



REGIONAL
SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME

12 November 1984

*Determination of methylmercury in
selected marine organisms by gas chromatography*

Reference Methods For Marine Pollution Studies No. 13

Prepared in co-operation with



FAO



IAEA

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) 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.

For bibliographic purposes this document may be cited as:

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

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 Present. UNEP, 1983.

This issue of the Reference Method for Marine Pollution Studies No. 15 was prepared in co-operation with the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA). It includes comments received from the FAO/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 this reference method is gratefully acknowledged.

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1. SCOPE AND FIELD OF APPLICATION

This reference method describes a gas chromatographic analysis for the determination of methylmercury (MeHg) in biological material after liberation of MeHg with a strong acid from a homogenized sample. Detection limit 0.001 mg kg^{-1} FW.

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/1158), pp. 124. FAO, Rome.
- KAMPS L. R. and McMAHON B. (1972) Utilization of the Westoo procedure for the determination of methylmercury in fish by gas-liquid chromatography. J.AOAC 55/590-595.
- 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.
- WESTOO, G. (1974) Methodology for mercury and methylmercury for food contamination monitoring. Working paper for FAO/WHO expert consultation to identify the food contaminants to be monitored and to recommend sampling plans and methodology. Rome, 7-11 October, 1974. Rome, FAO-ESN:MDN/74/17.

3. PRINCIPLES

An aliquot of the sample, prepared according to UNEP/FAO/IAEA (1984) is homogenized and the methylmercury (MeHg) in biological material is liberated with a strong acid (HBr). The MeHg is then extracted with an organic solvent (benzene) and the MeHg separated from interfering impurities by extraction with an aqueous ethanolic solution containing a thiol-compound (cysteine). After liberating the MeHg from the thiol-compound with a strong acid and extracting again with an organic solvent the extract is analysed by gas-liquid chromatography.

4. REAGENTS

For this analysis use only distilled water and reagents of recognized analytical quality.

4.1 Demineralized distilled water or water of equivalent quality, free from mercury.

4.2 Concentrated HCl ($d_{20^{\circ}\text{C}} = 1.19 \text{ g l}^{-1}$), suprapure or similar.

4.3 Hydrochloric acid, 6 N.

Mix equal volumes of distilled water (4.1) with concentrated HCl (4.2).

4.4 Sodium bromide.

4.5 Sodium sulphate, anhydrous. Dried overnight at 450°C .

NOTE: Sodium sulphate may be contaminated with substances which cause interferences. Carefully check this reagent.

4.6 Cysteine solution.

1.00 g cysteine hydrochloride ($1 \text{ H}_2\text{O}$)
0.775 g sodium acetate ($3 \text{ H}_2\text{O}$)
12.5 g anhydrous sodium sulphate
about 50 ml ethanol, mercury free

Dissolve the reagents in about 50 ml of distilled water (4.1) and dilute to 100 ml with ethanol. Prepare a fresh solution every 3 days.

4.7 Benzene. Distil with a Widmer column (5.12).

4.8 Standard solutions of methylmercury.

4.8.1 Stock solution: $100 \mu\text{g Hg l}^{-1}$. Weigh 0.1254 g methylmercury chloride to the nearest 0.0001 g. Transfer to a 1000 ml volumetric flask. Bring to volume with distilled water (4.1) and mix.

NOTE: This solution is stable for years.

4.8.2 Calibration solution: $1.00 \mu\text{g Hg l}^{-1}$. Dilute 1.00 ml of the stock solution (4.8.1) in a 100 ml volumetric flask and bring up to volume with distilled water (4.1).

NOTE: This solution must not be older than 2 days.

4.8.3 Stock solution for gas chromatography: $100 \mu\text{g Hg l}^{-1}$. Weigh 0.1254 g methylmercury chloride to the nearest 0.0001 g. Transfer to a 1000 ml volumetric flask. Bring to volume with benzene (4.7) and mix.

NOTE: This solution is stable.

4.8.4 Standard solutions: 0.05, 0.10, 0.20, 0.30 ng Hg μ l⁻¹.

Using volumetric flasks (5.9), dilute appropriate aliquots (e.g. 0.05, 1.10, 0.20, 0.30 ml) of the stock solution (4.8.3) in benzene (4.7) so that with 5 μ l the following amounts can be injected into the gas chromatograph: 0.25, 0.5, 1.0, 1.5 ng Hg. Keep the solutions well closed to avoid evaporation.

NOTE: This solution is stable for several months.

