REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME

12 November 1984

Determination of total mercury in selected marine organisms by cold vapour atomic absorption spectrophotometry

Reference Methods for Marine Pollution Studies No. 8 Rev. 1

Prepared in co-operation with

FAO IAEA IOC

UNEP 1984

Note: This document has been prepared in co-operation between the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA), the Intergovernmental Oceanographic Commission (IOC) of UNESCO and the United Nations Environment Programme (UNEP) under projects FP/ME/0503-75-07, ME/5102-81-01, FP/5102-77-03 and FP/5101-84-01.

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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 Regional Seas Programme is the assessment of the state of marine environment and of its resources, 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:

International Laboratory of Marine
Radioactivity
International Atomic Energy Agency
c/o Musée Océanographique
MC98000 MONACO

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

1/ UNEP: Achievements and planned development of 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 (Rev. 1) of the Reference Method for Marine Pollution Studies No. 8 was prepared in co-operation with the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA) and the Intergovernmental Oceanographic Commission (IOC) of UNESCO. It includes comments received from IOC's GIPME Group of Experts on Methods, Standards and Intercalibration (GEMSI), from the FOA/UNEP/IAEA Experts Consultation Meeting on Reference Methods for the Determination of Chemical Contaminants in Marine Organisms (Rome, 4-8 June 1984) and from a number of scientists who reviewed and tested the method. The assistance of all those who contributed to the preparation of Revision 1 of this reference method is gratefully acknowledged.

CONTENTS

1. SCOPE AND FIELD OF APPLICATION

This reference method describes the determination of total mercury in biological material by atomic absorption spectrophotometry after the organic matter has been decomposed by wet chemical digestion under pressure. Detection limit is 0.01 $mgkg^{-1}$ total mercury fresh weight.

2. REFERENCES

BERNHARD, M. (1976) Manual of methods in aquatic environment research. Part 3. Sampling and analyses of biological material. FAO Fish.Tech.Pap. No. 158 (FIRI/T158), pp. 124. FAO, Rome.

ISO (1979) Water quality - Determination of total mercury by flameless atomic absorption spectrophotometry - after digestion with permanganate - persulfate solution (Draft proposal ISO/DP 5666/I.2.)

UNEP/FAO/IAEA (1984) Sampling of selected marine organisms and sample preparation for trace metal analysis. Reference Methods for Marine Pollution Studies No. 7 Rev. 2, UNEP, Geneva.

UNEP/FAO/IAEA (in preparation) Guidelines for monitoring chemical contaminants in marine organisms. Reference Methods for Marine Pollution Studies No. 6, UNEP, Geneva.

3. PRINCIPLES

An aliquot of the sample, prepared according to UNEP/FAO/IAEA (in preparation) is decomposed in a pressure container in the presence of nitric acid at 120-150°C. Then the mercuric ion is reduced with an excess of $SnCl_2$ to metallic mercury which is volatilized by aeration and then the total mercury determined as monoatomic vapour at a wavelength of 253.7 nm.

4. REAGENTS

All reagents, including the distilled water, should be of recognized analytical quality, with as low as possible Hg concentration. All reagents must be checked for Hg contamination by analyzing blanks.

4.1 Demineralized distilled water with a mercury content below detection limits of this method.

4.2 Nitric acid ($d_{20°C}$ = 1.4 gl^{-1}).

4.3 Hydrochloric acid ($d_{20°C}$ = 1.19 gl^{-1}).

4.4 Sulphuric acid ($d_{20°C}$ = 1.84 gl^{-1}) diluted in an equal volume of distilled water (4.1).

CAUTION: Add the acid to the water slowly and with constant stirring to avoid spattering of concentrated acid.

4.5 Potassium permanganate solution (50 gl^{-1}).

4.6 Stannous chloride/hydroxylamine hydrochloride solution: Prepare this solution at least weekly by mixing 10 ml H_2SO_4 (4.4) with about 60 ml distilled water (4.1). After allowing to cool to room temperature, dissolve 3 g NaCl, 3 g hydroxylamine hydrochloride and 5 g $SnCl_2$ and bring the volume to 100 ml with distilled water (4.1). Alternatively, use 4% W/V Sodium Borohydride solution in 3% sodium hydroxide (if preferred for mercury reduction).

