منظمة الأغذية والزراعة للأمم المتحدة
联合国粮食及农业组织
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
ORGANISATION DES NATIONS UNIES POUR L'ALIMENTATION ET L'AGRICULTURE
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LONG-TERM PROGRAMME FOR POLLUTION MONITORING AND RESEARCH

IN THE MEDITERRANEAN SEA (MED POL - PHASE II)

REPORT OF AN EXPERTS CONSULTATION MEETING ON REFERENCE METHODS FOR THE DETERMINATION OF CHEMICAL CONTAMINANTS IN MARINE ORGANISMS

Rome, 4 - 8 June 1984

Convened jointly with the
United Nations Environment Programme
and the International Atomic Energy agency

UNEP/WG.118/Inf. 14

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INTRODUCTION:

UNEP's Regional Seas Programme Activity Centre in co-operation with FAO and IAEA has developed a series of Reference Methods for the determination of cadmium, zinc, lead, copper, mercury, arsenic, selenium, methyl mercury, PCBs and DDTs in selected marine organisms.

The methods are intended for use by the participating laboratories in the various Regional Seas Programmes in order to achieve comparable results on a world wide basis.

The methods are not considered as final and will be revised periodically in the light of new developments in methodologies and analytical instrumentation.

As the methods are also intended for the Mediterranean, FAO in collaboration with IAEA and UNEP have decided to organise a testing exercise in the framework of the MED POL activities.

In May 1983, MED UNIT had informed all MED POL National Co-ordinators that a testing exercise would take place for Reference Methods nos. 7-14, asking them at the same time to nominate laboratories in their respective countries directly. Analysts from 18 laboratories volunteered to participate in the testing of one or more Reference Methods.

The IAEA Laboratory in Monaco prepared and sent to the participants the reference samples for the exercise. The analysts were asked to analyse these samples using both their own method and the corresponding Reference Method. They were also asked to analyse fresh samples so that Reference Methods 7 and 12 could be tested. Forms were also sent to the participants on which they had to provide their comments.

The consultation meeting was organised to a) discuss the results of the testing exercise and b) to review and revise the Reference Methods in the light of the comments received.

Agenda item 1: Opening of the Meeting

The meeting took place at FAO headquarters in Rome, from 4 to 8 June 1984. It was attended by twenty three participants from the Mediterranean, other regions, FAO, UNEP and IAEA, specialists in the field of analysis of chemical contaminants in marine organismes. A list of all the participants is given in Annex I.

Mr. A.H. Lindquist, Director of the Fishery Resources and Environment Division, welcomed the participants on behalf of FAO and opened the meeting. He indicated FAO's interest in the MED POL activities and referred to the need for commonly accepted analytical methods.

Agenda item 2: Scope and purpose of the Meeting

Mr. G.P. Gabrielides, Senior Fishery Officer (Marine Pollution) explained the scope and purpose of the meeting referring in detail to the testing exercise and the expected results.

Mr. S. Civili, Marine Scientist, representing UNEP pointed out that the Reference Methods are for use in the MED POL Monitoring Programme, and that they are being recommended by UNEP to Mediterranean Governments as mandatory for legal purposes.

Mr. S. Aston, First Officer at the IAEA, Monaco Laboratory, expressed the hope that the discussions would facilitate the revision of the Reference Methods and mentioned that from now on the Monaco Laboratory has responsibility for the development, testing and intercalibration of the methods.

Agenda item 3: Election of Officers

Mr. G.C. Pappalardo was elected Chairman, Mr. M. Picer, Vice-Chairman and Mr. S. Aston, Rapporteur. Mr. G.P. Gabrielides acted as Technical Secretary to the meeting.

Agenda item 4: Adoption of the Agenda

The Agenda shown in Annex II was unanimously adopted.

Agenda item 5: Organisation of work

It was decided that during the discussions on Agenda items 6, 7, 8 and 9, a small informal group of participants, specialists in the analysis of chlorinated hydrocarbons, should meet to initiate discussions on Agenda item 12 and prepare recommendations for the plenary.

Agenda items 6, 7, 8, 9, 10, 11 and 12: Reference Methods 11, 8, 9, 10, 7, 12 13 and 14

During the course of the discussions it was pointed out that there is no such thing as the "perfect method" or the "best method". However, the need was recognised for Reference Methods to achieve comparable results, with the understanding that these Reference Methods will be revised in the light of new analytical developments and/or instrumental advances.

The improvements made to the existing Reference Methods are, of course, based on the presently available knowledge and experience.

Under Agenda items 6 through 12 Reference Methods 11, 8, 9, 10, 7, 12, 13 and 14 were considered, and their improvement discussed in detail. The participants agreed that atomic absorption spectrophotometry (AAS), which is widely used by participating laboratories, is a very good method for heavy metal analyses, including mercury. It was pointed out that for methyl mercury (Method 13) AAS could be used instead of gas chromatography, and this technique is used by some laboratories. However, it was agreed that gas chromatography is equally good for Method 13. The modifications recommended for these Reference Methods (excluding no. 9, see below) are given in Annexes III, IV, V, VI and VII except for Method 14 for which an improved text is provided in Annex VIII.

The results from the testing exercise on reference materials supplied by the Monaco Laboratory (mussel and fish tissues) for Cd, Cu, Pb, Zn and Hg and for chlorinated hydrocarbons were discussed as part of the process of revising the Reference Methods. It was agreed that, while the results suggested that improvements could be achieved, the existing Reference Methods produced results which were at least as compatible as those obtained when individual laboratories used their own established methods.