4.9 Teramlic alcohol, antifoam B emulsion (e.g. Fisher Corp.) or similar.

4.10 Working matrix: Prepare a sufficiently large amount of biological tissue to last several determinations (e.g. 300 g FW) according to (9.1). Homogenize with a stainless steel blender or other homogenizer (5.1).

Test for homogeneity by running 5 extractions (9.1) and analyses (10.3). If the coefficient of variation is less than 20% the working matrix is ready for use. Otherwise homogenize the working matrix again or prepare a new working matrix.

NOTE: The working matrix should be prepared from specimens of the same species which will be analysed.

5. APPARATUS

5.1. Homogenizer (e.g. Polytron).

5.2 Bulb pipettes, 1.00 ml, 2.00 ml, 3.00 ml, 4.00 ml, 5.00 ml, 10.00 ml, 15.00 ml, 50.0 ml and graduated pipettes, 1.0 ml, 5.0 ml and 10 ml.

5.3 Graduated cylinder, 100 ml.

5.4 Erlenmeyer flasks, 200 ml and 2000 ml.

5.5 Separation funnels, 75 ml capacity.

5.6 Test tubes with ground joints and stoppers, 5 ml and 15 ml capacity.

5.7 Centrifuge tubes, 200 ml capacity, complete with ground glass stoppers.

5.8 Centrifuge tubes, 10-15 ml capacity.

5.9 Volumetric flasks, 10 ml, 100 ml, 200 ml, 500 ml and 1000 ml capacity.

5.10 Centrifuge, 600 g or faster, at least 200 ml capacity.

5.11 Mechanical shaker for mixing solutions.

5.12 Widmer distillation apparatus complete with:

- Flask, round bottom and ground joint 29/32, 3 l capacity.
- Heating mantle for this 3 l flask with energy regulator, 500 W.
- Adapter with two ground joints 29/32 and one ground joint 14/23, with thermometer.
- Coil condenser with ground joint 29/32, coil length 20 cm.
- Distillation column, Widmer, with ground joints 29/32, effective length 20 cm.

5.13 Gas chromatograph with electron capture detector (^3H or ^{63}Ni) and recorder, complete with:

- 5 x 1/8" glass column containing lithium chloride and 5% phenyl diethanolamine succinate (PDEAS) on Chromosorb W, AW, DMCS, or similar, 60/80 mesh;
- a supply of carrier gas (gas flow rate 60-75 ml $\text{N}_2 \text{ min}^{-1}$) purified by a Molecular Sieve.

Preparation of the column and conditioning:

Dissolve 0.5 g lithium chloride and 1.5 g phenyl diethanol diethylamine succinate in 2.5 ml ethanol and 50 ml acetone in a round bottom flask. Add 10 g Chromosorb W, 60/80 mesh, AW, DMCS or similar. Evacuate the flask until all air bubbles have disappeared. After 10 min. transfer the mixture into a glass filter funnel and remove the liquid by suction. Air-dry on filter paper. Fill the column with the dry Chromosorb. Heat the column under low gas flow rate (25-30 ml $\text{N}_2 \text{ min}^{-1}$) at 210°C for 18 hours WITHOUT attaching the end of the column to the detector. Then bring the gas chromatograph to normal conditions i.e.,

- Gas flow 60-70 ml $\text{N}_2 \text{ min}^{-1}$
- Column temperature: 175°C
- Injection temperature: 200°C
- Detector temperature: 205°C (for ^{63}Ni detector use 220°C)

and saturate the column with MeHg by injecting large amounts (about 10 ng) of MeHg standard solution (4.8.4) repeatedly. CAUTION: In order not to contaminate the detector it is important to ensure that it is at operational temperature (205°C) before connecting the column while the carrier gas is flowing. Check the efficiency of the newly prepared column by injecting 0.10 ng Hg as MeHg. The peak height should be at least 20% of the maximal observable peak height. If the sensitivity is not sufficient or the peaks are broadened the column can be improved by keeping it at working temperature injecting (about

40 ng Hg per injection) repeatedly benzene solutions of MeHg iodide or methoxyethyl-mercury. These solutions are prepared by adding sodium iodide to aqueous solutions of MeHg chloride or methoxyethyl-mercury hydroxide and extracting with benzene. This solution is stable for only two hours. The benzene layer is then dried with sodium sulphate.

- 5.14 Chromatographic syringes 5 μ l.
- 5.15 Weighing bottles with ground stoppers.
- 5.16 Drying oven (105°C).
- 5.17 Stainless steel tweezers.
- 5.18 Desiccator.
- 5.19 Freeze dryer.
- 5.20 Analytical balance.

NOTE: All glassware to be used in analytical procedure should be cleaned first by ordinary laboratory procedures and then by rinsing with 1 N ammonium hydroxide solution, followed by rinsing with distilled water and ethanol.

