REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME

October 1987

The determination of methylmercury, total mercury and total selenium in human hair

Reference Methods For Marine Pollution Studies

No. 46 (draft)

Prepared in co-operation with

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PREFACE

The Regional Seas Programme was initiated by UNEP in 1974. Since then the Governing Council of UNEP has repeatedly endorsed a regional approach to the control of marine pollution and the management of marine and coastal resources and has requested the development of regional action plans. The Regional Seas Programme at present includes ten regions and has over 120 coastal States participating in it. (1), (2)

One of the basic components of the action plans sponsored by UNEP in the framework of the Regional Seas Programme is the assessment of the state of the marine environment and of its resources, and of the sources and trends of the pollution, and the impact of pollution on human health, marine ecosystems and amenities. In order to assist those participating in this activity and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of Reference Methods and Guidelines for marine pollution studies are being developed and are recommended to be adopted by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared in co-operation with the relevant specialized bodies of the United Nations system as well as other organizations and are tested by a number of experts competent in the field relevant to the methods described.

In the description of the methods and guidelines the style used by the International Organization for Standardization (ISO) is followed as closely as possible.

The methods and guidelines, as published in UNEP's series of Reference Methods for Marine Pollution Studies, are not considered as final. They are planned to be periodically revised taking into account the development of our understanding of the problems, of analytical instrumentation and the actual need of the users. In order to facilitate these revisions the users are invited to convey their comments and suggestions to:

Marine Environmental Studies Laboratory
International Laboratory of Marine Radioactivity
International Atomic Energy Agency
c/o Musee Oceanographique
MC98000 MONACO

which is responsible for the technical co-ordination of the development, testing and intercalibration of Reference Methods.

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(1) UNEP: Achievements and planned development of the UNEP's Regional Seas Programme and comparable programmes sponsored by other bodies. UNEP Regional Seas Reports and Studies No. 1 UNEP, 1982.

(2) P. HULM: A Strategy for the Seas. The Regional Seas Programme: Past and Future UNEP, 1983.

This issue of the Reference Method for Marine Pollution Studies No. 46 (draft) was prepared in co-operation with the World Health Organisation (WHO), and the International Atomic Energy Agency (IAEA). It includes comments received from the WHO/UNEP Meetings on the Biological Monitoring of Methylmercury in Mediterranean Populations (Zagreb, 17-21 September 1984) and on Health Effects of Methylmercury in the Mediterranean Area (Athens, 15-19 September 1986), as well as from a number of scientists who reviewed and tested the method. The assistance of all those who contributed to the preparation of the issue of this reference method is gratefully acknowledged.

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I. DETERMINATION OF METHYLMERCURY IN HUMAN HAIR

1. SCOPE AND FIELD OF APPLICATION

This Reference Method describes the determination of methylmercury in human hair by gas liquid chromatography. It is designed for biological monitoring of selected individuals and population groups with a possible intake of methylmercury exceeding the recommended Provisional Tolerable Weekly Intake (PTWI) through contaminated seafood, as part of a project on the evaluation of methylmercury in Mediterranean populations and related health hazards. The method, however, is also applicable to other regions.

2. REFERENCES

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WESTÖÖ, G. (1968) Determination of methylmercury salts in various kinds of biological material. Acta Chim. Scand., 22, 14-17.

3. PRINCIPLE

The method involves direct determination of methylmercury by gas liquid chromatography. The sample is disintegrated in a solution of sodium hydroxide, methylmercury is extracted from an aliquot of the solution into toluene and, after purification, a small volume is injected into a chromatographic column, filled with polyethyleneglycol succinate on Diatomite AW. Methylmercury in the gaseous mixture is detected with an electron capture detector and its amount determined by comparing the peak height with those of appropriate standards.

4. SAMPLING

Code number, name, age of donor and date of sampling are recorded by a marker on a transparent plastic sampling bag 12x5 cm. Hair may be cut using scissors, from several sites, preferably in the occipital area, and as close as possible to the scalp [1], in an amount which corresponds to about 2 grams, (amounts are best estimated by comparing them with a prepacked sample of known weight) but the minimum amount required is about half a gram.

The hair is collected on a sheet of white paper with proximal ends on the same side [2] and subsequently transferred with tweezers, or by folding the paper and letting hair slide into the bag. The proximal ends are identified [3] by a line, drawn on the outside of the bag with a marker.

Ten hairs, if long, are plucked off the scalp, wrapped in a sheet of plastic foil and placed in the bag with the rest of the sample, to enable later sectional analysis, if required [4].

The bag is then stapled at several points to immobilize the hair as much as possible, and then closed by folding the open end and stapling.

The completed Recording Form (Annex I) is fastened to the bag in the same way. Samples, collected at the same location and/or time are packed together and forwarded to the analytical laboratory. It is important to have information about longer breaks in the regular consumption of seafood suspected to have a high concentration of MeHg, for the period when the hair taken was formed, (assuming a growth rate of 1 cm per month).

Differences in the concentrations of mercury in hair of the same subject have been reported depending on its sampling site on the body and also in various areas of the scalp. These differences might in part be accounted for by the variability of analytical results and/or by local contamination; if true, they are not expected to be large enough to influence the interpretation of data in the context of the scope as outlined in 1 above.

NOTES:

1) With regular consumption of seafood there will be no major variation in concentrations of Hg or MeHg within a few mm from the scalp and hence, minor differences in this distance will not affect the comparability of results.

2) Does not apply for short and/or curly hair.

3) In long hair Hg and MeHg concentrations at the far end are influenced by repeated washing, application of cosmetic preparations etc. Thus distal end may no longer correctly reflect the exposure. The ends of very long hair are cut off to a length of about 10 cm.

