Evidence of the direct adsorption of mercury in human hair during occupational exposure to mercury vapour.

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Abstract

We have found clear evidence of direct adsorption of mercury in human hair after the occupational exposure to mercury vapour. We have performed both longitudinal analysis of human hair by Laser Ablation ICP-MS and speciation analysis by Gas Chromatography ICP-MS in single hair strands of 5 individuals which were occupationally exposed to high levels of mercury vapour and showed acute mercury poisoning symptoms. Hair samples, between 3.5 and 11 cm long depending on the individual, were taken ca. three months after exposure. Single point laser ablation samples of 50 µm diameter were taken at 1 mm intervals starting from the root of the hairs. Sulfur-34 was used as internal standard. The ratio $^{202}Hg/^{34}S$ showed a distinct pattern of mercury concentration with much lower levels of mercury near the root of the hair and high levels of mercury near the end of the hair. In all cases a big jump in the concentration of mercury in hair occurred at a given distance from the root, between 32 and 42 mm depending on the individual, with a high and almost constant concentration of mercury for longer distances to the root. When we took into account the rate of hair growth in humans, 9 to 15 mm/month, the jump in mercury concentration agreed approximately with the dates when the contamination occurred with the new growing hair showing much lower mercury concentration. In some cases the concentration of mercury at the tip of the hair was ca. 1000 times higher than that near the root. Additionally, speciation studies confirmed that mercury in all hair samples was present as inorganic mercury. The only explanation for these results was the direct adsorption of mercury vapour in hair at the time of exposure.

Keywords

Mercury poisoning, Hair analysis, Laser Ablation ICP-MS, GC-ICP-MS.

Introduction

Between November 19th and December 3rd 2012 seven workers doing maintenance work in a heat exchanger from a multinational zinc manufacturer located in Avilés (Asturias, Spain) were taken to hospital with acute mercury poisoning. In the next few days it was established that more than 50 workers from the same subcontracting company were exposed to dangerous mercury levels when cutting pipes in a heat exchanger. The total mercury levels in blood for these 50 workers were well in excess of the recommended biological limit values for occupational exposure of 10 ng Hg/ml of blood [1] and reached levels between 500 and 900 ng Hg/ml for the seven workers which showed acute poisoning symptoms [2]. Fortunately, none of these workers died after exposure in contrast to another similar accident that occurred in Japan in 1993 where three workers died after acute mercury poisoning [3]. During February-March 2013 several of the workers which showed acute mercury poisoning approached the University of Oviedo for an independent assessment of their mercury exposure levels. It was decided to carry out longitudinal hair analysis by Laser Ablation-ICP-MS as previous studies [4] have shown that, after acute exposure to inorganic mercury, mercury accumulates in hair and the longitudinal concentration profile of mercury can give information about the dates of exposure. These data would be complementary to the blood and urine analyses which were carried out routinely at different time intervals after their exposure.

The total concentration of mercury in human hair has been traditionally employed to assess environmental exposure [5]. Particularly, the exposure to methylmercury through the diet can be assessed by measuring total mercury in hair [5] which concentration is ca. 250 times higher than that in blood [6]. Additionally, occupational exposure to mercury vapour [7] or oral mercury poisoning with HgO [4] could be also detected in hair. Laser Ablation ICP-MS has been employed for the determination of mercury in human hair [4,8] and sulphur was employed as internal standard in both publications. The constant

concentration of sulphur in hair (ca. 5% w/w) makes it suitable for internal standardisation and correction for the variability of laser ablation sampling. Both single point [8] and line scans [4] were employed and, given the small diameter of the human hair, spot sizes below 50 µm were used [4,8].

In this work we have applied the methodology described previously [4,8] to the study of the longitudinal variation of mercury in single hair strands of occupationally exposed workers. Mercury was detected at mass 202 and sulphur-34 was employed as internal standard. Quantification of mercury was not attempted by LA-ICP-MS as only the mercury/sulphur signal ratio was employed to assess mercury incorporation in hair as a function of the distance to the root of the hair. Additionally, after laser ablation the hair samples were digested, derivatized and measured by GC-ICP-MS to gain speciation information.

Experimental

Hair samples

Samples were collected by extracting 2-4 hairs from the back of the head of the workers with the help of stainless steel flat tip tweezers. Hair samples were taken from 5 of the individuals who suffered from acute mercury poisoning. Samples 1 to 3 were taken on 26/02/2013 while samples 4 and 5 were taken on 12/03/2013. The hairs were stored immediately in plastic zip-lock bags and identified with the name of the worker and the date of collection. For analysis, hair samples were mounted on 25 x 50 mm microscopic glass slides and fixed with two-sided tape. The hair samples were cut every ca. 4 cm while mounting on the slides to fit in the laser ablation chamber. The distance to the root was established with the coordinates of each laser ablation point. No pre-treatment of the hair samples was performed.

Solid-liquid extraction in hair samples for GC-ICP-MS analysis

The direct digestion of the samples in the glass slides were performed by focussed microwaves in a microwave unit Explorer Hybrid from CEM Corporation (Matthews, NC, USA). The hair samples were treated with 25% tetramethylammonium hydroxide (TMAH) (Sigma-Aldrich) for 4.5 min at a fixed power of 35 W. After digestion the resultant supernatant was transferred to a vial containing 4 mL of acetic acid/sodium acetate buffer (pH 4). For derivatization, 0.8 mL of a 2% w/v sodium (tetra-n-propyl)borate (LGC-Standards, Wesel, Germany) in Milli-Q water and 1 ml of hexane (Sigma-Aldrich) were added for liquid-liquid extraction. The derivatization and extraction into hexane was accomplished by five minutes of manual shaking. Then, the sample was centrifuged (5000 rpm for 5 min), and the organic layer was transferred to a glass vial and stored at -18 ºC until analysis. Just before the GC-ICP-MS injection of the samples an additional step of pre-concentration under a gentle stream of nitrogen was carried out in a dedicated unit (Minivap, Supelco, Bellefonte PA). The samples were pre-concentrated until a final volume of approximately 20 µL.

