REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME MARCH 1991

Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons

Reference Methods For Marine Pollution Studies No.12 Rev.2

Prepared in co-operation with

FAO IAEA IOC

UNEP 1991

Note: This document has been prepared jointly by the Intergovernmental Oceanographic Commission (IOC), the International Atomic Energy Agency (IAEA), the Food and Agriculture Organization of the United Nations (FAO) and the United Nations Environment Programme (UNEP) under project FP.5102-88-03.

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PREFACE

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

One of the basic components of the action plans sponsored by UNEP in the framework of the Regional Seas Programme is the assessment of the state of the marine environment and of its resources, and of the sources and trends of the pollution, and the impact of pollution on human health, marine ecosystems and amenities. In order to assist those participating in this activity and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of Reference Methods and Guidelines for marine pollution studies are being developed as part of a programme of comprehensive technical support which includes the provision of expert advice, reference methods and materials, training and data quality assurance (3). The Methods 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.

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

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

(3) UNEP/IAEA/IOC: Reference Methods and Materials: A Programme of comprehensive support for regional and global marine pollution assessments. UNEP 1990.

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

Marine Environmental Studies Laboratory
IAEA Marine Laboratory
19, Avenue des Castellans
MC 98000 MONACO

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

Rev. 1 of the Reference Method for Marine Pollution Studies No. 12 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.

Rev. 2 of this method was updated according to the decisions of the joint meeting of the IOC/UNEP Group of Experts on Methods, Standards and Intercalibration (GEMSI) and the IOC/UNEP/IMO Group of Experts on the Effects of Pollution (GEEP), held in Moscow 15-19 October 1990.

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1. SCOPE AND FIELD OF APPLICATION

This publication describes the sampling and sample preparation procedure suitable to obtain uncontaminated samples of mussels, shrimps and fish for analyses of chlorinated hydrocarbons by gas chromatography.

2. REFERENCES

BERNHARD, M. (1975) 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.

UNEP/FAO/IAEA (1991). Guidelines for monitoring chemical contaminants in marine organisms. Reference Methods for Marine Pollution Studies No. 6.

UNEP/IOC/IAEA/FAO (1990). Contaminant monitoring programmes using marine organisms: Quality Assurance and Good Laboratory Practice. Reference Methods for Marine Pollution Studies No. 57.

3. PRINCIPLES

Specimens of organisms selected and collected according to UNEP/FAO/IOC/IAEA (1991) are enclosed in metal containers and transported to the analytical laboratory either as cooled fresh (-2^{o} to 4^{o}) or deep-frozen (-18^{o}C) samples. There the specimens are dissected under "clean conditions" and subsamples are prepared for the analyses of chlorinated hydrocarbons. The reader is also advised to consult UNEP/IOC/IAEA/FAO (1990) for guidance on quality assurance and good laboratory practice before commencing field collections of organisms.

4. REAGENTS

NOTE: Only distilled water and reagents or recognized analytical quality, with as low as possible chlorinated hydrocarbon concentrations should be used. All reagents must be checked for contamination by analyzing blanks. If residues are present the reagents have to be cleaned, e.g. by extracting with hexane and/or by baking off the chlorinated hydrocarbons at temperatures above 260° overnight. Solvents can be cleaned by distillation from all-glass apparatus.

4.1 Demineralized distilled water produced over permanganate (0.1 g $KMnO_4$ per litre) or water of equivalent quality, free from interferring chlorinated hydrocarbons.

4.2 Ethanol 95 per cent, pesticide analysis grade.

4.3 Hexane, pesticide analysis grade.

4.4 Detergent, free of chlorinated hydrocarbons.

4.5 Uncontaminated "open-ocean" subsurface (below 1 m depth) sea water.

5. APPARATUS

5.1 Thermally-insulated boxes (such as ice boxes for camping with good air-tight seals) preferably with metal, glass or porcelain insert cooled with commercially available cooling bags (ice packs), refrigerator, or similar, for transporting specimens during warm periods. For storage and transport of mussels the boxes must be equipped with a metal grid in the bottom in order to avoid that the mussels are being submerged when moistened during transport and storage.

NOTE: Cooling bags must be checked for contamination with chlorinated hydrocarbons.

