REGIONAL SEAS

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

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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 present Reference Method is designed to provide a set of guidelines to assist specialists in pollution and public health to design and implement monitoring programmes based upon the measurement of chemical contaminants in marine biota. It is strongly recommended as essential reading material prior to organizing a monitoring programme. It forms the basis of the strategy currently employed by UNEP's Regional Seas Programme, the GIPME programme (Global Investigations of Marine Pollution, jointly sponsored by IOC and UNEP) and of several programmes organized by FAO (particularly in their contribution to the MEDPOL programme).

The production of this method was coordinated by FAO (at the Coordinating Unit for the Mediterranean Action Plan). The original draft was prepared by Dr. Graham Topping, The Scottish Office Agriculture and Fisheries Department Marine Laboratory, Aberdeen, Scotland, together with substantial contributions from Dr. Jack Uthe, Marine Chemistry Division, Physical and Chemical Sciences Branch, Department of Fisheries and Oceans, Halifax, Canada. The excellent work of these authors is gratefully acknowledged. The manual was reviewed and revised by the IOC/UNEP Group of Experts on Methods, Standards and Intercalibration, GEMSI. Final editing was performed at MESL, Monaco.

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GUIDELINES FOR MONITORING CHEMICAL CONTAMINANTS IN THE SEA USING MARINE ORGANISMS

1. SCOPE AND FIELD OF APPLICATION

This manual provides guidelines on the use of chemical contaminant measurements in marine organisms for *environmental trend monitoring programmes and public health monitoring programmes:* It describes the steps required for planning and implementing these monitoring programmes from a strategic and practical point of view. Information in this manual corresponds to the basic strategy currently applied in the regional monitoring programmes of the United Nations bodies responsible for the production of this document.

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3. INTRODUCTION

Organisms accumulate many contaminants from their environment (i.e., from seawater, suspended particulate matter, sediments, and food). Field and laboratory studies have shown that contaminant concentrations in some marine plants and animals reflect concentrations in their environment. Scientists use this process (termed bio-accumulation) to assess marine contamination resulting from human activity (e.g., pipeline discharges, dumping from ships).

There are problems with using biota as bio-accumulators (bio-indicators). For example, individuals of a species exposed to the same contaminant concentration contain different contaminant concentrations after the same exposure time. These deviations reflect individual differences in factors such as age, sex, size, and physiological and nutritional states. Also, various species show different contaminant concentrations following identical exposure: differences in elimination rates may partially account for this.

These factors must be considered when planning a monitoring programme in order to control their effects on contaminant concentrations (reduce the variances). Variance reduction is necessary to detect smaller differences in mean contaminant concentrations in monitoring programmes.

Many other problems affect the success of monitoring programmes (NAS 1990, Uthe et al. 1991). Uthe et al. described a monitoring programme as a series of operational steps: from planning and design; through sample collection, measurement, data handling,

and data analysis; to interpretation (translating data into information for managers). Problems at any step can drastically affect the success of the programme. Each step of a monitoring programme requires quality management to ensure production of high quality data (Uthe and Chou [1988] give examples of problems at various steps in monitoring programmes for trace metals).

Uthe et al. (1991) also noted: 1. Once a protocol (a set of rules for a procedure) is selected, it must be rigorously followed; and, 2. Programmes must be designed for efficient statistical analysis.

This document provides guidance on programme design to scientists who manage marine pollution monitoring programmes, specifically those supported by the UNEP, IOC, and FAO. NAS (1990) discusses marine environmental monitoring in depth and is recommended to the readers of this text.

The guidelines in this report cover the following aspects with respect to ***Environmental Trend Monitoring Programmes*** and ***Public Health Monitoring Programmes***:

1. Aims of monitoring programmes, preparatory information, and training;
2. Pilot studies, planning, execution, and interpretation;
3. Designing a monitoring programme from the pilot study;
4. Selection of contaminants;
5. Selection of species;
6. Location of sampling sites;
7. Period and frequency of sampling;
8. Size of sample;
9. Selection of tissue; and,
10. Storage and preparation of tissue homogenates for analysis.

Although measurement, in particular the measurement of contaminants, is important, it will not be addressed here because other UNEP Reference Methods For Marine Pollution Studies deal with it. Readers should read relevant analytical documents (*e.g., UNEP/IOC/IAEA 1990* "Contaminant Monitoring Programmes Using Marine Organisms: Quality Assurance and Good Laboratory Practice" Reference Method No 57, Uthe et al. 1991a), which deal with all aspects of work that influence data quality.

4. DEFINITIONS

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

TERM	DEFINITION
Accuracy, precision, limit of detection	See definitions in Appendix 2 of Reference Method No 57.
Anthropogenic	Derived from human activity.
Bio-indicator, Bio-accumulator	A species that accumulates a contaminant in its tissues in amounts proportional to the concentrations of the contaminant in the local environment (i.e., in its water, sediment, and food).
Compliance	A judgement of acceptability related to regulations (e.g., of a fishery product with respect to its contaminant content) using data collected by surveillance programmes. For example, in statistical terms, compliance may be judged against the value where a 5% probability is allowed of being wrong (i.e., accepting a lot with a true value above the regulated limit - see ISO/REMCO [1993]).
Contamination	In the marine environment, a situation where either the concentrations of some natural substances (e.g., metals) are clearly above normal values, or the concentrations of man-made substances (e.g., p,p'-DDT) are found, *but which do not necessarily cause deleterious effects (called "pollution", see definition below).*
Depuration	Holding living specimens in clean water to remove unwanted material from them (e.g., pseudofaeces in mussels).
Hot spot	An area of the sea where there is a significantly elevated level of contamination.
Monitoring	A programme of repeated measurements of contaminants in marine samples to determine spatial distributions or temporal trends in contaminant concentrations.

