Guidelines for monitoring chemical contaminants in the sea using marine organisms

Reference Methods For Marine Pollution Studies No. 6

Prepared in co-operation with

FAO  IOC  IAEA

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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 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:

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IAEA Marine Environment 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.

(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, 1984.


(3) UNEP/IAEA/IOC: Reference Methods and Materials: A Programme of comprehensive support for regional and global marine pollution assessments. UNEP 1990.
The present document was prepared at the initiative of FAO, the Food and Agriculture Organization of the United Nations as part of its contribution to the Regional Seas Programme and in particular the Mediterranean Action Plan. The assistance of Dr. G. Topping with this work is particularly appreciated. The document was subsequently edited at IAEA's Marine Environmental Laboratory and reviewed by GEMSI, the IOC/UNEP Group of Experts on Methods, Standards and Intercalibration. The assistance of all those who participated in this work is gratefully acknowledged.
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1. SCOPE AND FIELD OF APPLICATION

This publication provides guidelines for monitoring chemical contaminants in the sea using measurements in marine organisms. It describes strategies for applying such measurements to the protection of public health, the assessment of the geographical distribution of contaminants and the evaluation of time trends in contamination which in turn can demonstrate the effectiveness of measures designed to control potential sources of pollution.

2. REFERENCES

The following are useful publications to consult in relation to the design, planning and conduct of marine pollution monitoring programmes using marine organisms:


3. INTRODUCTION

Marine organisms can accumulate contaminants from seawater, suspended particulate matter, sediments and their food. It has also been demonstrated, through field observations and experimental studies, that the concentration of some contaminants in tissues are related to the concentrations in the surrounding environment. This process, termed bio-accumulation, has been used by scientists to assess the marine contamination which has been caused by man's activities (eg. marine disposal of wastes by pipeline discharges and dumping from ships).

There are however certain difficulties in using bio-accumulators, or bio-indicators as they are sometimes known, for this purpose. For example, individuals of the same species exposed to the same concentration of contaminants for the same period of time will not accumulate the substances at the same rate. This is related to such factors as age, sex, size and physiological state of the individual. Similarly, different species do not bio-accumulate to the same level when they are exposed to the same concentration of contaminant in sea water, and often have different rates of contaminant elimination.

Therefore, careful consideration must be given to the above factors when a monitoring programme is designed in order to reduce (or allow for) the effects of natural variability.

This document provides guidance on the design of such programmes and is intended for scientists who are responsible for marine pollution monitoring programmes. It is particularly aimed at programmes which fall under the auspices of the UNEP, IOC and FAO.

The guidelines presented in this report cover the following aspects of marine pollution monitoring programmes:

- aims
- pilot studies
- criteria for the selection of contaminants, organisms and locations to be studied
- size of sample
- frequency of sampling operations
- tissue selection.

Although an important component of these programmes is the analysis of contaminants in samples, this matter will not be addressed in detail in this document since other UNEP Reference Methods For Marine Pollution Studies cover this topic. Readers of this document are therefore advised to have the relevant analytical documents to hand (see UNEP/IOC/IAEA 1990); particularly "Contaminant monitoring programmes using marine organisms: Quality Assurance and Good Laboratory Practice" Reference Method No 57, since this deals with all aspects of work which influence the quality of data.
4. DEFINITIONS

Before discussing the programmes for which these guidelines may be used, it is necessary to define some of the more important terms which are used in this report.

<table>
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<th>Term</th>
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<tr>
<td>Accuracy, precision limit of detection</td>
<td>See definitions in Appendix 2 of Reference Method No 57.</td>
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<tr>
<td>Anthropogenic</td>
<td>Derived from human activity</td>
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<td>Contamination</td>
<td>in the context of the marine environment this term describes a situation where either the concentrations of some natural substances (eg. metals) are clearly above normal values, or the concentrations of man-made substances (eg. DDT) is detectable but which do not necessarily cause deleterious effects (referred to as pollution, see definition below).</td>
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<tr>
<td>Bio-indicator</td>
<td>A species which accumulates a contaminant in its tissue in amounts that are proportional to the levels of the contaminant in the local environment (ie. water, sediment and food).</td>
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<td>Hot spot</td>
<td>An area of the sea where there is a significantly high level of contamination</td>
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<td>Pollution</td>
<td>The Group of Experts on Scientific Aspects of Marine Pollution defines pollution as &quot;the introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) which results in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of seawater and reduction of amenities.&quot;</td>
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<tr>
<td>Monitoring</td>
<td>A programme of repeated measurements of contaminants in marine samples which is carried out for a specific purpose eg. annual measurements of mercury in the edible tissue of fish to provide information on the potential annual intake of mercury by consumers. A study of mercury in fish which examines levels in different species would not be classed as monitoring. If, however, this study was repeated in subsequent years these sets of data would be classed as 'monitoring data'.</td>
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5. AIMS OF MONITORING PROGRAMMES