The meeting expressed the wish to repeat the analysis of the two reference materials supplied by the Monaco Laboratory using the modified versions of Reference Methods Nos. 8, 11 and 14. The participants felt that, with respect to Reference Method No. 9, insufficient expertise and experience was available to allow revision at this stage. It was recommended that expert opinion be sought from further afield to allow this method to be revised. The meeting felt that, while considerable modification of Reference Method No. 14 had been achieved, it recommended to UNEP and its co-operating agencies that further research was necessary to improve certain aspects of the method. This includes the distribution of specified packing materials for GC columns to participating laboratories, together with standards and reference materials for intercalibration.

Agenda item 13: Reference Method No. 6

Under this Agenda item a suggested outline for the preparation of Reference method No. 6 (Guidelines for monitoring chemical contaminants in marine organisms) was considered. The meeting agreed that this outline provided a suitable basis on which to proceed and presented to the secretariat several useful comments which will be taken into consideration in the drafting of the method.

Agenda item 14: Other matters

Under this Agenda item Mr. S. Aston explained to the participants the procedures to be followed in the development, testing and intercalibration of the Reference Methods.

Upon request by a number of participants, Mr. S. Civili provided the meeting with information on a)the development status of the MED POL-Phase II monitoring programme and b)the research component of MED POL-Phase II.

Agenda item 15: Adoption of the report and closure of Meeting

The meeting, after adopting the report, was closed on Friday afternoon the 8th of June, 1984.

ANNEX I

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ANNEX II

AGENDA

1 Opening of the consultation

2. Scope and purpose

3. Election of officers

4. Adoption of the Agenda

5. Organisation of work

6. Reference method No. 11

7. Reference method No. 8

8. Reference method No. 9

9. Reference method No. 10

10. Reference methods Nos. 7 and 12

11. Reference method No. 13

12. Reference method No. 14

13. Reference method No. 6

14. Other matters

15. Adoption of the report and closure of Meeting

ANNEX III

REFERENCE METHOD NO. 11

Proposed changes

- Lines 51-57

To be deleted and replaced with:
"liquid pressure decomposition. Detection limits of the method will vary with the individual instruments used, typical ranges of the detection limits are as follows (in $mgkg^{-1}$)

- with flame atomization

Fresh weight	Cd	0.1-0.2
	Cu	0.5-1.0
	Pb	5-8
	Zn	2.5-5

- With electrothermal atomization

Fresh weight	Cd	0.0005-0.001
	Cu	0.01-0.03
	Pb	0.01-0.05
	Zn	Not normally used

- Line 82 — Replace "electrochemical" with "electrothermal".

- Line 110 — Replace "50 (?)" with "10".

- Line 113 — Insert after "$CdCl_2$ "prepared from $CdCl_2.2.5H_2O$".

- Lines 122, 144, 166, 188 & 199 — Replace "frequently" with "daily".

- Lines 119, 163 185 & 197 — Replace "5.20" with "5.4".

- Lines 207, 209 606, 607 & (Table 1) — Replace "variance" with "variation".

- Line 231 — Replace "delivery" with "deliver".

- Line 232 — Insert new line, "Micropipettes or automatic sampler for injection into eletrothermal device (see operator's manual)".

- Line 247 — Replace "electrodic" with "electrodeless".

- Line 260	Add "Nitrogen of adequate purity or any other gas or gas mixture specified by the AAS manufacturer".
- Lines 327, 339, & 341	Replace "5" with "0.5".
- Line 395	Replace "120-150° C for at least 6 hours" with "140±2°C for three hours".
- Line 398	Replace sentence "If the solution ....." with "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".
- Line 400	Replace "120-150°C" with "140±2°C".
- Lines 405	Add "Analyse the samples as soon as possible".
- Lines 407, 408 and 408	Delete these lines.
- Line 430	Insert "before starting a set of analyses it is important to check the calibration of the instrument using standard solutions".
- Line 431	Replace "6" with "8".
- Lines 432	Replace with "Place 1.00 g FW or equivalent to not more than 0.2 g DRY WEIGHT".
- Line 434	Replace clause "sixth vessel" with "vessels 6-8, these vessels will be the blanks". Add "In the case that high blank values are found, check for cleanliness of reagents and apparatus, but also calculate the blank value by standard additions".
- Lines 466 & 467	Replace "substandard" with "working matrix".
- Line 468	Replace "matrix substandard" with "working matrix".
- Line 486	Replace "acetylen" with "acetylene".
- Line 522	Replace "307.6" with "213.9".
- Line 529	Replace "eletrothermal" with "electrothermal".
- Lines 531-522	Replace with "10.7.1. Determination of optimal conditions for the atomization programme: for each matrix determine the optimal parameters of the atomization programme (10.7.2) by changing the time intervals and temperatures of the four

steps (drying, charring, atomizing and cleaning). This should be done by reference to the manufacturer's manual, followed by a careful and critical assessment of the recommended parameters in relation to those determined experimentally using suitable matrix materials".

- Line 531 — After "Determination" insert "of".

- Line 557 — After "micropipette" insert "or preferably, if available, with an automatic sample injector".

- Line 572 — Add "When using electrothermal devices, the use of an automatic (or manual) background compensation system is recommended".

- Line 581 — Add sentence: "When using electrothermal atomizers, the presence of the biological matrix often causes sensitivity decreases; then the blank evaluations must be made by an addition to the blank matrix".

- Lines 582-583 — Replace "mass of analyzed metals" with "read-out" and viceversa.

- Lines 639-640 — Replace with following text:
"If the quality control checks reveal a fluctuation in the standard deviation or the accuracy of the results 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 substandard (select alternative reference material for analysis); contamination of equipment e.g. glassware, operator error(s)".

General note on units -μgkg^{-1} to be replaced with $mgkg^{-1}$ throughout the document.

Table 1 — Change "substandard" to "working matrix" and add extra lines for 3 blanks instead of 1.

