UNITED NATIONS ENVIRONMENT PROGRAMME
MEDITERRANEAN ACTION PLAN
MED POL

# MED POL PHASE III

# PROGRAMME FOR THE ASSESSMENT AND CONTROL OF POLLUTION IN THE MEDITERRANEAN REGION

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## PREFACE

The MED POL-phase III Programme was adopted by the Extraordinary Meeting of the Contracting Parties to the Barcelona Convention held in Monpellier from 1 to 4 July 1996. The Programme, as approved, included three main components: Assessment of pollution; Pollution control; and Supporting measures. Monitoring was recognised as a fundamental tool for the implementation of the Programme.

As a result, after the formal approval of the fundamental principles of the Programme in Montpellier, the MED POL Secretariat, in cooperation with the competent UN Agencies and Organizations and the experts of the region, elaborated more detailed guidelines for the implementation of the monitoring activities. A number of technical Meetings took place and, as a result, the operational details related to trend monitoring, biological effects monitoring and compliance monitoring were presented to the Contracting Parties Meeting held in Tunis in 1997 where thy were approved as an essential tool for the formulation and implementation of national monitoring programmes. The present document assembles all those documents approved by the Contracting Parties in relation to the description of MED POL Phase III and its main operational aspects.

## 1. BACKGROUND

**1.1** The MED POL Programme, designed initially as the environmental assessment component of the Mediterranean Action Plan, has been operational since 1975. Its first phase (MED POL-Phase I) was implemented from 1975 until 1980 and it comprised seven basic baseline studies covering the major marine pollution problems in the Mediterranean. In 1981, the Contracting Parties to the Barcelona Convention approved a new ten-year long-term programme (MED POL-Phase II, 1981-1990) which included two main components, monitoring and research. In 1991, the Contracting Parties decided to extend MED POL-Phase II until 1995. In 1995, it was further extended to 1996 to allow the completion of the programme as well as the formulation of the next phase.

**1.2** In fact, during the implementation of MED POL-Phase II, the need was felt to bring the MED POL Programme closer to the other components of the Mediterranean Action Plan and in particular to the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-Based Sources, which entered into force in 1983, and the more recent Coastal Areas Management Programme (CAMP). As a result, preparations were made to refocus the MED POL Programme and thus prepare a new phase of the programme (MED POL-Phase III, 1996-2005). In addition, global events such as the Rio de Janeiro Summit, Agenda 21 and the Contracting Parties meeting in 1995 outlined a different and more integrated dimension in the approach to marine pollution control programmes, i.e. towards sustainable development.

**1.3** As early as 1989, a meeting of experts took place to evaluate the main pollution data gathered until then through MED POL (UNEP(OCA)/MED WG. 5/3). Four experts prepared specific reports on sources of pollution (UNEP(OCA)/MED WG.5/Inf.3), micro-organisms in coastal areas (UNEP(OCA)/MED WG.5/Inf.4), heavy metals in coastal and reference areas (UNEP(OCA)/MED WG.5/Inf.5) and petroleum and chlorinated hydrocarbons in coastal and reference areas (UNEP(OCA)/MED WG.5/Inf.6), which presented the available data, showed the existing gaps, and made suggestions for the improved collection and use of data.

**1.4** In addition, several meetings and consultations took place within and outside the Secretariat with the scientific community and the United Nations bodies involved in the Programme; in particular, large forums such as the ICSEM/IOC/UNEP Workshops on pollution of the Mediterranean Sea were utilized to discuss with the scientific community the major achievements and shortcomings of the Programme and to propose new approaches. Two review papers, "Monitoring Strategies of Marine Pollution" and "Pollution Problems in the Mediterranean and Relevant Research Strategies", were presented and widely discussed during the Xth CIESM/IOC/UNEP Workshop held in Perpignan (1990). The papers summarized and critically analysed the work carried out within the framework of MED POL in the field of monitoring and research and proposed follow-up activities. During the XIth CIESM/IOC/UNEP Workshop on Pollution held in Trieste (1992) another review paper "The Data Quality Assurance Programme of MED POL" presented the new strategy of MED POL as to data quality assurance and the prospects in the specific field. Discussions on the subject of the new phase of MED POL were also held in the course of all Inter-Agency Advisory Committee Meetings of MED POL.

**1.5** In 1992, the Bureau of the Contracting Parties asked the Secretariat to organize the preparation of an in-depth evaluation of the MED POL Programme by scientists/experts external to the MAP office with a view to using this evaluation in the drafting of Phase III of MED POL. Five consultants worked during 1993 and an evaluation was prepared and presented to the

Eighth Ordinary Meeting of the Contracting Parties in October 1993 (UNEP(OCA)/MED IG.3/Inf.6). During the latter Meeting, the Contracting Parties formally agreed that a Phase III of MED POL should be prepared covering the period from 1996 to 2005 and, to this effect, they set a number of basic objectives and principles to be used in its preparation (UNEP(OCA)/MED IG.3/5, Annex IV).

**1.6** The meeting of experts on the preparation of MED POL-Phase III was held in Izmir from 20 to 23 June 1994 with the partial financial support of the Government of Turkey. Twenty experts from the Mediterranean and elsewhere attended the meeting, together with representatives of United Nations agencies and international organizations (UNEP(OCA)/MED WG.75/3). The meeting, after reviewing and discussing the achievements and shortcomings of Phases I and II of the MED POL Programme, prepared a draft MED POL-Phase III Programme, which was submitted for approval to the Joint Meeting of the Scientific and Technical Committee and the Socio-Economic Committee (Athens, 3-8 April 1995). Due to lack of time, this document was not considered by the Joint Meeting and delegations were requested to provide comments to the Secretariat in writing. After reviewing the comments received and taking into account the results of the informal consultation meeting on MED POL-Phase III (Athens, 13-15 December 1995), the document was revised to bring it in line with the Action Plan for the Protection of the Marine Environment and the Sustainable Development of the Coastal Areas of the Mediterranean (MAP-Phase II), approved by the Contracting Parties in June 1995. Finally, the revised document was first submitted to the Meeting of the MED POL National Coordinators (Athens, 18-22 March 1996), who discussed it in detail and agreed on its content, and subsequently transmitted to the Meeting of MAP Focal Points (Athens, 6-10 May 1996) who approved it. The present document is the final text adopted by the Contracting Parties at their Extraordinary Meeting held in Montpellier from 1 to 4 July 1996.

**1.7** Several technical meetings of Governments experts were held following the adoption of the MED POL Phase III Programme in order to formulate and approve operational details of the various components of the Programme. As a result, the Contracting Parties adopted the technical details related to the compliance monitoring, the trend monitoring and the biological effects monitoring at their Ordinary Meeting held in Tunis in 1997.

## 2. INTRODUCTION

**2.1** The organization of a programme for monitoring of the sources, levels and effects of contaminants, as well as the research related to this monitoring, was one of the cornerstones of the Mediterranean Action Plan (MAP) adopted by the governments of the Mediterranean countries in February 1975. With the adoption, in 1976, of the Barcelona Convention by the same governments, and the subsequent adoption of the Protocols to this Convention, the objectives and methodologies of the programme were gradually modified in order to respond to the expanding goals set by the governments.

**2.2** The declared objectives of the first phase of the Programme, MED POL-Phase I (1975-1980), were:

- to formulate and carry out a coordinated pollution monitoring and research programme taking into account the goals of the Mediterranean Action Plan and the capabilities of the Mediterranean research centres to participate in it;

- to assist national research centres in developing their capabilities to participate in the Programme;

- to analyse the sources, levels, pathways, trends and effects of pollutants relevant to the Mediterranean Sea;

- to provide the scientific/technical information needed by the Governments of the Mediterranean States and the EEC for the negotiation and implementation of the Convention and its related Protocols; and

- to build up consistent time-series of data on the sources, pathways, levels and effects of pollutants in the Mediterranean Sea and thus contribute to the scientific knowledge of the Mediterranean Sea.

**2.3** While the first phase of the Programme focused on strengthening national capabilities in order to enable all countries to participate in the Programme and on the development of methodologies needed to implement it, the next phase of the Programme[1] (MED POL-Phase II, 1981-1996) had more general and broader objectives to provide the Parties to the Barcelona Convention with:

- information required for the implementation of the Convention and the Protocols;

- indicators and evaluation of the effectiveness of the pollution prevention measures taken under the Convention and the Protocols;

- scientific information that may lead to eventual revision and amendment of the relevant provisions of the Convention and the Protocols, and for the formulation of additional protocols;

- information that could be used in formulating environmentally sound national, bilateral and multilateral management decisions essential for the continuous socio-economic development of the Mediterranean region on a sustainable basis; and

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[1] *Long-term programme for pollution monitoring and research in the Mediterranean (MED POL)-Phase II*. UNEP Regional Seas Reports and Studies No. 28. Rev.1. UNEP, 1986.

- periodic assessment of the state of pollution of the Mediterranean Sea.

**2.4** During the second phase of MED POL:

- the gains of the first phase were consolidated by considerable strengthening of national institutional capabilities through: training; provision of equipment; development of suitable sampling and analytical techniques, quality assurance programmes including intercalibration exercises, equipment maintenance and other forms of assistance;

- the monitoring of the levels and effects of contaminants was intensified, and gradually focused on monitoring related to compliance with the pollution control measures adopted by the Contracting Parties, through agreements with governments involving about 80 national institutions in practically all Mediterranean countries;

- the research programme contributing to the improved understanding of the requirements for pollution control measures was considerably broadened, and implemented through more than 500 research contracts with national institutions in practically all Mediterranean countries;

- a detailed survey (inventory) of pollutants from land-based sources, as defined by the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-Based Sources (LBS Protocol), was initiated;

- consistent databases resulting from monitoring, research and survey activities, and other sources, were built-up and used in the preparation of studies, analyses and assessments of specific environmental pollution problems;

- a regional assessment of the environmental state of the marine and coastal environment was prepared (1989 and 1995);

- a regional assessment of the possible implications of expected climate changes was prepared (1992 and 1995), and 11 detailed site-specific studies were carried out on the implications of these changes, with concrete recommendations for the possible mitigation of the negative effects;

- in-depth analyses ("assessment documents") of 13 specific problems related to the control of individual contaminants (or group of contaminants) covered by the LBS Protocol were prepared and used as the basis for the formulation of control measures subsequently adopted by the Parties to the Convention; and

- significant input was made from all activities listed above into the Coastal Area Management Programme (CAMP) carried out within the framework of the Action Plan.

**2.5** The Eighth Ordinary Meeting of the Contracting Parties to the Barcelona Convention (Antalya, 12-15 October 1993) reaffirmed the objective of the Mediterranean Action Plan (MAP) since its establishment to act as an instrument of regional cooperation covering the concerns both of the environment and of development, and approved a set of recommendations (UNEP(OCA)/MED IG.3/5, Annex IV) on the general strategy to be followed in MAP, as well as the action to be taken under specific components of MAP in order to implement this strategy.

**2.6** Recognizing that:

- in line with UNCED and Agenda 21, further emphasis is to be given to those MAP activities

contributing to the implementation of the sustainable development concept; and that

- MED POL, as the scientific and technical component of MAP, provides the scientific basis for decision-making related to marine pollution in the region in the process of achieving sustainable development;

recommendation 7.2 of the Antalya meeting called for the development of MED POL-Phase III and specified the fields in which it should assist the Contracting Parties, with the following overall objectives:

- organization of a Mediterranean coordinated marine pollution monitoring and research programme, concentrating on contaminants and pollutants affecting the quality of the marine and coastal environment, as well as the health of man and of the living resources in the Mediterranean and interpretation/ assessment of the results of the programme as part of the scientific basis for decision-making in the region;

- generation of information on the sources, levels, amounts, trends (trend monitoring) and effects of marine pollution, development of capabilities for assessing the present and future state of the marine environment within the Mediterranean region as an additional component of the scientific basis upon which the formulation of proposals for preventive and remedial action can be based;

- formulation of proposals for technical, administrative and legal programmes and measures for the prevention and/or reduction of pollution;

- strengthening and, when necessary, development of the capabilities of the national institutions, in accordance with the circumstances and the country requesting it, so as to implement monitoring and research of pollution of the marine environment; and

- assistance, as appropriate, to Contracting Parties for the implementation of the recommendations adopted with a view to the assessment of their effectiveness; this assistance will allow the competent authorities to verify the recommendations adopted taking into account data of a satisfactory standard.

**2.7** The Ninth Ordinary Meeting of the Contracting Parties (Barcelona, 5-8 June 1995) approved the Action Plan for the Protection of the Marine Environment and the Sustainable Development of the Coastal Areas of the Mediterranean (MAP-Phase II). Chapter 3 of MAP-Phase II, which deals with the assessment, prevention and elimination of marine pollution, gives the framework for MED POL-Phase III. In addition, the adoption of the amendment to the 1980 LBS Protocol by the Conference of Plenipotentiaries held in Syracuse on 6-7 March 1996, also provides MED POL with the legal framework of pollution control for the Mediterranean, thus indicating the main programme strategy to be followed.

**2.8** The main strategic change in the MED POL Programme is therefore the shift of the emphasis from pollution assessment to pollution control, which brings the programme close to the objectives of the LBS Protocol and MAP-Phase II and makes it an effective tool for achieving sustainable development. The Programme also includes monitoring for compliance purposes, especially as far as the control measures adopted are concerned.

## 3. OBJECTIVES OF MED POL-PHASE III (1996-2005)

**3.1** The objectives of MED POL-Phase III were formulated taking into consideration the experience gained during MED POL-Phases I and II, as well as the documents adopted by the Ninth Ordinary Meeting of the Contracting Parties (Barcelona, 5-8 June 1995), namely, MAP-Phase II, the Barcelona Resolution, the Priority Fields of Activities (1996-2005) and the amended Barcelona Convention and Protocols.

**3.2** The ultimate and overall objective of MED POL-Phase III (1996-2005) is the elimination of pollution[2] of the Mediterranean Sea from all activities that cause such pollution, in particular land-based activities, through the full implementation of the LBS Protocol. MED POL-Phase III provides the basis for action related to assessment, prevention and elimination of marine pollution and relates such action to other components of MAP-Phase II in the perspective of sustainable development.

The specific objectives of MED POL-Phase III are in particular:

(a) the assessment of all (point and diffuse) sources of pollution, the load of pollution reaching the Mediterranean Sea, and the magnitude of the problems caused by the effect of contaminants on living and non-living resources, including human health, as well as on amenities and uses of the marine and coastal regions;

(b) assistance to countries, including capacity-building, in the development and implementation of national action plans for the elimination of marine pollution, in particular from land-based activities;

(c) the assessment of status and trends in the quality of the marine and coastal environment as an early warning system for potential environmental problems caused by pollution;

(d) the formulation and implementation of action plans, programmes and measures for the prevention and control of pollution, for the mitigation of impacts caused by pollution and for the restoration of systems already damaged by pollution; and

(e) the monitoring of the implementation of the action plans, programmes and measures for the control of pollution and the assessment of their effectiveness.

**3.3** In view of the broad cross-sectoral mandate of MED POL with heavy emphasis on pollution control from all sources, in particular from land-based sources and activities, and taking into account the fact that the control of marine pollution is one of the central issues to be resolved within the framework of MAP-Phase II in order to enable the sustainable development of the Mediterranean region, the new phase of MED POL will require intensified interaction between MED POL and practically all

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[2] In the context of this document, "pollution of the marine environment" is interpreted according to the definition adopted in the United Nations Convention on the Law of the Sea and in the Barcelona Convention (as amended in 1995) as:

*the introduction by man, directly or indirectly, of substances or energy into the marine environment, including estuaries, which results or is likely to result in such deleterious effects as harm to living resources and marine life, hazards to human health, hindrance to marine activities, including fishing and other legitimate uses of the sea, impairment of quality for use of sea water and reduction of amenities.*

other components of MAP, and with the Coastal Area Management Programme (CAMP) in particular. Therefore, in outlining the possible elements of MED POL's new phase, an attempt is made in the present document to link the specific objectives and activities proposed for MED POL-Phase III with those adopted for the other components of MAP-Phase II.

**3.4** In addition to reflecting the links between MED POL-Phase III and the other components of MAP-Phase II, the Programme was also prepared with due regard for the concepts and recommendations contained in Agenda 21 as they bear on activities relevant to MED POL, specifically those contained in Chapter 17 of Agenda 21[3].

**3.5** The stated goals shall be achieved through the implementation of interdependent and linked (see Figures 1 and 2) activities grouped in three basic MED POL-Phase III programme elements (assessment of pollution-related problems; pollution control; and supporting measures), all contributing to the ultimate goal of MED POL-Phase III and MAP-Phase II. The rationale of these activities, their specific objectives and means of implementation are described in Sections 5-8 of the present document.

**3.6** The development of suitable measures for the prevention, abatement and control of pollution from all sources, and continuous assessment of the effectiveness of their implementation, are the central goals of the new phase of MED POL. All other activities are subsidiary to these goals and contribute to their more efficient achievement. By concentrating on these goals, MED POL-Phase III is expected to provide critically important inputs into practically all other components of MAP-Phase II, notably CAMPs (giving due consideration to pollution problems associated with coastal development), and thus make a significant contribution to the sustainable development of the Mediterranean region.

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[3] Chapter 17 of Agenda 21, adopted by the United Nations Conference on Environment and Development (Rio de Janeiro, 3-14 June 1992), lists 33 objectives and more than 180 types of activity that are recommended under the heading *Protection of the oceans, all kind of seas, including enclosed and semi-enclosed seas, and coastal areas and the protection, rational use and development of their resources.*

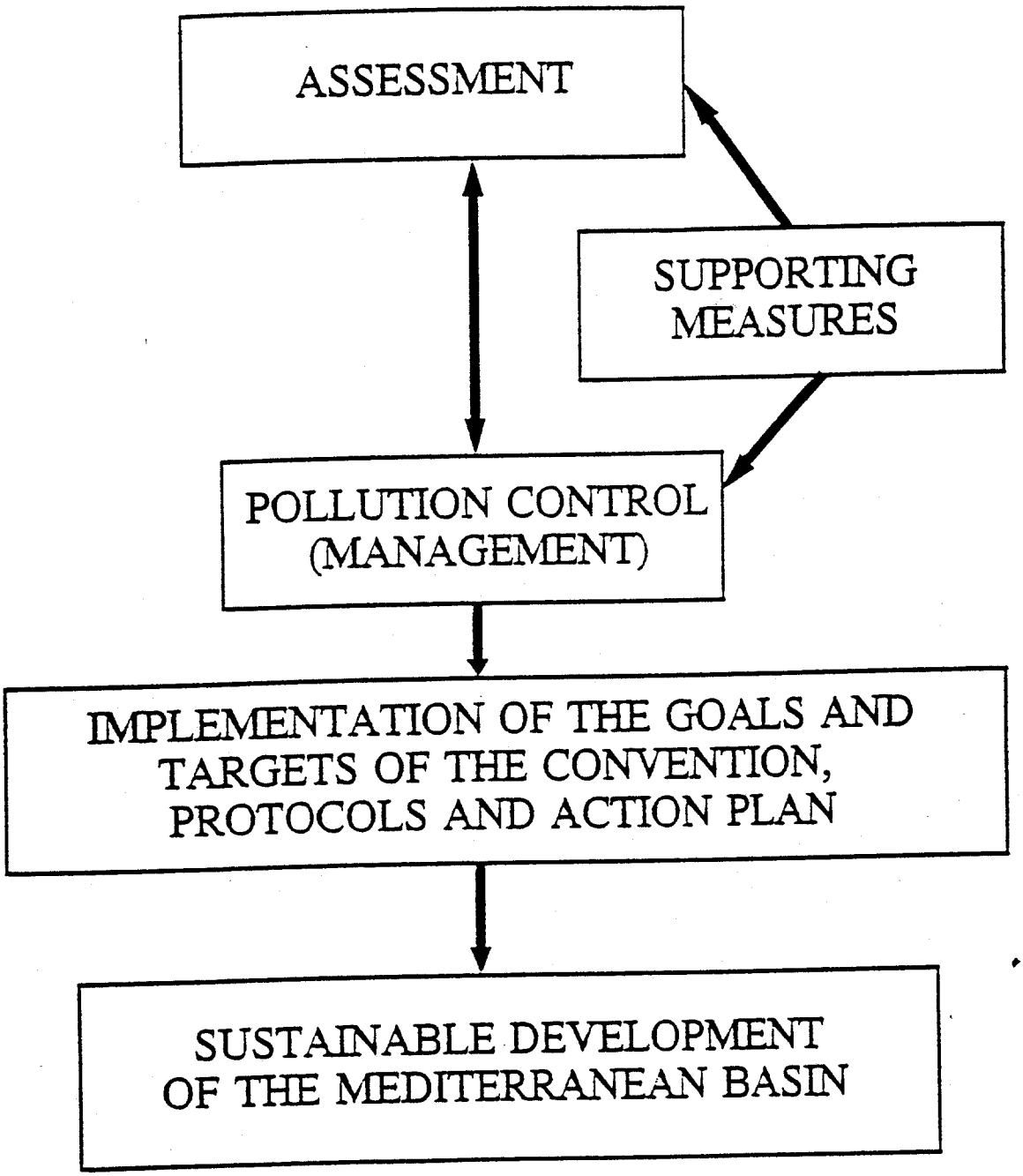


Figure 1: Relationship of MED POL-Phase III to the goals of the Mediterranean Action Plan emphasizing the feed-back relationship between assessment and pollution control.

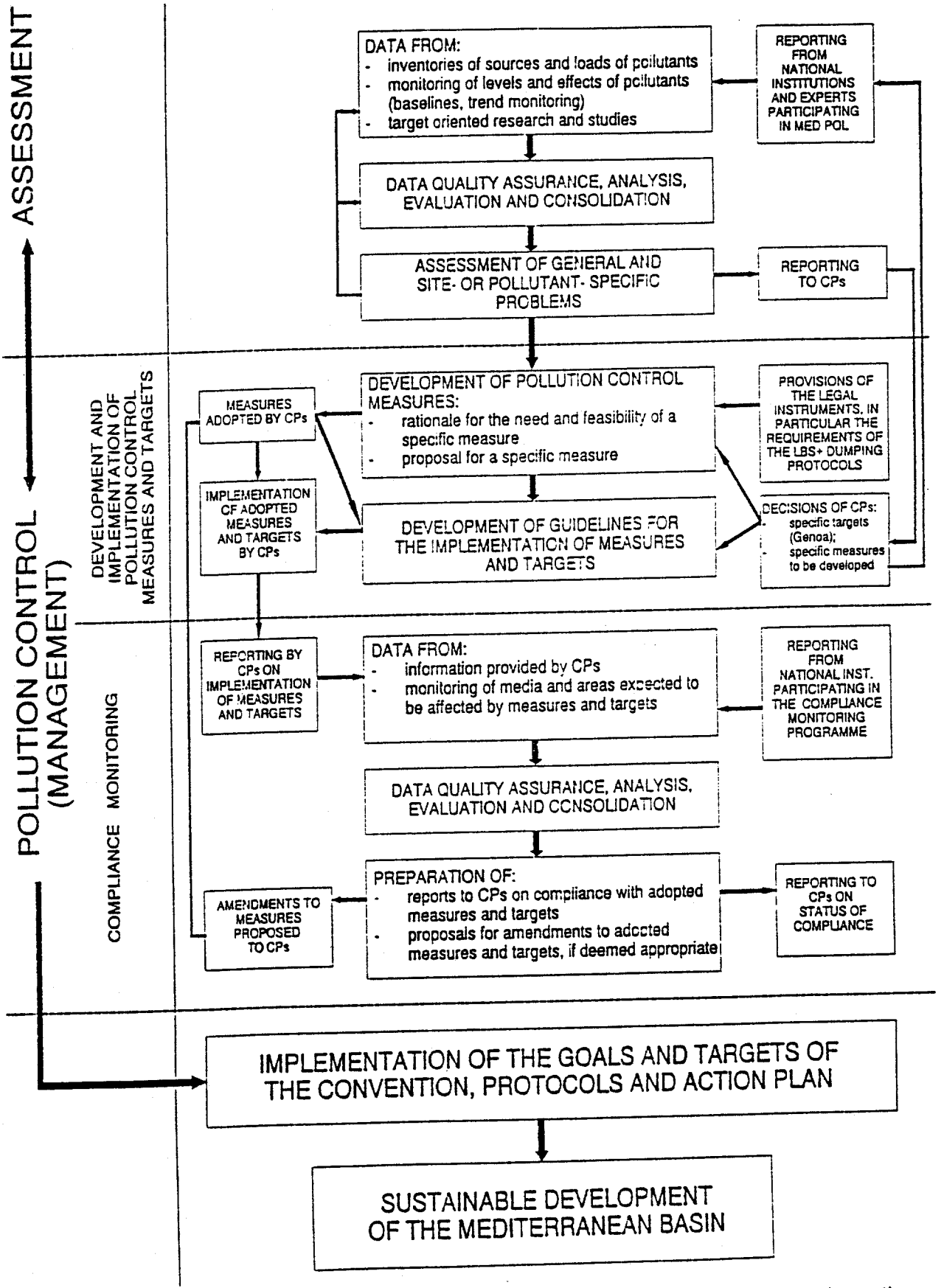


Figure 2: Simplified flow chart showing the more important links between the substantive activities of MED POL-Phase III.

## 4. MODALITIES OF COOPERATION BETWEEN THE CONTRACTING PARTIES AND THE SECRETARIAT REGARDING MED POL

**4.1** In order to ensure the efficient coordination of national efforts related to MED POL and a streamlined communication channel between the *Secretariat of MAP* and the national structures designated by the Contracting Parties to participate in MED POL, each Contracting Party designates a person or office as the *National Coordinator for MED POL*. Their mutual responsibilities shall be as follows:

**Responsibilities of the National Coordinators for MED POL**

**4.2** The National Coordinators for MED POL should actively promote MED POL-related activities in their respective countries and should maintain close and continuous contact with the MED POL Collaborating Institutions, other national agencies involved in the implementation of MED POL, as well as the Secretariat. In order to maximize the National Coordinators' efficiency, the Contracting Parties should establish, as appropriate, national mechanisms (e.g. Intersectoral Coordination Committees, Technical Committees, Scientific Advisory Groups) to assist the National Coordinators in the fulfilment of their duties. Furthermore, the Contracting Parties should endeavour to involve the National Coordinators in MAP-related decision-making at the highest possible levels[4].

**4.3** Responsibility for implementing MAP II of the Barcelona system lies with the MAP Focal Points and consequently also the implementation of MED POL. It is the responsibility of the MAP Focal Points to assist MED POL National Coordinators in the implementation of MED POL.

**4.4** The specific responsibilities of the National Coordinators shall be:

(a) to ensure the implementation of all activities of the national monitoring programme of MED POL covering compliance and trend monitoring;

(b) to ensure selection and designation of *National MED POL Collaborating Institutions* and coordinate their activities relative to all MED POL activities;

(c) to serve as the channel for all formal communications between the Secretariat and the National MED POL Collaborating Institutions, while for technical matters Institutions may be contacted directly by the Secretariat;

(d) to ensure the collection and evaluation of the data and information provided by the National MED POL Collaborating Institutions and to transmit these data and information annually, as well as their evaluation, to the Secretariat according to agreed formats and schedules;

(e) to ensure preparation and submission of reports on dumping activities relevant to the Dumping Protocol and on implementation of the LBS Protocol;

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[4] Should the Contracting Parties establish National Committees for MAP, the National Coordinators for MED POL should be members of such Committees.

(f) to organize the preparation of national surveys and/or inventories of point and non-point land-based sources of pollutants relevant to the LBS Protocol, including those relevant to airborne pollutants;

(g) to organize the preparation of national reports on the state of the marine and coastal environment areas, to be prepared every four years with the first report being prepared by the year 2001;

(h) to follow the progress achieved in the implementation of national MED POL-related activities and to report to the Secretariat thereon according to agreed formats and schedules;

(i) to participate in or be represented at the meetings of the MED POL National Coordinators; and

(j) to review the MED POL-related technical and policy documents and proposals prepared by the Secretariat before their submission to the Contracting Parties, and to advise the Contracting Parties and the Secretariat on how to handle these documents and proposals.

**Responsibilities of the National MED POL Collaborating Institutions**

4.5 As far as the national monitoring programmes are concerned, individual responsibilities shall be determined by the National Coordinators for MED POL in consultation with the Secretariat, as appropriate; such responsibilities shall be reflected in the monitoring agreements signed between the Secretariat and the National Coordinators for MED POL, as appropriate. The Institutions shall report to the Secretariat through the respective Coordinator according to agreed formats and schedules, and shall participate in the ongoing mandatory Data Quality Assurance programmes organized by the Secretariat.

**Responsibilities of the Secretariat**

**4.6** The specific responsibilities of the Secretariat shall be:

(a) to coordinate and harmonize the work carried out within the framework of the agreed national MED POL programmes in close cooperation with specialized bodies of the United Nations system supporting or participating in the Programme; this shall be done in close consultation and cooperation with National Coordinators for MED POL, National MED POL Collaborating Institutions, MAP's Regional Activity Centres and international and intergovernmental specialized organizations;

(b) to evaluate and analyse the data stored in the Secretariat's database received through the National Coordinators for MED POL;

(c) to organize Data Quality Assurance programmes with or through the relative competent United Nations specialized agencies participating in the Programme, as appropriate;

(d) to organize and implement training and capacity-building activities when needed and requested by developing countries;

(e) to convene the periodic meetings of the MED POL National Coordinators and any other *ad hoc* groups of experts called to:

- assist in the analysis, evaluation, and integration of data and information made available through the National Coordinators for MED POL or other sources; and

- review and advise on the technical and policy documents prepared by the Secretariat and the United Nations specialized agencies;

(f) to prepare, jointly with or through the relevant competent United Nations specialized agency or agencies participating in the Programme, whenever appropriate, technical and policy documents, including guidelines, for the Contracting Parties based on data and information received through the National Coordinators for MAP, through MED POL Collaborating Institutions, through other research Institutions and open scientific literature. These technical and policy documents include:

- reports on the state and trends in the environmental quality of the marine and coastal areas; and

- proposals for action plans, programmes and measures for pollution control, including those that may prevent or abate the environmental degradation of these areas, or contribute to the restoration of the areas affected by degradation; and

(g) to provide the Contracting Parties, and other interested bodies with information available on the state of the Mediterranean environment.

## 5. ASSESSMENT OF POLLUTION-RELATED PROBLEMS

Basis for action

**5.1** A scientific assessment of pollution-related problems of the Mediterranean region is one of the basic prerequisites for development of a rational approach towards the sustainable development of the region. Such an assessment, together with information provided through the other components of MAP-Phase II, is the sound foundation for the decisions and recommendations of the Contracting Parties to the Convention to adopt action plans, programmes and measures suitable and applicable in the Mediterranean region[5].

Objectives

**5.2** The specific objectives of this programme element shall be:

(a) to identify the sources, assess the present levels and keep under periodic review the trends in the load of contaminants reaching the Mediterranean Sea from marine and land-based sources including point and non-point sources and airborne contaminants. This will constitute an inventory of sources of pollution required as basic information for the implementation of the LBS and other Protocols[6];

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[5] Articles 5, 6, 7, 8 and 11 of the Barcelona Convention (1995).

[6] Paragraph 17.35 of Agenda 21.

(b) to assess, in areas under direct influence of pollution sources (e.g. coastal waters, estuaries), the levels and trends of contaminants and their potentially harmful effects on marine life and human health, and the negative effects on fisheries and aquaculture[6];

(c) to assess, in areas not under direct influence of identifiable point or non-point sources of pollution ("reference areas"), the magnitude of parameters which may serve as indicators for the general trend in the environmental quality of larger areas[6];

(d) to evaluate the anthropogenic loads of pollutants and to assess their potential harmful effects on the marine environment, taking into consideration and comparing (on a sub-regional basis) with background levels of relevant substances;

(e) to identify and assess potential short- and long-term threats to the Mediterranean environment;

(f) to provide the Contracting Parties, and other interested parties, with information available on the state of the Mediterranean environment.

Activities

**5.3** The stated objectives shall be achieved through:

(a) monitoring/studies/surveys, as appropriate, of levels, loads, pathways, and distribution of contaminants and their effects;

(b) monitoring of trends in the levels and effects of contaminants[7] (see Annex);

(c) target-oriented research in support of monitoring activities[8];

(d) analysis and evaluation (at a national, sub-regional or regional level) of pollution related data from surveys, baseline studies and monitoring organized within the framework of MED POL;

(e) preparation of reports on the assessment of specific pollution-related problems of the Mediterranean region including recommendations for action, if deemed appropriate[9];

(f) preparation of national reports on the state of the marine and coastal environment, to be prepared every four years with the first report being prepared by the year 2001;

(g) preparation of short and concise reports on the state of pollution of the Mediterranean environment for each meeting of the Contracting Parties, specifically highlighting the changes and trends identified since the submission of the last report[10]; and

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[7] Article 12 of the Barcelona Convention (1995).

[8] Article 13.3 of the Barcelona Convention (1995).

[9] The recommendations may lead to the development of proposals for concrete pollution control measures, as described in Section 6A of the present document.

[10] Paragraph 17.106 (d) of Agenda 21.

(h) preparation by the Secretariat for the 2001 meeting of the Contracting Parties, of a consolidated report on the state of the Mediterranean environment[11].

**5.4** The monitoring shall concentrate on the assessment of trends in pollution-related problems in order to provide a solid basis for the appraisal of the environmental health of the Mediterranean as a whole, and to serve as an early warning system for the problems that may be encountered in the future (see Annex), as well as the preparation of inventories of point and non-point sources of pollution, particularly the land-based sources, and the monitoring of the pollution loads reaching the Mediterranean from these sources.

**5.5** In some instances, data from monitoring programmes alone will not be sufficient for the assessment of pollution-related problems and their long-term implications. Therefore, in such cases, monitoring data will have to be supplemented by well-defined target-oriented research as indicated by the Contracting Parties.

5.6 Although the overall assessment for the Mediterranean will be organized by the Secretariat of MAP, there is also a need for national assessments in order to decide on national management measures.

Means of implementation

**5.7** The assessment of pollution-related problems will require a high degree of coordination and close cooperation between the Secretariat of MAP[12], the National Coordinators for MED POL, the National MED POL Collaborating Institutions, the specialized agencies of the United Nations system supporting or participating in MED POL, as well as other specialized intergovernmental and international organizations[13]. The modalities of their cooperation are described in Section 4 of the present document.

**5.8** Data and information relevant to the monitoring of trends in the levels and effects of contaminants, as well as to the inventories of pollution sources and loads (paragraphs 5.3(a) and (b) and 5.4), will be generated and provided to the Secretariat by the National MED POL Coordinators and by the National MED POL Collaborating Institutions as described in Section 4.

**5.9** Target-oriented research (paragraphs 5.3(c) and 5.5) will be based on research projects selected by the Secretariat in cooperation with the relative United Nations specialized agency participating in the Programme. For such projects, research contracts will be signed by the Secretariat or agency and the National MED POL Collaborating Institutions, in consultation with the relevant

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[11] Reports of this nature were published in 1990 and 1996.

[12] In the context of this document, UNEP's Coordinating Unit for the Mediterranean Action Plan in Athens is identified as the Secretariat of MAP.

[13] - United Nations Environment Programme (UNEP)
- Food and Agriculture Organization of the United Nations (FAO)
- United Nations Educational, Scientific and Cultural Organization (UNESCO)
- World Health Organization (WHO)
- World Meteorological Organization (WMO)
- International Atomic Energy Agency (IAEA)
- Intergovernmental Oceanographic Commission (IOC)
- World Conservation Union (IUCN)

National Coordinators for MED POL. The Collaborating Institutions may receive financial support from the Trust Fund to cover part of the cost of the research carried out by them.

**5.10** Assistance to developing countries will be needed in the form of training of their national experts and technical assistance (equipment, consumables, Data Quality Assurance) to their national institutions, in order to enable them to participate effectively in the programme element[14].

## 6. POLLUTION CONTROL

**6.1** A scientific assessment of pollution-related problems of the Mediterranean region is only the first step towards action to prevent, abate and control pollution and its effects. Therefore, the substantive focus of MED POL-Phase II gradually shifted from assessment of the problems related to pollution to the development of proposals for concrete pollution control measures. Taking into account the data and information obtained in the previous phases of MED POL and relying on a permanent system for keeping the present assessment up to date through activities envisaged in Section 5 of the present document, MED POL-Phase III will further emphasize the development of action plans, programmes and measures for the control of pollution and compliance with those adopted by the Contracting Parties, as its central activities.

### A. Development and implementation of pollution control measures[15]

Basis for action

**6.2** Pollution from land-based sources was recognized in the very early stages of MAP as the major problem for the Mediterranean region. The adoption of the LBS Protocol (1980), its entry into force (1983), and its amendment (1996), provided the legal basis for the development of action plans, programmes and measures for the control of pollution from land-based sources and activities in accordance with the Protocol.

**6.3** Although the control of pollution from land-based sources remains a major objective of MAP-Phase II, the control of pollutants from other sources and activities is not neglected, as exemplified by the adoption of protocols associated with the Barcelona Convention which deal with pollution from dumping and emergency situations, as well as with offshore exploration and exploitation[16].

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[14] Article 13.3 of the Barcelona Convention (1995).

[15] In the context of this document, *pollution control measures* are broadly interpreted as a combination of technical (technological), economic, legal and administrative policies, measures and practices contributing to the:

- prevention and mitigation of pollutants' impact on human health and on the quality of the marine and coastal environment, including their living and non-living resources, and amenities;
- general decrease of pollution load reaching the Mediterranean Sea;
- rehabilitation of marine and coastal environment damaged by the present impact of pollution; and
- achievement of sustainable development.

[16] *Protocol concerning Cooperation in Combating Pollution of the Mediterranean Sea by Oil and Other Harmful Substances* (adopted in 1976, entered into force in 1978); *Protocol for the Prevention of Pollution of the Mediterranean Sea by Dumping from Ships and Aircraft* (adopted in 1976, entered into force in 1978, amended in 1995); *Protocol for the Protection of*

Objectives

**6.4** The specific objectives of this programme element shall be:

(a) to develop action plans, programmes and measures for the control of pollution as required by the Barcelona Convention and its Protocols and by the decisions and recommendations of the Contracting Parties; and

(b) to implement the action plans, programmes and measures for the control of pollution adopted by the Contracting Parties.

