MEDITERRANEAN ACTION PLAN

Consultation Meeting on the Evaluation
of MED POL Phase II Monitoring Data

Athens, 12-13 January 1990

REPORT OF A CONSULTATION MEETING ON THE EVALUATION
OF MED POL PHASE II MONITORING DATA

(Athens, 12-13 January 1990)

In cooperation with

FAO   WHO   IAEA
_____
U N E P
Athens, 1990

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<u>Report</u>

1. Monitoring data collected in the framework of MED POL Phase II are submitted to the Coordinating Unit for the Mediterranean Action Plan (MEDU) since 1983. In its continuing efforts to review and assess these data, MEDU organized a small consultation meeting for their evaluation and potential utilization. The invited participants to the meeting were the representatives of the MED POL co-operating Agencies FAO, IAEA and IOC and two UNEP consultants both of which work at the DAFS Marine Laboratory in Aberdeen, Scotland. Both consultants are actively involved in ICES activities; Mr. Topping is chairman of the Marine Chemistry Working Group and organised a number of inter-calibration exercises for heavy metals in biota, while Mr. Fryer is a member of the group responsible for the statistical aspects of trend monitoring. The list of participants appears as Annex I.

2. The consultation took place at the premises of the Co-ordinating Unit in Athens on the 12th and 13th January 1990. After welcoming the participants Mr. L. Jeftic, Senior Marine Scientist at MEDU, explained, among other things, that the meeting is not concerned with all MED POL data but only with the chemical contaminants and more specifically with heavy metals, organohalogens and petroleum hydrocarbons. The floor was then taken in turn by each UN staff member present to provide information to the consultants on all relevant aspects. For example, the FAO representative Mr. G.P. Gabrielides explained the types of data available at MEDU pointing out that the majority of them referred to heavy metals (mainly mercury and cadmium) and chlorinated hydrocarbons in marine biota, the IAEA representative Mr. J.W. Readman explained the MED POL data quality assurance programme and Mr. A. Aksel, Computer Operations Officer at MEDU, provided information on data storage, processing and presentation used at MEDU.

3. Mr. Topping, after briefing the participants on the work done at ICES, presented a draft document on guidelines for monitoring chemical contaminants in marine organisms for comments by the participants. This document is reproduced as Annex II. At the end of the first day it was decided that the consultants should examine the data sets and decide whether they could be utilized for the assessment of spatial differences and temporal trends and ultimately for management decisions. If the data were not found suitable, the reasons should be explained and recommendations should be made for the improvement of the situation.

4. After studying the data the consultants decided to use the data from Yugoslavia and especially the mercury data from the Split area as an illustrative example. The comments and recommendations of the consultants are included in their report which is reproduced as Annex III.

5. The meeting was closed on Saturday January 13, 1990.

ANNEX I

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<u>ANNEX II</u>

GUIDELINES FOR MONITORING CHEMICAL CONTAMINANTS
IN THE SEA USING MARINE ORGANISMS

(Draft document submitted by G. Topping)

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1. INTRODUCTION

Marine organisms can accumulate contaminants within their tissues from sea water, suspended matter and sediments. It has also been demonstrated, through field observations and experimental studies, that the concentration of contaminants in some of the tissues reflect 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 in a group of individuals of the same species which are exposed to the same concentration of contaminants for the same period of time, the individual organisms will experience different levels of bio-accumulation; 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 excretion of contaminants following the same period of exposure.

It is clear, therefore, that if a programme of measurements of contaminants in marine organisms is carried out - to assess the current level of contamination in one area of the marine environment (generally referred to as either 'a baseline study' or 'a pilot study') or to assess changes in the level of contamination with time at any one site (generally referred to as 'trend monitoring') - then careful consideration must be given to the above factors, and the other variables which influence bio-accumulation, when the programme is designed.

This document is intended to provide such guidance for scientists who are responsible for the design and implementation of marine pollution monitoring programmes within the framework of Regional SEas projects. It is particularly aimed at programmes which fall under the auspices of the intergovernmental Oceanographic Commission (IOC) and the united Nations Environment Programme (UNEP). The document is not intended for the analytical or administrative staff associated with this work.

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

aims

criteria for the selection of contaminants, organisms and locations to be studied

numbers of individual organisms to be collected at any one time and the frequency of sampling operations

type(s) of tissue to be analysed.

Although an important component of these programmes is the analysis of contaminants in samples, this matter will not be addressed in this document since it is dealt with in some detail in other documents in the Reference Method Series sponsored by some of the UN agencies concerned with marine pollution studies. Readers of this document are therefore advised to have the relevant analytical documents to hand (see list of documents at the back of this publication); particularly "Quality Assurance and Good Laboratory Practice in relation to Marine Pollution Monitoring Programmes", Reference Method No., since this one deals with all aspects of work which influence the quality of data, including analytical data quality.