4) It is suggested that this part of sampling be postponed to a later stage and applied to cases for which the results indicated high exposure.

5. PRETREATMENT OF SAMPLE

Some authors recommend the use of hair as collected (McMullin et al., 1982; Chittleborough, 1980); it appears preferable, however, to analyze material from which adhering dust and grease have been removed. Of several solvents, or combinations thereof (hexane, alcohol, acetone, water, diethylether, detergents), the use of water and acetone are recommended by IAEA and WHO.

Hair is cut with stainless steel scissors into segments as short as possible (or powdered in a teflon homogenizer after freezing in liquid nitrogen), transferred to a wide test tube (Fig.1,a) or beaker and washed successively with acetone – water – water – water – acetone. Each time the sample is covered with the solvent (10-25ml depending on the amount), mechanically shaken (or stirred at frequent intervals) for 10 min and the solvent carefully decanted. The hair remaining in the tube after the last acetone wash is left to dry, protected from dust and draughts, in the open container and then transferred into a clean test tube or envelope.

NOTE: Significant extraneous contamination of samples with methylmercury is not likely to occur at any stage of the procedure. Extreme precautions have to be taken, however, to avoid contamination when determining inorganic mercury.

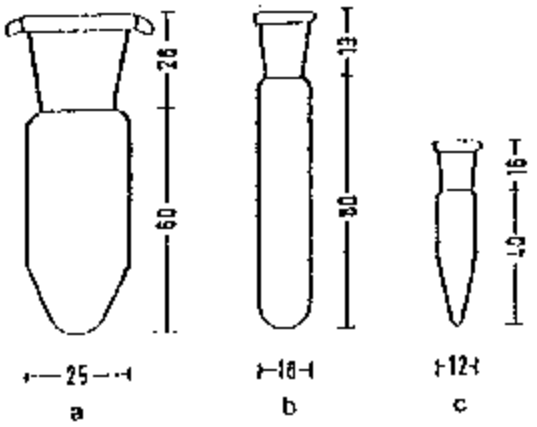

FIGURE 1: Test tubes for the decomposition of samples and extraction of methylmercury: 25ml (a), 10ml (b), 3ml (c).

6. REAGENTS

6.1 Sodium hydroxide, 7.5M, 250ml, kept in polyethylene or polypropylene bottle.

6.2 Cysteine hydrochloride, 1%, 100ml.

6.3 Sodium chloride, 1%, 100ml.

6.4 Copper sulphate, 1M in 2M sulphuric acid: to 60-80ml water in a beaker add carefully 10.ml of sulphuric acid (d=1.84 g/ml), dissolve in it 25g of copper sulphate pentahydrate and dilute to 100ml with water.

6.5 Potassium bromide, 4M, 100ml.

6.6 Sodium thiosulphate, 0.05M. 100ml.

6.7 Toluene, 500ml, chromatographic grade.

6.8 Benzene, 250ml, chromatographic grade.

6.9 Activated magnesium silicate (florisil, e.g. Merck 9,5514), 100g.

6.10 Sodium sulphate, anhydrous, 100g.

NOTE: Reagents 6.1-6.6 are aqueous solutions prepared with double distilled water from a quartz still. All chemicals and solvents should be of highest purity. The volumes and amounts should be sufficient for the analysis of about 100 hair samples.

7. STANDARD SOLUTIONS

7.1 Methylmercury (MeHg) stock standard solution, 10 µg ml^{-1} in benzene, 100ml: Weigh accurately 56.2mg of MeHg chloride, transfer into a 50ml volumetric flask containing 30–40ml benzene, shake until dissolved and add benzene to the mark. Using a Eppendorf type pipette, withdraw 1.00ml of the above solution, transfer it into a 100ml volumetric flask and dilute with benzene to the mark.

7.2 Methylmercury (MeHg) working standard solution, 100ng ml^{-1}, 60ng/ml^{-1} and 20ng/ml^{-1}. Using a high precision pipette transfer 0.5, 0.3 and 0.1ml of the solution under 7.1 into 50ml volumetric flasks and dilute with benzene to the mark.

NOTE: ALL OPERATIONS INVOLVING BENZENE SHOULD BE CARRIED OUT IN A WELL-VENTILATED HOOD. SKIN CONTACT WITH BENZENE MUST BE AVOIDED AS IT IS A KNOWN CARCINOGEN.

8. APPARATUS

8.1 Semi-micro analytical balance.

8.2 Centrifuge 5/50ml capacity.

8.3 Waterbath.

8.4 Mechanical shaker.

8.5 Transfer pipettes, Eppendorf type, 50µl, 100µl, 250µl, 500µl, 1000µl, 2 sets.

8.6 Volumetric flasks, 10ml, 25ml, 10 each, 50ml, 100ml, 4 each.

8.7 High precision micro syringes, 5µl, 10µl, 6 each, 1µl, 100µl, 3 each.

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* Benzene is a better extractant for MeHg than toluene. Because of its toxicity, however, it is used only in the final stage when small volumes are involved. See also NOTE under 7.2.

8.8 Test tubes with ground stoppers, 3ml, 10ml, 40ml (Fig. 1 a,b,c), 20 each.

8.9 Gas chromatograph with electron capture detector.

8.10 Recorder or equivalent reading instrument.

8.11 Chromatographic columns, glass, 1.6m long, 2mm dia, filled with 5% polyethylene glycol succinate (PEGS) on Chromosorb W, AW, DMCS or Chromosorb W, HP, 100-120 mesh.