5.2 All-metal or glass specimen containers (household ware) big enough for temporary storage and transport of samples (6). These should be previously cleaned with distilled water and detergent (4.4) and then rinsed twice with ethanol (4.2) and air dried. Alternatively, aluminum foil (household ware) heated overnight at more than 260°C to eliminate contamination from organics and chlorinated hydrocarbons can be used also.

5.3 Pyrex or porcelain dishes as working surface.

5.4 Pyrex dish or similar with a centimeter scale attached underneath to serve as a length-measuring scale (ruler or board).

5.5 Glass or non-rusting metal containers with airtight screw-caps for storing the sample after sample preparation (7). These containers and their lids should be thoroughly washed with distilled water and detergent (4.4), then rinsed twice with ethanol (4.2), air dried, rinsed again with clean hexane (4.3), drained and dried in an oven at 60°C in a well ventilated room. Plastic screw caps should be avoided or at least protected with an insert made from Aluminium foil (previously baked at 260°C overnight) and rinsed with hexane (4.3). Alternatively, teflon caps or liners can be employed.

5.6 Deep-freezer (-18°C).

5.7 Scraper (figure 1), a strong rust-free knife, or similar, for collecting mussels.

5.8 Glass bottle or carboy (20-50 L) with clean sea water (4.5) needed to moisten live mussels during storage and transport (6.2).

5.9 Stainless steel knives or similar, for dissecting specimens.

5.10 Tweezers of stainless steel or other non-rusting material.

5.11 Analytical balance (100-200 g) with a precision of 0.001 g or better for weighing specimens, subsamples and reagents; preferably a "top-loading" balance.

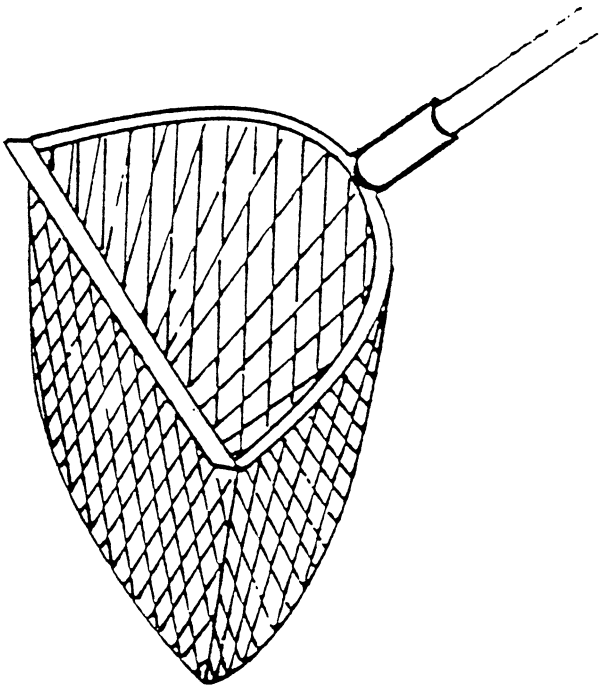

FIGURE 1: SCRAPER FOR COLLECTING MUSSELS

5.12 Glass washing bottle containing glass-distilled water (4.1). Before filling the bottle with water it must be heated overnight at more than 260°C.

5.13 Glass washing bottle for 95% ethanol (4.2). Before filling with ethanol the bottle must be heated overnight at more than 260°C.

5.14 Small aluminium foil to be used as "weighing aluminium".

5.15 Large metal knife for cutting samples from large fishes.

5.16 Stainless steel blender or other tissue homogenizer made from glass or porcelain.

5.17 Drying over (260°C).

5.18 Refrigerator, +4°C - 2°C (required for 6.2, 6.3, 6.4).

6. SAMPLING AND TRANSPORT

6.1 Presampling preparation

Clean the thermally-insulated boxes (5.1), the specimen containers (5.2), the measuring board (5.4) (for large fishes), the large rust-free knife (5.7, 5.15) and the scraper (5.7) (for mussels) with detergent (4.4) and rinse with distilled water or, alternatively, with clean open-ocean sea water (4.5) followed by ethanol (4.2).

6.2 Sampling of mussels

Scrape wild mussels from their attachments with the clean scraper or the rust-free knife (5.7).