2. DEFINITIONS

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

Term	Definition
Pollution	The widely accepted definition is that given by GESAMP (Group of Experts on Scientific Aspects of Marine Pollution), namely 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.
Pollutant	A substance which causes pollution.
Contaminant	A substance, which may or may not occur naturally in the marine environment, which does not cause a deleterious effect when it is present at a concentration below a specific level. However, if this level is exceeded the contaminant is classed as a pollutant. In theory, therefore, all contaminants are potential pollutants.
Monitoring	A programme of repeated measurements of contaminants, or pollutants, 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 'one-off' study of mercury in fish which is done to examine the levels of mercury in different species would not be classed as monitoring but as a 'pilot' or 'baseline' study. If, however, this study was repeated in subsequent years these sets of data would be classed as 'monitoring data'.

Pilot or	A programme of measurements of contaminants in marine
Baseline	samples, in an area not previously studied, to
study	establish the current levels of contamination in this area. This programme is a pre-requisite to the commencement of a monitoring programme in the area since the information collected in this study enables the investigator to design the detailed sampling programme for the monitoring work. Without such information the investigator may be unable to judge which contaminants, organisms and locations to select for the monitoring programme.

Anthropogenic	Man-made

Accuracy,	See definitions in Appendix 2, "Quality Assurance and
precision	Good Laboratory Practice in relation to Marine
limit of	Pollution Monitoring Programmes", Reference Method
detection	No. 57

3. AIMS OF MONITORING PROGRAMMES

Three main aims are normally identified for monitoring programmes involving the collection and analysis of marine organisms; they are:

to compare contaminant levels in edible marine organisms against permissible limits of contaminants in foodstuffs and to provide data to calculate the potential amount of contaminant taken in by the consumer during the ingestion of such foodstuffs (public health monitoring).

to check the levels of contamination in different parts of the marine environment in order to identify areas of the sea ('hot spots') where levels of contaminants are approximately an order of magnitude higher than levels in 'clean' or uncontaminated areas. Such measurements are 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 assess changes in concentrations of contaminants in organisms over a period of time at particular locations to judge whether levels are increasing or decreasing as a result of changes in the quantity and quality of wastes being discharged.

Before any monitoring programme can begin it is important to conduct a relevant pilot study in the area to establish the current levels of contaminants in the marine organisms and identify those contaminants which require further investigation, to provide relevant information on the future sites to be examined, and the sources of variability of contaminant levels in organisms at these sites.

4. PILOT STUDY

It has been stated above that a pilot study is a pre-requisite to the commencement of a monitoring programme since without such measurements it is very difficult to design a monitoring programme. Provided they are carefully designed, pilot studies can assist the investigator in the following way:

a) In relation to public health studies they can reveal which edible species are currently displaying unacceptable levels of contamination and which therefore merit further investigation in the form of a monitoring programme to provide an appropriate data bank.

b) They can identify which areas of the marine environment are sufficiently contaminated to warrant monitoring. In order to provide this information it is essential that the pilot study covers both the areas which are likely to be contaminated (in general those areas adjacent to estuarine waters receiving inputs of wastes from pipelines, rivers and dumping vessels) and those areas which, from a hydrographic and input viewpoint, are unlikely to be significantly affected by these discharges (ie sites located well offshore from industrialized areas or those located in inshore areas next to less populated and industrialized areas.

Whenever possible sampling sites in pilot studies should be located, and uniformly positioned, along the likely gradient of contamination to assess the extent of the contamination in the organisms selected for study.

c) They can provide some indication of the variability of contaminant levels in individuals of the same species from the same populations (and location). This type of information is essential to any investigator wishing to establish a programme of trend monitoring since without it he may not be able to judge whether his sampling and analytical work will be sufficiently detailed and comprehensive to permit the monitoring of changes of contaminant levels in his samples with time against the natural fluctuations that may exist in any population of organisms.

To obtain this information it will be necessary to collect a representative sample of the population of organisms (sufficient numbers and sizes/ages/lengths of organisms) to judge which size, or size range, and how many individuals of this size (or size range) are required in future samples to minimise this variability from sample to sample.

d) They can identify which tissues of organisms, particularly fish and large shellfish, are the most appropriate ones to use in subsequent monitoring programmes since not all tissues reflect changes in the ambient contamination with respect to time and degree of exposure.