Pilot Study — Measurement of contaminants in marine samples from an area not previously studied to investigate the current levels of contamination. This is a prerequisite to a monitoring programme because the information collected enables the investigator to design a sampling programme suited to the specific aims of the programme. Without such information the investigator may be unable to judge which contaminants, species, and locations to select for the monitoring programme.

Pollution — The Group of Experts on Scientific Aspects of Marine Pollution defines pollution as "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."

Quality Assurance — All procedures that a laboratory carries out to ensure that it produces data of the appropriate quality to meet the defined aims of its monitoring programme. Quality Assurance essentially has two elements - quality control and quality assessment. These last two terms are defined in UNEP Reference Method No 57.

Sample — A collection of a predetermined number of specimens of the same species simultaneously from one site.

Specimen — One unit (individual, tissue) selected to fulfil a sample requirement.

Surveillance — A programme designed to survey contaminant concentrations in edible fish tissues upon which to judge compliance related to national standards. Certain surveillance programs are also monitoring programmes.

5. ENVIRONMENTAL TREND MONITORING PROGRAMS

5.1 AIMS OF MONITORING PROGRAMMES, INCLUDING PREPARATORY INFORMATION AND TRAINING

The two principal aims of environmental monitoring programmes involving the collection and analysis of marine organisms are to:

1. Measure contaminant concentrations over time at selected locations to decide if they are changing in relation to their inputs (i.e., Trend Monitoring). Such measurements help to assess the efficiency of pollution controls; and,

2. Compare contaminant concentrations in different geographical areas (Spatial Monitoring). Such measurements help determine if current waste discharges are producing unacceptable contamination concentrations (i.e., they are causing, or likely to cause, marine pollution problems).

Investigators must write down specific aims for each monitoring programme before starting field studies. These aims help limit the number of parameters, species, and sites to be investigated. They also determine the resources required for the programme. There are two distinct aspects of aims:

Environmental management - Are standards complied with? What is the spatial area of contamination? What are the changes of concentrations with time in relation to changes in contaminant inputs?

Environmental science - Statistical significance of differences in contaminant concentrations - representative sampling of the population -selection of analytical methods with the required accuracy and precision.

The importance of developing realistic and specific aims (goals) for a monitoring programme cannot be stressed too highly. For example, a trend monitoring programme that can only detect a linear trend of 20% a year is pointless in an area where the expected rate is well below this. All goals must be defined within a statistically sound framework.

The first steps in developing aims are to select contaminants and species of interest in the study area and to find out what is known about them in the literature. (See Item 4. Pilot Study.) The Project Manger must choose all required biological and chemical measurements, train the individuals making these measurements, and develop a thorough quality management framework to ensure comprehensive quality assurance for all steps in the programme. This may require training in other procedures besides chemical analysis (e.g., preparing adequate and clear sample site descriptions, species identification, biological measurements [e.g., length, weight, sex], frozen sample handling, number handling, statistical analysis), along with quality control for each.

5.2 PILOT STUDY

A pilot study is simply an initial study designed to give the head investigator enough information with which to develop environmental monitoring programmes. Usually, the initial pilot study will survey contaminants of concern within the study area (country) using a common species. Mollusca, in particular bivalves, are species of choice. This study may be combined with other pilot studies (e.g., estimate specimen-to-specimen variances for the contaminants, identify sample handling and storage problems, measure contaminant concentrations in different tissues). These help the investigator design specific aims and develop an efficient monitoring programme. An efficient pilot study (see Appendix 1 for guidance) can provide the following information:

1. It can identify contaminated areas in the marine environment requiring monitoring;

2. It can estimate the variation in contaminant concentrations in specimens of the same species from the same location (population). Planning a trend monitoring programme requires this information. This requires a special sampling project at one or more sample sites (e.g., a control and contaminated area). Without it, the statistician cannot judge whether the sampling and analytical work will be able to detect changes in contaminant concentrations with time against this natural variation;

3. It can identify the best tissue to use in monitoring programmes. Usually all tissues do not reflect changes in environmental contaminant concentrations; and,

4. It may identify local areas of elevated contamination in the study area (hot spots). This will help the investigator rank areas and contaminants for action and study.

A pilot study also might include some biological effect measurements. This may be as simple as recording certain weights (e.g., whole animal, soft tissue weights), or as complicated as determining changes in community structure and populations. Linking contaminant concentrations with biological effects is an important step in a complete pollution assessment. Such a linkage makes a strong case for incorporating these contaminants within a monitoring programme and for taking immediate measures to regulate discharges. The Reference Method Series (see UNEP/IOC/IAEA, 1990) describes some biological effect procedures.