Activities

**6.5** The stated objectives shall be achieved by:

(a) providing an assessment of the magnitude and intensity of the problem, which is to be tackled by the measures ("assessment document"), including a scientifically sound rationale for pollution control measures, taking into account ecotoxicological criteria and the precautionary principle[17];

(b) formulation of proposals for action plans, programmes and measures for the control of pollution, taking into account article 4.4 of the Barcelona Convention (1995)[18] and the feasibility of implementing the measures in the Mediterranean region;

(c) formal adoption of the proposed action plans, programmes and measures, or of their amended versions, by the Contracting Parties;

(d) development of technical guidelines for the implementation of adopted action plans, programmes and measures; and

(e) implementation by the Contracting Parties of the adopted action plans, programmes and measures for the control of pollution.

Means of implementation

**6.6** A high degree of cooperation and coordination will be required among the Secretariat, the Contracting Parties, the National Coordinators for MED POL, as well as the relevant Regional Activity

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*the Mediterranean Sea against Pollution resulting from Exploration and Exploitation of the Continental Shelf, the Seabed and its Subsoil* (adopted in 1994).

[17] Article 4.3 of the Barcelona Convention (1995).

[18] Article 4.4 of the Barcelona Convention (1995) states that:
In implementing the Convention and the related Protocols, the Contracting Parties shall:
(a) adopt programmes and measures which contain, where appropriate, time limits for their completion;
(b) utilize the best available techniques and the best environmental practices and promote the application of, access to and transfer of environmentally sound technology, including clean production technologies, taking into account the social, economic and technological conditions.

Centres of MAP, the specialized agencies of the United Nations system (see footnote 13) as well as the relevant international and intergovernmental organizations to implement the activities listed above.

**6.7** The priorities for the formulation of action plans, programmes and measures, as well as the timetable for the development of proposals, shall be determined by the Contracting Parties.

**6.8** Based on the decisions of the Contracting Parties, the Secretariat will coordinate the preparation of the assessment documents, the formulation of proposals for action plans, programmes and measures, and the technical guidelines for their implementation.

**6.9** Consultants and *ad hoc* meetings of experts may be used by the Secretariat for the preparation of the assessment documents, the proposals for action plans, programmes and measures, and the guidelines for their implementation.

**6.10** The meetings of the MED POL National Coordinators shall review, and revise as necessary, the drafts of the assessment documents, the proposals for action plans, programmes and measures and the guidelines for their implementation, before they are submitted for the consideration of the Contracting Parties.

**6.11** The implementation of the adopted action plans, programmes and measures shall be the responsibility of the individual Contracting Parties[19].

**6.12** Assistance shall be provided through the Secretariat to developing countries requesting training of their national experts, or technical and legal advice to their national institutions, in order to ensure timely and effective implementation of the adopted action plans, programmes and measures for the control of pollution[20].

## B. Compliance control

Basis for action

**6.13** Compliance with the provisions of MAP-Phase II, the Barcelona Convention and its Protocols (in particular the LBS and Dumping Protocols), and specifically with the decisions and recommendations adopted by the meetings of the Parties to the Convention[21], is the key to successful environmental protection of the Mediterranean Sea. The most relevant decisions and recommendations pertinent to the abatement, prevention and control of pollution are:

(a) the relevant targets of the Genoa Declaration, adopted by the Contracting Parties in 1985[22], to be achieved as a matter of priority during the second decade of the Mediterranean Action Plan;

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[19] Regarding potential financial support for the implementation of the adopted measures, see paragraph 7.8.

[20] Article 13.3 of the Barcelona Convention (1995), and Article 10 of the LBS Protocol (1996).

[21] Article 27 of the Barcelona Convention (1995).

[22] Genoa Declaration. UNEP(OCA)/IG.56/5.

(b) the specific action plans, programmes and measures adopted by the Contracting Parties in the context of the implementation of the LBS Protocol[23]; and

(c) the relevant decisions of the Contracting Parties and especially paragraph 6 of the Barcelona Resolution adopted by the Conference of Plenipotentiaries (Barcelona, 9-10 June 1995).

Objectives

**6.14** The specific objectives of this programme element shall be:

(a) to monitor, on a continuous basis, the implementation and to assess the effectiveness of the implementation of action plans, programmes and measures for the control of pollution adopted or recommended by the Contracting Parties;

(b) to identify problems experienced by the Contracting Parties in the implementation of the action plans, programmes and measures, and formulate proposals that may assist in overcoming those problems[24]; and

(c) to keep the Contracting Parties regularly informed about the status of the implementation of the adopted action plans, programmes and measures[25].

Activities

**6.15** The stated objectives shall be achieved through:

(a) analysis and evaluation at a national, subregional or regional level of data and information generated by the Contracting Parties on the status of implementation of the adopted or recommended action plans, programmes and measures for the control of pollution[26];

(b) compliance monitoring programmes[27] carried out by National MED POL Collaborating Institutions (see Section 4 and the Annex);

(c) analysis and evaluation of data and information received through the National Coordinators for MED POL from national compliance monitoring programmes (see Section 4);

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[23] The common measures adopted so far are included in MAP Technical Reports Series No. 95.

[24] Paragraph 17.25 (b) of Agenda 21.

[25] Paragraph 17.35 (b) of Agenda 21.

[26] Article 26 of the Barcelona Convention (1995); Articles 4, 5 and 6 of the Dumping Protocol (1995); and Article 13 of the LBS Protocol (1996).

[27] Article 12 of the Barcelona Convention (1995), and Article 8 of the LBS Protocol (1996).

(d) target-oriented research in support of national compliance monitoring programmes[28]; and

(e) preparation of consolidated reports for the Contracting Parties on the status of the implementation of the action plans, programmes and measures, including recommendations on the ways and means to improve the efficiency of their implementation.

**6.16** The type of data and information that will be expected from the Contracting Parties, may include, *inter alia*:

(a) the status of the adopted or recommended action plans, programmes and measures (paragraph 6.13(b)) in relation to the relevant national legislation and national administrative procedures and practices[29];

(b) information on the permits issued for dumping of waste[30];

(c) the experience gained with the implementation of the action plans, programmes and measures for the control of pollution and dumping permits, and the permits provided for by the LBS Protocol;

(d) the results of the time series of measurements and observations (see Annex) regarding the levels and effects of contaminants in media directly affected by the measures (e.g. effluent waters, recipient waters);

(e) the major marine and land-based sources of marine pollution (including airborne) on the national territories, including coastal waters, and estimates of the amounts of contaminants reaching the marine environment from these sources; and

(f) with regard to land-based pollution, information relevant to the monitoring of the status of the biological diversity, and on threats to specially protected areas, which may come from sources of pollution outside their control[31].

Means of implementation

**6.17** The Secretariat will coordinate all activities envisaged under the programme element. Close cooperation with and among the Contracting Parties and their institutions designated to participate in the programme element is the basic prerequisite for its successful implementation.

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[28] Article 13.2 of the Barcelona Convention (1995), and Article 9 of the LBS Protocol (1996).

[29] Article 27 of the Barcelona Convention (1995) and Article 13 of the LBS Protocol (1996).

[30] Articles 5 and 6 of the Dumping Protocol (1995).

[31] Article 21 of the SPA protocol.

**6.18** Data and information on the status of the adopted or recommended action plans, programmes and measures, and on the experience gained with their application (paragraphs 6.16(a), (b) and (c)) will be provided to the Secretariat by the Parties to the Convention, or on their behalf by the designated National Coordinators for MED POL, without cost to the Trust Fund.

**6.19** Data and information on the results of the time series of measurements and observations, and on the marine and land-based sources of pollution (paragraphs 6.16(d) and (e)) will be provided to the Secretariat by the Contracting Parties through the National Coordinators for MED POL. The costs involved are expected to be shared between the Trust Fund and the relevant national institutions on the basis of agreements between the Secretariat and the relevant national authorities (see Section 4).

**6.20** Target-oriented research (paragraph 6.15(d)) will be based on research projects selected by the Secretariat in cooperation with the relevant United Nations specialized agency participating in the Programme. For such projects, research contracts will be signed by the Secretariat or agency and the National MED POL Collaborating Institutions, in consultation with the relevant National Coordinators for MED POL. The Collaborating Institutions may receive financial support from the Trust Fund to cover part of the cost of the research carried out by them.

**6.21** Assistance shall be provided through the Secretariat to developing countries requesting training of their national experts, or technical advice or assistance (equipment, consumables and Data Quality Assurance[32]) to their national institutions participating in monitoring the effectiveness of the implementation of pollution control measures and reporting on national compliance with these measures.

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[32] See paragraph 7.7 and the Annex.

## 7. SUPPORTING MEASURES

**7.1** References have been made in Sections 5 and 6 of the present document to some of the measures supporting the substantive MED POL activities, but in view of their importance for the MAP as a whole, they are described in these sections of the document in a more comprehensive way.

### A. Assistance (capacity-building)

Basis for action

**7.2** MED POL-Phase III cannot be implemented in a meaningful way without a strong national institutional basis supported with adequate financial resources, equipment and experts. While the situation in developed countries of the Mediterranean region seems adequate to deal with the implementation of the MED POL Programme, the capacity of the developing countries will need further strengthening.

Objective

**7.3** The objective of the programme element is:

- to facilitate the full participation of all Contracting Parties in MED POL, including the implementation of the action plans, programmes and measures for the control of pollution and the recommendations adopted by the Contracting Parties[33].

Activities[34]

**7.4** The stated objective shall be achieved by providing countries requesting assistance with:

(a) technical advice on the most suitable institutional arrangements that may be needed for the implementation of the MED POL programme;

(b) advice and technical assistance in all aspects of design and implementation of national MED POL programmes;

(c) advice on legal[35], technical[36] and fiscal[37] policies, strategies, and practices that may contribute to the implementation of the action plans, programmes and measures for the control of pollution and targets adopted by the Contracting Parties;

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[33] Paragraphs 17.6 (k), 17.9, 17.14, 17.17, 17.23, 17.35 (f), 17.38 (f), 17.40 and 17.104 of Agenda 21.

[34] References to the legislative authority for specific assistance measures are given in the relevant paragraphs of Sections 5 and 6 of the present document.

[35] E.g., review of the adequacy of existing national legislation, proposals for adjustments in national legislation, proposals for new legislation.

[36] E.g., clean production technologies, minimization of waste.

[37] E.g., user fees, charges for violating pollution control measures, pricing policies and practices, fiscal incentives, possible loans and grants from international financial institutions.

(d) individual and group training (e.g. seminars, workshops) of national experts (administrators, technicians, scientists) in all subjects related to the MED POL Programme;

(e) equipment and material donated to the National MED POL Collaborating Institutions;

(f) guidelines, manuals, documents and reference publications relevant to the implementation of the MED POL Programme; and

(g) assistance in maintaining the analytical equipment used in national pollution monitoring programmes.

Means of implementation

**7.5** Provision of the assistance shall be coordinated by the Secretariat, involving as necessary the relevant RACs of MAP, the specialized agencies of the United Nations system, as well as other international and intergovernmental organizations and programmes ready to offer or provide such assistance. The cost of the assistance will normally be at the expense of the Trust Fund, but the Secretariat will also solicit direct bilateral assistance (without cost to the Trust Fund) from countries and financial institutions ready to provide such assistance.

**7.6** Assistance may also be provided by the Secretariat to the MED POL National Coordinators needed to fulfil their role as defined in Section 4.

**7.7** Activities related to Data Quality Assurance (DQA)[38] will continue to be provided to MED POL Collaborating Institutions through the appropriate United Nations specialized agencies. The DQA programme will include all the elements necessary to achieve good quality data. Such elements cover all aspects of the monitoring programme ranging from sampling to data interpretation. The DQA programme should be mandatory and form an integral part of each national monitoring programme (see Annex, paragraphs 10 and 11).

**7.8** The implementation of MED POL may be eligible for financial support (loans or grants) from international or regional financial institutions and mechanisms on a regional or country basis. The Secretariat will explore the possibilities for such support and will assist interested and potentially eligible countries to formulate project proposals aimed at accessing these resources[39].

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[38] Data Quality Assurance is a mechanism for ensuring that the quality of the data is sufficiently reliable for its intended application.

[39] The Global Environment Facility (GEF), the European Union and the World Bank's Mediterranean Environment Programme are at present the three most promising mechanisms that may support MED POL-related activities.

## B. Data and information management[40]

Basis for action

**7.9** The nature and quality of data and information provided through MED POL is of crucial importance for the soundness of the scientific and technical rationale underlying the decisions of the Contracting Parties. Therefore, great emphasis should be placed on appropriate data and information management procedures and techniques.

Objectives

**7.10** The management of MED POL data and information shall have a twofold objective:

(a) to make available to the Contracting Parties on a continuous basis reliable data and the information required for the development and effective implementation of action plans, programmes and measures for the control of pollution; and

(b) to assist all components of MAP, and the Coastal Area Management Programmes (CAMPs) in particular, with data and information on the sources, levels (concentrations), trends and effects of contaminants in the Mediterranean region.

Activities

**7.11** The stated objectives shall be achieved through the following activities of the Secretariat:

(a) collection of data and information resulting from MED POL activities;

(b) quality control (validation) of collected data and information;

(c) storage of validated data and information in appropriate databases maintained at the Secretariat or in the country concerned;

(d) analysis and reduction, if appropriate, of the validated data at a national or regional level;

(e) preparation of synthesis reports (evaluations) on general and specific MED POL related issues; and

(f) distribution (exchange) of data, information and synthesis reports to the Contracting Parties and their subsidiary bodies, RACs, National Coordinators for MED POL, National MED POL Collaborating Institutions, meetings organized within the framework of MAP, and other individuals and organizations, as appropriate, and in accordance with the policy that will be adopted by the Contracting Parties (see paragraph 7.14).

Means of implementation

**7.12** The National Coordinators for MED POL and the National MED POL Collaborating Institutions shall be the primary source of data and information supporting the development and implementation of MED POL.

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[40] Data and information management in the context of this document is understood as involving acquisition, quality control, analysis, evaluation, storage, retrieval and exchange (dissemination) of data and information.

**7.13** The collection, quality control, analysis and evaluation of the data and information will be carried out nationally with the help of the Secretariat if needed, or by the Secretariat (with the help of the relevant organizations of the United Nations system, outside experts, and *ad hoc* meetings of experts, as necessary), for data stored at the Coordinating Unit.

**7.14** The Secretariat's databank will include only data useful for the establishment of trends. The Secretariat, with the assistance of relevant experts, will develop a proposal for data and information distribution policy to be submitted for adoption to the Contracting Parties. The guiding principle of that policy should be that access to the data and information received by the Secretariat will normally be free for the parties involved in MED POL.

**7.15** The reports of the Secretariat to the Contracting Parties will be transmitted through the subsidiary bodies of the Contracting Parties.

## C. Coordination and cooperation

Basis for action

**7.16** MED POL, as one of the basic and most complex MAP activities, linked with virtually all other MAP activities, requires a well-coordinated approach in order to ensure the harmonious cooperation and interaction of, and inputs from, the various actors contributing to its implementation.

Objective

**7.17** The objective to be achieved through the coordination of MED POL activities is:

- to ensure the full implementation of Contracting Parties' decisions relevant to MED POL through the highest degree of efficient cooperation among the Secretariat, national structures, international organizations, and individuals participating in the implementation of MED POL[41].

Activities

**7.18** The stated objective shall be achieved by:

- guidance provided directly or indirectly by the Secretariat to all parties involved in MED POL; and

- close cooperation among all parties involved in MED POL.

Means of implementation

**7.19** The Secretariat shall coordinate all MED POL activities, in close cooperation with the National Coordinators for MED POL[42], the national MED POL Collaborating Institutions, the specialized agencies of the United Nations system, as well as other international and intergovernmental organizations ready to offer or provide support.

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[41] Paragraph 17.10 of Agenda 21.

[42] The modalities of cooperation are described in Section 4.

**7.20** The Meeting of the MED POL National Coordinators, as the subsidiary body of the Contracting Parties, will continue to review periodically the progress of MED POL, evaluate its results and advise the Contracting Parties on the strategies to be followed for its implementation.

**7.21** The MED POL National Coordinators may establish *ad hoc* expert groups to advise them on specialized topics.

**7.22** The coordination of the inputs from the specialized organizations of the United Nations system into MED POL will continue to be ensured through direct working level contacts with these organizations and periodic Inter-Agency Advisory Committee (IAAC) Meetings on MED POL.

**7.23** The periodic meetings of the heads of RACs with the Secretariat will continue to be used to ensure: (a) the coordination between complementary activities of MAP implemented by the Secretariat and the respective RACs; and (b) the inputs of MED POL into activities carried out by RACs.

## 8. IMMEDIATE PRIORITIES FOR THE IMPLEMENTATION OF MED POL-PHASE III

**8.1** In view of the fact that MED POL-Phase III encompasses a wide range of important activities that require urgent implementation, the MED POL budget should be increased substantially. However, since such an increase does not appear feasible through the MED Trust Fund, it is considered necessary to seek outside funding (see paragraph 7.8). Until such outside assistance is possible, it is recommended that, during the initial stage of its implementation, the MED POL Programme concentrate on a number of priority activities.

**8.2** The following activities (not in order of priority), which are in line with the Priority Fields of Activities adopted by the Contracting Parties (Barcelona, 1995), are recommended:

(a) Formulation, including the setting of priorities, and implementation of regional, subregional and national action plans, programmes and measures for the control of land-based pollution.

The implementation of the LBS Protocol will be the cornerstone of MED POL-Phase III. This implementation will be based on national and regional action plans, programmes and measures. In order to formulate such action plans, programmes and measures, MED POL-Phase III will establish the priorities in accordance with those set out in the annexes to the LBS Protocol, taking into account the characteristics of substances provided in Annex I to the Protocol.

(b) Formulation and implementation of a programme of coastal zone trend monitoring on a regional basis.

On the basis of the experience gained through MED POL-Phases I and II and in view of the objectives of MED POL-Phase III, national monitoring programmes will have to be designed or redesigned in order to satisfy national needs and enable the results of the programme to be used as a management tool for controlling marine pollution. A number of fixed coastal stations from the national programmes will be selected for inclusion in a regional monitoring network for the establishment of trends in the Mediterranean. This programme will provide information that can be used for the assessment of the overall quality status of the Mediterranean Sea, as well as for the effectiveness of control measures taken.

(c) Identification of sources (especially major "hot spots") and assessment of loads of pollution.

The preparation of inventories of point and non-point sources of pollution, particularly the land-based sources, and the monitoring of the pollution loads reaching the Mediterranean from these sources, is considered a high priority since such information is necessary for making management decisions. Within this context, a list of major pollution "hot spots" in the Mediterranean will be prepared and relevant action plans (with economic aspects and timetables) for reduction and elimination of pollution will be developed and implemented.

(d) Assistance to countries for the formulation, development and implementation of national monitoring programmes.

Although considerable progress was achieved during MED POL-Phase II, in many cases, national monitoring programmes have not yet produced the expected results, either because the programme was not designed properly or was not implemented fully (temporal and spatial gaps) and the data were not of the required quality. Through MED POL-Phase III, assistance will be provided for the formulation of appropriate monitoring programmes to developing countries requesting it, as defined in paragraph 7.4.

(e) Assistance to countries (including capacity-building) for the implementation and enforcement of adopted pollution control measures.

It is clear that without the proper implementation of the control measures the success of the programme will be jeopardized. In accordance with paragraph 6.12, assistance will be provided to facilitate the implementation and enforcement of measures to developing countries requesting it as defined in paragraph 7.4.

(f) Eutrophication and biological effects to be considered as priority subjects for research.

From MED POL-Phase II results it appears that eutrophication is becoming a major problem at regional level. As a result, special attention should be given to this problem and extra efforts are required for its solution. Being very complex and involving a number of processes, more research is required to understand its causes, effects, geographical distribution and trends and eventually propose remedial action.

The Contracting Parties (Antalya, 1993) have decided to introduce monitoring of biological effects in the MED POL Programme. This is not possible unless reliable techniques that can be used routinely are developed. Research is required to assist in the final selection of the techniques and in developing and testing the methodologies.

## ANNEX

## MONITORING OF THE LEVELS AND EFFECTS OF CONTAMINANTS IN THE CONTEXT OF MED POL-PHASE III

**1.** Two basic types of monitoring are identified within the framework of the MED POL-Phase III Programme: compliance and trend monitoring. Surveys will also be carried out in order to complement the monitoring data and facilitate decision-making for management purposes.

**2.** Compliance monitoring is defined as the collection of data through surveillance programmes to verify that the regulatory conditions for a given activity are being met e.g. concentration of mercury in effluents. In the case of identifying an instance of non-compliance, appropriate enforcement can be established which can be escalated until compliance is achieved.

**3.** Trend monitoring is defined as the repeated measurement of concentrations or effects over a period of time to detect possible changes with time. This type of monitoring will provide information that can be used for the assessment of the state of the environment and the effectiveness of pollution control measures taken. If the effectiveness of measures is deemed inadequate, additional activities may be initiated such as the formulation of new measures or the revision of existing ones, etc.

**4.** Depending on the matrices and parameters included in the programme, monitoring will be carried out for the following purposes:

**Compliance monitoring**

- **Compliance monitoring of health-related conditions** (eg. sanitary quality of bathing areas and waters used for aquaculture, quality of seafood). This type of monitoring has a national significance, but data may also be used for regional assessments;

- **Compliance monitoring of effluents** to determine whether the adopted common measures concerning concentrations of contaminants in effluents (e.g. mercury, cadmium) are complied with; and

- **Compliance monitoring in "hot spot" areas** to determine whether the environmental quality objectives or limit values set are complied with (e.g. DDT in water).

**Trend monitoring**

- **Coastal zone trend monitoring**, through a regional network of selected fixed coastal stations, of parameters that contribute to the assessment of trends and the overall quality status of the Mediterranean Sea. As explained under Section 8, this type of monitoring will be carried out on a regional basis;

- **Trend monitoring in "hot spot" areas** (intensively polluted areas) and high risk areas that are likely to become heavily polluted, are subject to harmful seasonal phenomena such as algal blooms, or where control measures have been taken. This type of monitoring will be designed as necessary at the subregional level, and will be carried out on a national basis, and the data will be utilized for taking management decisions at a local level, including the assessment of the effectiveness of the control measures taken;

- **Trend monitoring of loads** (e.g. from land-based sources of pollution in general or from identified sources, pollutants transported by atmosphere, pollutants carried by rivers) and assessment of loads originating from non-point sources. Data from this type of monitoring will be utilized locally but also for regional assessments; and

- **Trend monitoring of biological effects** at different organizational levels, including molecular, cellular, physiological, behavioural, community and ecosystem levels, can also be used as an early warning system. This type of monitoring can be included in national monitoring programmes as well as in the regional trend programme.

**5.** In addition, surveys will be carried out to complement the monitoring data:

- surveys of health-related effects (e.g. occurrence of illnesses in bathers exposed to contaminated waters and sand and in consumers of seafood) will be carried out on a routine basis;

- surveys of point and non-point land-based sources of pollution needed for the development, compilation and maintenance of inventories, will be carried out; and

- baseline and trend surveys through international and multinational cruises of the whole Mediterranean Sea will be conducted at periodic intervals (once every five or ten years) in order to contribute to the assessment of the overall quality status of the Mediterranean Sea.

**Matrices to be monitored**

**6.** The matrices (one or several) included in monitoring programmes will depend on the objective and purpose of the monitoring. The most common matrices which could be included in monitoring programmes are:

(a) effluents reaching the marine environment from industrial plants, municipal sewerage systems and agriculture drainage channels;

(b) waters, sediments and biota (which also include individuals, populations and communities of marine mammals and sea birds) of marine coastal zones and estuaries which are, or are likely to be, under the direct impact of identifiable point or non-point source(s) of pollution;

(c) atmosphere through which pollutants may enter the marine environment and thus affect its quality; and

(d) humans who may be affected by pollutants through direct or indirect exposure to polluted marine media, or products (e.g. food) derived from such media.

**Parameters or indicators to be monitored**

**7.** Parameters or indicators to be monitored will vary from case to case, i.e. will be site and problem specific. They may include one or several of the following types of physical, chemical or biological parameters or health-related indicators:

- physical and chemical properties of the monitored abiotic media;

- the concentration of a specific chemical compound or group of compounds in a given matrix;

- marine ecosystem health on molecular, cellular, individual organism, community and ecosystem levels (e.g. bioassays, teratogenic or genetic changes if appropriate, biomarkers, histopathology, physiology, population structure);

- sanitary quality of media used by people (e.g. microbiological quality of bathing waters), or for food production (e.g. quality of waters used for and by aquaculture);

- ecological effects of coastal aquaculture (land-based and marine facilities);

- health effects on humans exposed to contaminated media (e.g. bathers) or products (e.g. contaminated shellfish) derived from such media;

- marine litter.

**8.** In case of compliance monitoring, the selection of the parameter(s) to be monitored is determined by the pollution control measure whose compliance is being monitored.

**Programme design**[43]

**9.** For both compliance and trend monitoring, it is essential that the question being posed is both testable and specific, i.e. within a statistical context. The question must relate to a specific environmental compartment, i.e. water, suspended material, sediment or biota. The sequence then is:

- to identify meaningful levels of change and the confidence limits of that change that are to be detected (e.g. with what precision can a 20 per cent loss in number of species of a benthic sediment-living community be detected?);

- to obtain knowledge of special and temporal variability of the element being sampled from a desk study or pilot study;

- application of power analyses is essential in order to rationalize the programme[44];

- selection of elements of the programme taking into account logistic constraints[45];

- define data quality objectives and decide *a priori* on the statistical methods to be applied in analysing the data; and

- to select sampling sites and sampling frequency based on the foregoing information.

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[43] See Guidelines for monitoring chemical contaminants in the sea using marine organisms. UNEP Reference Methods for Marine Pollution Studies No. 6.

[44] See Peterman, R.M. and M'Gonigle, M., Statistical Power Analysis and the Precautionary Principle, Marine Pollution Bulletin, Vol. 24, No. 5, pp. 231-234, 1992.

[45] See also new experimental designs (Underwood, Aust. J. Mar. Sci. 1993).

# APPENDIX I

# COMPLIANCE MONITORING PROGRAMME AS PART OF MED POL PHASE III

## TABLE OF CONTENTS

## BACKGROUND

Following the first phase of the implementation of the MED POL Programme (MED POL - Phase I) from 1975-1980, the Contracting Parties to the Barcelona Convention approved a ten-year long-term Programme (MED POL - Phase II, 1981-1990) consisting of a monitoring and research component. In 1991 the Contracting Parties extended MED-POL Phase II until 1995 and the Programme was subsequently extended until 1996 to enable its completion and the formulation of the next phase.

In 1992 the Bureau of the Contracting Parties requested the Secretariat to organise the preparation of an in-depth evaluation of the MED POL Programme by experts and scientists external to the MAP office, with the intention to use this evaluation in the drafting of Phase III of MED POL. This evaluation was presented to the Eighth Ordinary Meeting of the Contracting Parties in October 1993 (UNEP, 1993a). During this meeting the Contracting Parties formally agreed to the preparation of MED POL Phase III, covering the period 1996-2005, and set a number of basic objectives and principles for its preparation (UNEP 1993b, Annex IV).

The meeting of experts on the preparation of MED POL Phase III held in Izmir, in June 1994, after reviewing and discussing the achievements and shortcomings of Phases I and II of the MED POL Programme, prepared a draft Programme for MED POL Phase III which was submitted for approval to the Joint Meeting of the Scientific and Technical Committee and the Socio-Economic Committee in April 1995. The document was not considered by the Joint Meeting due to lack of time and consequently the delegations were requested to provide comments to the Secretariat in writing. After reviewing the comments received and taking into account the results of the informal consultation meeting on MED POL III (Athens, December 1995), the document was revised to bring it in line with the Action Plan for the Protection of the Marine Environment and the Sustainable Development of the Coastal Areas of the Mediterranean (MAP Phase II) which was approved by the Contracting Parties in June 1995. The revised document was submitted to the Meeting of MED POL National Coordinators (Athens, March 1996), the Meeting of MAP Focal Points (Athens, May 1996) and finally the Extraordinary Meeting of the Contracting Parties (Montpellier, 1-4 July 1996), where it was adopted (UNEP, 1996).

According to the Annex of the MED POL Phase III Programme, two basic types of monitoring will be organised, compliance and trend monitoring. The contents of the document refer to the compliance monitoring and is proposed as a guide for the Contracting Parties for the implementation of this type of activity, taking into consideration that compliance monitoring will be applied for compliance with the national regulatory conditions.

The present document was presented to Meeting of MED POL National Coordinators held at Delphi in May 1997, and has been revised according to the comments made at that meeting with a view to its submission to the Tenth Ordinary Meeting of the Contracting Parties.

# 1. INTRODUCTION

The uncontrolled discharge of liquid waste into the Mediterranean Sea over past decades caused such severe damage to the marine environment that all Mediterranean countries decided to combat the situation by ratifying and implementing the Barcelona Convention and its related Protocols. The Mediterranean Action Plan (MAP), which was the outcome of this common determination, constitutes the essential mechanism for identifying problems and implementing related management techniques.

During MAPs first decade, the problem of inadequate pollution control programmes was recognized as one of the main reasons for the deterioration of the marine environment: discharge of liquid waste without proper treatment, failure to carry out environmental impact assessment studies (EIA), miscalculation of the environmental capacity of the Mediterranean Sea, unsatisfactory operation of existing wastewater treatment plants, etc.

MAP has now entered its third decade and the need for efficient control of the main pollution sources around the Mediterranean Sea has become evident: ratification of the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources (LBS Protocol) and the Genoa Declaration constitute the first steps in this direction.

The Coordinating Unit for the Mediterranean Action Plan (UNEP/MAP) is actively involved in this common effort and has prepared this document to serve as a tool for proper compliance monitoring control of effluents and the ambient marine system, including "hot spot" areas.

The purpose of this document is to provide practical instructions and guidance for the collection and evaluation of the information needed for compliance monitoring of effluents and the ambient marine system.

The technical aspects can be found in the attached list of publications and supplementary technical documents (i.e. handbooks and manuals).

The importance of evaluating the environmental capacity of a water body, the elaboration of regional planning and assessment, the development of quality criteria and quality objectives, and the proper handling of monitoring data are highlighted as these aspects are frequently ignored or underestimated.

As this document represents a first step towards the elaboration of pollution control programmes, further action involving greater technical detail will have to be initiated in the near future in relation to effective implementation of the LBS Protocol.

Some indicative ideas are listed below:

- management techniques for industrial and urban pollution sources (river loads);
- development and implementation of quality criteria for effluents and the ambient marine environment;
- measures to control diffuse sources of pollution;
- continuous up-to-date evaluation of point sources of pollution;
- preparation of an attainability analyses document;
- elaboration of a document for the compliance monitoring of sediments;
- establishment of a body for enforcement of the Protocols;
- identification of sensitive and "hot spot" areas in the Mediterranean;
- development and elaboration of inspection procedures.

Certain countries have already elaborated and implemented some of these measures, but what are lacking are well-defined operational methods suitable to the special conditions of the Mediterranean environment.

# 2. COMPLIANCE CONTROL

## 2.1 Basis for action

Compliance with the provisions of MAP-Phase II, the Barcelona Convention and its Protocols (in particular the LBS and Dumping Protocols), and specifically with the decisions and recommendations adopted by the meetings of the Parties to the Convention[1], is the key to successful environmental protection of the Mediterranean Sea. The most relevant decisions and recommendations pertinent to the abatement, prevention and control of pollution are:

(a) the relevant targets of the Genoa Declaration, adopted by the Contracting Parties in 1985[2], to be achieved as a matter of priority during the second decade of the Mediterranean Action Plan;

(b) the specific action plans, programmes and measures adopted by the Contracting Parties in the context of implementation of the LBS Protocol[3], and

(c) the relevant decisions of the Contracting Parties, especially paragraph 6 of the Barcelona Resolution adopted by the Conference of Plenipotentiaries (Barcelona, 9-10 June 1995).

## 2.2 Objectives

The following are the specific objectives of this programme element:

(a) to monitor on a continuous basis the implementation of action plans, programmes and measures for the control of pollution adopted or recommended by the Contracting Parties, and to assess the effectiveness of their implementation;

(b) to identify problems experienced by the Contracting Parties in implementing these action plans, programmes and measures, and to formulate proposals that may assist in overcoming them[4]; and

(c) to keep the Contracting Parties regularly informed about the status of implementation of the action plans, programmes and measures adopted[5].

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1 Article 13.3 of the Barcelona Convention (1995) and Article 10 of the LBS Protocol (1996).

2 Genoa Declaration. UNEP (OCA)/IG.56/5.

3 The common measures adopted so far are included in MAP Technical Reports Series No. 95

4 Paragraph 17.25 (b) of Agenda 21.

5 Paragraph 17.35 (b) of Agenda 21.

## 2.3 Activities

The stated objectives will be achieved through:

(a) analysis and evaluation at a national, subregional or regional level of data and information generated on action plans, programmes and measures for the control of pollution[6];

(b) compliance monitoring programmes[7] carried out by national MED POL collaborating institutions;

(c) analysis and evaluation of data and information from national compliance monitoring programmes transmitted through the National Coordinators for MED POL;

(d) target-oriented research in support of national compliance monitoring programmes; and

(e) preparation of consolidated reports for the Contracting Parties on the status of implementation of the action plans, programmes and measures, including recommendations on ways and means to improve the efficiency of their implementation.

## 2.4 Monitoring of the levels and effects of contaminants in the context of MED POL Phase III

1. Two basic types of monitoring are identified within the framework of the MED POL - Phase III Programme namely compliance monitoring and trend monitoring. Surveys are also being carried out in order to complement the monitoring data and facilitate decision-making for management purposes.

2. Compliance monitoring is defined as the collection of data through surveillance programmes to verify that the regulatory conditions for a given activity are being met e.g. concentration of mercury in effluents. If a case of non-compliance is identified, appropriate enforcement can be put into effect and escalated until compliance is achieved.

3. Trend monitoring is defined as the repeated measurement of concentrations or effects over a period of time to detect possible changes with time. This type of monitoring will provide information that can be used to assess the state of the environment and the effectiveness of the pollution control measures taken. If the effectiveness of these measures is deemed inadequate, additional action may be taken, for example, the formulation of new measures or the revision of existing ones, etc.

4. Depending on the matrices and parameters included in the programme, monitoring will be carried out for the following purposes:

(a) compliance monitoring;

(b) trend monitoring.

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[6] Article 26 of the Barcelona Convention (1995); Articles 4, 5 and 6 of the Dumping Protocol (1995); and Article 13 of the LBS Protocol (1996).

[7] Article 12 of the Barcelona Convention (1995), and Article 8 of the LBS Protocol (1996).

### 2.4.1 Compliance monitoring

- **Compliance monitoring of health-related conditions** (e.g. sanitary quality of bathing areas and waters used for aquaculture, quality of seafood). This type of monitoring is of national scope, although data may also be used for regional assessments. A comprehensive approach to microbiological and health-related monitoring of recreational and shellfish-growing areas is set out in considerable detail in documents WHO/UNEP (1994) and (1996).

- **Compliance monitoring of effluents** to determine whether there is compliance with the common measures adopted concerning concentrations of contaminants in effluents (e.g. mercury, cadmium); and

- **Compliance monitoring in "hot spot" areas** to determine whether there is compliance with the environmental quality objectives or limit values set (e.g. DDT in water).

### 2.4.2 Programme design[8]

For both compliance and trend monitoring, it is essential that the question being posed is both capable of being tested and specific, i.e. within a statistical context. The question must relate to a specific environmental compartment, i.e. water, suspended material, sediment or biota. The sequence to be followed is:

- to identify meaningful levels of change and the confidence limits of that change to be detected (e.g with what precision can a 20 per cent loss in number of species of a benthic sediment-living community be detected?);

- to obtain knowledge of special and temporal variability of the element being sampled from a desk study or pilot study;

- to apply power analyses in order to rationalize the programme[9];

- to select elements of the programme taking into account logistic constraints[10];

- to define data quality objectives and decide *a priori* on the statistical methods to be applied in analyzing the data; and

- to select sampling sites and sampling frequency based on the foregoing information.

### 2.4.3 Data quality assurance programme

After a scientifically-based national monitoring programme has been designed, a data quality assurance (DQA) programme is required in order to ensure reliability of the data. The programme must cover all aspects of the data quality assurance required, including:

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[8] See Guidelines for monitoring chemical contaminants in the sea using marine organisms. UNEP Reference Methods for Marine Pollution Studies No. 6.

[10] See also new experimental designs (Underwood, Aust. J., Mar. Sci. 1993)

- trained staff;
- appropriate facilities, sampling and measuring equipment and other consumables;
- regular calibration, maintenance, and servicing of the equipment;
- sampling that conforms to sampling design;
- sample handling procedures, including, for example, transportation, preservation, storage, tissue dissection, bone grinder, homogenization, sub-sampling (sub-sampling includes all steps up to measurement);
- regular checks of the accuracy and precision of routine measurements, by analyses of appropriate reference materials (where available) and documentation of the results on control charts;
- external quality assessment (e.g. participation in intercalibration exercises);
- standard operating procedures (written protocols with precise descriptions of all elements of the measurement and quality control procedures);
- recording of all calculations such as data translation and transcription prior to final documentation (record books and/or computers); and
- data evaluation procedures (e.g. converting data into a report).

The results obtained by sampling, measurement and observation must not only be of sufficient analytical (accuracy and precision) quality but must also meet the requirements of the objectives[11] and be comparable on a Mediterranean-wide basis.

[11] Experience with quality assurance programmes, largely based on the practices of MED POL, is described in Contaminant Monitoring Programmes using Marine Organisms: Quality Assurance and Good Laboratory Practice (Reference Methods of Marine Pollution Studies No. 57, UNEP 1990).

# 3. WATER USES (PRESENT AND PLANNED) AND THE ASSESSMENT OF THEIR IMPORTANCE

## 3.1 Maintenance of the ecosystem

Many of the principal human uses of marine waters depend upon successful maintenance and enhancement of the existing ecosystems or, in a few cases, upon creating and continuing new and artificial ecosystems for specific purposes.