GENERAL HINTS: Replace "substandard" with "working matrix" throughout the document. Change units of gkg^{-1} to $mgkg^{-1}$ throughout the document.

ANNEX IV

REFERENCE METHOD NO. 8

Proposed changes

Title page and - Line 5	Replace "flameless" with "cold vapour".
- Line 47	Replace "0.05 g" with "0.01 mgkg $^{-1}$ total mercury". Delete "per Kg.".
- Line 88	Delete "or water of equivalent quality,....".
- Lines 103, 106 249	Replace "sulphate" with "hydrochloride".
- Line 108	Insert "or 4% W/V Sodium Borohydride solution in 3% sodium hydroxide (if preferred for mercury reduction)".
- Lines 109 & 110	Delete both lines.
- Line 112	Replace "4.8" with "4.7".
- Line 114	Replace "4.8.1" with "4.7.1".
- Line 119	Replace "4.8.2" with "4.7.2.".
- Line 123	Replace "at least weekly" with "daily".
- Line 129	Replace "4.9" with "4.8".
- Lines 133, 136 469, 585, 615 & Table 1	Replace "variance" with "variation".
- Line 138	Replace "4.10" with "4.9".
- Line 180	Replace "electrodic" with "electrodeless". Replace "sulpha-chrom" with "sulphuric acid-permanganate" and replace "4.11" with "4.10".
- Line 240	Replace "analytic" with "analytical".
- Lines 245 & 247	Replace sentence with "wash with a mixture of sulphuric acid and permanganate (4.10) prepared immediately before use".
- Line 308 & 323	Replace "5" with "0.5".
- Line 337	Replace "plastic" with "teflon".
- Line 359	Insert after 1 g FW "or 0.2 g DRY WEIGHT".

- Line 371 — Replace "120-150°C for at least 6 hours" with "140°C for 3 hours".

- Line 375 — Insert full stop after "complete", then insert "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".

- Line 395 — Insert "Before starting a set of analyses it is important to check the calibration of the instrument using standard solutions".

- Line 401 — Replace "6" with "8".

- Line 402 — Replace "substandard" with "working matrix".

Replace "about 1 g FW" with "1.00 g FW".

- Line 403 — Replace "sixth vessel" with "sixth to eighth vessels, these vessels" also, replace "substandard" with "working matrix".

- Line 404 — Replace "blank" with "blanks".

- Line 426 — 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.

- Line 429 — Insert "Then immediately add 1 drop of potassium permanganate solution (4.5)".

- Line 433 — Replace "sulphochrom" with "sulphuric acid-permanganate mixture".

- Lines 445 & 446 — Replace "prepare a graph of mass...." with "prepare a graph of instrument read-out against the corresponding mass of Hg".

- Line 487 — Replace "dryed" with "dried".

- Line 502 — Replace with following text:
"If the quality control checks reveal a fluctuation in the standard deviation or the accuracy of the results by more than 5%, check the following factors: stability of stock solutions (prepare new solutions); instrument drift or inadvertant changes in operational parameters, contamination of the substandard (select alternative reference material for analysis); contamination of equipment e.g. glassware; operator error(s)".

Table 1 — Change "substandard" to "working matrix" and add extra lines for 3 blanks instead of 1.

GENERAL HINT: Use $mgkg^{-1}$ instead of μgkg^{-1} throughout the document.

ANNEX V

REFERENCE METHOD NO. 10

Proposed changes

- Title	Replace "Flameless" with "Hydride Generation".
- Line 54	Replace "0.05" µg Se/Kg" with "0.05 mg $Sekg^{-1}$".
- Line 112	Replace "Reduce" with "Reducing".
- Lines 139 & 421 & Table 1	Replace "variance" with "variation".
- Line 166	Replace "six to nine" to "at least 9".
- Lines 181 & 182	Replace "hollow cathode" with "electrodeless".
- Line 187	Replace "electrodic" with "electodeless".
- Lines 187 & 188	Replace these lines with "NOTE: a hollow cathode Se lamp may also be used".
- Line 301	Replace "about 1 g FW" with "of 1.00 g FW or not more than 0.2 g equivalent DRY WEIGHT".
- Lines 316 & 318	Replace "If the solution is not clear ...." with "If the solution is not clear or has 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".
- Line 321	Replace "an equal amount of concentrated HCl" with "an amount of concentrated HCl (4.3) calculated to ensure that the final solution will be 5 M with respect to HCl".
- Line 322	Replace "oxydes" with "oxides".
- Lines 329 330 331	Replace with: "Analyze the sample as soon as possible".
- Line 348	Insert "Before starting a set of analyses it is important to check the calibration of the instrument using standard solutions".
- Line 353	Replace "G" with "g".
- Lines 354, 355 & 357	Replace "substandard" by "working matrix".
- Line 355	Replace "sixth vessel" with "sixth to eighth vessels, these vessels" also, replace "substandard" with "working matrix".

- Line 359 — Insert this missing line "(4.8.2) to the second vessel, 0.2 ml to the third vessel, 0.3 ml to the".

- Line 377 — Delete "and add 1 ml of diluted sulphuric acid (4.5)".

- Line 399 — Replace "prepare a graph of Se....." with "prepare a graph of instrument read-out against the corresponding mass of Se".

- Line 444 & 445 — Replace with following text:
"If the quality control checks reveal a fluctuation in the standard deviation or the accuracy of the results by more than 5%, check the following factors: stability of stock solutions (prepare new solutions); instrumental drift or inadvertant changes in operations parameters, contamination of the substandard (select alternative reference material for analysis); contamination of equipment e.g. glassware, operator error(s)".

Table 1 — Change "substandard" to "working matrix" and add extra lines for 3 blanks instead of 1.