After the pilot study, the investigator must prepare detailed instructions for specimen collection, storage, and analysis for each monitoring programme. These will define the information required to meet each specific aim (including its statistical requirements). Time spent on planning specific, efficient programmes will ensure the best use of a laboratory's resources and the highest chance of success. Priorities must be decided. It is usually better to conduct a small programme with a specific, high priority focus, specific aims, and clear goals (benchmarks). Goals are required for regularly assessing the monitoring programme and deciding its future. This may result in a

recommendation to cancel the programme, reduce its effort, or increase its funding and effort.

5.3 DESIGNING A MONITORING PROGRAMME

There are several items to be considered in the detailed planning of an environmental monitoring programme:

1. Which contaminants are to be measured?

2. Which species are to be selected?

3. Where are the samples to be collected?

4. When is sampling to be done and how frequently?

5. How many specimens (of what size) are to be taken for each sample?

6. Which tissue(s) are to be taken for analysis?

The head investigator, field biologist, analytical chemist, and statistician must consider each question during the detailed designing and planning of the monitoring programme (e.g., prepare sampling instructions, estimate workload, prepare the necessary instruction sheets for the field staff, develop safeguards to ensure sample integrity, develop analytical quality control needs and data handling methods). All participants in the study must be fully trained in their roles within the programme and recognize how their efforts unite with those of the other participants. The investigator must:

1. Design a sampling programme: select sufficient numbers of specimens with the required biological attribute (usually size, but others, e.g., sex may be used); define sample sites; specify sampling frequency and period; and design sample handling methods. This is based on the results of the pilot study and any other relevant information. A statistically sound base is required for sample design. Once the sampling programme design is complete, instruction sheets (sampling instructions) must be issued to field staff. Training is probably required at this point to ensure consistent sample collection and handling;

2. See that samples are collected, stored, and transported in a way that ensures sample integrity (i.e., sample continuity [see point 4], no loss and gain of contaminants before analysis). Advice on this can be found in the UNEP Reference Methods series. Again it will be necessary to prepare instruction sheets for field and laboratory staff.

3. Arrange for sample processing and storage (Uthe and Chou [1988] have described problems encountered in sample handling) and arrange for sample analysis by methods

with the required accuracy, precision, and lower limits of detection. Collaboration between the head investigator and all participants is essential for success. The investigator and the analyst should consult UNEP Reference Method No 57, which gives directions on Quality Assurance, if they are in doubt how to achieve and maintain analytical data quality; and,

4. Ensure that there is a proper documentation system for tracing (tracking) samples from sampling to final data recording. The investigator must make sure that all team members are aware of, and comply with, the documentation system (see Appendix 2 for more details on this matter).

5.4 SELECTION OF CONTAMINANTS

The contaminants to be studied are determined by: 1. The aims of the monitoring programme; 2. The findings of the pilot study; and, 3. The analyst's ability to measure these contaminants with the required accuracy and precision. In practice the last factor dictates which contaminants can be included.

It is necessary that the statistician specify the accuracy, precision, and limits of detection for all measurements. This defines the necessary standards of analysis needed to detect changes in contaminant concentrations. It is pointless to consider monitoring contaminant concentrations using an analytical method with an inadequate level of precision, accuracy, or lower limit of detection.

If the analytical methods do not meet the required standards, the analyst must either set up methods that meet these standards or abandon the programme to prevent waste. However, an analytical method that has the theoretical performance to meet the aims does not guarantee success. It must be proven within the analyst's laboratory. Also, other factors have to be considered in obtaining the required analytical data quality. These are discussed in "Quality Assurance and Good Laboratory Practice in Relation to Marine Pollution Monitoring Programmes," UNEP Reference Method No 57. Again, investigators should obtain a copy for analysts at the start of the work.

It is obvious that the principal investigator also must ensure that all other techniques associated with analysis are of the required quality (e.g., species selection and other biological measurements, sample handling, including dissection and storage) to yield high quality. They must be proven along with the analytical methods discussed above.

It may be appropriate to measure other contaminants that meet regional and international needs. This should be considered only if additional data are useful to the laboratory or part of Regional Studies, and does not impede the laboratory's main aims. Appendix 3 lists contaminants, identified by the International Council for the Exploration of the Seas and the Joint Monitoring Group of the Oslo and Paris Commission, for monitoring work in the North Sea and adjacent waters, or recommended for the MED

POL programme (Category I and II substances). Also, certain chemical methods and instruments measure additional contaminants at small incremental cost.

The final selection of core contaminants should be related to knowledge of their sources and inputs to the study area and their transport and persistence. Two examples: 1. An extensive monitoring programme for pesticides along a desert coastline would be unwarranted; and, 2. Many organophosphorus pesticides persist in sediments, but are rapidly metabolized by organisms. It would be pointless to measure them in biota, but important to analyse sediments when studying pollution (biological effects) by them.

5.5 SPECIES SELECTION

5.5.1 Spatial and trend monitoring

Sampling species with the following attributes yields the most reliable data on contaminant trends in organisms (Butler et al. 1971, Haug et al. 1974):

1. A simple relationship exists between contaminant concentrations in the species and average concentrations in the surrounding environment;

2. The species accumulates the contaminant without being affected by the concentrations encountered in its environment (other than in polluted areas);

3. The species is sedentary and, thus, represents the collection area;

4. The species is widespread and abundant in the study region, to allow comparisons among different areas;

5. The species lives long enough so that more than one year-class can be sampled, if desired;

6. The species is large enough to yield sufficient tissue for analysis;

7. The species is easy to collect and hardy enough to survive unfavourable conditions (e.g., harbours) or within the laboratory (e.g., for depuration before analysis, for studies of contaminant dynamics).