The ecosystem includes all of the biological and non-biological (geological, physical, and chemical) components of the environment and their highly complex interaction. Studies of ecosystems must include all that is within the body of water as well as imports into and exports from it. Research in such situations has shown that the biotic elements include producers of organic material, several levels of consumers, and decomposers. In the least complex situation, these act at rates controlled by the abiotic factors to transfer energy and recycle materials.

## 3.2 Uses of the marine system to be protected

Coastal marine waters serve a wide variety of exceptionally important human uses. Many of these uses yield significant local benefits such as the production of shellfish, as well as recreational activities. Other uses involve regional benefits due to the global unity of the marine system, because local factors influence, and are influenced by, water quality at distant points.

Many human uses of marine waters directly depend on the nature and quality of the biological, chemical, and physical systems present. Efforts to protect and enhance these uses will be limited mainly by our ability to understand and protect the environmental conditions that are essential for the biota.

### 3.2.1 Human health

Ideally, criteria for coastal waters in regard to human health should be sets of quantitative exposure-response relationships between environmental exposure factors and effects on the pollution groups exposed. When dealing with human subjects, it is often difficult to establish even a basic cause-effect relationship, and even more difficult to obtain a graded response.

Seawaters are becoming increasingly contaminated chemically and microbiologically and may be a health hazard for man. One of the scientific approaches to demonstrating the relationship between water quality and disease is an epidemiological survey.

Monitoring of water quality is one of the means of assessing the potential risk. However, the recovery of pathogens from bathing waters does not necessarily indicate that the incidence of disease will be significantly increased.

### 3.2.2 Amenities, aesthetics and recreation

The aesthetic qualities of water relate to the general principles laid down in common law. They concern the beauty and quality of water and their concept may vary according to the individual in question. It is not possible to develop any rationale for these qualities by quantifying definitions; nevertheless, decisions on quality factors best reflect the public interest.

Aesthetic qualities provide general rules for protecting water against environmental damage; they represent minimal requirements for freedom from pollution and are essential to the enjoyment of a nation's water resources.

The enjoyment of amenities greatly depends not merely on the availability of an activity, but on the aesthetic satisfaction it affords. Aesthetic satisfaction can be a very positive force in promoting public health and well being. It is experienced through the senses of sight, smell, taste and touch.

As an optimum, when developing criteria to protect aesthetic quality there must be knowledge of the relationship between quality and other environmental factors, how it is detected by the senses, and the related degree of adverse or favourable reaction. In seeking such information, it is obviously necessary to be sure that the population whose reaction is to be assessed is reasonably representative of those whose interests the criteria adopted are intended to protect. In many coastal areas, this may mean ensuring an appropriate balance between the reactions of residents and non-residents, whose requirements and sensitivity may differ.

### 3.2.3 Aquaculture and fisheries[12]

The basis for major marine and coastal fisheries is the capture of wild species produced in estuaries, coastal waters and oceans. The quality and quantity of the available supply of useful species are controlled by the nature and efficiency of the several ecosystems upon which each species depends for its life cycle. Serious pollution at any point in the lower reaches of a river, an estuary, or inshore ocean might, therefore, interrupt the necessary pattern and reduce catches.

Estuaries play a particularly useful role as far as fisheries are concerned. They serve as spawning grounds, nursery areas, havens for parasites and predators, as well as highly productive and rich sources of food. As recipients of waste both from rivers entering them and cities and industries along their shores, estuaries are naturally more susceptible to immediate damage by pollution than any other part of the marine system. Although these inshore stretches of water are exceptionally vulnerable to physical and chemical damage, open waters along the coast can also be harmed, by waste disposal.

Pollutants can be detrimental to fisheries by reducing the numbers of species as a result of mortality directly caused by toxicity, smothering, intolerable heat, or other deadly changes. Species may also decrease when a pollutant causes sublethal stress that significantly interferes with feeding, movement, reproduction, or some other essential function.

### 3.2.4 Tourism

Tourism constitutes a major economic activity in a number of countries. Its expansion will continue and is desirable both for the economy and for the social well being of the community. In addition, it can promote job creation and regional development.

Environmental resources are a major element of tourism and a healthy environment is a vital ingredient for tourist areas. Unrestrained growth of tourism would diminish the quality of tourist areas and possibly their income-earning capacity.

The competent authorities should ensure that decisions on tourism development plans are based on the fullest information available concerning their environmental implications. An environmental impact assessment should be carried out for major tourism developments so as to evaluate potential damage to the environment in the light of the growth in tourism envisaged and peak demand.

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[12] UNEP, 1987

In terms of residual waste, the most widespread problem in resort communities is water pollution through the discharge of inadequately treated effluents. Water bodies, which are among the most attractive resources for tourism development, are also frequently used for the cheap and convenient disposal of sewage.

### 3.2.5 Industrial water uses

Industry uses water for many purposes, for example, cooling, cleaning of equipment, production, washing, etc. Using water for the final disposal of industrial effluents is another important factor that must be mentioned.

Seawater is mostly used for receiving industrial effluents and the criteria for establishing ambient and effluent quality standards are mentioned elsewhere. The use of seawater in industrial units themselves is rather limited, and only concerns cooling, washing of floors, etc. In these cases, no special environmental quality standards higher than those applied for bathing and fishing purposes are required. Special precautions are only taken to protect cooling towers, etc, from the salinity of seawater, and the washing of floors causes no special problems.

### 3.2.6 Commercial water uses

Ports, ship reception facilities and transport are the main fields of commercial activity related to seawater use. Marinas, on the other hand, are usually included in the tourism sector.

Seawater is mainly used to wash and clean ships' equipment and for the discharge of ship effluents. This form of use has seldom been taken into consideration in development plans, hence the present bad conditions to be found in almost all ports and reception facilities in the Mediterranean. It is only recently that regulations for port reception facilities have started to be applied in an effort to limit marine pollution caused by ships.

Criteria for the implementation of ambient and effluent quality standards for sea traffic have yet to be analyzed and assessed.

# 4. QUALITY CRITERIA

## 4.1 Environmental capacity[13]

Throughout history, the sea has been used to receive human waste. Only recently has such use been questioned because of the possible loss on restricted use of marine resources. The recognition that such marine pollutants as artificial radionuclides can jeopardize human health through the consumption of seafood or through exposure on beaches, and disasters such as that at Minamata Bay (mercury poisoning), have resulted in restrictions being placed upon the release of certain substances into the marine environment. However, to the scientific community some of these restrictions appear to be arbitrary because they are not based upon up-to-date concepts. There needs to be an awareness that the marine environment has to be treated as a resource for society as a whole and that the capacity of waters and sediment to receive waste must be assessed continually if resources are to remain renewable.

Various terms are used to describe the extent to which the environment is able to accommodate waste without unacceptable effects. One such term is "environmental capacity". This is a property of the environment and can be defined as the environment's capacity to accommodate a particular activity or rate of activity without any unacceptable impact.

## 4.2 Water quality criteria - standards

Water quality criteria specify the concentrations of water constituents which, if not exceeded, are expected to result in an aquatic ecosystem suitable for water use at a higher level. These criteria are based on scientific facts obtained from experimental or *in situ* observations that depict the responses of organisms to a defined stimulus or material under identifiable or regulated environmental conditions for a specified period of time.

The aim of water quality criteria is not to ensure the same degree of safety or survival and propagation at all times to all organisms within a given ecosystem. The intention is not only to protect essential and significant life in water, as well as the direct users of water, but also to protect life that is dependent on living in water for its existence or may intentionally or unintentionally consume any edible portion of such life.

The word "criterion" should not be used interchangeably with or as a synonym for the word "standard". Criterion means a constituent concentration or level associated with a degree of environmental effect upon which scientific judgement may be based. As currently utilized in connection with the water environment, it means a designated concentration of a constituent that, when not exceeded, will protect an organism, an organism community, or a prescribed water use of quality at an adequate level of safety. In some instances, criterion may in fact be a narrative statement rather than a constituent concentration.

On the other hand, a standard connotes a legal requirement for a particular reach of water or an effluent. A water quality standard may use a water quality criterion as a basis for regulation or enforcement, but the standard may differ from a criterion because of prevailing local natural conditions, such as naturally occurring organic acids, or because of the importance of a particular stretch of water, economic considerations, or the level of safety that may be sought for a particular ecosystem.

Quality criteria have been designed to provide long-term protection. They thus constitute a basis for effluent standards, but it is not intended that they should become effluent standards.

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[13] United States Environmental Protection Agency (US/EPA), 1972

### 4.2.1 Suggested procedure for establishing criteria

(a) to undertake a critical review of the relevant documentation;

(b) to determine the physical, chemical and biological characteristics, including variability in space and time, that influence the desired use or property of the environment. This can be achieved in part through preliminary field observations and laboratory experiments. Such data, together with judicious use of mathematical modeling techniques, will limit the number of variables to be considered;

(c) to establish the relative importance of each characteristic, usually to within an order of magnitude. This again can be achieved in both the field and laboratory and will further limit the number of variables to be considered;

(d) to determine the amount of stress being inflicted on the water mass to be protected. This should be expressed in appropriate units (e.g. concentration, mass, volume, energy, number of organisms). This will help to define the magnitude of the problem;

(e) to determine the chemical and physical fate and distribution of the stress in the system taking into account time factors. This will require chemical, physical and/or biological analyses of various compartments in the system as well as hydrological data;

(f) to determine the portions of the population or use in the area to be protected (or chosen for study) that are subject to each of several different levels of risk. This information will concern several different levels of risk and will be needed when deriving standards from the criteria. It requires an estimation of the rates of input to defined portions of the system;

(g) to determine the exposure/response relationship relevant to the local system in question. This is a fundamental and almost universally applicable procedure and will involve determination of the most vulnerable point in the system (e.g. top predator, man, fish, life stage, required food organism, enzyme, physiological process);

(h) to make experimental exposures in the laboratory and/or field whenever possible so as to establish a family of exposure response curves reflecting the effects of expected variations in conditions and pollutant input on observed response;

(i) to estimate the effects of several degrees of target response on trophic levels immediately above and below target. This will provide a first estimate of the probability of remote effects in the ecosystem and requires consideration of patterns of biomagnification.

## 4.3 Control of discharges based on environmental quality objectives

Various methods have been employed to control the discharge of polluting materials into a body of water. The oldest method is probably the imposition of identical limits on all discharges. This method is often called "uniform emission standards". It is now being superseded in some countries by control based upon a reference to the environmental or ambient quality levels necessary to maintain the receiving water in a fit state for its legitimate and required uses.

The "environmental quality objectives" system is based on the philosophy of controlling discharges so that the quality of the receiving water body at any specified place is suitable for its established legitimate uses. The procedure for the control of discharges based on environmental quality objectives is illustrated in Figure 1.

The upper left part of the diagram concerns the derivation of the environmental quality levels taking into consideration the area of the water body and local uses. The quality objectives for a specific use will be similar throughout the Mediterranean and the process of deciding upon appropriate quality levels in individual cases will be simplified if uses are classified and criteria and quality objectives attached to each use. This is indicated in the upper right part of the diagram.

The next stage is to decide what conditions and restrictions must be applied to the discharge in order to attain the required quality levels. There are two variables to be considered: the discharge point and the pollution load of the effluent. In general, the longer the pipeline in the sea the greater the acceptable polluting load of the effluent. For a defined bathing water area, there will be a seaward limit and the pipeline should discharge beyond this limit. For any given point of discharge, the concentration of faecal coliforms in the effluent must be such that the dilution, dispersion and death-rate of the indicator will reduce the faecal coliform concentration at the boundary of the bathing area to within the limit.

The controlling authority carries out sampling and analysis both of the effluent to ensure that discharge is within the prescribed limits and of the sea water to confirm that the environmental quality within the defined zone meets the use objectives (Compliance monitoring procedure) (Figure 2 supplements Figure 1; they illustrate the control mechanism).

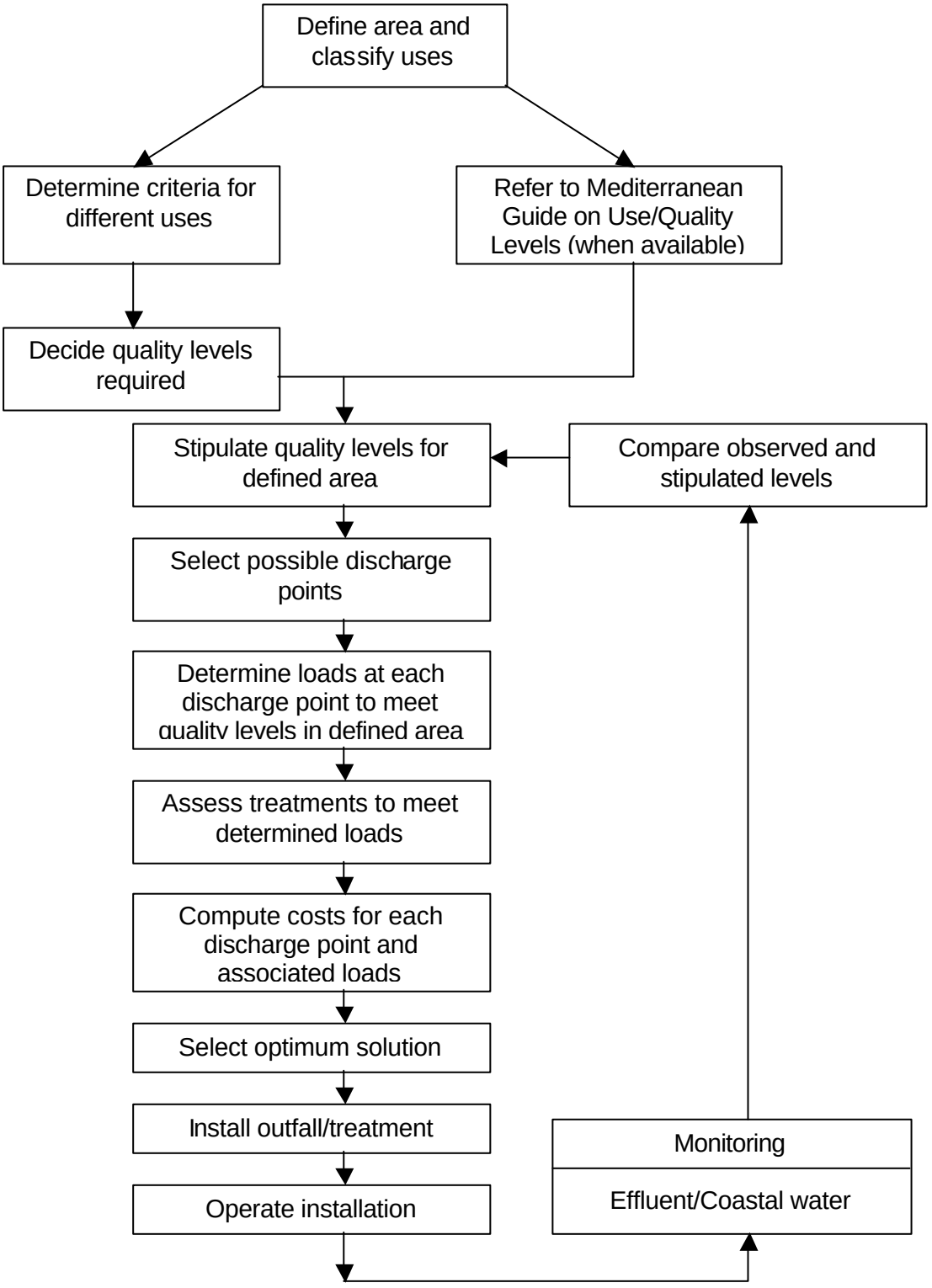


Figure 1

Diagram illustrating procedure for control of discharges by environmental quality objectives based on water use (WHO/UNEP, 1979 and UNEP/WHO, 1996

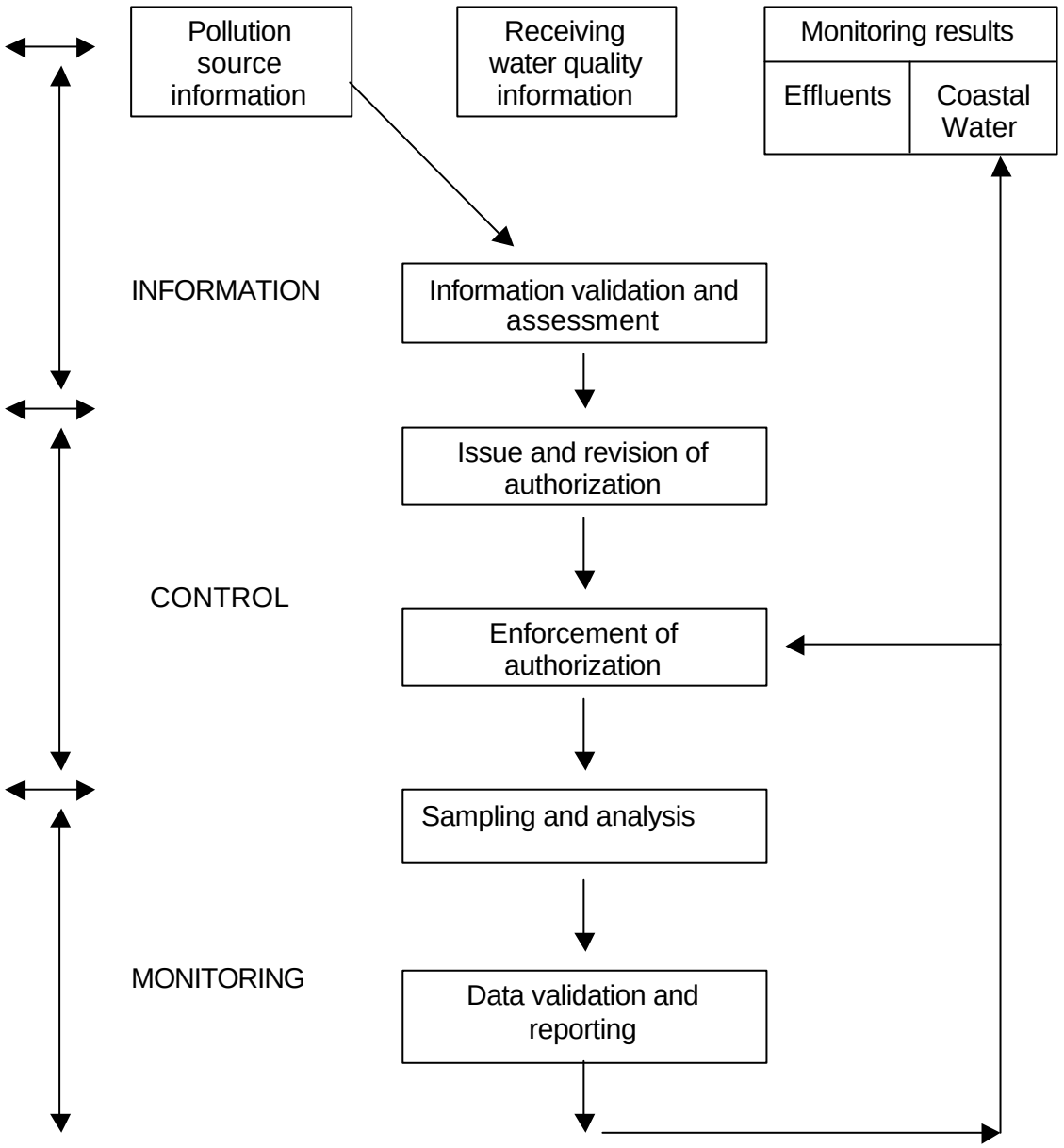


Figure 2.

Diagram illustrating the executive functions of coastal pollution control (WHO/UNEP, 1979 and UNEP/WHO, 1996)

# 5. POLLUTION SOURCES

Land-based pollution sources can be classified into two main types: point sources and diffuse (non-point) sources.

## 5.1 Point sources[14]

Point sources are those from which pollutants are continuously or discontinuously discharged into the receiving water body from a single point. Examples of this type of source are:

(a) Sewer outfalls, including outfalls of municipal or industrial sewage, stormwater outfalls and combined outfalls: They may either discharge into the immediate coastal areas from points above or below sea level, or enter the marine environment away from the coastline via a submarine pipeline;

(b) Rivers: polluted rivers discharging in coastal areas may be important carriers of pollutants originating from points located inland, far away from the sea;

(c) Coastal lagoons: These may be also important sources of pollution, particularly if they act as final recipients of wastes;

(d) Solid waste and sludge disposal and dumping sites: Solid wastes and sludge disposed of directly into the sea, whether from specific points on land or from barges or ships, can be considered as a point source of pollution;

(e) Accidents and leakages: Discharges of pollutants into the sea as a result of incidental or continuous leakage, or arising out of terrestrial accidents, such as an explosion in a coastal refinery, are also included in the category of point sources.

## 5.2 Diffuse (non-point) sources

Sources from which pollutants do not flow into the receiving water from a single point but are spread along the coast are considered diffuse sources. They can be classified as:

(a) Run-off: stormwater which flows in an uncontrolled way into the sea, or leachate reaching the sea from dumping sites in the vicinity of the coastline, are the main examples of diffuse sources;

(b) Small outfalls: untreated sanitary outfalls that are present in large numbers along the coast behave as diffuse sources;

(c) Airborne pollution: there is evidence that considerable quantities of lead and possibly other trace metals, DDT, PCBs, low molecular weight petroleum hydrocarbons and other organic substances are transported to the open ocean by the atmosphere, either as particles or in the gas phase (Duce, *et al*. 1976; SCEP, 1979; FAO, 1971, GESAMP, 1989). The sources contributing to airborne pollution are thus also diffuse sources.

[14] WHO/UNEP, 1994

# 6. COMPLIANCE MONITORING PROGRAMME

## 6.1 Scope of activity

The aims of a programme to monitor land-based sources of marine pollution for compliance purposes should be:

(a) to complete the baseline studies necessary to survey the types and amounts of pollutants discharged or dumped into the coastal marine environment in any given area;

(b) to compile and regularly update an inventory of land-based sources of marine pollution, including data on the probable fate of the pollutants;

(c) to carry out effluent quality control where criteria or standards already exist and to assess the control measures being implemented;

(d) to compile data on which to base decisions on the promulgation and implementation of control measures where such measures do not already exist;

(e) to draw up a database to be used for the environmental impact assessment of any future coastal development.

The outline given in Figure 3 (modified from Mancy Allen, 1978) could be followed when planning an effective compliance monitoring programme . The main flow-chart is shown on the left side of the figure, while the right side contains information regarding the considerations to be taken into account when making a suitable decision. As can be seen in the outline, several factors affect decisions on the planning of a programme, among which financial restrictions may be the most important. A realistic decision on monitoring should always be financially feasible, and the compliance monitoring programme prepared accordingly.

When planning a compliance monitoring programme determining the parameters to be measured is very important. Generally speaking, these will depend on the types of sources present and the pollutants discharged. The determination can be based on data from existing monitoring programmes as well as on the water uses that must be protected. For example, the Mediterranean States agreed on priority parameters for pollution source monitoring in the region within the framework of the Long-term Programme of Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II). These parameters were essentially designed for a coordinated regional programme, and the final choice in any particular area would depend mainly on local circumstances. However, consideration must be given to both the precision required and the precision obtainable because these factors may affect the significance, performance, and cost of the monitoring programme.

## 6.2 Monitoring area

One essential prerequisite of any compliance monitoring programme, and the preparations therefore, is to assess the problem. Prior to establishing the programme, the impact of actual and potential pollution on the various uses of the coastal waters in question should be determined through the acquisition of relevant data (area assessment). The area assessment should include both landward and seaward descriptions of the area, and the data obtained should be noted either on a fact sheet, or on a descriptive map, or on both, depending on the circumstances.

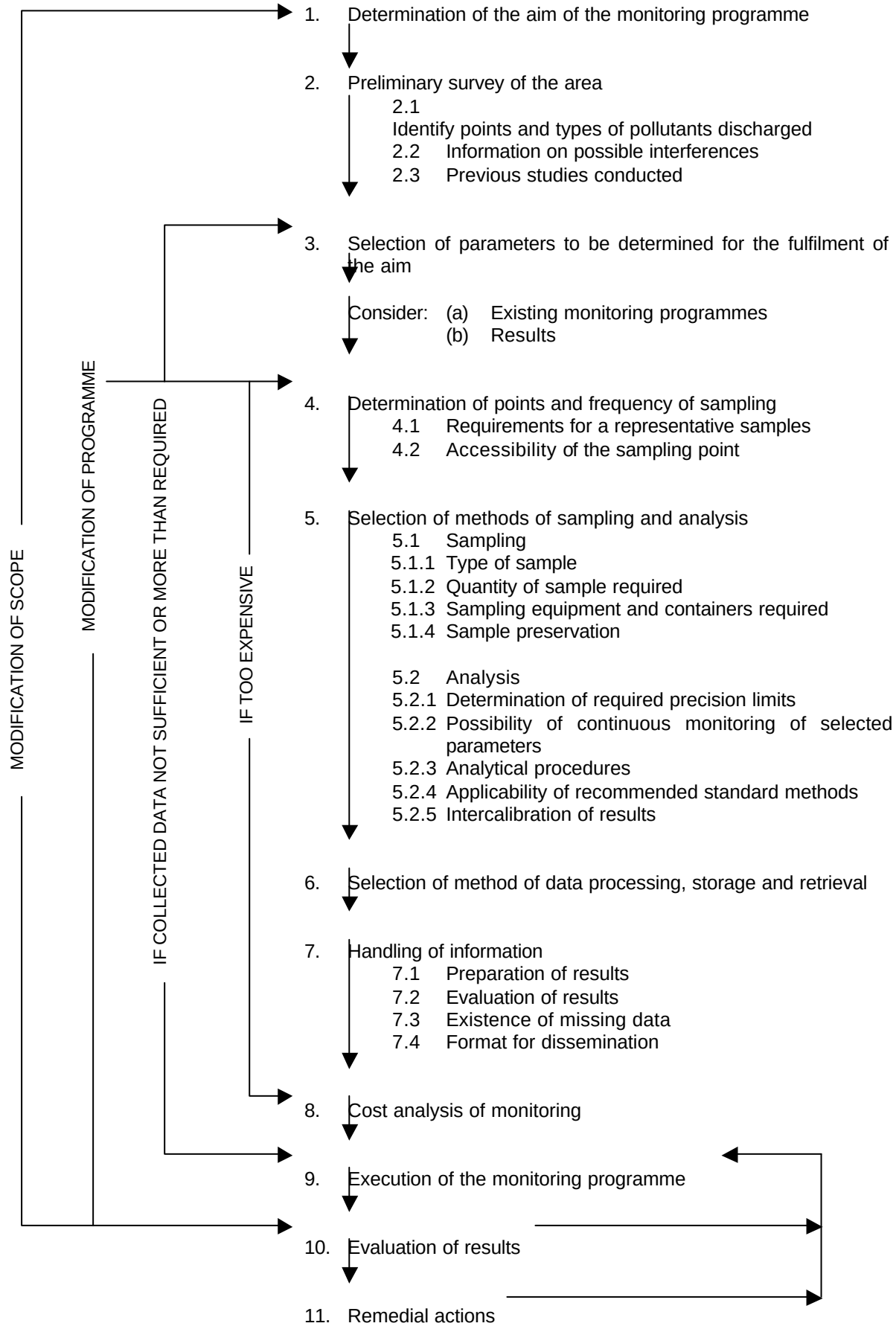


Figure 3.

Suggested flow diagram of an effective compliance monitoring programme
(Mancy, 1978 and WHO/UNEP, 1994)

### 6.2.1 Area assessment

From the landward side, the following should be noted, wherever appropriate to the aims and objectives of the programme:

- land use: categories of land use within the general area, including use of immediate coastal areas, e.g. industrial, residential, forestry, agricultural, recreational or mixed;

- run-off: identification of rivers and streams, including location, flow and individual monthly discharge into the sea, as well as areas where erosion is known to occur;

- wastewater discharges and outfalls: outfall sites, beach and offshore, including type, e.g. industrial, domestic or mixed, and total daily flow. Industrial discharges should be specified;

- wastewater treatment: location of treatment plants, capacity in m per day, and degree of treatment; sludge production and disposal;

- dumping sites: identification of dumping sites in the vicinity of the beach, indicating whether for solid waste, sewage disposal or both, and giving volume of deposit per year;

- coastline: sand, rock, gravel, cliffs. Also, whether shallow or deep water.

From the seaward side, the following should be noted, again wherever appropriate to the aims and objectives of the programme:

- shellfish areas: sites and types of shellfish should be indicated on a map, and information on catches (tonnage per year) should be given;

- fishing grounds: sites, types of fish and, if possible, information on catches, tonnage, etc should be indicated;

- protected areas: information about fish in marine parks and other similar protected areas;

- dumping sites: determination of locations, material and amount dumped;

- marine biota: general information about marine fauna and flora, wildlife and nature reserves should be provided.

The following meteorological and oceanographic observations will also have to be made, wherever relevant:

- winds: drawing up of seasonal wind roses;

- precipitation and air temperature: annual precipitation in tabular form. The same table to include average monthly air temperatures;

- currents and tides: description and seasonal fluctuation of currents, tidal cycles where applicable;

- salinity and temperature: based on existing studies the data should be sufficient to provide information on water column stratification and its seasonal variations;

- depth contours: from nautical charts;

- buoys and other navigational aids: these, as well as any other important obstacles such as wrecks or rocks, should be indicated.

Since a monitoring programme is a prerequisite for compliance monitoring, it is understood that all the above data should be available prior to compliance monitoring, unless specific conditions require additional data and consequently modification of the monitoring programme.

### 6.2.2 Maps

The use of adequate maps and nautical charts is an essential prerequisite for such a programme. The first step to be taken is to draw up detailed maps of the areas selected for monitoring. These should incorporate as much as possible of the information collected during the area assessment, in particular:

(a) sewage outlets and any waste or other discharge points;

(b) inshore and offshore solid waste dumping sites;

(c) local currents in the coastal waters relative to point sources and beach locations.

The most recent geodetic and nautical maps of the coastal area to be studied should be obtained. The nautical charts are generally of major interest. The situation and use of each map will normally define the appropriate scale. A map of practical size could be the European A3 format (approximately 42 x 60 centimetres). Many copying machines allow for direct reduction from A3 to A4, resulting in economic reproduction and presentation of results.

Each map should be clearly identified by location, coordinates, scale and orientation. This must be done before any copying or reproduction is effected. Identification should include:

(a) location: use the name of a typical town or conspicuous landmark. Always indicate the country;

(b) coordinates: give the approximate latitude and longitude of the location;

(c) scale: this should be graphed, e.g. in divisions of 100 metres or in kilometres, not numerical, as the latter may change with enlargement or reduction;

(d) orientation: indicate N for north, or give lines for latitude and longitude of the main location;

(e) date: give date of preparation of map, if available.

## 6.3 General design

Prior to actual implementation of the compliance monitoring programme, it is essential to decide on:

(a) the matrices to be monitored;

(b) the parameters to be monitored in each matrix;

(c) the number and location of sampling points;

(d) the frequency of sampling.

The extent of the programme will depend entirely on already-existing resources and on extra resources that can be made available to meet the required demand. These resources consist of:

(a) trained manpower for sampling and analysis;

(b) laboratory facilities (apparatus, equipment and materials);

(c) transport facilities.

It should be borne in mind that in practically all cases the essential minimum is dictated by the provisions of international conventions or other similar legal instruments. In most countries, in order to conform to local requirements, national legislation provides for coverage over and above this minimum.

## 6.4 Preparation of preliminary report

A brief and concise report stating clearly the aims of the compliance monitoring programme and including information collected during the preliminary survey, a summary of previous studies and related maps, should be prepared. This report should form the basis for finalization of the compliance monitoring programme.

## 6.5 Sampling

Sampling techniques should be determined with great care as even with the most sensitive analytical techniques it is not possible to obtain more accurate and dependable results than the collected sample can provide.

It is not possible to provide specific sampling instructions that would be suitable and applicable under all conditions. Because of this, only general principles are outlined in the following sections.

The most important principle in sampling is to enable the analysis to be made on samples that are "representative" of the water concerned. In other words, the sample and its source should have the same composition. Furthermore, the sample should be a true representation of the variations in the characteristics of the source over time. Sampling should be performed in a systematic way in order to minimize discrepancies.

Selection of the sampling point location, as well as the frequency of sampling for the determination and monitoring of land-based marine pollution sources, depend mainly on the sensitivity required as well as the resources allocated for the compliance programme. There is a basic difference between the selection of sampling methodology for application by all Mediterranean countries on a common basis and the selection of a methodology in order to comply with national or even local requirements.

### 6.5.1 Matrices and locations

In programmes aimed at the determination of land-based pollution and compliance, details will have to be determined in the light of the situation existing in each particular locality. These will necessary differ according to land use and related activities, as well as water use, in the area in question.

In a regional compliance monitoring programme aimed at the determination of land-based marine pollution originating from all Mediterranean countries, mandatory monitoring would be restricted to major sources of pollution, while at the same time allowing for additional components to enable national and local requirements to be met (compliance with local legislation).

In keeping with these general principles, the matrices to be monitored and the location of sampling points should be as explained below.

#### 6.5.1.1 Point sources[15]

When samples are to be collected from a point source, the homogeneity of the system should first be verified and, if possible, sampling points should be located where homogeneous distribution of the parameters to be measured is observed. This is not always possible, especially if there are undissolved materials whose density is different to that of the water or if the extent of chemical and/or biological reactions varies in different parts of the system.

When the system is heterogeneous, the number and location of samples to be collected should be adjusted accordingly so that the results are representative. Variations in the homogeneous character of a system over time should be checked because seasonal variations, etc. are possible. Sampling locations near the boundaries of water systems, such as the banks of rivers or the walls of pipes and channels, should be avoided unless these locations are of special interest. The following principles should be adhered to in relation to the different types of point sources:

- Outfalls

The collection of samples from an outfall (domestic sewage or industrial effluent) is described in detail in document WHO/UNEP, 1994. EEC Directives on the following could also be used as a model for:

(a) urban wastewater: collecting systems, discharges to receiving waters, reference methods, parameters to be measured, limit values etc, necessary for compliance control;

(b) industrial effluents: limit values, industrial sectors, frequency of sampling, quality objectives, etc. for cadmium in effluents, as a guide for compliance control.

Document US/EPA, 1984 could also serve as general guidance for the basic inspection procedures.

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[15] WHO/UNEP, 1994

- Rivers and streams

Monitoring stations on rivers should be established, provided that the river satisfies one of the conditions below:

(a) the average flow exceeds 100 $m^3$/sec;

(b) the watershed exceeds 100 $km^2$;

(c) it is thought to be heavily polluted.

A monitoring station on a river should be located outside the limits affected by tides and waves, at a point downstream from the last effluent discharge at a distance sufficient to obtain homogeneous distribution. If there is any possibility of non-homogeneous distribution of quality at the chosen location, experimental tests of the nature and magnitude of any heterogeneity should be made. If the results indicate that the river is of homogeneous character, one position for sampling will be enough, otherwise either the location of the sampling point should be transferred to a location of a homogeneous character or samples should be taken from several additional locations in addition to the original one selected so that the overall characteristics can be represented. For major rivers, even if they are homogeneous, it is advisable for more than one sample to be taken from different depths on the same cross section, forming a sampling point grid if necessary. In this case, the effect of variations of flow rate at the different points should be taken into consideration when preparing composite samples or estimating the overall input of any specific pollutant into the receiving water. When a limited number of samples needs to be taken in order to determine existing pollutants, if equipment is available, it is recommended that an "integrated" sample be taken from top to bottom in midstream, or from side to side at mid-depth, in such a way that the sample is integrated according to flow. If only a grab or catch sample can be collected, this is best taken in mid-stream at mid-depth (APHA, 1990). On the other hand, for velocity measurements, which are essential in order to determine the flow and, consequently, the total amount of pollutant discharged into the receiving water, sampling should be effected at a point located at 0.6m of the total depth measured from the bottom or, to increase accuracy, at points located 0.2m and 0.8m of the total depth (Linsley, 1964), taking the average of these. Special attention is necessary when dealing with rivers that have a tendency to flood or a seasonally- varying stratification.

Bridges over rivers are easily accessible and convenient sampling points. However, before any decision is taken regarding their use, it should be verified that samples collected from them are valid and representative. Sampling from areas where stagnation may occur and from areas located near the inside bank of a curve in the stream which may not be representative of the main channel should be avoided.

- Solid waste and sludge disposal

Although it is not recommended practice, solid wastes and sludge can, in some countries, be dumped into a receiving water either legally (with an authorization) or illegally, directly from the coast or from barges used for the purpose.

In the case of authorized dumping, the amount of waste should be determined either by weighing the load on specially allocated scales or, if this is not possible, by estimating the amount by volume. All municipalities or other institutions dumping solid wastes and sludge in this way should be obliged to provide information in an appropriate format on the amount and composition of the material dumped. Random sampling is usually carried out by taking one sample for every 500 tons of municipal solid wastes and one sample for every 10 tons of industrial solid wastes, taking into consideration the waste's origin and classification. Samples should be collected carefully from different parts of the solid waste load, trying to be as representative as possible. In cases where it is proven that a declaration by a particular industry is not correct, all loads coming from that industry should be examined.

The sampling of solid wastes and sludge from unauthorized dumping into receiving water is very difficult, if not impossible. The only possible way of controlling unauthorized dumping and estimating the possible amount is source control. To achieve this, all sources of hazardous wastes should be obliged to fill in a declaration form giving information about the amount, properties and place of disposal of hazardous wastes. The accuracy of the information given in the declaration should be verified through random inspection.

- Major accidents

Major accidents undoubtedly contribute towards marine pollution. If detailed information about the characteristics of the material flowing into the sea as the result of an accident is available, an estimation of the volume of the material in question reaching the sea is enough to determine the amount of pollutant. If an analysis of the material leaking is not available, samples should be collected from the accident site and affected areas.

### 6.5.1.2 Diffuse sources

Sampling from diffuse sources is a very complicated process for which a generally acceptable procedure is not available. In such cases, the following approaches are suggested:

(a) collection of a representative sample and estimation of the overall effect;

(b) determination of the concentrations of selected pollutants in various parts of the receiving marine environment in combination with salinity or other tracers, extrapolating to zero salinity and flow estimations;

(c) utilization of information obtained from similar situations for which accurate load calculations are available;

(d) in the case of urban waste, calculation of the population equivalent on the basis of previous experience.