Transfer a suitable number of undamaged mussels, collected according to UNEP/FAO/IOC/IAEA (1991), into the clean thermally-insulated boxes with grid on the bottom (5.1). Collect a clean sea water sample (4.5) in a suitable container (5.8) from the sampling site to keep the mussels moist if a long transport (more than 2 hours in hot climates) is envisaged. Keep the mussels moist with the clean sea water (4.5) without submerging them. If the mussels have to be transported and stored before sample preparation (7) for more than 24 hours, deep-freeze them (5.6). Place a suitable number of the specimens in a clean specimen container (5.2) or a large enough aluminium foil (5.2) taking care that the shells do not puncture the foil, then squeeze out the air and close the sample by folding airtight. Place the enclosed sample into another aluminium foil (5.2) together with a specimen identification note (see Appendix), close the aluminium-foil airtight again and deep-freeze.

This represents the "specimen sample".

NOTE: The transport of mussels collected near the laboratory will not present special transport and storage problems. Mussels, especially when gathered from the intertidal zone, will survive aerial exposure for 24 hours. Mussels submerged in sea water during transport will open their valves, start pumping water and excreting waste products, while during aerial exposure their valves will remain closed and their metabolic rate is greatly reduced; therefore their submersion in sea water during transport should be avoided.

6.3 Sampling of shrimps and small to medium-size fishes

Place a suitable number of the undamaged specimens selected, according to UNEP/FAO/IOC/IAEA (1991), on a fishing vessel, fish market, etc., in a clean specimen container (5.2) or a large enough aluminium-foil. Squeeze out the air and close the sample by folding the aluminium foil airtight. Place the enclosed sample into another aluminium foil (5.2) together with a specimen identification note (see Appendix) and close the aluminium foil airtight. Deep freeze (5.6) the enclosed specimen whenever possible. Use a refrigerator (5.18) or a cooled thermo-insulated box (5.1) only if the storage period is not too long (48 hours in hot climate).

This represents the "specimen sample".

6.4 Sampling large-size fishes

Determine the length, the body weight and sex (gonads) of the specimen selected according to UNEP/FAO/IAEA (1991).

Separate with a clean metal knife (5.15) a portion of at least 100 g of muscle tissue. This portion must be at least 5cm thick so that during sample preparation (7.5) contaminated and dirty tissue can be sliced off. Place each portion into a separate clean aluminium foil (5.2), squeeze out the air and fold airtight. Place it together with the specimen identification note (see Appendix) into a second aluminium foil (5.2), close it airtight and deep-freeze (5.6), whenever possible. Use a refrigerator (5.18) or a cooled thermo-insulated box (5.1) only if the storage period is not too long (48 hours in hot climate).

This represents the "specimen sample".

7. SAMPLE PREPARATION

7.1 Preparatory activities

If necessary, partially thaw deep-frozen samples (6) by placing them overnight in a refrigerator at -2°C to 4°C (partially frozen samples are easier to cut than completely thawed or even fresh samples).

Clean the knives (5.9), the length-measuring devices (5.4), the tweezers (5.10), and the blender (5.16) with detergent (4.4), rinse first with distilled water (5.12) and then with ethanol (5.13).

Remove sufficient aluminium foil to serve as "weighing aluminium" (5.14) from the roll, arrange it loosely so that air can reach all surfaces. Place it into an oven (5.17) together with the dishes (5.3) and the containers (5.5). Bake off all organic contaminants by heating it overnight at more than 260°C.

Clean hands carefully with detergent (4.4) and dry them. If possible a clean room should be used for preparatory activities.

7.2 Sample preparation of mussels

Scrape off all foreign materials attached to the outer surface of the shell with a cleaned knife (knife no. 1) (5.9) used only for this purpose. Handle the mussels as little as possible.

Rinse each mussel with distilled water (4.1) or alternatively with clean sea water (5.8) and let the water drain off.

Pull out the fibre-like byssus which extrudes from between the closed shells on the concave side of the shells.

Weight (5.11) the whole mussel to the nearest 0.2 g and note the weight.

Insert a second clean knife (knife no. 2) (5.9) into the opening from which the byssus extrudes and cut the posterior adductor muscle by turning the knife as indicated in figure 2, then cut in the other direction and open the mussel. Do not try to break the mussel open with the knife: if the two muscles are cut the mussel will open easily. Check if the byssus has been eliminated completely; if not, remove the remainder with clean tweezers (5.10).