Since this pilot study has been successfully completed and the results evaluated the investigator should then prepare a clear and unambiguous statement on the aims of the monitoring programme. Only by doing this will it be clear what information will be required to meet these aims, and consequently what criteria should be laid down to obtain the required quantity and quality of data. Time spent on defining aims, and on the planning of a statistically significant sampling and analytical programme, will inevitably produce a more efficient and effective programme which will make the best use of the laboratory's most important resource (ie staff time). It is generally sensible to initially aim for a programme which satisfies essential, rather than desirable, objectives. It is easy to expand such a programme if the necessary resources are available. Finally, it is necessary to review the programme of work, on a regular basis, to assess how well the aims are being met. This may lead to a reduction of effort on sampling and analyses, and the time gained can be usefully employed on other aspects of marine pollution studies, or it might identify the need to put in more effort.

5. DESIGNING A MONITORING PROGRAMME

In designing a monitoring programme to meet the aims of the laboratory's programme there are a number of factors to be considered:

a)   which contaminants should be measured;

b)   which organism(s) should be selected;

c)   where should the samples be collected;

d)   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 who will have to do this evaluation, design and plan the sampling work, prepare the necessary guidance sheets for the staff who do this work and discuss with the analysts the precautions to be taken by field and laboratory staff in the storage and processing of samples prior to their analysis.

Specifically, the investigator will have to do the following:

1)   Design a representative 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 management needs and the need to ensure that it will provide a statistically sound basis on which to judge changes in contaminant levels in space or time. Once this has been done, guidance sheets should be prepared and issued to the staff who will carry out this field work.

2) Ensure that samples are collected, stored and transported to the laboratory in a way which minimises losses and gains of contaminants prior to analysis. Guidance on these aspects can be obtained by consulting the relevant documents in the Reference Methods series. Again it will be necessary to prepare suitable guidance documents for the relevant field and laboratory staff.

3) Arrange for the processed samples to be analysed using methods which provide the required accuracy and precision for the purposes of the investigation. Experience has shown that close collaboration between the investigator and the analysts is essential if this part of the work is to be done efficiently and effectively. The investigator and the principal analyst should consult the Reference Method document giving guidelines on Quality Assurance if they are in any doubt about what is required to achieve and maintain the required quality of analytical data.

4) Ensure that there is an adequate system of documentation to allow samples to be traced from the time of collection to the completion of the analyses and that all relevant staff are aware of, and comply with, the procedures (see Appendix 3 for more details on this matter).

Each of the factors a - f will now be considered in more detail.

6. SELECTION OF CONTAMINANTS TO BE MEASURED

The selection of substances to be monitored will be determined by the findings of the pilot study (ie which contaminants, present at significant levels above the background values, justify further study), the aims of the monitoring programme and the ability of the analyst to measure the substances in question with the accuracy and precision that are required to achieve the aims. In practice it is this last factor which largely determines whether a particular contaminant or group of contaminants can be included in the monitoring programme.

Therefore before embarking on a monitoring programme it is essential that the investigator discusses with the analyst(s) the required analytical performance characteristics (accuracy, precision and limit of detection) of the analytical procedure to ensure that they meet the necessary standards eg it would be inappropriate to consider the monitoring of temporal changes in levels of mercury in fish flesh using an analytical method which had a poor level of precision.

If the performance characteristics of the analytical method which had been used in the pilot study do not meet the required standard for monitoring purposes, there are two options that can be pursued - either the analyst must select a method which does meet the standard or, if for some reason (eg there is a statutory requirement to use a particular method) this is not possible, the investigator must abandon the proposed monitoring programme. Any other course of action will merely result in wasted effort. However, it should be stressed that the use of an analytical method which in theory has

the required performance characteristics to meet the aims does not guarantee success for an investigator since there are other factors which have to be taken into account in obtaining the required quality of analytical data. These factors are discussed in some detail in "Quality Assurance and Good Laboratory Practice in relation to Marine Pollution Monitoring Programmes", Reference Method No, and investigators are strongly advised to obtain a copy of this document and discuss it with their analyst before embarking on a monitoring programme.

In addition to selecting contaminants on the basis of the results of the pilot study which meet the aims of the laboratory's marine pollution programme, it may be possible for the investigator in include other contaminants which meet regional and international needs. This latter work should only be considered if the additional data arising from it is useful to the laboratory, or is part of the laboratory's commitment to Regional Studies and it does not jeopardise the main aims of the monitoring programme. A list of contaminants, identified by other organisations (International council for the Exploration of the Seas, Oslo and Paris Commission's Joint Monitoring Programme for the North Sea and adjacent waters is given for information in Appendix 1).