NOTE: If PEGS is not available, DEGS (diethylene glycol succinate) should be used.

9. PROCEDURE

The physical properties of hair and its chemical composition (as compared to fish muscle which is the material most widely analyzed for MeHg), in particular the high percentage of sulphur containing amino acids in its matrix, require a different approach to desintegration and substantial further modifications of the extraction procedure devised by Westöö (1968). The measurement system is basically the same as used in the analysis of seafood.

9.1 After the chromatographic column has been conditioned, adjust the gas flow (60 ml h^{-1}) and temperatures to the optimum (column 130°C, injector 160°C, detector 250°C).

9.2 Inject 5ul aliquots of the working MeHg standard solutions (7.2) and check the sensitivity of the detector and linearity of response.

9.3 Disintegrate 50-100mg of hair sample by covering it with 2ml of 7.5 M NaOH (6.1) in a 25ml test tube (Fig.1,a), add 1ml cysteine hydrochloride (6.2) and place the test tube with contents in a water bath at 90°C for 30 min; stir the test tube repeatedly. Cool and transfer the solution into a 10ml volumetric flask; wash the test tube several times with small volumes (1ml) of 1% NaCl (6.3) and add washings to the solution in the flask; dilute to the mark with NaCl and mix.

9.4 Transfer a 1ml aliquot of the solution to a conical test tube. Acidify with 2ml $CuSO_4$-H_2SO_4 solution (6.4), add 2ml 4M KBr (6.5) followed by 3ml of toluene (6.7). Equilibrate for 10 min and centrifuge.

9.5 Withdraw 2ml of the organic solution and transfer it to a 10ml test tube (Fig.1,b).

9.6 Strip MeHg from the organic solvent by equilibrating for 5 min with 0.6ml 0.5M thiosulphate (6.6). Centrifuge.

9.7 Withdraw 0.5ml of the aqueous phase with an Eppendorf pipette and transfer it into a conical 3ml test tube (Fig.1,c).

9.8 Add 0.3 ml of H_2SO_4-$CuSO_4$ solution followed by 0.3ml KBr and 0.5ml benzene (6.8). Equilibrate for 10 min and centrifuge. Transfer the benzene phase to a new 3ml test tube.

9.9 Start taking chromatograms with MeHg working standard solutions in 5µl, followed by a 5µl sample. Continue alternating samples and standard solutions. If necessary, adjust the aliquots taken (and/or the volumes of benzene in the final extraction) so as to get the deflection of the recorded pen (or the reading on the display) between 20 and 80% of the full scale.

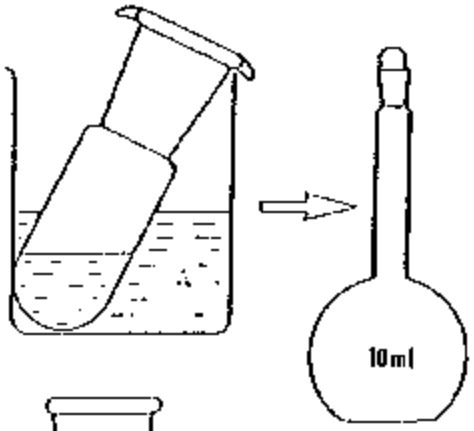
NOTE: A series of ten samples daily is assumed in normal working conditions provided that the analyst has become familiar with the procedure.

9.10 If the MeHg peak is not clearly separated proceed with purification as follows: Add 250 mg Florisil (6.9) and 50mg anhydrous sodium sulphate (6.10) to the sample extract in the test tube. Shake and let settle. Inject a new 5µl aliquot and inspect the chromatogram.

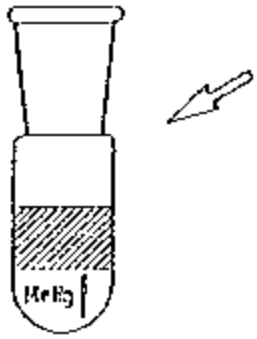
NOTE 1: With typical MeHg concentrations for exposed populations (>1 µg/g) the treatment with Florisil is usually not necessary. It is effective in removing interferences but 10-15% of MeHg may be lost.

NOTE 2: After three or four injected samples, peaks begin to tail. Restore the performance of the column by injecting a 5µl aliquot of a saturated solution of $HgCl_2$ in benzene.

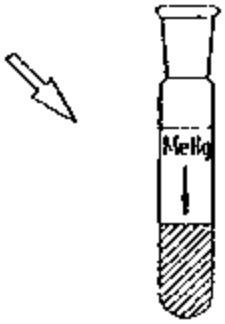
10. SCHEME FOR DETERMINATION OF METHYLMERCURY IN HAIR



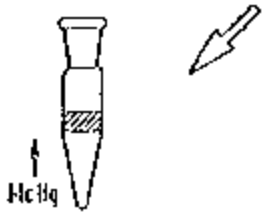
10.1 50-100mg sample + 2ml NaOH (6.1) + 1ml cysteine (6.2); keep at 90°C 30 min, stir frequently; cool, dilute to 10ml with NaCl (6.3) in a volumetric flask.



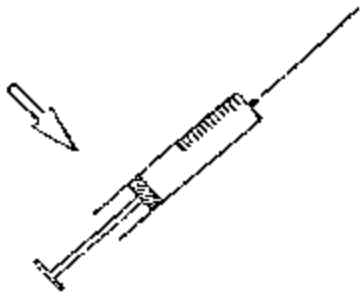
10.2 1ml aliquot+2ml H_2SO_4–$CuSO_4$ (6.4) + 2ml KBr (6.5) + 3ml toluene (6.7). Equilibrate 10 min, Centrifuge. Transfer 0.5ml aqueous phase. Withdraw 0.5ml and join aqueous phases.