GENERAL HINTS:

Use $mgkg^{-1}$ instead of μgkg^{-1} throughout the document.

ANNEX VI

REFERENCE METHODS NOS. 7 AND 12

Proposed changes

R.M. 7 - page 4 2nd para. - Line 2	Insert "undamaged".
6.3 para. - Line 1	Insert "undamaged".
R.M. 12 - page 4 - Line 179	Insert "undamaged"
- page 5 - Line 207	Insert "undamaged"
R.M. 7 - page 5 7.2 para.	Delete "Precision of weighing: 0.1 per cent of its total weight".
R.M. 7 - page 5 7.1 para.	Add to note "If possible a clean room should be used for preparatory activities".
R.M. 12 - page 6 - Line 260	Add "If possible a clean room should be used for preparatory activities".
R.M. 7 - page 10 7.5 para.	Insert at the end of 2nd para. "NOTE: it has been recognised that differences in trace metal concentrations may exist between different muscles in large fish, therefore as much information as possible on the actual sample should be recorded".
R.M. 12 - page 10 - Line 443	As above, but for "trace metal" substitute "chlorinated hydrocarbons".
R.M. 12 - page 7 - Line 288	Replace "ethanol" with "distilled water or clean sea water".
Table 1	R.M. 7 and 12 Some table format for both reference methods, draft table to be adopted.

ANNEX VII

REFERENCE METHOD NO. 13

Proposed changes

- Line 208	Add "600 g or faster, at least 200 ml capacity".
- Line 226	After "(3H)" add "or 63 Ni".
- Line 230	After "DMCS" add "or similar". Change "205" to "200".
- Line 234	Delete "13A 30/60 (Varian)".
- Line 251	Add "for 63 Ni detector use 220°C".
- Lines 233 & 248	Replace "N2" with "carrier gas".
- Line 245	Add new line: "CAUTION: In order not to contaminate the detector first heat the detector then simultaneously column and injection past while the carrier gas is flowing".
- Line 251	Detector kept at room temperature.
- Line 254	Reconnect detector. (First heat detector to operation temperature - column and injection.....?
- Line 254	After "repeatedly" insert "Heat detector to operational condition (205°C) and connect the column to it".
- Line 262	After "with benzene" add "This solution is stable for only two hours".
- Line 265	Replace "pipettes" with "syringes".
- Line 362	Replace "water" with "aqueous".
- Lines 479 & 480	Delete these lines.
- Lines 487-495	Replace with: "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 substandard (select alternative reference material for analysis); contamination of equipment eg. glassware, operator error(s)".

GENERAL HINT: Change units of $\mu g kg^{-1}$ to $mg kg^{-1}$ throughout the document.

ANNEX VIII

REFERENCE METHOD NO. 14

Revised text

1. Scope and field application

This Reference Method describes primarily the determination of DDTs and PCBs in marine organisms by gas chromatography. Several other halogenated pesticides may be present in samples and many of these could also be determined by these methods. However, every analyst must test his own recovery and analytical reproducibility for every residue quantified. Not all residues will be stable to all of the clean up procedures applied for the analysis of PCBs and DDTs. Thus some additional information is given on the stability of some common pesticides in these procedures.

Recognizing that not all participants in the UNEP Regional Seas Programmes are equipped with advanced high resolution chromatography capability the manual presents a procedure based on packed column technology but encourages participants to increase the resolution and accuracy of their determinations as newer technology becomes available to them.

2. References

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Zell, M. and Ballshmitter, K. 1980. Baseline studies of global pollution: III. Trace analysis of polychlorinated biphenyls (PCB) by ECD glass capillary gas chromatography in environmental samples of different trophic levels. Fresenius. Z. Anal. Chem. 304, 337-349.

3. Principles

From an aliquot of the sample prepared according to guidelines given in Reference Method no. 12 a lipid extract is prepared using hexane or petroleum ether as solvent. Extracts are then treated with concentrated H_2SO_4 to destroy some of the interfering lipids and then further cleaned and fractionated into classes of chlorinated hydrocarbons by elution from a silica gel absorption column. Residues are then analysed by gas chromatography by comparison with standards. Confirmation of the DDT series is accomplished by a dehydrochlorination procedure. To reduce analytical variability the use of internal standards, common chromatographic materials and specific methods for peak quantifications are urged.

4. Reagents

All reagents, including the distilled water should be of demonstrated analytical quality, whose use creates as low as possible a response on the electron capture detector. All reagents must be checked for their ECD response by analysing complete procedural blanks. If contaminants are detected the solid reagents must be cleaned by extracting them with pure solvents and/or evaporating the chlorinated hydrocarbons by heating overnight at 260° to 300° C. All solvents should be distilled in glass quality and pretested for their suitability for pesticide analysis. They may require redistillation in the laboratory on a routine basis.

4.1 Demineralized distilled water produced by distillation over permanganate (0.1 g $KMnO_4$ per litre) or equivalent quality, demonstrated free from intefering substances.

4.2 Ethanol, 96%

4.3 Hexane, or petroleum ether (40°-60°C)

4.4 Acetone, diethyl ether, iso-octane
(all distilled-in-glass quality)

4.5 Detergent

4.6 Concentrated H_2SO_4

4.7 Potassium hydroxide

4.8 Concentrated hydrochloric acid

4.9 Cleaning solution made of concentrated sulphuric acid saturated with potassium dichromate

4.10 Anhydrous Na_2SO_4

4.11 Very high purity silica gel for chromatography such as Merck Kieselgel 60 0.040-0.063 mm size

4.12 PCB standards - Prepare stock solutions of Aroclor 1254 and Aroclor 1260 by dissolving 100 mg standard in 100 ml iso-octane
Store stock solutions in sealed glass ampoules

4.13 DDT standards - Prepare a stock solution of the DDT series (pp'DDT, o,p DDT, pp'DDE, o,p DDE, ppDDD) by dissolving 100 mg of each standard in iso-octane
Store stock solution in sealed glass ampoules

4.14 Other standards - should be prepared if other residues are to be quantified in these procedures

Working solutions from these standard stock solutions should be prepared on a regular basis and stored in clean glass volumetric flasks tightly capped with non-contaminating materials such as teflon or glass. Extreme care must be taken to ensure that standards have not changed concentration through solvent evaporation.