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

8.1 Oven-drying

A clean weighing bottle (5.15), with its ground stopper removed, is placed into a drying oven (5.16) set at 105°C. It is important to use the tweezers (5.17) 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 (5.18) to cool.

Weigh the empty bottle with its stopper in place on the analytical balance (5.20). Note the weight. Place 1-2 g of the specimen sample (7) in the weighing bottle and close with the stopper. Weigh it again and note the weight.

Place the weighing bottle containing the subsample in the drying oven set at 105°C. remove the stopper with tweezers and place the stopper also 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 it and the 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 weighings is less than 0.5% of the total weight; calculate the dry weight (DW) and record the fresh weight (FW) 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 (5.20) subsample of the specimen sample (7) in a clean 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 two 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. EXTRACTION AND CLEAN-UP

9.1 Sample

Homogenize (5.1) about 5 g of tissue (note the exact weight in the protocol) in a 200 ml centrifuge tube (5.8). Add 60 ml of distilled water (4.1) and then 14 ml of concentrated HCl (4.2) and 10 g NaBr (4.4). Mix and then add 70.0 ml benzene (4.7), shake mechanically (5.11) for 5, or 15 minutes vigorously by hand. Centrifuge (5.10) and then transfer 50.0 ml of the benzene extract into a separating funnel (5.5). Add 6.0 ml cysteine solution (4.5) with a pipette (5.2) and shake vigorously for 2 minutes. Centrifuge. If too much emulsion or foam is present add an antifoam agent (4.9). The emulsion can also be broken by vigorous stirring with a glass rod. Centrifuge again. Transfer 2.0 ml of the clear aqueous layer into a 15 ml test tube with ground joint (5.6). Acidify with 1.2 ml 6 N HCl (4.3), add 0.5 g NaBr (4.4) and extract with 4.0 ml benzene by shaking for 2 min. Transfer the extract into a 5 ml test tube (5.6). Dry with anhydrous sodium sulphate (4.5).

This benzene extract represents the test solution used in the gas chromatographic analysis.

9.2 Blank

Follow procedure (9.1) replacing the 5 g tissue sample with 5 ml of distilled water (4.1). If the blank gives a peak at the retention time of MeHg (1.5 min) check all reagents and solutions for impurities.

NOTE: The extraction and clean-up should be carried out in one run in order to minimize loss of MeHg. If the procedure has to be interrupted briefly then it should be discontinued only after the first centrifugation, i.e. when the MeHg is bound to cysteine. Longer interruption (i.e. overnight) should only occur when the sample is in benzene solution. Usually the final benzene extract (i.e. the test solution) is stable and can be stored for several weeks, but an extract kept longer may lose MeHg.

10. GAS CHROMATOGRAPHIC ANALYSIS

10.1 Standardization

Set up the gas chromatograph to normal operating conditions (5.13) and inject (5.14) 5 μ l benzene (4.7) as a solvent blank followed by 5 μ l each of the standard solutions (4.8.4) containing 0.05, 0.1, 0.2 and 0.3 ng Hg μ l⁻¹ in order to obtain standards of 0.25, 0.5, 1.0, 1.5 ng Hg. Plot peak height against amount of mercury injected. A straight line cutting the x-axis at the most at 0.05 ng Hg from zero should be obtained. Otherwise, check the gas chromatograph for malfunctioning or check the benzene (4.7) for impurities.

10.2 Calibration

Add 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml of the calibration solution (4.8.2) into 5 different 200 ml centrifuge tubes (5.8). Add to each 60 ml of distilled water (4.1) and then 14 ml of concentrated HCl (4.2) and 10 g NaBr (4.4). Mix and then add 70.0 ml benzene (4.7), shake mechanically (5.11) for 15 min., or 5 min. vigorously by hand. Then transfer 50.0 ml of the benzene extract into a separating funnel (5.5). Add 6.0 ml cysteine solution (4.5) with a pipette (5.2) and shake vigorously for 2 minutes. Transfer 2.0 ml of the clear aqueous layer into a 15 ml test tube with a ground joint (5.6). Acidify with 1.2 ml 6 N HCl (4.3), add 0.5 g NaBr (4.3) and extract with 4.0 ml benzene by shaking for 2 min. Transfer the extract into a 5 ml test tube (5.6). Dry with anhydrous sodium sulphate (4.5).

Prepare a subsample of the working matrix and extract it as described under (9.1).

Inject (5.14) 5 μ l of each of the extracts containing the different calibration solutions (4.8.2) and the extract of the working matrix and plot peak heights obtained against Hg content.