4.7 Mercury standard solutions.

4.7.1 Mercury stock solution: 1 g Hgl^{-1}. Weigh 1.354 g mercuric chloride ($HgCl_2$) to the nearest 0.001 g. Transfer to a 1000 ml volumetric flask and dissolve in about 10 ml nitric acid (4.2). Dilute to volume with distilled water (4.1) and mix.

4.7.2 Mercury standard solution: From the stock solution (4.7.1) prepare, in volumetric flasks (5.3) by appropriate dilutions using micropipettes (5.20), a mercury standard solution which in 0.1 ml contains the lowest amount of mercury standard to be used for standardization (10.1). Prepare this standard solution daily using as diluent 1 ml nitric acid (4.2) diluted in 250 ml of distilled water (4.1).

NOTE: The concentration of the Hg standard depends on the Hg levels anticipated in the samples to be analyzed.

4.8 Working matrix: Prepare the working matrix by homogenizing a sufficiently large sample (e.g. 300 g of fresh weight) of the same tissue and species which will be analyzed. Test the homogeneity of the working matrix by analyzing 5 subsamples for their mercury content (10), including mineralization (digestion) (9). If the coefficient of variation of the five analyses is less than 20% the working matrix is ready for use. Otherwise homogenize the working matrix until the above coefficient of variation is obtained or prepare a new working matrix.

4.9 Detergent, for laboratory use.

4.10 Sulphuric acid-permanganate mixture: 4 volumes sulphuric acid (4.4) and 1 volume potassium permanganate solution (4.5).

5. APPARATUS

5.1 Eight or more Teflon digestion vessels of at least 25 ml capacity, single or in a "digestion block".

NOTE: If smaller digestion vessels are used, the amounts digested (9) must be accordingly reduced to avoid explosions.

5.2 Oven or hot plate for the digestion vessels, working temperature 120-150°C, with double independent temperature controls.

NOTE: Oven or hot plate must have two independent temperature controls so that an overheating and consequent explosion can be avoided.

5.3 Six to nine 25 ml, several 10 ml, 100 ml, 500 ml and 1 litre volumetric flasks (borosilicate glass).

5.4 Micropipettes to delivery accurately: 0.01 ml.

5.5 Stainless steel, glass or Teflon tissue homogenizer.

5.6 Pipettes of 1, 2, 10 ml capacity.

5.7 Wash bottle with distilled water (4.1).

5.8 Analytical balance (100-200 g) with a precision of 0.001 g for weighing reagents; preferably a 'top-load' balance.

5.9 Atomic absorption spectrophotometer (AAS) with a hollow cathode Hg lamp and background correction or, alternatively, a mercury analyzer.

NOTE: Background correction may not be necessary if it can be shown that the matrix effect is negligible.

NOTE: Lower detection limits can be reached using an electrodeless discharge lamp.

5.10 Signal indicator, recorder or similar.

5.11 A tall-form aeration flask with a volume suitable for the sample size and compatible with the rest of the apparatus, bearing a calibration mark corresponding to the optimum filling level, the dead volume of which has been reduced to a minimum and through which the gas current flows under optimum conditions. For this, the aeration tube should be fitted either with a finely drawn-out point, a ball pierced with holes, or a glass frit (porosity between 100 and 250 µm). It is necessary to confirm that a series of different flasks all give the same results. After each use, treat the aeration flask with the sulphuric acid - permanganate mixture (4.10) to oxidize any traces of Sn(II) that it may contain.

5.12 Measuring cell with windows (e.g. quartz) transparent to UV (253.7 nm), the length and diameter of which are suitable for the spectrophotometer being used.

5.13 Equipment which eliminates any condensation of water vapour in the measuring cell. Any appropriate system may be adapted for this: an infra-red lamp, an electrical heating element, a rod heater, continuously passing air around the cell, etc.