10.3 4ml toluene phase + 0.6ml $Na_2S_2O_3$(6.6). Equilibrate 10 min., centrifuge. Transfer 0.5ml aqueous phase. Withdraw 0.5ml and join aqueous phases.



10.4 1ml aqueous phase+0.3ml KBr+0.3ml H_2SO_4–$CuSO_4$+0.5ml benzene (6.8). Equilibrate.



10.5 Inject 5µl organic phase.

11. QUALITY CONTROL

At present no certified reference material is available for MeHg in hair and accuracy has to be assured by other means of analytical quality control. Two alternatives are given below:

11.1 Interlaboratory hair reference sample (IHRS): Before beginning the actual monitoring, 2 3 hair samples of at least 50g each with MeHg contents between 5µg g^{-1} and 20µg g^{-1} should be provided by the organizers. Aliquots (2-3g) should be distributed to two or three experienced participating laboratories to determine their MeHg content. Upon reaching agreement between the results, these samples will serve as reference materials for the project.

11.2 Matrix reference sample (MRS): A hair sample (100g) with a low MeHg (<0.05µg g^{-1}) and total mercury (<0.5µg g^{-1}) should be collected by each of the participating laboratories for internal quality control. It should be obtained from individuals whose diets do not include seafood and who are not associated with any type of activities, or environments, involving mercury. The hair sample is washed according to the instructions given in Section 5 above, and checked for MeHg using the proposed (or an equivalent) procedure. Total mercury (and selenium) should also be determined in this sample.

11.3 Together with the analyzed samples a similar aliquot of IHRS is processed in the same way (under Section 9.). The result obtained for this sample is a measure of the accuracy achieved in the series. Comparing the peaks for this sample with those obtained for the working standard solution aliquots, IHRS also indicates matrix influences and the yield of the method.

11.4 Matrix reference sample is used as a replacement for the interlaboratory reference sample for checking the performance of the separation and detection system in the presence of the hair matrix. By spiking aliquots of matrix reference sample (MRS) in the disintegration stage (9.3) with known amount of MeHg (e.g. accurately measured volumes of the MeHg stock standard solution, 7.1) approaching to those expected in analyzed samples, control is secured over the functioning of the column and detector under the influence of the matrix. The peaks of MeHg in the spiked MRS are compared with those obtained with equal amounts of MeHg in the working standard solution, injected subsequently.

NOTE: Methylmercury added to the MRS is not entirely equivalent to that bound in the samples. If the decomposition of the sample is complete, however, identical behaviour during processing can be assumed.

12. CALCULATION OF RESULTS

The concentration of MeHg in the sample is obtained from experimental data as follows:

$$\text{MeHg (in } \mu g\ g^{-1}) = f \cdot \frac{h_x \cdot a_s \cdot V_x}{h_s \cdot m_x \cdot v_x},$$

where,

f = a dilution factor dependent on the volume ratios of the organic and aqueous phases in the successive stages of processing,

h_x = the peak height for the sample,

a_s = the amount of MeHg standard added (in ng),

V_x = the volume of the final organic sample solution (in µl).

h_s = the peak height for the injected MeHg standard,

m_x = the amount of the sample processed (in g),

v_x = the aliquot of the sample solution injected (in µl).

A combined test report on all three determinations described in this Reference Method (methylmercury, total mercury and selenium) is given on pages 21 to 22.

II. DETERMINATION OF TOTAL MERCURY IN HUMAN HAIR

1. SCOPE AND FIELD OF APPLICATION

This Reference Method describes the determination of total mercury in human hair by atomic absorption spectrophotometry. It is designed for biological monitoring of selected individuals and population groups in the Mediterranean region with a possible intake of mercury exceeding the recommended Provisional Tolerable Weekly Intake (PTWI) through contaminated seafood. The method is also designed to enable (a) comparison with previous data and (b) the necessary correlation between total mercury and methylmercury in the subjects monitored.

2. REFERENCES

BOCK, R. (1979), A Handbook of Decomposition Methods in Analytical Chemistry, International Text Book Company Ltd., Glasgow/London.

UNEP/FAO/IAEA/IOC (1984) Determination of total mercury in selected marine organisms by flameless atomic spectrophotometry. Reference Methods for Marine Pollution Studies No. 8, Rev. 1., UNEP, Geneva.

3. PRINCIPLE

Determination of total mercury is based on cold vapour atomic absorption spectrometry after complete mineralization of the sample in a reflux system. Decomposition with an oxidizing acid mixture is described in detail. In subsequent phases of the analysis the reference method is followed as used for determining total mercury in selected marine organisms (UNEP/FAO/IAEA/IOC, 1984).

NOTE: If teflon bomb digesters are available, they may be used instead of the reflux digesters described below. Their use is described in the above mentioned Reference Method No. 8.

4. REAGENTS

4.1 Nitric acid (d = 1.4g ml^{-1}).

4.2 Sulphuric acid (d = 1.84g ml^{-1}).

4.3 Hydrogen peroxide, 30%.

4.4 Potassium permanganate solution, 1% in distilled water.

4.5 Hydroxylammoniumchloride, 1% solution in distilled water, prepared daily.

All reagents should be of suprapur or equivalent purity. They are usually packed in 250 ml quantities. 100 determinations would require approximately 200ml of HNO_3, and 50ml each of sulphuric acid and hydrogen peroxide.

5. EQUIPMENT AND APPARATUS

The laboratory should be adapted for trace analytical work, possibly equipped with a laminar flow hood.

