Mercury and selenium in workers previously exposed to mercury vapour at a chloralkali plant

Dag G Ellingsen, Roy I Holland, Yngvar Thomassen, Marit Landro-Olstad, Wolfgang Frech, Helge Kjuus

Abstract
The concentrations of total mercury (B-Hg), inorganic mercury (B-IHg), and methyl mercury (B-MeHg) in whole blood, urinary mercury (U-Hg), and selenium in urine (U-Se) and whole blood (B-Se) were determined in 74 chloralkali workers previously exposed to Hg vapour, and compared with 51 age matched referents. Dental amalgam state, fish consumption, and exposure related indices were studied with regard to the determined elements. A significant relation between the surface of dental amalgam and U-Hg (Pearson's r = 0.63, p < 0.001) was found among the referents. Mean U-Se was significantly lower (p < 0.001) among the subjects previously exposed to Hg (34.1 nmol/mmol creatinine) compared with that for the referents (42.6 nmol/mmol creatinine). A significant negative relation between the cumulative Hg dose and U-Se was also found. The mechanisms and the clinical significance of these findings are not clear. No relation between current U-Hg and previous occupational exposure to Hg was found among subjects in whom exposure had ceased more than one year before the study.

Workers in the mercury (Hg) based chloralkali industry are exposed to Hg vapour. Liquid Hg is used as a cathode in the electrolysis of brine during production of chlorine and caustic soda. Leakage and maintenance work may lead to the release of Hg vapour into the working atmosphere.

The release of Hg vapour from dental amalgam restorations has been suggested as a source of exposure, and several authors have reported an association between the amount of dental amalgam and urinary Hg concentration (U-Hg). A decrease in the concentration of blood and plasma Hg was found after removal of dental amalgam fillings. Some authors have suggested an association between the amount of Hg in the human brain and dental amalgam fillings.

The main source of exposure to dietary Hg is the consumption of fish contaminated with methyl Hg (MeHg). Inorganic Hg (IHg) in foodstuffs is poorly absorbed in the gastrointestinal tract and it is generally assumed that the exposure is low, contributing little to the total Hg uptake in the general population. Hg vapour is believed to be the predominant form of Hg in the atmosphere.

The interaction between selenium (Se) and inorganic Hg is not completely understood. The acute nephrotoxic effect of mercuric Hg in rats is decreased by the simultaneous administration of Se, and it has been suggested that Se may be an effective protective agent against the acute toxicity of Hg. Several authors have found decreased urinary Se concentrations (U-Se) among subjects under ongoing exposure to inorganic Hg, whereas others have reported higher concentrations. Postmortem studies on retired Hg miners and dentists have shown significant amounts of Hg and Se in different tissues compared with unexposed referents several years after cessation of exposure, and a roughly 1:1 molar ratio between the elements has been found.

The aim of the present investigation was to study the concentrations of different Hg species and Se in whole blood and urine, and the possible relation between previous exposure to Hg and these concentrations among former chloralkali workers. The roles of Hg exposure from dental amalgam restorations and the consumption of fish were also considered.
Material and methods

SUBJECTS
The study was performed during 1989 as a part of a comprehensive study on possible chronic onward health effects among chloralkali workers previously exposed to Hg vapour in a chloralkali plant that was shut down in 1987.

Details on study design and exposure assessment have been published separately.20 Briefly, male workers under 65 years of age with at least one year of exposure to Hg vapour were eligible for inclusion in the study. The referents were randomly selected among men working in a nitrate fertiliser plant in the same industrial complex as the chloralkali plant and frequency matched for age. The participation rate for the dental examination was 79.6% (n = 74) among the chloralkali workers v 83.6% (n = 51) among the referents.

The exposed subjects were on average 44.9 (range 24.2-64.8) years old, and 58.1% were current smokers. The mean consumption of smoking tobacco was 44 (range 0-150)g/week. The mean age of the referents was 45.8 (range 24.3-63.7) years, and 68.6% were current smokers. Their mean consumption of smoking tobacco was 55 (range 0-250)g/week.

ASSESSMENT OF EXPOSURE
Since 1948 the workers at the plant were biologically monitored by the determination of U-Hg. A cumulative U-Hg dose (cumulative U-Hg) could thereby be calculated for each worker, based on more than 2300 U-Hg measurements performed among the exposed participants during their time of employment in the plant. The cumulative U-Hg dose expresses the sum of the individual mean U-Hg for each year employed. The subjects in the present study had been exposed to Hg vapour for on average 7.9 (range 1.1-36.2) years, and the exposure had on average ceased 12.4 (median 8.5, range 1-35) years before the study.