There are three principal aims of monitoring programmes which involve the collection and analysis of marine organisms; they are:

- to compare contaminant levels in the edible tissues of marine organisms against national limits and to provide data to calculate the potential amount of contaminant taken in by consumers (ie Public Health monitoring).

- to compare the levels of contamination in different geographical areas (Spatial Monitoring). Such measurements are often made to assess whether the current discharges of wastes are producing unacceptable levels of contamination ie they are causing, or likely to cause, marine pollution problems.

- to measure the levels of contaminants over time at particular locations to judge whether they are changing in relation to the inputs of contaminants (ie Trend Monitoring). Such measurements are made to assess the efficiency of measures taken to reduce pollution.

Investigators should write down the specific aims of each monitoring programme before commencing any field measurements. These aims are needed to narrow the list of parameters, species and sites to be investigated. There are two distinct aspects of aims:

**Environmental management** - Are standards complied with? What is the spatial extent of contamination? What are the changes of levels with time in relation to changes in inputs of contaminants?

**Environmental science** - Statistical significance of differences in levels of contaminants - representative sampling of the population - selection of analytical methods with the required accuracy and precision.
6. PILOT STUDY

This assists the investigator in the design of an efficient monitoring programme for each specific aim. Provided a pilot study is carefully planned (see Appendix I for guidance), it can provide the following information:

a) In relation to public health studies, it can identify the relevant edible species, particularly the ones which contain elevated levels of regulated contaminants and therefore merit further investigation to determine the need for additional regulatory action, such as input controls or restriction on the harvesting or consumption of fish/shellfish.

b) It can identify which areas of the marine environment are sufficiently contaminated to warrant monitoring.

c) It can provide an indication of the variability of contaminant levels in individuals of the same species from the same population and location. This information is essential to an investigator wishing to establish a programme of trend monitoring. Without it, he may not be able to judge whether his sampling and analytical work will be sufficiently detailed to detect changes in contaminant levels with time against the natural fluctuations that may exist in any population of organisms.

d) It can identify which tissues of organisms, particularly fish and large shellfish, are the most appropriate ones to use in specific monitoring programmes since not all tissues reflect changes in the levels of contaminants in the environment to which the organism is exposed.

e) It can identify, and sometimes quantify, inputs of contaminants to the study area. This will help the investigator to select which contaminants should be given priority, if the resources for monitoring are limited, and in which areas contaminated organisms are likely to be found.

A pilot study can easily be expanded in order to accommodate measurements of biological effects. These effects may include changes in community structure and populations or adverse changes in the biochemistry of organisms (for example, acetyl cholinesterase depression by organophosphorus pesticides). Linkage of "levels" with "effects" is an important step in a complete pollution assessment. When effects are noted on a pilot scale, associated with specific contaminants or groups of contaminants, a strong case can be made for incorporating such contaminants in a full-scale monitoring programme and for taking immediate measures for their control and abatement. Details of some biological effects measurements are included in the Reference Method Series (see UNEP/IOC/IAEA, 1990).

Once a pilot study has been successfully completed, and the results evaluated, the investigator should prepare a protocol for each specific monitoring programme for the collection and analysis of samples. This protocol will specify what information is required to meet the specific aims, and the criteria to obtain the required quantity and quality of data. Time spent on the planning of a statistically significant sampling and analytical programme, will inevitably produce a more efficient programme which makes the best use of the laboratory's most important resource (ie staff time). Initially, it is generally sensible to conduct a programme which satisfies essential, rather than very ambitious, aims. It is relatively easy to expand this basic programme if extra resources become available. Finally, it is necessary to review the monitoring programme on a regular basis, to assess how well the aims are being met. This review may result in a reduction of effort on sampling and analyses, and the time gained can be usefully employed on other aspects of marine pollution studies. However, it might identify the need to put in more effort.
7. DESIGNING A MONITORING PROGRAMME

There are a number of factors to be considered in the planning of a monitoring programme which is to meet specific aims:

a) Which contaminants should be measured?
b) Which organism(s) should be selected?
c) Where should the samples be collected?
d) When should the sampling be done and how frequently should it be carried out?
e) How many individual organisms should be collected on each sampling occasion and which size(s) should be included in each sample?
f) Which tissue(s) of the organism(s) should be selected for analysis?