As can be seen from the four possible methods outlined above, only the first two require actual sampling, while the other two are based purely on estimates. The collection of a representative sample in order to make an overall estimate can easily be achieved if the diffuse source is in the form of small outfalls. If this is so, one of the outfalls should be chosen arbitrarily and the results obtained extended to all the others. In the case of a "runoff", it is recommended that a channel at least 50m long perpendicular to the direction of the runoff be constructed, and samples collected from the outlet of this channel. It is considered that a 50m-length collection channel would be sufficient in most cases.

Selecting the location of sampling points in the receiving marine environment in order to apply approach (b) above depends entirely on local conditions. However, the following general principles can still be applied:

(a) a grid of sampling points should be formed covering all the marine environment immediately affected;

(b) the depth from which the sample is to be collected should be decided according to local conditions. However, it is recommended that, at points where the depth exceeds 10m, at least three samples (one below the surface, one at mid-depth, and one at 1m above the bottom) should be collected.

### 6.5.2 Sampling frequency

The frequency of sampling should be determined in such a way as to represent adequately the true quality and variation, but at the same time it should not exceed the minimum essential requirements in order to avoid unnecessary effort and cost.

The best solution to the question of frequency is the use of instruments that measure continuously and automatically. This is not always possible, however, due to the unavailability of adequate instrumentation and the high cost involved.

A decision on the frequency of sampling can only be taken only after available data have been examined and the variation of characteristics has been evaluated.

When systematical data are not available, the following sampling programme should be followed, at least for major sources:

(a) hourly sampling during one 24-hour period in each quarter (season) to assess daily cyclic effects;

(b) daily sampling during seven consecutive days in each season, to determine any weekly cyclic effects;

(c) weekly samples to delineate seasonal effects and to determine how less frequent sampling would have affected the results.

After a one-year trial period on the basis of the above programme, an evaluation should be made to permit a decision on a suitable sampling frequency that provides the required confidence limit of the means.

If the parameters to be determined show systematic trends or cyclic variations, the time of sampling should be considered in addition to the number of samples. Both should be chosen in such a way as to reflect the actual situation. Whatever the results of the above-mentioned analysis, the frequency of sampling should not be less than once per month. For practical reasons, whenever applicable, the sampling frequency may be adjusted to fit other monitoring programmes, such as the compliance monitoring of the quality of coastal recreational and shellfish-growing areas.

### 6.5.3 Reference methods[16]

Four decades ago, adequate analytical techniques were not widely available to allow chemists to quantify contaminants causing pollution and to assess their impact. As a result of the increased concern to measure potential pollutants in the marine environment, techniques were rapidly adapted from other areas of pure and applied chemistry and a large number of methodologies and data sets began to appear in the scientific literature.

For the more inexperienced scientists, keeping abreast of the scientific literature on methodology is a daunting challenge and it would be difficult to test the many hundreds of methodological modifications (not always improvements) published each year. Most conventional textbooks cannot be re-edited sufficiently rapidly to keep up with the pace of these developments. Clearly a more dynamic and flexible approach to this issue is required. The UNEP Reference Methods for Marine Pollution Studies series was established in 1983 as an attempt to address this issue and to provide a mechanism for testing, optimizing and updating methodologies and communicating them to marine scientists throughout the world.

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[16] UNEP/IAEA/IOC, 1990

By providing a flexible mechanism for technical support, adapted to real environmental problems, United Nations agencies are endeavouring to keep marine environmental scientists well-armed to face these challenges, not alone, but as part of a global team with a common aim.

The Reference Methods programme provides a wide-ranging series of methods and guidelines for marine pollution studies. Each method is self-contained and follows, as closely as possible the format and terminology recommended by the International Standardization Organization) (ISO). The methods are designed to be applicable throughout the world and to produce data of sufficient accuracy, reliability and precision to allow meaningful interpretation for the purposes of regional marine pollution studies, as well as interregional comparisons (and so to contribute to UNEP's Global Environmental Monitoring System (GEMS)).

The Reference Method Catalogue (UNEP/IAEA/IOC, 1990) gives a full listing of methods now available and those currently being prepared or tested. Many of the methods are interrelated to form a structured series of texts on monitoring strategy, sampling techniques, analysis, quality assurance and data interpretation. Each text is self-contained and can be updated without altering the rest of the series. The reader should make sure he has the latest edition of each method he or she requires.

In document US/EPA (1974) there is a recommendation on sampling and preservation of samples according to measurement.

# 7. COMPLIANCE MONITORING IN THE MARINE ENVIRONMENT

## 7.1 Substances regulated under the Barcelona Convention

### 7.1.1 Regulated substances

A system that is used in all marine conventions is to regulate the use and/or discharges of certain substances and materials that are known, or at least suspected, to be harmful to the marine environment. The usual procedure has been to define a set of criteria on which to base the selection of a number of substances that should be regulated. Typical criteria were: toxicity, persistence and bioaccumulation. Substances showing high toxicity together with high persistence or ability to bioaccumulate were "banned", which means that they should be eliminated from discharges. A list of such substances was usually referred to as the "Black List" although the term is no longer used. Other substances of environmental concern are identified, although they are considered as being less harmful. The text of the convention usually allows these substances to be discharged, although their discharge should be minimized. Similarly, a list of such substances was usually referred to as the "Grey List".

The regulations in the text of the Barcelona Convention (as it came into force in 1978) are rather general. For example, Article 8 states: "The Contracting Parties shall take all appropriate measures to prevent, abate and combat pollution of the Mediterranean Sea Area caused by discharges from rivers, coastal establishments or outfalls, or emanating from any other land-based sources within their territories". However, the amended Convention which has not yet entered into force is more precise. The text of the LBS Protocol which came into force in 1983 is somewhat more specific (Articles 5 and 6), whereas in the 1996 amended Protocol the categories of substances, their characteristics, and the sectors of activity that need to be taken into account in the preparation of action plans, programmes and measures for the elimination of pollution from land-based sources and activities are included.

### 7.1.2 Measures adopted by Contracting Parties

From the viewpoint of human health, the LBS Protocol is the most important. In view of the considerable economic and legal implications of this Protocol, the text itself is similar to the Convention in that it provides a framework for prevention and control measures, with progressive implementation. To date, the Contracting Parties have adopted joint measures under the Protocol (WHO/UNEP, 1995).

## 7.2 Media in which contaminants should be monitored

The ICES in its role as scientific adviser to PARCOM and partner with PARCOM in the North Sea Task Force (NSTF), responded to a request to recommend to PARCOM and NSTF a scheme to describe in which media the different contaminants or hazardous substances should preferably be monitored. The scheme should also assign sampling priorities among various media in order to make the monitoring more cost effective. The advice given appears below.

It is important to stress that the information contained in the tables should not be used alone but should always be combined with the explanatory text.

The matrices considered included sea water, sediments, and biota, as included in the current Joint Monitoring Programme (JMP). The matrices were selected as those most appropriate for the provision of the most information in relation to each monitoring purpose. They were selected on scientific grounds and did not take any account of the relative costs or convenience of the alternative choices.

In some cases, no matrix was recommended, either because the monitoring of a particular contaminant was not appropriate to the monitoring purpose or because advice could not be given for technical reasons.

The reliability of the information provided by a monitoring programme and its consequent value depend upon the attention paid to quality assurance at all stages of the measurement programme (sample collection, storage, preparation, preconcentration, analysis, standardization and interpretation). Participating laboratories should be required to adopt appropriate procedures in this area.

### 7.2.1 Compliance monitoring of health-related conditions

Table 1 provides advice on the contaminants and matrices that should be included in a regional or broader survey to assess the possible hazards to human health caused by the presence of selected contaminants in marine foodstuffs. In several cases, primary and secondary choices of matrix are given.

There may be areas of contamination which could give rise to localized increases of concentration in foodstuffs. Such situations are unlikely to be detected or adequately described by large-scale surveys and are better approached through specially designed and targeted monitoring exercises by national or local authorities. In such circumstances, the relevant authorities should assess the most important exposure pathway by which the contaminant reached the public through marine foodstuffs. The monitoring programme should be directed at that pathway and not be constrained by the advice given in Table 1 in relation to broader scale surveys.

Compliance monitoring of health-related conditions (e.g. sanitary quality of bathing areas and waters used for aquaculture, quality of seafood) is of national importance, but data may also be used for regional assessments. A comprehensive approach to microbiological and health-related monitoring of recreational and shellfish-growing areas is shown in detail in documents WHO/UNEP (1994) and WHO/UNEP (1996).

### 7.2.2 Compliance monitoring of sea water (Table 2)

The use of water analysis to reflect current levels of marine contamination is attractive in that it concerns the important aqueous phase, the environment in which both biota and sediment exist. The requirements for precision and accuracy of analysis at low concentrations limit the number of determinants that could be considered in offshore waters in relation to mercury, cadmium, copper, zinc and lead, all at secondary matrix level. Even in these cases, it would be essential for each laboratory to establish in-house quality control procedures and for rigorous assessments to be made to establish comparability among laboratories, with particular attention to lead.

In near-shore waters, concentrations may be somewhat more variable and subject to anthropogenic influences, and chromium and nickel analyses might also be considered. The same quality assurance precautions would be needed. In near-shore waters, it is necessary to take account of any correlation between contaminant concentrations and salinity, and of the influence of the concentration and composition of suspended matter on the dissolved contaminants.

Sea water is not a matrix of choice for CBs, as the octane: water partition coefficients indicate that the compounds would be predominantly associated with sediment or biota.

The concentrations of arsenic naturally present in sea water make it difficult to discriminate between anthropogenic influences and natural processes, therefore, sea water is not indicated as an appropriate matrix.

In some sea areas (usually small and isolated areas), the inputs of contaminants are sufficiently large to cause marked elevations of contaminant concentrations in sea water, or changes in concentrations could be expected. In such areas, it might be appropriate for national authorities to give more prominence to water analysis in monitoring programmes. The monitoring of sea water at a more regular frequency than once every five years could be justified:

(a) in areas with enhanced levels of contaminants, and
(b) in areas where changes could be expected as a result of known reduction in inputs for example.

A distinction must be made between near-shore waters, where marked salinity gradients may be found and which are more likely to be influenced by riverain or land-based inputs of contaminants, and offshore waters, where gradients are usually substantially less marked and which are more remote from the above-mentioned inputs of contaminants. In document IMCO/FAO/UNESCO/WMO/IAEA/UN/UNEP (1980), Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP), Marine Pollution Implications of Coastal Area Development. Rep. Stud. GESAMP, (11): 114 p., there is a synoptic table of a preliminary programme of oceanographic observations that could be used for a compliance monitoring programme for sea water.

Table 1

In relation to the assessment of possible hazards to human health
(Chassard-Bouchaud, 1993)

| Matrix | Contaminant | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CBs | ã-HCH | Hg[5] | Cd | Cu[3] | Zn[3] | As[4] | Cr[3] | Ni[3] | Pb | MeHg | TBT[3] | Chlordane | Planar CB | PCDD/ PCDF | DDT[3] | Diel-drin | PAH | PCC | Tria-zines[3] | PPDE[4] | PBB[4] |
| Shellfish | P | P | P | P | | | | | | P | P | P | | P | P | | P | P | P | | | P |
| Fish muscle | | | P | | | | | | | | P | | | | | | | | | | | |
| Fish liver | S[2] | S[2] | | S[2] | | | | | | | | | | S[2] | S[2] | | S[2] | | S[2] | | | S[2] |

P: primary matrix
S: secondary matrix

Notes and qualifications:

1. If fish liver is not a consumed fisheries product, no analysis is needed.
2. If fish liver is not a consumed fisheries product and there remain human health concerns, transfer attention to fish muscle.
3. These contaminants are not normally of concern in respect to the consumption of fisheries products.
4. Arsenic is present in seafood in measurable concentrations, but its chemical form makes it of little concern with respect to human health.
5. Hg should be understood to include methyl-mercury compounds. In countries where public health regulations refer to methyl-mercury rather than
6. total mercury, samples may be analyzed for methyl-mercury.
6. Too little is known about the toxicity to assess potential hazard.

Table 2

In relation to the assessment of existing level of marine pollution (i.e. contamination)
(Chassard-Bouchaud, 1993)

| Matrix | Contaminant | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CBs | ã-HCH | Hg | Cd | Cu | Zn | As | Cr | Ni | Pb | TBT | MeHg | Chlordane | Planar CB | PCDD/ PCDF | DDT | Diel-drin | PAH | PCC | Tria-zines | PPDE | PBB |
| Near-shore water | | P | P[1] | P[1] | P[1] | P[1] | | P[1] | P[1] | P[1] | S[1] | | | | | | | | | P | | |
| Offshore water | | S | S[1] | S[1] | S[1] | S[1] | | | | S[1] | | | | | | | | | | | | |
| Surficial sediments[2] | P | | P | P | P | P | P[5] | P | P | P | P | P | P | P | P | P | P | P | P | | P | P |
| Shellfish | S[3] | S[3] | S[1] | S[1] | | S[1] | | | | S[1] | P | P | S[3] | S[3] | S[3] | S[3] | S[3] | S[3] | S[3] | | S[3] | S[3] |
| Fish muscle | | | T[1.4] | | | | S[1.4] | | | | | S[4] | | | | | | | | | | |
| Fish liver | S[4] | | | T[1.4] | | | | | | T[1.4] | | S[4] | S[4] | S[4] | S[4] | S[4] | S[4] | S[4] | S[4] | | S[4] | S[4] |

P: primary matrix
S: secondary matrix
T: tertiary matrix

Notes and qualifications:

1. Potential addition/alternative to sediment measurements in areas where sediment conditions are not wholly favourable.
2. Should be accompanied by total organic carbon measurements, size fractionation (<63 m), and description of the sediment type. Sampling should be carried out following current ICES guidelines.
3. Could be carried out on an opportune basis, as may provide additional information on distribution.
4. Sedentary species only (e.g. flatfish).
5. The signal-to-noise ratio for discriminating between anthropogenic and natural influences is extremely low.

### 7.2.3 Compliance monitoring of sediments[17]

#### 7.2.3.1 Introduction

Although methods for the chemical and biological characterization of water-borne contaminants are applied in regulatory and monitoring programmes in many countries, methods for the assessment of sediments are less widely or uniformly established.

Sediments may act as a sink for, and source of, toxic chemicals through sorption of contaminants to particulate matter. The effects of surface water contamination become integrated over time and space and create a hazard to aquatic communities (both pelagic and benthic), which is not directly predictable from observations of contaminant concentrations in the water column. Sediments can serve as historical records of change due to both man-made pollution and natural environmental causes. For example, lake sediments reflect surface water quality more consistently than do flowing rivers even though there may be seasonal changes in the lake environment, e.g. metal cycling in hypolimnetic waters.

Effects on benthic organisms are of concern because in many ecosystems the sediment community plays an important role in the recycling of detrital material to the pelagic community. In addition, benthic organisms are a critical component of a variety of aquatic food webs. There is thus a need for sediment quality objectives that may be used as a scientific basis for the development of standards to protect ecosystems from the effects of sediment contamination, and to manage contaminated sediment in the long term. Consequently, the main objectives are:

(a) to consider the methods available for use in developing environmental quality objectives (or criteria) for sediments, and to reach consensus on the methods most appropriate for this purpose;

(b) to recommend the most appropriate test methods to assess:

  (i) the toxicity of sediments; and
  (ii) the toxicity of a particular chemical or group of chemicals for sediment-dwelling organisms.

#### 7.2.3.2 Useful methods

A number of potentially useful methods exist, namely:

- equilibrium partitioning;
- interstitial water quality;
- spiked sediment toxicity;
- reference concentrations;
- apparent effects threshold;
- screening level concentrations;
- sediment quality triad;
- tissue residue.

[17] OECD, 1992

Each method is evaluated with respect to the following characteristics:

(a) Chemical specificity: can the method be used to derive a concentration for a specific chemical?

(b) Causality: are the observed effects caused by the specific chemical?

(c) Chronic effects: does the method consider chronic toxicity endpoints?

(d) Bioaccumulation: does the method consider food chain accumulation and ingestion of contaminated sediment for (i) benthos, (ii) fish?

(e) State of development: is the method ready for use (tested, validated, used)?

(f) Bioavailability: how generally applicable is the method across sediment types? Are sediment quality objectives a function of the bioavailable phase?

(g) Applicability: is the method applicable to bedded sediments or suspensions?

(h) Recommendation: on the basis of the foregoing evaluations, can the method be recommended for use in deriving sediment quality objectives?

The results are shown in Table 3.

#### 7.2.3.3 Objectives of sediment toxicity tests

It is important always to be clear about the objectives of contaminated sediment studies as the objectives are vital to selection and/or development of appropriate test systems.

There are a number of reasons for developing and utilizing sediment toxicity tests:

(a) to assist in setting quality standards for individual compounds;

(b) to assess the impact of discharges of sediments associated with receiving waters, such as sediment disposal associated with dredging activities;

(c) to assess the persistence of toxicity in sediments following the alteration, amelioration or cessation of toxic discharges;

(d) to predict the impact on sediment-dwelling organisms exposed to new substances that may be released into the environment;

(e) to estimate the degree to which toxicity is responsible for low benthic species diversity in impacted systems.

Table 3

Evaluation of the present state of development of eight methods for deriving sediment quality objectives

| Elements/characteristics Method | Chemical specificity | Causality | Chronic effects | Bioaccumulation benthos/fish | State of development | Bioavailability | Applicability (a) | Can be recommended |
|---|---|---|---|---|---|---|---|---|
| Equilibrium partitioning | ++ | ++ | + | +/-- | ++b) | ++b) | BS* | yes |
| Interstitial water quality | ++ | ++ | + | -/-- | - | + | B | yes |
| Spiked sediment toxicity | ++ | ++ | +c) | +/-- | +d) | +e) | BS* | yes |
| Reference concentrations | ++ | -- | -- | +/ | ++ | -- | BS | no |
| Apparent effects threshold | ++ | -- | -/+f) | -/-- | ++ | +e) | B | no |
| Screening level concentrations | ++ | -- | + | -/-- | - | + | B | no |
| Sediment quality triad | -- | -- | + | -/-- | + | - | B | no |
| Tissue residue | * | * | * | */-- | -- | * | B*S* | no |

Key:
++ High - Low
+ Medium -- Very low
(a) To bedded sediments (B), suspended sediments (S).
(b) Well developed for organic chemicals and being developed for metals and other chemicals.
(c) For freshwater organisms only.
(d) Methods and guidelines need to be developed.
(e) If concentrations are properly normalized to reflect biological availability, e.g. by organic carbon or acid volatile sulphides.
(f) Chronic effects on benthic organisms in field situations can be considered.
+ There is potential for development.

### 7.2.3.4 Sediment characterization

A wide range of parameters may be relevant in characterizing the sediment associated with solid phase tests; they depend on the purpose of the investigation. The following key parameters are frequently necessary for interpretation of the toxicity test results:

- **Particle size distribution.**
- **Dissolved oxygen.**
- **Organic carbon content.**
- **Total ammonium concentration.**
- **Acid volatile sulphides (AVS).**
- **pH.**

The following may also be relevant on a site-specific basis:

- biochemical oxygen demand;
- chemical oxygen demand;
- nitrate/nitrite;
- chloride;
- sulphate;
- redox (Eh) potential;
- dissolved organic carbon (pore water);
- conductivity;
- salinity;
- hydrogen sulphide;
- suspected or spiked chemical contaminants**.**

### 7.2.3.5 Sampling and storage of test sediment

Sediment for toxicity tests should be fresh and handled in a way that minimizes alterations that may affect the toxicity to organisms exposed in the laboratory. When sediment samples undergo toxicity tests, parallel samples are often subjected to chemical, physical and/or biological investigations. The combined objectives of the particular investigation, therefore, determine the sampling design, including the equipment used, sampling points, depth of sediment and time of sampling.

Sediment for biological investigations (evaluation of benthic community structure) are usually processed (screened) and preserved on-site, whereas samples for chemical and physical characterization are handled and stored according to procedures more or less specific to the particular test. For the purpose of sediment toxicity evaluations, it is important to obtain sediments with as little disruption as possible to allow for realistic laboratory evaluation of *in situ* conditions.

### 7.2.3.6 Assessing contaminated sediments

The ability to define contaminants responsible for toxicity in contaminated sediments provides a unique opportunity for insights concerning remedial and regulatory activities, including:

(a) identification of discharges responsible for sediment contamination resulting in toxicity;

(b) identification of unsuspected contaminants responsible for toxicity in sediments;

(c) identification of point versus non-point source impacts resulting in toxic sediments;

(d) evaluation of disposal options for dredged materials.

### 7.2.3.7 Water quality criteria approach

The water quality criteria approach compares the concentrations of individual contaminants present in sediment interstitial water with water quality criteria (WQC). Existing WQC have been developed from a broad range of toxicological studies using a wide range of aquatic organisms. These criteria have been used in the regulatory context to specify contaminant levels that, if not exceeded, will protect 95 per cent of aquatic life from adverse effects.

A major assumption of the approach is that water column organisms used to develop WQC have the same sensitivities as infaunal benthic organisms. It is also assumed that the major route of contaminant exposure is from the interstitial water and exposure from ingestion of contaminants on sediments is not significant.

The principal advantage of this approach is that it relies on existing toxicological databases used to develop WQC. It only requires the additional measurement of the contaminant concentration in the interstitial water.

On the other hand, the approach has several disadvantages: (i) WQC are available only for a limited number of contaminants; (ii) the toxicological data used to develop WQC were from sediment-free bioassays so there is no consideration of the effect that soluble or particulate organic matter, present in interstitial water may have on contaminant bioavailability; (iii) the potential to increase contaminant body burden through ingestion or direct contact with sediment contaminants is not taken into account; and (iv) suitable methods are still being developed for the isolation and measurement of contaminant concentrations in interstitial water.

### 7.2.4 Compliance monitoring of biota

Marine organisms are commonly used to monitor chemical contaminants in the sea. It is well known that they can concentrate toxicants by uptaking them from water and sediment as dissolved or particulate matter, which enter their organisms via gills, the digestive tract or tegument epithelia. Toxicants are then stored in various tissues and organs, among which a target is generally determined to be used as the main indicator. Elimination and excretion occur via several routes.

It is difficult to find the right species for monitoring purposes. Environmental indicators are suitable for the observation of long-term development in an ecosystem, as well as for planning and controlling effects of anthropogenic activities.

### 7.2.4.1 Definitions

Figure 4 shows a proposal for the classification of bioindication (Hertz, 1991).

Bioindication means the time-dependent, sensitive response of measurable quantities of biological objects and systems to anthropogenic influences on the environment. In general, a distinction can be made between:

- bioindication as a qualitative method for the detection of the presence of pollutants, and
- biomonitoring as a more quantitative method for the determination of the effects of the pollutants present.

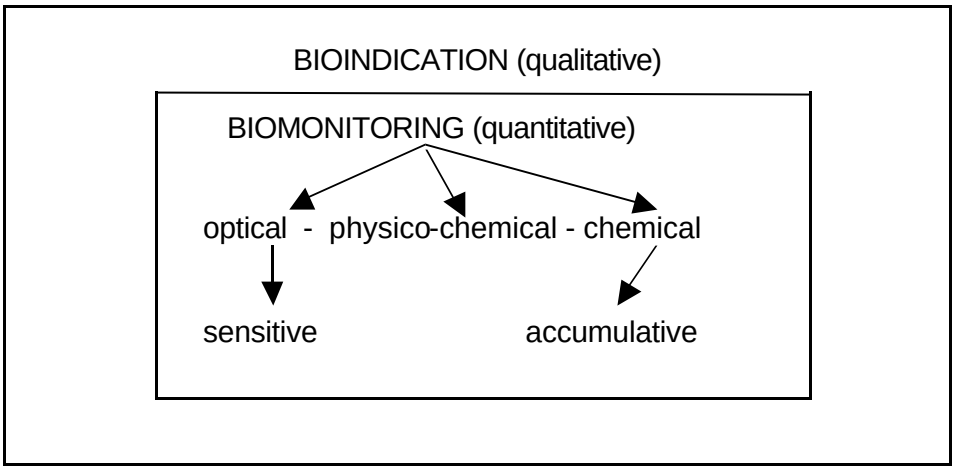


Figure 4.

Classification of bioindication (from Hertz, 1991)

"Biomonitors are organisms which can be used for the recognition and quantitative determination of anthropogenically induced environmental factors". For the detection and recognition of water pollution, biological organisms which respond sensitively and specifically to a given pollutant can be used. In addition, organisms that readily amass the polluting components without changing their chemical nature may be used as accumulators. This classification into sensitive and accumulative biomonitors is now well-accepted terminology.

### 7.2.4.2 Sensitive biomonitors

They are used in aquatic ecosystems as integrators of the pollution stresses caused by contaminants in order to provide early warning systems. They can be divided into two categories:

- ecological surveys, and
- toxicity testing.

Ecological surveys

These may use indicator species or assessments based on the composition of biological communities and numerical diversity. By making comparisons between affected and control areas, ecological surveys can indicate the health of a water body exposed to pollutant loadings.

Toxicity testing

This is used to obtain basic information about the general toxicity of effluents expected to be introduced into an ecosystem. A great number of toxicity tests have been performed to answer various questions.

### 7.2.4.3 Selection of contaminants

Among the many possible chemical species that could be considered, the bioaccumulation of heavy metals has been the most extensively studied. They are important polluting elements in many biological systems and correspond to the following trace metals: arsenic, cadmium, chromium, copper, lead, mercury, nickel, tin and zinc.

Many other chemical substances are measured for monitoring purposes: DDT and other chlorinated pesticides, polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons.

The selection of substances to be monitored should be based on the following considerations:

- the aims of the monitoring programme (see Table 4);
- the findings of the pilot study (which contaminant present at a significant level will justify further study?);
- the ability of the analyst to measure these substances with the required accuracy and precision.

### 7.2.4.4 Selection of organisms

The choice of the test organisms must be guided by several criteria:

- the abundance of the species;
- their geographical range: organisms must be ubiquitous so that the comparisons can be made between areas, countries, continents and possibly hemispheres;
- whether or not they constitute an important link in the food chain;
- the organisms accumulate the contaminant without being affected by the levels encountered;
- the organisms are sessile and thus representative of the area of collection;
- the organisms are sufficiently long-lived, to allow sampling of more than one year class if desired;
- the organisms are of a reasonable size, to give adequate tissue for analysis;
- the organisms are easy to sample all through the year;

- the organisms are easy to handle in experimental work, robust enough to survive in the laboratory, allowing research on the uptake, storage and elimination of contaminants;

- the organisms must offer the possibility of working *in situ* on the population level and with native communities;

- the organisms exhibit high concentration factors;

- the organisms are tolerant of brackish waters, to allow comparisons to be made between estuarine and offshore sites.

Table 4

Chemical substances commonly measured in marine organisms for compliance monitoring purpose (UNEP/FAO/IAEA,1993)

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'-DT and p,p' - DDT.

Chlorinated pesticides other than DDT
Aldrin, Alpha-Chlordane, Trans-Nonachlor, Dieldrin, Heptachlor, Heptaclor epoxide, Hexachlorobenzene, Lindane (gamma-BHC) 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 Fluorene, 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

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

| Category I (mandatory) | Category II (optional) |
|---|---|
| total mercury | total arsenic |
| organic mercury | radionuclides |
| cadmium | polynuclear aromatic hydrocarbons |
| halogenated hydrocarbons | |

# 8. COMPLIANCE MONITORING IN "HOT SPOT" AREAS [18]

## 8.1 Definitions

"Hot spot" areas are:

(a) **Point sources** on the coast which potentially **affect** human health, ecosystems, biodiversity, sustainability or the economy in a significant manner. They are the **main points where high levels of pollution loads** originating from domestic or industrial sources are being discharged;

(b) Defined **coastal areas** where the **coastal marine environment is subject to pollution** from one or more point or diffuse sources on the coast which potentially **affect** human health in a significant manner, ecosystems, biodiversity, sustainability or the economy.

## 8.2 "Hot Spot" indicators (primary)

- biochemical oxygen demand (BOD), chemical oxygen demand (COD);
- nutrients (phosphorus, nitrogen);
- total suspended solids;
- oil (petroleum hydrocarbons);
- heavy metals;
- persistent organic pollutants;
- radioactive substances (whenever applicable);
- litter;
- microorganisms (faecal coliforms, E.coli, faecal streptococci);
- organisms (e.g macroalgae for the soluble phase, mussels for the particulate phase and a detritus feeder for the sediment phase).

## 8.3 Compliance monitoring in "hot spot" areas

Compliance monitoring in "hot spot" areas would follow the basic steps referred to in previous chapters for regular areas, except that it would require an extended and more frequently repeated programme. It would demand more resources and would often involve an element of research, e.g. on the dispersion and fate of pollutants in the marine environment after discharge. Ultimately, a series of extended monitoring programmes may lead to new achievements in terms of pollution control, which may then result in a redefinition of the scope of activities, new programming, new choice of monitoring parameters, etc.

[18] WHO/UNEP, 1997

# 9. ANALYTICAL QUALITY CONTROL[19]

## 9.1 General

The role of the analytical laboratory is to provide qualitative and quantitative data to be used in decision-making. To be of value, the data must accurately describe the characteristics or the concentration of constituents in the sample submitted to the laboratory. In many cases, an approximate answer or incorrect result is worse than no answer at all because it will lead to faulty interpretations.

Decisions made using water and wastewater data are far-reaching. Water quality standards are set to establish satisfactory conditions for a given water use. The laboratory data define whether that condition is being met, and whether the water can be used for its intended purpose. If the laboratory results indicate a violation of the standard, action is required on the part of pollution control authorities. With the present emphasis on legal action and social pressures to abate pollution, the analyst should be aware of his responsibility to provide laboratory results that are a reliable description of the sample. Furthermore, the analyst must be aware that his professional competence, the procedures he has used, and the reported values may be used and challenged in court. To meet this challenge satisfactorily, the laboratory data must be backed up by an adequate programme to document the proper control and application of all the factors that affect the final result.

## 9.2 Quality control programme

Because of the importance of laboratory analyses and the resulting action, a programme to ensure the reliability of the data is essential. It is recognized that all analysts practice quality control to varying degrees, depending somewhat upon their training, their professional pride, and awareness of the importance of the work they are doing. However, under the pressure of the daily workload, analytical quality control may easily be neglected. Consequently, an established, routine control programme applied to every analytical test is important in assuring the reliability of the final results.

## 9.3 Analytical methods

The need to standardize of methods within a single laboratory is readily apparent. Uniform methods in collaborating laboratories are also important so that the methodology does not constitute a variable in comparison or joint use of data among laboratories. Uniformity of methods is particularly important when laboratories are providing data to a common data bank, or when several laboratories are cooperating in joint field surveys. A lack of standardization of methods raises doubts as to the validity of the results reported. If the same constituent is measured by different analytical procedures within a single laboratory, or in several laboratories, the question of which procedure is superior arises, and why the superior method is not used throughout.

## 9.4 Control of analytical performance

### 9.4.1 Precision and accuracy

Precision refers to reproducibility among replicate observations. In an analytical quality control programme, it is determined not on reference standards, but by the use of actual water samples that cover a range of concentrations and a variety of interfering materials usually encountered by the analyst. Obviously, such data should not be collected until the analyst is thoroughly familiar with the method and has obtained a reproducible

[19] US/EPA, 1973, UNEP/IOC/IAEA/FAO, 1990 and OECD, 1996

standard curve. For colorimetric analyses, the initial standard curve should include a blank and a series of at least eight standards encompassing the full concentration range to be used for routine sample analyses. Subsequently, at least two standards (a high and a low) should be analyzed to verify the original standard curve. For other measurements, such as pH, conductivity, turbidity, etc., instruments should be standardized according to the manufacturer's instructions and sound, scientific practices.

Accuracy refers to a degree of difference between observed and known, or actual, values. Again, accuracy should be determined on actual water samples routinely analyzed, and preferably on the same series as those used in the precision determinations.

### 9.4.2 Evaluation of daily performance

Once valid precision and accuracy data are available on the method and the analyst, systematic daily checks are necessary to ensure that valid data are being generated.

In order to prove that reproducible results are being obtained (i.e., precision of the method), it is necessary to run replicate samples. Although the frequency of such replicate analyses is, by nature, dependent on such factors as the original precision of the method, the reliability of the instrumentation involved, and the experience of the analyst, it is good laboratory technique to run duplicate analyses at least 10 per cent of the time. The resulting data should accord favourably with the known precision of the method. If they do not, the system is not under control and the results are subject to question.

A most convenient way of recording the obtained precision and accuracy data is through the preparation of quality control charts. Plotting of the data systematically is a response to the question of whether or not the laboratory analyses are under control, and is useful in observing the development of positive or negative trends.

### 9.4.3 Quality control charts

Quality control charts were originally developed for the control of production processes where large numbers of items were being manufactured and inspected on an essentially continuous basis.

There are various systems currently available for plotting data in the form of cumulative sum charts. One system that has been in continuous use is that of Anon. (1969). It has proved very useful in monitoring the validity of data generated by a contracting laboratory and is currently being used routinely to record intra-laboratory performance in technical operations daily.

#### 9.4.3.1 Shewhart quality control charts

Shewhart (1931) chart concepts and other statistical techniques have refined and quantified the search for quality in manufacturing. Although originally developed to control production processes where large number of articles were being manufactured and inspected on an essentially continuous basis, the same concepts have been readily adapted to laboratory operations where the analyst produces comparatively fewer results on an intermittent basis.

#### 9.4.3.2 Precision control charts

These charts are developed by collecting data for many samples, a minimum of 15 to 20, run in duplicate under assumed controlled conditions.

#### 9.4.3.3 Accuracy control charts

As in the above system, these charts developed by collecting data for many samples, a minimum of 15 to 20, but on spiked samples (preferably) or standards under assumed controlled conditions. Again, these data should be generated over an extended period of laboratory time, and be representative of normal operating conditions.

### 9.5 Data handling and reporting

To obtain meaningful data on water quality, the laboratory must first collect a representative sample and deliver it unchanged for analysis. The analyst must then complete the proper analysis in the prescribed fashion. Having accomplished this step, one other important step must be completed before the data are of use. This includes the permanent recording of the analytical data in meaningful, exact terms, and reporting it in proper form to some storage facility for future interpretation and use.

## 10. ATTAINABILITY ANALYSES[20]

Consideration of the suitability of water body for a given use is an integral part of the water quality standards review and revision process. This is intended to assist States in answering three central questions:

1. What are the aquatic protection uses currently being achieved in the water body?
2. What are the potential uses that can be attained based on the physical, chemical and biological characteristics of the water body? and,
3. What are the causes of any impairment of the uses?

Attainability analyses therefore are methods and approaches that can be used to address the above questions as related to the protection of the marine environment.

The data and information collected from the water body survey provide a basis for evaluating whether it is suitable for a particular use. It is not envisaged that each body of water would necessarily have a unique set of uses. Rather the characteristics necessary to support a use could be identified so that water bodies processing those characteristics might be grouped together as likely to support particular uses.

The complexity of an aquatic ecosystem does not lend itself to simple evaluations, so there is no single formula or model that will provide all the answers. Thus, the professional judgment of the evaluator is the key to the interpretation of the data collected.

The most common desktop evaluations of use attainability are statistical analyses of water quality monitoring data to determine the frequency of violation of criteria for the designated aquatic use. Statistical evaluations of contraventions of water quality criteria should consider the confidence intervals for the number of violations that are attributable to random variations (rather than actual water quality deterioration).

For example, in the case of a monitoring station with 12 dissolved oxygen observations per year with a standard of 5 mg/l DO, if statistical analyses of the DO observations indicate that the upper and lower confidence limits for the frequency of random violations of the 5 mg/l DO standard cover a range of one to four violations per year, a regulatory agency should be cautious in deciding whether actual use impairment has occurred unless more that four violations are observed annually.

The development of a manual on attainability analyses should be a priority in a compliance monitoring programme.

Among the tools applicable to use of attainability analyses, particularly chemical evaluations, is use of indices. Many water quality indices have been developed. The Denius water quality index is presented here as an example to show its applicability.

This index includes 11 variables and has a scale that decreases with increased pollution, ranging from 0 to 100. The index is computed as the weighted sum of its subindices. The 11 variables included in the index are: dissolved oxygen, biochemical oxygen demand, escherichia coli, alkalinity, hardness, specific conductivity, chlorides, pH, temperature, coliform, and colour. This index is unique in that the calculated water quality index could be matched to specific water uses. Denius proposed different descriptor language for different index ranges depending on the specific water use under consideration, as illustrated in Figure 5. The index values can be derived from the following formula:

---

[20] US/EPA, 1983

$$Q = \frac{5(DO) + 214(BOD)^{-0.642} + 400(5E.Coli)^{-0.30} + 300\,(Coli)^{-0.30} + 535\,(SC)^{-0.3565} +}{5 \quad + \quad 2 \quad + \quad 4 \quad + \quad 3 \quad + \quad 1}$$

$$\frac{+ 62.9\,(Cl)^{-0.207} \quad + 10^{\,1.974 - 0.00132(HA)} + 54\,(ALK)^{-0.178} + 10^{0.235\ pH + 0.440}}{+ \quad 5 \qquad\qquad\qquad 1 \qquad\qquad 0.5 \qquad\qquad\qquad 1 \quad +}$$

$$\frac{+ 8\,(Ta-Ts) + 224 + 128\,(C)^{-0.288}}{2 \quad + \quad 1}$$

Note: If the pH is between 6.7 and 7.3, 100 should be substituted for the pH expression. If the pH is greater than 7.3, the pH expression should be 10.

| | | |
|---|---|---|
| DO | = | dissolved oxygen in per cent saturation |
| BOD | = | biochemical oxygen demand in mg/l |
| E.Coli | = | escherichia coli as E.coli per ml |
| Coli | = | coliform per ml |
| SC | = | specific conductivity expressed in microohms per cm at 25 C |
| Cl | = | chlorides in mg/l |
| HA | = | hardness as ppm $CaCO_3$ |
| ALK | = | alkalinity as ppm $CaCO_3$ |
| pH | = | pH units |
| Ta | = | actual temperature |
| Ts | = | standard temperature (average monthly temperature) |
| C | = | colour units |

Once the quality unit is determined based on the above calculation, a comparison with Figure 5 should reveal the quality of the water for a specific use.