8. The species exhibits high bio-accumulation factors, to allow analysis without preconcentration.

9. The species tolerates brackish water, to allow comparisons between estuarine and offshore sites. However, offshore animals may show surprisingly high contaminant concentrations and burdens (total amount of contaminant in the animal or tissue); and,

10. The species must be easy to identify with certainty (Phillips et al. 1980). This was a problem in some early programmes where *Mytilus trossulus* was an unrecognized species in sample collections (McDonald and Koehn 1988).

These attributes tend to limit the useful species to a group of moderately large, abundant, widespread, well-studied, inter-tidal species, mainly mollusca. Filter-feeding mollusca tend to reflect contaminants in the water column: deposit feeders are also influenced by sedimentary contamination. Sediment working both by organisms and water currents will average short-term variations in contaminant loading. Appendix 4 lists species that some scientists suggest for use in monitoring various metallic and organochlorine contaminants in rocky and muddy intertidal areas in United Kingdom waters.

In practice, availability, scientific knowledge, and an understanding of the ability of a species to reflect environmental contaminant concentrations, determines which species are useful as monitors. If the latter information is unknown, it must be obtained either from the scientific literature (e.g., Phillips 1980) or by a pilot study. Final selection should be made in consultation with a knowledgeable biologist. Common mussels, (*Mytilus edulis, M. trossulus, M. californianus*, and *M. galloprovincialis*) are generally suitable for spatial and trend monitoring programmes in temperate coastal waters. Oysters have been suggested in tropical waters, but their speciation may be a problem (Phillips et al. 1980).

Other species can be used for environmental monitoring purposes, provided they accumulate contaminants in relation to environmental contaminant concentrations. Several studies have employed fin fish (e.g., Misra et al. 1993, ICES, 1989, 1991). Aquatic plants have also been used.

5.6 LOCATION OF SAMPLING SITES

5.6.1 Spatial Environmental Monitoring

Hot spots usually exist in estuarine and coastal areas where anthropogenic wastes are discharged. The offshore areas where hot spots are most likely to occur are waste dumping sites and oil platforms. However, Uthe and Chou (1987) found higher cadmium concentrations and burdens in scallops (*Placopecten magellanicus*) collected offshore compared with concentrations in equivalently-sized nearshore scallops, including ones from a cadmium-contaminated area. They suggested nutrition and growth factors accounted for this. Therefore, caution is needed when comparing inshore and offshore populations.

A decision to monitor contaminant concentrations in hot spots should be taken only after consideration of the discharges to them. If the pilot study leads to action to reduce inputs, it is appropriate to monitor to see if the new controls result in lower contaminant concentrations in organisms. If no regulation of inputs is considered, monitoring is justified only to update the results of the pilot study, or where there is knowledge that discharges might increase in the future. Other sampling sites may be included in the programme to include clean and moderately contaminated areas. All sampling should be done by scientific personnel using research or chartered boats instead of fishermen, to ensure sample continuity and integrity.

Precise sampling locations must be recorded, because small spatial changes (resulting in large biological or environmental change) may strongly influence the final data (i.e., "mussels from the harbour wall" must specify the exact point on the harbour wall). It is essential to record details identifying the sample site, including a map and a photograph (with identifying marks and scale).

5.6.2 Trend Monitoring

In hot spots, once contaminant inputs are reduced, contaminant concentrations in monitoring organisms should decrease over a fairly short interval. One can employ a simple technique (e.g., the use of pooled samples), if a large decrease is expected. In situations (e.g., more distant areas), where smaller changes are expected, more complex methods are required to detect them with statistical confidence (i.e., the variance must be reduced). Methods are available for reducing variance by using measured biological factors (e.g., length, weight, sex, fat content) and contaminant concentrations in individual specimens (Misra et al. 1993; Uthe et al. 1991a&b). These techniques are much more expensive to employ; therefore, monitoring for small magnitude changes must be carefully justified before using them. Usually, it is better to use a single pool, remembering that only very large changes will be detected.

5.7 PERIOD AND FREQUENCY OF SAMPLING

5.7.1 Spatial and trend monitoring

For spatial monitoring, all samples should be collected during a short period (within 1-4 weeks) to yield an overview of contaminant concentrations in the study area. This also helps to ensure that all specimens are in a similar physiological state. If one expects large annual changes in the quantity and composition of inputs, annual or biennial samplings are appropriate. Experience has shown that changes in contaminant inputs often affects only the immediate discharge area (hot spot). It is these areas where more frequent monitoring should be conducted.

For trend monitoring, the frequency of sampling must reflect: 1. The time scales over which the changes must be detected; 2. The degree of confidence required in the measurement of these changes; and, 3. The available laboratory resources. A monitoring programme in which the proposed work is less than that needed to detect the desired changes in contaminant concentrations is frustrating and wastes resources. Also, if the original programme aims cannot be met with the available resources, they must be replaced with ones that can be.

It is sensible to sample an area once every five years or so if no change in inputs is expected. More frequent sampling can be justified if more data is required for other purposes (e.g., to reassure the public that contaminant concentrations are not changing).

