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MEDITERRANEAN ACTION PLAN

Meeting of experts to review the MED POL
Biomonitoring Programme

Malta, 29 September - 1 October 1997

**REPORT OF THE MEETING OF EXPERTS TO REVIEW THE
MED POL BIOMONITORING PROGRAMME**

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Introduction

1. The Meeting of Experts to review the MED POL Biomonitoring Programme was held at the Foundation for International Studies, University of Malta, Valletta, Malta from 29 September to 1st October 1997, following the invitation of the Ministry for Foreign Affairs and the Environment and the Euro-Mediterranean Centre on Insular Coastal Dynamics.
2. Experts from the following Contracting Parties to the Barcelona Convention attended the Meeting: Albania, Algeria, Cyprus, Egypt, European Community, France, Greece, Israel, Italy, Lebanon, Libya, Malta, Monaco, Morocco, Slovenia, Spain, Syria, Tunisia and Turkey.
3. The list of participants is attached as Annex I to this report.

Agenda item 1. Opening of the Meeting

4. H.E. George Vella, Deputy Prime Minister and Minister for Foreign Affairs and the Environment opened the Meeting and welcomed the participants to his country. He said that one of the major objectives of the MED POL programme is to establish and enhance marine pollution monitoring programmes in the Mediterranean members states. Such MED POL monitoring programmes have been ongoing for the past 20 years and they have yielded a number of notable results including the identification of some "hot spots" of marine pollution, the training of personnel, intercalibration exercises, and the availability of cash grants for the purchase of equipment by the less developed countries.

The Minister said that proper monitoring should include the right mixture of chemical and biological parameters, that is, the assessment of the quality of the environment also depends on the responses of the organisms to pollution or other contaminants and stressors. However, we are aware of the difficulties which need to be overcome in order to develop such a comprehensive monitoring programme.

He was sure that such limitations would be discussed by the workshop and wished the Meeting every success in the deliberations.

5. During the opening session, Mr Leslie Agius, Acting Director, Foundation for International Studies, Mr Gabriel P. Gabrielides, MAP Senior Programme Officer and Mr Charles Galdies, Euro-Mediterranean Centre on Insular Coastal Dynamics also made statements.

Agenda item 2. Election of officers

6. The Meeting unanimously elected Mr Victor Axiak (Malta) as Chairperson, Mr Aldo Viarengo (Italy) as Vice-chairperson and Ms Amel Chaffai (Tunisia) as the Rapporteur.

Agenda item 3. Adoption of the Agenda and organisation of work

7. The Meeting adopted the proposed agenda contained in document UNEP(OCA)/MED WG.132/1 which appears as Annex II to this report.

Agenda item 4. Background and scope

8. Mr G.P. Gabrielides, Senior Programme Officer reviewed the activities that have taken place in the framework of the MED POL programme since the Contracting Parties took the decision that biological effects monitoring should be initiated to complement the chemical monitoring already going on in the framework of the MED POL programme. The emphasis was placed on training of scientific personnel while many laboratories made an effort to acquire the necessary equipment. When a sufficient number of laboratories were able to undertake the recommended biomonitoring techniques, a pilot biomonitoring programme was undertaken during 1996 the results of which will be discussed by the Meeting.

9. It is expected that, on the basis of the experience gained so far, the Meeting would agree on a battery of techniques and the species recommended for use. The Meeting should in fact prepare a programme for the development of biomonitoring which should also contain quality assurance activities and an assistance component to enable all countries to participate in the programme when it is launched on a Mediterranean wide scale.

Agenda item 5. Review of the results of the 1996 pilot biomonitoring programme including evaluation of techniques

10. Document UNEP(OCA)/MED WG. 132/3 was presented by Mr A. Viarengo who was also the author as the technical coordinator of the pilot biomonitoring programme. After presenting the basic concepts of the programme and the techniques and species used, he concluded that he was optimistic as most of the laboratories involved were able to identify correctly the deleterious effects of pollution on molluscs and fish sampled from different sites situated on a gradient of pollution. Another important point was that most of the research teams involved in the programme utilised as a common base the set of biomarkers suggested for the programme and used in the training courses. Moreover, the laboratories improved the programme by using additional stress indices.