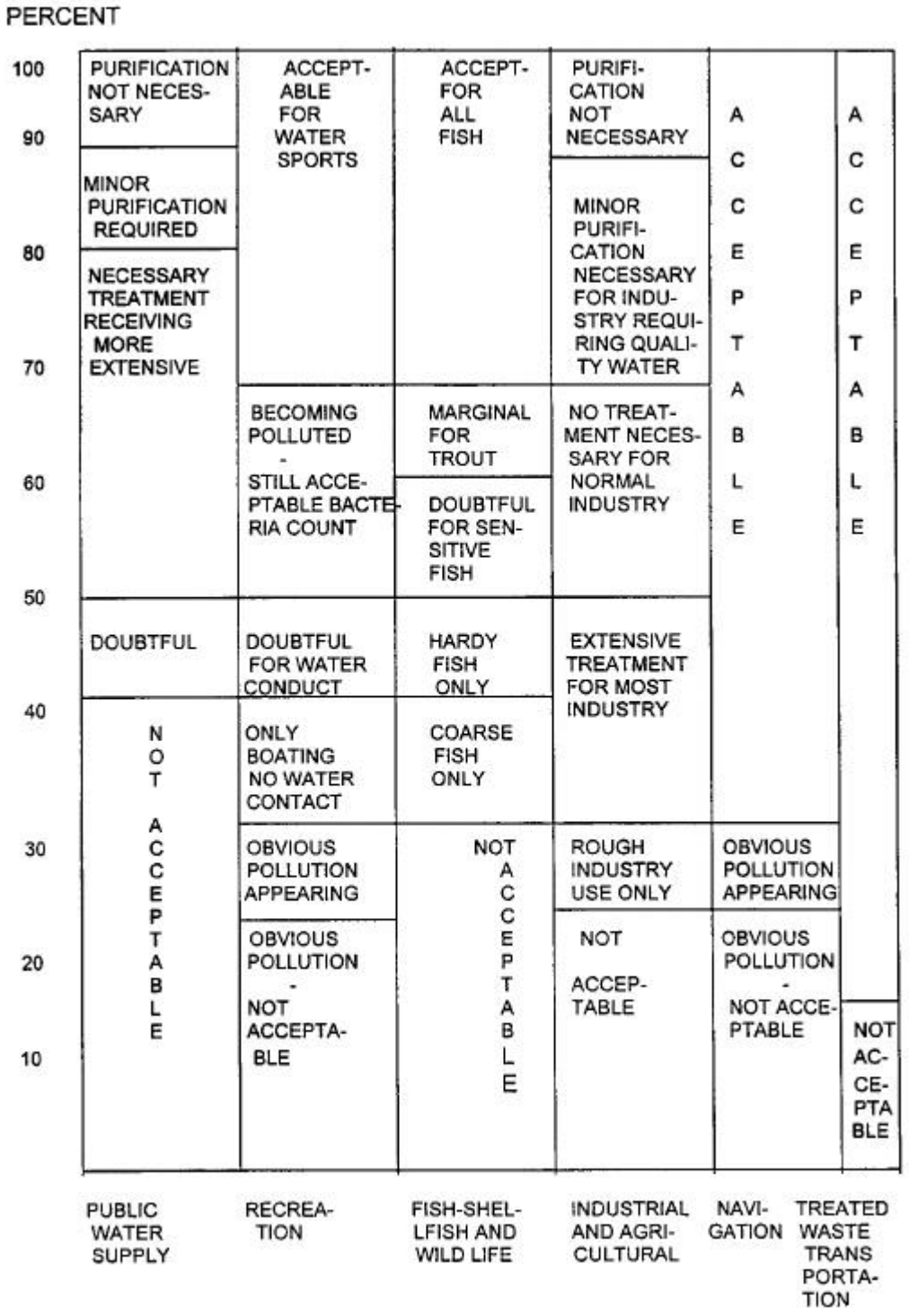


Figure 5.

General rating scale for the quality unit (US/EPA,1983)

Another useful index is the contamination index, which helps to assess the contribution of anthropogenic sources of metal contamination in sediments over time. The Wedepohl ratio compares the amount of metal in the sediment sample with the concentration in an average shale (or sandstone). If, for example, scientists have measured silicon and aluminum, then have correlated metals with Si/Al ratios, a contamination factor (Cf) may be computed as follows:

$$Cf = (Co-Cp)/Cp$$

where: Co surface sediment concentration

Cp= predicted concentration, derived from the statistical relation between the Si/Al ratio and the log metal content of old, pre-pollution sediments.

Thus, Cf < 0 when the observed metal concentration is less than the predicted value; Cf = 0 when observed and predicted are the same; Cf > 0 when the observed is greater than the predicted value.

The contamination index ($C_l$) is found by adding together contamination factors for metals in a given sediment.

Then,

$$C_l = \sum_{n=1}^{n} Cf = \sum_{n=1}^{n} (Co-Cp)/Cp$$

The toxicity index ($T_l$) is related to the contamination index and is expressed by the following equation:

$$T_l = \sum_{i=1}^{i} (M_1/M_i) \cdot Cf_i$$

where: $M_i$ = the "acute" any time criterion for any of the metals,
but : $M_1$ is always the criterion value for the most toxic of the metals.

The "acute" any time criterion is defined as the concentration of a material that may not be exceeded in a given environment at any time. When evaluating toxicity indices, sampling stations should be characterized by their minimum salinities. This is because the toxicity of metals is often greater in freshwater than in saltwater.

A more detailed discussion of the development of the contamination index may be found in the US/EPA publication, Chesapeake Bay: A Profile of Environmental Change (1983a) and A Framework for Action (1983c).

## 11. ENFORCEMENT[21]

It is important to emphasize that enforcement is but one component of environmental quality management (EQM). As such, it must be consistent with the other components. For example, if legislation, development of standards, and permit conditions are not clear and unambiguous to both the discharger and to the regulatory agency, enforcement will be difficult, if not impossible.

One characterization of the components of EQM is:

- perception of an environmental quality problem;
- data collection, analysis, development of strategies to "solve" the problem;
- legislation and elaborating regulations;
- development and promulgation of standards;
- issuance of permits;
- application of environmental instruments to induce initial compliance;
- enforcement of permit conditions against non-complying activities.

There should be feed-back from each component of the EQM cycle to other components. It is also important to emphasize that all levels of government are involved in and carry out activities with respect to environmental management.

One of the important questions with respect to EQM and its the enforcement component is the allocation of management tasks among the levels of government. In addition, an integral problem of environmental quality management is the allocation of resources among the components of the EQM cycle and within the enforcement component.

Multiple actors are involved in each component of environmental quality management, including enforcement. An illustrative list of actors and their roles is given below:

Public agencies: as regulatory bodies at all levels of governments of general jurisdiction and special agencies, such as the water authorities in the United Kingdom, the Genossenschaften in Germany, air quality management districts in the United States, and the river basin agency in France. Their role consists of:

- elaborating regulations;
- setting standards and developing guidelines;
- issuing permits, making inspections;
- monitoring discharges, checking accuracy of data collected at discharges (i.e. self-monitoring data);
- imposing sanctions for non-compliance;
- developing cooperative agreements with public and private dischargers;
- assisting in environmental audits;
- publicizing the performance of dischargers both good and bad, maintaining and providing access to information on discharging activities;
- developing and operating a complaint response system;
- promoting cleaner process technologies.

[21] OECD, 1985

Public agencies as dischargers: same role as for private entities/ activities.

Courts:

- determining whether or not a discharging activity has been in compliance;
- determining whether or not standards are "fair", or "reasonable";
- determining whether or not the regulatory agency has performed its designated functions;
- imposing judicial sanctions.

Private sector, e.g., industrial activities, agricultural operations, mining operations, forest products operations, institutional operations. They are or should be involved in:

- elaborating regulations;
- setting standards and developing guidelines;
- self-monitoring of quality of input raw materials; self-monitoring of discharges;
- developing cooperative agreements with regulatory bodies performing environmental audits.

Trade associations

- submitting evidence for elaborating regulations, standard setting proceedings, performing research on pollution control and process modification technology;
- participating in the development of guidelines for environmental audits.

Insurance companies

- requiring environmental audits as a condition of providing insurance coverage;
- establishing various standards of operations for activities before providing insurance coverage.

Public interest groups: e.g. environmental groups

- elaborating regulations;
- endorsing;
- monitoring of performance of the private sector and public agencies;
- participating in joint groups with the private sector and public bodies in developing standards and monitoring procedures;
- initiating court proceedings against private and public polluting activities, as well as against public regulatory agencies.

Enforcement can be improved by developing nine courses of action:

- at the level of regulations;
- at the level of permits;
- improving monitoring;
- developing cooperative agreements;
- developing environmental auditing;
- strengthening controls and sanctions;
- devising incentive measures;
- enhancing information and publicity;
- increasing agency capacity.

Because the contexts of enforcement do not all involve the same elements, these suggestions do not necessarily apply to all contexts or countries. Moreover, governments should fix their own enforcement priorities.

Mediterranean countries cover a broad spectrum of stages of political, social and economic development, and the most appropriate form of organizing controls will vary accordingly. Experience in different countries, however, does provide some general guidance. One major consideration is the extent to which the responsibilities are apportioned between central and local government.

Central government determines national policy, enacts legislation and retains overall, ultimate control. It has been found advantageous at central government level to arrange for formal consultation and liaison among the ministries involved in various aspects of coastal pollution such as health, industry, tourism, fisheries, local affairs, navigation and marine matters.

The extent to which central government itself carries out executive duties or delegates them to local or regional authorities will be influenced by the resources and technical capabilities of the latter. It must also be borne in mind that municipalities are usually responsible for sewerage and disposal. They are dischargers and it might not be deemed appropriate for an authority to issue authorizations to itself and enforce them.

Supply of information covers the collection and processing of existing information, as well as the gathering of additional information and access to routine monitoring data. Evaluation of the data that governs the conditions to be attached to the authorization may be carried out at the information stage and then transmitted to the control stage at which the authorization is issued. The discharge is monitored to ascertain the extent of compliance with the authorization, and the receiving water is also monitored to confirm its quality. Monitoring consists of collecting samples, transporting them to a laboratory and analyzing them. The analytical results are fed back to the control authority normally responsible for enforcement and to the information collection stage. At regular intervals, the data will be scrutinized and at agreed intervals the conditions of the authorization will be reviewed. An annual report may be prepared and published.

The collection and interpretation of the data is a complex operation calling for a high degree of technical skill. In some countries, facilities may exist for this work to be carried out regularly. Where, there are management authorities for inland waters, these may have or acquire the necessary competence. For many countries, data collection and evaluation may best be carried out by single specialized institutions serving the whole country.

# 12. ORGANIZATION

The organizational requirements for an effective pollution management programme cover a wide range of activities that must be undertaken in order to achieve practical results in combating water pollution with the least expenditure of time and money. The following elements have frequently been included in national programmes:

- establishment of a coastal water control organization;
- management of wastewater facilities (collection, treatment and disposal);
- monitoring of coastal waters and effluents;
- research.

## 12.1 Establishment of a coastal water control organization

The main tasks of a coastal water control organization are:

- collection of information;
- decision on and approval of pollution control policy;
- implementation of policy;
- monitoring of results achieved.

All these elements generally fall under the responsibility of one organizational body, but in some cases they may be spread over one or more different administrative structures. The advantage of combining all these aspects under the same organization is enhanced synchronization among the various technical departments and a single pattern of thought, thus avoiding controversy among several agencies responsible.

### 12.1.1 Collection of information

Knowledge of the existing situation is very important for the development of an appropriate water pollution control policy and strategy, in order to enable decision-makers to base the policy on precise and realistic data without making theoretical estimations.

The information should cover the condition of the coastal and inland waters (i.e. rivers) in the water catchment area, an estimation of the hydraulic and pollution loads of all pollution sources (land-based and offshore), and the content of development plans for the region in order to predict the future impact on the environment.

This procedure should be executed within the shortest possible period of time so as to prevent any inconsistency between the start and the end of the data collecting operation. Simple, quick and precise methods of data collection and interpretation are therefore very important. In this context, the introduction and implementation of computerized systems is strongly recommended.

The data collected should be renewed at regular intervals in order to keep it updated as changes occur. Here again, the importance of computer systems should be highlighted.

### 12.1.2 Decision and approval of pollution control policy

After the data have been collected, the main outlines of the pollution control strategy should be planned and analyzed. The scientists, managers, technicians, etc. responsible for the technical aspects of environmental measurements should elaborate a strategy based on simple and reliable control methods, taking into account all possible data and the related environmental impact. A well-designed and argued technical plan involving the least possible financial expenditure has the greatest chance of being approved by the decision-

makers. It should be emphasized in this context that highly sophisticated control methods with a higher risk of failure and greater financial cost should be avoided.

Due to the complexity of environmental problems and the multitude of technical, financial, social and political aspects involved in any pollution control strategy, the decision-makers involved should consider all possible implications deriving from the proposed policy. Cooperation between the regional authorities and governmental and international agencies is thus essential when reviewing the adequacy of the policy to be implemented.

### 12.1.3 Implementation of policy

After the policy has been approved, the water control organization is responsible for its implementation. A high level of education and experience among the manpower employed is a basic prerequisite for successful implementation. In addition, discipline on the part of public opinion, industry, communities, etc., convinced of the need for the policy, as well as cooperation between them and the implementing body responsible, is the second condition to be fulfilled in order to achieve permanent results. A simple and effective policy that leads to rapid and visible improvements in the marine environment is the most persuasive argument.

Implementation of the policy consists of the following general steps:

- legal cover (regulations, laws, etc);
- technical measures (i.e. installation of treatment plants, changes in industrial production, etc.);
- advertising campaigns aimed at the public.

These measures should be taken simultaneously in order to obtain the aforementioned cooperation and acceptance of the policy by interested bodies.

### 12.1.4 Monitoring of results achieved

Once a policy has been applied correctly, the expected results will subsequently be achieved. Nevertheless, the conditions of the policy's implementation need to be monitored constantly as practice has shown that even the best strategy for combating pollution will fail if there has not been continuous monitoring of the conditions.

The relevant monitoring department of the organization will be responsible for the regular inspection of industrial processes, wastewater installations, agricultural activities, etc. detecting failures, lack of maintenance, operating problems, etc. A high level of professional experience on the part of the monitoring personnel is essential for this procedure in order to ensure that the organization's authority remains at the topmost level. The second task of a monitoring mechanism is to monitor water quality and effluents.

## 12.2 Management of wastewater facilities (collection, treatment and disposal)

The administrative organization of wastewater installations is an essential part of the successful implementation of a pollution control plan in addition to the efficient engineering aspect. Experience has shown that even facilities of very sophisticated design have failed to work successfully because of bad administrative organization and support.

No one type of administrative organization can be recommended as being universally suitable because economic, political and geographical conditions vary from one country to another. The following are some of the major factors that have to be taken into account when drawing up an organizational scheme suitable for a particular case:

- existing organization of water supply;
- size of the area;
- development plans in the area;
- regional organizational scheme in the country.

## 12.3 Monitoring of coastal water and effluents

Monitoring the quality of coastal water and effluents is usually part of the control programme of a water pollution control authority and is frequently the latter's administrative responsibility.

This aspect of monitoring programmes covers effluent sampling and analysis. The control authorities undertake sampling and analysis not only of effluents in order to verify compliance with the prescribed limits but also of sea water to ensure that water uses are adapted to the prevailing conditions. This is mentioned separately, however, as water quality monitoring is also used for purposes other than pollution control.

Not only is a water quality monitoring programme essential for continuous estimation of sea water conditions before the effects of any pollution incite the authorities to take action, but it is also of valuable assistance to the control bodies. This knowledge is an important constituent of the information required in order to decide upon and implement pollution control strategies and estimate the impact on the marine environment of any development plans.

## 12.4 Research

Research programmes in support of coastal pollution control management are always oriented towards applied technical methods, eschewing theoretical considerations, which are mostly the responsibility of universities and other scientific institutions. The organizational scheme for establishing appropriate research programmes varies according to the administrative structure in each country.

From the organizational point of view, an autonomous scientific-technical body, acting as scientific adviser to the water pollution control organization is the optimal solution. In some cases, the authorities' technical needs are covered by contracts with research bodies (universities, institutes, etc) which carry out the scientific work on their behalf. This is the least expensive solution and is widely used in order to save costs.

An applied research programme must cover the following elements:

- pilot-plant studies on water pollution control techniques;
- development of full-scale projects for wastewater treatment methods;
- elaboration of new cost-effective sampling and analysis techniques;
- cost-benefit analysis of applied technical pollution control measures.

The operational pattern for rapid execution of such a programme should be as follows:

(a) task definition and priorities set by the water pollution control organization;

(b) timetable for programme completion;

(c) approval of completed intermediate work phases by the supervising authorities.

## 13. STEPS ILLUSTRATING PROCEDURE FOR MONITORING OF COMPLIANCE CONTROL

Summarizing the various principles and guidelines set out in the previous chapters, the following is a brief description of the steps needed to implement a compliance control monitoring programme.

Where appropriate, it is indicated whether the technical information that is a prerequisite for such control is contained in UNEP documentation.

1. Identification of the monitoring programme's aims.

2. Definition of the area and classification of uses:
   need to develop quality criteria in relation to effluents and the ambient marine environment, as well as how to determine assimilative capacity.

3. Information from previous studies conducted.

4. Survey of the area (WHO/UNEP, 1994) - area assessment:

   4.1 Landward side:
   - land use;
   - run -off;
   - wastewater discharges and outfalls (identification of sources of pollution and pollutants discharged);
   - waste treatment;
   - dumping sites;
   - coastline morphology.

   4.1 Seaward side:
   - shellfish areas;
   - fishing grounds;
   - bathing waters;
   - protected areas;
   - dumping sites.

5. Programme design and execution:

   5.1 Matrices and locations:

   5.1.1 Point sources:
   (WHO/UNEP, 1979)
   - outfalls:
     need to develop and elaborate inspection procedures;
   - rivers and streams:
     need to develop river basin management;
   - solid waste - sludge disposal;
   - major accidents.

   5.1.2 Diffuse sources:
   lack of measures for the control of diffuse sources of pollution.

   5.1.3 Health-related conditions
   (WHO/UNEP, 1994, 1995 and 1996).

5.1.4 Sea water
(UNEP/IOC/IAEA/FAO, 1990).

5.1.5 Sediments:
need to prepare a document on compliance monitoring in sediments.

5.1.6 Biota
(UNEP/FAO/IAEA, 1993, UNEP/IAEA/IOC, 1990
UNEP/IOC/IAEA/FAO, 1990
WHO/UNEP, 1994, 1995 and 1996).

5.2 Selection of parameters.

5.3 Determination of points and frequency of sampling
(UNEP/IAEA/ICC, 1990).

5.4 Selection of methods of sampling and analysis
(UNEP/IAEA/IOC, 1990).

5.4.1 Sampling:
- type of sampling;
- quantity of samples required;
- sampling equipment and containers required;
- sampling preservation.

5.4.2 Analysis:
- determination of required precision limits
(UNEP/IOC/IAEA/FAO, 1990);
- possibility of continuous monitoring of selected parameters;
- analytical procedures (analytical quality control)
(UNEP/FAO/IAEA, 1993);
- applicability of recommended standard methods
(UNEP/IAEA/IOC, 1990);
- intercalibration of results
(UNEP/IAEA/IOC, 1990).

5.5 Selection of method of data processing, storage and retrieval
(UNEP/FAO/IAEA, 1993).

5.6 Handling of information
(UNEP/FAO/IAEA, 1993).

5.7 Cost analysis:
A document on the principles of cost analysis for the execution of a programme is lacking.

5.8 Execution of the programme.

5.9 Evaluation of the results (attainability analyses):
development of an attainability analyses document.

6. Enforcement of laws and regulations in cases of violation of effluent standards and quality objectives: development of a document on the enforcement of protocols and national laws and regulations.

7. Remedial actions:

elaboration of a document on the integral assessment of the state of pollution in an entire area and the necessary remedial action to be taken, commencing with regional planning, reclassification of uses, revision of effluent standards and quality objectives, and ending with enforcement.

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# APPENDIX II

# A REGIONAL SITE-SPECIFIC TEMPORAL TREND MONITORING PROGRAMME

## TABLE OF CONTENTS

## BACKGROUND

Following the first phase of the implementation of the MED POL Programme (MED POL - Phase I) from 1975-1980, the Contracting Parties to the Barcelona Convention approved a ten-year long-term Programme (MED POL - Phase II, 1981-1990) consisting of a monitoring and research component. In 1991 the Contracting Parties extended MED-POL Phase II until 1995 and the Programme was subsequently extended until 1996 to enable its completion and the formulation of the next phase.

In 1992 the Bureau of the Contracting Parties requested the Secretariat to organise the preparation of an in-depth evaluation of the MED POL Programme by experts and scientists external to the MAP office, with the intention to use this evaluation in the drafting of Phase III of MED POL. This evaluation was presented to the Eighth Ordinary Meeting of the Contracting Parties in October 1993 (UNEP, 1993a). During this meeting the Contracting Parties formally agreed to the preparation of MED POL Phase III, covering the period 1996-2005, and set a number of basic objectives and principles for its preparation (UNEP 1993b, Annex IV).

The meeting of experts on the preparation of MED POL Phase III held in Izmir, in June 1994, after reviewing and discussing the achievements and shortcomings of Phases I and II of the MED POL Programme, prepared a draft Programme for MED POL Phase III which was submitted for approval to the Joint Meeting of the Scientific and Technical Committee and the Socio-Economic Committee in April 1995. The document was not considered by the Joint Meeting due to lack of time and consequently the delegations were requested to provide comments to the Secretariat in writing. After reviewing the comments received and taking into account the results of the informal consultation meeting on MED POL III (Athens, December 1995), the document was revised to bring it in line with the Action Plan for the Protection of the Marine Environment and the Sustainable Development of the Coastal Areas of the Mediterranean (MAP Phase II) which was approved by the Contracting Parties in June 1995. The revised document was submitted to the Meeting of MED POL National Coordinators (Athens, March 1996), the Meeting of MAP Focal Points (Athens, May 1996) and finally the Extraordinary Meeting of the Contracting Parties (Montpellier, 1-4 July 1996), where it was adopted (UNEP, 1996).

According to the Annex of the MED POL Phase III Programme, two basic types of monitoring will be organised, compliance and trend monitoring. Trend monitoring will be carried out at four levels, coastal zone trend monitoring, trend monitoring in pollution hot spot areas, trend monitoring of loads and trend monitoring of biological effects.

The present document was reviewed by an informal consultation held in Athens on 10-11 April 1997 (UNEP(OCA)/MED WG.128/3) and by the Meeting of MED POL National Coordinators (Delphi, Greece, 20-23 May 1997) (UNEP(OCA)/MED WG.127/4). It refers to the first two types of trend monitoring of chemical contaminants in biota and sediments and does not make a differentiation between them. In the document it becomes apparent that initially such an exercise could take place in areas where the expected changes in contaminant levels are high. Such areas may be polluted sites where control measures have been taken and an improvement is anticipated. Another point which must be noted is the fact that the level of the contaminant and its trend refers to the individual stations where the samples were taken. In this respect, the programme could be termed Asite-specific@ but, as in the case of the normal monitoring programme for levels, a statistical approach could be used to reflect the trends on a regional basis.

# 1. INTRODUCTION

The first phase of MED POL included baseline studies which would generate data which were insufficiently available at the time. However since only very few laboratories were able to perform such types of analyses, during the first phase of MED POL (1975-1980) emphasis was placed on strengthening and upgrading the technical capabilities of national laboratories, mostly in developing countries, so that all countries would be able to participate in the Programme, and on the development of the methodologies needed to implement the Programme. Analytical instruments and laboratory materials were provided while an extensive training programme was pursued. In addition, a quality assurance programme was launched which included the development of standard analytical techniques, the maintenance of instruments, intercomparison exercises, etc.

In view of the inexperience of many laboratories, and difficulties inherent to the Programme, the data collected during the first phase of MED POL could not be considered of high quality, largely due to the validity and comparability of the data and the uneven and inadequate geographical coverage of the Mediterranean Sea.

During MED POL Phase II, monitoring was organised on a national level. Each national monitoring programme aimed to cover monitoring of levels of pollutants in the marine environment (nearshore and offshore areas) and monitoring of sources of pollution including inputs through the atmosphere. The areas monitored were usually near the coast, especially those affected by pollution. Assistance to laboratories in the developing countries continued. The QA programme was enhanced with on-job training and split sample exercises. Data were provided to the Secretariat on an annual basis.

MED POL Phase II recommended a number of priority parameters to be monitored, (UNEP, 1986), frequencies and species.

This approach was soon abandoned as it was necessary to redesign each national monitoring programme on the basis of local needs and conditions. For example, in certain cases organohalogens were not monitored as they did not pose a problem in certain countries. The frequency of sampling in most cases changed from seasonal to annual.

Not all national monitoring programmes run smoothly. In many cases there are temporal gaps, despite the effort made to collect samples in such a way as to be able to use the data for the identification of trends, and in certain cases geographical gaps. Furthermore, the data stored in the MEDU data bank were not screened properly, until only very recently. In addition, while an effort was made to use all the data on the Mediterranean level, this proved fruitless as the data originated mostly from polluted areas and did not cover large parts of the Mediterranean. According to the evaluation of the MED POL Programme by external experts and scientists (UNEP, 1993a), this was due to insufficient infrastructure of the participating institutes and limited experience of analytical procedures, or due to limited specification of the work to be done in monitoring agreements. The evaluation concluded that the results of MED POL Phase II could not provide a complete and representative description of the state of the marine environment in the Mediterranean and could not allow an estimate of the balance of inputs.

Statistical analysis for trends of MED POL monitoring data for heavy metals and halogenated hydrocarbons in a MAP/MED POL study carried out in 1992 by Dr Robert Fryer, statistician and member of the ICES Working Group on statistical aspects of monitoring, revealed that due to the inconsistent collection, preparation and chemical analysis of data, objective investigation of between-year variation in contaminant levels is virtually impossible and that due to

an insufficient number of pools on each sampling occasion and/or an insufficient number of years sampled, only very large trends in contaminant levels are likely to be detected (Fryer, 1992). The results of the above study were not unexpected as the programmes were not designed accordingly.

In the present effort, trend monitoring, involves the selection of a number of fixed coastal stations from the national monitoring programmes, to be included in a regional monitoring network for the establishment of temporal trends in the Mediterranean. If this type of monitoring takes place in areas under the direct influence of pollution sources, the efficiency of control measures may be assessed.

# 2. PROGRAMME DESIGN

## 2.1 Introduction

A monitoring programme of trends in the levels of contaminants over a number of years, should provide a simple description of any observed variation with time in the environmental levels of contaminants, for example an upward or downward trend.

An obstacle to the above is the occurrence of variation between years caused by other sources of change such as biological variables, i.e. seasonal changes in the physiology and behaviour of organisms, and environmental variables i.e., changes in sediment composition, climatic changes etc. (see Carlberg, 1993; Phillips and Rainbow, 1994 chapters 5, 6 and 8). Furthermore, variations in analytical methods due to variations in sample and data handling may also be a source of between year variation. Large sample-to-sample variations in contaminant concentrations and small differences in mean contaminant levels between samples are found to occur due to sources of random between year variation. Therefore, in order therefore to detect environmental trends, particularly small changes, it is necessary to take into account all other factors contributing to variations, in the design of the monitoring programme and where possible take appropriate measures to ensure that these factors are kept constant or controlled.

A trend monitoring programme should include an indication of the level of change in contaminant concentration that the programme is expected to detect and the desired probability with which the change should be correctly detected. It is obvious that the exercise becomes simpler when the expected change is sufficiently high. The probability of having a correct answer from the programme must be investigated prior to the start of the programme and if found not to be satisfactory, the programme should be redesigned or the objectives of the programme should be reconsidered. Once a programme design is formulated it must be rigorously followed, including quality control and assessment methods, in particular those necessary to ensure analytical precision and accuracy, since any analytical trend that is not detected and corrected will be ascribed to the trend in environmental contaminant levels.

A detailed design of an environmental trend monitoring programme therefore should include the following:

- Description of the objective of the trend monitoring programme
- Determination of the stations to be selected for monitoring
- Determination of the contaminants to be measured
- Selection of the sampling matrices
- Determination of the species to be utilized
- Selection of tissues for analysis of contaminants in biota
- The timing and frequency of sampling
- The number of samples and size of specimens to be taken for each sample
- Determination of sampling and analytical methodology

Methods should subsequently be formulated for quality control and assessment. This document also includes relevant measures for assistance to participants in the regional trend monitoring programme.

## 2.2 The objective of the Trend Monitoring Programme

As stated above, the general objective of the trend monitoring programme is to provide an assessment of a change with time in the environmental levels of chemical contaminants. In particular a Trend Monitoring Programme should allow the identification of a specific temporal trend in the contaminant level with a given confidence. In order to specify the objective fully, the

manager has to give parameters for the confidence limits and the minimum trend that should be identified. Examples for these are given in the Annex.

## 2.3 Selection of monitoring stations

A number of fixed coastal stations from the national monitoring programmes will be selected by the MED POL National Coordinators in each country, to be used in the trend monitoring programme.

In order to select the location of appropriate stations for the detection of contaminant trends, the knowledge of the ecological dynamics in a specific coastal area as well as of its seasonal and annual patterns is necessary. In this context, the support of dynamic information derived from satellite remotely sensed data could be very useful. As a matter of fact, satellite sensors, could provide spatial and temporal patterns relevant to some sea surface parameters (suchas temperature, chlorophyll-like pigments, suspended matter) which are directly influenced by river discharges - as well as by plant discharge or coastal runoff in general - sea dynamics, seasons, biology productivity, etc..

The following criteria will determine the sites to be selected for trend monitoring:

- The selection of a site will satisfy the managerial objectives of the programme;

- The site will allow the detection of the change in contaminant level that the trend monitoring programme is expected to detect (as described in the accompanying Annex), through the selection of a realistic number of samples (see section 2.9 on the number of samples required for trend monitoring). This means that it is preferable to select sites where the expected change is sufficiently high so that the number of samples required will be realistic. Such sites may be polluted areas where control measures have been taken and a decrease in contaminant levels is expected;

- The site will allow the selection of a sufficient number of biota required for the trend monitoring programme, which fulfil the criteria for the selection of organisms for the purpose of monitoring chemical contaminants;

- The site will be suitable for sediment down-core analysis, particularly as regards sedimentation rates and bioturbation intensity.

## 2.4 Contaminants to be measured

The selection of contaminants depends on legislative requirements and managerial objectives. Analytical capability, as shown by the results of interlaboratory comparisons, quality control tests and limit of quantification is also considered.

On a Mediterranean scale and on the basis of the past MED POL monitoring data the following contaminants could be selected for measurement in the temporal trend monitoring programme:

a) - Total mercury in sediment and biota
   - Cadmium in sediment and biota

The above may be considered as priority contaminants, for which that trend monitoring would be carried out at most, if not all, selected stations.

b) - Total arsenic in biota
   - Zinc in sediment and biota

- Copper in sediment and biota
- High molecular weight halogenated hydrocarbons in sediment and biota
- Polynuclear aromatic hydrocarbons in biota.

The choice of monitoring the above and other contaminants according to the needs of the monitoring programme will be site dependent and will depend upon whether there is a past or present input of sufficient scale.

In the case of sediments, parameters for normalising the results should be addressed.

Total mercury in marine biota will be determined by flameless atomic absorption spectrophotometry (for a detailed analytical methodology of total mercury determination in marine organisms see UNEP/FAO/IAEA/IOC, 1984a).

Total arsenic in marine biota will be determined by hydride generation atomic absorption spectrophotometry (for a detailed analytical methodology of total arsenic determination in marine organisms see UNEP/FAO/IAEA/IOC, 1985).

Total cadmium, total zinc and total copper in marine biota will be determined by atomic absorption spectrophotometry (for a detailed analytical methodology of total cadmium, zinc and copper determination in marine biota see UNEP/FAO/IAEA/IOC, 1984b).

Total mercury in marine sediments will be determined by flameless atomic absorption spectrophotometry (for a detailed analytical methodology of total mercury determination in sediments see UNEP/IAEA, 1985a and UNEP/IOC/IAEA, 1995).

Total cadmium in marine sediments will be determined by flameless atomic absorption spectrophotometry or graphite furnace atomic absorption spectrophotometry when Cd is too low in concentration to be determined by FAAS (for a detailed analytical methodology of total cadmium determination in sediments see UNEP/IAEA, 1985b and UNEP/IOC/IAEA, 1995).

Total copper in marine sediments will be determined by flame atomic absorption spectrophotometry or graphite furnace atomic absorption spectrophotometry when Cu is too low to be determined by FAAS (for a detailed analytical methodology of total copper determination in sediments see UNEP/IAEA, 1985c and UNEP/IOC/IAEA, 1995).

Total zinc in marine sediments will be determined by flame atomic absorption spectrophotometry (for a detailed analytical methodology of total zinc determination in sediments see UNEP/IAEA, 1986 and UNEP/IOC/IAEA, 1995).

High molecular weight halogenated hydrocarbons in sediment and biota and polynuclear aromatic hydrocarbons in biota will be determined by gas chromatography. PCBs and DDTs and other halogenated hydrocarbons will be analysed by high resolution capillary gas chromatography. If not available, on a provisional basis analysis can be carried out by packed column gas chromatography but the upgrading is highly recommended. For a detailed analytical methodology of high molecular weight halogenated hydrocarbons and polynuclear aromatic hydrocarbons see UNEP/FAO/IOC/IAEA, 1986, UNEP/IOC/IAEA, 1988, UNEP/IOC/IAEA, 1992 and UNEP/IAEA/IOC/FAO, in preparation).

## 2.5 Selection of the sampling matrices

For the time being there is not sufficient expertise to undertake with certainty statistical trend monitoring in all matrices. Most of the available experience is in the use of biota which has a number of theoretical and practical advantages over the analysis of either natural waters or sediments. Most biomonitors exhibit contaminant concentrations which permit relatively simple measurement compared to seawater analysis which is costly and difficult to obtain high quality data especially for trace metals. In addition, the high contaminant variability in time and space make it difficult to obtain reliable trend information.

Most contaminants of concern in aquatic ecosystems tend to associate preferentially with suspended particulate material, rather than being maintained in solution, although this varies in extent between individual contaminants. Sampling of surficial sediment samples has the advantage of a simple methodology and more general availability. Sediment down-core analysis in order to identify past time trends requires more elaborate sampling material and expertise. Attention should be paid to site selection in relation to sedimentation rates and bioturbation intensity, favoring sites with high sedimentation rates and low bioturbation intensity.

Biota and sediments are therefore considered as the primary matrices for the sampling of contaminants for trend monitoring purposes, presenting the advantage of integrating contamination over time. Biota and sediments are primary matrices for the measurement of total mercury, cadmium, zinc, copper and high molecular weight halogenated hydrocarbons.

Polynuclear aromatic compounds are best monitored for trends in biota. Arsenic is a difficult contaminant to be monitored for trends; sediment profiles were proven unsatisfactory while the use of biota is still being studied.

The use of sediments and biota for marine pollution trend monitoring should ideally be part of an integrated monitoring programme which includes otgher compartments of the environment (e.g. SPM, seawater, interstitial water). This will help in the interpretation of the monitoring data.

## 2.6 Biota

### 2.6.1 Species to be selected for the measurement of contaminants

The trend monitoring programme will carry out measurements for contaminants in species most closely fulfilling the objectives of the programme while at the same time selecting species adhering to the greatest extent possible to the following criteria:

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

- The species accumulates the contaminant;

- The species is sedentary and thus represents the collection area;

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

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

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

- The species is easy to collect and hardy enough to survive unfavourable conditions or within the laboratory;

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

- The species tolerates brackish water, to allow comparisons between estuarine and offshore sites;

- The species must be easy to identify with certainty.

The following benthic or demersal species were used in the past for MED POL monitoring purposes:

- Bivalves
  *Mytilus galloprovincialis*, or
  *Mytilus edulis*, or
  *Perna perna*, or
  *Donax trunculus*
  The latter three species were suggested as alternative species if *M. galloprovincialis* did not occur in the area

- Demersal fish
  *Mullus barbatus*, or
  *Mullus surmuletus*, or
  *Upeneus mollucensis*
  The latter two species were suggested as alternative species if *M. barbatus* did not occur in the area.

  Sparidae were also used.

For efficient, cost-effective trend monitoring, particularly in sites which are not directly affected by large pollution discharges or yearly marked changes in discharges due to regulation, it may me useful to focus on one or two species selected as trend monitors. Mollusc species show many of the characteristics involved in the criteria for selection of species for the purpose of contaminant monitoring, and in particular, reflecting the environment=s response to changes in inputs. Common mussels are suitable for trend monitoring programmes in temperate coastal waters, reflecting contaminant loads in the water column when in good physiological condition. Deposit feeders reflect contaminant loads in the sediment.

### 2.6.2 The tissues selected for analysis of contaminants in biota

It must be stressed that once decided the same tissue should be used at all times and at all stations. If the selected species is a mollusc, the whole soft tissue may be used for analysis as it is a relatively simple process providing sufficient material.

In the case of crustaceans, the use of the digestive gland (hepatopancreas) which concentrates metallic and organic contaminants has proven to give satisfactory results for trends. However, if the managerial objective includes human health concerns, the whole edible tissue should be used.

For fish, muscle may be a suitable tissue for most purposes although it is usually selected for public health concerns, or where liver and kidneys may not provide sufficient tissue for analysis. Most toxic metals accumulate in liver and kidneys. Fatty tissues accumulate

hydrocarbons and organochlorines.

Other tissues may also be used especially target organs of specific contaminants.

### 2.6.3 Timing and frequency of sampling

It is a considerable demand on resources to sample and analyse biota and sediments several times every year, where this is not essential. For this reason sampling of biota for trend monitoring of contaminants could generally take place once every year, while sampling of sediments for trend monitoring of contaminants could take place over a larger time frame, depending upon contaminant influx and environmental physiochemical considerations.