METHODS
An interview and a short clinical examination were carried out for all participants, and urine specimens from two consecutive days were collected. The first sample was a morning urine first void specimen from the day before examination. The subjects had been instructed to store the urine sample in a refrigerator. The second sample was from the morning of the examination day. The urine samples were collected and stored in NUNC polypropylene tubes (20 ml). A blood sample was obtained from the cubital vein and stored in a tube (10 ml) containing heparin (Venoject). The blood and urine samples were frozen and stored at -20°C until analysis. As urine was sampled on two consecutive days, the mean U-Se and U-Hg has been used in the data presentation.

Dental examination
A clinical dental examination was carried out by a dentist (MLO) in a regular dental surgery. A complete oral assessment was established for all participants. The amalgam restorations were recorded, both according to number of surfaces and by grading each restored surface with a score from 0.5 to 6.0, according to its size. Restorations of the occlusal, buccal, and lingual/palatal surfaces of the molars could be assigned a score from 0.5 to 6.0, and those of the proximal surfaces of the molars from 0.5 to 4.0. Restored surfaces in other teeth were scored from 0.5 to 3.0. The scores of the proximal surfaces of the molars and premolars were adjusted according to their extensions in the apical direction, which was determined from copies of x-ray films. By addition of the scores for all the amalgam surfaces, the value “amalgam points” was produced as an expression of the total amalgam surfaces. Occlusal points represent the score of the occlusal surfaces.

Se in whole blood (B-Se)
B-Se was measured by electrothermal atomic absorption spectrometry. A Perkin-Elmer 5100 atomic absorption spectrophotometer equipped with a Zeeman-effect based background corrector, an HGA-600 graphite atomiser, an AS-60 automatic sampler, and a Perkin-Elmer Se electrode discharge lamp were used. Pyrolytic graphite coated tubes without platforms were used and calibration was made against human whole blood Seronorm trace element 906 (Nycomed Ltd, Oslo, Norway) with a known content of Se; Se was added to this material to cover the concentration range of 0-2.5 μmol Se/l. After thawing, the whole blood samples were diluted 1:5 with an aqueous matrix modification solution containing 0.25% nickel nitrate and 0.2% triton X-100. B-Se was measured in three replicates of 20 μl diluted whole blood specimen. The detection limit was 0.02 μmol Se/l (2 SD). The accuracy and precision of the measurements were monitored by use of Seronorm trace element whole blood quality assurance materials, batches 905 and 906. The within day variation for these samples was typically 4% and the between day variation was slightly higher (typically 5%-6%). The average Se concentrations found in batch 905 were 1.26 (SD 0.06) μmol Se/l (n = 34) and in batch 906 1.24 (SD 0.08) μmol Se/l (n = 33). The certified values were 1.23 and 1.22 μmol Se/l respectively.

Se in urine (U-Se)
U-Se was measured by hydride generation atomic absorption spectrometry. A Perkin-Elmer Model 2100 atomic absorption spectrophotometer equipped with a continuous flow hydride genera-
tion system and a Perkin-Elmer Se electrode discharge lamp was used. The urine samples were decomposed after the International Union of Pure and Applied Chemistry recommended procedure with nitric, perchloric, and sulphuric acids, and each urine sample was measured from three replicate readings of 2 seconds. The detection limit of the method was 0.005 μmol Se/l (2 SD). The within and between day variations were typically 4% and 6%, respectively for Seronorm trace elements human urine, batch 108. The average concentration measured in this quality control material (0.60 (SD 0.03) μmol Se/l, n=8) was in accordance with the value recommended by the producer (0.62 μmol Se/l).

Hg in urine (U-Hg)
U-Hg was measured by direct SnCl₂/NaOH cold vapour atomic absorption spectrometry using a batch system coupled with an LDC Model 1235 mercury monitoring system. Each urine sample was measured from two replicate 125 μl injections into the reagent reservoir. The detection limit of the method was 2 nmol Hg/l (2 SD). The accuracy and precision of the measurements were ensured with a human quality assurance urine, Seronorm trace element 108. The within day and between day variations of this material were typically 1%. The average Hg concentration measured in Seronorm trace element 108 (245 (SD 2) nmol Hg/l, n =15) was in accordance with the certified value of 250 ± 10 nmol Hg/l.

