Determination of Mercury and Creatinine in Urine

Analytical 11

1st Edition

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Euro Chlor

Euro Chlor is the European federation which represents the producers of chlorine and its primary derivatives.

Euro Chlor is working to:

- improve awareness and understanding of the contribution that chlorine chemistry has made to the thousands of products, which have improved our health, nutrition, standard of living and quality of life;
- maintain open and timely dialogue with regulators, politicians, scientists, the media and other interested stakeholders in the debate on chlorine;
- ensure our industry contributes actively to any public, regulatory or scientific debate and provides balanced and objective science-based information to help answer questions about chlorine and its derivatives;
- promote the best safety, health and environmental practices in the manufacture, handling and use of chlor-alkali products in order to assist our members in achieving continuous improvements (*Responsible Care).*

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Prior to 1990, Euro Chlor's technical activities took place under the name BITC (Bureau International Technique du Chlore). References to BITC documents may be assumed to be to Euro Chlor documents.

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RESPONSIBLE CARE IN ACTION

Chlorine is essential in the chemical industry and consequently there is a need for chlorine to be produced, stored, transported and used. The chlorine industry has co-operated over many years to ensure the well-being of its employees, local communities and the wider environment. This document is one in a series which the European producers, acting through Euro Chlor, have drawn up to promote continuous improvement in the general standards of health, safety and the environment associated with chlorine manufacture in the spirit of *Responsible Care.*

The voluntary recommendations, techniques and standards presented in these documents are based on the experiences and best practices adopted by member companies of Euro Chlor at their date of issue. They can be taken into account in full or partly, whenever companies decide it individually, in the operation of existing processes and in the design of new installations. They are in no way intended as a substitute for the relevant national or international regulations which should be fully complied with.

It has been assumed in the preparation of these publications that the users will ensure that the contents are relevant to the application selected and are correctly applied by appropriately qualified and experienced people for whose guidance they have been prepared. The contents are based on the most authoritative information available at the time of writing and on good engineering, medical or technical practice but it is essential to take account of appropriate subsequent developments or legislation. As a result, the text may be modified in the future to incorporate evolution of these and other factors.

This edition of the document has been drawn up by the Analytical Working Group to whom all suggestions concerning possible revision should be addressed through the offices of Euro Chlor.

Summary of the Main Modifications in this version

Section	Nature
All	New specific document based on parts of ANAL 3-7 and appendixes of Health recommendations

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1. INTRODUCTION

In parallel with all the technical and organisational prevention measures taken in the chlor-alkali industry to further reduce the mercury emissions and protect the health of the workers (see ***Env. Prot. 11- Code of Practice - Mercury Housekeeping***), it is necessary to organise a good biological monitoring programme (see ***HEALTH 2 - Code of Practice: Control of Worker Exposure to Mercury in the Chlor-Alkali Industry)***.

Because it is non invasive and it reflects average exposure during the previous 2-4 months, measurement of mercury in urine is advised, although it is of limited use for detecting short term high exposures.

This recommendation describes the methods developed for this monitoring, starting from the precautions to be taken for the sampling.

2. URINE SAMPLING

In the occupational setting, it is not practicable to collect 24 hour or even 12 hour specimens of urine and measurements are made on spot samples. The level of mercury in urine is affected by dilution or concentration of the urine, as may occur with a high or low fluid intake respectively. To minimise this effect, mercury concentrations should be corrected for creatinine content of the urine and expressed as µg/g creatinine.

In addition, to avoid possible problems with diurnal variations, it is recommended that samples are given at approximately the same time of the day. A sample taken prior to commencing work or at the end of the shift after showering has the advantage of reducing possible contamination.

Snap samples of urine are collected by voiding directly into 250 ml - 500 ml glass or polypropylene bottles taking care to avoid contamination from hands and clothing, especially if sampling in the workplace. Polythene bottles are not suitable for collection as it is possible for mercury contamination to occur through the walls of the container.

Ideally the samples should be analysed immediately (within 48 h) after collection but where this is not possible they should be stabilised by the addition of ~14 M nitric acid so that the concentration of the acid in the samples is 5% v/v.

In order to minimise bacterial degradation, the stabilised samples should be stored in a refrigerator (4°C).

For longer storage (more than 1 week), the sample can be stored at - 20 °C.

3. DETERMINATION OF MERCURY IN URINE

3.1. Objective and area of application

The objective of the proposed method is the determination of total mercury in urine.

The lower limit of detection, based on the threefold overall standard deviation of the blank on the reagents, must be determined by each laboratory but should be at least 0.08 µg/sample volume (fluorescence method is more sensitive than AAS). The maximum sample volume is 50 ml, giving lower limits of detection of 0.002 mg Hg/litre for liquids.