Carrying out sampling of biota during a period in the year when contaminant concentrations are not being significantly affected by changes in physiological mechanisms, is essential for consistency of sampling. Such periods of minimal change are generally related to periods outside the spawning cycle and when food supply is relatively constant. Food supply and the spawning period are known to cause changes in total body weight, lipid concentration, lipid composition and therefore contaminant levels. In order to avoid such variations it is recommended that sampling take place in the pre-spawning period. In order to obtain comparable data from the various sampling stations it is necessary to establish the pre-spawning period at all these stations in order to ensure that samples are taken at correct occasions.

### 2.6.4 The number of specimens (individuals) to be included in each sample

The number of specimens needed to detect important trends depends on the type of the trend, the magnitude of the trend and the variability in the data. In order therefore to choose an appropriate number of specimens, the statistical power of the monitoring programme should be considered through power studies which examine the types and magnitude of changes that will be detected for a given number of specimens. This process is described in the attached Annex.

The number of specimens in each fish sample collection should be sufficient to allow the sample to be collected in a length-stratified manner (age-related), i.e., the size of the fish should include as wide a length-range as possible and there should be an equal number of individuals in each length-grouping. The agreed length-stratification for a particular species should be strictly adhered to each year. The number of specimens within a sample of mussels should be sufficient to allow as wide a size-range as possible.

#### 2.6.4.1 Pooling of specimens of biota

It may be necessary to pool (bulk) fish tissues, particularly in the case of fish livers and mussel and other shellfish tissues, in order to provide sufficient quantities of material for chemical analysis.

Pooling can distort the statistical analysis of log-transformed data by increasing the yearly mean concentration values and decreasing the power of tests to detect trends (Nicholson *et al.*, 1989).

Nicholson *et al.* (1989) however have shown that in general pooling does not influence trend identification (i.e. differences between years and associated regression coefficients will be unaffected, although trends may be less precisely estimated than from unbulked data), if pooling is consistent between years, i.e. if samples consist of the same number of pools, which contain the same number of specimens.

Keeping the same number of individuals in the pool between years is the most important

aspect, i.e. in the pool, for a given length class, the number should be the same each year. It is also important to maintain the same number of pools each year (preferably based on length-stratification of the sample if possible).

## 2.7 Sediments

Sediments have an important role in environmental monitoring as they are considered the sink of most contaminants. Marine sediments are closely inter-related to several other compartments of the marine environment. Therefore, their use in monitoring should ideally be part of an integrated monitoring programme which includes other compartments of the marine environment, such as, water, suspended particulate matter and biota. In addition, it is essential to enhance the comparability of results with other sedimentary data sets for the same contaminants. Factors which can be considered include water content, organic carbon, total extractable lipid content, grain size distribution, etc. Apart from the normalization techniques, harmonization of baseline studies as well as strict quality assurance/control programme are also essential.

Any monitoring programme, regardless of the actual matrix concerned, should be based on a statistically sound design. In particular, the probability of detecting a certain intensity in the variation of contaminant level over time should be estimated through the use of statistical power analysis. This approach, well established for the planning of the monitoring of contaminants in other matrix (such as biota), is well documented in literature. In particular, values which allow to estimate the uncertainty that the programme is going to face (component of variance) has not been documented for past programmes relevant to sediment.

Uncertainty can be expressed with respect to the quantity of interest (in this case long-term temporal variation) through an error model. This model should account for the quantities (known or assumed) that affect the estimated value for the underline trend in a specific time-point. A sensible model should account for:

a) small-scale spatial variability;

b) short-term temporal variation which may occur also in undisturbed surface sediment (because of biological activity or any other reason);

c) short-term analytical variability;

d) long-term analytical variability.

The key-factor is to obtain relevant and reliable estimates for (a) and (b) above. Pragmatic reasons (i.e. cost of sampling) suggests that the sampling effort (in time and space) required to estimate these components cannot be adopted in routine monitoring. Past research may suggest on the importance of these quantities. A pilot study may be a good option to estimate these components and careful judgement should be adopted to decide whether these components may apply for areas other than those they originate from.

There are as yet no concrete guidelines for the determination of the number of sediment samples to be used in the trend monitoring of contaminants in sediments. Further developments are expected from the ICES Working Group on Environmental Assessment and Monitoring Strategies and the ICES Working Group on Statistical Aspects of Environmental Monitoring.

# 3. SUPPORTING MEASURES

The success of the trend monitoring programme will depend largely on the development of adequate supporting measures, consisting of methods for quality assurance and assistance to

the participants in the monitoring programme.

## 3.1 Quality Assurance

Quality assurance of the monitoring programme refers to those procedures which are developed to ensure that analytical results are valid, traceable, reproducible, representative, complete and accurate, i.e. close to the true value; as well as measures developed to assess performance. Methods of quality assurance collectively consist of methods for quality control and quality assessment.

### 3.1.1 Quality Control Methods

The design of quality control methods involves the development of procedures for each step of the trend monitoring programme which contribute to the eventual production of quality data, and procedures to ensure that each participating laboratory will produce comparable data.

Quality control methods will involve the following:

a) Standard sampling and measurement procedures, including:

- species selection (i.e. methods to ensure the ability to distinguish between two native species belonging to the same genus)

- sample handling (including methods of storage, transportation, preservation, analytical sub-sampling, dissection, homogenization, bone grinding of tissue and sampling, sieving and grinding of sediments)

- biological measurements (methods for measurement of length, total weight, organ weights, methods for the determination of age, sex and fat and water content)

- chemical measurements (methods for the analysis of chemical contaminant residues)

Guidelines and recommended procedures for the storage and pre-treatment of samples following their collection are given in UNEP/FAO/IAEA (1984) and UNEP/FAO/ IAEA/IOC (1984c) dealing with heavy metals and halogenated hydrocarbons respectively.

Important procedures for consistent sampling, sample preparation and chemical analysis for trend monitoring of contaminants in biota are described in UNEP/FAO/IOC/IAEA (1993), Uthe, J.F. (1994) and Uthe *et al.* (1991). The role of consistency in the sampling process is described in Fryer (1993).

Guidelines and recommendation procedures for sediment sampling, handling etc., are given in UNEP/IOC/IAEA (1995), Mudroch and Ascue (1995), Mudroch and MacKnight (1994).

b) Data handling procedures, including methods of data translation, data transcription and keeping records of calculations, methods for long-term storage of data in log-books or computer files and methods of data reporting.

Data handling procedures are described in UNEP/FAO/IOC/IAEA (1993), UNEP/IOC/IAEA/FAO (1989), Uthe, J.F. (1994) and Uthe *et al.* (1991).

c) Use of certified reference materials (CRMs) of identical or similar matrix as the sample analysed and covering the concentration ranges likely to be encountered in the measurements, in order to obtain accurate and precise data following routine measurements. Available reference materials, for the choice of appropriate CRMs are listed in IAEA (1995).

d) Regular analysis of reference materials throughout the monitoring programme in order to ensure that analytical performance is maintained.

e) Regular mandatory participation of the laboratories involved in the trend monitoring programme in intercomparison exercises organized by the IAEA-MEL, in order to ensure the comparability of the data being produced among the participants in the programme.

Intercomparison exercises will involve the analysis, through methods used in their normal monitoring work, by each participating laboratory, of blind samples containing unknown concentrations of a specified analyte or samples of known but undisclosed concentrations of the analyte.

f) Regular calibration, servicing and maintenance of all the equipment. According to the needs of the programme and resources available, the IAEA-MEL will backup laboratories in this field, namely through supply of calibration standards to laboratories.

### 3.1.2 Quality Assessment Methods

Quality assessment methods will be developed for the assessment of the performance of individual laboratories participating in the monitoring programme over time and in relation to the other laboratories participating in the programme

The assessments will be based on the analyses of CRMs and other reference materials. The quality assessment methods will include rules for the selection of reference materials and the frequency of their analyses when carrying out interlaboratory assessments of performance with respect to the quality of trend data.

Methods will be developed for the regular documentation on control charts of the results of analyses of the same reference material over a period of time, in order to obtain information on whether the results are within the acceptable limits of accuracy and precision and how this information is comparable to the results of other laboratories participating in the programme.

These measures should also provide important information for the design of the trend monitoring programme.

## 3.2 Other forms of assistance

The necessary training will be provided namely through group training courses, to ensure that all laboratories are able to participate in the trend monitoring programme and in the relevant quality control and quality assurance activities.

Where problems continue to exist in the sampling process or in the analysis of

contaminants, or both, the necessary assistance will be provided on a case-by-case basis for any participant requiring such assistance.

Where continuing analytical problems have been established for a participant, a methodology for split-sampling and analysis of contaminants by another laboratory will be formulated.

In order to ensure the comparability of monitoring data on a regional scale, analysis of split samples will be organized in support ot national trend monitoring programmes in need.

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# ANNEX

## Power studies of a programme design for temporal trend monitoring in biota

Table of contents

## INTRODUCTION

The present annex aims to explain how to carry out a power study for the design of a monitoring programme. The explanation follows the applied statistics which have been developed for the planning of programmes using biota to detect temporal trends of contaminants in the environment as well as following general guideline for UNEP's Regional Seas programmes. The presentation is relevant to the statistical methodology itself, therefore any environmental issues for the programme design (such as site and species selection) are out of the scope of the present annex and are reported in the main part of the document.

In a broad sense, power studies encompass activities which link together the specification, the optimization and the effectiveness (i.e. how good the programme is) of a monitoring programme through the use of statistics. Power studies may be used for the planning of programmes as well as for the assessment of already-collected monitoring data. The present annex is relevant to the latter, thus the power study may be directed either at estimating the effectiveness of a programme which follows a specific *ad-hoc* protocol as well as having a certain laboratory performance, or the identification of the number of samples that should be collected to detect an important trend, or the estimation of which intensity (i.e. how strong the variation of contaminant concentration is over time) in trend can be detected by a programme etc.

The exercise can be roughly summarized in the following steps:

a) make clear the purpose of the study and "translate" it into a statement (monitoring objective) that can be handled through the use of statistical methods;

b) gather all the information which is required for the study and perform the actual calculation;

c) compare the result of the study with any expected (or required) values which may have been set prior to the beginning of the study. If the result is not satisfactory, decide on any possible revision of the protocol of the programme. An example may explain the issue: the investigator wants to decide the number of samples to be collected to achieve a target value in the effectiveness of the programme and reaches the conclusion that the adopted protocol cannot provide this value regardless of the number of samples, so the programme' s protocol should be revised to search for any optimization.

A power study is based on a detailed **monitoring objective**. In particular the objective must account for:

a) the trend itself that should be detected (magnitude and shape over time);

b) design information such as programme duration, number of specimens to be collected and the possibility of pooling of specimens in composite sample;

c) managerial aim relevant to the effectiveness of the programme or in other words how much the investigator wont to be sure about having a correct answer from the programme;
d) information on the uncertainty which affects the estimated value for level of contaminant in the environment. This uncertainty arises from analytical performance of chemical analysis and variability in the population of monitoring organisms.

Point a) above describes trend in term of magnitude and shape of variation of contaminant level over time. The former refers to the maximum difference in contaminant level in the time-series. The latter accounts for how this variation occurs over time. For example

variation may be easily described by upward or downward shape such as a straight line or an exponential variation. Other shapes, such as random variation, may indeed occur.

The annex is organized as follows:

- the first section explains how to formulate monitoring objectives about the trend in a statistically sound way;

- the second section deals with monitoring objectives, in particular it presents how the objective should be formulated and which information it should include;

- the third section explains the power calculation: it presents the relevant quantities and emphasizes such issues as how many samples should be collected and what is the maximum allowed level of error to detect an important trend;

- the fourth section provides examples of power studies for certain monitoring objectives.

## 1. FORMULATION OF OBJECTIVES IN TEMPORAL TREND MONITORING

This section is relevant to an important part of applied statistics: the test of hypothesis, which can be found in any text book (such as Zar, 1984 and Sokal and Rohlf, 1995).

The investigator has in mind a question such as : *is there a trend in contaminant level over time?* To search evidence about the answer from the monitoring data this person formulates two mutually exclusive (i.e. if one is true the other is false and vice versa) hypotheses:

- the first denotes "no difference" in contaminant level over time and is called **null hypothesis** ($H_0$);

- the second denotes "difference" and is called **alternative hypothesis** ($H_1$),

subsequently the investigator tests $H_0$ against $H_1$ through the use of a proper statistical test. If $H_0$ is rejected in favor of $H_1$ then the investigator concludes that a trend in contaminant level over time exists. It is important to note that the non-rejection of the null hypothesis does not necessarily mean that the hypothesis is true: it denotes that there is not enough evidence to conclude that it is false.

Example: how to formulate the hypothesis

The investigator searches evidence for a linear trend of contaminant level over time. This trend is written as: $y = a + bt$, where b is the variation per time unit (or slope of $y$ versus $t$).

$b$=0 denotes no trend of $y$ over $t$ (i.e. a flat trend) whereas $b \neq 0$ denotes trend.

The hypotheses are :
$H_0$: $b$=0 and $H_1$: $b \neq 0$.

## 1.1 Error in hypothesis testing and power

The investigator should realize that a true null hypothesis occasionally will be rejected, which means that a trend is erroneously identified when in fact it does not exist. This error occurs with probability á and its value is chosen by the investigator itself. A common value (but not mandatory) is á=0.05 but values up to á=0.1 are statistically accepted. á=0.05 means that the investigator is keen to accept to wrongly reject a true null hypothesis 5% of the time. The probability of accepting a true null hypothesis is 1-á. On the other hand if $H_0$ is in fact false the test may not detect it. This error occurs with probability â. **The power of the test is the probability of rejecting the null hypothesis when it is false and therefore should be rejected, and is defined as 1-â** (Zar, 1984). In trend monitoring â=0.1 % (i.e. power= 90%) may be considered an acceptable value, but this is not mandatory.

The following table summarizes the probabilities of having correct or wrong answers from the test for trend (showing in parenthesis the above-mentioned typical values for probabilities):

| Decision taken | Null Hypothesis true | Null Hypothesis false |
|---|---|---|
| Accept Null Hypothesis | 1-á (95%) | â (10%) |
| Reject Null Hypothesis | á (5%) | power=1-â (90 %) |

It is important to remark that both errors (á, â) cannot be minimized for a given programme and intensity for the trend: the smaller á is chosen the larger â will be. Should both errors be small, then only a strong trend is likely to be detected.

# 2. OBJECTIVES OF MONITORING PROGRAMMES

The design of a programme is based on a monitoring objective which is:

a. sufficiently detailed;

b. expressed in a measurable way;

c. formulated in a way to allow handling through statistical techniques (statistically sound formulation).

An objective which is formulated in the proper way allows to link monitoring questions, programme specification and effectiveness and to focus the attention on the problem to be solved. Those designing the programme will formulate the objective according to their questions. Typical questions may be how many samples are required to detect an important trend?

## 2.1 Steps in designing monitoring programmes

This section provides examples of power studies. The actual calculation is described in section 4.

Objectives may include statements which can be arbitrary. These are relevant to the duration of the programme and the intensity of the trend. The former may be either the minimum number of years which is required to have a reliable answer from the programme or the time-

span in which any answer should be provided. The latter refers to the intensity which is expected. If there is no interest in any of these quantities they must be intended to be statistical requirements, i.e., they must be components of the power study.

A proper power study should explore several aspects of the design. For example the planner should look at the sensitivity of power to the values of the other parameters (which reflects the specifications of the programme), identify the minimum value for trend that is likely to be detected an so forth. An appropriate way to look to these dependencies may be to go through the following examples. The order of these example is not important and specific needs should suggest how to organize power studies.

**EXAMPLE 1**

Suppose that the manager of an international monitoring programme has gathered information on components of errors from several laboratories and different areas, such as those reported inTable 2. This person wants to see the power that the local programmes are expected to have. Values inTable 2 have been used for demonstration.

The objective of the power study is formulated as follows:

*Identify the power of a monitoring programme to detect a trend of 10 % per year in a 10 year programme, at different levels of analytical and sampling variability and number of specimens collected*.

Comment. The choice of the magnitude for the trend is arbitrary. The choice of the duration of the programme may follow managerial constrains.

The calculation is presented in example 4.1.

**EXAMPLE 2**

Suppose that the investigator of one laboratory has reliable estimates for components of error. The investigator intends to reduce the cost for analysis, therefore the effect of pooling specimens into a composite sample is explored. Values in Table 2 have been used for demonstration.

The objective of the power study is formulated as follows:

*Identify the power of a monitoring programme to detect a trend of 10 % per year in a 10 year programme at different levels of pooling and number of specimens collected and one level of analytical and sampling variability.*

Comment. The choice of the magnitude for the trend is arbitrary. The choice of the duration of the programme may follow managerial constrains.

The calculation is presented in example 4.2.

**EXAMPLE 3**

Section 1.1 presented the topic relevant to errors in hypothesis testing. This example explores the trade-off between á-error and minimum detectable trend. Suppose that the investigator intends to identify which magnitude in trend is likely to be detected at different values of á (i.e. different level of risk in false rejection of $H_0$). Values inTable 2 have been used for demonstration. The example has been tailored around the number of specimens collected.

The objective of the power study is formulated as follows:

*Identify the minimum detectable trend to identify a linear trend during 10 years at different levels of á and number of specimens collected, with 90 % power.*

Comment. The choice of the duration of the programme may follows managerial constrains.

The calculation is presented in example 4.3.

**EXAMPLE 4**

Suppose that the investigator intends to identify the target value for ø required to detect important trends.

The objective of the power study is formulated as follows:

*Identify the maximum value for total error to detect a trend of 10 % per year in a 10 year programme, with á=0.05 and 70 % power.*

Comment. The choice of the magnitude for the trend is arbitrary. The choice of the duration of the programme may follow managerial constrains.

The calculation is presented in example 4.3.

## 3. COMPUTATION OF STATISTICAL POWER

This section explains how to calculate the statistical power. Part 3.1 describes which quantities are involved; part 3.2 reports on the calculation of power for the test for a linear trend. Appendix 1 describes the statistical methods employed in part 3.2. Appendix 2 explains how values for error can be obtained from monitoring data.

### 3.1 Which quantities are involved in the calculation of power

The probability of having a correct answer from the programme (i.e. 1-á or 1-â as described in section 1) depend on:

- the signal-to-noise ratio of contaminant variation over time;

- the specifications of the programme such as: duration, number of specimens collected and degree of pooling into a composite sample.

In particular its value increases with:

- duration (hereafter denoted as *T* in years);

- number of specimens (*R*),

whereas its value decreases with:

- noise

- degree of pooling, i.e., how many specimens are included in a composite sample (I) prior to its analysis.

The magnitude of the trend as well as its shape over time should be accounted for when the value for intensity of the signal is calculated. In fact theory (Fryer and Nicholson, 1993) shows that certain shapes of trend are detected easier than others.

Noise denotes the uncertainty between the measured level of contaminant in the environment and its underline value (which is unknown). For programmes following UNEP guidelines (UNEP/FAO/IOC/IAEA, 1993) noise is conveniently defined as the total residual variance (ø5) about the mean:

$$ø5 = ó_y5 + ó_w5/R + ô_y5 + I\,ô_w5/R \quad (1)$$

where *R* is the number of specimens collected and *I* is the number of specimens which may be pooled in each composite sample. The components for error are:

$ó_y$. Random between year sampling variability. This component accounts for the error between the contaminant level measured in the monitoring population and its underline level in the environment. It arises from uncontrolled sources which affect the entire population. Simply, this component attempts to quantify "how well" the monitoring population estimates the level of contaminant in the environment;

$ó_w$. Random sampling variability within a year. This is the variability which is left in the annual data after the effect of any co-variables (such as size) has been accounted for. This component arises from individual variability in the accumulation features within the monitoring population;

$ô_y$. Random between-year analytical variability. This quantity estimates the bias over a long-term period (say one year), following ISO 5725 its value is calculated as: $ô_y^2 = Z_R^2 - Z_r^2$, where $Z_R^2$ is the reproducibility variance and $Z_r^2$ is the repeatability variance;

$ô_w$. Random within-year analytical variability. This quantity is the precision of the laboratory within a small time-period;

The formulation of total variance with four components arises from the simplest specification for the programme (i.e. all specimens for one year are collected in one occasion and analysed in the same time). Different programme specifications will lead to a different formulation of total variance.

Components of error may be estimated from monitoring data provided this being documented. In particular $\hat{o}_y$ and $\hat{o}_w$ of chemical analysis must be known. The process goes through the following steps:

a) Calculate ø5. This is the residual variance of mean yearly values regressed on years. Unless a certain pattern being the best fit (such as linear, exponential), it is not appropriate to impose a "formula" to data. Therefore a generic function (such as locally weighted smooth curves), which fits mean yearly values regardless any desired pattern being imposed, should be used to calculate ø5.

b) calculate $ó_w$5 from the within year variance by correcting it for $\hat{o}_w$ (by difference).

c) calculate $ó_y$5 from ø5 by correcting it for $ó_w$5, $\hat{o}_y$ and $\hat{o}_w$.

Published values, which have been calculated following the above approach, are reported in Table 2. Components of error have been computed on logarithmic scale. This type of transformation have been used (Anon, 1989; Anon, 1991 and Zangrandi, 1996) with monitoring data (contaminants in marine organisms) to satisfy the assumption required by the method (see appendix 1).

## 3.2 Power of the test for a linear trend

The previous section (3.1) shows that the signal must be defined also with respect to the shape of variation of contaminant level over time. Therefore the shape of the trend should be decided prior to planning any test for a trend.

It is sensible to aim to detect a linear trend as this shape is among the easiest to be identified (Nicholson and Fryer, 1992). Therefore a programme which is not likely to detect a linear trend will not identify other more difficult shapes.

Power is a function of:

- á-value;
- duration of the programme (*T*);
- signal-to-noise ratio (**b*/Ø*).

Power values, programme duration and signal-to-noise ratio (computed for á= 0.05) have been combined in Table 1. Provided that the statistical requirements of the test are satisfied (see Appendix 1), Table 1 can be used to solve monitoring objectives (see example 4.4).

Table 1 may be insufficient (for example if **b*/Ø* is out of the Table or á?0.05 is chosen) or tedious to use on a routine basis, thus the computation can be carried out with the proper probability distribution. Power can be calculated from a non-central F distribution on 1 and *T*-2 degrees of freedom, with non-centrality parameter ä (see Zar, 1984).

The quantity ä accounts for the signal-to-noise ratio as well as for the number of years. For a linear trend ä takes the form (Nicholson *et al.*, 1996):

ä= b5(T-1)T(T+1)/12ø5 (2)

*power* = 1 - prob F(F1-á,1,*T*-2,ä), (3)

where F1-á is the 100(1-á)th percentile of a central F-distribution on 1 and *T*-2 degrees of freedom. These probabilities can be obtained from statistical tables (fpr a full description of the procedure see Cohen, 1977), or they can be computed with packages such as SAS, SPLUS, STATABLE (and perhaps others). The manuals of the packages are sufficiently detailed to allow the actual computer function to be written easily.

## Table 1

Test for a linear trend. Values of **b*/ø* corresponding to different powers (columns) and numbers of years (rows). á=0.05 (from Nicholson *et al.*, 1996, modified)

| | **0.50** | **0.60** | **0.70** | **0.80** | **0.90** | **0.95** | **0.99** |
|---|---|---|---|---|---|---|---|
| **5** | 0.906 | 1.035 | 1.176 | 1.344 | 1.584 | 1.786 | 2.175 |
| **6** | 0.616 | 0.700 | 0.791 | 0.899 | 1.051 | 1.178 | 1.421 |
| **7** | 0.459 | 0.520 | 0.586 | 0.664 | 0.773 | 0.864 | 1.037 |
| **8** | 0.360 | 0.408 | 0.459 | 0.520 | 0.604 | 0.674 | 0.806 |
| **9** | 0.293 | 0.332 | 0.373 | 0.422 | 0.490 | 0.546 | 0.653 |
| **10** | 0.245 | 0.278 | 0.312 | 0.352 | 0.409 | 0.455 | 0.544 |
| **11** | 0.209 | 0.237 | 0.266 | 0.300 | 0.348 | 0.388 | 0.462 |
| **12** | 0.181 | 0.205 | 0.230 | 0.260 | 0.301 | 0.335 | 0.400 |
| **13** | 0.159 | 0.180 | 0.202 | 0.228 | 0.264 | 0.294 | 0.350 |
| **14** | 0.141 | 0.160 | 0.179 | 0.202 | 0.234 | 0.261 | 0.311 |
| **15** | 0.127 | 0.143 | 0.161 | 0.181 | 0.210 | 0.233 | 0.278 |
| **16** | 0.114 | 0.129 | 0.145 | 0.163 | 0.189 | 0.211 | 0.251 |
| **17** | 0.104 | 0.117 | 0.132 | 0.148 | 0.172 | 0.191 | 0.228 |
| **18** | 0.095 | 0.107 | 0.120 | 0.136 | 0.157 | 0.175 | 0.208 |
| **19** | 0.087 | 0.098 | 0.110 | 0.125 | 0.144 | 0.160 | 0.191 |
| **20** | 0.080 | 0.091 | 0.102 | 0.115 | 0.133 | 0.148 | 0.176 |
| **21** | 0.078 | 0.084 | 0.094 | 0.106 | 0.123 | 0.137 | 0.163 |
| **22** | 0.069 | 0.078 | 0.088 | 0.099 | 0.115 | 0.127 | 0.152 |
| **23** | 0.065 | 0.073 | 0.082 | 0.092 | 0.107 | 0.119 | 0.141 |
| **24** | 0.060 | 0.068 | 0.077 | 0.086 | 0.100 | 0.111 | 0.132 |
| **25** | 0.057 | 0.064 | 0.072 | 0.081 | 0.094 | 0.104 | 0.124 |

**Table 2**

Values for components of error (logarithmic scale) relevant to mercury contamination in fish from an international monitoring programme for three levels of variability (Anon, 1995)

| variability | $ó_w$ | $ó_y$ | $ô_y$ | $ô_w$ |
|---|---|---|---|---|
| Low | 0.08 | 0.22 | 0.09 | 0.04 |
| Medium | 0.26 | 0.28 | 0.13 | 0.05 |
| High | 0.52 | 0.42 | 0.24 | 0.10 |

## 4. EXAMPLES OF POWER STUDIES FOR CERTAIN MONITORING OBJECTIVES

This section describes the calculation relevant to the examples of objectives provided in section 2.1. Because of the routine calculation which is required to prepare figures 1 to 3 those interested in repeating the example as an exercise should perform at least part of the calculation with a calculator:

a. If Table 1 is used, calculate ø;

b. If the power is calculated from a non-central F distribution (either using software or statistical tables), calculate ø and ä.

Relevant values for components of error are essential for power studies. Examples 1,2 and 4 are based on the only up-to-date published values (Table 2). These components are relevant to an international programme as well as having the following features:

- the wide range of their values arises from different analytical performances from several laboratories or because of different species being employed. The range of a single laboratory is likely to be smaller;

- $ó_y$ dominates the total variance. This component is not affected neither by R nor by I (see formula 2), therefore, looking at figures. 1, 2 and 3, it may be wrongly concluded that the number of specimens to be collected is not important. The decision on the size of the sample should take into consideration the heterogeneity of the monitoring population whit respect to, for example, sexual development or any other factor that may not be taken into consideration (or it is difficult to see) while collecting the specimens.

### Example 4.1

For convenience the calculation has been made only for three combinations of errors inTable 2 (along the rows), for *I*=1 (no pooling) and á=0.05.

a. calculate ä for different values of *R* (number of specimens), by substituting values accordingly in equations (1) and (2);

b. calculate power from non-central F distribution on 1, *T*-2 degrees of freedom and non-centrality parameter ä.

The result is shown in figure 1.

Remark. The study aims to explore extreme scenarios of monitoring performance, so it is assumed that low sampling variability occurs with low analytical variability and high sampling variability occurs with high analytical variability. In the first case (fig. 1 top) power is very high whereas in the second instance the level for power is unacceptable (fig. 1, bottom). A more realistic picture in given by a medium level of variability (Table 2, center). In this case the programme is likely to have a power ? 80 % (fig.1, middle).

**Example 4.2**

The computation follows example 4.1 except for the calculation of ä which takes into account different combinations of *I* and *R*.

The result is shown in figure 2.

Remark. Following a strict statistical consideration, the study suggests that pooling does not affect power, provided that a reasonably large number of specimens are collected (i.e., *R*>20). The reason for this depends on the large value for $ó_y$ which dominates ø (Table 2). $ó_y$ does not change with *R* or with *I* (see formula 1), therefore any optimization of the programme should be directed to reducing $ó_y$.

**Example 4.3**

a. Calculate from the non-central F distribution the value of ä at á, and 1, *T*-2 degrees of freedom which leads to power=90 %;

b. Calculate *b* from ä for different values of *R* (by substituting values accordingly in equations (1) and (2)).

The result is shown in figure 3.

Remark. The example explores the trade-off between á, â and minimum detectable trend. For simplicity â has been held constant. The choice of á and target value for â depends on the managerial aim of the programme and the risk (in managerial terms) of an á or a â error. A programme for general trend surveillance (i.e. no trend direction is expected) where the programme itself is not meant to be a regulatory tool, may have á and â of the same magnitude (in fig. 3 á=â=10 %). In a programme to monitor a recovery measure, an á-error is less problematic than a â-error, so a larger á allows the detection of smaller trends.

**Example 4.4**

The following example describes the use of Table 1.

The monitoring objective is satisfied (for *T*=10 and power 70 %) if:

**b*/ø* $ 0.312; then the maximum error is:

ø # 0.320. This value is compared with those that arise from the components inTable 2 (for *R*=25 and *I*=1):

- low variability ø5=0.0165, ø=0.128

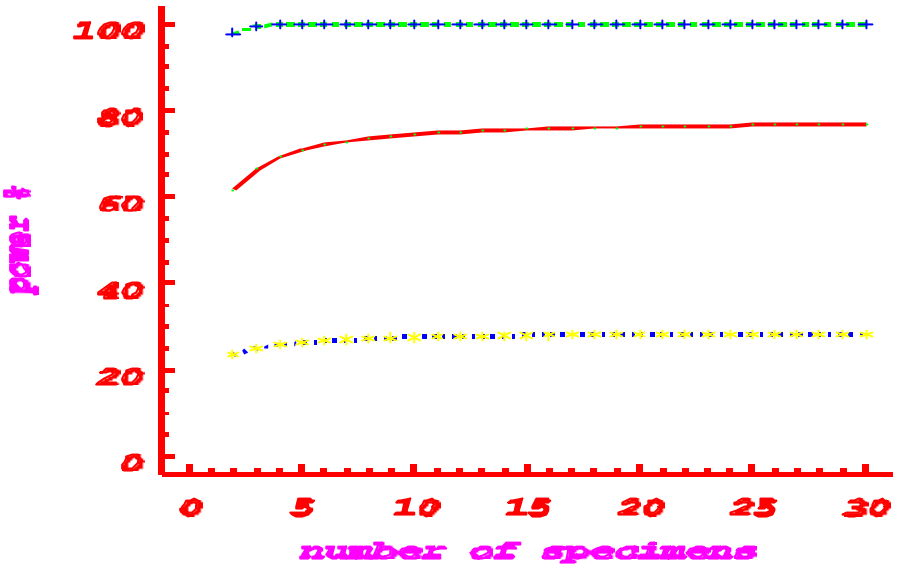


Figure 1. Example 1. Power (%) for the test for a linear trend versus number of specimens per year for different levels of error. á=0.05. No pooling (I=1). Values for *b*, *T* and errors, as in the text.

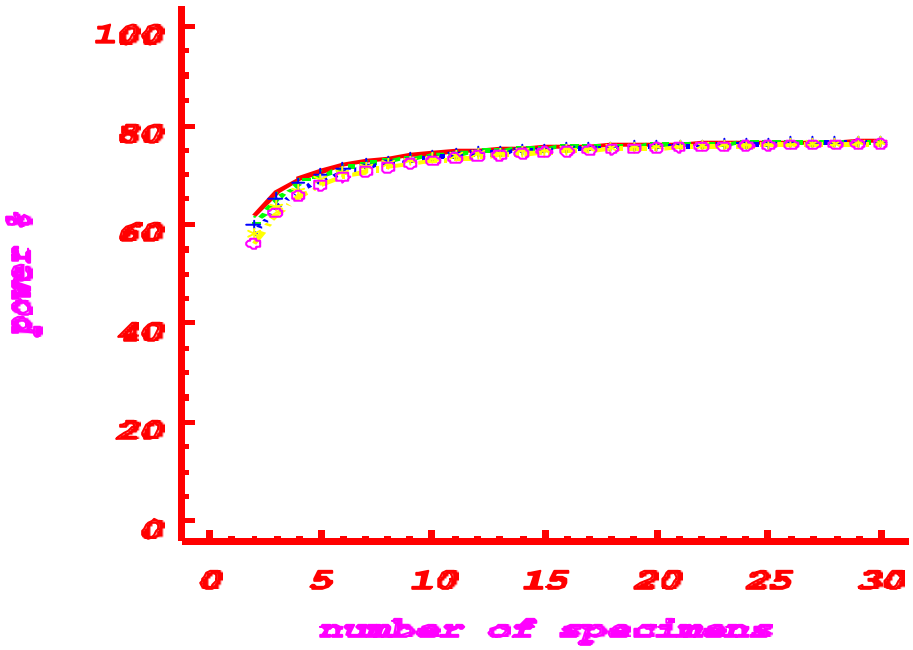


Figure 2. Example 2. Power (%) for the test for linear trend versus number of specimens per year for different degree of pooling ( : I=1, +: I= 5, x: I=30, o: I=15). á value=.05. Values for *b*, *T* and errors, as in the text.

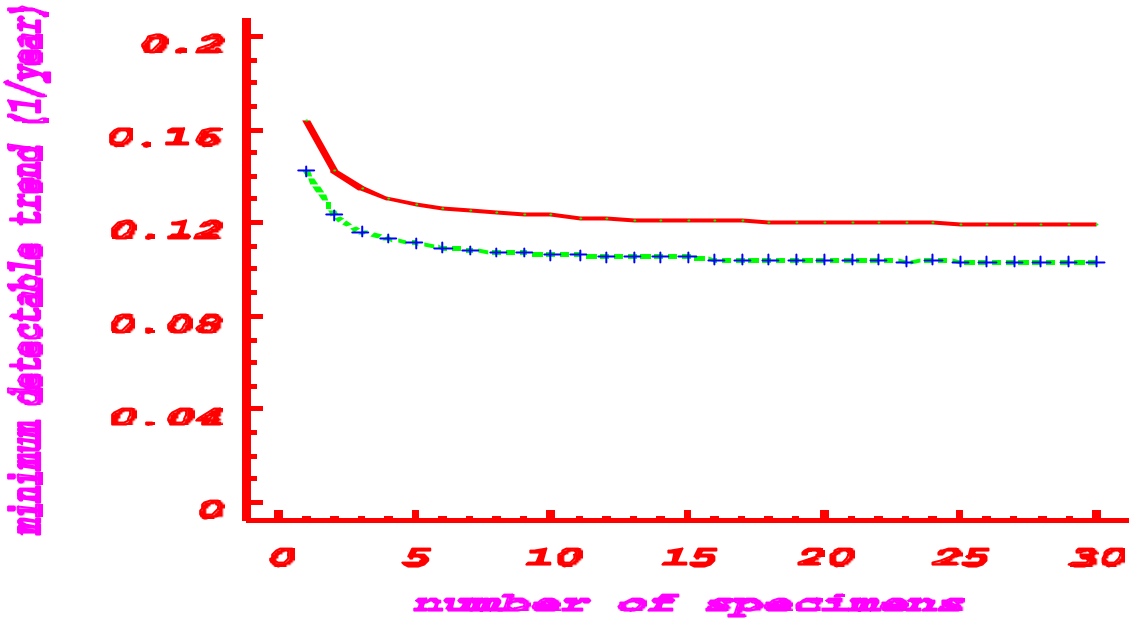


Figure 3. Example 3. Minimum detectable trend (year $^{-1}$) versus number of specimens per year for different á values (ò: =.05, +: = .10). Target power=90%. No pooling (I=1). Values for *T* and errors, as in the text.

- medium variability ø5=0.087 ø=0.296,

therefore medium variability will lead to the target value being satisfied.

Suppose now a more strict value for power, say 90 %. From Table 1:

*b*/ø $ 0.409; then

ø # 0.244. Therefore only low variability (Table 2) will satisfy the target value for ø

## Appendix 1. Description of the statistical method

Let $y_t$ be the mean value of contaminant concentration in the year *t*. A linear trend for the expected value of $y_t$ is written as:

$E[y_t] = a + bt$.

Assume that $y_t$ are independent and normally distributed (Zar, 1984) about the linear trend with constant variance:

$Var[y_t] = ø5$.

Then the evidence for a linear trend is given by the value for *b* (see example 1) which is calculated by regressing $y_t$ on years. The null hypothesis:

$H_0$.: $b$=0

is tested against the alternative hypotheses:

$H_1$.: $b$?0

by F-test on 1 and $T$-2 degrees of freedom (Zar, 1984).

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# APPENDIX III

# MANUAL ON THE BIOMARKERS RECOMMENDED FOR THE MED POL BIOMONITORING PROGRAMME

## PREFACE

The scope of this manual is to serve as an initial source of technical reference for laboratories interested to start routine biological-effects pollution monitoring. A number of biomarkers have been considered in this manual, starting from those capable of giving a general indication of biological stress due to pollution. These biomarkers of stress (*general stress indices*) include the assessment of damage to genetic and subcellular components. Both the elevation of enzymatic activity of the mixed function oxygenase system (MFO) and the induction of metallothionein (MT) protein synthesis also termed as biomarkers of exposure (*specific stress indices*), are considered.