Instrumentation

A CETAC-LSX-213 laser system (Cetac Technologies, Omaha, USA) with helium as carrier gas was employed. Laser energy was 20% of nominal and the repetition rate selected was 10 Hz. Measurements were performed by single point analysis with a spot size of 50 µm and a total of 100 laser ablation shots per point (ca. 10 s ablation times). A 10 seconds delay was selected from the start of the measurements to get background data before the ablation peak. Single point measurements were carried out every 1 mm from the root to the tip of the hair. The laser ablation was coupled to an Agilent 7500ce (Agilent Technologies, Tokyo, Japan) ICP-MS instrument. Masses 34 for sulphur and 202 for mercury were measured with 0.1 s integration time using the time-resolved software of the instrument. A total acquisition time of 60 seconds was selected. Raw data were taken to Microsoft Excel for further treatment. Experimental conditions employed in the LA-ICP-MS analyses are given in Table 1.

The chromatographic separation of the propylated forms of MeHg, EtHg and Hg(II) was accomplished with a gas chromatograph model Agilent 6890N (Agilent Technologies, Tokyo, Japan) fitted with an split/splitless injector and a DB-5MS capillary column from Agilent J&W Scientific (cross-linked 5% diphenyl, 95% dimethylsiloxane, 30 m × 0.53 mm i.d. × 1.0 μm). The gas chromatograph was coupled to the Agilent 7500ce ICP-MS using a laboratory-made transfer line. The volume of injection for the four samples was 2 μL of the preconcentrated solution.

Data treatment

Peak areas were calculated both for ^{34}S and ^{202}Hg for each data point after background subtraction using the first and the last 10 seconds in each measurement point to calculate the baseline. This procedure is illustrated in Figure 1 for one of the data points taken at 20 mm from the root of hair 1. The reconstructed baselines, shown as the two dashed lines in Figure 1, were subtracted from the whole data set. After background subtraction peak areas both for ^{202}Hg (red line) and ^{34}S (black line) were calculated by the trapezoidal method using the whole data set. The ratio of peak areas $^{202}Hg/^{34}S$ was used in the longitudinal analysis of the data. For the data point shown in Figure 1 the ratio of peak areas was 0.0122.

Results and discussion

Suitability of ^{34}S as internal standard.

The use of ^{34}S as internal standard for the determination of mercury in single hair strands was suggested previously [4,8]. In order to check its suitability and to study the stability of ^{34}S measurements with time we have taken all peak area measurements for ^{34}S performed on the 5 hair samples and plotted them in Figure 2. Please note that every hair sample was measured on different days and under slightly different experimental conditions. As can be observed, for each hair sample the measured areas were fairly constant during a measurement session apart from a few outliers. However, differences in

sensitivity from one sample to another were quite large with more than one order de magnitude difference between hairs 3 and 4. Unfortunately, none of the hair samples were measured again on a different day so we do not know whether these differences were due to measurement conditions in the ICP-MS or actual sulphur concentration differences between hair samples of different individuals. In any case, the suitability of ^{34}S as internal standard for each hair sample is demonstrated. However, it is worth stressing that these measurements cannot be used to compare mercury concentrations in the different hair samples.

Ablation peak profiles.

We observed two general types of peak profiles in all measured hairs. For data points near the root of the hair the peak profiles for all hairs were similar to those shown in Figure 1. It seems that the concentration of sulphur and mercury were constant throughout the diameter of the hairs. On the other hand, peaks further away from the root in three of the hair samples (hairs 1, 2 and 4) showed a distinct and very intense double peak for mercury such as that shown in Figure 3 for hair 1 at 51 mm from the root. Please note that, for this point, the area of the mercury signal is about 1000 times higher than that in Figure 1 for the same hair sample and with similar sensitivities and peak profiles for sulphur. Figure 4 shows a detail photograph of hair 5 after ablation. All the other hairs showed similar images. As can be observed, the different ablation spots occur at exactly 1 mm intervals. The ablation starts at the upper surface of the hair, goes through its core and finally ablates the lower surface of the hair which is fixed to the glass slide by a two-sided tape. The final effect of the ablation process is the cutting of the hair in two at the ablation spot. When observing the mercury peak profile in Figure 3 it seems to indicate that this element is concentrated in the surface of the hair with lower concentrations towards its core. The surface of the hair was ablated both at the beginning and at the end of the laser ablation burst of 100 shots which explains the double peak obtained.

Finally, Figure 5 shows the mercury peak profiles for hairs 2 and 4 both at a zone of low mercury concentration near the root of the hair (left axis) and at another zone of high mercury concentration near the tip of the hair (right axis). As can be observed, the peak profiles are very similar to those already discussed for hair 1.

Longitudinal analysis of mercury in hair.

Figure 6 shows all the results obtained for the 5 hair samples in terms of ratio of peak areas ($^{202}Hg/^{34}S$) vs. distance to the root (mm). Please note that the results are expressed in logarithmic scale to show better the differences between the different hair samples. In all samples, the ratio of peak areas between mercury and sulphur is low near the root of the hairs (values between 0.01 and 0.1) and then there is a drastic increase in the ratio of peak areas at certain distances from the root depending on the particular hair sample. Hairs 1, 2 and 4 show near three orders of magnitude of difference in the ratio of peak areas near the tip of the hair in comparison to data near the root. The sample from hair 3 was probably too short to see the increase in concentration of mercury and the results for hair 5 need some explanation. Hair 5 was ca. 300 mm long and only the first 100 mm were measured. The owner kept the hair bundled in a ponytail which could have prevented the mercury vapour to attach to the hair in the same way as for hairs 1, 2 and 4 where the hair was kept loose. Anyway, we still observe a jump in the concentration of mercury in hair 5 about 42 mm from the root with some random spikes at longer distances where parts of the hair may have been more exposed than others to mercury vapour.