It is the principal investigator, together with a knowledgeable statistician and biologist, who will have to do this evaluation, design and plan the sampling work, prepare the necessary instruction sheets for the field staff, discuss with the analysts the precautions to be taken by staff in the storage and processing of samples prior to their analysis.

Specifically, the investigator will have to do the following:

(i) Design a sampling programme for the organisms of interest; selecting sufficient numbers, and sizes, of individuals at each site at appropriate intervals of time to take into account the inherent variability of contaminant levels in the organisms. This work will be done on the basis of the results obtained from the pilot study and any relevant information from other similar studies. Sampling must be designed to provide a statistically sound basis on which to judge changes in contaminant levels. Once this sampling programme has been designed, instruction sheets should be prepared and issued to the field staff.

(ii) Ensure that samples are collected, stored and transported to the laboratory in a way which minimizes losses and gains of contaminants prior to analysis. Guidance on this can be obtained by consulting the relevant documents in the UNEP Reference Methods series. Again it will be necessary to prepare instruction sheets for field and laboratory staff.

(iii) Arrange for the processed samples to be analyzed using methods which have the required accuracy and precision. Experience has shown that close collaboration between the principal investigator and the analysts is essential if this work is to be successful. The investigator and the principal analyst should consult the UNEP Reference Method No 57 which gives guidelines on Quality Assurance, if they are in any doubt about how to achieve and maintain the required quality of analytical data.

(iv) Ensure that there is an adequate system of documentation to allow samples to be traced from the time of collection to the recording of analytical data. The investigator should ensure that all relevant staff are aware of, and comply with, the system of documentation (see Appendix 2 for more details on this matter).

Each of the factors a - f will now be considered in more detail.
8. SELECTION OF CONTAMINANTS

The selection of substances to be monitored will be determined by a) the aims of the monitoring programme, b) the findings of the pilot study (ie which contaminants, present at significant levels above the background values, justify further study), and c) the ability of the analyst to measure these substances with the required accuracy and precision. In practice the last factor will often determine whether a particular contaminant or group of contaminants can be included in the monitoring programme.

It is essential that the principal investigator and the principal analyst agree to the required accuracy, precision and limit of detection for the measurements to ensure that the necessary standards of analysis are achieved eg. it would be inappropriate to consider measurements of specific changes in contaminant levels using an analytical method which had an inadequate level of precision.

If the analytical method used in the pilot study does not meet the required standard for the specific monitoring purposes, the analyst must select another method which meets the required standard. If for any reason this is not possible (eg. there is a statutory requirement to use a particular method) the investigator should abandon the proposed monitoring programme. Any other action will merely result in wasted effort, since the aims will not be met using an inadequate analytical method. However, it must be stressed that the use of an analytical method which, in theory, has the required performance characteristics to meet the aims does not necessarily guarantee success. Other factors have to be taken into account in obtaining the required quality of analytical data. These are discussed in some detail in "Quality Assurance and Good Laboratory Practice in relation to Marine Pollution Monitoring Programmes", UNEP Reference Method No 57. Investigators are strongly advised to obtain a copy of this document for analysts at the outset of the work.

In addition to selecting contaminants to meet the aims of the laboratory's marine pollution programme, it may be appropriate to include other contaminants which meet regional and international needs. This should only be considered if the additional data is useful to the laboratory, or if it is part of the laboratory's commitment to Regional Studies, and does not jeopardize the main aims of the laboratory's monitoring programme. A list of contaminants, identified by some organizations (International Council for the Exploration of the Seas, Oslo and Paris Commission's Joint Monitoring Group) for monitoring work in the North Sea and adjacent waters as well as those recommended (category I and II substances) for the MED POL programme are given, for information, in Appendix 3.