7. SELECTION OF ORGANISMS FOR MONITORING PURPOSES

Spatial and trend monitoring

The ideal characteristics of organisms, which can be used as bio-indicators in trend monitoring, are:

The organism should accumulate the contaminant without being affected by the levels encountered;

The organism should be sedentary in order to be representative of the area of collection;

The organism should be abundant in the study region, in order to allow comparisons between different areas;

The organism should be sufficiently long-lived to allow sampling of more than one year class if desired;

The organism should be of a reasonable size, giving adequate tissue for analysis;

The organism should be easy to sample and hardy enough to survive in the laboratory, allowing (if desired) defecation before analysis and laboratory studies of uptake of contaminants;

The organism should tolerate brackish water, to allow comparisons to be made between estuarine and offshore sites;

The organism should exhibit high concentration factors, allowing direct analysis without pre-concentration;

A simple relationship should exist between contaminant residues in the organisms and the average concentrations in the surrounding seawater or sediments, depending on the purpose of the programme.

These conditions 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 conditions in the water column, whilst deposit feeders will respond to sediment chemistry. The working of the sediments both by organisms and water currents will cause an averaging of relatively short-term variations in contaminant loading. Water chemistry, however, will more closely respond to effluent discharges and dispersal conditions at the time of sampling. Filter-feeders are therefore more likely to provide the information required to fulfil the objectives of the monitoring programme concerned with water quality. In Appendix 2 a list of recommended organisms is given which scientists in the United Kingdom have suggested may be used for the assessment of contamination by a range of metals and organochlorine compounds under the contrasting conditions of rocky and muddy inter-tidal areas.

In practice the selection of an organism or organisms, for trend monitoring purposes, is influenced by local knowledge of the availability of the organism and its ability to act as a bio-indicator. If this information is not obtained from the pilot study it must be sought from the many publications on this topic. The use of the common mussel, Mytilus species (edulis, californianus and galloprovincialis), in global mussel watch programmes suggests that this species is the one that scientists in general would recommend for marine monitoring programmes concerned with trend studies.

7.1   Public Health programmes

The selection of organisms for these programmes is determined largely by the findings of the pilot study. If this study has revealed that some edible species show evidence of significant contamination which approaches or exceeds statutory limits for contaminants in foodstuffs then these species should be included in any subsequent public health monitoring programme.

Since some permissible limits of contaminants in foodstuffs are extremely low (eg Cd) the analytical method for this work must have the necessary performance characteristics, particularly a high degree of accuracy and a detection limit which is ca 1/10 of the permissible concentration of the contaminant in the foodstuff. These criteria are necessary if the analyst is to have confidence in the results of his(her) analyses since he(she) may have to have to advise that some foodstuffs, which exceed permissible limits, should not be sold to the general public.

8. LOCATION OF SAMPLING SITES

8.1 Spatial and trend monitoring

The selection of sites for monitoring will be based on the findings of the pilot study. The decision to monitor contaminant levels in 'hot spots' should only be taken 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 include these areas in the monitoring programme. The aim of this work being to assess changes in contaminant levels so that one can judge whether the new controls on inputs have been effective. If no action is to be taken on the regulation of discharges then subsequent monitoring programmes will clearly be aimed at updating the information collected in the pilot study for these areas rather than trying to whether there have been any increases or decreases of contaminant levels with time.

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

Other sampling sites should include estuarine, coastal and offshore areas to provide coverage of both clean and moderately contaminated areas in the programme. The purpose of this work is either to ensure that there is no deterioration in water quality in these areas or to provide warning of any deterioration. Sampling of fish 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.

8.2 Public Health Programmes

Samples of fish and shellfish can either be collected from the fish markets or from fishing vessels or research ships which are operating in traditional fishing areas. In both cases what is required is a representative sample of the species normally consumed by the general public. Since 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, and since the retailer and the consumer do not adopt any stringent dissection procedures, other than from a public health viewpoint, it is not necessary for the scientist in the filed to use the careful sampling and pre-treatment procedures that are used in other monitoring programmes.

In some countries there may be officials, known in the UK as fishery officers, who have special knowledge of edible species of fish and shellfish and the areas from which they are caught by commercial fishing boats. Investigators may find it helpful to discuss their proposed monitoring programme with such experts since they may be able to offer assistance in the design of the collection programmes.

The final decision on where samples are to be collected must rest with the investigator. He (she) will determine whether information is required on potential contaminant intake by the consumer (in which case samples will be taken from the fish markets) or whether it is more appropriate to determine if edible species are continuing to be exposed to contamination in the fishing areas (in which case the sampling must be done by scientific staff who will take the necessary precautions to obtain representative samples to achieve this aim).