If we assume a hair growth rate of between 9 and 15 mm per month depending on the individual [9] the “jumps” in mercury concentration for samples 1, 2, 4 and 5 are in good agreement with the dates when the accident took place (end of November - beginning December 2012). The higher relative concentrations of mercury appear in the part of the hair that was already exposed at the time of the accident while the new hair which grew afterwards showed much lower mercury content. This can only be explained if mercury vapour was adsorbed into the hairs in a non-reversible way during the accident. This adsorbed

mercury stayed in the hair and could not be removed even by repeated washing. Please note that the hair samples were taken between three and three and a half months after the accident. Luckily 4 out of the 5 individuals tested had hair long enough to see the change in mercury concentration along their hairs.

Mercury speciation

A typical GC-ICP-MS profile for mercury in hair for a person which has not been occupationally exposed to mercury is shown in Figure 7. As can be observed, the main peak corresponds to methylmercury with a minor peak for inorganic mercury. This behaviour is typical when mercury appears in hair as a consequence of the presence of mercury in the diet. The GC-ICP-MS profile for three of the hairs studied (hairs 1, 2 and 4) are shown in Figure 8 (A, B and C respectively). As can be observed now, the main peak corresponds to inorganic mercury in all cases with a small peak for methylmercury. These results confirm the non-reversible adsorption of mercury vapour on these human hair samples found by longitudinal analysis using Laser Ablation.

Conclusions.

The fact that mercury vapour can be irreversibly adsorbed on human hair was suggested by Wilhelm *et al* [7] when analysing hair of practising dentists using mercury amalgams. Experiments to test this fact were devised by Li *et al* [10] who proposed mercury in hair as an indication of occupational exposure to mercury vapour. Our results here confirm these previous studies and show that mercury concentrations in exposed hair can be up to 1000 times higher than the mercury which does go into the hair as a consequence of ingestion or lung absorption. The jumps in the concentration of mercury, for 4 of the analyzed hairs, appeared at distances from the root which were well in agreement with the dates of mercury contamination and allowed us to demonstrate the cause-effect relationship of the intoxication. GC-ICP-MS studies confirmed the presence of inorganic mercury in the hair samples. Another conclusion of this work is that data on mercury concentrations in hair after occupational exposure

should be taken with extreme care as the possibility of hair contamination by direct adsorption of mercury vapour from the atmosphere can not be ruled out.

Acknowledgements.

This research was supported by the MICINN (project CTQ-2012-36711, co-funded by FEDER).

References.

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307 disease and health status”. *Chem. Soc. Rev*. 2011, **40**, 3915-3940.
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309 occupational exposure to mercury vapor: Simulated experiments and field data
310 analysis”. *Sci. Total Environ*., 2011, **409**, 4484-4488.
311

312 **Table 1**. Experimental conditions of the LA-ICP-MS instrument.

313

Experimental parameters ICP-MS Agilent 7500ce	
RF power	1500 W
External Ar flow	15 $L \cdot min^{-1}$
Carrier Ar flow	0.75 $L \cdot min^{-1}$
Acquisition parameters ICP-MS Agilent 7500ce	
Mode	Time resolved analysis
Points per amu	1
Integration time per point	0.1 s
Measured masses	$^{34}S^{+}$, $^{202}Hg^{+}$
Instrumental parameters LA CETAC LSX-213	
Laser Energy (5.6 mJ máx)	20 %
Repetition rate	10 Hz
Spot size	50 µm
Ablation mode	Single point
Helium flow	0.80 $L \cdot min^{-1}$

314

315

Figures.

Figure 1. Laser ablation profile for ^{202}Hg (red line, right axis) and ^{34}S (black line, left axis) measured at 20 mm from the root of hair 1. The baseline data is shown as white points (sulphur) or gray points (mercury). The reconstructed baseline is shown as a black dashed line for both isotopes.