Results indicated that EROD activity in molluscs is not as powerful as in fish and therefore in the future it should only be used on fish liver.

11. Individual laboratories were then allowed to present their results. The Spanish data were presented by Ms A. Torreblanca (University of Valencia) and Ms C. Porte (C.I.D., Barcelona). The techniques of acetylcholinesterase, metallothioneins, cytochrome P-450 and stress proteins were used on samples collected from Barcelona,

Ebro delta, Alboraiia, Cullera and Denia. The results of EROD activity in *Mullus barbatus* were much better than those for *Serranus cabrilla*.

12. The Monaco programme included metallothioneins, EROD, lysosomal membrane stability (as determined by glutathion and neutral red assay) and stress-on-stress. The results were presented by Mr P. Rolland who discussed the issue of caging indicating the advantages and disadvantages. The species used were *Dicentrarchus labrax* and *Mytilus galloprovincialis*. This programme also formed part of the work carried out in the framework of the RAMOGE Agreement.

13. The Maltese data presented by Mr V. Axiak concern EROD in *Serranus cabrilla* sampled from two sites but no significant differences were established. Metallothioneins was measured in *Patella rustica*. Another biomarker was used by the laboratory which is specific for TBT. This is imposex used on *Murex trunculus*. The results were encouraging for this specific contaminant.

14. The Israeli pilot programme was executed by two laboratories (University of Tel Aviv and IOLR, Haifa). EROD and CYP1A1 were measured by Mr A. Yawetz in *Lithognathus mormyrus* and lysosomal membrane stability in *Patella coerulea* collected from Haifa bay. The anticipated reference site of Shikmona was found to be more polluted than the station at the mouth of Kishon river. Preliminary results concerning the utilisation of the DNA alkaline unwinding technique were also presented.

Metallothionein and P-450 1A1 m-RNA results were presented by Mr M. Tom who in parallel with results concerning a field study he presented experimental data in order to achieve a better understanding of the induction mechanisms.

15. Mr A. Viarengo presented the Italian results stressing the importance of using general stress indices like lysosomal membrane stability. The technique "stress-on-stress" was easy to use and did not require any sophisticated equipment. Data have also been presented showing that lipofuscin and lysosomal lipid accumulation represent two powerful general stress indices.

16. In the discussions that followed, participants stressed the need to use a battery of tests. However, even though many believed that there should be flexibility in choosing the techniques and the species, it was agreed that at least there should be consensus on a core of techniques. This core of techniques should include the four already recommended for use ie two general stress indices (lysosomal membrane stability and DNA alteration) and two specific stress indices (metallothioneins and EROD). Additional biomarkers could be added to satisfy special interests or requirements, among these being acetylcholinesterase activity, imposex, P-450 1A1 m-RNA, CYP1A1 protein, lipofuscin and lipid lysosomal accumulation. The technique stress-on-stress was also proposed as it was easy to use by all laboratories utilising mussels.

17. In discussing the choice of species, participants proposed a number of species with which they had some experience. It was also pointed out that there is no single species that can be found in all regions of the Mediterranean. However, it was

agreed, that for comparison purposes an effort should be made to utilise common species. *Mytilus galloprovincialis* was present in all the areas except the Eastern Mediterranean while *Mullus barbatus* could be found almost everywhere and this fish had an advantage over others as it was the major species in the chemical monitoring programme. The results for EROD induction have proved to be very satisfactory. Finally, the Meeting agreed on the table shown below.