5.14 Equipment to absorb Hg vapour leaving the measuring cell (e.g. 0.25% iodine in 3% KI solution).

NOTE: Figure 1 shows an example of the way in which the various components are assembled and the principle of the operation of the apparatus in the case of a system using an open circuit. Other satisfactory arrangements may also be utilized. The entrainment gas may be air, nitrogen or argon, and an absorbing solution other than that given in 5.14 may be used. It is possible to use an aeration system based on a closed circuit system although this system is less sensitive than the system using open circuit. A commercially available kit may also be used.

5.15 Weighing bottles with ground stoppers.

5.16 Desiccator.

5.17 Drying oven (105°C).

5.18 Stainless steel tweezers.

5.19 Freeze-dryer.

NOTE: Glassware used for the first time in the analytical procedure (10) must be treated as follows:

- wash with diluted nitric acid (4.2);
- wash with a mixture of sulphuric acid and permanganate (4.10) prepared immediately before use;
- wash with stannous chloride/hydroxylamine hydrochloride solution (4.6) to remove all deposits of manganese dioxide;
- finally, wash several times with distilled water (4.1).

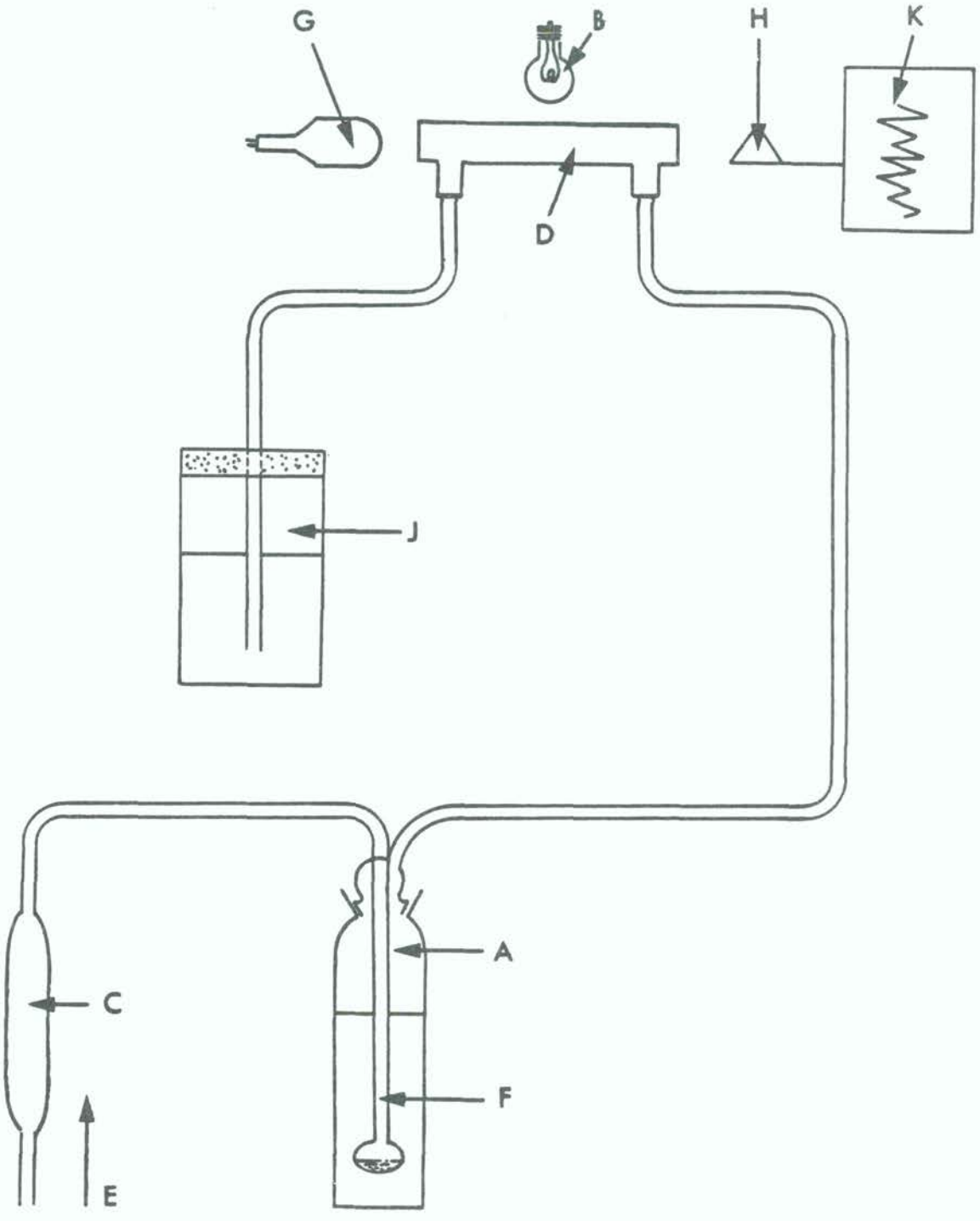

FIGURE 1 : EXAMPLE OF A SYSTEM USING OPEN CIRCUIT

A - Aeration flask for test solution; B - Arrangement for heating of the optical cell; C - Rotameter (1 litre air/minute); D - Quartz optical cell; E - Compressed air (1 litre/minute); F - Aeration tube with a perforated bulb at the end; G - Mercury hollow cathode lamp, or other lamp as appropriate; H - Spectrophotometer detection system; J - Absorber for mercury vapour containing iodine/iodide solution (5.14); K - Recorder.

6. SAMPLING

For a sampling plan follow UNEP/FAO/IAEA (in preparation) and for sampling of marine organisms follow UNEP/FAO/IAEA (1984).

7. SAMPLE PREPARATION

For sample preparation follow UNEP/FAO/IAEA (1984).

8. DETERMINATION OF DRY WEIGHT

NOTE: Results expressed on the basis of the dry weight (DW) of the analyzed tissues are more reliable than results expressed on the basis of its fresh weight (FW) because the continuous loss of water from the fresh biological samples does not allow an accurate determination of FW. However, results expressed on the basis of FW are easier to interpret as most transfer models of pollutants are based on FW.

Freeze-drying is preferred to oven drying, if a freeze drier is available.

8.1 Oven-drying