3.2. Principle

The sample is digested with an oxidising agent, for example one of the following :

- concentrated nitric acid
- sodium chlorate with hydrochloric acid
- sodium bromide/bromate in concentrated hydrochloric acid

to ensure that all of the mercury is present as soluble mercury II ions.

When using sodium chlorate with hydrochloric acid for oxidation, the formed chlorine has to be reduced by addition of ascorbic acid (if fluorescence detection method is used).

The mercury ions are then reduced to elemental mercury by the addition of an acidic solution of stannous chloride (if there is a risk of deposits, sodium borohydride can also be used).

The mercury vapour is then purged from the sample and determined using an analytical technique such as atomic fluorescence or atomic absorption spectrometry (wavelength of mercury is 254 nm).

3.3. Apparatus

- Atomic absorption spectrophotometer/atomic fluorescence spectrometer.
- Volumetric flasks - 250 (± 0.15) ml and 1000 (± 0.4) ml
- Measuring cylinders - Stoppered 50 (± 1) ml and 1000 (± 10) ml
- Auto-pipette - 5 (± 0.05) ml
- Auto-dispenser - 5 (± 0.05) ml
- Pipettes - 10 (± 0.02) ml
- Burette - 25 (± 0.03) ml

or equivalent laboratory equipment.

3.4. Reagents

Some laboratory suppliers now offer a range of reagents of guaranteed low mercury content especially for trace mercury analysis:

- Tin II Chloride - 25% in Hydrochloric Acid, 20% w/v
- Standard Mercury Solution 1000 mg/l, (Purity + 5 mg/l)
- Oxidising agent (as above – nitric acid / sodium chlorate and hydrochloric acid, etc)

- Ascorbic acid (solid or in solution)

Note 1: All reagents must be used within 1 year of opening unless otherwise stated.

Note 2: To minimise contamination, use auto-dispensers for the stannous chloride and the nitric acid to permanently remove the need for pipettes. It is also recommended that all the glassware used should be retained exclusively for this determination.

3.4.1. Example of preparation of reagent

12.5 % v/v Tin II Chloride Reagent:

Transfer 2.5 litres of the 25 % v/v tin II chloride solution to a 5 litre plastic container using the 1000 ml measuring cylinder. Add 2.5 litres (± 25ml) of water using the 1000 ml measuring cylinder, and mix well. This solution is prepared monthly, or more frequently, if required.

10 % v/v Nitric acid Reagent Blank:

Add 0.5 litres (± 5 ml) of deionised water to a 1 litre plastic container. Carefully add 100 ml (± 5 ml) of the oxidising agent to a 100 ml measuring cylinder and mix well. This solution is prepared as required.

The method of preparation can be adapted according to the equipment and reagents used.

Note: alternative oxidising agent solutions can be obtained, for example by mixing in hydrochloric acid sodium chlorate (100 g/l) or 0.01 N potassium bromide/bromate (in this case, excess bromine is removed by addition of hydroxylamine hydrochloride).

3.4.2. Example of preparation of standards

Standard Mercury Solution A (10 mg/l):

Pipette 10 ml (+ 0.02 ml) of standard mercury solution (1000 mg/l) into a 1000 ml volumetric flask, containing approximately 500 ml of deionised water. Make up to the mark with deionised water and mix well. Then transfer to a 500 ml stoppered amber glass bottle. This solution contains 10 mg/l of mercury and is stable for a month.

Standard Mercury Solution B (0.1 mg/l):

Pipette 10 ml (+ 0.02 ml) of solution A into a 1000 ml volumetric flask, containing approximately 500 ml of deionised water, make up to the mark with deionised water and mix well. This solution contains 0.1 mg/l of mercury and is stable for one day.

3.5. Calibration

A full calibration is performed at a frequency appropriate to the analysis (prior to performing the analysis of a series of samples), prepared as below.

The Linear Correlation Coefficient obtained should be 1.00 ± 0.01.

An example is shown below.

3.5.1. Calibration standards

- To five 250 ml volumetric flasks, containing approximately 50 ml of water, add 25.0 ml of the oxidising agent using the auto-dispenser, followed by 0 (blank), 5.0, 10.0, 15.0 and 20.0 ml of Solution B (0.1mg/l Hg) by high precision pipette.
- The flasks are then made up to the mark with deionised water and mixed well. The flasks should be labelled 0 (blank), 2, 4, 6 and 8 ppb (µg/l) Hg respectively.
- Wash out a sample vessel with the standard, then fill it up and place it on the auto-sampler turntable. Repeat for each standard.
- Analyse according to the analysis procedure outlined in the instrument manual.
- A copy of the calibration curve must be stored in a records system for future reference.