Total mercury in whole blood (B-Hg)
B-Hg was measured after a nitric/perchloric acid digestion (maximum temperature 150°C, Tecator digestion system 40) and dilution to volume with 3 M HCl by the same instrumentation as used for the replicate urine. The digested blood solutions were measured from two 250 μl injections into a reagent reservoir of SnCl₂/HCl. The detection limit of the method was 3 nmol Hg/l (2 SD). The accuracy and precision of the measurements were ensured from Seronorm trace element human whole blood 904 and 905. The within day and between day precision of the measurements for these materials were typically 4% and 6% respectively. The average Hg concentrations measured (904: 24 (SD 2) nmol Hg/l, n = 8, 906:75 (SD 4) nmol Hg/l, n = 8) were in accordance with the values recommended by the producer of 20 and 70 nmol Hg/l respectively.

Methyl Hg (B-MeHg) and inorganic Hg (B-IHg) in whole blood
Mercury species were released by adding 0.6 M hydrochloric acid to the blood samples, and extracted as diethylcarbamate complexes (at pH 7) into toluene. After derivatisation with butyl magnes-
Table 1 Concentrations of Hg and Se in 74 exposed subjects and 51 referents†

<table>
<thead>
<tr>
<th></th>
<th>Mean (nmol/l)</th>
<th>Median (Range)</th>
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<tbody>
<tr>
<td>B-Hg</td>
<td>26.0</td>
<td>25.0 (12.0–61.0)</td>
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<td>28.3</td>
<td>26.0 (15.0–64.0)</td>
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<tr>
<td>B-MeHg</td>
<td>4.6</td>
<td>4.2 (0.2–13.9)</td>
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<td>6.3</td>
<td>5.2 (1.4–21.1)**</td>
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<td>B-IHg</td>
<td>1.7</td>
<td>1.4 (0.2–5.8)</td>
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<td>1.5</td>
<td>1.5 (0.2–4.0)</td>
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<td>U-Hg</td>
<td>1.8</td>
<td>1.4 (0.3–6.1)</td>
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<td>1.3</td>
<td>1.2 (0.3–3.0)*</td>
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<tr>
<td>B-Se</td>
<td>1.60</td>
<td>1.61 (1.07–2.27)</td>
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<td>1.57</td>
<td>1.57 (1.07–1.99)</td>
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<tr>
<td>U-Se</td>
<td>34.1</td>
<td>30.5 (7.0–144.0)</td>
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<td>42.6</td>
<td>39.0 (23.0–116.0)**</td>
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*p < 0.05; **p < 0.01; ***p < 0.001.
†B-MeHg and B-IHg were determined for 72 exposed subjects (E) and 50 referents (R).

Table 2 shows the four indices of dental amalgam assessment and fish consumption expressed as number of fish meals each month in the two groups under study. No significant differences between the exposed subjects and the referents were found.

The strongest relation between the amount of dental amalgam and U-Hg among the referents was found between amalgam points and U-Hg (Pearson’s r = 0.63, p < 0.001). Figure 2 shows this relation. All amalgam surfaces, occlusal surfaces, and occlusal points correlated slightly less well with U-Hg (r = 0.60, p < 0.001; r = 0.55, p < 0.001; r = 0.58, p < 0.001 respectively). The correlation between amalgam points and U-Hg among the subjects previously exposed to Hg was lower than among the referents (r = 0.41, p < 0.001). No significant association was found between the dental amalgam indices and B-IHg or B-Hg among the referents. Four referents with no dental amalgam fillings had on average 2.2 ng/g (range 0.8–3.1) B–IHg. Twenty per cent of the Hg in whole blood among the referents was in the inorganic state. A negative association between age and amount of dental amalgam was found among both referents and exposed subjects.

The significant relation between the number of fish meals each month and B-Hg (r = 0.40, p = 0.003) among the referents, was not found for B-MeHg (r = 0.27, p = 0.07). Figure 3 shows the association among the referents between number of fish meals each week, B-MeHg, and B-Hg respectively. Referents reporting eating less than one fish meal each week (mean 1.8 fish meals each month, range 1–2) had on average 5.2 ng/g B–MeHg and 22.3 nmol/l B-Hg. Referents who fished in their leisure time (n = 28) did not have higher B-Hg or B-MeHg compared with the other referents.