Seasonal variations in food supply, reproduction, behaviour, and other physiological functions cause changes in tissue weights, fat content and composition, and water content. These influence tissue contaminant concentrations. Sampling should be during the pre-spawning period to minimize these variations. The timing of the spawning period varies with species and location. A knowledgeable biologist should be consulted.

5.8 SIZE OF SAMPLE

5.8.1 Spatial and trend monitoring

If either the analytical resource or the amount of sample material is limited, all specimens in a sample are often pooled for analysis. If the specimens were not sorted (e.g., selecting a small size range), no information will be obtained on the effect of animal size on contaminant concentrations. Site-to-site differences can still be assessed with some level of confidence by pooling enough specimens to reduce the pool-to-pool variance and analysing each pool several times. This minimal technique (***Single Pool Technique***) pools sufficient specimens (e.g., 20-25) to justify an assumption that the analytical variance is now the major variance. (See Appendix 5 for further guidance). If contaminant concentration is significantly related to animal size (i.e., size explains much of the animal-to-animal variation in contaminant concentrations), select a small size range to reduce number of animals required.

For monitoring, to estimate the pool-to-pool variance, it is better (Van der Meer 1989, 1990) to use a few equivalent pools at each site, instead of one large pool. The number of specimens per pool and the number of pools per sample site will be determined by the size of the change considered significant (***Multiple Pool Technique***).

Analysis of individual animals (***Individual Specimen Technique***) is costly and recommended only for studies in which it is essential to detect small changes (e.g. of the order of 10-25%) in contamination. Sampling a small size range may reduce the contaminant variance; however the effects of the other biological and physiological factors

cannot be easily controlled or considered. Techniques and measurements that enable the statistician to reduce the effects of these factors on the variance can be employed (Misra et al. 1993, Uthe et al. 1991a&b), if it is essential to reduce the variance further.

Selection of one of these three techniques for an environmental monitoring programme must be made with great care, in consultation with a statistician who can weight the costs and benefits of each *with respect to the specific situation being studied.* The basic rule is, "The smaller the change in mean contaminant concentration that one wishes to detect with a fixed level of statistical confidence, the greater the costs in both time and resources." It is our opinion that the Single Pool Technique is sufficient for environmental monitoring in most areas, with the others needed only in specific, critical areas.

5.9 SELECTION OF TISSUE

5.9.1 Spatial and trend monitoring

For mollusca, usually the whole soft tissue is analysed; in large crustaceans, the digestive gland (hepatopancreas) is often used because it concentrates both metallic and organic contaminants. Uthe and Chou (1987) noted the importance of removing this gland immediately (within 1 minute) after death to prevent autolysis and cadmium loss from the tissue. It is essential that investigators determine the effect of their post-mortem procedures on tissue contaminant concentrations. Procedures may have to be modified to prevent such changes (e.g., freeze drying tissue can only be used if the laboratory has shown that freeze drying does not cause loss of volatile contaminants, such as organochlorine pesticides).

For fish, muscle is usually the tissue of choice (e.g., ICES 1989), probably because of its public health concern. However, the laboratory must use storage and handling procedures that do not alter contaminant concentrations (e.g., the problem of "drip" , i.e. the loss of fluid from thawing tissue, which can be as great as 40% of the tissue mass, from frozen fish muscle tissue [Uthe and Chou, 1988]). Sampling muscle tissue must be carefully defined (e.g., take: 1. The entire musculature comprising the red and white muscle from both sides of the fish; 2. Only the white muscle; or, 3. A portion of the white muscle from a defined site within the musculature). Liver and kidney tissues have also been used (ICES 1991) because most toxic trace metals accumulate in them. Fatty tissues accumulate the organochlorines and hydrocarbons of concern.

5.9.2 Normalization procedures

Tissue contaminant data can be reported on a dry weight basis (i.e., $\mu g \cdot g^{-1}$ dry wt.) or a wet (undried) weight base (e.g., $\mu g \cdot g^{-1}$ wet wt., generally used for public health data). Because drying is a common part of many 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 should record wet weight:dry weight ratios.

For lipophilic organic contaminants (e.g., organochlorine pesticides) concentrations are often expressed in $\mu g \cdot g^{-1}$ fat wt. (where the fat content is measured or estimated, e.g., as Hexane-Extractable Organic Matter). This results in some normalization for seasonal or spatial differences in the fat content of the organisms. Both dry and fat weight measurements also must be subject to quality control.

6. PUBLIC HEALTH MONITORING PROGRAMMES

Most of the preceding sections also apply to public health monitoring. Therefore, the following will discuss only specific items of public health monitoring.

6.1 AIMS OF MONITORING PROGRAMMES

The aims of public health monitoring programmes are: 1. To compare contaminant concentrations in the edible fish tissues with national and foreign regulations (determine compliance); and, 2. To estimate the exposure of fish consumers to contaminants.

A pilot study is needed in places where little is known about contaminant concentrations in local fishery products. Based on knowledge gathered from published information, it measures selected contaminant concentrations in pooled samples of edible fish tissue. The pools are prepared from specimens collected at the "near-consumer" level (e.g., sampling off fishery vessels when they return to port, sampling in fish plants). Based on a combination of published information, fisheries statistics, coastal maps showing areas receiving contaminant inputs and nearby fisheries, and a listing of contaminant regulations (e.g., FAO 1989), the head investigator selects contaminants and 1-2 commercial fish species (to use as "surveillance" species) for the pilot study.