3.5.2. Quality controls checks

It is highly recommended that quality control checks are included in the method of analysis, for example:

- Participation in a round-robin scheme
- Analysis of a sample with a known concentration of mercury (calibration sample) with each batch of analysis
- An instrument drift check
- Repeat analysis of a sample as a precision check
- Analysis of a spiked urine sample (anolyte addition)

3.6. Procedure

3.6.1. Sampling and sample preservation

Snap samples of urine are collected by voiding directly into small (120 ml) polypropylene, polycarbonate or polystyrene bottles, taking care to avoid contamination from hands and clothing, especially if sampling in the workplace.

Other sample bottles should be tested before being put into use - for example, polyethylene bottles are not suitable for collection as it is possible for mercury contamination to occur through the walls of the container.

Samples are analysed on the day of sampling.

If for any reason they are not analysed on the day of sampling, the samples must be stored in the refrigerator for analysis on the following day. If they cannot be analysed the following day, discard the samples and request new samples; alternatively add nitric acid and freeze the samples immediately at minus 20°C, which allows them to be kept for up to one month..

3.6.2. Analysis procedure

- Mix the sample by swirling and then transfer, by auto-pipette, 5.0 ml of sample into a 50 ml stoppered measuring cylinder, containing 5.0 ml of the oxidising agent added from the auto-dispenser.

- Mix and allow standing for about 5 minutes, then make up to the 50 ml mark with deionised water and mix well.

- Wash out an analysis vessel with a portion of the sample, then fill the vessel with the remainder of the sample and place it on the auto-sampler turntable.

- Analyse according to the analysis procedure outlined in the instrument manual, taking into account the aliquot of sample taken (normally 5 ml) and the final volume.

- After all analysis for the day is complete, the instrument must be flushed with 10 % nitric acid blank by running it as a sample. This is done to prevent mercury contamination of the system.

- The system must then be flushed clear of acid with water by running it as a sample. This is done to prevent excess wear on the tubing.

In the case of large batch samples, automatic systems (available from a number of suppliers) can be used to increase the efficiency.

3.7. Calculation

The results may be in ppb (µg/l) total mercury in urine (according to the instrument used).

Note: These results are then adjusted according to the creatinine concentration determined in each sample obtained using the method described below, in order to express the mercury as µg Hg/g Creatinine as follows:

$$\frac{\text{Total Mercury (µg/l)}}{\text{Creatinine (g/l)}} = \text{µg Mercury / g Creatinine}$$

4. DETERMINATION OF CREATININE IN URINE

4.1. Reference

The procedure is that given by H Varley in Practical Clinical Biochemistry 1954.

4.2. Principle

Creatinine combines with picric acid in the presence of hydroxyl ions to produce a red coloured complex. The creatinine concentration is determined from the absorbance at 520 nanometres compared to that from standard creatinine solutions.

4.3. Apparatus

- Analyser validated to medical standards, like the Beckman Synchron CX3 Delta Analyser, enabled for creatinine chemistry
- Auto-sampler sample cup trays called sectors
- Stoppered cylinders - 25 ml (for tolerances see individual glassware)
- Automatic pipette - 1 ml (check volume weekly)
- Disposable pipette tips - 1 ml
- Disposable sample cups to fit CX3 auto-sampler sectors - 2.0 ml and 0.5 ml

4.4. Reagents (ex. Beckman Synchron)

- Picric Acid 0.05 M - Beckman
- Alkaline Buffer Solution (0.188 M Sodium Hydroxide buffered with Sodium Borate and Sodium Phosphate) – Beckman (Expiry dates of individual reagents are stated on the bottles).
- Prepare the creatinine reagent by mixing one bottle of picric acid reagent with one bottle of alkaline buffer reagent. Label this as "mixed creatinine reagent" (sign and date the label). This solution is stable for 30 days.
- Creatinine Standard 3 mg/dl (purity not stated by manufacturer) - Sigma Aldrich Ltd. Expiry date is one year after receipt of standard.
- Synchron CX calibrator solution 1 (1.0 mg/dl creatinine solution) - Beckman, order code 465908. Stable until expiration date on the label if stored unopened at +2oC to 8oC. After opening, stable for one month at room temperature or until the expiration date if sooner.
- Synchron CX calibrator solution 2 (8.0 mg/dl creatinine solution) - Beckman, order code 465909. Stable until expiration date on the label if stored unopened at +2oC to 8oC. After opening, stable for one month at room temperature or until the expiration date if sooner.
- Synchron probe wash concentrate - Beckman.
- Preparation of wash solution - add one bottle of wash concentrate to 10 litres of deionised water.