	Biomarker	Recommended species	Tissue used
Specific stress	EROD	<i>Mullus barbatus</i> (<i>Dicentrarchus labrax</i> for caging) but if not available <i>Mugil sp.</i>	Liver
	Metallothioneins	<i>Mullus barbatus</i> (<i>Dicentrarchus labrax</i> for caging) but if not available <i>Mugil sp.</i>	Liver
		<i>Mytilus sp.</i> but if not available <i>Patella sp.</i>	Digestive gland (for molluscs) Hepatopancreas (for limpets)
General stress	Lysosomal membrane stability	<i>Mullus barbatus</i> (<i>Dicentrarchus labrax</i> for caging) but if not available <i>Mugil sp.</i>	Liver
		<i>Mytilus sp.</i> but if not available <i>Patella sp.</i>	Digestive gland (for molluscs) Hepatopancreas (for limpets)
	DNA alteration	<i>Mullus barbatus</i> (<i>Dicentrarchus labrax</i> for caging) but if not available <i>Mugil sp.</i>	Liver
		<i>Mytilus sp.</i> but if not available <i>Patella sp.</i>	Digestive gland (for molluscs) Hepatopancreas (for limpets)

18. It was pointed out by one delegation that the induction of EROD activity using CYP1A1 protein or m-RNA provides essential information and therefore this technique could be introduced by participating laboratories in the future.

19. The Monaco delegation provided detailed information on the advantages and disadvantages of caging. Caging can be used instead of sampling free-living organisms; it can also be used as a complementary technique. The laboratories in Monaco are at the disposal of the participants for any additional information on the subject.

Agenda item 6. Data quality assurance

20. Mr Viarengo presented the results of the intercomparison exercises for which he was responsible. The results were considered satisfactory and it was agreed that an effort should be made to organise intercalibration exercises for all four recommended techniques on an annual basis.

21. Another issue related to the quality of data is that of the proper training of the personnel utilising the biomonitoring techniques. The needs of training should be assessed especially as regards the level and the duration. The representative of the Secretariat pointed out that long-term training could not be provided through MED POL and that other programmes should be found, such as bilateral governmental support schemes.

22. Mr G.P. Gabrielides informed the Meeting that a letter was addressed to all MED POL National Coordinators requesting them to designate the laboratories in their countries which would participate in each type of monitoring including biomonitoring in the framework of MED POL Phase III. As soon as this information is received the Secretariat will organise consultancy missions to these laboratories to investigate their capabilities and assess their needs in as far as equipment and training are concerned. On the basis of the reports of the missions a programme for capacity building will be prepared and presented to the Governments or to any funding Organisations. He also stressed that as this is a cooperative programme he would expect advanced laboratories to assist laboratories in less developed countries to upgrade their technical capabilities so that they could fully participate in the programme.

23. In response to the above, the representative of Monaco informed the Meeting that within the framework of the RAMOGE programme on the monitoring of the marine environment using biological effects techniques, video tapes will be prepared for the analytical procedures for each of the proposed biomarkers and the cassettes would be made available to all interested laboratories. She also mentioned that during the next meeting of the RAMOGE commission it will be proposed to promote the organisation of training courses within the North-South concept of cooperation.

24. The delegate from Tunisia invited the Secretariat to organise a Training course at the University of Sousse, Chotte Mariam. She mentioned that this laboratory

could be the Focal Point for training scientists from the South on biomonitoring techniques.

25. The representative of the Euro-Mediterranean Centre on Insular Coastal Dynamics, Mr C. Galdies informed the Meeting that they have a long experience in organising training courses and that they would be willing to undertake the logistics for the organisation of any training courses or intercomparison exercises on biomonitoring techniques which the Secretariat would decide to have.

Agenda item 7. Review of manual on biomonitoring techniques

26. Mr G.P. Gabrielides presented document UNEP(OCA)/MED WG.132/6 which is the draft manual on the recommended biological effects techniques. He explained that the manual was prepared at the request of the new comers into the programme to assist them in their effort to implement biomonitoring techniques in their laboratories. Even though the first draft was prepared by Mr C. Galdies the document was distributed to all the scientists participating in the programme for comments all of which were incorporated. The document is presented to the Meeting for final comments.