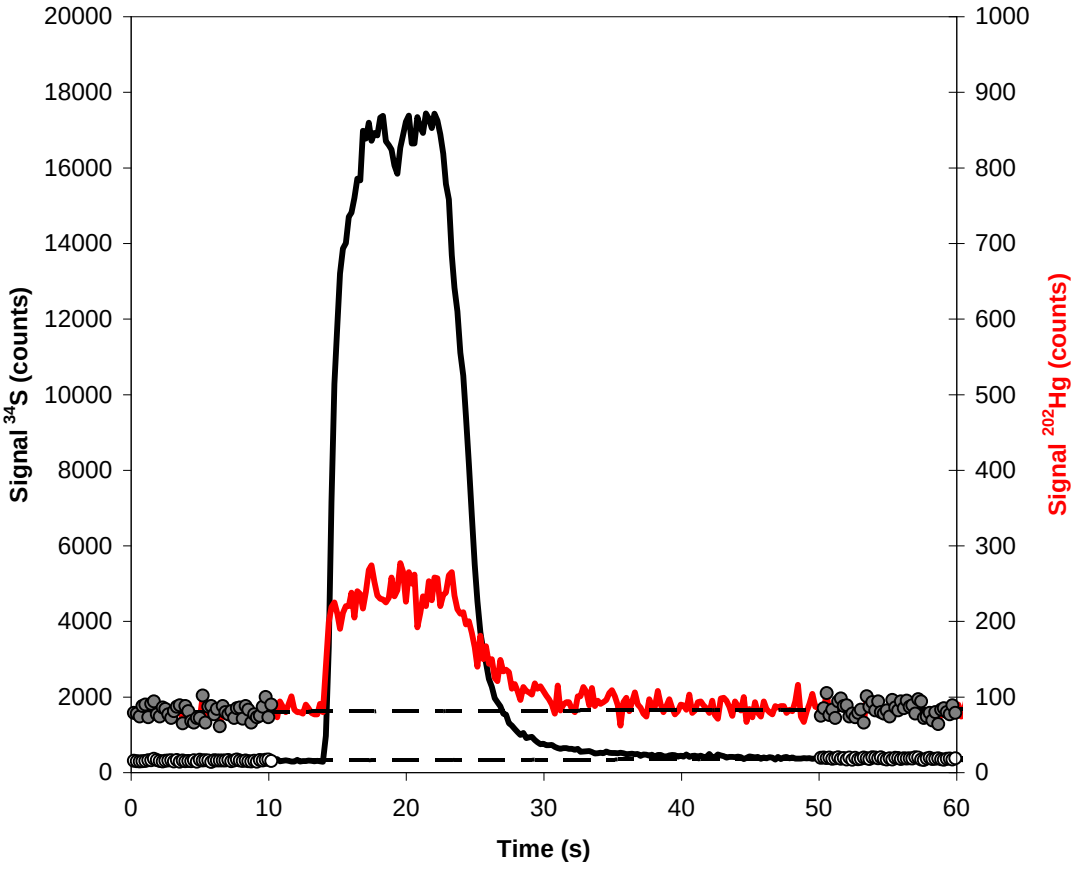


Figure 2. Peak area measurements for ^{34}S performed on the 5 hair samples and plotted versus the distance to the root for each hair sample.

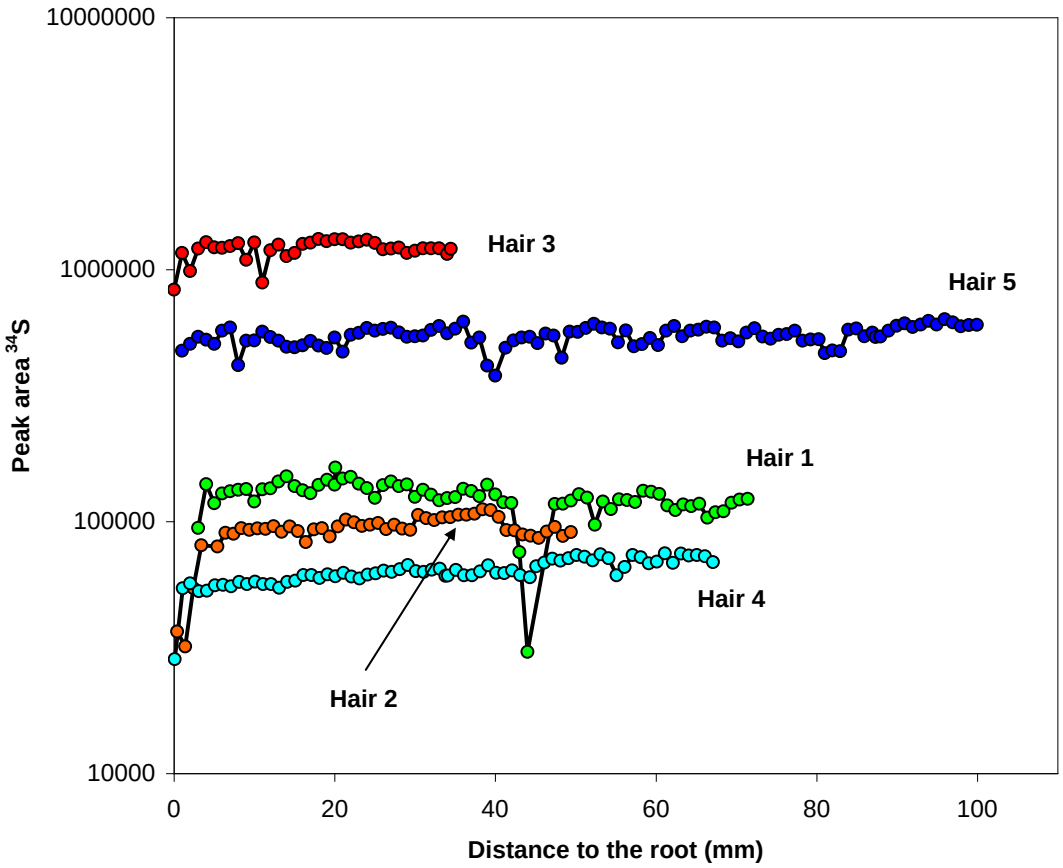


Figure 3. Laser ablation profile for ^{202}Hg (red line, right axis) and ^{34}S (black line, left axis) measured at 51 mm from the root of hair 1.

