Dear participants of COPHES/DEMOCOPHES,

The analytical method for total mercury in hair determination has been developed, validated and ISO/IEC 17025 accredited (accreditation number 223/LE460, Spanish National Accreditation Body) in the National Centre for Environmental Health of Spain (CNSA-ISCIII) under the direction of Dr. A. Castaño. You can use this SOP for the generation of your own laboratory SOP.

This analytical method is comprehensively described with the intent that they can be used as SOPs within every laboratory without further briefing of the staff.

You can use these SOPs for the generation of your own laboratory SOPs.

Mercury

Application	Determination in scalp hair
Analytical principle	Thermal decomposition-gold amalgamation-atomic absorption spectroscopy

Summary

The method described here permits the assay of total mercury in human hair samples both for general population and exposed workers.

Hair samples are weighed and introduced into the sample boat without any pretreatment. The sample is introduced in the Direct Mercury Analyzer where it is initially dried and then thermally decomposed in a continuous flow of oxygen. Combustion products are carried off and further decomposed in a hot catalyst bed. Mercury vapors are trapped on a gold amalgamator and subsequently desorbed for quantification. The mercury content is determined using atomic absorption spectrophotometry at 254 nm.

The quantitative determination of mercury is achieved from a calibration curve obtained from human hair reference materials analyzed in the same way as the hair samples.

Mercury

Imprecision: less or equal to 9,4% depending on the concentration of mercury.

Accuracy: Recovery rate, r≥98% (depending on the concentration of mercury).

Quantification limit: 0,01 ng Hg per mg hair.

Mercury

Mercury is a naturally occurring element distributed throughout the environment by both natural and anthropogenic processes. It is persistent in the environment and found in various chemical forms: elemental mercury, inorganic mercury (Hg^{2+} compounds) and organic mercury (mainly methylmercury, MeHg). [1]

In spite of its potential risks, mercury continues to be used in a variety of products and processes all over the world because of its unique properties. Elemental mercury is used in artisanal and small-scale mining of gold and silver, chlor-alkali production, vinyl chloride monomer production, in manometers, thermometers, electrical switches, fluorescent lamp bulbs and dental amalgam fillings. Mercury compounds are used in some batteries, pharmaceuticals, paints and as laboratory reagents and industrial catalysts. Mercury can be released to air, water and soils during production and uses or after disposal of the mercury-containing products and wastes. Mercury is also released during natural processes, such as volcanoes and leaching from certain soils. [2]

Mercury is a toxic, persistent pollutant that bioaccumulates and biomagnifies through food webs. [2] People are exposed to methylmercury mainly through their diet, especially through the consumption of freshwater and marine fish. [3] People may be exposed to elemental or inorganic mercury through inhalation of ambient air during occupational activities and from dental amalgams. [4] Exposures to elemental mercury or inorganic mercury forms can also occur due to use of some skin-lightening creams and soaps, the presence of mercury in some traditional medicines, use of mercury in cultural practices, and due to various accidental mercury spills in homes, schools or other locations. [5]

General population is exposed to some low levels of mercury. The occurrence and severity of adverse health effects depend on the chemical form of mercury, the dose, the age or developmental stage (considering fetus as the most susceptible), or the duration and the route of exposure. [6]

The primary targets for toxicity of mercury are the nervous system, the kidneys and the cardiovascular system, being developing organ systems (such as the fetal nervous system) the most sensitive to toxic effects of mercury. Effects on the nervous system are the most sensitive toxicological endpoint observed following exposure to elemental mercury and methylmercury, while damage to the kidneys is the key end-point in exposure to inorganic mercury compounds. [6]

Based on risk assessments and other considerations, several countries and international organizations have established reference levels for daily or weekly methylmercury or mercury intakes which, based on available data and research, are estimated to be safe (or without appreciable risk to health). The reference intake levels for methylmercury exposures range from 0.7 to 2 µg methylmercury per kilogram body weight (µg/kg body weight) per week. [7]

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established provisional tolerable weekly intakes (PTWIs) for total mercury at 5 µg/kg body weight and for methylmercury at 1.6 µg/kg body weight. [8]