A clean weighing bottle (5.15) with its ground stopper removed, is placed into a drying oven (5.17) set at 105°C. It is important to use the tweezers (5.18) every time the glass is touched, to avoid leaving fingerprints and particles of dirt on the weighing bottle. After 2 hours at 105°C place the stopper and the bottle separately into a desiccator (5.16) to cool.

Weigh the empty bottle with its stopper in place on the analytical balance (5.8). Note the weight. Place 1-2 g of the subsample of the specimen sample (7) in the weighing bottle and close with the stopper. Weigh it again and note the result.

Place the weighing bottle containing the subsample in the drying oven set at 105°C, remove the stopper with tweezers (5.18) and also place the stopper in the oven.

After 24 hours replace the stopper on the bottle, remove the bottle with stopper from the drying oven, open the bottle and place bottle and stopper in a desiccator to cool.

Weigh the bottle with stopper in place and note the weight.

Repeat the drying cycle until the difference between subsequent weighing is less than 0.5% of the total weight; calculate the dry weight (DW) and DW/FW ratio.

NOTE: Biological materials containing large amounts of lipids cannot be oven-dried to constant weight and must, therefore, be freeze-dried (8.2).

8.2 Freeze-drying

Place a 1-2 g exactly weighed subsample of the specimen sample (7) in the clean sample container suitable for freeze-drying and freeze-dry (5.19) for 24 hours. Weigh the subsample exactly and freeze-dry for another 24 hours. Determine again the weight of the subsample. If the difference between the 2 weighings is less than 0.5% determine the DW and DW/FW ratio. Otherwise repeat the drying cycle until the difference between successive weighings is less than 0.5%.

9. MINERALIZATION OF THE BIOLOGICAL MATRIX

NOTE: Explosion hazard!! If too high amounts of organic material are placed in closed digestion vessels, (e.g. instead of the amount in fresh weight the same amount in dry weight is used) the vessels may burst with great energy (explode). An explosion may also occur if the vessels are overheated. Therefore, the entire digestion procedure must be carried out with the appropriate precautions required for working with acids under pressure. For example, the digestion operation must be carried out under a closed fume hood. Defective teflon bottles must be discarded and bottles which have been used for a certain length of time must be replaced before there is any risk of bursting. To avoid overheating the oven or the hot plate (5.2) must be equipped with a second thermostat which intervenes above the digestion temperature (e.g. 160°C).