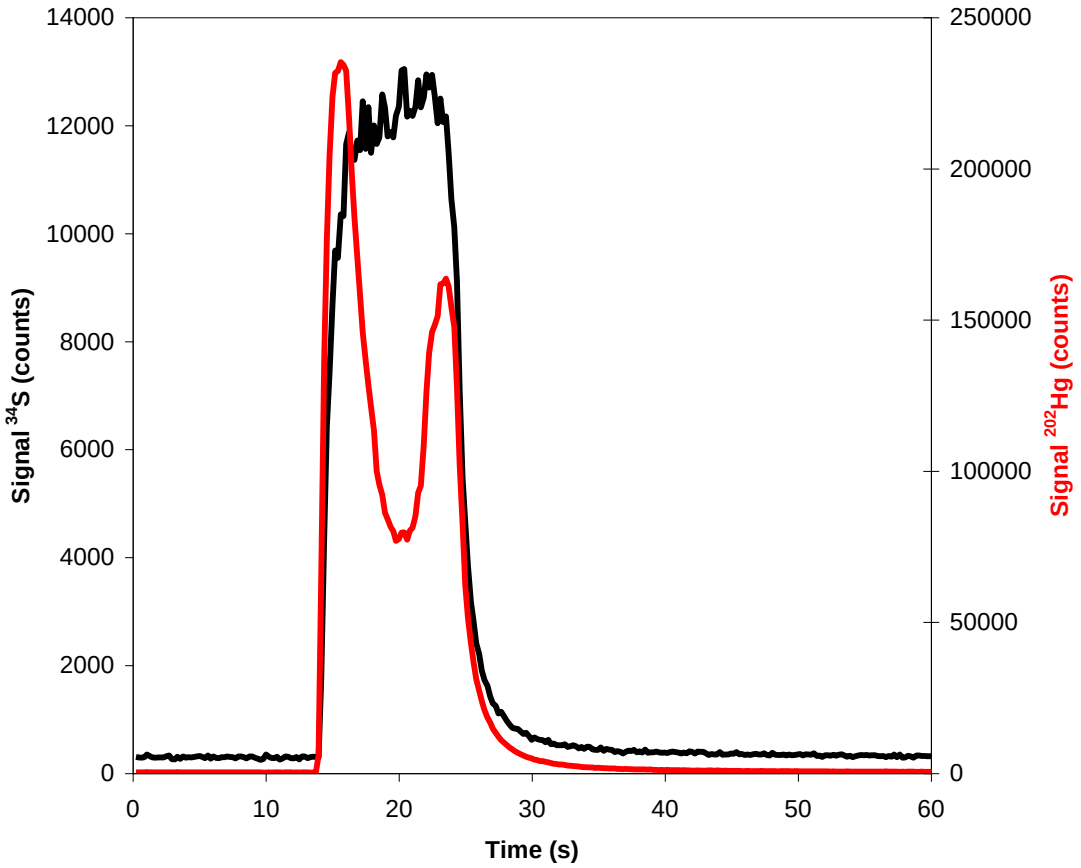


Figure 4. Photograph of hair 5 after ablation.

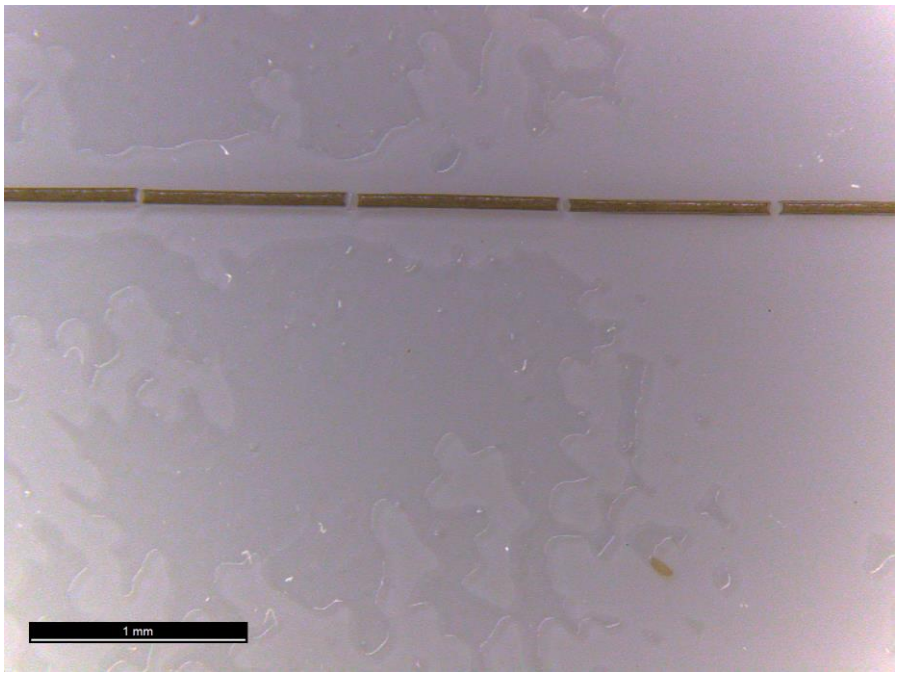
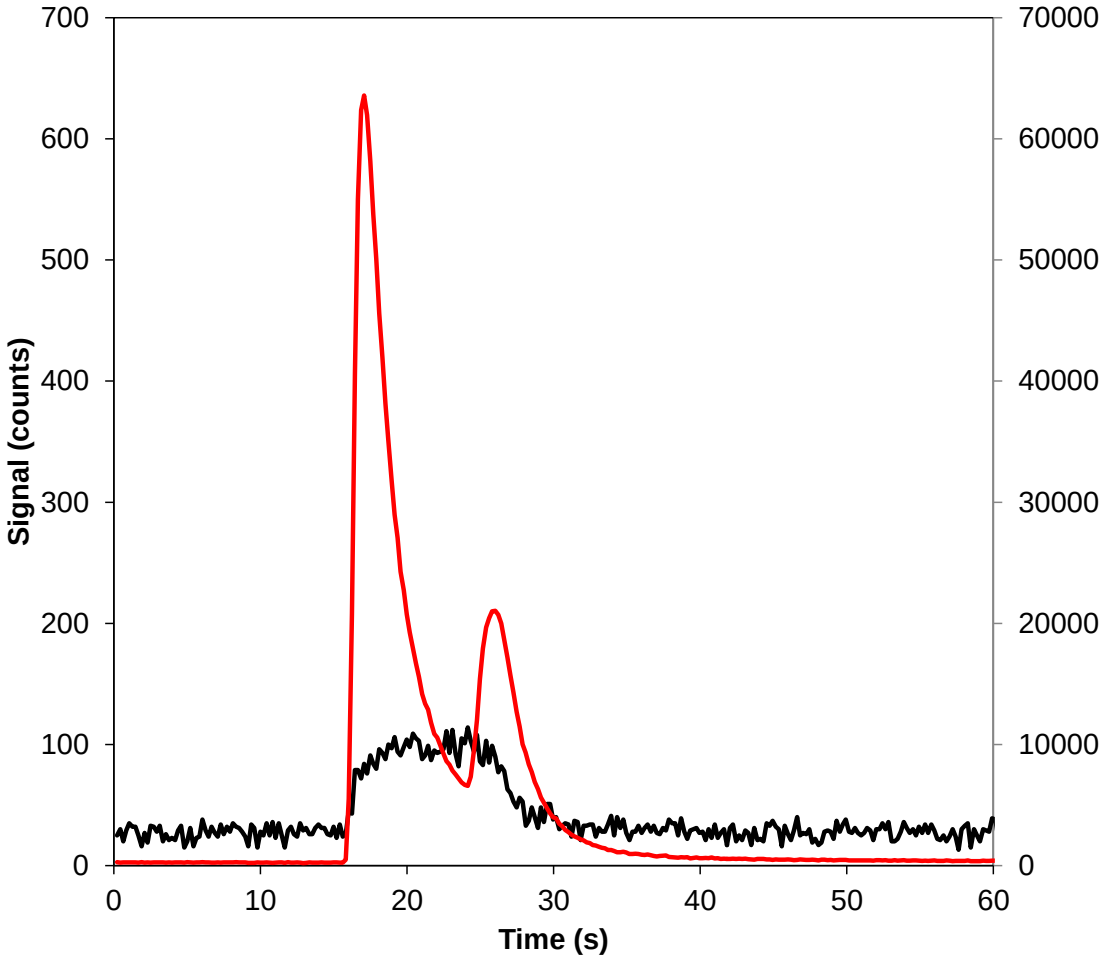