Table 3 shows univariate relations between some of the variables. Among the referents B-Se was positively correlated with B-MeHg (r = 0.38, p = 0.006) and negatively correlated with the current consumption of smoking tobacco (r = −0.40, p = 0.004, not shown).

Multiple stepwise linear regression analysis was used for further analysis of the concentrations of...
Table 3 Pearson’s correlation coefficients for selected univariate relations among 74 exposed subjects and 51 referents†

<table>
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<tr>
<th></th>
<th>U-Hg</th>
<th>B-Hg</th>
<th>B-MeHg</th>
<th>B-IHg</th>
<th>B-Se</th>
<th>U-Se (log)</th>
<th>Age</th>
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<td>B-MeHg</td>
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*p < 0.05; **p < 0.01; ***p < 0.001.
†B-MeHg and B-IHg were determined for 72 exposed subjects and 50 referents.

Figure 3 Relation between total blood mercury (B-Hg), methyl Hg in blood (B-MeHg), and number of fish meals each week among 51 referents (median and range).

elements in the two groups. The model used among the referents included as independent variables; age, amalgam points, number of fish meals each month, current consumption of alcohol (l/month), and current consumption of smoking tobacco (g/week). None of these variables contributed significantly to the models for U-Se(log), B-MeHg, or B-IHg. The amalgam points and fish meals each month were the only significant predictors for U-Hg and B-Hg respectively. Both current consumption of smoking tobacco and age were correlated with B-Se (multiple R = 0.50, p = 0.001; B-Se = 1.41 + 0.0048 age - 0.0011 tobacco consumption). Age was not included when B-MeHg was added to the model, but tobacco consumption remained (multiple R = 0.54, p < 0.001; B-Se = 1.52 + 0.018 B-MeHg - 0.0011 tobacco consumption) in the model. Consumption of fish meals each month was correlated with age among the referents in the present study (r = 0.40, p = 0.003).

Similarly, all exposed subjects (n = 74) were included in a stepwise multiple linear regression analysis of B-Se and U-Se(log), with the independent variables among the referents as well as time since cessation of Hg exposure and cumulative U-Hg. B-Se was negatively correlated with current tobacco consumption (B-Se = 1.66 - 0.0013 tobacco consumption, multiple R = 0.29, p = 0.01). U-Se(log) was associated with time since cessation of exposure and cumulative U-Hg. (U-Se(log) = 2.02 - 0.15 cumulative U-Hg (log) - 0.004 cessation of exposure, multiple R = 0.38, p = 0.004). Spearman rank correlation between untransformed U-Se and cumulative U-Hg was r = -0.25 (p = 0.03). Figure 4 shows the relation between cumulative U-Hg and U-Se.

In a further analysis 18 subjects who were still working in the building where production had ceased more than one year before the study were
excluded. These 18 subjects had mean U-Hg and B-Hg of 3·4 (range 1·0–6·1) nmol/mmol creatinine and 27·0 (range 20·0–38·9) nmol/l respectively. Their mean concentration of B-IHg was 2·3 (range 0·2–5·0) ng/g. The exposure among the remaining subjects had on average ceased 16·1 (median 18·0, range 1–35) years before the examination. The model included the variables used among the referents as well as time since cessation of exposure and cumulative U-Hg. Current U-Hg concentration was correlated with amalgam points and age (U-Hg = 1·95 + 0·007 amalgam points – 0·02 age; multiple R = 0·65, p < 0·001). The number of fish meals each month and amalgam points were both positively related to B-Hg (multiple R = 0·47, p = 0·001). Fish meals each month were also positively correlated with B-MeHg (multiple R = 0·37, p = 0·006). Cumulative U-Hg and years since cessation of exposure did not significantly contribute to any of the models.

Discussion

The comparability between the exposed subjects and referents has been discussed elsewhere. The participation rate was about 80% for both groups and it is not assumed that the variables studied should have influenced the participation rate in the present study. Hence, it may be assumed that the study populations are representative for a group of workers previously exposed to Hg vapour, and referents selected from a population of industrial workers with regard to Se and Hg state. The accuracy of the analytical procedures used for the determination of the elements in biological fluids in the present study is also regarded as acceptable.