NOTE: A laboratory in which metallic mercury or its salts had been handled at any time in the past is not suitable for determining mercury at low levels. It is also advisable that concentrated mercury solutions (stock standard solutions) be prepared and diluted in a separate room.

In addition to general apparatus and glassware (pipettes, volumetric flasks, etc.) listed for determination of methylmercury (Chapter I, Section 8, 8.1-8.8) the following special apparatus is required.

5.1 Twelve flasks for decomposition of samples, 25ml, round bottom, with standardized ground glass joints, 19mm dia. (Fig. 2,d).

5.2 A digestion unit (e.g. unit as used in routine semi-micro Kjeldahl determination of nitrogen) with temperature control between 80 and 300°C, for simultaneous decomposition of 6 samples.

5.3 Six Condensers (Fig. 2,a).

5.4 Attachment (Fig. 2,b) permitting separate collection of condensate.

5.5 Atomic absorption spectrometer for UV and visible spectral range (Fig. 3,h).

5.6 Mercury hollow cathode lamp (Fig. 3,f).

5.7 Absorption cell, 150mm long, 10mm dia. with silica windows (Fig. 3,g).

5.8 Aeration flasks, 50ml (Fig. 3,a).

5.9 Recorder (Fig. 3,j).

[illegible] should be cleaned with [illegible] diluted

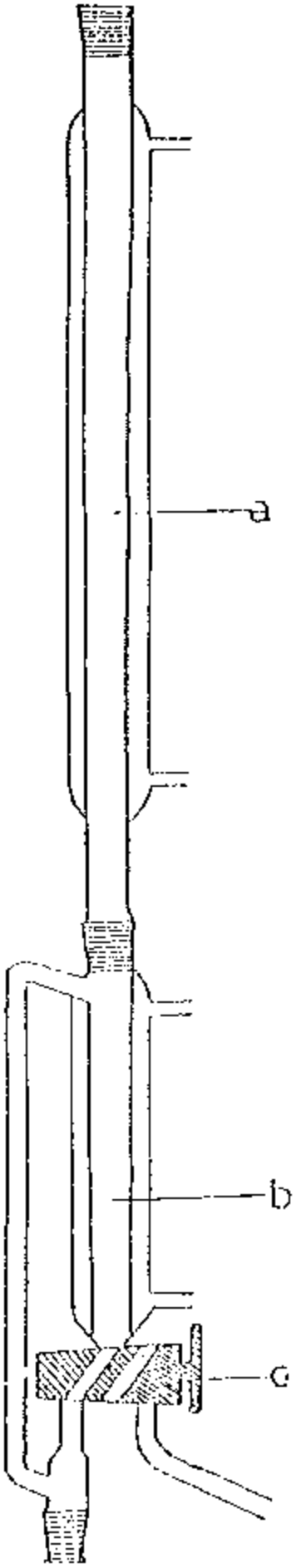


FIGURE 2: Decomposition assembly for total mercury in biological material.