Figure 5. Peak profiles for ^{202}Hg in hair 2 (A) and hair 4 (B) in two different zones in the hair. Red line corresponds to a high concentration profile near the tip of the hair (right axis) whereas the black line corresponds to a low concentration profile near the root of the hair (left axis).

A) Hair 2.



B) Hair 4.

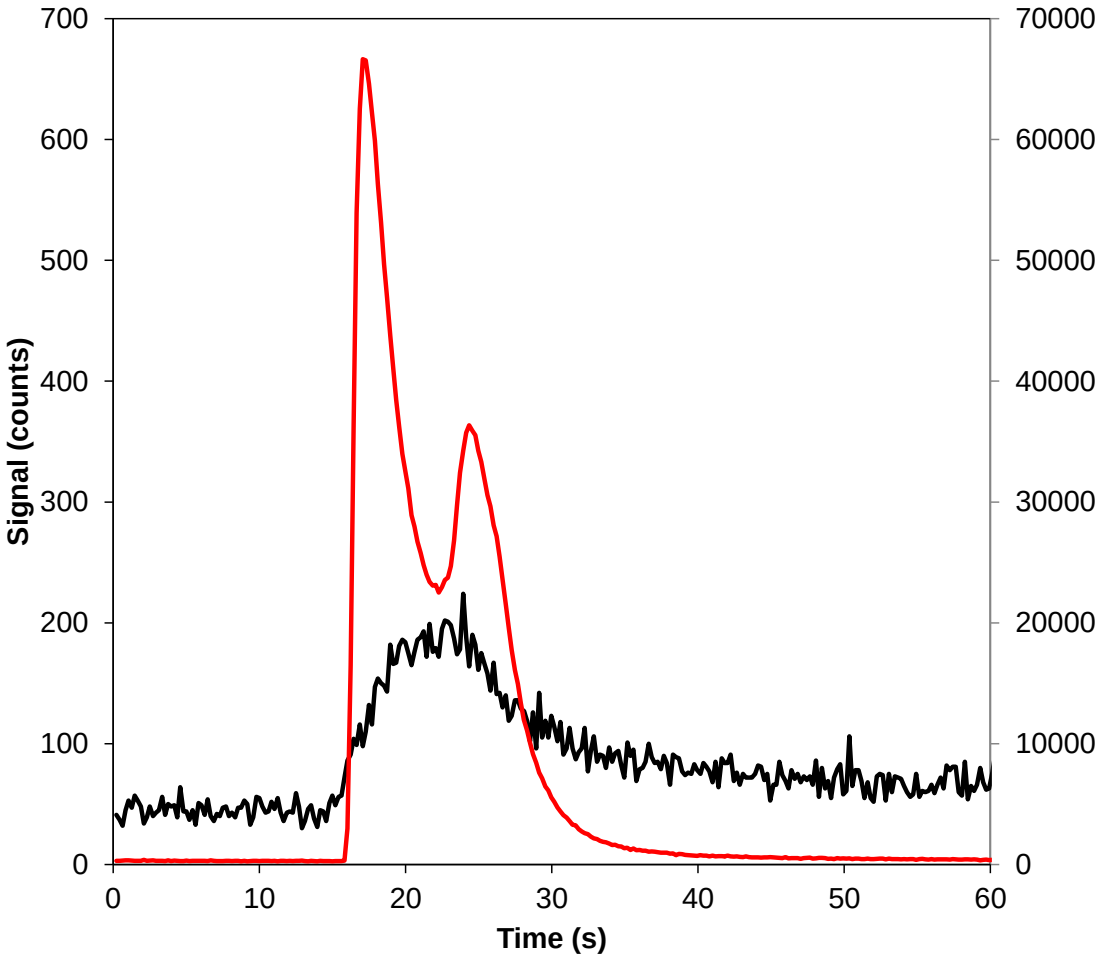

700
600
500
400
300
200
100
0
70000
60000
50000
40000
30000
20000
10000
0
0
10
20
30
40
50
60
Signal (counts)
Time (s)

Figure 6. Ratio of peak areas ($^{202}Hg/^{34}S$) measured for the 5 hair samples at different distances to the root for each hair sample.