The final selection of contaminants should also be related to knowledge of their likely sources (eg. an extensive monitoring programme for pesticides along a desert coastline would be unwarranted) and information from scientific literature on their transport and persistence in the environment. Such information will also help to identify which environmental compartment should most usefully be monitored. As an example, organophosphorus pesticides are rapidly metabolized by many marine organisms but are rather persistent in sediments. It would be pointless to monitor them in biota but highly relevant to monitor their biological effects.
9. SELECTION OF ORGANISMS

9.1 Spatial and trend monitoring

Experience has shown that the most reliable data on contaminant trends in organisms are obtained by sampling organisms which have the following characteristics:

- A simple relationship exists between contaminant residues in the organisms and the average concentrations in the surrounding seawater or sediments.
- The organism accumulates the contaminant without being affected by the levels encountered.
- The organism is sedentary and thus representative of the area of collection.
- The organism is widespread in the study region, to allow comparisons between different areas.
- The organism is sufficiently long-lived, to allow sampling of more than one year class if desired.
- The organism is of a reasonable size, to give adequate tissue for analysis.
- The organism is easy to sample and robust enough to survive in the laboratory, allowing (if desired) depuration before analysis and, if needed, studies of uptake of contaminants.
- The organism exhibits high concentration factors, to allow direct analysis without pre-concentration.
- The organism is tolerant of brackish water, to allow comparisons to be made between estuarine and offshore sites.

These characteristics restrict the useful organisms to a range of fairly large, abundant, widespread, inter-tidal organisms, mainly molluscs. Filter-feeding molluscs are more likely to reflect contaminants in the water column, whilst deposit feeders will also be influenced by sediment chemistry. The working of the sediments both by organisms and water currents will cause an averaging of short-term variations in contaminant loading. Water chemistry, however, will more rapidly respond to effluent discharges and dispersal conditions at the time of sampling. Filter-feeders are therefore more likely to provide the information required to fulfill the objectives of a monitoring programme concerned with water quality. In Appendix 4, lists are given of organisms which some scientists in the United Kingdom have suggested may be used for monitoring a range of metals and organochlorine compounds in either rocky or muddy inter-tidal areas in UK waters.

In practice the selection of an organism, for monitoring purposes, is determined by its availability in the study area and its known ability to act as a bio-indicator. If this latter information is not known it must be obtained from either the scientific literature (e.g. Phillips 1980), or the pilot study. Final selection should be made in consultation with a knowledgeable biologist. Common mussels, (Mytilus edulis, M. californianus and M. galloprovincialis), that are used in global mussel watch programmes are generally suitable for spatial and trend monitoring programmes in coastal waters.
Other species of shellfish, and fish, can be used for spatial and trend monitoring purposes provided the organism can be shown to accumulate the specific contaminant(s) and that the concentrations of the contaminant(s) are in proportion to the concentrations in either water or sediment or food.

9.2 Public Health programmes

If the pilot study has revealed that edible species from the local fishery contain levels of contaminants which approach or exceed statutory limits for contaminants in foodstuffs, then these organisms should be included in any subsequent public health monitoring programme.

Since permissible limits of some contaminants (eg. Cd) in foodstuffs are extremely low, the analytical method for this work must be capable of producing the required data quality. A high degree of accuracy, and a detection limit which is ca 1/10 of the permissible concentration of the contaminant in the foodstuff, are essential for this work. These criteria enable the analyst to have confidence in the results that are provided to managers for regulatory purposes.
10. LOCATION OF SAMPLING SITES

10.1 Spatial and trend monitoring

Hot spots are usually found in estuarine and coastal areas where anthropogenic wastes are discharged. The offshore areas where hot spots are most likely to occur are those used for the dumping of wastes from ships or those in the vicinity of offshore oil platforms.

A decision to monitor contaminant levels in 'hot spots' should be taken only after careful consideration of the discharges to these areas. If, as a result of the pilot study, the relevant authorities decide to reduce inputs then it would be appropriate to monitor to judge whether the new controls have been effective in reducing levels in organisms. If no action is to be taken on the regulation of discharges then monitoring is only justified if there is a good reason to update the information collected in the pilot study.

Other estuarine, coastal and offshore sampling sites may be included in the programme to provide coverage of both clean and moderately contaminated areas. All sampling should be done by scientific personnel operating from research or chartered vessels, rather than by fishermen, to ensure that contamination of the samples during and after collection is kept within acceptable limits.

For long-term monitoring programmes, the precise locality of sampling sites should be registered as very small spatial variation may strongly influence the final data (i.e., "mussels from the harbour wall" should specify which point in the harbour wall). In some cases it may be useful to photograph the sites, particularly where intertidal organisms are taken.

10.2 Public Health Programmes

In some countries there may be officials who are knowledgeable about the edible species of fish and shellfish caught by commercial fishermen. Investigators may find it helpful to discuss their proposed monitoring programme with such officials since they can often offer valuable advice in the design of the collection programmes.