9.1 Cleaning of the digestion vessels before and between digestions

Clean the digestion vessels (5.1) with detergent (4.9), if necessary, and rinse with distilled water (4.1), then proceed with the digestion procedure (9) without adding the sample (blank runs). If the blank values are greater than detection limit repeat the blank runs.

9.2 Predigestion experiments

Determine, by digestion procedure (9.3), for every new matrix the minimum amount of concentrated nitric acid (4.2) necessary to destroy completely the organic matter by adding to about 1 g FW sample (7) increasing amounts of nitric acid (from 1 ml to not more than 6 ml).

9.3 Digestion (mineralization) procedure

Place an exactly weighed sample (7) of about 1 g FW or 0.2 g DRY WEIGHT in each of the digestion vessels (5.1), one vessel being charged with an exactly weighed amount of the working matrix (4.9) to check the efficiency (11) of each digestion.

Add concentrated HNO_3 in amount needed for digestion (9.2), cover the vessels and close them tightly.

Let the samples in the vessels predigest at room temperature for at least one hour (preferably overnight).

Place the vessels in a preheated oven or on a hot plate (5.2) at 140°C for 3 hours.

Remove the vessels from the oven (hot plate), let them cool to room temperature and then open them. If the solution is not clear or has a yellow-brownish colour, the digestion is not complete. In which case, take a new sample (7) and repeat the experiment as described in 9.3 ensuring that the digestion conditions are followed exactly until the solution is clear. When the solution is clear, transfer the contents of each vessel into clean 25 ml volumetric flasks (5.3) and bring up to volume with distilled water (4.1).

The contents of the volumetric flasks represent the test solutions.

10. ANALYTICAL DETERMINATION OF TOTAL MERCURY

10.1 Standardization of the Hg determination

Before a new matrix is analyzed, and at periodic intervals as specified in the quality control procedure (12.3), carry out a digestion procedure (9.3) with 8 digestion vessels all but three charged with the working matrix (4.9) in order to standardize (calibrate) the method and the apparatus used. Before starting a set of analyses it is important to check the calibration of the instrument using standard solutions.

Prepare an appropriate standard solution (4.8.2) so that 0.1 ml of the standard added to 25 ml of the test solution (9.3) will result in a final Hg concentration in the test solution approximately double than the lowest Hg concentration anticipated in the samples to be analyzed.

Prepare a series of 8 clean digestion vessels (9.1). Add an exactly weighed aliquot of 1.00 g FW of the working matrix (4.9) to five vessels (5.1). No working matrix is added to the sixth to eight vessels, these vessels will be the blanks. Report the exact weights in the protocol. The first vessel should contain the working matrix (4.9) only, without any additions. With a micropipette (5.4) add 0.1 ml of the standard solution (4.8.2) to the second vessel, 0.2 ml to the third vessel, 0.3 ml to the fourth vessel and 0.4 ml to fifth vessel. Then add the predetermined amount of (9.2) of concentrated nitric acid to all six vessels and carry out the digestion procedure (9.3) and the Hg determination according to 10.2.

Determine the Hg concentration in the six digestion vessels and construct a standardization (calibration) curve. Verify that the concentrations to be

analyzed are in the straight part of the curve. If they are not, change the amounts to be analyzed by appropriate dilutions of the test solution (9.3) and of the standard solution (4.7.2).

10.2 Aeration and determination

Set up the AAS (5.9 to 5.14) for Hg analysis, adjust the controls of the apparatus and the gas flow using an aeration flask (5.11) filled with distilled water (4.1) to the calibration mark. Wait until the apparatus and the gas flow stabilize. Divert the gas flow and replace the aeration flask (5.11) with another flask to which just before replacement 2 ml of stannous chloride solution (4.6) has been added. Alternatively if a hydride generation system is available sodium borohydride (4.6) may be used instead of stannous chloride. In that case, follow the manufacturer's instructions on hydride generation.