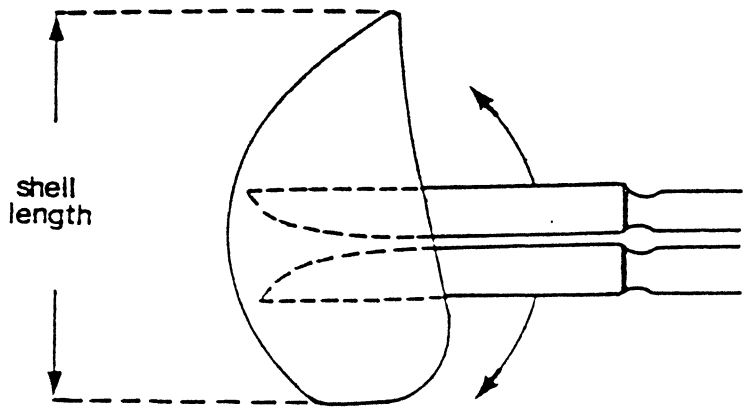

FIGURE 2: CUTTING THE TWO ADDUCTOR MUSCLES

Rinse the soft part of the mussel in its shells with distilled water or clean sea water (5.13).

Loosen all tissue with the second clean knife (knife no. 2) (5.6) remove the soft tissue from the shell with a pair of clean tweezers (5.10) without touching the outer part of the shells, and let all the water drain off.

(a) Single specimen sample: Weigh a clean empty specimen container (5.5) on the balance (5.11) and note the weight. Then put the soft part of the mussel in it and reweigh. Note the fresh weight of the soft part. Close the container airtight, label it with the sample preparation code and deep-freeze (5.6).

Determine the length of one shell (figure 2) by placing it with the inner part facing the cm scale (5.4). Note the length of the shell together with the total weight and the weight of the soft part of the mussel in the protocol.

(b) Composite sample: Fill a specimen container (5.5) of known weight with at least 10 soft parts of mussels prepared as described above. Reweigh the specimen container and note the composite fresh weight of the mussels. Homogenize the specimens in a cleaned blender (5.16) and return the homogenate in the sample container (5.5). Note the total weight again and recalculate the fresh weight of the homogenate. Label the sampler container (5.5) with the sample code.

Place several sample containers (5.5) in a clean aluminium foil (5.2), include an identification note with the containers sample codes, seal the foil airtight and deep-freeze (5.6).

This represents a "tissue sample".

NOTE: Comparing the weight of the container holding the mussel homogenate at the time of its preparation with the weight of the same container before the sample is used for analysis of chlorinated hydrocarbons, the moisture lost during storage can be calculated. This loss must be taken into account when calculating the concentration of chlorinated hydrocarbons in the analysed sample.

NOTE: By comparing the weight of the container with the homogenate, at this point, with the weight of the container before the analysis is carried out, the loss of moisture during long storage can be determined.

7.3 Sample preparation of shrimps

(a) Single specimen sample: Determine the length of the shrimp from rostrum to uropod; (see figure 3) using the appropriate length measuring device (5.4). Weigh the shrimp after placing a clean "weighing aluminium" (5.14) on the balance (5.11) and note it, length and fresh weight.