The use of appropriate internal standards is encouraged in order to improve the overall accuracy of the analysis. These standards should be selected such that they do not occur in samples, they do not interfere with the analysis of the contaminants to be quantified, they should be stable to all the procedures applied to the samples and they should have similar chemical behaviour to the contaminants of interest so that they are recovered with the contaminants. They should be added to samples in a concentration range to produce a similar response on the electron capture detector to the contaminants contained in samples. Compounds eluting in the PCB fraction which have proved useful as internal standards are specific isomers of the PCBs which do not commonly occur in the environment (eg. 2, 5, 2', 6' tetra chlorobiphenyl). For the DDT fraction 1,1 dichloro - 2,2- diphenylethylene has been used successfully. Other compounds may be more applicable to various systems. Preliminary range finding analysis will be necessary to determine the amount of internal standards to add to samples.

5. Apparatus

Gas chromatograph (GL) with electron capture detector preferably Ni-63 complete with glass GC columns. The column specified for the quantification procedure described in this manual was chosen because the characteristics of resolution of standard mixtures of PCBs has been thoroughly calibrated and the response factors generated for each major PCB peak created by several standard PCB mixtures have been published. All participants are requested to use the column specified in this manual unless they are able to quantify individual PCB isomers by high resolution chromatography procedures.

5.1 High purity carrier gas for the gas chromatograph including molecular traps to remove trace contaminants and moisture.

5.2 Rotary evaporator

5.3 Kuderna-Danish or similar concentrator and heater

5.4 Soxhlet extraction apparatus and heaters

5.5 Glassware including boiling flasks, ground glass stoppers, beakers, erlenmeyer flasks, chromatography columns, separatory funnels, weighing bottles, pipettes, syringes, tissue grinders

5.6 Drying oven (temperature range up to at least 300°C) for determining sample dry weights, baking of contaminant residues from glassware and reagents
A muffle furnace is better for baking materials at greater than 300°C, if required

5.7 Centrifuge and tubes (at least 600 g)

5.8 Freezedryer and porcelain mortar (for procedure 8.2.1)

5.9 Analytical balance with a precision of 0.0001 g

5.10 Stainless steel tweezers and spatulas

5.11 Dessicator (completely cleaned with no grease)

5.12 Glass wool precleaned by extraction and oven baking

5.13 Supply of clean dry nitrogen

5.14 Silica gel column

The silica gel should be pre-extracted in the soxhlet apparatus to remove any contaminants. It is then dried at low heat in an oven. Activation is achieved by heating the dried gel at 250°C for 2 hours. It is then partially deactivated with 3% water by weight and stored in a tightly sealed jar with ground glass stopper. The water should be well mixed into the gel and the mixture should be allowed to equilibrate for 1 day before use. The activation deactivation procedure should be repeated weekly. A 1 cm I.D. glass column with teflon stopcock is plugged with precleaned glass wool. A column with a scintered glass disc could also be used. One gram of silica gel is weighed out into a beaker and covered with hexane. A slurry is made by agitation and is poured into the glass column. The gel is allowed to settle into an even bed and any gel adhering to the column sides is rinsed down with hexane. The solvent is drained to just above the gel bed. It should be rinsed by a further 5 ml of hexane and drained again. The gel should never run dry. Individual columns should be prepared immediately before use, a new column of silica gel used for each sample.

6. Sampling and sample preparation

Sampling and sample preparation should be carried out in accordance with UNEP/FAO/IAEA Reference Methods No. 6 (in preparation) and No. 12.

7. Preparative analytical procedures

7.1 Cleaning of glassware:

Scrub all glassware vigorously with brushes in hot water and detergent. Rinse five times with tap water and twice with distilled water. Rinse with acetone or ethanol followed by hexane or petroleum ether. Bake overnight in an oven at 300°. All glassware should be stored in dust free cabinets and tightly sealed with precleaned aluminium foil when not in use. Ideally glassware should be cleaned just before use. If necessary to remove tough organic residues the glassware must be soaked for at least two hours in the acid cleaning solution (4.9), thoroughly rinsed with tap and distilled water and then put into the drying oven.

7.2 Extraction of samples

7.2.1. Extraction procedure for freeze dried samples

Select a 50 to 100 g fresh weight subsample from the sample. Weigh this subsample and freeze dry it. When the subsample appears to be dry, weigh it and freeze dry it again for 24 hours. Reweigh it. If the difference between the two dry weights is greater than 5% continue freeze drying. Special care must be taken to ensure the freeze drier is clean and does not contaminate the samples. The freeze drying procedure should be tested by drying 100 g Na_2SO_4 as a blank and extracting this as a sample. Pulverize the freeze dried subsample carefully in a cleaned mortar. Accurately weigh about 5 to 20 g of this pulverized material, note the weight extracted (Ws), and place it into a precleaned extraction thimble in a soxhlet apparatus. The size of the subsample should be adjusted so that about 1 g or "lipid" will be extracted. Smaller subsamples could be used if residue concentrations are

expected to be high. Add about 200 ml of hexane or petroleum ether to the extraction flask, a few carbon boiling chips and extract the sample for 8 hours cycling the solvent through at a rate of 4 to 5 cycles per hour. If internal standards are used they should be added to the solvent in the flask before extraction. Extract an empty thimble containing the freeze dried NaSO4 as a procedural blank.