6.2 PILOT STUDY

The pilot study can:

1. Provide information on contaminant concentrations in edible tissues of the surveillance species and the need for continuing surveillance;

2. Identify marine areas sufficiently contaminated to require fishery controls and, possibly, a monitoring programme to assist management of contaminated commercial fisheries; and,

3. Suggest the need for determining contaminant concentrations in other commercial species.

Once a pilot study has been evaluated, the investigator must prepare protocols for future public health monitoring programmes. Each protocol will specify the information needed to meet the specific aims, and the criteria to obtain the required data quantity and quality.

6.3 DESIGNING A MONITORING PROGRAMME

Similar factors must be considered in the planning of a public heath monitoring programme as were considered in the design of environmental monitoring programmes. The investigator must:

1. Design a sampling programme for commercial species of interest; selecting enough specimens of various sizes for each pooled sample to ensure the sample represents the population of the commercial catch. Sampling must be designed to provide a statistically sound basis on which to judge contaminant concentrations in the sample against national regulations (determine compliance). Instruction sheets must be prepared and issued to the field staff. A statistician must be consulted. Many countries have already developed sampling protocols as part of their public health monitoring programmes. The principal investigator must be aware of all public health monitoring protocols of countries where the fish are to be marketed.

2. Ensure that samples are collected, stored, and transported to the laboratory in a way that minimizes losses and gains of contaminants before analysis. Advice on this is found in the UNEP Reference Methods series. Again it is essential to prepare instruction sheets for field and laboratory staff.

3. Arrange for the samples to be processed and analysed using methods that have the required accuracy and precision.

4. Ensure there is an suitable system of sample documentation and continuity from specimen collection to recording analytical data.

6.4 SELECTION OF CONTAMINANTS

The selection of contaminants for study will be determined by: 1. The findings of the pilot study (i.e., which areas and species required further study); 2. The need to meet national and foreign regulations; and, 3. The need to inspect other commercial fish species.

Because regulated limits for many contaminants (e.g., cadmium, dieldrin) in fishery products are low, the analytical methods must provide the required data quality. The head investigator, the statistician, and the analyst must agree to the degrees of accuracy and precision required and the limits of detection to ensure compliance of the product. However, less than values (a bane of most environmental trend studies) that are significantly lower than regulated concentrations are acceptable in compliance studies. In practice, measured concentrations are often simply judged against regulated values and rapid analytical methods with detection limits one-tenth the regulated values may be acceptable (e.g., a detection limit of 0.01-0.1 $\mu g\ Hg \cdot g^{-1}$ may be acceptable for determining compliance with a regulation specifying 1.0 $\mu g \cdot g^{-1}$). A system of quality assurance framed within national legal requirements must be operational.

6.5 SELECTION OF ORGANISMS

The results of the pilot study can be used to suggest other fishery products for study. If the pilot study has identified hot spots, other commercial fish species from these areas may require study. Shellfish species, in which the visceral tissues are commonly eaten, warrant special attention because visceral tissues generally accumulate toxic metal and organic contaminants.

6.6 LOCATION OF SAMPLING SITES

6.6.1 Commercial Fish Stock Studies

With the fishing industry and local fishery experts, it should be easy to develop a programme for surveying the major stocks of commercial fisheries. Fish and shellfish samples can be obtained from the fish markets or from fishing vessels or research ships that are operating in commercial fishing areas. The basic requirement is to take a representative sample of the commercial catch from each site and analyse the usual edible tissues. Some countries may specify the exact sampling procedures for public health monitoring.

Usually, fishermen do not take precautions during handling and storage other than to ensure catches are fit for sale. The fish merchant and the consumer usually do not use careful dissection procedures, other than from a public health viewpoint. The scientist will use careful sampling and pretreatment procedures to minimize inadvertent contamination. This may result in some difference between contaminant concentrations in scientific and commercial samples.

6.6.2 Contaminated Fishery Monitoring

A commercial fishery may be stopped because the concentration of a contaminant exceeds national regulations. Alternately, the fishery may continue with the product being exported to countries with a less stringent regulation. Sometimes fishing is controlled only in small areas of the fishery (i.e, hot spots). Based on the worth of the affected fishery, various monitoring programmes may be set up, ranging from a simple, infrequent surveillance sampling of the hot spots for less valuable species, to full monitoring programmes that are used in extremely valuable fisheries: 1. To complement pollution control activity; 2. To minimize areas of impact; and, 3. To reopen fisheries at the earliest possible moment (e.g., Uthe et al. 1987)

6.7 PERIOD AND FREQUENCY OF SAMPLING

Unless there is a seasonal fishing pattern for some species, samples may be taken at any time of the year. However, biology and behaviour (e.g., reproduction and migration) may require sampling in predetermined periods (e.g., when one expects highest seasonal contaminant concentrations). A typical monitoring programme in offshore fish stocks might be a survey every 5 years. A more frequent sampling programme (e.g., annual) may be needed in hot spots or where concentrations approach or exceed regulations. Increased sampling should be limited to the species and contaminants that caused the concern.