Samples of fish and shellfish may be obtained from the fish markets or from fishing vessels or research ships which are operating in traditional fishing areas. The basic requirement is a representative sample of the species normally consumed by the general public. It should be noted, however, that some countries may specify the exact sampling procedures for public health monitoring.

Commercial fishermen do not usually take any special precautions during the collection, storage, transport and off-loading of their catches, other than to ensure that they are presentable enough for sale. The retailer and the consumer do not normally adopt any stringent dissection procedures, other than from a public health viewpoint. The scientist, however, will use careful sampling and pre-treatment procedures to ensure that contamination is kept within acceptable limits. These different approaches to sampling may lead to differences in the amount of contaminants found in the samples. In general the scientific samples will be less contaminated than those taken from fish markets, fishing boats and fish retailers.

The final decision on where and how to collect samples for public health monitoring will depend on whether information is required on actual contaminant intake by the consumer (in which case samples will be taken from the fish markets or fish retailers) or whether the aim is to determine which edible species and areas are exposed to contamination (in which case the sampling must be done by scientific staff).
11. PERIOD AND FREQUENCY OF SAMPLING

11.1 Spatial and trend monitoring

For spatial monitoring, collections should be made over a short interval of time (within weeks rather than months) to enable a synoptic comparison of concentrations of contaminants at different sites. This also helps to ensure that organisms are in the same physiological state. If major annual changes in the quantity and/or composition of inputs are anticipated it would be appropriate to conduct an annual or biennial sampling. Experience has shown that the effects of changes in inputs of contaminants are often confined to the area in the immediate vicinity of the discharge. It is these areas where more frequent monitoring should be conducted.

For trend monitoring, the frequency of sampling will a) reflect the time scales over which the changes are required to be detected, b) the degree of confidence required in the measurement of these changes, and c) the available laboratory resources. Investigators should note that there is nothing more frustrating and time-wasting than a programme in which the proposed work is well below the minimum standard required to detect the desired changes in contaminant levels. If, for any reason, the resources are insufficient to meet the specific aims of the programme, then the programme should be canceled and replaced with one which has less ambitious aims but which can be carried out successfully with available resources.

If no changes in inputs are expected, then it would be sensible to restrict sampling to ca 5 yearly intervals. A more frequent sampling programme can only be justified if there is a need to provide more regular data for other purposes eg. to reassure the general public that levels of contaminants are not changing.

Seasonal variations in food supply, and the spawning cycle, are known to cause changes in total body weight, as well as lipid concentration and composition and, these may influence contaminant levels in the tissues of some organisms. In order to minimize these variations, it is suggested that sampling be undertaken at the pre-spawning period.

11.2 Public Health monitoring

Unless there is a seasonal fishing pattern for some species, samples may be taken at any time of the year. Ideally all species should be sampled at the same time so that a synoptic picture of the contaminant levels can be obtained. A typical monitoring programme might consist of a survey every 5 years. A more frequent sampling programme (ie annual) will be needed if the results of the pilot programme show that concentrations of contaminants in foodstuffs approach or exceed permissible limits for foodstuffs. Increased sampling should be confined to the particular species and contaminants which give cause for concern.
12. SIZE OF SAMPLE

12.1 Spatial and trend monitoring

Ideally, the investigator will have established the relationship between contaminant levels and size of organisms from the results of the pilot study. It is good practice to select a particular size or size range to minimize the variance of contaminant levels from sample to sample. The number of individuals required for each sample will be determined by the magnitude of the change that is considered to be significant in relation to the specific aims. The smaller the difference the greater the number of individuals required for each sample. (See Appendix 5 for further guidance)

If the relationship between size of organism and contaminant level has not been obtained from the pilot study then a sufficient number of individuals should be collected at one of the sampling sites to cover the size range of organisms in the population, to establish the variability of contaminant levels with size. This is a minimum requirement since, ideally, this sampling procedure should be done at all sites. The information on variability at one site will allow the investigator to make comparisons with other sites where individuals of a limited size range are collected.

If either analytical resources or sample material is limited it may not be practical for the individuals from each site to be analyzed separately. In this case, individuals should be combined to make one sample (often referred to as 'pooled' samples). For 'pooled samples', no information will be obtained on the variation of contaminant levels with size but the data can be used to assess site to site differences with some level of confidence, provided that a number of replicate analyses are done on each of the 'pooled' samples, and the 'pooled' samples consist of individuals from the same size range.