7.2.2 Extraction procedure without freeze drying

Select a 25 to 100 g fresh weight subsample and place in a blender. Add 3 times the weight of anhydrous sodium sulfate and blend at high speed for 1 or 2 minutes or until well blended and the sample appears to be dry. Transfer the mixture to precleaned extraction thimble, add internal standards and extract with about 200 ml hexane or petroleum ether for 8 hours in the soxhlet apparatus cycling 4 to 5 times per hour. Extract the same amount of sodium sulfate as the procedural blank.

Note: The blender must be constructed of contamination free materials and precleaned.

7.3 For both extraction procedures the extracts are concentrated on a rotary evaporator to about 10 ml. The temperature of the water bath must not exceed 30°C. Dry extracts by passing them through a pasteur pipette plugged with glass wool and containing about 1 g of anhydrous sodium sulfate and collect in the graduated tube of a Kuderna-Dansih concentrator. Concentrate extract to near 1 ml with the concentrator anf adjust extract volume to exactly 1 ml by a gentle stream of clean dry nitrogen. Record accurate volume.

7.4 E.O.M. determination

Solvent extractable organic matter is determined in the following manner. On the weighing pan of an electrobalance evaporate a known amount of the extract (5 to 10µl) and weigh the residue to ±µg.

The quantity of EOM is :

$$\text{E.O.M.} \ \frac{(\text{mg})}{\text{g}} = \frac{\text{weight of residue (mg) X volume of extract (ml) X } 10^3}{\text{amount evaporated (µl) X quantity of sample extracted (g)}}$$

Note that extreme care must be taken to ensure balance and pans are clean, dry and stable to obtain accurate readings of ± 1 µg. A small hot plate is used to warm pans and forceps and thus keep instruments dry after solvent cleaning. If no electrobalance is available a known volume of the extract can be transferred into a clean preweighed beaker. The solvent is evaporated with dry nitrogen gas until a constant weight of ± 5 mg is reached. Calculate the amount of "lipids" in the sample taking into account the volume of the lipid extract which was dried.

7.5 Sulfuric acid clean-up

Take an aliquot of the concentrated extract, containing about 200 mg of "lipid", and add to glass centrifuge tube containing sufficient concentrated sulfuric acid (about 1 ml or more if necessary). In order to hydrolyze the "lipids" agitate the tube for several minutes to ensure complete hydrolysis.

Extract should be colourless. Centrifuge to separate the phases and transfer the solvent supernatant into another graduated tube. Reduce the volume of the extract by evaporating the solvent with clean dry nitrogen to 0.5 ml in preparation for the column chromatography separation.

7.6 Column chromatography

Two types of chlorinated hydrocarbons which interfere with the analysis of PCB and DDT are chlorinated terpenes (camphechlor, toxaphene) and chlordane-related compounds. Both of these types are very complex mixtures and it is impossible to improve the resolution of the GC column to separate all the individual isomers from PCB compounds. There is not a perfect method available to separate the PCBs from all components in toxaphene. The method below will however give PCBs in the first fraction and about 90% recovery of the toxaphene and chlordane in the second (DDT) fraction.

The extract aliquot treated with acid and reduced to 0.5 ml is applied to the silica gel column. It is carefully eluted with 7 ml of hexane and the first fraction collected. Then the column is eluted with 10 ml 25% (v/v) diethylether in hexane and the second fraction is collected. Fraction one will contain the PCBs, DDE and some other pesticides such as hexachlorobenzene. Fraction two will contain the DDTs, DDDs, most of the toxaphene and chlordane components and some other pesticides such as hexachlorocyclohexane.

The column chromatography procedure must be calibrated with standard mixtures for the recovery of all the compounds of interest.

7.7 Other pesticides

Several other pesticides will be recovered in these procedures if they are present in samples. But many residues will not be stable to treatment with acid or base. Table 1 is meant only as a guide for predicting the recovery of various residues in these procedures.

7.8. Determination of fresh weight/dry weight (FW/DW) ratio

The constant weight of a weighing flask is determined by repeated weighing. A subsample of 1-2 g is introduced into the flask and heated in an oven at 105°C for 24 hours. The flask is then transferred to a dessicator to cool and then weighed again. The procedure is repeated until a constant weight is reached. The (FW/DW) ratio is then computed. For freeze dried samples the moisture content must be corrected into the weight of the sample extracted.

8. Gas chromatographic determinations

8.1 G.C. columns

The stationary phase recommended for analyses of PCB and DDT compounds is a silicone oil OV-101. This is applied on the support, Chromosorb W HP. For 6 ft columns with internal diameter of 4 mm, the particle size of the support should be 80-100 mesh.

Table 1: Stability of different compounds to different treatments

Compound	Treatment		Transformation Product
	H_2SO_4	(KOH/EtOH)	
HCB	+	+	
1,1-dichloro-2,2-diphenyl-ethene (internal standard)	+	+	
Y-HCH Lindane	+	-	
2,5,2', 6' -tetrachlorobi-phenyl (internal standard)	+	+	
β-HCH	+	-	
Aldrin	+	-	
p,p'DDE	+	+	
Heptachlor epoxide	-	-	
Dieldrin	-	+	
0,p'-DDE	+	+	
o,p'-DDT	+	-	o,p' -DDE
p,p'-DDD	+	-	p,p' -DDMU
p,p'-DDT	+	-	p,p' -DDE
PCBs	+	+	
Toxaphene	+	-	
Phthalate esters	-	-	

\+ Stable against treatment
\- Not stable against treatment

8.2 Gas chromatographic conditions

The samples should be analysed on a gas chromatograph equipped with an electron capture detector. The glass column should be filled with acid-washed and dusted Chromosorb W HP 80-100 mesh coated with 5% OV-101.