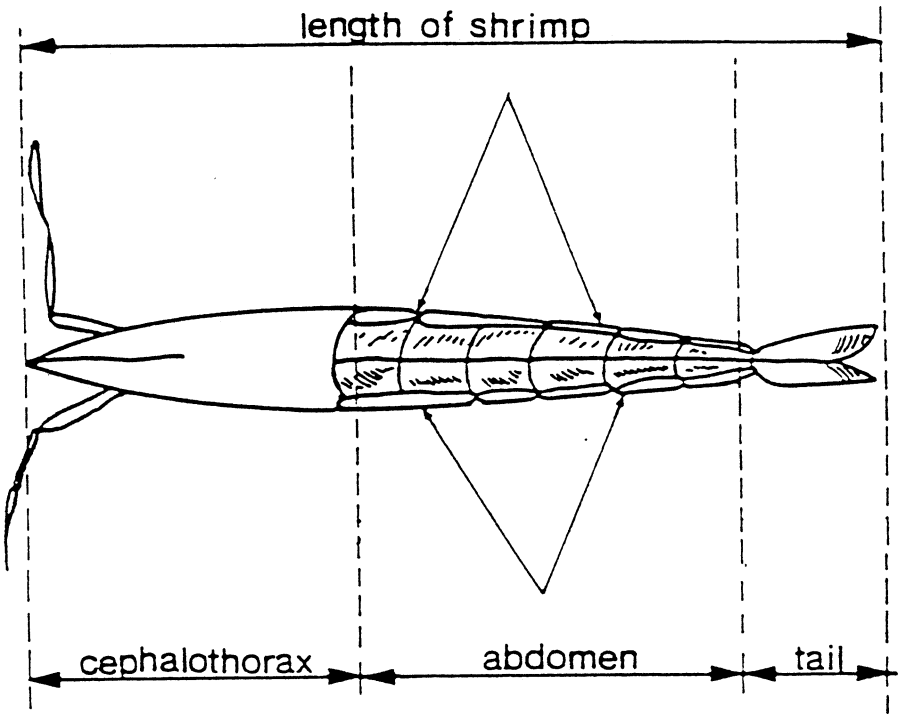

FIGURE 3: SCHEMATIC DIAGRAM OF A SHRIMP (arrows indicate where to cut after the legs have been removed)

Separate the abdomen from the cephalothorax and the "tail" (telson and uropod) with a first knife (5.9) (knife no. 1) taking care that no viscera remain in the abdomen (figure 3). Cut off all legs. Turn the abdomen with the ventral side up and cut with a knife (5.9) along the edges of the sterinites (the "ventral exoskeleton"); lift the sterinites off with a pair of tweezers (5.10) and discard.

Loosen with a second clean knife (5.9) (knife no. 2) the abdomen muscle and lift it from the exoskeleton with a clean pair of tweezers (5.10).

Transfer the muscle with a clean pair of tweezers (5.10) into a preweighed (5.11) container (5.5), determine and note the fresh weight of the muscle. Close the container airtight, label it with the sample code and deep-freeze (5.6).

(b) Composite sample: Start sample preparation as described for single specimen sample above taking care to record length, fresh weight, tail muscle weight and the sex of each specimen separately. Reduce the tail muscle(s) of the large specimens to the weight of the smallest tail muscle. A composite sample should not contain less than 6 tail muscles from 6 different specimens of the same sex. Homogenize the tail muscles in a blender (5.16). Transfer the homogenate into a suitable clean container (5.5) which has been weighed empty. Close the container airtight, label it and weigh the container with the homogenate. Note the weight of the homogenate together with the other data in a protocol and deep-freeze (5.6) the container.

This represents a "tissue sample".

NOTE: The concentration of chlorinated hydrocarbons in a composite sample should represent the mean value of concentrations of single specimens. In order to avoid over-representation of large specimens the weight of the tail muscles of all specimens to be included in the composite sample should be reduced to that of the tail muscle of the smallest specimen. As there might be differences in the chlorinated hydrocarbon content of male and female specimens, use them in separate composite samples.

NOTE: Comparing the weight of the container with the homogenate at this point with the weight of the container before the analysis is carried out, the loss of moisture during long storage can be determined.

7.4 Sample preparation for small to medium size fish

(a) Single specimen sample: Determine the fork length (see figure 4) to the nearest mm on the length-measuring board (5.4). Weigh (5.11) the fish on clean "weighing aluminum" (5.14) with an accuracy of 0.1% of its weight and note both the length and fresh weight of each specimen separately.

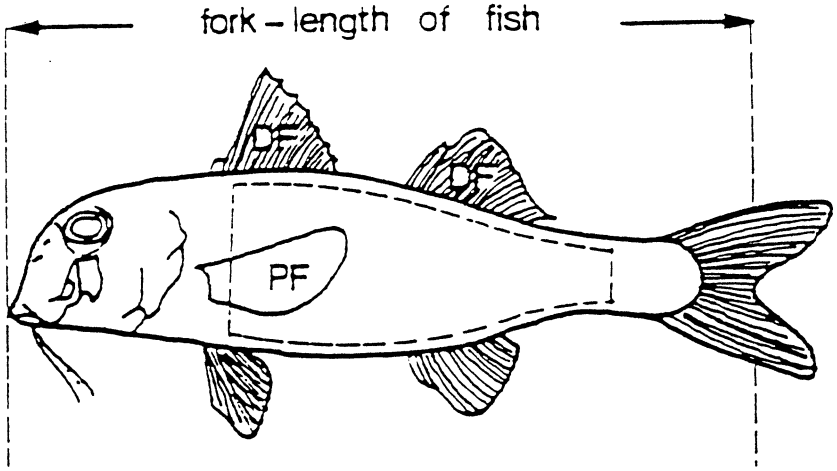

FIGURE 4: SCHEMATIC DIAGRAM OF FISH
(PF-pectoral fin, DF=dorsal fin, dashed line shows where the cuts should be made)