The potential use of the last two biomarkers is considerable, since these biomarkers are able to show the cellular response to heavy metals (MT) and aromatic organic xenobiotic compounds (MFO), both of which are considered as being major pollutants in coastal areas. Such biomarkers seem to offer the best information on the biological response of the animals to **the two classes** of toxic pollutants therefore reporting an *early warning signal* that environmental damage is in progress. They have been carefully characterised in a number of marine organisms and proved to be suitable to identify a biological response induced by the effects of the chemical pollutants. However, due to the complex nature of these biological responses, extreme caution should be exercised when coming to interpret monitoring results from field stations. These responses have to be assessed in the light of the physiological status of the test organisms at the time of sampling which can be ascertained by the measurement of the two general stress indices mentioned above (genetic damage and alteration of lysosomal activity). This manual also attempts to address a number of technical pitfalls and whenever possible, suggests ways how to enhance the certainty of biomonitoring results.

### Important note on health safety

Safety deserves special attention. Most of the chemicals and equipment listed in the following sections are relatively harmless, provided they are not abused. Disposable items should be used wherever possible, as should safety items such as gloves, lab coats and special waste disposal containers for carcinogenic substances.

### Acknowledgements

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## 1. INTRODUCTION

Upon exposure to harmful contaminants, marine organisms start manifesting a number of symptoms that are indicative of biological damage, the first ones appearing after a short while at the subcellular level. These *'sublethal'* effects, when integrated, often converge to visible harm for the organisms and to the whole population at a later stage, when it is too late to limit the extent of biological damage resulting from environmental deterioration.

Most of these symptoms have been reproducibly obtained in the laboratory and the various biological mechanisms of response to major xenobiotics are now sufficiently well understood. The use of *biomarkers*[1] has, since then, came into common practice by ecotoxicologists who used these responses as 'early warning' pollution monitoring tools to signal the onset of harmful effects at the cellular and sub-cellular levels. The following discussion briefly describes a number of common sublethal effects, exhibited by marine organisms as well as their correct application as biomarkers of biological harm resulting from marine pollution.

### 1.1 Mutagenic processes

Abnormal DNA replication, transcription as well translational processes of RNA (Eichorn, 1973) often represent symptoms following the interaction between xenobiotics and the nuclear material. *In vitro* studies show that heavy metals, for example, can easily alter the complementing hydrogen bonding capability of nucleic acids and thus facilitate the onset of the above processes.

Metabolic by-products of bioaccumulated xenobiotics can also generate harmful effects on the integrity of genetic material. Within the eukaryotic cell, organic xenobiotics are biotransformed into several mutagenic and carcinogenic metabolites by cytochrome P-450 enzymes and the epoxide hydrase function (see 1.3 below). These metabolites are highly reactive by-products with an affinity for nucleophilic sites on cellular macromolecules, like DNA. The interaction of these reactive substances with DNA can lead to the formation of DNA lesions. Unless inhibited by certain types of contaminants, such as heavy metals, specialized nuclear enzymes are able to correct these lesions thus minimizing misexpression of the genome. Inefficient repair of the genome also characterizes one of the first steps which lead to chemical carcinogenesis and malignancy. This could have a serious implication if such processes are detected within the reproductive tissues of marine organisms, for this would negatively affect its reproductive success.

So far, the study of genotoxicity has mainly been confined to human and other mammalian systems. Direct correlation of those genotoxic effects onto other, different organisms, is not advisable since these may be influenced by quite different mechanisms, such as differential accumulation capabilities, differences in metabolic pathways or complements of enzymes which are characteristic of different phyla.

It was only recently that the successful use of standard methods to determine genotoxic damage has rendered it possible to substantiate mutagenic effects in marine invertebrates (Shugart, 1988) and reveal a direct correlation between chromosomal abnormalities, nuclear enzyme inhibition and other related events with the bioaccumulation of heavy metals (Dixon, 1983) and polycyclic aromatic hydrocarbons (PAHs) (Bolognesi *et al.*, 1991).

The benefits of using these responses to monitor genotoxic risks is self-evident, and

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[1] Two other categories fall under biological effects monitoring: (1) bioassay testing and (2) ecosystem responses

are creating the need for both the systematic screening of major marine pollutants for their DNA-altering potentials as well as unmasking suspected pollution sources. In fact they have been instrumental in detecting such pollution hazards along specified coastal stretches in the Mediterranean (Scarpato *et al.*, 1990; Bolognesi *et al.*, 1991).

1.2 Cytoplasmic injuries

As known, eukaryotic cells respond to common pollutants by exhibiting a series of cellular changes that ultimately can lead to cellular death. Such changes involve alterations of plasma membrane and the activity of the different intracellular compartments.

One subcellular component which has proved to be very sensitive to the presence of contaminants is the lysosomal vacuolar system. The importance of lysosomes lies in their normal degradative role of cellular and extracellular macromolecules by means of hydrolytic enzymes segregated within them (such as proteases, lipases, nucleases, phosphatases, etc.). Damage to lysosomes often involve loss of integrity of their membranes, this leading to functional alteration and eventually to a release of their hydrolytic enzymes into the cytosol. Many environmental contaminants, including aromatic hydrocarbons, carbon tetrachloride, asbestos, silica, aminoazobenzene derivatives, beryllium, metals and viruses are known to be sequestered into lysosomes under certain conditions (Moore, 1985). As mentioned, overloading of the lysosomes leads to membrane destabilisation and as extreme consequence to cellular necrosis due to the release of degradative lysosomal enzymes. Undoubtedly, a negative consequence of the alteration of the lysosomal vacuolar system would be a perturbed intracellular digestion, rapidly affecting the nutritional status of the cell and eventually of the organism.

The evaluation of lysosomal injury has now become widely accepted as a sensitive index of cellular health, where the destabilisation of the lysosomal membrane bears a quantitative relationship to the magnitude of the stress response. *In vitro* (Moore, 1990) and *in vivo* (Lowe *et al.*, 1992) investigations on lysosomal damage have been successfully carried out along pollution gradients using both teleosts and invertebrates, and have been correlated with total tissue burdens and benthic sediments for a range of contaminants.

1.3 Alteration of normal biochemical pathways

1.3.1 Induction of cytochrome P450-dependent mono-oxygenase system

This multi-enzyme complex consists of a group of hemoproteins associated with the smooth endoplasmic reticulum. Among the wide range of different types of reactions, they are able to catalyse metabolically-important reactions as well as the oxidative hydroxylation of organic aromatic compounds such as hydrocarbons, O-, N- and S-dealkylation reactions, N-oxidation, sulphoxidation and deamination reaction. In a nutshell, the role of cytochrome P450 is to convert lipophilic, endogenous and xenobiotic organic substances into water-soluble compounds (biotransformation) to facilitate their elimination from the body.

Of particular interest to pollution monitoring is that in vertebrates the P450 gene expression and enzymatic activity can be markedly induced by a number of chemical compounds which belong to common classes of environmental pollutants, including PAHs, polychlorinated dibenzo-p-dioxines and dibenzofurans, polyhalogenated biphenyls and other halogenated organic compounds such as pesticides and herbicides. The induction of the P450 isoenzymes has therefore attracted the interest of ecotoxicologists for its application as a field sublethal bioassay that is diagnostic for PAH exposure. Their interest also focused on the biotransformation of these xenobiotics which, unlike normal endogenous compounds, tends to

be deleterious since their transformation could lead to a more chemically reactive species (Heidelberger, 1973). Studies show that these intermediate metabolites are able to bind to DNA and promote mutagenesis.

Cytochrome P450 activity in marine vertebrates, and to some extent also in invertebrates, has proven to be one of the most sensitive indicators of environmental contamination (Payne, 1977) as this assessment is based on a sound knowledge of the properties and regulation of this enzyme complex (Stegeman, 1989). Toxicity studies demonstrate that this biomarker is a more sensitive indicator of pollution stress than other physiological variables such as osmoregulation and energy metabolism (Nikunen, 1985). This system responds relatively rapidly to a variety of organic environmental pollutants as well as to complex mixtures including municipal and industrial effluents. Induction can be detected fairly rapidly and elevated activities of P450 can persist for weeks after a contaminant exposure has ceased (Kloepper-Sams and Stegeman, 1989).

In field situations, P450 activities in fish (EROD) were shown to give sensitive responses to a PAH pollution gradient over a broad area in the north-western Mediterranean region, validated by a concomitant PAH concentration in the sediments (Garrigues *et al.*, 1990). Preliminary investigations in the Mediterranean to measure benzo(a)pyrene-mono-oxygenase (BaPMO) activity in *Mytilus galloprovincialis* seem to indicate the possibility of using these molluscs for the detection of PAH pollution gradients (Selli *et al.*, 1994). However, further research is required to fully understand this response in molluscs.

### 1.3.2 Increased cytosolic levels of metal-binding proteins

Another biological response occurring at the subcellular level is the induced rate of synthesis of a class of metal-binding proteins, known as metallothioneins (MT), following exposure to heavy metals. This is considered to be a sublethal detoxification response, but the induced synthesis of these proteins could result in biological costs which reduce the fitness of the individual.

Extensive studies using many different organisms (Hamer, 1986) indicate that these proteins have three major physiological roles: (1) *Detoxification of heavy metals that penetrate into the cells at toxic concentrations* (Goering and Klaassen, 1984), which is strongly supported by the inducibility of MT genes by heavy metals accumulated within the cell (Hamer *et al.*, 1985); (2) *Internal homeostasis of Cu and Zn* by (a) keeping intracellular concentrations of free Zn and Cu cations at a low level by binding excess metals in a non-toxic form as well as (b) acting as a storage of Cu and Zn for later use and reactivation of *apo*proteins that require these metals for their activity (Brouwer *et al.*, 1986) and (3) *Participation in metabolic functions,* including scavenging of free radicals (Thornalley and Vasak, 1985; Brouwer and Brouwer, 1998) and protection against damage due to ionising radiation (Karin, 1985) and, as recently proposed, regulation of gene expression (Roesijadi *et al.*, 1998).

The use of this biomarker for biomonitoring the environmental metal impact has now been validated and a number of biomonitoring exercises using this index have been conducted in various areas within the Mediterranean. (Viarengo *et al*., 1988a; Pavicic *et al.*, 1991; Galdies, 1995; UNEP, 1997a; 1997b; Tambutté *et al*., 1998).

## 1.4 Utilisation of sub-lethal responses as diagnostic tools

### 1.4.1 Monitoring criteria and sample acquisition

Sampling of biological material to monitor sublethal responses has its special requirements. Allowance should be given to a number of biotic and abiotic factors which can potentially influence, and thereby disturb, the analysis. The investigator has to make sure that changes in the magnitude of the responses are only due to temporal pollution fluxes rather than to any other source of variation; to do this, the stress indices have to be measured in the same test species at the same time and place (UNEP/FAO/IAEA, 1993). Inconsistent sampling tends to add noise to the data and makes it harder to identify meaningful trends. Temporal monitoring should also take into account fluctuations in the population density in a way not to interfere with statistical sampling. Allowance should also be given to geographical variation which might affect the physiology of bioindicator species.

Values of T, pH and possibly salinity of the water, where the animals are collected should be provided. The site location should be clearly indicated by geographical co-ordinates.

Once collected, samples are to be handled in the same way as in previous sampling occasions and are to be adequately stored to prevent degradation of their biochemical entities or activities. Studies show that most biochemical parameters can only survive short periods of time at ambient temperatures and for this reason, either dry ice or liquid nitrogen could be required for temporal storage of animals or tissues prior to storage in a deep freeze at -80°C.

It is important to point out that the utilization of animals, caged for brief period of time (weeks) in farms that guarantee animals with standard characteristics, instead of wild animals represents a well recognised tool to reduce the effects of biotic and abiotic factors on the physiological status of the selected sentinel organisms.

### 1.4.2 Choice of species

The choice of the test organism must be guided by several criteria including its abundance and geographical distribution along the Mediterranean coasts, longevity (to allow sampling of more than one-year class if desired), ease of sampling all year round, whether it is amenable to laboratory investigations. It is also important that the animal physiology and biochemistry should be quite well known. Having done this, one soon realises that there is available a limited choice of marine vertebrates and invertebrates[2]. Based on previous research work in the field, a number of species have been identified.

Teleosts such as combers (*Serranus* spp.) tend to be sensitive to PAH pollution (Narbonne *et al.*, 1991) and are therefore chosen as test organisms to monitor the activity of their cytochrome P-450. The selection of this hermaphroditic species provides the investigator with some advantages, both in terms of eliminating sex-linked variations in the magnitude of the response as well as due to its sedentary mode of life.

Seabass (*Dicentrarchus labrax*) also responds well towards PAH contamination in terms of P450 activity. Sexually immature representatives (weighing up to 75±17g) are preferred to eliminate sex-type differences in enzyme activity levels. This fish is reared in aquaculture farms and therefore represents an ideal species for caging experiments in biomonitoring programmes.

Striped mullet (*Mullus barbatus*) has also been successfully used and proved to be an

[2] Apart from animals, investigations are being carried out to use similar responses in marine plants, such as *Posidonia oceanica*

excellent bioindicator to monitor P-450 activities (Mathieu *et al.*, 1991). It has been categorised as belonging to the high hepatic xenobiotic-metabolising activity fish group, showing a higher enzymatic activity than do combers, seabass and other fish. Mullets exhibit an increased enzyme activity from October to February which decreases just before spawning. Sex-type differences in P450 activity is also exhibited by this species, with males showing a higher P-450 activity than females during the reproductive period.

*Mytilus galloprovincialis* is the recommended mollusc but if not available *Patella* sp. can be used. The recommended species for caging is *Mytilus galloprovincialis*. In particular molluscs are recommended to evaluate lysosomal membrane stability, metallothionein content and DNA alterations.

Sessile marine molluscs such as mussels for biological monitoring are generally preferred because they exhibit the necessary criteria that qualifies them as good bioindicators (Viarengo and Canesi, 1991). They can bioaccumulate a number of contaminants and are particularly responsive to the major classes of environmental pollutants. However, mussels are not widely distributed in the Mediterranean and therefore other species have to be selected where absent or when spatial biomonitoring data is to be compared. Other drawbacks for those wanting to use these molluscs include their low level of both their MFO activity as well as its responsiveness to organic xenobiotics. As a comparison, the level of benzo(a)pyrene hydroxylase activity is increased by only about 50% in mussels from polluted areas (Suteau *et al.*, 1988) but by at least three times in fish collected from the same area (Addison and Edwards, 1988). With regard to MT measurements, mussel tissues (such as gills) prove to be a good indicator of Cd, Cu and Hg, but not of Zn pollution (George and Olsson, 1994).

Patella species have been recently utilized in the framework of the MED POL biomonitoring programme, in coastal areas were mussels are not present (Israel, Malta), obtaining encouraging results for the biomarkers utilized.

One other thing regarding invertebrates is that although many aspects of metabolising systems are similar, there nevertheless exist significant differences between various invertebrates even within the same phyla. For example, the ability of synthesizing MT proteins in response to Cd is clearly not uniform among molluscs and is due to interspecific differences in bioaccumulation (Langston *et al.*, 1989).

General stress indices (such as genetic and subcellular injuries) seem to be ubiquitous in all eukaryotic cells, thus precluding problems when coming to choose the test species. Obviously, care should always be taken to try to establish a good signal-to-noise ratio as much as possible.

### 1.4.3 Interpretation of results: some practical considerations

A prerequisite for using biomarkers is the knowledge of their normal physiological fluctuation range but also their real meaning. Consideration has to be given to any background variability in order to obtain a good signal-to-noise ratio.

It is important to note that the strong variations of environmental parameters may influence the physiological status of the sentinel organisms. It has been observed that the integrity of the lysosomal membrane can be destabilised by non-chemical stressors such as hypoxia, hyperthermia, osmotic shocks, etc. (Moore, 1985). These factors were found to modulate the response of the P-450 enzymes to environmental pollutants. Changes in salinity, temperature, and oxygen availability are among the most significant factors influencing mono-

oxygenase activity (Goksoyr and Forlin, 1992) and metallothionein levels in mussel (Viarengo *et al.*, 1988b) and in fish. (Engel, 1988).

A good example of a major physiological factor influencing the activity of some of these biomarkers in vertebrates, particularly mono-oxygenase activity, is a change in the levels of reproductive hormones. In many fish species, mono-oxygenase activity usually decreases shortly before or during spawning season. Differences in liver mono-oxygenase activities between fish sexes has also been recorded (Stegeman *et al.*, 1986). Reproductive stress may also pose urgent requirements of some essential metabolic precursors, such as essential metals like Cu and Zn which in turn may stimulate the appropriate storage, transport, and release of these metals to metabolically-important sites. This process exactly fits one of the functions of metallothionein proteins (see section 1.3.2), and justifies its transient elevated synthesis during such kind of physiological states.

One may therefore conclude that confident application of biomarkers in environmental monitoring very much depends upon the understanding of the regulatory processes involved and characterisation of the magnitude and timing of these changes. For these reasons, as mentioned above, the “caging system” represents the more appropriate approach to reduce the biological variability of the animals sampled in different sites along the coast.

### 1.4.4 Effects due to complex mixtures of pollutants

Apart from the above a/biotic factors, biomarkers can also be affected by thousands of different chemicals present in field contaminated areas; the results obtained are due, therefore, to the interactions of the different pollutants with the studied biomarkers (such as additive, synergic and/or antagonistic effects). It is very difficult to reproduce realistically these complex mixtures in the laboratory; but readers can at least glimpse at the interactive effects between the biomarkers and major type of contaminants by referring to the diagram below (Fig. 1).

Studies suggest that the presence of organic xenobiotics in the sampling environment can reduce the synthesis of metallothionein (Viarengo *et al.*, 1988b). We have already seen that these organic xenobiotics tend to bioaccumulate in lysosomes and destabilise the integrity of the lysosomal membrane thus affecting the catabolic rate of cellular proteins, including the integrity of the biomarkers (Viarengo *et al.*, 1987).

Moreover, concentrations down to nanomolar levels of $Cu^{2+}$, $Hg^{2+}$ and $CH_3Hg^{+}$ in the reaction mixture are able to significantly inhibit P450 activity as detected by a catalytic assay whereas at micromolar concentrations, the enzyme is totally inhibited (Viarengo *et al.*, 1994). This has important implications in the utilisation of MFO activity as a tool for the evaluation of marine pollution due to organic xenobiotics and can explain the low correlation sometimes observed between P450 activity levels and the amount of organic xenobiotic compounds accumulated in fish liver cells.

Another significant interference is caused by the presence of bioaccumulated tributyltin (TBT) compounds. The leaching of this biocide from antifouling paints is

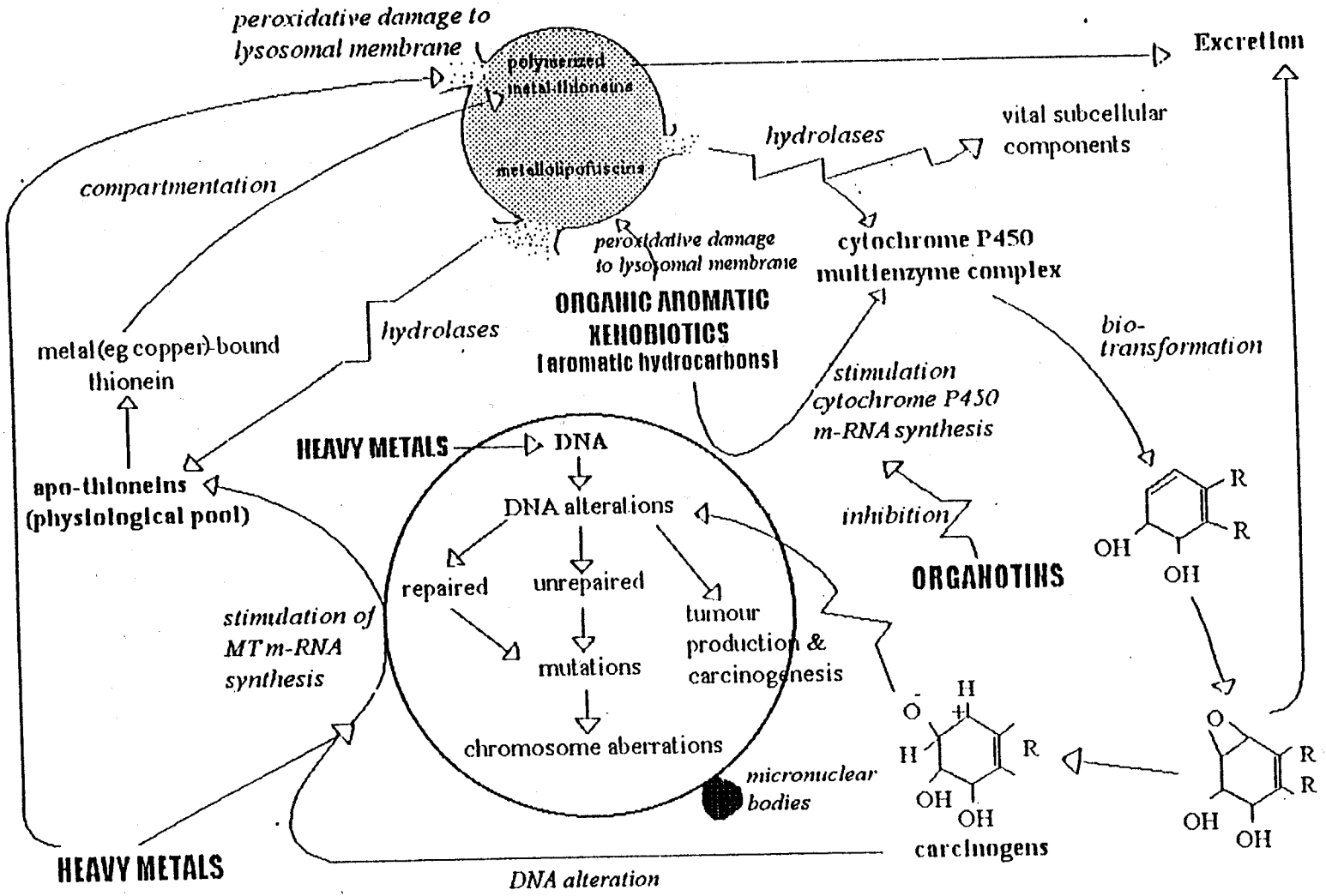


Fig. 1 Synergistic effects of a mixture of pollutants on various metabolic pathways. Sequence of events is initiated by cell exposure to different types of pollutants (see text for discussion).

considered to be a major pollution problem in enclosed areas where there are significant yachting and shipping activities. Studies show that mono-oxygenase activities can be reduced in the presence of even minimal levels of TBT, indicating considerable effects of TBT on this enzyme complex (Cassar and Axiak, 1994). This strongly suggests that exposure of fish to this widespread marine contaminant may alter the induction response to other environmental contaminants, particularly PAHs.

### 1.5 Conclusion

Some may say that the implication of these interactive effects vis-à-vis the interpretation of those stress indices under field situations which are not specific to any particular class of contaminants, such as the changes in the lysosomal hydrolase latency and mutagenic events, is not critical. Rightly so. However, specific stress indices, like the MFO system and MT which may be susceptible to interactive effects, are to be interpreted with caution when applied to field situations having mixtures of both organic and inorganic xenobiotics. **However, it is important to emphasize that a correct approach of biomarker utilization in biomonitoring programmes consists of the use of a battery of biomarkers: both biomarkers of stress such as DNA damage and lysosomal membrane stability evaluation and biomarkers of exposure i.e. metallothionein, EROD and eventually acetylcholinesterase activity.**

It is hoped that this brief introduction highlighted both the intricacies of utilising these sublethal responses as biomarkers of biological harm, as well as pitfalls often encountered if results are not interpreted correctly. Analysis of isolated homeostatic mechanisms is never encouraged, and a general picture should always be considered and translated into the overall state of health of the organism, and eventually of the population.

## 2. EVALUATION OF LYSOSOMAL MEMBRANE STABILITY

### 2.1 Background

It is very difficult to evaluate the molecular changes affecting the permeability of the lysosomal membrane. These analyses require extensive purified lysosomal membrane preparations and their examination at molecular level. An easier way to assess this parameter is to examine whether its normal physiological function has been altered or disrupted following exposure to pollutants.

One tool which links both descriptive morphology and biochemistry to observe such pathological alterations is cytochemistry. Apart from permitting the use of very small samples of tissue, this technique is ideal to detect changes in particular target cells and tissues.

Cytochemistry has been successfully applied to assess lysosomal integrity by visualising the hydrolytic enzymes within the lysosomes, and has proved to be a rapid and sensitive investigative tool for evaluating the effects of organic xenobiotics and other injurious agents at very low intracellular concentrations. This generalised response occurs in all cell types ranging from fungi to vertebrates, so that such cytochemical test can be applied on a fairly widespread basis.

### 2.2 Assessment of lysosomal membrane stability: cytochemical assay on cryostat sections

2.2.1 Principle

The following protocol is a cytochemical procedure for the determination of lysosomal membrane stability, based on the evaluation of the activity of N-acetyl-â-hexosaminidase, a lysosomal enzyme. Lysosomal destabilisation is measured as the increased permeability of the substrate (naphthol AS-BI N-acetyl-â-glucosaminide) visualized by the reaction with the enzyme into the lysosomes in presence of diazonium salt. The preparation of tissues for the examination of cell structures requires the use of specialised methodology to produce high-quality stained sections. In this section all observations are related to frozen material, and this preparative technique will be described.

2.2.2 Solutions and chemicals

Lysosomal membrane labilising buffer (Solution A)

- 0.1 M Na-citrate Buffer - 2.5% NaCl w:v, pH 4.5

Substrate incubation medium (to be prepared just 5 minutes before use) (Solution B)

- 20 mg of naphtol AS-BI N-acetyl-â-D-glucosaminide (Sigma, N4006) are dissolved in 2.5 ml of 2-methoxyethanol (Merck, 859) and made up to 50 ml with solution A, containing also 3.5g POLYPEP (Sigma, P5115; low viscosity polypeptide to act as a section stabiliser).

Diazonium dye (Solution C)

- 0.1M Na-phosphate buffer, pH 7.4, containing 1 mg/ml of diazonium dye Fast Violet B salts (Sigma, F1631) (Note: saturated solution)

  Other dyes can be utilised such as:
  Fast Garnet GBC (Sigma)
  Fast Red Violet LB (Difco)
  Fast Blue BB (Sigma)
  Fast Blue RR (Sigma)

Fixative (Solution D)

- calcium formol: 2% Ca-acetate w:v + 10% Formaldehyde v:v
  Mounting Medium: aqueous Mounting Medium (Vector Laboratories H1000) or Kaiser glycerin gelatin

- Liquid Nitrogen

2.2.3 Preparation of tissue

Rapidly excise 5 small pieces (3-4$mm^3$) of the organ/tissue (usually digestive gland of mollusc or fish liver) obtained from five different animals and rapidly place them on an aluminium cryostat chuck (i.e. aligned in a straight row across the center).

While dissecting, leave the chuck on ice and then place it for 40 seconds in a small

plastic box containing pre-cooled N- hexane[3] at -70°C using liquid nitrogen. Seal the chuck with 4-5 pieces of Parafilm and immediately store at -80°C. (At this temperature the tissue preparations maintain their integrity for months).

Using a Bright's Cryostat or other equivalent equipment (cabinet temperature below -25°C), cut 10 ìm thick sections using a 15° knife angle. Transfer the sections to "warm" slides (at room temperature) to flash-dry them. The slides can be stored in the cryostat (for at least 4 hours).

2.2.4 Enzymatic determination of membrane stability

Place the sections in a Hellendal jar containing solution A for different times (0, 3, 5, 10, 15, 20, 30, 40 minutes) at 37°C in order to find out the range of pre-treatment time needed to completely labilise the lysosomal membrane (i.e. labilisation period). In the last five minutes use shaking water-bath.

Transfer the set of slides to solution B and incubate the slides for 20 minutes at 37°C in a Hellendal jar preferably in a shaking water-bath.

Wash the slides in filtered sea-water at room temperature or with a saline solution (3% NaCl) at 37°C for 2 to 3 minutes. Transfer the slides to solution C containing the diazonium coupler for 10 min at room temperature. Rapidly rinse the slides in running tap water for 5 minutes. Fix the sections for 10 minutes in solution D at 4°C (or mount directly with glycerol gelatin), rinse in distilled water and mount in aqueous mounting medium.

2.2.5 Interpretation of results

View the slides under a microscope and divide each section into four areas (quarters) for statistical interpretation (see Fig. 2).

Lysosomes will stain reddish-purple due to the reactivity of the substrate with N-acetyl-â-hexosaminidase. The average labilisation period (LP) for each section corresponds to the average incubation time in the acid buffer that produces maximal staining reactivity. LPs for the other samples (in this case n=5) are similarly obtained.

Now analyze one quarter of each set of sections pertaining to the same animal and find out the section quarter showing maximal staining (from which the LP value is derived). Repeat this analysis for the remaining three quarters of the first specimens analyzed. The mean of the results obtained represents the LP value of specimen 1. The same procedure is adopted for the other sections present on the slides derived from the other four remaining animals. Finally, a mean value of lysosomal membrane stability of the sample will be calculated utilizing the 5 data obtained from the 5 animals analyzed.

Compare test samples with those taken from reference area and determine gradient of cytotoxicity. Reduction in the LP along the expected pollution gradient would indicate cellular stress due to pollution.

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[3] Hexane cooling prevents the formation of ice in the tissue and hence it reduces any structural damage to the subcellular components

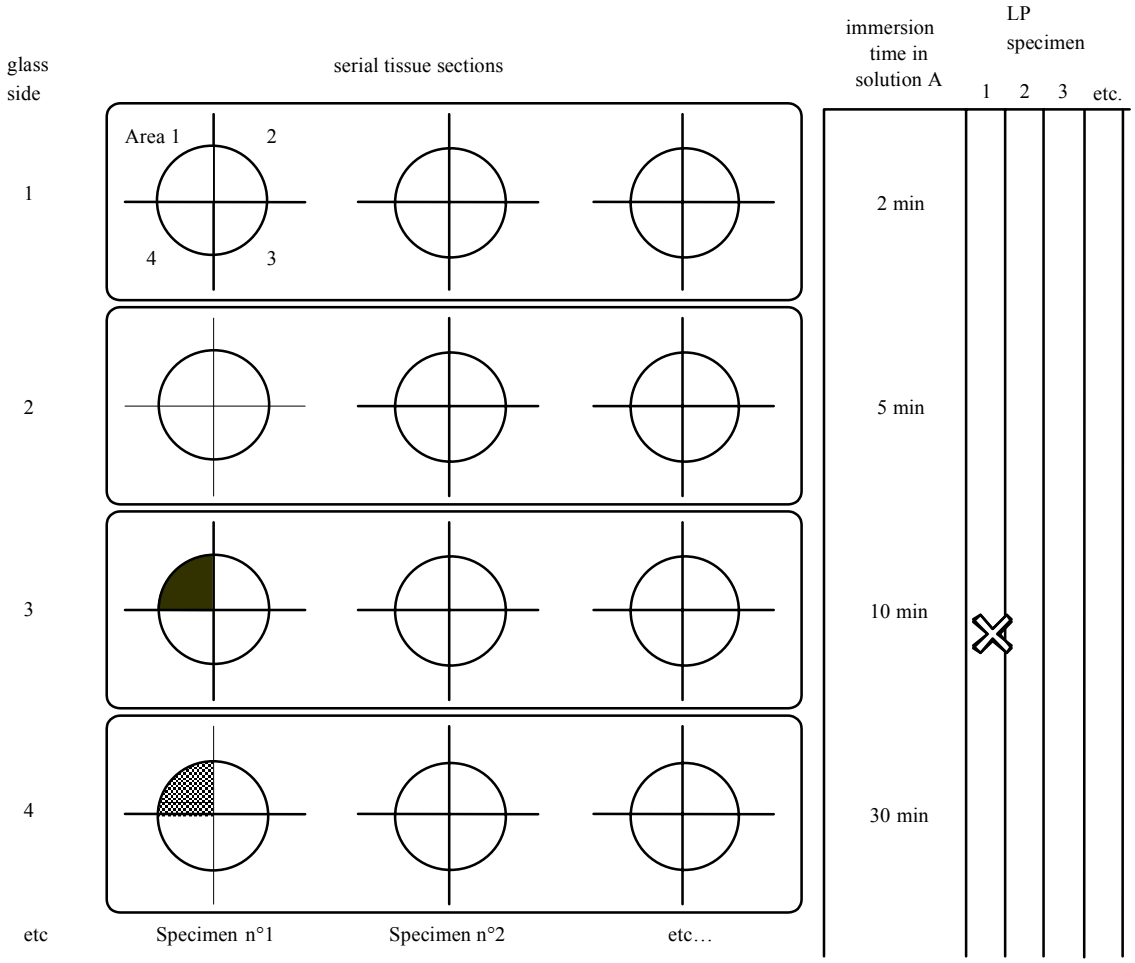



Fig. 2 Staining Intensity:

Area 1 (10 min) > Area 1 (30 min) > Area 1 (15 min) ...etc.
Area 2 (10 min) > Area 2 (30 min) > Area 2 (20 min) ...etc.
Area 3 (15 min) > Area 3 (20 min) > Area 3 (30 min) ...etc.
Area 4 (15 min) > Area 4 (30 min) > Area 4 (20 min) ...etc.

LP value for specimen 1 = mean of 4 areas = 12.5 minutes

Any decrease in staining intensity in successive sections following that with maximal staining may be due to loss of enzyme by diffusion from fully labilised lysosomes. If there are two peaks of staining intensity, then consider only the main staining peak as the LP. This may be due to differential latent properties of the lysosomal hydrolase concerned.

"0" time will be utilised only to verify the correct lysosomal enzyme activity and it will not be considered in the evaluation of the maximal staining intensity peak.

Intervals of 3 or 5 min are generally satisfactory for most test situations. The data can then be statistically tested using the Mann-Whitney U-test (Speigel, 1961) and compared with reference data. For mussel digestive gland, timing intervals of 3, 5, 10, 15, 20, 30 and 40 minutes are normally utilized.

2.3 Determination of lysosomal membrane stability in living cells: neutral red retention

assay

Neutral red is a lipophilic dye and as such will freely permeate the cell membrane. Within cells the compound becomes trapped by protonisation in the lysosomes and accumulated in these organelles, where it can be visualised microscopically. The degree of trapping of this lysosomotropic marker depends on the pH of the lysosome as well as the efficiency of its membrane associated proton pump (Segien, 1983).

The acid environment of lysosomes is maintained by a membrane $Mg^{2+}$-ATPase dependent $H^+$ ion proton pump (Ohkuma *et al.*, 1982), the neutral red retention assay reflects on the efflux of the lysosomal contents into the cytosol following damage to the membrane and, possibly, impairment of the $H^+$ ion pump (Lowe *et al.*, 1992). So any impairment of this latter system will result in a reduction of the dye retention assay. Studies indicate that, similarly to the cytochemical method described above, the neutral red retention assay is sensitive to the main classes of chemical pollutants (Lohse, 1990). The following protocol has been specifically adapted to be used on mussels.

2.3.1 Chemicals and solutions

- Physiological saline
  20 mM (4.77g) Hepes
  436 mM (25.48g) NaCl
  53 mM (13.06g) $MgSO_4$
  10 mM (0.75g) KCl
  10 mM (1.47g) $CaCl_2$

  Dissolve these in 1 liter of distilled water. Gas for 10 minutes (95%$O_2$:5% $CO_2$) and adjust to pH 7.3 with 1M NaOH. Store solution in refrigerator, but use it at room temperature.

- Neutral Red dye
  Prepare stock solution by dissolving 20 mg of neutral red powder in 1 ml di-methyl-sulfoxide (DMSO). Transfer 5 ìl of stock solution into 995 ìl of physiological saline (working solution). Keep neutral red in the dark and in fridge when not ulilized. The working solution must be prepared freshly before analysis.

2.3.2 Practical evaluation

The following procedures is according to Lowe *et al.* (1992) and Lowe *et al.* (1995).

Fill the eppendorf tubes with sigmacote (SIGMA) for 10-30 minutes, then return sigmacote to container (it is reusable). Put 2 ìl of Poly-L-Lysine (SIGMA), diluted 1 to 10 with distilled water, on a microscope slide and spread out with a cover slip. Leave to dry in a humidity chamber.

Insert scissors half way along the ventral surface of the mussel and partially disclose the valves to allow the insertion of the hypodermic syringe. Drain the water from the shell. Fill an hypodermic syringe with 0.5 ml of physiological saline and then aspirate 0.5 ml of haemolymph from the posterior abductor muscle of the mussel. After obtaining the haemolymph sample, discard the needle and expel the content in a siliconised eppendorf tube.

Dispense 40 ìl of haemolymph-saline mixture on the slide, in the same position where

the poly-l-lysine was added and incubate in humidity chamber for 30 minutes to allow the cells to attach. Carefully drain the excess solution from the slide by placing the slide on its side and letting the liquid run off. Add 40 ìl of the neutral red working solution and leave in a humidity chamber for 15 min (maintained 15-16°C during the analysis). Apply a coverslip and inspect the preparation under a microscope.

Look at the slides every 15 minutes for the first hour then every 30 minutes for the next two hours thereafter. Determine the time at which 50% of the lysosomes in the cells leaches out neutral red in the cytosol. Derive a mean value for each specimen and then a global mean for all specimens pertaining to the same pool. Compare samples from monitored field sites with those taken from reference field sites and determine gradient of cytotoxicity. An increase in leaching rates would indicate cellular stress due to pollution.

Results:

| Samples | 0 | 15 | 30 | 45 | 60 | 90 |
|---|---|---|---|---|---|---|
| control | + | + | + | + | + | + |
| treated | + | + | ± | - | - | - |

Key:
+ more than 50% of the lysosomes in the cells retaining neutral red
- less than 50% of the lysosomes in the cells retaining neutral red

## 3. EVALUATION OF GENOTOXIC EFFECTS

### 3.1 Alkaline filter elution method

The following protocol, commonly known as the alkaline filter elution method (AFE) is a widely used method to determine the extent of genetic damage in a wide range of marine organisms (Erickson *et al*., 1980). DNA single strand breaks or weak points in the alkali are identified by measuring the rate at which single-stranded DNA passes through a membrane filter of known porosity under alkaline denaturing conditions.

The sensitivity of the method depends on the complexity of the DNA, which differs considerably among the different taxa. Thus, DNA from a lower taxon will eluate faster than one of a higher one, even if completely undamaged. One good advantage in using this method is that it allows the determination of genotoxic damage in live animals, and in many instances, small tissue biopsies may be sufficient. Additionally, microfluorometric DNA determination (Cesarone *et al*., 1979) increases the sensitivity and reproducibility of the alkaline elution method.