Store all reagents at room temperature (21-25°C). Use distilled or de-ionised water.

4.5. Calibration

The analysis equipment must be calibrated each time before analysing a series of samples according to the instrument manual.

It is highly recommended that quality control checks are included in the method of analysis, for example:

- Participation in a round-robin scheme
- Analysis of a sample with a known concentration of creatinine (calibration sample) with each batch of analysis
- An instrument drift check
- Repeat analysis of a sample as a precision check
- Analysis of a spiked urine sample (anolyte addition)

4.6. Procedure

- Pipette 1 ml of sample into a clean 25 ml stoppered cylinder and dilute to 20 ml with deionised water. Stopper and mix well.
- Analyse the samples according to the instrument manual.
- If the result is <1.0 or >25.0 mg/dl Creatinine, repeat the determination. If it is still outside the acceptable range then the result is not fit for purpose and a new sample should be requested.

4.7. Calculation

The results obtained from the analyser are in mg/dl Creatinine, they are converted to g/l as follows (the factor 20 coming from the dilution):

$$\textbf{Creatinine g/l} = \frac{\textbf{Reading (mg/dl) x 10 x 20}}{\textbf{1000 x 1}}$$

This simplifies to:

$$\textbf{Creatinine g/l} = \frac{\textbf{Reading (mg/dl)}}{\textbf{5}}$$

Note: This result is then used in conjunction with the total mercury figure obtained as above as follows:

$$\frac{\textbf{Total Mercury (µg/l)}}{\textbf{Creatinine (g/l)}} = \textbf{µg Mercury / g Creatinine}$$

Please note that if the creatinine result is not between 0.35 and 3.5 g/l, the final mercury result will be checked, and if the result is higher than normal for that employee then a new sample will be requested.

5. APPENDIXES

Appendix 1 - Calculation of the limit of detection and control of blank values

6. REFERENCES

- ***Env Prot 11 - Code of Practice - Mercury Housekeeping***
- ***HEALTH 2 - Code of Practice: Control of Worker Exposure to Mercury in the Chlor-Alkali Industry***
- ***Anal 3-7 - Standardization of Methods for the Determination of Traces of Mercury***
- ***Standardisation of Methods for the Determination of Traces of Mercury; Part II - Determination of Total Mercury in Materials Containing Organic Matter, published in Analytica Chimica Acta: Anal. Chim. Acta, 84, (1976), 231-157***

Appendix 1: Calculation of the limit of detection and control of blank values

It is essential when carrying out high sensitivity analysis at concentration levels close to the limit of detection, to monitor and control the blank values in order to ensure that the results obtained are meaningful and that a realistic value for the limit of detection is quoted depending on the blank values which each individual laboratory can achieve.

➢ Calculation of limit of detection

The limit of detection (LD) is given by the expression:

$$LD = st_{(P,f)}$$

where
- s = standard deviation
- t = student's confidence coefficient (here 3.25)
- P = probability (here 99.5% one sided)
- f = degree of freedom (here, for 10 measurements, 9)

➢ Monitoring and control of blank values

For the method of analysis in use carry out a series of ten blank determinations following the appropriate procedure. Calculate the standard deviation of a single blank and use it to calculate the confidence interval (CI) from the following expression:

$$CI = \overline{x} \pm st(P,f)$$

where
- $\overline{x}$ = mean of the blank values
- s = standard deviation
- t = student's confidence coefficient (here 2.26)
- P = probability (here 95% two sided)
- f = degree of freedom (here, for 10 measurements, 9)

Carry out a daily blank which should be within the CI. If it is significantly higher than x + st, check the reagents used in the procedure and replace any with high mercury levels. If the blank still exceeds the upper limit or if it is significantly lower than x - st, re-determine the confidence interval and recalculate the limit of detection.

➢ Accuracy statistics

The following *precision* statistics is based and with reference to the Dr. Drexler last round robin:

Impurity	*Dimension*	*c*	*k*	*n*	s_L
Hg	g/l	11.7	53	1	3.29
		29.6	53	1	6.05
Creatinine	g/l	0.81	27	1	0.062
		1.26	27	1	0.122

c : overall mean value;
k : number of laboratories after rejection of 3 outliers;
n : number of replicates;
s_L : between laboratory standard deviation.

The *trueness* is proven looking at the obtained overall mean values and the reference value (and its tolerance range) as reported by Dr. Drexler. For the exact formulation of a statement on the trueness we should know more on the origin/calculations of the reference value and the tolerance range.

- Reference

I.M. Kolthoff et al, Quantitative Chemical Analysis, Fourth Edition, Errors in Quantitative Analysis, The Macmillan Company, Collier Macmillan Ltd, London.

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