6.8 SIZE OF SAMPLE

The size(s) of organisms taken for a sample must be based on catch information and marketing patterns. In the simplest case, sufficient numbers of specimens from the commercial fishery (representing the usual size distribution in the commercial fish) are taken for one pooled sample. If a range of sizes is sold, then these different sizes might be analysed (e.g., small, medium, and large). The number of specimens per sample must be sufficient to minimize variation (e.g., 25 for the single pool, 5-10 individuals from each size range of fish and large shellfish [crabs, lobsters], 50 for small shellfish [mussels, shrimps]).

6.9 SELECTION OF TISSUE

Only edible tissue are analysed for contaminants, however, their selection is dependent upon consumption customs, which can be expected to differ among countries. National regulations will frequently specify the tissues to be analysed.

Appendix 1

GUIDANCE ON THE PLANNING OF A PILOT STUDY

Desk Study

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

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

This review can often provide data on the current contaminant concentrations in water, sediments, and biota and, occasionally, information on inputs of contaminants to the area via rivers, pipelines, and dumping from ships. It also may 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 rivers and the sea. These latter data should be verified by contacting the local or national authority that has responsibility for regulating discharges to rivers and coastal waters. This authority also should be approached for information on the past and present discharges to the area.

For public health work, the investigator must identify the fish and shellfish species that are caught for human consumption and the relevant national and foreign regulated limits for contaminants in marine foodstuffs. Information on commercial catches can be obtained from either local fishermen or their representative organisations, or local or central governmental fisheries departments. Information on food regulations can be obtained from international agencies (FAO, 1989), local environmental health departments, or the national government department responsible for food safety.

This review should enable the principal investigator to identify the suite of contaminants and the fish and shellfish species that must be given priority in the pilot study for public health purposes. It also will 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, additional study is needed to identify the locations where samples will be collected.

Identifying Sampling Sites

The pilot study must cover all areas that are likely to be contaminated and others that, from a hydrographic and input viewpoint, are unlikely to be significantly affected (i.e., sites located far from industrialized areas or those located in inshore areas next to less populated and

industrialized areas). Comparisons between inshore and offshore samples of certain species (e.g., shellfish) must be carried out with caution.

The concentrations 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 for discharges containing solids, the settlement of solid material to the seabed sediments; and,
- the physical and chemical processes in the sea (i.e., 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 a hydrographic expert to decide the best locations for sampling in relation to known inputs.

Assuming the principal investigator can provide the hydrographer with the relevant information on inputs, and that the hydrographer 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 probable 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 probably will not be subject to contamination (i.e., 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 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 a sampling grid along the salinity gradient. It is 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.

Specimen Size

The concentration of some contaminants can vary with size in many species. It is important in spatial and trend monitoring to reduce this source of variability in the data to detect

differences in mean contaminant concentrations between sites and with time (see Appendix 5). If this relationship is unknown before starting monitoring, it must be established by the pilot study.

To do this, the investigator must collect a representative sample of each population of species at selected sampling sites. This sample must include sufficient numbers of specimens to cover the size range of the species usually captured. The investigator should consult a knowledgeable biologist for guidance on the size ranges a statistician for an appropriate length-stratified sampling plan.

Selection of Tissue

There is considerable scientific literature on the accumulation of contaminants by various tissues of different species (e.g., Phillips, 1980). It is advisable for the investigator to check this aspect for the species to be examined in the pilot study. It is also advisable to consult a knowledgable biochemist or biologist to decide the best procedure for tissue dissection, to ensure that there is no possibility of one tissue contaminating another.

The investigator should investigate the relationship between the contaminant concentration, tissue, and size of organism by analysing tissue from different-sized specimens, rather than by analysing pooled samples; even if the latter consist of several individuals of a small size range. However, if analytical resources are limited, it may be necessary for him to establish this relationship by analysing pooled samples. A statistician's advice should be sought.

Appendix 2

DOCUMENTATION OF DATA

Use of the following guidelines should provide adequate documentation to allow a laboratory to track samples from sample collection to the project completion by recording the appropriate data in logbooks or in computer files.

Documentation

1. Descriptions of the sampling strategy, methods of sample collection, sample codings, procedures for storage, and pre-treatment and analytical procedures, plus a list of ancillary observations for each site;

2. Sample documentation (description of species, numbers of specimens collected for each sample, weights of tissue taken for analysis [individual tissue or homogenate], plus ancillary data on organisms [e.g., length, weight, and age, as required]);

3. Description of analytical procedures, including details of accuracy, precision, and limit of detection for each measurement;

4. Description of working standards used on each occasion, calculations of results, and data handling;

5. Description of quality control and quality assessment and evidence that these procedures have been applied at all steps and have provided acceptable data;

6. A secure system for the long term storage of data either in logbooks or electronically is essential. Duplicate records should be kept separately in case one set is lost.

Advice should be sought on storing electronic media (e.g., computer tapes and discs) to ensure long-term stability of data files.

Data Storage

Even experienced personnel make simple arithmetic errors in calculating results or translation and transcription errors (e.g., the retyping of data sets). Thus, a check must be made for arithmetic errors before compiling tables of results. All tables, etc. also must be checked carefully. Once this has been done, it is appropriate to carry out a preliminary assessment of the quality of the data, before 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 must be made to ensure that no errors have been made in data handling, from the first sample coding, through all measurements, translations, and transcription to the final report. Methods for controlling data handling errors are given in Uthe et al. (1991a).