The column is isothermally held at 200°C to give p,p'-DDT a retention time relative to aldrin of 3.03. The injector is held at 220°C and the detector at 275°C for (Ni-63 only). The carrier gas flow is set to 60 ml/minute.

8.3 Column preparation

The following procedure is recommended for the preparation of the columns for the gas chromatograph.

Fill the column with concentrated HCl and leave it for one hour.

Wash with water, then with acetone and finally with toluene.

Fill the column with a toluene solution of hexamethyldisilazane (HMDS, 10%).
Warning: use fume hood and do not touch the HMDS reagent.

Wash the column with toluene, then with methanol and finally with acetone.

Dry the column either with air, using a water jet air pump, or in an oven.

Put about 10 mm glass wool in the outlet end of the column and weigh the empty column.

Attach a funnel to the inlet of the column and fill at least half a coil with the filling material before the other end is connected to a vacuum pump (water jet air pump).

Fill the column by gentle tapping. If the filling material gets stuck before the column is filled, disconnect the water jet air pump and put the column in an oven (~100°C) for a few minutes and then continue with the warm column.

When the column is full, put about 10 mm glass wool in the inlet end.

Weigh the filled column and label it.

The column has to be conditioned before it is connected to the detector. Connect the inlet to the injector in a chromatograph (but not to the detector) and apply a carrier gas flow of about 60 ml/min and heat the column to allowed maximum temperature for the stationary phase (250°C). The column should be conditioned overnight.

8.4 Column test

When the column has been connected to the detector, the carrier gas flow is set to 60 ml/min for a column with 4 mm internal diameter. The column performance is then measured by the 'number of theoretical plates' for a specific compound and can be done according to the following procedure:

Set injector and detector temperatures at 200 and 300°C respectively, and the column oven temperature at 180°.

Inject p,p'-DDT standard, and measure the retention time (t_r). Adjust the column temperature to get a pp'-DDT retention time relative to aldrin of 3.03.

Measure the width of the DDT-peak at its half height ($b_{1/2}$) in minutes and the retention time (t_r) also in minutes.

Calculate the number of theoretical plates with the formula:

$$N = 5.54 \left(\frac{t_r}{b_{1/2}}\right)^2$$

A measure independent from column length is the height equivalent to a theoretical plate (HETP):

$$HETP = \frac{L}{N}$$

where L is the column length.

Adjust the flowrate of the carrier gas to obtain optimum performance. HETP should be as low as possible.

8.5 Calibration of the electron capture detector

The procedures taking place in the ECD are not fully known yet. A simplified model is that β-particles from the radioactive source (^3H or ^{63}Ni) collide with atoms in the carrier gas. Secondary, much slower electrons are then expelled and these give a current in an applied electric field. When a compound, capable of withdrawing these electrons, is eluted from the column, the detector registers a response.

The signal thus measured is proportional to the concentration of electronwithdrawing compounds in the detector to a certain limit. When a considerable part of the electrons is captured, the probability for a molecule to find an electron is diminished and the proportionality is lost. This means that the response curve (response as function of the injected amount) will be linear for low amounts and then curve off.

The linear range for the ECD differs between instruments but covers normally from the detection limit to 10-100 times the detection limit. It is therefore essential to know the shape of the calibration curve and it can be constructed after injection of known amounts of the compound of interest. The samples are then diluted/concentrated to give responses within the linear range.

8.6 Sample quantification

DDTs

The concentration of the various DDTs are calculated from the peak heights of the chromatogram obtained after acid treatment and silica gel separation.

PCBs

PCBs are a complex mixture of compounds that cannot all be resolved on the chromatographic system. The method for quantification suggested in this manual is based on quantification of the major peaks resolved from standard aroclor mixtures. Peaks should be measured independently as per the sample chromatogram and related to the response factors derived from the detector response to standard aroclor and the data presented in the following tables 2 and 3 and Fig. 1 and 2.

After comparison of the retention times of peaks in the sample chromatogram to those in the corresponding standard, the peak heights are measured and related to the peak height in the standard, the residue is quantified according to the formula:

$$\frac{V}{M} \cdot \frac{h \cdot h'_{is}}{h_{is} \cdot h'} \cdot c = \text{result in } \mu g/g \text{ (ppm) on fresh weight basis}$$

V = total extract volume (ml)
M = fresh weight of the sample (g)
h = peak height of the compound in the sample (mm)
h' = peak height of the compound in the standard (mm)
h_{is} = height of the internal standard peak in the sample (mm)
h'_{is} = height of the internal standard peak in the standard (mm)
c = concentration of standard (μg/ml)

The standard chosen should be the one that resembles the sample most. If an intermediate chlorination is found a mixture of Arochlor 1254 and 1260 could be prepared and response factors adjusted accordingly. The total PCB amount is obtained after summation of the concentrations from each peak eluting after DDE.

Table no. 2: Composition of Aroclor 1254 (FDA Lot No. 71-698;52.2% total Cl determined)

Peak R_{DDE} (X100)	Mean wt %	Chlorine atoms	
		No.	% of total
47	7.1	3	8
		4	92
54	2.7	4	100
58	1.2	4	100
70	14.7	4	57
		5	43
84	18.6	4	4
		5	96
98	8.3	4	100
104	14.1	4	6
		5	94
125	15.6	4	40
		5	60
146	9.0	5	15
		6	85
160	ND*	6	100
174	7.4	6	100
203	1.3	6	100
232	ND	6	100
280	ND	6	100
332	ND	6	100

*ND = not determined. HECD Cl responses were too low for accurate measurement.