The US EPA has developed Reference Doses (RfDs) for mercuric chloride of 0.3 µg/kg body weight/day and methylmercury 0.1 µg/kg body weight/day and a Reference Concentration (RfC) for elemental mercury of 0.3 µg/cubic metre. [9]

Exposures can be estimated by measuring pollutant levels in various human matrices, such as hair, blood or urine, which are useful tools for human exposure assessment and surveillance tools for monitoring mercury exposure in individuals and populations. [10]

The presence of mercury in blood indicates recent or current exposure to mercury. There is a direct relationship between mercury concentrations in human blood and consumption of fish contaminated with methylmercury. [11] Urine mercury levels are usually considered the best measure of recent exposures to inorganic mercury or elemental mercury vapor because urinary mercury is thought to indicate most closely the mercury levels present in the kidneys. [12]

Hair is generally the preferred choice to document methylmercury exposure as it provides a simple, integrative and non-invasive sample. Once incorporated in the hair, mercury does not return to the blood, thus it provides a good long-term marker of exposure to methylmercury. Most mercury in hair is in the form methylmercury, especially among populations that consume fish. Hair incorporates methylmercury during its formation and shows a relatively direct relationship with blood mercury levels, providing an accurate and reliable method to measure methylmercury intake levels. [13]

Numerous analytical methods are available for analysis total mercury in human hair, including cold vapor atomic fluorescence spectrometry; inductively coupled plasma mass spectrometry and cold vapor atomic absorption spectrometry. Direct solid introduction techniques, where no sample pretreatment is required, have shown very little chemical waste and the potential for contamination is lowered. In addition, the amount of hair required for analysis can be reduced and therefore the throughput is increased. The principle combines combustion, gold amalgamation of mercury and atomic absorption spectrometry detection. [14]

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Mercury

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Application	Determination in scalp hair
Analytical principle	Thermal decomposition-gold amalgamation-atomic absorption spectroscopy

Contents

1. General principles

Hair samples are weighed and put into the sample boat without any pretreatment. The sample is introduced in the Direct Mercury Analyzer where it is initially dried and then thermally decomposed in a continuous flow of oxygen. Combustion products are carried off and further decomposed in a hot catalyst bed. Mercury vapors are trapped on a gold amalgamator and subsequently desorbed for quantification. The mercury content is determined using atomic absorption spectrophotometry at 254 nm.

The quantitative determination of mercury is achieved from a calibration curve obtained from human hair reference materials analyzed in the same way as the hair samples.

There are different configurations for the Direct Mercury Analyzer depending on the trademark and model used. For the procedure described here, and standard version equipped with two measuring cells of different path length flow has been used:
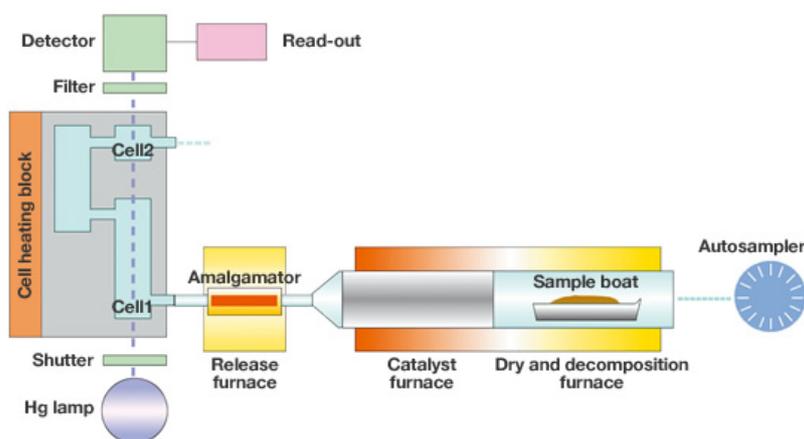
The guidance values for the working ranges of the two measuring cells are: 0-20 ng Hg (low range) and 20-1000 ng Hg (high range).