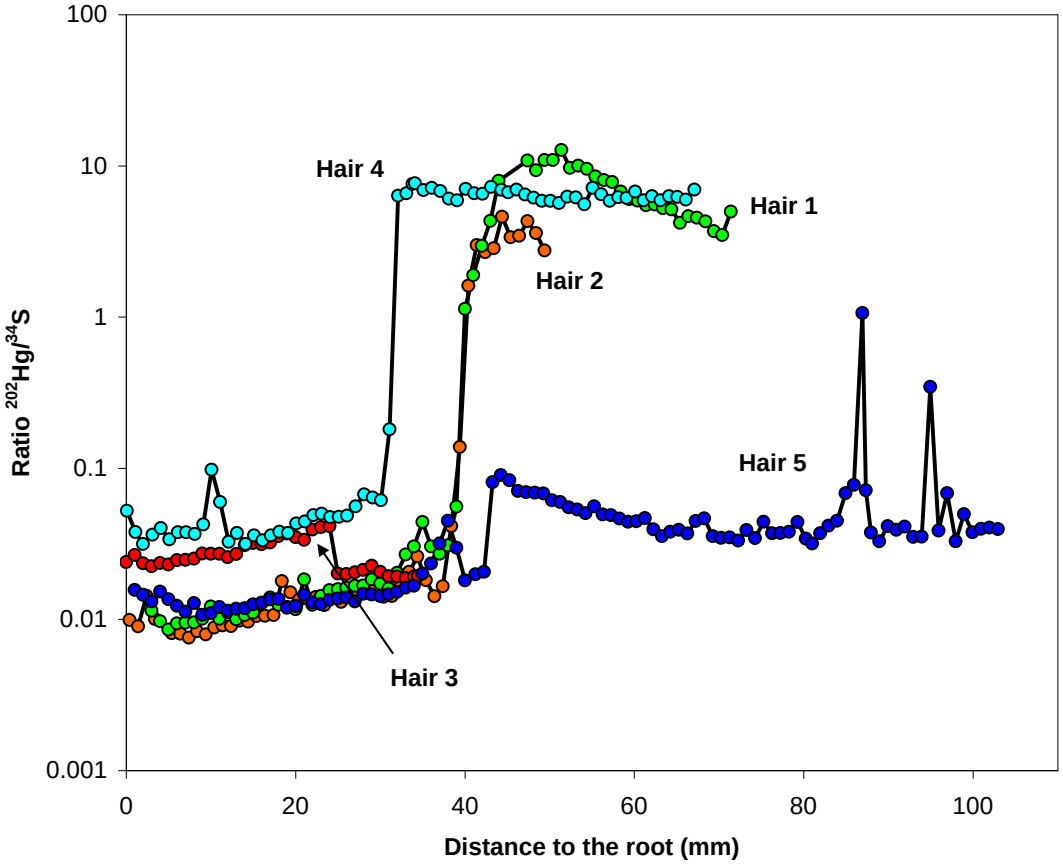


Figure 7. GC-ICP-MS chromatogram of mercury detected at mass 202 from the hair of a non-occupationally exposed individual.

