}^{-1}$  creatinine, respectively.

Table 3. Receiver operator characteristic (ROC) analysis of various mercury measurements to discriminate between dental workers and controls.

	Area under curve	95% confidence interval	Criterion value of highest accuracy
Fingernail	0.929***	0.891–0.957	0.51 $\mu\text{g g}^{-1}$
Urine	0.826*	0.774–0.820	1.0 $\mu\text{mol mol}^{-1}$
Toenail	0.794*	0.740–0.841	0.27 $\mu\text{g g}^{-1}$
Pubic hair	0.744	0.687–0.796	0.49 $\mu\text{g g}^{-1}$
Head hair	0.735	0.655–0.768	0.63 $\mu\text{g g}^{-1}$

\*\*\*  $p < 0.001$  compared with all other biological materials; \*  $p < 0.05$  compared with head hair.

## Discussion

The results of the urine analysis confirm that exposure to mercury for almost all dentists is substantially below the HSE's current biological monitoring Health Guidance Value. In fact, the majority of dentists had urine concentrations significantly less than our laboratory reference range ( $2 \mu\text{mol mol}^{-1}$  creatinine). The results are comparable with a recent Swedish study that reported an average urine mercury concentration of  $3 \mu\text{mol mol}^{-1}$  creatinine in dental workers (Langworth *et al.* 1997).

Good correlation between head and pubic hair concentrations indicate that there was either negligible surface contamination of head hair in the environment examined or that the washing procedure used removed any external contamination. The latter reason appears less likely since inorganic mercury irreversibly binds to hair and can only be removed by washing with weak acids (Morton *et al.* 2002a, b). However, the relatively poor correlation between the hair and urine results highlights that the mercury sources may be different or that toxicokinetic considerations may be important. It is known that the principal routes of excretion for inorganic mercury are urine and faeces (Clarkson *et al.* 1988), but urine is a minor route of excretion for methyl mercury, which can be found in dietary elements such as fish. Methyl mercury is accumulated in hair at the time of formation and reflects the concentration in blood (Kershaw *et al.* 1980). This suggests that mercury in hair could be both from dietary and exogenous exposure. Exogenous exposure could be from sources such as shampoos, cosmetics and other environmental influences, all of which may mask any low level exposure to inorganic mercury. A recent study concluded that between 70% and 100% of mercury in hair may be present as methyl mercury, and that hair samples can be considered good indicators of human exposure to methyl mercury, usually from fish consumption (Vasconcellos *et al.* 1988).

It can be seen from this study that mercury levels in all the measured biological materials are significantly higher in dentists compared with the unexposed population. A similar but smaller Scottish study carried out on hair and nail samples in 1973 showed that the hair (both pubic and head) and nails (both finger and toe) of dentists had significantly higher concentrations of mercury than unexposed persons, and that fingernail data gave the highest concentration of mercury at 62 p.p.m. (Lenihan *et al.* 1973). This study hypothesized that fingernails can gather external contamination, particularly from the handling of amalgam. The high discriminatory ROC value found for fingernails and the significant difference from toenails in our study suggest that there is a remaining problem with direct contact of mercury with the fingers. The relationship between fingernail mercury and the specific work tasks of dental workers is worthy of further investigation.

A recent study reported a mean toenail mercury concentration of 0.94 p.p.m. in dentists, around twice that found in non-dental health professionals, and that in all subjects fish consumption was a primary exposure factor associated with toenail mercury levels (Joshi *et al.* 2003). A study of mercury in head hair from New York dentists in 1985 showed a mean concentration of 2.98 p.p.m., roughly four times the value in our study; a smaller longitudinal element to this study suggested a

three-fold decrease in head hair mercury levels from 1972 to 1985 (Scarlett *et al.* 1988). Head hair mercury levels similar to those found in our study were reported in another study of US dentists (Francis *et al.* 1982). A German study reported average head hair concentrations in dentists that were twice that found in referents, a similar result to the present study (Ott *et al.* 1991). Interestingly, this study reported that head hair levels in dentist were a quarter of those found in workers in the chloralkali industry, where very large amounts of mercury are used in the electrolytic process; the authors noted that hair mercury concentrations in dentists were correlated with fish consumption.

A study of trace elements in female toenails in the US ( $n = 127$ ) in 1982 and in 1988 showed mean mercury concentrations of  $0.87$  and  $0.67 \mu\text{g g}^{-1}$ , respectively (Garland *et al.* 1993), which are higher than we found in unexposed persons. Lower levels of mercury in hair and nails were reported in a survey carried out in non-occupationally exposed Swedish inhabitants, with mean levels of  $0.261 \mu\text{g g}^{-1}$  (range  $0.053$ – $0.927 \mu\text{g g}^{-1}$ ) for head hair ( $n = 114$ ) and  $0.122 \mu\text{g g}^{-1}$  (range  $0.028$ – $0.311 \mu\text{g g}^{-1}$ ) for fingernails (Rodushkin and Axelsson 2000). Other published ranges of hair mercury levels include unexposed 'normal' concentrations of  $0.5$ – $10 \mu\text{g g}^{-1}$  in 1992 in the US (Rodushkin and Axelsson 2000) and a mean of  $0.3 \mu\text{g g}^{-1}$  (range  $0.03$ – $0.802 \mu\text{g g}^{-1}$ ) for 40 unexposed subjects in 2000 (Katz and Katz 1992). A recent study involving more than 29 000 dentists reported mercury toenail concentrations of  $0.91 \pm 1.47 \mu\text{g g}^{-1}$  compared with control concentrations of  $0.45 \pm 0.40 \mu\text{g g}^{-1}$  (Yoshizawa *et al.* 2002). The mean values, although higher than those reported in the present study, are consistent with the ranges quoted here.

Hair and nail analysis have a useful place in biomonitoring, particularly in estimating long-term or historical exposure for some trace elements. Hair measurements also have the capability of 'time-profiling' exposure through segmental analysis. However, largely due to the need for pre-analytical sample preparation and possible contamination problems from external sources, urine has usually been used in occupational biological monitoring strategies to measure mercury exposure (Mason *et al.* 2001). Urine mercury reflects cumulative exposure over 2–4 months. Blood mercury levels respond immediately to inhaled mercury vapour and are reported to reflect exposure over several days to 2 weeks, making them well suited to monitoring acute exposure despite requiring an invasive sample. However, it must be noted that (i) urinary mercury determinations may not fully reflect the internal dose of dietary methyl mercury; (ii) the potential losses of mercury through bacterial reduction may indicate the need for stabilization of samples depending on storage conditions between collection and analysis; and (iii) there is a diurnal variation in urinary mercury excretion, although this has been suggested as relatively unimportant in the total variability when using creatinine-corrected results in untimed urine samples (Calder *et al.* 1984, Mason and Calder 1994). Urine is likely to remain the matrix routinely used to monitor occupational exposure to inorganic mercury, but analysis of other biological material may be necessary in some studies, especially where the importance of dietary intake of methyl mercury needs to be considered.

## Acknowledgements

The authors would like to thank Warren Cairns, Barry Smith and Monica Martinez for their contributions to the project.

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