Rinse the fish with ethanol (5.13) and place it on the clean surface of the dish (5.3) used as working surface. Then remove the pectoral fin and cut the skin of the fish with a first knife (5.9) near the dorsal fins, starting from the head to the tail as shown in figure 4.

Cut near the gills across the body, along the ventral edge from the gills to the tail and finally across the body near the tail. These four cuts should be carried out first on one side only taking care not to cut too deep in order to avoid cutting into the viscera and thus contaminating the fillet (muscle). It is advisable that a second person hold the fish by the head and tail during this operation.

Pull the skin from the fillet with a pair of tweezers (5.10), taking care that the outer skin does not contaminate the fillet.

With a second clean knife (5.9), cut the fillet from the vertebral column (backbone) starting from the cut near the gills. Lift the fillet with a second clean pair of tweezers (5.10), so that the fillet will not touch the working surface (5.3) or other parts of the fish.

Weigh the fillet in a clean container (5.5) and note the fresh weight.

If one fillet does not yield enough material for analysis put the fish, skin-side upwards, on a clean portion of the working surface (5.3) or on a new working surface (5.3) or other parts of the fish.

Weigh the fillet in a clean container (5.5) and note the fresh weight.

If one fillet does not yield enough material for analysis put the fish, skin-side upwards, on a clean portion of the working surface (5.3) or on a new working surface and remove the second fillet from the other side of the same fish, as described above, add it to the first sample and record their total weight.

Close the container airtight. Identify the container with a code number and/or label, record all data in the protocol and deep-freeze (5.6)

Determine and note the sex of fish by examining the gonads.

(b) Composite sample: Start sample preparation as described for single specimen sample above taking care to record the length, the fresh weight and fillet weight of each fish separately. Determine and note by examining the gonads the sex of each specimen separately.

Reduce the fillet(s) of the large specimens to the weight of the smallest fillet. A composite sample should not contain less than 6 fillets from 6 different specimens of the same sex. Homogenize the fillets in a blender (5.16). Transfer the homogenate into a suitable clean container (5.5) which has been weighed empty. Close the container airtight, label it and weigh the container with the homogenate. Note the weight of the homogenate together with the other data in a protocol and deep-freeze (5.6) the container.

NOTE: It has been recognized that differences in chlorinated hydrocarbon concentrations may exist between different muscles in large fish, therefore as much information as possible on the actual sample should be recorded.

This represents a "tissue sample".

NOTE: The concentration of chlorinated hydrocarbons in a composite sample should represent the mean value of chlorinated hydrocarbon concentrations of single specimens. In order to avoid overrepresentation of large specimens, the weight of the fillets of all specimens to be included in the composite sample should be reduced to that of the fillet of the smallest fish. As there might be considerable differences in the chlorinated hydrocarbons content of male and female specimens, use them in separate composite samples.

NOTE: Comparing the weight of the container with the homogenate at this point with the weight of the container before the analysis is carried out, the loss of moisture during long storage can be determined.

7.5 Sample preparation of large-size fish

If necessary, thaw partially, e.g. overnight, in a refrigerator (-2 to 4°C) the subsample taken in the field during sampling (6.4).

Rinse the subsample with ethanol (5.13) and place it on a clean working surface (5.3). Remove any skin and bone that may be present. Cut off thin slices from all surfaces with clean knives (5.9) and discard them. Repeat the operation with a second clean knife in order to obtain a clean uncontaminated block of homogeneous tissue.

Transfer the tissue into an airtight container (5.5), close it, label it, weigh it, note all data in the protocol and deep-freeze (5.6).

This represents a "tissue sample".