Mix by gently shaking the contents of the replacement aeration flask, wait for 30 seconds and then restore the gas flow through the aeration flask. Then immediately add 1 drop of potassium permanganate solution (4.5). The entrainment of the mercury vapour through the measuring cell (5.12) produces a recorder tracing which begins to decrease. If the peak area is being measured, do not interrupt the tracing until the signal has returned to its initial value. After each test, clean the aeration flask with freshly prepared sulphuric acid-permanganate mixture (4.10) and then rinse with distilled water (4.1).

11. EXPRESSION OF RESULTS

From the height of the peak obtained from the test solution, determine, by reference to the standardization (calibration) curve (10.1) and making allowance for the blank determination, the concentration of Hg in the test solution. In the case of an apparatus with digital read-out or a maximum response indicator, prepare a graph of instrument read-out against the corresponding mass of Hg.

NOTE: If the signal generation is slow, consider peak area instead of peak height.

Check if the digestion vessel containing the working matrix of the digestion series (9.3) yielded a result within 10% of the result obtained in the homogeneity test (10.1). If it did not, check the digestion procedure (9.3) for errors and repeat it with the same samples until a satisfactory result is obtained.

Using the result obtained with the known concentration of test solution calculate the Hg concentration of sample, taking into account the exact weight of the sample placed in each digestion vessel (9.3). Express this concentration both in $mgkg^{-1}$ FW and in $mgkg^{-1}$ DW utilizing for the latter the results of (8).

12. ESTIMATION OF PRECISION AND ACCURACY

12.1 Precision

Estimate the precision of the entire analytical procedure (9 to 10) by digesting 5 subsamples from one original sample by calculating standard deviation (S) and coefficient of variation (CV) (CV=S.100/mean). If the coefficient of variation is greater than 10%, check the procedure for possible errors and contamination.

NOTE: The working matrix test (10.1) can be used for estimation of precision.

12.2 Accuracy

Analyze a certified standard with a matrix similar to the material under study together with your own working matrix (4.9) using this reference method. Calculate the mean and the standard deviation for the certified standard and the working matrix. If the value given for the certified standard is within the interval of your mean $\pm$ standard deviation, your method has the required accuracy and the working matrix can be used as standard for checking the accuracy of your procedure. If not, check procedure for errors.

NOTE: Standards are distributed as dried material so reduce the aliquot for digestion (9.3).

NOTE: In addition, by participating in intercalibration exercises involving several analytical laboratories, the accuracy of the method as used by the analyst can be checked and compared with the accuracy obtained by other participants in the exercise.

12.3 Quality control

Analyse periodically, at least once a week or whenever the routine has been interrupted for more than a week, the working matrix (4.9) standardizing the method according to (10.1) in order to guarantee the precision and accuracy of your results.

If the quality control checks reveal a fluctuation in the standard deviation or the accuracy of the results by more than 10%, check the following factors: stability of stock solutions (prepare new solutions); instrument drift or inadvertant changes in in operational parameters, contamination of the working matrix (select alternative reference material for analysis); contamination of equipment, e.g. glassware; operator error(s).

13. TEST REPORT

Fill in the test report (table 1) giving full details in every column. Attach sampling and sample preparation protocol (UNEP/FAO/IAEA (1984).

Table 1 : Test Report on Total Mercury Concentration in Biological Material

1. Sample code: _____

2. Determination of dry weight by freeze-drying ___ or in oven ____

   2.1 Duration of drying: _____ hours

   2.2 Date of drying (day, month, year): _____

   2.3 DW/FW ratio: _____

3. Mineralization (digestion)

   3.1 Duration of mineralization: _____ hours

   3.2 Temperature used for mineralization: _____ °C

   3.3 Date of mineralization (day, month, year) _____

   3.4 Anomalies observed which may influence test results:

   _____

   _____

   _____

4. Standardization (calibration) using working matrix

   4.1 Date (day, month, year): _____

   4.2 Result:

digestion vessel	1	2	3	4	5	6	7	8 (blank)
added FW (mg)						-	-	-
added stand. sol. (ml)	-	0.1	0.2	0.3	0.4	-	-	-
units of recorded signal								
$mgkg^{-1}$ FW								