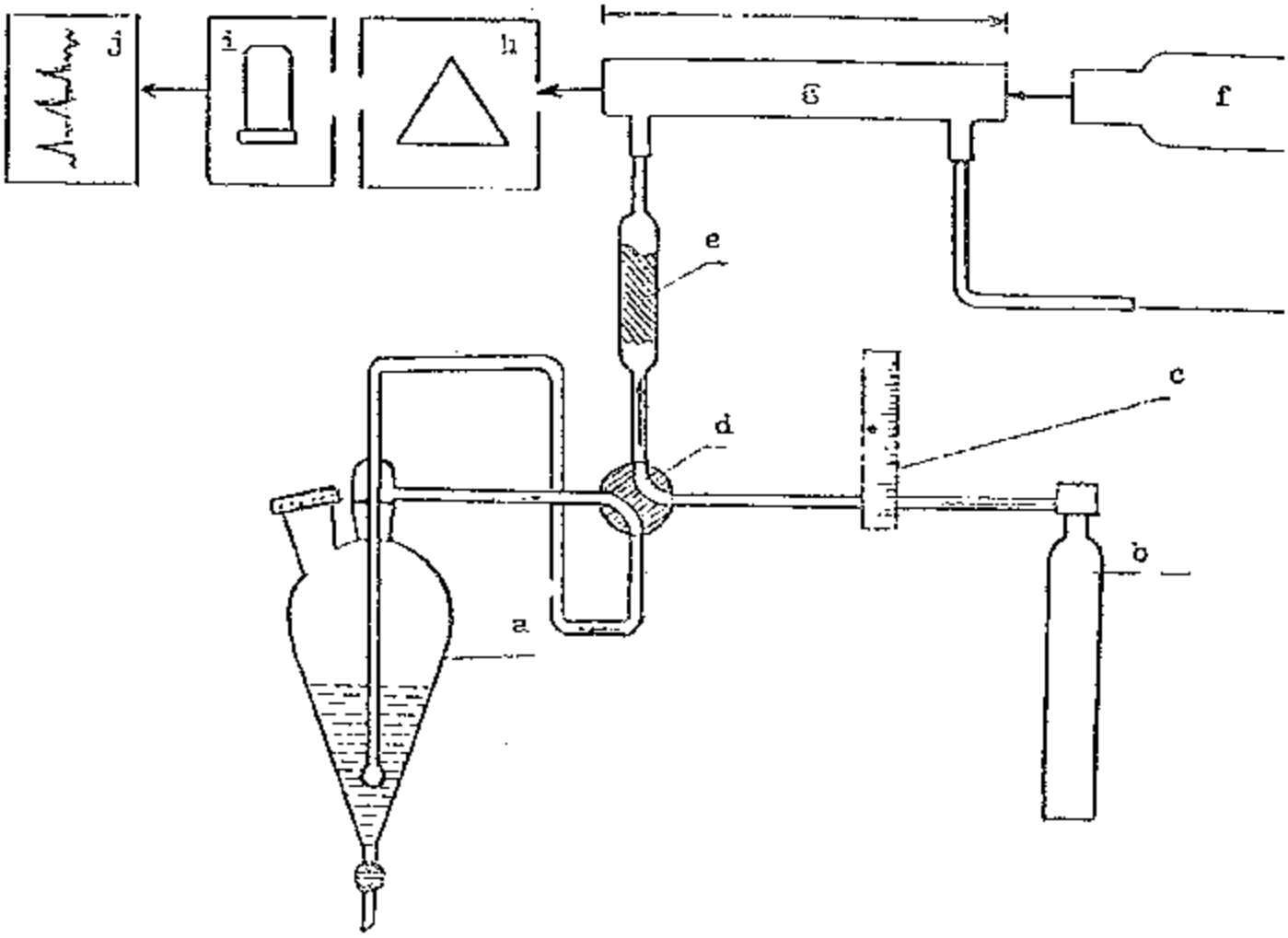


FIGURE 3: Assembly for determination of total mercury a) aeration flask, b) nitrogen cylinder, c) flowmeter, d) two-way stopcock, e) trap with magnesium perchlorate, f) mercury lamp, g) absorption cell, h) spectrometer, i) detector, j) recorder.

6. SAMPLING AND PRETREATMENT OF SAMPLE

Hair samples should be taken and pretreated in the same manner as described for determination of methylmercury (Chapter 1, Sections 4 and 5).

7. PROCEDURE

7.1 Weigh accurately 100-200mg of hair sample, prepared according to the instructions for MeHg and transfer it into a decomposition flask.

7.2 Add 2ml of nitric (4.1) acid, assemble the decomposition apparatus (Fig. 2) and let the hair disintegrate at 50°C for 1 h.

7.3 Add carefully 0.5ml concentrated sulphuric acid (4.2) through the condenser and increase the temperature slowly until fumes of sulphuric acid begin to evolve.

7.4 Cool and let the condensate drain into the decomposition bottle. Close the stopcock and add 0.5ml of 30% H_2O_2 (4.3). By carefully reopening the stopcock add hydrogen peroxide dropwise into the mixture in the decomposition bottle.

7.5 Close the stopcock and expel nitrogen oxides by keeping the mixture for 30 min at 90°C.

7.6 Slowly increase the temperature and evaporate to fumes of sulphuric acid. Cool.

7.7 After the solution becomes clear and colourless (if not repeat the treatment under 7.4 and 7.5) open the stopcock and let the condensed liquid drain into the decomposition flask. Transfer the solution from the decomposition flask into a 25ml volumetric flask.

7.8 Reattach the flask to the apparatus and add through the condenser 2 small portions (2ml) of water. By boiling, let the condensing vapour wash the unit. Add the condensate to the sample solution in the volumetric flask.

7.9 Add $KMnO_4$ solution (4.4) dropwise until a faint permanent pink colour is obtained, then destroy excess with some drops of hydroxylammoniumchloride ($HONH_3Cl$) solution (4.5). Make up with water to the mark.

For standardization and analysis follow the procedures described in Reference Method for Marine Pollution Studies No. 8, (Determination of total mercury in selected marine organisms by cold vapour atomic absorption spectrophotometry) in this series, Sections 10, 11 and 12, using an appropriate standard reference material for quality control or a matrix reference sample/interlaboratory reference sample as described in section 1, 11. (p.9).

III. DETERMINATION OF SELENIUM IN HUMAN HAIR

1. SCOPE AND FIELD OF APPLICATION

This Reference Method describes the determination of selenium in human hair (and other indicative tissues) by gas liquid chromatography and is designed for biological monitoring of selected individuals and population groups in the Mediterranean region with a possible intake of methylmercury exceeding the recommended Provisional Tolerable Weekly Intake (PTWI) through contaminated seafood. The data are intended to establish a possible correlation between methylmercury intake and levels of selenium in the subjects monitored.

2. REFERENCES

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3. PRINCIPLE

The sample is ashed with magnesium nitrate. 4-nitro-1,2-diamino-benzene reacts with selenium (IV) to give 5-nitro-2,1,3-benzo-selenodiazole extractable into toluene or benzene (Poole et.al., 1977; Cappon and Smith, 1978). Selenium in the solvent phase is determined by gas liquid chromatography using an electron capture detector.

The above method has been selected because selenium is determined in conjunction with methylmercury, both of which require competence in gas chromatographic techniques. Reliable result for total selenium, however, will also be obtained by the following techniques:

a) Atomic absorption spectrophotometry after transformation of selenium into gaseous selenium hydride followed by its thermal decomposition, to produce atomic vapour in a heated absorption cell of the spectrophotometer (Watkinson, 1979; Dedina and Rubeska, 1980).

b) Fluorimetric method exploiting the intense fluorescense of the naphtoselenodiazol formed in the reaction between 2,3 diamino-naphtalene with Se (IV). This method was recently recommended on the basis of comparative studies but requires a high quality spectrofluorimeter (Analytical Methods Committee, 1979).

c) Activation analysis, based on either 17 sec $^{77}Se^{m}$ or 120.4 day ^{75}Se (Saced and Thomassen, 1979).