12.2 Public Health monitoring

The size(s) of organisms to be sampled should be based on information on consumption patterns. If a range of sizes is sold, then these different sizes should be analyzed. The number of individual organisms in each sample will be influenced by the importance of the species as a foodstuff, the availability of scientific manpower and the need to sample sufficient numbers of each species and of each size category to cover the range of values encountered in a typical population or catch. Generally, a sample of 5-10 individuals from each size range of fish and large shellfish (crabs, lobsters) and ca 50 individuals for smaller shellfish (eg. mussels, shrimps) would be sufficient.
13. SELECTION OF TISSUE

13.1 Spatial and trend monitoring

For invertebrates, whole soft tissue (less viscera) should be taken for analysis.

For fish, muscle is the most useful tissue for most purposes. However, liver and kidney tissues have been used for studies of fish and the digestive gland of large crustaceans. In general, whole soft tissue is taken for smaller shellfish.

13.2 Public Health monitoring

Only edible tissue need be analyzed for contaminants - usually this means muscle tissue for fish and large crustaceans and whole soft tissue (less viscera, ie guts, gills and gonad) for small shellfish.

Every opportunity should be taken to collect data on the size (or length) and age of the species. This may be relevant to subsequent decisions on regulatory action.

13.3 Normalization procedures

It is usual to report all tissue data on a dry weight basis (ie. g contaminant/g (dry weight)). However, some literature values use wet weight which may be required for public health studies. Since drying is a common part of most analytical protocols (see RM. No. 7 "Sampling of selected marine organisms and sample preparation for trace metal analysis" and RM. No. 12 "Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons"), the reader is advised to record wet/dry weight ratios on a routine basis.

In the case of lipophilic contaminants, such as chlorinated hydrocarbons, contaminant concentrations are often expressed in terms of g contaminant/g HEOM (where HEOM is Hexane Extractable Organic Matter, principally lipid). This procedure enables a certain degree of normalization for seasonal or spatial variations in the lipid content of sentinel organisms and facilitates the comparability of data.
Appendix 1

GUIDANCE ON THE PLANNING OF A PILOT STUDY

Desk Study

It is important to determine what is known about contaminants in the proposed study area, before any field work is done. Some of this information can be found by reviewing the relevant scientific journals and other published material (e.g. books, conference proceedings).

Annual reports of other marine institutes, local and central government and industrial research laboratories are also useful sources of data, as are unpublished scientific reports from these organizations. If these latter sources provide useful data, it is good practice to contact scientists from the relevant organizations to identify whether there is any other unpublished data or information, which might be useful to the investigator.

This review can often provide data on the current levels of contamination in water, sediments or biota and occasionally information on inputs of contaminants to the area via rivers, pipelines or dumping from ships. It may also reveal the type of industry and agriculture located in the coastal region, the range and scale of potentially toxic substances used by them, and possibly information on their discharges to the rivers and sea. These latter data should be verified by contacting the local or national authority, which has responsibility for regulating discharges to rivers and coastal waters. This authority should also be approached for information on the past and present discharges to the area.

For public health work, the investigator should identify which fish and shellfish species are caught for human consumption, and whether there are relevant permissible limits for contaminants in marine foodstuffs. Information on commercial catches can be obtained from either the local fishermen or their representative organizations or the local or central government fisheries department. Information on food standards can be obtained from the local environmental health department or the central government department responsible for food safety. It is difficult to be more specific about the exact sources of the above information in each country since they do vary from country to country.

This review should enable the principal investigator to identify the group of contaminants, and specific fish and shellfish, which should be given priority in the pilot study for public health purposes. It will also give some general guidance on the species to be selected for spatial and trend monitoring purposes. However, before the principal investigator can plan this latter work he needs to do some additional desk work to identify the locations where samples should be collected.

Identifying sampling sites

It is essential that the pilot study covers the areas which are likely to be contaminated and the areas which, from a hydrographic and input viewpoint, are unlikely to be significantly affected (ie sites located well offshore from industrialized areas or those located in inshore areas next to less populated and industrialized areas).
The level and extent of contamination in coastal and estuarine waters is determined by:

- the rate of input of contaminants
- the location of the individual inputs
- the composition of the waste - whether the contaminants are in solution, attached to solids or associated with mixtures of solid and liquid
- the dilution and dispersion of wastes following discharge, and in the case of discharges containing solids, the settlement of solid material to the sea bed sediments
- the physical and chemical processes in the sea (ie adsorption and desorption of substances between dissolved and particulate phases of seawater).