9. PERIOD AND FREQUENCY OF SAMPLING

9.1 Spatial and trend monitoring

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, consequently, contaminant levels in the tissues of some organisms. In order to minimise these variations, it is suggested that sampling be undertaken at the pre-spawning period.

Collections should be made over a short interval of time (weeks rather than months) to ensure as synoptic a picture as possible and to enable comparisons of concentrations of contaminants at different sites. This helps to ensure that organisms are in the same physiological state (ie lipid metabolism, spawning etc.).

It is appropriate to monitor on a 2-3 year basis unless major changes in the quantity and/or composition of inputs are anticipated, which are considered to influence levels of contaminants in organisms, in which case it might be appropriate to conduct an annual monitoring programme. Experience has shown that in most cases the effects of changes in inputs of contaminants are confined to the area in the immediate vicinity of the discharge. It is these areas where the more frequent monitoring should be targeted.

Each laboratory will clearly establish the frequency of sampling which meets the aims of its programme. This frequency will reflect the time scales over which the changes are required to be detected, the degree of confidence required in the measurement of these changes, and the resources it has at its disposal. Investigators should note that there is nothing more futile than a programme in which the proposed work is well below the minimum required to observe the desired changes in contaminant levels. If, for any reason, the resources at the disposal of the laboratory are insufficient to meet the aims of the proposed monitoring programme then it should be cancelled and replaced with one which has less ambitious aims but one which can still be carried out successfully with the available resources.

9.2 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. However, available

resources may allow more frequent surveys to be conducted; assuming that the results indicate that this is required. A more frequent sampling programme (ie annual) will be needed if concentrations of contaminants in foodstuffs are found during the pilot study to approach or exceed permissible limits for foodstuffs. However, this increased sampling should be confined to those species and contaminants which give cause for concern following the evaluation of the results of the pilot study.

10. SIZE AND NUMBER OF INDIVIDUALS IN SAMPLE

10.1 Spatial and trend monitoring

Ideally, the investigator will have established the relationship between contaminant levels and size of organisms during the pilot study. If this is the case then it is usual to select a particular size or size range for monitoring programmes to reduce variance of contaminant levels from sample to sample. The number of individuals required for each sample will be determined by the differences in contaminant levels one wishes to be able to differentiate (ie the smaller the difference the greater the number of individuals required for each sample). See Appendix 4 for further guidance on this aspect.

If the necessary work on relationship between size of organism and contaminant level has not been done in the pilot study then the number of individuals collected in the monitoring programme should cover the size range of organisms encountered at the sampling site in order to establish the variations of contaminant levels with size. Although this approach need not be taken at each of the sampling sites it should be carried out at least at one site in order to allow comparisons with other sites where samples consist of individuals of a limited size range. If analytical resources are limited it may not be possible for the organisms from each site to be analysed individually. If this is so then individuals should be combined to make one sample. In this case, no information will be derived on the variation of contaminant levels with size but the results can be used to compare data from site to site with some level of confidence (this assumes that a number of replicate analyses are performed on the bulked sample to allow differences to be detected above and beyond those produced by sample variation).

10.2 Public Health monitoring

The size(s) of organisms to be sampled should be based on information on consumption patterns. If small and large sizes are sold to the general public, this should be reflected in the sample. The number of individual organisms in each sample may be influenced by the importance of the species as a foodstuff, the availability of scientific manpower and the need to sample sufficient 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 could be collected for each size range of fish and large shellfish (crabs, lobsters) and ca 50 individuals for smaller shellfish (eg mussels, shrimps).

11. SELECTION OF TISSUE

11.1 Public Health monitoring

Only edible tissue need be analysed for contaminants - usually this means muscle tissue for fish and large crustaceans and whole soft tissue (less viscera) for other shellfish.

Every opportunity should be taken to collect data on the size (or length) and age of the species. This may assist in any decision making at a later data.

11.2 Spatial and trend monitoring

For invertebrates, whole soft tissue (less viscera) should be taken for analysis. N.B. For the analysis of lipophilic contaminants (eg organochlorine compounds) it is important to measure the lipid content of each sample in order to ensure that comparisons of data on a lipid basis can be made for different regions and times of lipid concentrations in organisms).

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

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APPENDIX 1

RANGE OF CHEMICAL SUBSTANCES MEASURED IN MARINE ORGANISMS BY
SCIENTISTS INVOLVED IN MARINE POLLUTION MONITORING PROGRAMMES

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, Heptaclor epoxide,
Hexachlorobenzene, Lindane (gamma-HCH) and Mirex.