(From SAWYER L.D)

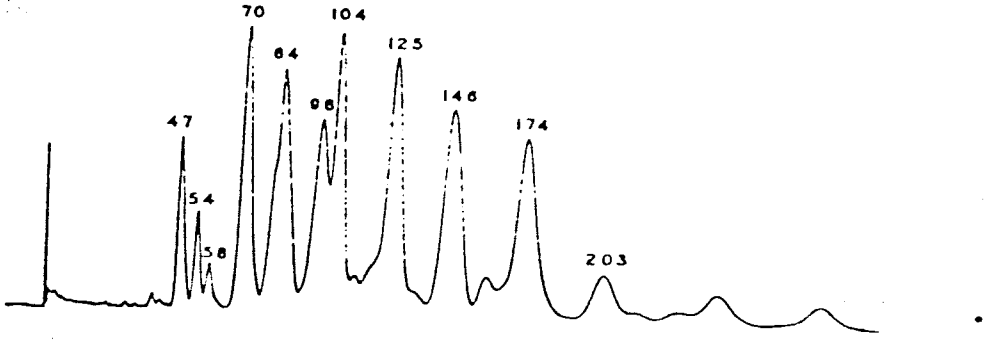

Fig. 1. Peak resolution of Aroclor 1254 at standardized GLC conditions. (SAWYER L.D.)

Table No.3: Composition of Aroclor 1260 (FDA Lot No. 71-699; 58.4% total Cl determined)

Peak R_{DDE} (X100)	Mean wt%	Chlorine atoms	
		No.	% of total
70	2.4	5	100
84	3.6	5	100
98 & 104	2.8	5	47
		6	53
117	4.4	6	100
125	11.0	5	9
		6	91
146	13.3	5	6
		6	94
160	5.5	6	36
		7	64
174	10.0	6	100
203	10.9	7	100
232 & 244	11.2	7	100
280	12.5	7	100
332	4.2	7	100
360 & 372	5.4	7	100
448	0.8	7	100
528	2.0	7	100

(From SAWYER, L.D.)

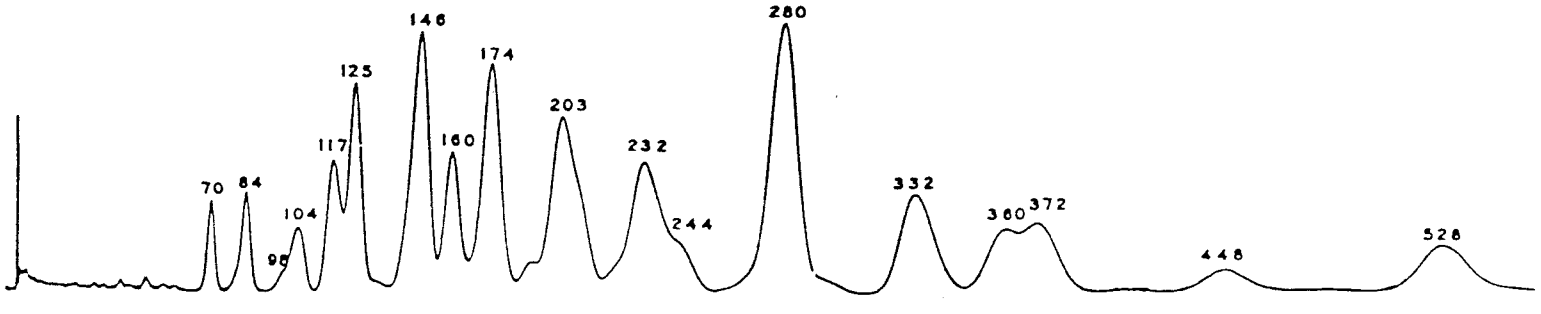

Fig. 2. Peak resolution of Aroclor 1260 at standardized GLC conditions. (SAWYER, L.D.)

9. Confirmation tests

Confirmation analyses of the pp'DDT and pp'DDD present in the sample are done by dehydrochlorination with alchoholic KOH solution. pp'DDT is transformed to pp'DDE and pp'DDD is converted to pp'DDMU. After GC analysis of fraction 2 add 1 ml of methanol and one pellet of KOH to the concentrator tube. Heat in a Kuderna-Danish concentrator for 30 minutes with a snyder column in place to avoid sample loss. Allow the mixture to cool and add 10 ml distilled water and 1 ml hexane. Extract by shaking vigorously. Centrifuge to separate phases. Remove hexane and pass it through a pasteur pipette plugged with glass wool and containing a few grams of Na_2SO_4. Reextract the KOH mixture with an additional 1 ml of hexane. Centrifuge and pass the hexane through the Na_2SO_4. Concentrate the combined extracts to appropriate volume for gc analysis.

Further confirmations could be achieved by laboratories having gas chromatographic mass spectrometry analyses.

10. Precision

Estimate the precision of the entire analytical procedure by extracting 5 subsamples from the same sample. If the relative standard deviation in the µg residue/kg fresh weight values is greater than 20% of the mean, then evaluate the procedure for possible errors and contamination.

By participating in intercalibration exercises involving several analytical laboratories, the relative agreement to mean values and precision of individual laboratories can be evaluated. All participants will be requested to participate in intercalibration exercises.

11. Recovery

All participants are encouraged to test their procedures for the recovery of standards added during the extraction step. This can be most effectively done by using internal standards in samples extractions but can be done in a general manner with parallel of blank extractions.

12. Test Report

Fill in the test report giving details in every column. Attach the relevant sampling and sample preparation protocol (UNEP/FAO/IAEA(1982)).