Unless the principal investigator has a good working knowledge of hydrography of the local area, it will be necessary to seek the help of an hydrographic expert to determine the optimum locations for sampling in relation to known inputs.

Assuming the principal investigator can provide the hydrographer with the relevant information on inputs, and that his colleague has a good understanding of the hydrographic characteristics of the area (direction, speed and variability of currents, salinity and temperature of the water masses, and the freshwater flows to the sea) it should be possible to calculate the theoretical dilution and dispersion of wastes at estuarine and coastal sites. This information can then be used to identify the locations where organisms are exposed to contamination and the adjacent areas where they will probably not be subject to contamination (ie clean or control areas).

If expert hydrographic advice is not available, the principal investigator should establish a sampling grid along the likely gradient of contamination; with sampling sites located at progressively increasing distances from the input (100m, 300m, 1000m, 3000m etc.). If a river is the principal source of contamination to the study area, the investigator can establish his sampling grid along the salinity gradient. It is relatively easy to calculate the dilution of river water, and the corresponding dilution of contaminants, by measuring the salinity at locations in an estuary and comparing these measurements with the salinity values of the water entering the estuary. For this calculation, the investigator assumes that river water has zero salinity and that the contaminants behave conservatively during mixing of freshwater and seawater.

Sample size

The concentration of some contaminants can vary with size of the organisms. It is important in spatial and trend monitoring to reduce this source of variability in the data to detect differences in contaminant levels between sites and with time (see Appendix 5). If this relationship is not known by the investigator prior to the commencement of monitoring, it will be necessary to establish it during the pilot study.
To do this, the investigator must collect a representative sample of each population of species at each sampling site. This sample should include sufficient numbers of individuals to cover the range of sizes/ages/lengths of individuals in each population. The investigator should consult a knowledgeable biologist for guidance on the range of sizes that might be expected for each species.

Selection of tissue

Although there is considerable scientific literature on the accumulation of contaminants by different tissues (eg. Phillips, 1980), it is advisable for the investigator to check this aspect for the specific organisms to be examined in the pilot study. It is also advisable to consult a biologist to determine the best procedure for dissection of organisms into their constituent parts, to ensure that there is no possibility of one tissue being contaminated by another.

Ideally, the investigator should investigate the relationship between the contaminant level, tissue and size of organism by analyzing tissue from individuals of different sizes rather than by analyzing pooled samples; even if the latter consist of a number of individuals of the same size or size range. However, if analytical resources are limited, it may be necessary for him to establish this relationship by analyzing pooled samples.
Appendix 2

DOCUMENTATION OF DATA

The adoption of the following guidelines by a laboratory should provide adequate documentation to allow it to trace samples from the collection stage to the completion of its analyses by providing a record of the appropriate data in logbooks or in computer files.

Documentation

(i) Descriptions of the sampling strategy, methods of sample collection, procedures for storage, and pre-treatment and analytical procedures, plus a list of ancillary site observations;

(ii) Sample documentation (description of organisms, numbers of individuals collected for each sample, weights of tissue taken for analysis (individual tissue or homogenate) plus ancillary data on organisms (length, weight and age);

(iii) Description of analytical procedures, including details of accuracy, precision and limit of detection;

(iv) Description of quality control and quality assessment and evidence that these procedures have been applied and have provided acceptable data;

(v) Description of working standards used on each occasion and calculations of results;

(vi) A secure system for the long term storage of data either in logbooks or computer files is essential. It is also advisable to have a duplicate set of records in case one is lost, mislaid or accidentally destroyed;

Advice should be sought on the correct method of storing computer tapes and/or discs to ensure the long-term stability of data files.

Storage of data

It has been shown that even the most experienced personnel can make simple arithmetic errors in calculating results. Thus, a check should be made for such errors before compiling tables of results. Once this check has been made it is appropriate to carry out a preliminary assessment of the quality of the data, prior to its evaluation and publication, to ensure that no erroneous results are included. This assessment can include a comparison of the results with existing data (i.e. data for the study area either previously collected by the laboratory or data published in the literature). Before consigning data to long term storage, a final check should be made to ensure that no errors have been made in transcribing the data (i.e. the re-typing of data sets by typists or data processors can sometimes lead to such errors).
Appendix 3

EXAMPLES OF CHEMICAL SUBSTANCES MEASURED IN MARINE ORGANISMS FOR MONITORING PURPOSES
(SOURCE:

Trace metals
Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Mercury (Hg), Nickel (Ni), Tin (Sn), and Zinc (Zn).