#### 3.1.1 Equipment

- peristaltic pumps with multiple channels (flow rate 1-10 ml/h);
- spectrofluorometer: excitation: 360 nm / emission: 450 nm.;
- Fraction collector;
- pH meter able to measure pH>12;

- Filters (Millipore), Type GV 0.22 μm, GVWP 02500[4];
- inverted microscope;
- centrifuge;
- micro-syringe filter holder, luer inlet (XX30 02500)4
- micro-syringe stainless support screen4
- O-ring teflon filter sealing4
- Flat gasket, teflon[4]

3.1.2 Chemicals and Solutions

- Homogenisation buffer

  0.14 M NaCl
  1.47 mM $KH_2PO_4$
  2.7 mM KCl
  8.1 $Na_2HPO_4$
  0.1 M EDTA
  Bring the solution to pH 7.4 using NaOH.

- HANKS' balanced salts solution 2X

  273.8 mM NaCl
  10.73 mM KCl
  0.81 mM $MgSO_4 . 7H_2O$
  2.52 mM $CaCl_2 . 2H_2O$
  0.674 mM $Na_2HPO_4 . 2H_2O$
  0.88 mM $KH_2PO_4$
  8.33 mM $NaHCO_3$
  10.09 mM D-Glucose . $H_2O$

- Lysing solution

  2 M NaCl
  0.02 M EDTA
  0.2% N-laurylsarcosinate, sodium salt (Sigma L5125)
  bring solution to pH 10.2 using NaOH 1N.

- Washing solution

  0.02 M EDTA
  bring solution to pH 10.2 using NaOH 1N.

- Eluting solution

  0.04 M EDTA. Bring to pH 12.3 using tetraetylammonium hydroxide (Merck 822149.0250).

- Working BIS solution

Prepare 1.5 x $10^{-4}$ M of BIS solution by dissolving 8 mg of bisbenzimide (33258

[4] Millipore Corp. USA.

Hoechst: 2-[2-(4-hydroxy-phenyl)-6-benzimidazole]-6-(1-methyl-4-piperazyl)-benzimidazole trihydrochloride (Farbwerke Hoechst, Frankfurt, Germany), MW: 533.9) in 100 ml distilled water. Make 1 ml aliquots in Eppendorf tubes and store at -20°C. This solution remains stable for at least 1 week when stored at 4°C in dark glass bottles and wrapped in tinfoil. Prepare the working solution by add 100 ml of water containing 0.154 M NaCl and 0.015 M $Na_3$citrate (SSC buffer, pH 7.0) to 2 ml of BIS stock solution. The final solution is the working BIS solution.

- DNA standard

Calf thymus DNA was purchased from Sigma Chemical Company (St. Louis, Mo.), dissolved in sterile SSC, pH 7.0, sonicated for 10 s to increase the solubility, and diluted to a concentration of 1 mg/ml. This stock solution was diluted with SSC, pH 7.0.

3.1.3 Sample preparation from tissues of aquatic organisms

Avoid damaging DNA during handling procedures by using high EDTA concentrations (0.03-0.1 M). Always keep materials on ice and try to work fast.

3.1.3.1 For fish liver

Excise liver and wash in homogenization buffer to remove blood residues. Proceed immediately to the next step or store at -80°C. Homogenize the liver in the buffer using 1:5 w/v.

3.1.3.2 For mussel gill cells

Open mussels and remove gill cells. Isolate gill cells by enzymatic digestion with a solution of dispase (Boehringer Mannheim), 0.1 mg/ml in modified (2X) Hanks' Balanced Salt solution**,** for 10 min at 37°C. The cellular suspension obtained by filtration is centrifuged at 1,000 rpm for 10 minutes.

3.1.3.3 For mussel haemolymph

Introduce a hypodermic syringe in the large adductor muscle and draw out some haemolymph. Dilute the sample with equal volume of Hanks' solution 2X.

3.1.4 Sample application

Prepare the elution apparatus. Place a filter (0.22 µm pore size) on a stainless support screen set on a filter holder, then put an O-ring Teflon filter sealing and screw a stainless estension barrel.

Load sample onto filter (10-20 mg fish liver per filter; 1 to 2 x $10^6$ haemocytes or gill cells per filter). Count cell concentration using a counting chamber.

3.1.5 Lysing of cells

Wash the filters with 4.5 ml of lysing solution. Repeat washing using 2.5 ml of washing

solution at the same flow rate.

3.1.6 Elution of single stranded DNA

Perform the elution under reduced light conditions.

Elute DNA through Durapore filters (25 mm diameter, 0.2 µm pore size) placed on filter-holders (Millipore Corp. USA) with 10 ml of eluting solution at a flow rate of 0.05 ml/min (i.e. 2 ml. per fraction). Collect this volume in 5 tubes each containing 2 ml.

Recover the remaining DNA by removing the filter and immersing it in 4 ml of eluting solution. Cut it into small pieces. Shake vigorously.

Rinse the filter holder and tubes with 4 ml of eluting solution. This is denoted as 'dead' volume.

3.1.7 Microfluorimetric determination of DNA

Place 1 ml aliquots of each elution fraction, the DNA retained on the filter, and a wash of the filter holder in disposable glass test tubes. Neutralise each tube with 0.4 ml of 0.2 M $KH_2PO_4$, and add 0.6 ml of water to bring the volume up to 2 ml. Finally, add 1.0 ml of working BIS solution and vortex. Determine the increased fluorescence, due to binding of the fluorochrome to DNA, using a spectrofluorometer with the excitation wavelength set at 360 nm and the emission at 450 nm.

3.1.8 Calculation

3.1.8.1 Calculation of the slope of the elution curves (elution rate)

Elution slopes are calculated from semi-logarithmic plots of the fraction of DNA retained on the filter versus eluted volume or elution time and are expressed as the average rate of elution. The slope of the elution curve is a measure of the number of breaks in arbitrary units. Since the elution rate is faster for broken than for intact DNA, the amount of DNA retained on the filter is a measure of DNA single strand breaks. The elution rate decreases exponentially with elution time or volume (fraction number).

When the elution profile approaches a straight line we could use the first order kinetics equation.

For first order kinetics of alkaline elution:

$$y = ae^{-kv}$$

where:
y = the fraction of DNA retained on the filter after the elution of the volume v
a = the quantity of DNA present on the filter at the 0 solution volume
v = vol

$$\ln y = -kv + \ln a$$

and

$$K = -\ln y/v$$

The elution rate (K) could also be expressed versus t (the time of elution).

3.1.8.2 Calculation of Strand Scission Factor (SSF)

A value characterising the relative number of DNA-strand breaks, referred to as a "strand scission factor", was calculated by taking the absolute value of the $\log_{10}$ of the percentage of DNA retained in the treated sample eluted divided after a known elution volume by the percentage of DNA retained in the control sample eluted into the same amount of volume. Therefore, a strand scission factor of 0 indicates no DNA strand breaks. Values greater than 0 indicate a relative value for DNA breaks in the exposed cells (Meyn and Jenkins, 1983).

$$SSF = \log \frac{\text{(\% DNA eluted in 6 ml from test sample)}}{\text{(\% DNA eluted in 6 ml from control sample)}}$$

**Table 1**

Grid table showing presentation of arbitrary data

**Control sample**

| **DNA Standard: 1 µg** | | | **fluorescence** 160 | | | |
|---|---|---|---|---|---|---|
| **Fraction number** | **Volume (ml)** | **X value total volume (ml)** | | **Total fluorescence** | **Y (% retained)** | **ln Y** |
| **1** | 1.8 | 1.8 | 45 | 81 | 98.4 | 4.58 |
| **2** | 1.9 | 3.7 | 45 | 85 | 96.7 | 4.57 |
| **3** | 1.8 | 5.5 | 42 | 76 | 95.2 | 4.55 |
| **4** | 1.8 | 7.3 | 39 | 70.2 | 93.8 | 4.54 |
| **5** | 2.1 | 9.4 | 40 | 82 | 92.3 | 4.52 |
| | | | | | | |
| **Dead** | 4 | | 38 | 152 | | |
| **Filter** | 4 | | 1138 | 4552 | | |
| | | **Total Fluorescence** | | 5098 | | |
| | | **Total DNA (µg)** | | 31.8 | | |

**Treated or exposed sample**

| **DNA Standard: 1 µg** | | | **fluorescence** 160 | | | |
|---|---|---|---|---|---|---|
| **Fraction number** | **Volume (ml)** | **X value total volume (ml)** | | **Total fluorescence** | **Y (% retained)** | **ln Y** |
| **1** | 2.0 | 2.0 | 248 | 496 | 87.8 | 4.48 |
| **2** | 2.0 | 4.0 | 205 | 410 | 77.8 | 4.35 |
| **3** | 2.0 | 6.0 | 142 | 284 | 70.8 | 4.26 |
| **4** | 2.0 | 8.0 | 98 | 196 | 66.0 | 4.19 |
| **5** | 2.0 | 10.0 | 92 | 184 | 61.5 | 4.12 |
| | | | | | | |
| **Dead** | 4 | | 65 | 260 | | |
| **Filter** | 4 | | 562 | 2248 | | |
| | | **Total Fluorescence** | | 4078 | | |
| | | **Total DNA (µg)** | | 25.4 | | |

Example:

| For control sample | For treated or polluted samples: |
|---|---|
| **K = 0.0088** | **K = 0.0575** |
| Y = 70.8 | Y = 95.2 |

**SSF = log (70.8/95.2) = -0.129 at 6 ml of elution**

3.2 Determination of micronuclei frequency

3.2.1 Background

Micronuclei are small DNA-containing bodies which can be present near the cell nucleus during interphase resulting from both chromosome breakage and spindle dysfunction.

The type of mutations that could contribute to micronuclei production include:

a) mutations to kinetochore proteins, centromeres and spindle apparatus that could lead to unequal chromosome distribution or whole chromosome loss at anaphase;

b) unrepaired DNA strand-breaks induced by environmental and endogenous genotoxic agents which may result in acentric chromosome fragments.

Studies indicate that the relative occurrence of micronuclei can provide an indication of accumulated genetic damage throughout the life span of the cells even during short phases of contamination. These considerations suggest the suitability of this test to monitor the extent of genotoxic damage in marine organisms in a time-integrated manner. The following protocol has been devised to assess the frequency of micronuclei in cells.

3.2.2 Equipment

- centrifuge,
- optical microscope.

3.2.3 Chemicals and solutions

- *HANKS' balanced salts solution 2X (HBSS 2X)*

  273.8 mM NaCl
  10.73 mM KCl
  0.81 mM $MgSO_4 . 7H_2O$
  2.52 mM $CaCl_2 . 2H_2O$
  0.674 mM $Na_2HPO_4 . 2H_2O$
  0.88 mM $KH_2PO_4$
  8.33 mM $NaHCO_3$
  10.09 mM D-Glucose . $H_2O$

- *Dispase solution*
  Dispase I (neutral protease; grade I, Boehringer Mannheim, Germany) 0.1 mg/ml in *HBSS 2X*

- *methanol : acetic acid* (3:1)

- *3% Giemsa*

3.2.4 Method

3.2.4.1 Preparation of cell suspension

*Mussel haemolymph*

Samples of haemolymph are drawn from the posterior adductor muscle sinus by an hypodermic syringe. The samples are diluted with an equal volume of Hanks' Balanced Salt solution and spinned at 1,000 rpm.

*Gills of mussels*

Gills are taken off and cells are isolated by enzymatic digestion with a solution of Dispase (Boehringer Mannheim, Germany), 0.1 mg/ml in modified (20‰) Hank's Balanced Salt solution, for 10 min at 37°C. The cellular suspension obtained by filtration is centrifuged at 1,000 rpm for 10 min.

3.2.4.2 Slide preparation

Aliquots of cellular pellet of mussel gills and hemolymph are fixed in methanol:acetic acid (3:1) for 20 min, then spread on slides, air dried and stained with 3% Giemsa. The slides are coded and scored blind.

3.2.4.3 Slide scoring

Two thousands cells with preserved cytoplasm per mussel are scored under oil immersion at 1,000 x magnification. Due to the high interindividual variability of the MN

frequency, 8-10 animals must be analysed for each experimental point.

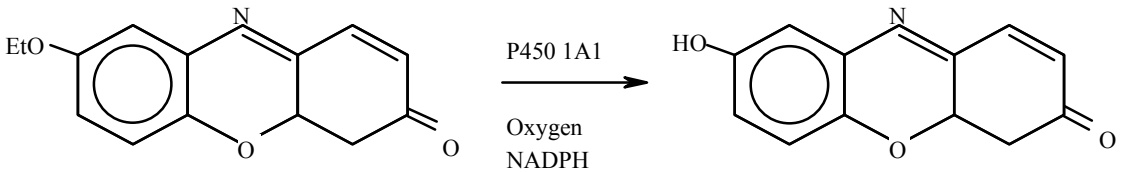
The following criteria have to be met during scoring:

- only intact cells are scored;
- chromatin structure and color intensity similar to that of the main nucleus;
- on the same optical plane as the main nucleus;
- round or oval;
- not fragmented (to exclude small stain particles and apoptotic cells);
- located within 4-fold the shortest axis of the nearest nucleus.

## 4. EVALUATION OF MIXED FUNCTION OXIDASE ACTIVITY

### 4.1 Background and principle

This section comprises the procedure to determine the activity of the cytochrome P450 multi-enzyme complex in fish as a biomarker of biological response to organic xenobiotics in the marine environment. It describes methods for preparing MFO containing subcellular fractions, determination of the catalytic activity of ethoxyresorufin O-deethylase (EROD) and estimation of the protein content. The catalytic estimation is based on the incubation of a substrate (ethoxyresorufin together with an enzyme preparation and cofactor (NADPH) in appropriate buffer, the fluorescence increase due to resorufin production is then evaluated, by a spectrophotofluorimeter.




*Ethoxyresorufin* *Resorufin*

This procedure has been tested both in the field and the laboratory as biomarker of exposure, mainly by PAH, PCB and chlorodibenzodioxins. It can also be applied to pentoxy- or benzyloxy-resorufin O de-alkylase (PROD and BROD) to indicate induction of other P450 isozymes (Burke and Mayer, 1983).

#### 4.1.1 Sampling

A problem of great importance in field sampling of fish is the collection and storage of samples until they can be processed in the laboratory. The hemoprotein degrades rapidly in intact tissue or subcellular fractions; even the use of liquid nitrogen for storage may affect enzyme activity (Forlin and Andersson, 1985). Samples that have been thawed or stored at -20°C are of no use in catalytic measurements.

General guidelines for collection of fish[5]:

[5] Report of the Workshop on Biological Effects Monitoring Techniques, Aberdeen, Scotland, 2-6 October 1995

- fish should be sampled outside the species-dependent spawning season and the gonadosomatic index should be recorded;
- fish should be sampled within a species-dependent defined length-range;
- either male (usually higher EROD levels than females) or female (higher induction ratio in comparison of high and low polluted sites) individuals should be used. Data from males and females should not be mixed;
- the numbers of individuals sampled must be representative for each site to enable appropriate statistical treatments (5-10 per site);
- only fish without external and internal visible diseases should be used for further processing, and
- bottom water temperature should be measured at the time and at the place of capture.

4.1.2 Equipment

- Top-pan balance weighing to 0.1 g
- Conventional dissection instruments
- Ice bucket
- Range of small beakers
- Electric drill capable of 2700 rpm
- Potter-Elvehjem teflon-glass homogenizer (5 or 15 ml)
- Measuring cylinder (10 or 25 ml capacity)
- Refrigerated ultracentrifuge
- Graduated tubes, 5-15 ml
- Pasteur pipettes
- Nalgene cryotubes
- Micropipettes with disposable tips to deliver 10, 25, 50, 100, 200,1000 µl
- Glass pipette to deliver 2 ml
- Fluorimeter

4.1.3 Solutions and chemicals[6]

- Solution A
  150 mM KCl
- Solution B
  10 mM HEPES containing 250 mM sucrose; 1 mM $Na_2EDTA$,
  adjust to pH 7.4 with KOH (34-36%, Prolabo)
- Solution C
  80% solution B + 20% glycerol (V/V)
- Solution D
  18.2% $KH_2PO_4$ (Prolabo) 50mM + 22.2% $Na_2HPO_4$ (Prolabo)

[6] All chemicals are from Sigma

200 mM bring to 100% with water, pH 7.44

- Storage of solutions and chemicals

+ 4°C Solutions A, B, C, D, G-6PDH
-20°C NADP, G-6-P, Resorufin
-80°C Ethoxyresorufin (preferably)

4.1.4 Preparation of samples for analysis

This section describes the steps for preparing S9 fraction and microsomal samples prior to the measurement of MFOs. It is always convenient to prepare in advance as many reagents and solutions. Most, like those required for protein determinations, are stable and will withstand freezing and thawing if kept in plastic bottles. It is usually not possible to prepare nucleotide co-enzyme solutions in advance, however, and since (usually) small amounts of these are needed and as they are relatively expensive, it is desirable to preweigh appropriate amounts of these, and keep them (cooled and desiccated) in small vials.

S9 fractions are prepared by centrifugation (e.g. 9.000 x g for 15 minutes). Microsomal fractions are prepared by ultra-centrifugation (e.g. 100,000xg for 90 mins) of homogenates from fresh or frozen tissues (such as liver or hepatopancreas). Cytochrome P-450 activity measurements can be made both in the S9 supernatant and in the 100.000xg resuspended microsomal fraction.

4.1.4.1 Tissue dissection and preparation for analysis

Kill fish by severing spinal cord at the level of the pectoral fins and by insertion of a scissors blade in the brain. Weigh fish with an accuracy of ± 1%.

Dissect out the liver and avoid rupturing the gall bladder, since bile may contain MFO inhibitors. Weigh the liver (± 1% accuracy) and place in a beaker on ice.

All subsequent operations should be performed at 4°C.

4.1.4.2 Homogenization of tissue

Mince weighed liver (ideally ≥ 1 g, weighed to ± 0.1 g) with scissors, rinse it in solution A and blot dry on tissue paper.

Adjust solution B by 0.1M PMSF (dissolved in ethanol).

Place the liver in a Potter glass homogeniser tube on ice and add solution B in ratio 5:1, v:w. Homogenize with 10 vertical strokes at high speed, keeping the tube cooled in ice. This produces the "crude homogenate".

4.1.4.3 Preparation of S-9 fraction and 100.000 x g pellet (microsomes)

Place the homogenate in centrifuge tubes and spin for 15 minutes at 9,000xg in a centrifuge. Collect the resultant supernatant (S-9), without the lipid phase, and subdivide it in small aliquots (100-200 µl) and store at –80°C. Utilize an aliquot of S9 for protein determination using the Bradford method (Bradford, 1976).

Alternatively, prepare 100.000 x g fraction (microsomes). Transfer S-9 fraction to an

ultracentrifuge tube and re-centrifuge again at 100.000 x g for 50 min at 4°C. Discard the supernatant (cytosol) and resuspend the microsomal pellet in 1 ml of sol. C.

Transfer quantitatively this suspension into the potter, re-homogenize with 5 hand strokes using a teflon tip, keeping the homogenizer cooled in ice. Transfer the homogenized suspension in a graduated tube and record its volume. Hold on ice.

This is the microsomal preparation that is now ready for quantification of protein concentration and enzymatic activities.

Freeze aliquots (100 and 200 ìl) in Nalgene 1.5 ml cryotubes and stored at -80°C or in dry ice if required for future reference.

4.1.5 Protein determination

This is in accordance with Bradford (1976).

1.5 ml polystirene spectrophotometer cuvettes are prepared containing various concentrations of Bovin Serum Albumine diluited with MilliQ water to a final volume of 20 microliter using 1, 2, 5, 10, 20 µg of BSA from a 1µg/µl stock solution.

10 µl of S9 are dispensed into the sample cuvettes. 10 µl of MilliQ water are added to obtain a final volume of 20 µl. 480 µl of MilliQ water are added to the reference and the samples. 500 µl of Pierce Protein Assay Reagent are dispensed in all the cuvettes.

The absorbance at 595 nm is read against a blank containing only the reagents without S9 supernatant. BSA calibration curve is plotted and the protein concentration is estimated according to the regression curve.

4.1.6 Ethoxyresorufin O-deethylase (EROD) determination (Suteau *et al.*, 1988)

4.1.6.1 Preparation

Add sequentially in a tube:

1/100 volume of ethoxyresorufin (from a stock of 123 ìM in DMSO)
1/10 volume of glucose-6-phosphate (25 mM in $H_2O$)
1/10 volume NADPH (25 mM in $H_2O$)

Bring the mixture to the desired final volume with solution D. Add glucose-6-phosphate dehydrogenase (G-6-PDH) to obtain a final concentration of 1 unit $ml^{-1}$. Warm the medium for 5 minutes at 30°C in a water bath.

4.1.6.2 Enzymatic reaction

While the above medium is warming, dispense individual S9 samples (10 to 100 ìg in solution C) into Falcon 2018 polypropylene tubes.

For each sample, set a time "zero" in duplicate and a time "five" in duplicate.

To set a time ‘zero’ reaction, add 2 ml cold acetone onto the S9.

Now transfer all the tubes to the water bath and every 20 seconds add 1 ml of the warm medium to the samples and the time ‘zero’ reaction using an Eppendorf Multipette fitted with a 50 ml syringe. Vortex the tubes immediately.

Stop the reaction after 5 minutes by adding 2 ml cold acetone except the time ‘zero’ reaction tube. Vortex again the tubes.

Centrifuge the samples for 5 minutes at 6000 x g to eliminate flocculated protein.

4.1.6.3 Quantification of the resorufin produced

Having checked the extinction coefficient of the resorufin standard, add 100 pmoles of resorufin to a new tube (using a 2 mM standard resorufin solution in di-methyl-sulphxide in 5 µl of a 1/100 dilution of solution C) and add 1 ml of reaction medium and 2 ml of cold acetone and vortex.

Measure the fluorescence using a spectrofluorimeter with an excitation wavelength of 537 nm and an emission wavelength of 583 nm.

Transfer the samples carefully into cuvettes leaving behind any precipitated protein.

Autoblank on the time ‘zero’ reaction tube.

4.1.7 Calculation of the activity

For the calculation of EROD activity expressed in pmoles of resorufin/min/mg protein you can use the following formula:

$$\text{EROD activity} = (IF_c \times c \times VF)/(IF \times V_c \times t \times P)$$

In which,

$IF_c$ is the fluorescence of the sample
IF is the fluorescence of the standard (nmol/ml)
C is the concentration of the standard (nmol/ml)
VF is the final volume of the mix (ml)
$V_C$ is the volume of the sample (ml)
t is the reaction time
P is protein concentration (mg/ml)

4.1.8 Interpretation of results

The EROD measurement is a convenient way of assessing P-450 1A1 catalytic activity and has gained widespread use in biomonitoring studies with fish. The catalytic assay can be viewed as a very useful primary test to identify biological responses due to PAH contamination. The occurrence of hepatic lesions should be recorded; it is a good idea to preserve representative sub-samples of hepatic tissue for future histological examination. Confirmation of increased EROD response can be obtained by determining PAH adducts[7] in fish as evidence that the EROD response is being mediated by organic aromatic xenobiotic compounds.

[7] PAH metabolites are not indicative of biological effects *per se* but can provide a sensitive marker of exposure to bioavailable levels in the environment. PAH bioavailability can vary markedly in different fish species living in environments similarly contaminated with PAHs

It is important to follow closely the proposed protocol as it is well known that standardization problems could arise due to: a) intercomparability of EROD activities in S-9 and microsomal fractions. This often tends to produce somewhat ambiguous results. b) the use of different protein estimation methods by different laboratories. c) a more pressing issue, is the use by different authors of different extinction coefficients for the reaction product - resorufin (phenoxazone) - which was found to vary between 20 to 73 $mM^{-1}$ $cm^{-1}$.

Therefore, if the task is to intercompare and assess the EROD values among various regional laboratories, then it is important to fully standardise the catalytic assay before any actual biomonitoring takes place.

4.1.9 Future developments

Ongoing research is taking place to detect immunochemically the inductive response of P450 using antibody probes for both protein levels and mRNA levels (Goksoyr *et al.*, 1991a). In this way, the amount of a specific antibody probe cross-reacting with the P450 protein is measured chemically.

Immunochemical detection of m-RNA can prove highly advantageous since catalytic activity of induced P-450 (Gooch *et al.*, 1989) may be inhibited by certain inducers (such as organochlorines). Consequently, analysis of catalytic activity alone might show no response, but strong induction can still be seen by immunochemical analysis of the P-450 protein or its mRNA. Apparently different types of inducers can also modulate the catalytic activity, as can endogenous compounds. In other cases the catalytic activity may be lost due to bad storage (e.g. in field sampling situations), or the sample or tissue may be too small to give measurable catalytic activity (as with fish eggs and larvae) (Goksoyr *et al.*, 1991b). In all of these cases, immundetection of P450 has been able to detect inductive responses that would not have been possible with catalytic measurements alone (Goksoyr *et al.*, 1991a).

## 5. EVALUATION OF METALLOTHIONEIN (MT) LEVEL

5.1 Background

Routine quantification of MT levels often proves to be problematical due to the lack of a measurable biological activity of this metalloprotein. This has forced investigators to explore unique structural features to be exploited for the evaluation of the MT concentration. Research efforts have relied for on estimates of (1) metal content bound to this protein (e.g. by competitive metal displacement or direct quantification techniques) and (2) physical (e.g. absorption measurements), and chemical (e.g. measurement of sulphyhydryl groups and immunochemical affinities) characteristics.

Each of these approaches has its own strength and weakness. One major disadvantage common to most of these methods is the indirect estimation of metallothioneins, which may lead to inconsistent results concerning the absolute value of the metallothionein concentration in the tissues. In addition, most of these procedures require expensive laboratory equipment (e.g. ultracentrifuges, AAS, chromatographic systems, etc.) and sample preparation and assay optimization require large commitments of time.

Biomolecular assay, such as the measurement of MT m-RNA, are providing some hope in developing a very sensitive technique (Swapan *et al.*, 1991). Specific immunoassays for MT are available, but the limited inter-species compatibility provides a challenge for future

development (Kay *et al.*, 1991).

Recently, investigators are adapting simpler but still accurate and sensitive techniques to quantify the levels of MT in biological tissues.

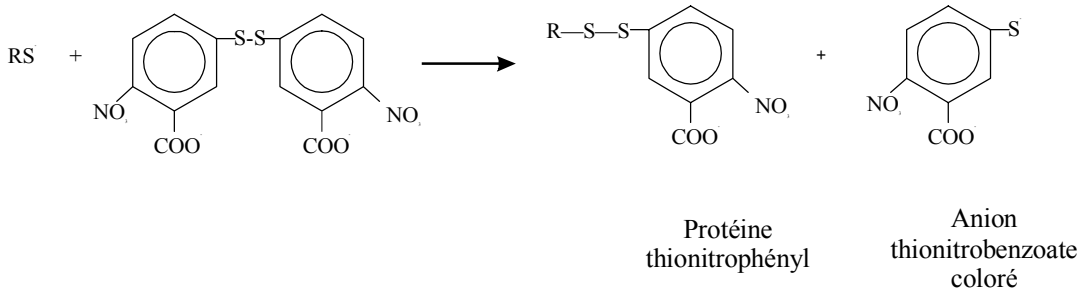
The following methodology is based on the estimation of the sulfhydryl content of MT proteins. This method has been reported to be a sensitive, time saving, and low-cost technique able to detect metallothionein content in the tissues of marine organisms (Viarengo *et al.*, 1997) and is currently being intercalibrated and standardized by a number of laboratories within the Mediterranean.

5.2 Spectrophotometric determination of the -SH groups using Ellman's reagent

5.2.1 Principle

The method here described consists of the ethanol/chloroform fractionation of the 30.000xg cytosolic containing fraction, to obtain a partially purified metallothionein fraction. Metallothionein concentration in the samples is then quantified by evaluating the SH residue content by a spectrophotometric method, using Ellman's reagent (DTNB: 5,5 dithiobis 2 nitrobenzoic acid) (Ellman, 1959). As known, metallothioneins are characterised by an extremely high cysteine content (about 20-30%) when compared to other proteins eventually present in the ethanolic extracts and therefore the metallothionein determination based on the SH detection allows a more selective evaluation of these metalloproteins.

Illustrated below is the reaction between DTNB and protein SH groups. The reaction produces stechiometric amounts of TNB (thionitrobenzoate), a yellowish compound with maximum absorbance at 412 nm.




The analytical procedure has been adapted to be used on mussels, although other organisms can be used.

5.3 Practical evaluation

To detect metallothionein content in biological tissues by the DTNB reaction, the samples have to be prepared under rigorous reducing conditions (0.01% β-mercaptoethanol) as described in the protocol. The ethanol/chloroform fractionation allows both the elimination of low molecular weight soluble thiols, which reacting with DTNB, could interfere with metallothionein quantification, and the partial concentration of metallothioneins whose level in the tissues of uncontaminated animals is often low. During ethanolic fractionation the addition of RNA as co-precipitant and acid is essential to allow a quantitative metallothionein recovery. A final "washing" of the metallothionein extracts eliminates thiol contaminants, such as reducing agents present in the cells (glutathione, cysteine, etc.) or thiols added during sample

preparation (â-mercaptoethanol). The concentrated MT pellet is resuspended in 0.25 M NaCl with addition of HCl and EDTA (to remove heavy metal cations still bound to metallothioneins), followed by the addition of a known amount of DTNB reagent in a high ionic strength medium (to completely denature metallothioneins). A calibration curve of reduced glutathione (GSH) or purified Cd, Zn thionein (commercially available) can be utilized to quantify the metallothionein content in mollusc tissues. Absorbance is evaluated at 412 nm.

5.3.1 Equipment

- Cooling high speed centrifuge (having swing- and fixed-type angle rotors)
- Spectrophotometer
- Motor driven teflon/glass Potter homogenizer with teflon tip.
- Freezer
- Nitrogen gas cylinder

5.3.2 Solutions and Chemicals

- Homogenization buffer

  Make up:

  0.5M Sucrose-20mM TRIS pH 8.6.
  Leupeptin stock solution (1 mg/ml) (SIGMA L2884).
  Ethanolic stock solution of Phenylmethylsulfonyl fluoride (PMSF) (58 mg/ml) (SIGMA P7626).

  To the desired volume of Sucrose-TRIS buffer add 3.0 ìl/ml leupeptin, 1.5 ìl/ml PMSF and 0.1 ìl/ml â-mercaptoethanol (equivalent to 0.01%) (MERCK 805740).

- GSH (SIGMA G 4521) stock solution: freshly prepared before analysis
- 0.25M NaCl
- 0.2M phosphate buffer pH 8 containing 2M NaCl (store at room temperature)
- DTNB (SIGMA D 8130)
- RNA (SIGMA R 7250) (100 mg/ml) store at –20°C.
- cold (-20°C) absolute ethanol
- chloroform
- 37% HCl
- 1N HCl/EDTA 4 mM (store ar room temperature)

5.3.3 Sample and enriched MT fraction preparation

5.3.3.1 Homogenization

Rapidly dissect out and blot the digestive gland on 3 µm filter paper . Weigh a pool of tissues belonging to at least 10 animals and homogenize about 1 g of tissue in 3 volumes of homogenizing buffer containing â-mercaptoethanol, PMSF and leupeptine, with 8 strokes in a motor driven teflon/glass Potter homogenizer.

5.3.3.2 Centrifugation

Centrifuge the homogenate at 30,000 x g for 20 minutes to obtain a soluble nuclei and mitocondria free fraction containing MTs. The centrifuge rotor must be kept at 0-4°C.

*Note on safety: equilibrate the tubes before centrifuging. Employ (16ml) PYREX© or COREX© glass tubes or high organic solvent resistant plastic tubes.*

5.3.3.3 Ethanolic precipitation

Precipitate the high molecular weight proteins present in the supernatant using cold (-20°C) absolute ethanol. To 1 ml of the 30,000xg supernatant add 1.05 ml of cold (-20°C) absolute ethanol and 80 ìl of chloroform. Vortex for few seconds. Centrifuge in a fixed angle or swinging rotor at 6,000xg for 10 minutes at 0-4°C. Collect the supernatant and measure the volume using a pipette.

To the 6,000xg supernatant add 40 ìl of 37% HCl and 10 ìl of a solution of RNA (1 mg/10 ìl) followed by 3 volumes of cold ethanol. Store at -20°C for 1 hour.

Re-centrifuge at 6,000xg for 10 minutes using the swinging rotor. Discard the supernatant and wash the pellet with an ethanol/chloroform/homogenizing buffer (cold -20°C) solution (87:1:12 v/v) without the addition of â-mercaptoethanol, PMSF and leupeptin.

Centrifuge for 10 minutes at 6,000xg using a swinging rotor. Remove supernatant and dry pellet under nitrogen gas stream for about 10 min.

*Note: tubes must be kept on ice during all steps.*
*Note on safety: Use (16ml) PYREX© or COREX© glass tubes or organic-acid solvent resistant plastic tubes.*

5.3.3.4 Resuspension of the MT enriched fraction

Add to the pellet 150 ìl of 0.25 M NaCl solution and 150 ìl of a solution made of 1N HCl containing 4 mM EDTA (destabilising solution). Put a glass stirrer into the tube and vortex a few seconds for a complete resuspension of the sample.

5.3.4 DTNB assay (Ellman's reaction)

5.3.4.1 Glutathione reference standard curve

Make up a glutathione stock solution at 1mg/ml concentration in 0.25 M NaCl. Store in ice. Prepare at least 3 GSH reference standard concentrations and a blank in accordance to Tab 5.1 on session § 5.3.4.2.

5.3.4.2 MT spectrophotometric evaluation

Just before analysis dissolve 0.43 mM (7.14 mg/42 ml) DTNB in 0.2 M phosphate buffer pH 8 containing 2M NaCl. Store the solution in darkness at room temperature.

Add to blank, standards and samples 4.2 ml DTNB solution. Centrifuge metallothionein samples at 3.000xg for 5 min. at room temperature.

Measure the absorbance, $ABS_{412}$ , using a spectrophotometer set at 412 nm utilizing reduced glutathione (GSH) as a reference standard.

| | GSH stock solution | 0.25M NaCl | 1N HCl 4 mM EDTA | DTNB soln | Final Volume |
|---|---|---|---|---|---|
| Test samples | - | 150 ì l | 150 ì l | 4.2 ml | 4.5 ml |
| Blank | - | 150 ì l | 150 ì l | 4.2 ml | 4.5 ml |
| Standard: | | | | | |
| Stnd 20 (14.4 nmol/ml) | 20 ì l | 130 ì l | 150 ì l | 4.2 ml | 4.5 ml |
| Stnd 40 (28.8 nmol/ml) | 40 ì l | 110 ì l | 150 ì l | 4.2 ml | 4.5 ml |
| Stnd 80 (57.8 nmol/ml) | 80 ì l | 70 ì l | 150 ì l | 4.2 ml | 4.5 ml |

5.3.5 Calculation and interpretation of result

5.3.5.1 Plotting of a standard curve

Express glutathione GSH reference standard as nmol/ml.

Plot a standard curve in which $ABS^{GSH}_{412}$ is a linear function of GSH concentration (nmol/ml).

$$\mathbf{ABS^{GSH}_{412} = \varepsilon\ [-SH]}$$

In which (unit of measure between brackets):
$\mathbf{ABS^{GSH}_{412}}$ , ($OD_{412}$), is the Optical Density of GSH samples at 412 nm ,
**ε**, ($OD_{412}$/nmol/ml), is a constant representing the extinction coefficient for GSH,
**[-SH]**, (nmol/ml), represents the concentration of sulphydrylic groups in GSH samples.

5.3.5.2 Quantitative determination of MT content

Interpolate $ABS^{MT}_{412}$ values obtained for metallothionein samples on the reference curve. The corresponding values found on the X-axis represents the concentrations of sulphydrylic groups belonging to metallothionein present in the samples. Taking into account the molecular features of mussel metallothionein (Mackay *et al.*, 1993), for which cystein residues are 21 and molecular weight is 8600 DA, the final volume of DTNB reaction (4.5ml) and the dilution factor of the homogenate (4), the concentration of mussel metallothionein (ng/g tissue w.w.) present in the samples can be obtained using the following formula:

$$\mathbf{[x/21]*8{,}600 * 4.5 * 4}\ ,$$

in which :
**x** (nmol/ml) represent the X-axis value coming from interpolation of $ABS^{MT}_{412}$ value on the reference curve.

Alternatively, determine graphically or by the use of a specific software (such as Microsoft Excell®) the equation of the line discussed on § 5.3.5.1. and use the following formula to determine metallothionein (ng/g tissue w.w.) content in the samples :

$$[[(ABS^{MT}_{412}/\varepsilon_{GSH})/21]*8{,}600] * 4.5 * 4 \quad (1)$$

in which :
$ABS^{MT}_{412}$ is the OD value red for metallothionein sample
$\varepsilon_{GSH}$ is a constant representing the extinction coefficient for GSH
21 is the number of cysteine residues of mussel metallothionein (Mackay *et al.*, 1993)
8,600 is the molecular weight of mussel metallothionein (Mackay *et al.*, 1993)
4.5 (ml) is the final volume of DTNB reaction
4 is the dilution factor of the homogenate.

By simplifying (1) it can be obtained:

$$(ABS^{MT}_{412}/\varepsilon_{GSH}) * 7.37 * 10^3 \quad (2)$$

A higher level of sulphyhydryl content (≡MT) relative to the basal pre-existing level in reference clean samples would *generally* indicate the presence of a metal pollution stress in the animals at the sampling location. However, for the reasons already discussed in the introductory part of this manual, high MT levels can also be related to other factors which can influence its synthesis. For a correct approach to the use of Metallothionein as biomarker of exposure to heavy metal pollution (Viarengo *et al.*, in press).

Coastal areas in which organisms show higher MT level than those belonging to reference unpolluted areas should be further investigated. In this case, chemical analysis of biota and sediments is necessary to identify the type of insulting metal in that particular area. Therefore it would be right to set aside a set of samples for metal analysis.

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