4. REAGENTS

The following volumes and amounts should be sufficient for about 100 determinations of selenium:

4.1 Hydrochloric acid solutions, 8M, 6M and 1M, 250ml each.

4.2 Ammonia, 1M, 250ml.

4.3 Magnesium nitrate hexahydrate, 250g.

4.4 4-nitro-1,2-diaminobenzene (4-NDB), 1% in 1M HCl, 100 ml.

4.5 5-nitro-2,1,3-benzoselenodiazole (5-NBSeD), 5g.

NOTE: Solutions of 4-NDB and 5-NBSeD have to be prepared weekly (50ml) and impurities removed by two equilibrations with 10ml toluene.

4.6 Sodium selenate (IV), 5g.

4.7 Toluene, 500ml.

4.8 Benzene, 500ml.

4.9 Florisil, 100g.

4.10 Sodium sulphate anhydrous, 50g.

5. STANDARD SOLUTIONS

5.1 Selenium stock standard solution: Dissolve 31mg of 5-NBSeD (4.5) in 100ml of toluene (4.7) and dilute in a volumetric flask to the mark with toluene.

5.2 Selenium working standard solution: Withdraw 100µl of the stock standard solution with a high precision micropipette or syringe and dilute to 100ml with toluene (4.7).

NOTE: If 5-NBSeD (4.5) is not available, a working selenium standard solution can be prepared as follows: To a solution (100ml) of sodium selenate (IV) (4.6) equivalent to 2µg Se ml^{-1} add 2ml of 4-NDB (4.4) and dip the bottle for 10 min in a water bath at 75°C. Extract the selenodiazole formed with 10ml toluene, discard the aqueous solution and wash the toluene phase with 5ml of 1M ammonia (4.2). Introduce anhydrous sodium sulphate (4.10) (50mg) and florisil (4.9) (250mg) to remove water and impurities. Take 5ml of clear solution and dilute to 100ml.

NOTE: Toluene can be replaced by benzene which is a better extractant; with care it can be used in laboratories with well ventilated hoods in spite of its toxicity.

6. APPARATUS

Basic analytical instrumentation and glassware as in previous chapters and the following additional equipment.

6.1 Four infrared lamps.

6.2 Electric furnace for temperatures up to 800°C.

6.3 Twenty silica crucibles, 15ml.

6.4 Gas chromatograph with electron capture detector.

6.5 Glass columns, 1.5m long, 3mm dia. filled with 3% OV-17 on Chromosorb AW.

6.6 Chromatorgraphic syringes, 5µl and 10µl, 4 each.

7. SAMPLING AND PRETREATMENT OF SAMPLE

Hair samples should be taken and pretreated in the same manner as described for determination of methylmercury (Chapter I, Sections 4 and 5).

8. PROCEDURE

8.1 Adjust the nitrogen flow-rate through the chromatographic column, and temperatures of the injector (220°C), column (190°C) and detector (270°C).

8.2 Weigh accurately 250mg of sample prepared according to the instructions for MeHg, into a 15ml silica crucible; wet the material with 2ml of saturated magnesium nitrate solution (4.3). Put the crucible with contents under an infrared lamp and evaporate carefully to avoid sputtering.

8.3 When completely dry increase the temperature by bringing the IR lamp closer until nitric oxides are no longer formed.

8.4 Transfer the crucible with contents to an electric furnace at 500°C for 30 min during which the material will turn into a white ash.

8.5 Take the crucible out of the furnace to cool. Dissolve the ash in 3ml 8M HCl (4.1) while warming to 90°C for 10 min to bring all selenium to oxidation state (IV).

8.6 Transfer the solution to a 10ml volumetric flask, wash the crucible with several 1ml aliquots of 6M HCl (4.1) and dilute to the mark with the acid.

8.7 Transfer 2ml of the solution to a 10ml test tube and equilibrate with 3ml toluene (4.7) to remove organic impurities. After separation of phases transfer the aqueous solution to a second 10ml test tube.

8.8 Add 1ml of 1% 4-NDB (4.4) and dip the tube for 10 min in a water bath at 75°C.

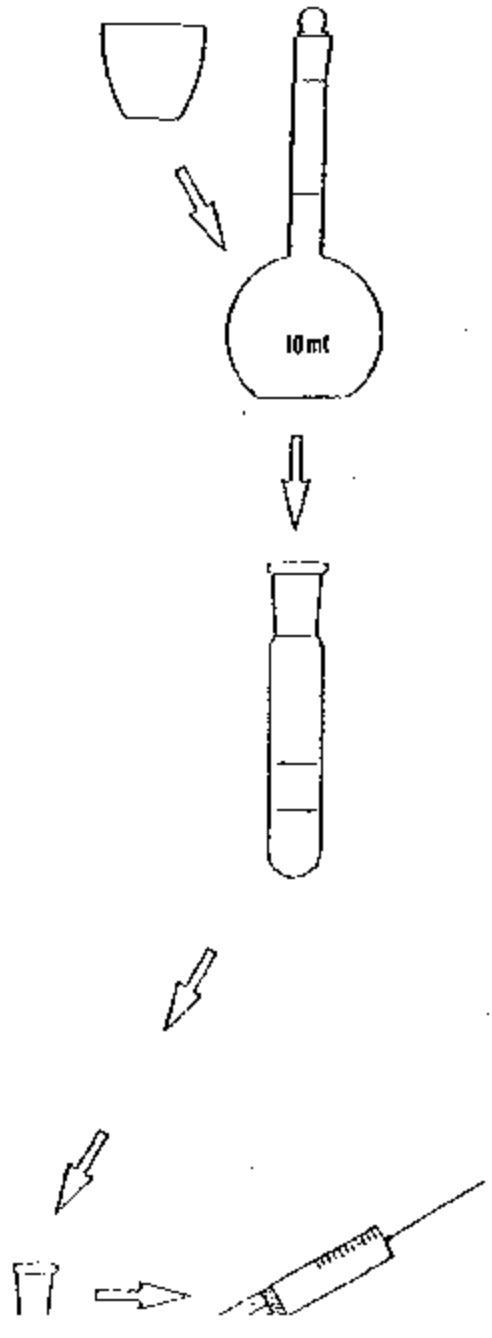
8.9 Extract the benzoselenodiazole formed by equilibration with 2ml benzene (4.8) for 5 min. Withdraw and discard the aqueous phase.