2. Equipment, chemicals and solutions

2.1. Equipment

Direct Mercury Analyzer (e.g. DMA-80 from Milestone)

Analytical balance (readability: 0,01 mg) (e.g. XP205 from Mettler)

Microlitre pipette, adjustable between 100 and 1000 µL (e.g. from Gilson)

PC system for data evaluation including the appropriate software (e.g. Easy-Doc from Milestone)

Scissors

Spatula

Graph paper (mm)

Polypropylene flasks

0,5 mL nickel combustion boats

1,5 mL quartz combustion boats

Antistatic tweezers

Sample transport tray

100 mL volumetric flask

Talc-free gloves

2.2. Chemicals

Oxygen gas

70% ethanol

37% hydrochloric acid

Ultrapure water (e.g. milli-Q water)

2.3. Solutions

Hydrochloric acid (0,37%):

1 mL of 37% hydrochloric acid is pippeted into a 100 mL volumetric flask. The volumetric flask is subsequently filled to its nominal volume with ultrapure water.

2.4. Calibration standards

Two hair reference materials of different mercury levels are used. Examples of these materials are:

NIES CRM No.13 (NIES-13): 4,42±0,20 ng/mg

Reference Material IAEA-086: 0,573 (0,534-0,612) ng/mg

3. Specimen collection

See COPHES Deliverable 3.1.: 'Define methods for sampling specimens, including choice of collectors, conditions of handling, preservation, sending, storing conditions in the lab and QC aspects for the biomarker selected'.

4. Sample preparation

Talc-free gloves should be worn when handling samples.

Remove the lock of hair from the bag in which the sample is provided using tweezers.

Place the hair on a work surface covered with a sheet of graph paper on which the portion to be sampled is to be cut, holding it in place with a paper clip on the end furthest from the scalp. The graph paper should be changed between samples.

Once held in place, the lock of hair should be extended using tweezers so that it forms an approximately straight line. A 3-cm sample should then be removed from the end closest to the scalp using scissors for subsequent analysis.

The segment removed should be placed in a polypropylene flask labeled with the sample code, and the flask sealed with a stopper labeled with the same code.

The remaining hair should be disposed as a conventional waste.

Those samples for which the end closest to the scalp is not known should be treated as follows:

- Samples shorter than 3 cm in length should be processed in their entirety.
- Samples longer than 3 cm in length are considered to be invalid and should therefore be rejected.

Remove the stopper from the flask containing the 3-cm hair sample and cut the hair into pieces 1-3 mm in length using scissors. The flask should then be stored in a sealed cupboard at room temperature until analysis.

All samples received by the laboratory should be stored at room temperature before and after the analysis.

The tweezers and scissors used above should be cleaned with 70% ethanol between samples.

5. Conditioning of the Direct Mercury Analyzer

Technical data:

Principle: Atomic Absorption Spectrometry.

Mercury detection system: Single beam spectrophotometer with sequential flow through two measurement cells.

Light source: low pressure mercury vapor lamp.

Wavelength: 253,65 nm.

Interference filter: 254 nm, 9 nm bandwidth.

Detector: silicon UV photo detector.

Work ranges (with automatic switch over): 1st low range 0-20 ng, 2nd high range 20-1000 ng.

Autosampler: built-in 40 positions.

Carrier gas: oxygen, inlet gas 4 bar (60 psi), flow rate app. 200 mL/min.

The technical data listed here were established for the configuration of the instrument used in this case.

Step 1: Preparation of the Direct Mercury Analyzer

The following operations should be carried out in accordance with the user manual: opening oxygen supply, Direct Mercury Analyzer start-up and data file creation.

Step 2: System cleaning

An empty position should be measured with the appropriate method (the measurements conditions listed here were established for the configuration of the instrument used in this case and they must be optimized for other instruments in accordance with the manufacturer's instructions):

Drying time: 10 s
Drying temperature: 200ºC
Decomposition time: 240 s
Decomposition temperature: 650ºC
Purge time: 60 s

Step 3: System background checking

Three empty nickel combustion boats should be analyzed with the previous method and checking that the absorbance (measured in terms of peak height) of the final samples is less than 0.003. (The acceptable system background should be established by the laboratory in accordance with the manufacturer's instructions).