Eighteen subjects previously exposed to Hg were still working in the building where production had ceased more than one year before the study. Their concentrations of B-Hg and B-IHg were comparable with those of the referents, indicating no current exposure. Their U-Hg, however, was higher than among the referents, which may reflect their kidney burden more than the one year postexposure. Cherian et al. suggested that U-Hg may be an index of renal concentration of Hg. The remaining exposed subjects had only slightly higher U-Hg than the referents. Among these the current B-Hg and U-Hg were not influenced by cumulative U-Hg or time since cessation of exposure.

Previous studies in Norway among workers occupationally unexposed to Hg have shown mean U-Se and U-Hg of 39 nmol Se/mmol creatinine and 1·75 nmol Hg/mmol creatinine,17 1·58 μmol Se/l in serum,24 and 24·8 nmol Hg/l in whole blood.24 Those results indicate that U-Se, U-Hg, B-Se, and B-Hg among the referents in the present study are comparable with concentrations found in previous Norwegian studies.

The participants delivered morning urine samples from two consecutive days, and a substantial variation was found for U-Hg between the days. Correction for urinary creatinine concentration reduced the variability. Other studies have found a reduction in the variability of U-Hg after correction for urinary dilution.27,28 As the analytical day to day variation in measurements of Hg at this concentration range is of the order of 5%, the calculated 35% variation between the days is presumably caused by a real day to day variation in urinary excretion; however, storage and sampling procedures may also contribute to this variation.

Several studies have shown a relation between the U-Hg and the amount of dental amalgam fillings. In our present study there was a highly significant association between the amalgam points and U-Hg (r = 0·63). The correlation coefficient was lower when the total number of amalgam surfaces was used. The relations between amalgam points and U-Hg from day 1 and day 2 were 0·57 and 0·62 respectively, indicating that the use of amalgam points and the mean individual U-Hg is a more appropriate expression of the biological relevance of the two variables than number of amalgam surfaces and one single value of U-Hg. The observation that mechanically active surfaces have a lower correlation with U-Hg than has total amalgam points is supported by similar observations made in another study.7 No association was found between the amalgam indices used in the present study and B-Hg or B-IHg among the referents; nor was there any relation between amalgam indices and B-Se or U-Se. This finding is by contrast with other studies, which have reported an association between B-Hg and amalgam restorations.7 Other
authors have found a poor correlation between B-Hg and the amount of amalgam restorations. It may be assumed that Hg vapour from dental amalgams should be measured as IHg in whole blood. Only 20% of total blood Hg among the referents was in the inorganic state. It has been suggested that chewing may influence the amount of Hg vapour in the mouth. Subsequent pulmonary uptake of Hg vapour may influence a possible relation between B-IHg and amalgam indices. The subjects in the present study were not instructed to avoid eating before a blood sample was taken, which may confound a possible association between amalgam restorations and B-IHg. It has been found that the removal of amalgam fillings reduces the concentration of Hg in whole blood and plasma.

A significant correlation between the number of fish meals each month and B-Hg, but not B-MeHg, was found among the referents. The B-MeHg among referents eating little fish was comparable with that among the remaining referents. One explanation may be that other sources of fish consumption exist, such as fish as sandwich fillings, which have not been recorded in the present study. Another possibility is that other sources of MeHg may exist—for example, meat from animals fed with fish products. The B-MeHg is low in the reference group, indicating a low total intake of foodstuffs contaminated with MeHg.

An association between B-Se and B-MeHg was found, indicating that fish products may be a common source of Se and MeHg. The number of fish meals each month was not, however, significantly correlated with B-Se. Other studies have suggested a relation between the consumption of fish and the amount of Se in serum. Current use of smoking tobacco was negatively correlated with B-Se among both the referents and the exposed subjects. Current smokers among all participants of the study had 0-09 μmol/l B-Se less (p < 0-01) than current non-smokers. Ellis et al reported lower concentrations of Se in serum and whole blood among smokers compared with non-smokers.

There was similar B-Se in the two groups but U-Se was lower among the exposed subjects compared with the referents. No other studies on Se in subjects previously exposed to Hg are known to us. Some studies have shown decreased U-Se among subjects under ongoing exposure to inorganic Hg, and one study has shown higher U-Se among exposed workers compared with the unexposed referents. The present study showed a weak negative correlation between cumulative U-Hg and U-Se. Different mechanisms of the interaction between Hg and Se have been suggested—for example, a redistribution of Se on exposure to Hg, competition for common binding sites between inorganic Hg and Se, and the formation of an Hg-Se complex. Animal models may further elucidate these mechanisms in the future.

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