8.10 Remove the impurities from the solvent phase by stripping with 3ml 1 M ammonia (4.2). Withdraw and discard the aqueous phase.

8.12 If many peaks appear in the selenium region add 250mg of Florisil (4.9) and 50mg anhydrous sodium sulphate (4.10); shake the mixture thoroughly. After clarification inject another 5ul aliquot, proceeded, and followed, by different volumes of working selenium standard solution to cover the relevant range, between e.g., 0.1 and 1.0ng.

The yield of the procedure is between 85 and 90% but is reduced by about 10% in the case of treatment with Florisil.

9. SCHEME FOR DETERMINATION OF SELENIUM IN HAIR



9.1 Sample, 150-250mg + 2ml $Mg(NO_3)_2$(4.3) evaporate, heat to 150°C, ignite at 500°C.

9.2 Dissolve ash in 3ml HCl (4.1), keep at 90°C 10 min; transfer into 10ml volumetric flask, wash crucible and dilute to volume with HCl (4.1).

9.3 Transfer 2ml into 10ml test tube (Fig.1, MeHg), add 1ml 4-NDB (4.4), keep 10 min. at 80 90°C, cool.

9.4 Extract 5-NBSeD formed into 1ml toluene (4.5), withdraw and discard aqueous phase.

9.5 Inject 5ul into the

10. CALCULATION OF RESULTS

The concentration of Se in the sample is obtained from experimental data as follows:

$$\text{Se (in } \mu g.g^{-1}) = f.\frac{h_x.a_s.V_x}{h_s.v_x.m_x}$$

where:

f ... the dilution factor, dependent on the fraction of sample sub-sampled in the test tube after dissolution (2/10 in the case of the 9.Scheme on p. 20)

h_x ... is the peak height for the sample (cm)

a_s ... is the amount of Se standard in ng

V_x ... is the volume of the final organic sample solution in µl

h_s ... is the peak height for the Se standard (cm)

v_x ... is the aliquot of the injected sample solution in µl

m_x ... is the amount of the processed sample in g

IV. REPORT OF THE ANALYSIS

1. Sample Code:_____Date of receipt:_____

   Date of analysis:_____

2. Laboratory:_____

   Analyst:_____

3. Results and estimated uncertainty%

   MeHg_____$^{+}$_____Hg tot_____$^{+}$_____Se_____$^{+}$_____

4. Data on the subject (Code):_____

   4.1 age_____

   4.2 sex_____

   4.3 profession_____

   4.4 pregnancy (if applicable)_____

   4.5 exposure:

   4.5.1 type of seafood consumed_____

   4.5.2 concentration of MeHg_____Hg_____Se_____

   4.5.3 origin of seafood_____

   4.5.4 frequency: daily_____weekly_____

   4.5.5 amounts _____

   4.5.6 exposure history:

   time between exposure and sampling_____

   exposure: continuous_____periodical_____

5. Sample details:

   5.1 date of sampling_____

   5.2 area, location_____

   5.3 cause of increased mercury levels:

   elevated natural levels of seafood_____

   local pollution (source)_____

6. Measurement system

6.1 Instrument, Model:

MeHg_____

Hg total_____

Se_____

6.2 Column:	MeHg	Se
length(m)	_____	_____
liquid phase	_____	_____
solid support	_____	_____

6.3 Temperature (°C):

injector	_____	_____
column	_____	_____
detector	_____	_____

6.4 Retention: Time (Sec):	_____	_____

7. Analytical procedure:	MeHg	Hg	Se
7.1 number of processed aliquots	_____	_____	_____
7.2 amount analyzed, mg	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
7.3 number of injected aliquots	_____	_____	_____
7.4 evaluation of peaks by peak height	_____	_____	_____
by intergration	_____	_____	_____
7.5 precision achieved:	_____	_____	_____
7.6 quality control: IHRS[a]	_____	_____	_____
MRS[b]	_____	_____	_____
WSS[c]	_____	_____	_____

8. Remarks and observations relevant to interpretation of results:

_____

_____

ANNEX I

RECORDING FORM FOR HAIR SAMPLING

To be completed by the person collecting hair samples:

1. Sample code: ..... Sampling date: .....

2. Data on donor:
   Name: .....
   Date of birth: .....
   Sex: .....
   Occupation: .....
   Employment: .....

3. Data on hair:
   Colour: .....
   Type: .....
   Treatment[x]: .....

4. Data on exposure[xx]:
   Type of seafood: .....
   Origin: .....
   Amounts consumed: daily: ..... weekly: .....

x Enquire about, and record any special treatment applied to the scalp/hair during recent months such as medicated shampoos (Selsun!), dyes, ointments, perm.

xx Data on exposure may be taken from the dietary survey if available, or obtained by interviewing the donor during sampling.

To be completed by the analyst:

Sample received on: .....

Quantity grams: .....

Analysed for: .....

Analysis completed on: .....

Results: MeHg: ..... Hg: ..... Se: .....

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