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	Flurene, Phenanthrene, 1-Methylphenanthrene and Anthracene.
4-ring compounds	Fluoranthrene, Pyrene and Benz(a)anthracene
5-ring compounds	Chrysene, Benzo(a)pyrene, Benzo(e)pyrene and Dibenz(a,h)anthracene.

The first provisional UK Red List - the 26 substances, selected from existing List 1
substances and the EC list of 129 substances, which are thought to represent the best
candidates for priority action

EC Directive Substance
adopted

+   Mercury
+   Cadmium
+   Gamma-hexachlorocyclohexane (Lindane)   *
+   DDT   *
+   Pentachlorophenol (PCP) *
+   Hexachlorobenzene (HCB)

+   Hexachlorobutadiene (HCBD)
+   Aldrin   *
+   Dieldrin *
+   Endrin   *
        Chloroprene
        3-Chlorotoluene
        Polychlorinated Biphenyls (PCBs)   *
        Triorganotin compounds *
        Dichlorvos   *
        Trifluoralin   *
+   Chloroform
+   Carbon Tetrachloride
        1,2-Dichloroethane
        Trichlorobenzene
        Azinphos-methyl   *
        Fenitrothion   *
        Malathion   *
        Endosulphan   *
        Atrazine   *
        Simazine   *

*   Substances which are likely to enter the aquatic environment through a variety
        of indirect routes

APPENDIX 2

LIST OF POSSIBLE ORGANISMS FOR THE ASSESSMENT OF CONTAMINATION

	Cd	Hg	Cu	Cr	Pb	Zn O/
Rocky substrate						
Mytilus edulis	+	+	?	+	+	+
Littorina littorea	+		+	?	+	+
Patella vulgata	+		+		+	+
Muddy substrate						
Scrobicularia plana (da Cos)	+	+	?	+	+	+
Macoma abalthica	+	+	?	+	+	+
Nereis diversicolor	+	+	+	+	+	+

Key: + = appears to act as good indicator
    ? = doubt about use as indicator

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 contaminant levels in the same part of the marine environment.

APPENDIX 3

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

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

2. 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);

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

4. Description of quality control and quality assessment and evidence that these procedures have been applied and have provided acceptable data;

5. Description of working standards used on each occasion and calculations of results;

6. 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 accidently 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 carried out 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 (ie 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 transcriptional errors have been made in transcribing the data (ie the re-typing of data sets by typists or data processors can sometimes lead to such errors).

APPENDIX 4

DETECTION OF DIFFERENCES IN LEVELS OF CONTAMINANTS IN MARINE ORGANISMS IN RELATION TO SPATIAL AND TREND MONITORING PROGRAMMES

Natural variability in contaminant levels in marine organisms both within, and between different, populations is an important factor to be taken into account when designing a programme to detect significant differences in contaminant levels with time or space. The ability of the investigator to determine whether differences in contaminant levels between samples are significant is dependent on the design of the sampling programme to minimise the effects of the natural variability, the precautions taken to ensure that sample handling variability is minimised (collection, storage and dissection of samples prior to analysis) and the ability of the analyst to minimise the measurement variability (ie good precision). In practice it is the first of these factors that determines the success or otherwise of monitoring programmes. Considerable progress having been made over the past few years in the improvement of analytical performance and sample handling to the point where they are no longer the limiting features in monitoring programmes.

It is necessary, therefore, to examine and quantify this natural variability for the organism(s) and contaminant(s) to be monitored, either during the pilot study or by commissioning a special investigation before the commencement of the monitoring programme. Samples of individual organisms which cover the range of sizes should be collected from one or more populations at the same location and analysed for the contaminants in question. If sufficient analytical resources can be spared this sampling should be repeated at other locations to provide information of variability between locations. On the basis of this work it should be possible to select the appropriate number and size (or range of sizes) of organisms to minimise variability from sample to sample. These future samples can either consist of a number of individuals which can be analysed separately or a number of individuals which can be bulked for examination. Before making any final decision on this matter the investigator should discuss the results of the special study, and proposals for future sample composition with a statistician.

To illustrate this problem it is appropriate to refer to the work done by Gordon, Knauer and Martin (1980) who investigated trace metal analysis of randomly selected individual organisms on two separate occasions. They found mean coefficients of variation of 18-40% and concluded that a sample of 20-100 individuals from each site was required to detect a concentration difference of 20% between sites. Differences of 40% could be detected by analysing only one third of this number of samples. These results may be compared to those obtained by Bryan, Langston and Hummerston (1980) for Scrobicularia plana, (da Costa) which indicate that a difference of 30-40% in metal concentration could be detected between samples from two sites by analysing three pooled samples each containing six animals.