If this is not the case, further nickel combustion boats should be analyzed until said value is obtained. If the desired background level is not attained after five nickel combustion boats have been analyzed, the system should be cleaned by

analyzing a hydrochloric acid solution (0,37%) in a quartz combustion boat, then cleaning the system and checking the background.

Step 4: Pre-measurement quality control

Two samples of NIES-13 certified reference material containing approx. 10 ng of mercury (approx. 2,26 mg of material) should be analyzed with the following parameters (guidance parameters that must be optimized for other instruments in accordance with the manufacturer's instructions):

> Drying time: 60 s
> Drying temperature: 200ºC
> Decomposition time: 150 s
> Decomposition temperature: 650ºC
> Purge time: 60 s

The concentration determined for the second reference material sample should be within the uncertainty range for this point described in the validation. If this is not the case, the measurement should be repeated until a value within said range is obtained. If such a value is not obtained after five attempts, the system should be recalibrated.

Once the previous four steps have been successfully completed, the Direct Mercury Analyzer is ready for sample analysis.

6. Analytical determination.

Sample weighing.

Both the combustion boats and the support used to weigh the hair samples should be handled using tweezers.

Place the combustion boat support on the balance. Place a nickel combustion boat on top of the support and tare the balance.

Open the flask containing the sample and transfer small portions of hair into the combustion boat using a spatula until a weight of between 3,0 and 3,5 mg is

reached. Place the combustion boat containing the sample onto the sample tray and note the sample code, weight and tray position in the weighing log. Three replicates should be prepared for each sample.

The spatula should be cleaned with 70% ethanol between samples.

To ensure that the analyzer is measuring correctly, a quality control sample consisting of a weight of reference material, which will vary randomly between the points included on the calibration curve, should be weighed every three samples (nine combustion boats).

Sample analysis.

The nickel combustion boats containing samples and quality controls should be placed in the Direct Mercury Analyzer auto sampler in the order in which they were weighed.

The samples and quality controls should then be programmed by entering their code and weight and selecting the method and last valid calibration of human hair. The parameters of the method are (guidance parameters that must be optimized for other instruments in accordance with the manufacturer's instructions):

> Drying time: 60 s
> Drying temperature: 200ºC
> Decomposition time: 150 s
> Decomposition temperature: 650ºC
> Purge time: 60 s

Under these conditions the analysis time for each sample is around five minutes.

Only those measurements performed between two quality controls whose values lie within the established range (assigned value of the Reference Material ± uncertainty in that level) are considered valid.

7. Calibration.

The calibration is performed with human hair reference materials NIES-13 and IAEA-086 in the range 1-100 ng Hg.

The following table includes the approximate weight of reference material which should be weighed for each calibration point in triplicate following the instructions from section 6:

ng Hg	Reference material	Weight
0	---	0.00 mg
1	IAEA 086	1.75 mg
2.5	IAEA 086	4.36 mg
5	IAEA 086	8.73 mg
10	NIES 13	2.26 mg
15	NIES 13	3.39 mg
20	NIES 13	4.53 mg
25	NIES 13	5.66 mg
35	NIES 13	7.92 mg
50	NIES 13	11.31 mg
100	NIES 13	22.62 mg

For the conditioning of the Direct Mercury Analyzer see steps 1-3 described in section 5. During the data file creation, it should be indicated this is a calibration file.

The calibration samples are then measured under the same parameters used for the samples (see section 6). From the resulting calibration graph the equation parameters and correlation coefficient r^2 are obtained. These parameters should comply with the ranges established in the validation of the method.

The frequency of calibration should be established by each laboratory. As a guidance value, a new calibration can be performed every three months. A new calibration should be also performed if the quality control samples values do not lie within the established range.

8. Calculation of the analytical result.

Once all samples of the analytical run have been analyzed, exit the analysis program, save the data, transfer them to the computer with the appropriate software and print the log using this software.