5. Test result and estimation of precision using subsamples of same sample

5.1 Date (day, month, year): _____

5.2 Result:

digestion vessel	1	2	3	4	5
added FW (mg)					
units of recorded signal					
$mgkg^{-1}$ FW					

FW: mean ____ $mgkg^{-1}$; Stand.deviation _____ coeff. of variation ___%

DW: mean ____ $mgkg^{-1}$;

DW/FW ratio: _____

6. Estimation of accuracy

6.1 Date (day, month, year): _____

6.2 Type of certified standard used: _____

6.3 Declared $mgkg^{-1}$ of certified standard: _____

6.4 Results:

digestion vessel	1	2	3	4	5	6	7	8
added certified (mg)					-	-	-	-
added working matrix (mg)	-	-	-	-				
units of recorded signal								
$mgkg^{-1}$ FW								

	mean	stand.dev.	coef.of variation
certified standard			
working matrix			

7. Anomalies observed during test and other remarks relevant to the interpretation of results:

_____

_____

_____

8. Intercalibration exercise (give details): _____

_____

_____

_____

_____

9. Full address of the institution which carried out the test:

_____

_____

_____

_____

10. Name(s) and signature(s) of the person(s) who carried out the test:

_____

_____

Date: _____

Attachment: Sampling and sample preparation protocol relevant to the analyzed sample.

LIST OF REFERENCE METHODS FOR MARINE POLLUTION STUDIES

LISTE DES METHODES DE REFERENCE POUR LES ETUDES DE POLLUTION MARINE

UNEP/WHO : Guidelines for monitoring the quality of coastal recreational waters. (Draft) Reference Methods for Marine Pollution Studies No. 1. UNEP 1982.

UNEP/WHO : Determination of total coliforms in sea-water by the membrane filtration culture method. Reference Methods for Marine Pollution Studies No. 2 Rev. 1, UNEP 1983.

PNUE/OMS : Détermination des coliformes totaux dans l'eau de mer par la méthode de culture sur membranes filtrantes. Méthodes de références pour les études de pollution marine No 2 rév. 1, PNUE 1983.

UNEP/WHO : Determination of faecal coliforms in sea-water by the membrane filtration culture method. Reference Methods for Marine Pollution Studies No. 3 Rev. 1, UNEP 1983.

PNUE/OMS : Détermination des coliformes fécaux dans l'eau de mer par la méthode de culture sur membranes filtrantes. Méthodes de références pour les études de pollution marine No 3 rév. 1, PNUE 1983.

UNEP/WHO : Determination of faecal streptococci in sea-water by the membrane filtration culture method. Reference Methods for Marine Pollution Studies No. 4 Rev. 1, UNEP 1983.

PNUE/OMS : Détermination des streptocoques fécaux dans l'eau de mer par la méthode de culture sur membranes filtrantes. Méthodes de références pour les études de pollution marine No 4 rév. 1, PNUE 1983.

UNEP/WHO : Determination of faecal coliforms in bivalves by multiple test tube method. Reference Methods for Marine Pollution Studies No. 5 Rev. 1, UNEP 1983.

PNUE/OMS : Détermination des coliformes fécaux dans les bivalves par le test des tubes multiples. Méthodes de références pour les études de pollution marine No 5 rév.1, PNUE 1983.

UNEP/FAO/IAEA : Guidelines for monitoring chemical contaminants in marine organisms. Reference Methods for Marine Pollution Studies No. 6, UNEP. (in preparation)

UNEP/FAO/IAEA/ IOC: Sampling of selected marine organisms and sample preparation for trace metal analysis. Reference Methods for Marine Pollution Studies No. 7 Rev. 2, UNEP 1984.

UNEP/FAO/IAEA/ IOC: Determination of total mercury in selected marine organisms by cold vapour atomic absorption spectrophotometry. Reference Methods for Marine Pollution Studies No. 8 Rev. 1, UNEP 1984.