Appendix 3

EXAMPLES OF CHEMICAL SUBSTANCES MEASURED IN MARINE ORGANISMS FOR MONITORING PURPOSES

Trace Metals

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

DDTs and Their Metabolites

p,p'-DDT, p,p'-DDE, p,p'-DDD (p,p'-TDE), o,p'-DDT, o,p'-DDE, and o,p'-DDD.

Other Chlorinated Pesticides other than DDT

Aldrin, α–Chlordane, Trans-Nonachlor, Dieldrin, Heptachlor, Heptachlor epoxide, Hexachlorobenzene, Lindane (γ-HCH), and Mirex (possibly Endosulfan [Thiodan] I and II and Endosulfan sulfate, Endrin, α-HCH, Toxaphene [Strobane])

Polychlorinated Biphenyls (PCBs)

Measurements are usually restricted either to a small number of individual chlorobiphenyls (known as PCB congeners) or to estimates of the total PCB content based on the use of technical mixtures as standards.

Polynuclear Aromatic Hydrocarbons (PAHs) These can include:

2-ring compounds Naphthalene, 1-Methylnaphthalene, 2-Methylnaphthalene, and 2,6-Dimethylnaphthalene.

3-ring compounds Fluorene, Phenanthrene, 1-Methylphenanthrene, and Anthracene, Acenaphthene.

4-ring compounds Fluoranthrene, Pyrene, and Benz[*a*]anthracene

5-ring compounds Chrysene, Benzo[*a*]pyrene, Benzo[*e*]pyrene, and Dibenz[*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 measurement in marine organisms.

category I (mandatory) - total and organic mercury, cadmium, and halogenated hydrocarbons.

category II (optional) - total arsenic, radionuclides, and PAHs.

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.

1. Bivalves

Mytilus galloprovincialis, or
Mytilus edulis, or
Perna pern, or
Donax trunculus

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

2. Demersal fish

Mullus barbatus, or
Mullus surmuletus, or
Upeneus molluccensis

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

3. Pelagic carnivore fish

Thunnus thynnus, or
Thunnus alalunga, or
Xiphias gladius

4. Pelagic plankton feeding fish

Sardina pilchardus

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

5. 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

	Cd	Hg	Cu	Cr	Pb	Zn	HH	PHC
Rocky substrate								
Mytilus edulis (common mussel)	+	+	?	+	+	+	+	+
Littorina littorea (gastropod)	+		+	?	+	+		
Patella vulgata (limpet, gastropod)	+		+		+	+		
Muddy substrate								
Scrobicularia plana (da Costa) (peppery furrow bivalve)	+	+	?	+	+	+		
Macoma balthica (bivalve)	+	+	?	+	+	+		
Nereis diversicolor (annelid)	+	+	+	+	+	+		

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 concentrations in the tissues of the two groups will reflect contaminant concentrations in the same part of the marine environment.

Appendix 5

DETECTION OF DIFFERENCES IN MEAN CONTAMINANT CONCENTRATIONS IN MARINE ORGANISMS IN RELATION TO SPATIAL AND TREND MONITORING PROGRAMMES

Natural variability in contaminant concentrations in marine organisms within and between populations of a species is an important factor to be considered when designing a programme to detect significant differences in mean contaminant concentrations over time or space. The ability to determine whether differences in mean contaminant concentrations are statistically significant is dependent on the difference between the estimated mean contaminant concentrations in the two populations and their respective variances. The investigator must determine the design of the sampling programme to minimise and estimate these variances, the precautions taken to ensure that sample handling variability is minimised (collection, storage, and dissection of samples before analysis), and the ability of the analyst to minimise the measurement variability (i.e., good analytical precision), and all other sources of error. In practice, it is the first of these factors that often determines the success or otherwise of monitoring programmes. Considerable progress has been made in improving both analytical performance and sample handling to the point where they may no longer be limiting features in monitoring programmes.

It is necessary, therefore, to examine and quantify this natural variability for the species and contaminant(s) to be monitored, either during the pilot study or by carrying out a special investigation before starting the monitoring programme. Individual specimens, spanning the size range, should be collected from one or more (if sufficient analytical resources are available) species at one or more sites, analysed for the contaminants in question, and intra- and intersite variances estimated. Based on this work it is possible to select the appropriate number and size (or range of sizes) of organisms to minimise the between-sample variance. These future samples can either consist of several individuals that can be analysed separately, or individuals that can be pooled for examination. Before making any final decision on this matter the investigator should discuss the results of this study (and proposals for future sample composition) with a statistician.

This problem was studied by Gordon et al. (1980), who investigated variability in trace metal concentrations in two populations of *Mytilus californianus*, through the analysis of randomly selected individual organisms on two separate occasions. They found coefficients of variation of 18-40% and concluded that 20-100 specimens a site (population) was required to detect a mean concentration difference of 20% between sites. Differences of 40% could be detected by analysing only one-third this number of specimens per site. These results may be compared to those obtained by Bryan et al. (1980) for *Scrobicularia plana* (da Costa), which indicated that a difference of 30-40% in metal concentration could be detected between sites by analysing three pools per site, each pool containing six animals.

See also Topping (1983) for statistical approach to the selection of numbers and sizes of fish in relation to trend studies.

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