8. SAMPLING AND SAMPLE PREPARATION PROTOCOL

Fill in the sampling and sample preparation protocol (table 1) giving full details in every column. This protocol should be attached to the test report on the determination of chlorinated hydrocarbons in the analyzed sample.

The following guidelines should be kept in mind when completing the protocol (the numbers refer to those used in table 1):

1.1 Use the scientific name for the species samples. If necessary indicate subspecies or variety.

1.2 Indicate the name under which the species is known locally.

1.3 Use any code adopted by your institution. Never use the same sample code for more than one sample.

3.2 For samples obtained on fish market, indicate the town (village) where the market is. For samples taken at standard sampling stations or areas, indicate the name (code) of the station or area.

3.3 If the sampling point does not coincide with a standard sampling station or area, it may be advisable to code (name) it, in particular when the sampling point is used more frequently (e.g. a particular fish market). Never use the same sampling point code for more than one sampling point.

3.4 and 3.5 Always indicate the longitude and latitude of the sampling point to the nearest minute. For samples obtained from fish market, enquire about their provenience and try to reflect it also as geographic co-ordinates. Circle either E or W and N or S, as appropriate.

3.6 Give any additional information which may be relevant for the interpretation of the results (e.g., sampling point in vicinity of outfalls or similar).

4.1 Indicate the difference between data given under 2 and 5.

4.2 Mark the storage conditions used. If none of them are applicable, give additional explanations in 4.3.

6.2 Identify sex of the specimen whenever possible. As for specimen length, determine shell length for mussels, fork length for fish and total length for shrimp as indicated in figures 2, 3, and 4. Specimen weight always refers to the fresh weight of the whole mussel, of the whole shrimp and of the whole fish. Note that sample weight, in the case of mussels, refers to the total weight of soft tissues. In the case of shrimp, the sample weight refers only to the fresh weight of the muscle, and in the case of fish, to the fresh weight of the fillet or of the combined weight of fillets removed from the same fish.

6.3 Whenever possible use six or more specimens of the same sex and size (age) in preparing composite samples. Mean length and weight refers to the arithmetical mean of the weight and length of individual specimens, as explained above. Always calculate the standard deviations.

Table 1: Sampling and Sample Preparation Protocol

1. Sample (specimen)

   1.1 Scientific name:_____

   1.2 Common name:_____

   1.3 Sample code:_____

2. Date of sampling: day_____; month_____; year_____

3. Sampling point

   3.1 Country:_____

   3.2 Type of sampling point:_____fish market;

   _____sampling area/station

   3.3 Sampling point code:_____

   3.4 Longitude:_____ _____'E or W

   3.5 Latitude: _____ _____'N or S

   3.6 Conditions at sampling point which may be relevant for the interpretation of results:

   _____
   _____
   _____

4. Sampling storage

   4.1 Duration of storage:_____hours;_____days

   4.2 Storage: deep-freezing_____; cooling_____

   4.3 Factors relevant to sample storage which may be important for the interpretation of results:

   _____
   _____
   _____

5. Date of sample preparation: day____; month___; year___

6. Sample preparation

6.1 Tissue type (kind)_____

6.2 Single specimen sample: sex___; specimen length_____cm;

specimen weight_____g; sample weight_____g

6.3 Composite sample:

- number of specimens _____; sex _____

- mean length of specimens _____cm; stand. dev.___

- mean weight of specimens _____g; stand. dev. ___

- total weight of composite sample _____g

- total net weight of homogenate _____g

6.4 Factors relevant to sample preparation which may be important for the interpretation of results:___

_____

_____

_____

7. Full address of the institution carrying out the sampling and sample preparation:

_____

_____

_____

_____

_____

8. Name(s) and signature(s) of the person(s) who carried out the sample preparation:

_____

_____

Date:_____

APPENDIX
Specimen identification note

A standard specimen identification note should contain the following data:

- sample code (the same code should be used in 1.3 of the Sampling and Sample Preparation Protocol; see Table 1);
- Species name (important, in particular, whenever storage of sample may create difficulties in determining the species);
- sampling date;
- sampling location (given as sampling point code, if possible; see 3.3 of Table 1);
- collector's (sampler's) name.

Example:

IMW 239
Crassostrea irridescens
18 May 1991
Fish Market in Punta Arenas, C.R.
J. Sericano

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United Nations Environment Programme

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