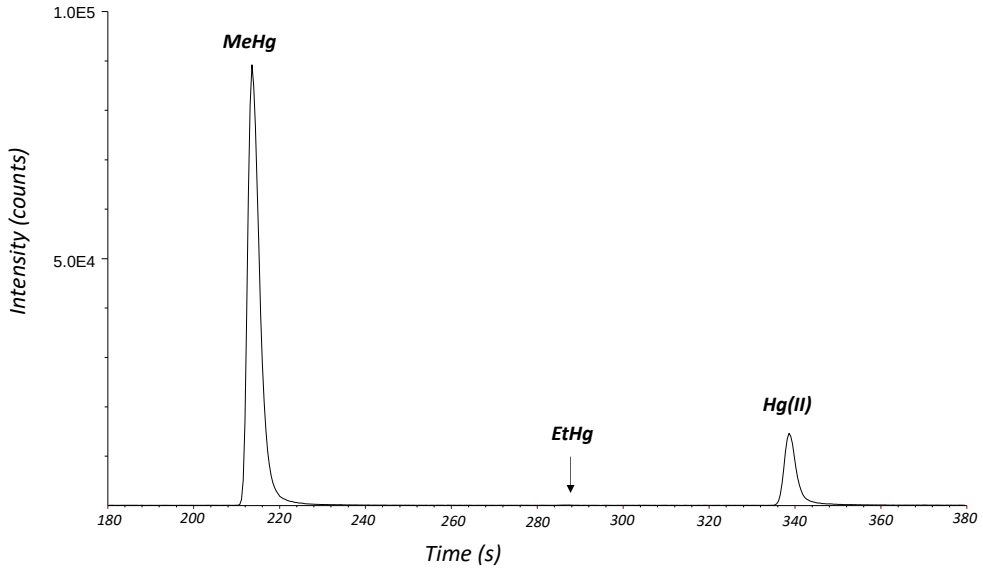
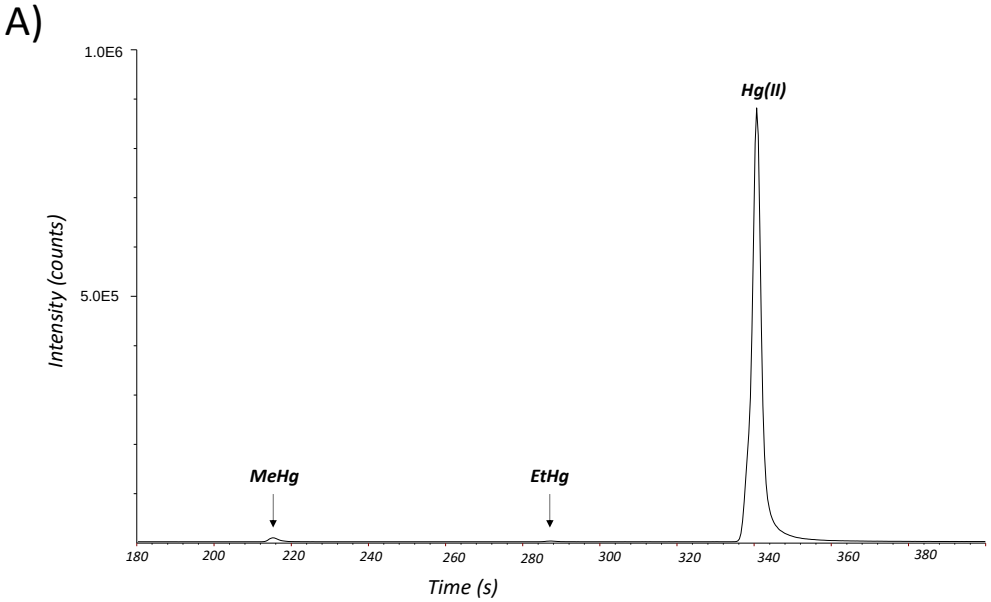
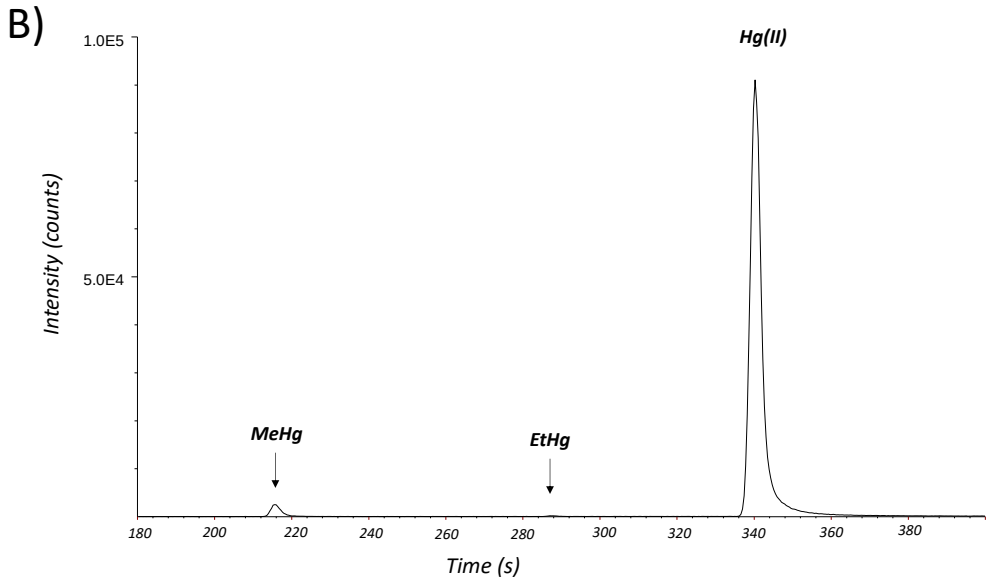
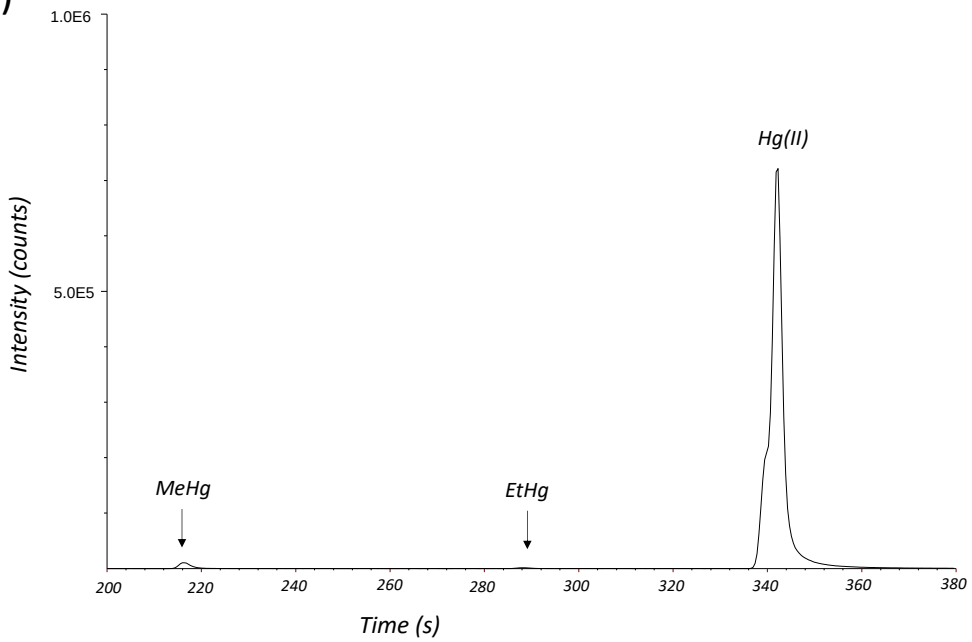


Figure 8. GC-ICP-MS chromatograms of mercury detected at mass 202 from the hairs 1 (A), 2 (B) and 4 (C).





C)


1.0E6
5.0E5
Intensity (counts)
Hg(II)
MeHg
EtHg
200
220
240
260
280
300
320
340
360
380
Time (s)

377

378

379