DDT and its metabolites
o,p'-DDD, p,p'-DDD, o,p'-DDE, o,p'-DDT, and p,p'-DDT.

Chlorinated pesticides other than DDT
Aldrin, Alpha-Chlordane, Trans-Nonachlor, Dieldrin, Heptachlor, Heptachlor epoxide, Hexachlorobenzene, Lindane (gamma-BHC), and Mirex (+ Endosulfan ?)

Polychlorinated biphenyls (PCBs)
Measurements are usually restricted to either a small number of individual compounds (known as congeners) or to the total concentration of PCBs.

Polyaromatic hydrocarbons
These can include:

2-ring compounds  Naphthalene, 1-Methylnaphthalene, 2-Methylnaphthalene, 2,6-Dimethylnaphthalene, and Acenaphthene.
3-ring compounds  Fluorene, Phenanthrene, 1-Methylphenanthrene and Anthraccene.
4-ring compounds  Fluoranthrene, Pyrene, and Benz(a)anthracene
5-ring compounds  Chrysene, Benzo(a)pyrene, Benzo(e)pyrene, and Dibenzo(a,h)anthracene.

For the purposes of the Long-term programme for pollution monitoring and research in the Mediterranean sea (MED POL - Phase II) the following chemical contaminants were identified for analysis in marine organisms.

category I (mandatory)  category II (optional)
total mercury  total arsenic
organic mercury  radionuclides
cadmium  polynuclear aromatic hydrocarbons
halogenated hydrocarbons
Appendix 4

A. LIST OF MED-POL SPECIES

For the purposes of the Long-term programme for pollution monitoring and research in the Mediterranean sea (MED POL - Phase II) the following species (nearly all edible), representing different ecotypes, are recommended for the monitoring of chemical contaminants in marine organisms.

a) Bivalves

   *Mytilus galloprovincialis*, or
   *Mytilus edulis*, or

b) Demersal fish

   *Perna perna*, or
   *Donax trunculus*

*M. edulis*, *P. perna* or *D. trunculus* can only be monitored as alternative species if *Mytilus galloprovincialis* does not occur in the area.

   *Mullus barbatus*, or
   *Mullus surmuletus*, or
   *Upeneus molluccensis*

*M. surmuletus* or *U. molluccensis* can only be monitored as alternative species if *Mullus barbatus* does not occur in the area.

c) Pelagic carnivore fish

   *Thunnus thynnus*, or
   *Thunnus alalunga*, or
   *Xiphias gladius*

d) Pelagic plankton feeding fish

   *Sardina pilchardus*
   Other clupeids should only be monitored as alternative species if *S. pilchardus* does not occur in the area.

e) Crustaceans

   *Parapenaeus longirostris*, or
   *Nephrops norvegicus*, or
   *Penaeus kerathurus*

*N. norvegicus* or *P. kerathurus* can only be monitored as alternative species if *P. longirostris* does not occur in the area.
### B. LIST OF POSSIBLE ORGANISMS FOR THE ASSESSMENT OF CONTAMINATION IN THE NORTH ATLANTIC REGION

<table>
<thead>
<tr>
<th>ROCKY SUBSTRATE</th>
<th>Cd</th>
<th>Hg</th>
<th>Cu</th>
<th>Cr</th>
<th>Pb</th>
<th>Zn</th>
<th>HH</th>
<th>PHC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mytilus edulis</em> (common mussel)</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Littorina littorea</em> (gastropod)</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Patella vulgata</em> (limpet, gastropod)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

### MUDDY SUBSTRATE

<table>
<thead>
<tr>
<th>MUDDY SUBSTRATE</th>
<th>Cd</th>
<th>Hg</th>
<th>Cu</th>
<th>Cr</th>
<th>Pb</th>
<th>Zn</th>
<th>HH</th>
<th>PHC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Scrobicularia plana</em> (da Costa) (peppery furrow bivalve)</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Macoma balthica</em> (bivalve)</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Nereis diversicolor</em> (annelid)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Key:**
- + = appears to act as good indicator
- ? = doubt about use as indicator
- HH = halogenated hydrocarbons
- PHC = petroleum hydrocarbons

**NOTES:** The organisms listed for muddy substrates are all deposit feeders, whilst those for rocky substrates are filter feeders or herbivores. It is unlikely that contaminant levels in the tissues of the two groups will reflect contaminat levels in the same part of the marine environment.