To calculate the total mercury concentration in each hair sample the following operations will be carried out: calculation of corrected ng Hg according to the formula from the validation (if necessary); concentration of each replicate from the corrected ng Hg; mean and standard deviation of calculated concentrations for the three replicates and uncertainty of the average value calculated from the formula in the validation procedure.

If the concentration of one of the replicates is not within the range set by the mean ± uncertainty, Dixon test will be applied to decide if the suspected value is discarded:

$$Q = \frac{X_{suspected} - X_{nearest}}{X_{highest} - X_{lowest}}$$

If Q is greater than or equal to 0,970 the suspected value should be rejected. If it is lower, the sample should be re-analyzed.

The calibration range for this assay is 1-100 ng Hg (guidance values which should be established for each laboratory). If the amount of mercury obtained in the sample is outside this range, the analysis should be repeated taking into account the following:

- If it is lower than the lowest mercury standard included in the calibration (1 ng Hg; guidance value), three new replicates should be weighed, on the basis of the concentration obtained, to ensure that the new determination is within the calibration range.

  In light of the organic content of the sample and capacity of the nickel combustion boats used, the maximum sample amount which can be introduced into the Direct Mercury Analyzer is 100 mg (guidance value).

- If the amount of mercury determined is higher than the highest point included on the calibration curve, three new replicates should be weighed, on the basis of the concentration obtained, to ensure that the new determination is within the calibration range. The sample should not weigh less than 1 mg (guidance value).

9. Standardization and quality control.

Two hair reference materials of different mercury levels have been used to perform the evaluation of the method: NIES CRM No.13 (4,42 ng/mg) and Reference Material IAEA-086 (0,573 ng/mg).

Quality controls consisting of a weight of reference material that varies randomly among the points included in the calibration curve are included every three samples (nine measurements). Only those measurements performed between two quality controls whose values lie within the established range (assigned value of the Reference Material ± uncertainty in that level) are considered valid (see section 6).

Two blind hair samples are measured each year as part of the internal quality control program.

External quality control is realized by participation in round-robin experiments (twice a year). As an example, our laboratory participates regularly in 'Quebec Multielement External Quality Assessment Scheme' (QMEQAS) organized by the Centre du Toxicologie – Institut National de Santé Publique (Canada).

10. Evaluation of the method

10.1. Precision

Two hair reference materials with different mercury levels have been used to determine the precision of the method: NIES CRM No.13 (4,42 ng/mg) and Reference Material IAEA-086 (0,573 ng/mg).

The different levels of ng Hg included in the calibration (see section 7) have been measured by triplicate in sixteen different days to establish the precision for each level which is included in the next table:

Concentration (ng Hg)	Precision (% CV)
1 ng	9,4
2,5 ng	5,5
5 ng	3,7
10 ng	2,2
15 ng	1,3
20 ng	2,1
25 ng	2,2
35 ng	1,6
50 ng	1,3
100 ng	1,0

10.2. Accuracy

Two hair reference materials with different mercury levels have been used to determine the precision of the method: NIES CRM No.13 (4,42 ng/mg) and Reference Material IAEA-086 (0,573 ng/mg).

The different levels of ng Hg included in the calibration (see section 7) have been measured to established the accuracy for each level. The relative recovery rates are summarized in the next table:

Concentration (ng Hg)	Relative recovery (%)	Range (%)	% CV
1 ng	80,3	60,8-117,5	13,7
2,5 ng	99,6	86,2-133,0	7,9
5 ng	106,7	99,8-144,0	6,3
10 ng	102,6	91,5-108,3	3,0
15 ng	100,3	97,3-104,1	1,9
20 ng	98,7	94,4-107,6	2,9
25 ng	100,1	95,1-104,7	2,3
35 ng	99,9	96,4-104,6	1,8
50 ng	100,2	97,1-102,5	1,3
100 ng	100,0	95,0-102,1	1,1

If the recovery rates (as in our case) do not include 100%, it is recommended to use a correction equation.