Check peak height of working matrix against previous values.

10.3 Analysis of sample

Start each series of sample analyses with a standardization (10.1), a calibration (10.2) and a blank (9.2).

Inject 5 μ l of the test solution (9.1) followed by 5 μ l of a standard solution (4.8.4) whose peak height does not differ more than 25% from that of the sample. Check the correct function of the gas chromatograph by comparing this peak height with the corresponding one obtained in previous standardizations (10.1).

Make duplicate injections of both the sample and the standard for each sample analysed.

NOTE: If MeHg levels are expected to be so high that the peak obtained lies beyond the linear part of the standardization curve, either less tissue must be extracted (9.1) or the test solution (9.1) must be diluted. If on the other hand the peak height is small the sensitivity of the procedure can be increased either by increasing sample size to a maximum of 10 g, or by employing a smaller amount of the cysteine solution and changing the subsequent volumes accordingly.

11. EXPRESSION OF RESULTS

Read the MeHg concentration corresponding to the peak height obtained from the calibration curve (10.2) and correct the weight difference with respect to the theoretical 5 g of tissue to be extracted. Correct also for differences in volumes used in the calibration (10.2) and in the sample extraction (9.1).

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 analysing 5 subsamples from one original sample. If the coefficient of variation is greater than 20%, check the procedure for possible errors and contamination.

NOTE: The standardization test (10.1) can also be used for the estimation of the precision of the gas chromatographic analysis.

12.2 Accuracy

- Participate in the intercalibration exercises and, in addition,
- include analyses of the working matrix (4.10).

12.3 Quality control

If the quality control checks reveal a fluctuation in the standard deviation or the accuracy by more than 5%, check the following factors: stability of stock solutions (prepare new solutions); instrumental drift or inadvertant changes 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 Methylmercury 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. Extraction and clean-up

3.1 Date (day, month, year): _____

3.2 Anomalies observed which may influence test results:

4. Gas chromatographic analysis

4.1 Date (day, month, year): _____

4.2 Standardization:

	Blank	0.25	0.5	1.0	1.5

peak height					

4.3 Calibration

	Blank	1 ml	2 ml	3 ml	4 ml	5 ml

peak height						

matrix standard						

peak height						

4.4 Analysis of sample

weight of sample (grams) _____ FW

FW/DW ratio: _____

peak height: _____

4.5 Result of analysis (mgkg^{-1} FW) _____

5. Estimation of precision

5.1 Date (day, month, year) _____

5.2 Results:

replicates	1	2	3	4	5
_____	_____	_____	_____	_____	_____
g sample	_____	_____	_____	_____	_____
peak height	_____	_____	_____	_____	_____
mean peak height _____	_____	_____	_____	_____	_____
mean conc. _____	_____	_____	_____	_____	_____
stand. deviation _____	_____	_____	_____	_____	_____
coeff. of variation _____	_____	_____	_____	_____	_____

6. Estimation of accuracy

6.1 Date (day, month, year) _____

6.2 Origin and type of certified standard used: _____

6.3 Declared mg MeHgkg^{-1} of certified standard: _____

6.4 Results:

replicates	1	2	3	4	5
_____	_____	_____	_____	_____	_____
g sample	_____	_____	_____	_____	_____
peak height	_____	_____	_____	_____	_____
mean peak height _____	_____	_____	_____	_____	_____
mean conc. _____	_____	_____	_____	_____	_____

7. Anomalies observed during test and other conditions which could influence the test 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 1984.
- UNEP/FAO/IAEA : Determination of DDTs and PCBs in selected marine organisms. Reference Methods for Marine Pollution Studies No. 14, UNEP 1982.

- 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 vapor atomic absorption spectrophotometry. Reference Methods for Marine 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/WHO : 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 vapor 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)
- UNEP/WHO : Determination of BOD_5 and COD in estuarine waters. (in preparation)
- UNEP/FAO : Acute toxicity tests. (in preparation)
- UNEP/UNESCO : Determination of total cadmium in estuarine waters and suspended matter. (in preparation)
- UNEP : Biological non-acute toxicity tests. (in preparation)
- UNEP/IOC : Determination of basic oceanographic and meteorological conditions. (in preparation)
- UNEP/IOC : Determination of standard physical and chemical parameters. (in preparation)

- UNEP/IAEA : Determination of selected trace metals in aerosols and in wet precipitation. (in preparation)
- UNEP/IAEA : Determination of halogenated hydrocarbons in aerosols and in wet precipitation. (in preparation)
- UNEP/WMO : Sampling of dry deposition. (in preparation)

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Oceans and Coastal Areas Programme Activity Centre
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