UNEP/FAO/IAEA : Determination of total arsenic in selected marine organisms by flameless atomic absorption spectrophotometry. (Draft) Reference Methods for Marine Pollution Studies No. 9, UNEP 1984.

UNEP/FAO/IAEA : Determination of total selenium in selected marine organisms by hydride generation atomic absorption spectrophotometry. Reference Methods for Marine Pollution Studies No. 10, UNEP 1984.

UNEP/FAO/IAEA/ IOC: Determination of total cadmium, zinc, lead and copper in selected marine organisms by flameless atomic absorption spectrophotometry. Reference Methods for Marine Pollution Studies No. 11 Rev. 1, UNEP 1984.

UNEP/FAO/IAEA : Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons. Reference Methods for Marine Pollution Studies No. 12 Rev. 1, UNEP 1984.

UNEP/FAO/IAEA : Determination of methylmercury in selected marine organisms. Reference Methods for Marine Pollution Studies No. 13, UNEP UNEP 1984.

UNEP/FAO/IAEA : Determination of DDTs and PCBs in selected marine organisms. Reference Methods for Marine Pollution Studies No. 14. UNEP 1982.

UNEP/IOC/IAEA : Monitoring of tar on marine beaches. Reference Methods for Marine Pollution Studies No. 15, UNEP. (in preparation)

UNEP/IAEA : Determination of DDTs, PCBs, PCCs and other hydrocarbons in sea-water by gas chromatography. (Draft) Reference Methods for Marine Pollution Studies No. 16, UNEP 1982.

UNEP/IAEA : Determination of DDTs, PCBs and other hydrocarbons in marine sediments by gas liquid chromatography. (Draft) Reference Methods for Marine Pollution Studies No. 17, UNEP 1982.

UNEP/IOC : Determination of total dissolved cadmium in sea-water by differential pulse anodic stripping voltammetry. (Draft) Reference Methods for Marine Pollution Studies No. 18, UNEP 1983.

UNEP/IOC : Determination of total mercury in estuarine waters and suspended matter by cold vapour atomic absorption spectrophotometry. Reference Methods for Marine Pollution Pollution Studies No. 19, UNEP 1983. (in preparation)

UNEP/IOC : Monitoring of petroleum hydrocarbons in sediments. Reference Methods for Marine Pollution Studies No. 20, UNEP. (in preparation)

UNEP/WHO : Determination of total coliforms in sea-water by multiple test tube method. Reference Methods for Marine Pollution Studies No. 21, UNEP 1983. (in preparation)

UNEP/WHO : Determination of faecal coliforms in sea-water by multiple test tube method. Reference Methods for Marine Pollution Studies No. 22, UNEP 1983. (in preparation)

UNEP/WHO : Determination of faecal streptococci in sea-water by multiple test tube method. Reference Methods for Marine Pollution Studies No. 23, UNEP 1983. (in preparation)

UNEP/WMO : Sampling of aerosols and wet precipitation for analysis of chemical pollutants. Reference Methods for Marine Pollution Studies No. 24, UNEP (in preparation)

SPC/UNEP : Coral Reef Monitoring Handbook. Reference Methods for Marine Pollution Studies No. 25, UNEP 1984.

UNEP/IAEA : Determination of total mercury in marine sediments and suspended solids by cold vapour absorption spectrophotometry. Reference Methods for Marine Pollution Studies No. 26, UNEP 1984. (in preparation)

UNEP/IAEA : Determination of total cadmium in marine sediments by flameless absorption spectrophotometry. Reference Methods for Marine Pollution Studies No. 27, UNEP 1984. (in preparation)

UNEP/IOC : Monitoring of petroleum hydrocarbons in sea-water. (in preparation)

UNEP/IAEA : Guidelines for monitoring of estuarine waters and suspended matter. (in preparation)

UNEP/WHO : Determination of faecal coliforms in estuarine waters, suspended matter and sediments. (in preparation)

UNEP/WHO : Determination of phosphorus in suspended matter and sediments. (in preparation)

UNEP/WHO : Determination of nitrogen in suspended matter and sediments. (in preparation)

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UNEP/UNESCO : Determination of total cadmium in estuarine waters and suspended matter. (in preparation)

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