In this case the recovery values do not include 100% in levels 1 ng and 5 ng (relative recovery ± CV do not include 100%). The mercury content obtained from the calibration curve in the range 1-7,5 ng can be corrected by applying, only in this interval, a correction formula to read off the actual value (if necessary, this correction equation must be established by each laboratory):

*Corrected value = 0,0132 * (read value)^2 + 0,8023 * (read value) + 0,3720*

After the application of the correction equation, the relative recovery rates are the following:

Concentration (ng Hg)	Relative recovery (%)	Range (%)
1 ng	102,5	86,5-133,3
2,5 ng	98,1	86,5-127,4
5 ng	100,5	94,1-136,6
10 ng	102,6	91,5-108,3
15 ng	100,3	97,3-104,1
20 ng	98,7	94,4-107,6
25 ng	100,1	95,1-104,7
35 ng	99,9	96,4-104,6
50 ng	100,2	97,1-102,5
100 ng	100,0	95,0-102,1

10.3. Uncertainty

The uncertainty for each of the level of mercury evaluated (after applying the correction equation) is included in the following table: [15]

Concentration (ng Hg)	Uncertainty (%)
1 ng	30,6
2,5 ng	18,3
5 ng	15,1
10 ng	7,8
15 ng	6,1
20 ng	7,6
25 ng	6,7
35 ng	5,8
50 ng	5,3
100 ng	5,1

If the uncertainty for a sufficient number of concentrations is available (as in this case) an equation can be determined to establish the uncertainty in each sample (measured as triplicate, see section 8):

$$Uncertainty\ (\%) = 25,99*(corrected\ value)^{-0,414}$$

10.4. Quantification limit

The quantification limit corresponds to the lowest value of the calibration curve: 1 ng Hg.

If a maximum weight of sample of 100 mg is considered, the quantification limit in terms of concentration is: 0,01 ng Hg/mg hair.

10.5. Sources of error

Considering that this method does not require any sample pretreatment, in addition to the determination with the Direct Mercury Analyzer, sample weighing is another key point in the analysis. Therefore, the laboratory should establish a calibration and verification program of the balance with the appropriate

frequency (e.g., calibration once a year and internal verification every two weeks).

It should be particularly careful with the presence of static electricity during handling of the hair sample to avoid problems during the weighing or inadvertent losses of sample. It therefore recommended the use of a system of elimination of static electricity during handling of the hair sample (weighing and introduction into the mercury analyzer).

Special attention should be paid to the initial system background checking (as described in section 5), to avoid memory effects due to the presence of mercury in the system from a previous group of samples. During the samples run with usual mercury concentrations, problems from memory effects do not usually occur; however, special attention should be paid to the variation in the measurements in triplicate and the concentration of quality control measurements, as described in the procedure.

11.   Discussion of the method

The method described here permits reliable and accurate determination of total mercury in hair samples at the concentration ranges of environmental and occupational exposure.

This method does not require any pretreatment or extraction of samples, so very little chemical waste and low potential of contamination is expected. The small amount of hair samples used and the short times of analysis allow a high throughput of samples.

An standard sample amount of 3,0-3,5 mg is recommended in the procedure, but the laboratory can establish its own value taking into account the equipment used, the development and validation of the method and the expected values in its samples (with the parameters included in this procedure, a limit of 0,3 ng/mg is reached for the referred standard sample weight).

A limit of quantification of 1 ng Hg has been established in this procedure, but lower levels can be achieved if necessary. With the configuration of two measurement cells described in this document, special attention to the recovery

rates must be paid for levels lower than 1 ng. Some instruments have also the possibility of a third measurement cell for lower concentrations.

The highest level of the calibration curve included in this method is 100 ng Hg, but calibration levels can be changed for the laboratory during the validation procedure. The mercury analyzer usually has the possibility of reaching levels up to 1000 ng Hg, but in general these are not necessary for determining mercury in hair samples and they have not been considered here.

We have determined precision, accuracy and uncertainty for each level of the calibration curve. Each laboratory should establish the levels for the method validation, but at least a concentration next to the limit of quantification should be included.

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