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

ANNEX III

CONSULTANTS' REPORT

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1. INTRODUCTION

The consultants Drs G. Topping and R. Fryer of the Marine Laboratory, Aberdeen, Scotland, visited MEDU on 12-13th January 1990, to have discussions with MEDU staff on the data collected through the MED POL concerning chemical contaminants in marine organisms. Their task was the following:

a) to examine the data sets from participants in the MED POL monitoring programme and to determine whether the data for contaminants in marine organisms were sufficiently comprehensive and detailed to allow an assessment of spatial difference sand temporal trends for different parts of the Mediterranean Sea area.

b) in the event that (a) could not be done, to identify and document the reasons why and to prepare guidance on how future monitoring should be conducted to allow this aim to be achieved.

2. ASSESSMENT OF DATA

Since a review of all the data sets held on MEDU's computer could not be carried out in the limited time available, it was agreed that the assessment should be done on the data set from one country. Yugoslavian data were chosen for this purpose since they were generally agreed to be some of the best data available on the MEDU computer.

Two questions were addressed in particular:

a) Was the sampling program adequate?

b) Assuming adequate quality control, could any conclusions be drawn from the data already collected?

The biota compartment was chosen for study since the work of the International Council for the Exploration of the Seas' (ICES) Working Group on the Statistical Aspects of Trend Monitoring (WGSATM) has concentrated on this compartment. According to the conclusions in the report of the National Monitoring Programme of Yugoslavia for 1983-1986 (MAP Technical Reports Series No. 23) there were no significant changes in Hg and Cd concentrations in mussels (Mytilus galloprovincialis) except in the Split area. Hence, the data from the Split area are used as an illustrative example.

The Hg data for station 1 in the Split area are given below:

Date	No of items homogenized in each sample	No of samples	Hg conc. ($\mu g\,gm^{-1}$ WW)
28/03/84	12	1	240
11/06/84	12	1	160
01/09/84	12	1	218
26/12/84	6	1	95

16/05/85	6	1	88
11/09/85	6	1	93
26/11/85	6	1	59
21/03/86	10	1	40
04/06/86	6	1	72
01/10/86	6	1	84
08/12/86	6	1	152
24/03/87	6	1	70
28/06/87	6	1	104
19/08/87	6	1	100
17/11/87	10	1	44

Two problems arise immediately in the interpretation and analysis of these data:

a) the data are not collected at the same time each 'season'. Metal concentrations in mussels vary naturally throughout the year; if the data are not collected at the same time each season (ie within, say, a period of a couple of weeks) then it is difficult to analyse the data for time trends because it is not possible to 'compare like with like'.

b) different numbers of mussels are homogenized in each sample. An appropriate statistical analysis of the data is then quite complex and requires the weights of each contributing tissue. However, if the same number of items are homogenized in each sample, this problem does not arise.

Assuming (unrealistically) that there is no seasonal variation in concentration levels and that the different numbers of homogenized items have no affect on the analysis, the data can be assessed for trends in time. An analysis of variance of log Hg concentration gives slight evidence of a change in concentration with time ($p < 0.10$), with concentrations in 1984 being greater than those in 1985-87. However, station 1 is directly influenced by the effluents from the chloralkali plant 'Jugovinil', where there have been positive efforts to improve sewage treatment. Thus, even at a station where large trends might be expected, the data collected is sufficient only to provide slight evidence of temporal trends.

There are two main reasons why it is difficult to detect trends in data such as these. First, the time series is very short (ie only four years data), so gradual changes in concentration levels can easily be 'lost' in the natural variability of samples. This situation will inevitably improve as the monitoring programme continues. The second reason is more fundamental and of great concern. Namely, very few samples were analysed on each sampling occasion; at station 1, only one sample was analysed on each sampling occasion, although there were four sampling occasions each year. This gives only very limited information about the variability of concentration levels within the population at any one time. Further, it means that there are very few degrees of freedom for any statistical procedure that attempts to assess trends. It is very important to take replicate samples on each sampling occasion.

3. RECOMMENDATIONS FOR SAMPLING STRATEGIES

Appropriate sampling strategies depend on the objectives of the study. Unfortunately, strategies that are optimal for one objective (ie to measure temporal trends) are not necessarily optimal for other objectives (ie to measure spatial trends). Most of the comments / recommendations below are applicable to all monitoring programmes; however, the emphasis is directed towards monitoring for temporal trend assessment purposes.

3.1   Replicate samples. The most fundamental principle of trend monitoring is to obtain replicate samples on each sampling occasion. In general, the more samples the better. However, clearly a balance must be struck between the benefits accrued from obtaining more samples and the increased costs of chemical analyses. For temporal trend monitoring, a sensible approach might be to reduce the number of sampling occasions (to once a year, say) and to take a larger number of samples on each occasion.

3.2   Sampling times. Sample at the same time each year (or season, depending on the frequency of sampling occasions).

3.3   Homogenization.   There are both advantages and disadvantages in homogenizing material for analysis. Homogenization loses information about the variability of concentration levels between individuals. However, homogenization is often necessary where items are too small to be analysed individually in order to provide a much larger quantity of material for analysis. An appropriate number of items to be homogenized for a sample depends on the organism in question (see the examples below). However, the number of individuals that are homogenized should be consistent on each sampling occasion. Some examples of recommended sampling strategies for temporal trend monitoring are given below.

All samples should be collected at the same time each year/season.

a)   Mussels (Mytilus spp). 5 samples should be collected at each location to be investigated, each sample being a homogenate of the tissue of 25 individuals.

b)   Fish muscle. A length stratified sample, consisting of 5 individuals from each of 5 length classes should be chosen. Each tissue is analysed. Thus, there are 25 samples on each sampling occasion.

c)   Fish liver. As for fish muscle, except that for some species, it is necessary to homogenize the 5 individuals in the smallest length class to produce a large enough quantity of material for analysis. Thus, there are 21 samples each year. It is important that the same protocol is followed each year; ie the 5 individuals from the smallest length class should either be homogenized or be examined as individuals; under no circumstances should one alternate this arrangement on successive years.

4. OTHER FACTORS AFFECTING DATA QUALITY

UNEP has expressed concern about the quality of data arising from monitoring programmes, particularly the accuracy and comparability of data produced by individual laboratories. To date it has dealt with this latter problem by encouraging laboratories to participate in intercomparison exercises, by arranging for less experienced analysts to be given training in analytical procedures and by arranging reference analytical methods to be produced and distributed to participants.

However, the acquisition of reliable and relevant data of the appropriate quality, for contaminants in marine samples, is dependent on four factors, which are:

a)   a meaningful sampling programme

b)   a suitable storage and pre-treatment procedure for samples following collection and prior to analysis

c)   the production of accurate and precise analytical measurements

d)   a data assessment procedure

All four factors must be given adequate consideration before and during any monitoring programme if the investigator is to achieve the overall aims of the programme. The term used to describe this approach to monitoring work is "quality assurance (QA)". A manual on QA is currently being produced under the auspices of UNEP/IOC/IAEA and on completion it will be distributed to all participants in MEDPOL.

The QA manual will provide general guidance on quality assurance and outline the approaches that should be taken by laboratories to achieve the specific aim(s) for each monitoring programme. Once this manual is available, it is essential that all MEDPOL laboratories use it during the conduct of monitoring work in the Mediterranean Sea area. In the interim period, laboratories who wish to improve data quality in relation to the main aims of the MED POL programme should follow the above guidelines for sampling strategy and adopt the following approach in relation to:

Collection of organisms
Storage and pre-treatment of organisms
Analytical work
Data assessment and documentation of data

5. COLLECTION, STORAGE AND PRE-TREATMENT OF SAMPLES

Ensure that the collection procedure does not result in contamination of the samples.

Select storage containers and conditions of storage that ensure that the sample does not deteriorate during storage and that they are not contaminated by the storage container.

In relation to the pre-treatment of samples (ie dissection of tissue, subsampling etc) prior to analysis, make sure that these tasks are done in an area of the laboratory which is free from contamination and that the dissection tools do not contaminate the sample.

Ensure that the staff doing the field work receive adequate training before they commence this work and that they are given written instructions on what to do on each sampling occasion. This is the responsibility of the manager of the monitoring work.

6. ANALYTICAL QUALITY ASSURANCE

Following the selection of analytical method, the analyst should check the performance of the method using a relevant certified reference material (if in doubt about which to use consult experts at ILMR).

Each laboratory should establish a programme of in-house quality control and assessment which includes the use of control charts, to monitor laboratory performance throughout the monitoring programme and provide a measure of long term variance of the analysis.

Laboratories should participate in any intercomparison exercises organised by ILMR.

Analysts should ensure that they have well-maintained instrumentation so that these instruments are always operating at maximum efficiency.

7. DOCUMENTATION, ASSESSMENT AND REPORTING OF DATA

Check the documentation procedures to ensure that they provide a good description and permit identification of samples from the time of collection to the time when the data is evaluated and stored on computer.