27. The delegate from Monaco recommended that the following should also be included in the Manual, (a) a section on evaluation and interpretation of the data which should take into account biological and seasonal variations, (b) a table showing the recommended techniques and the suitable species, (c) a list of the laboratories participating in the programme, (d) more information on the collection and treatment of the animals.

28. It was agreed that all those participants wishing to submit detailed comments should do so by the end of October 1997.

Agenda item 8. Recommendations for future action

29. The representative of the European Community took the floor and expressed his views on the outcome of the meeting suggesting that there should be a distinction of the recommendations which should be addressed to scientists and those to be addressed to policy makers such as the Meeting of the Contracting Parties. The policy makers must be convinced that a programme will provide results useful for decision making and for practical action.

30. As far as funding of the programme is concerned he said that concerning possible E.U. funding DG XI does not have money for this type of activity but other sources of the E.U. could be available, for instance within DG XII. The Euro-Mediterranean partnership programme provides 90% of the funds for bilateral cooperation while 10% is for regional cooperation.

Recommendations to participating laboratories

31. Any biomonitoring programme should include a battery of tests. The core techniques recommended and supported are: EROD, metallothioneins, DNA alteration and lysosomal membrane stability. In addition, participating scientists are free to add to the programme any other techniques which they consider relevant, such as acetylcholinesterase activity, imposex, P-450 1A1 m-RNA, CYP1A1 protein, lipofuscin and lipid lysosomal accumulation.
32. Participating laboratories should make an effort to utilise the same species for reasons of comparison. Fish and molluscs should be used. *Mullus barbatus* is the recommended fish but if not available *Mugil sp.* can be used. *Mytilus galloprovincialis* is the recommended mollusc but if not available *Patella sp.* can be used. The recommended species for caging are *Dicentrarchus labrax* and *Mytilus galloprovincialis*. See Table in paragraph 17.
33. Participating laboratories must also record basic environmental parameters such as the temperature in sea water at the time of sampling; also the sampling point should be clearly indicated. Other information which should be recorded are the size the number and the sex of the animals utilised.
34. Participating laboratories should use a standard procedure for the transport of the animals to the laboratory and for organ extraction. In addition, sample storage procedures should be similar.
35. The participation of laboratories in intercalibration exercises should be obligatory.
36. Whenever possible, fish and molluscs should be collected from the same site and at the same time to allow a correct interpretation of the battery of stress indices employed. Moreover, whenever possible, the same individuals should be used for the battery of biomarkers.
37. Caging can be used in addition or in substitution to the collection of free living animals. In this case the species recommended are: *Dicentrarchus labrax* (fish) and *Mytilus galloprovincialis*.
38. Sampling should not take place during the reproductive stage of a particular species.
39. Data analysis should be standardised as much as possible.

Recommendations to policy makers

40. In view of the fact that biomonitoring provides fundamental data useful for risk assessment and for an early warning system which will enable a timely formulation of governmental strategies to prevent irreversible alterations in Mediterranean coastal

ecosystems and human health, it is recommended that every support should be given to the programme. As a fundamental first step laboratories in the less developed countries should be assisted to be adequately equipped and the necessary training provided to enable their full participation in the programme.

Other recommendations

41. MED POL National Coordinators should designate as soon as possible the laboratories which will participate in the biomonitoring programme.
42. The Secretariat should investigate other sources of funding, in particular E.U.

Agenda item 9. Any other matter

43. No other matter was raised by the participants.

Agenda item 10. Adoption of the report of the Meeting

44. The English version of the draft Report of the Meeting was unanimously adopted by the participants. The french version will be circulated for comments.

Agenda item 11. Closure of the Meeting

45. Mr Gabrielides expressed the deep satisfaction of the Secretariat for the results of the meeting hoping that this programme would be promoted by all governments concerned. He added though that he did not expect substantial MTF funding and that other sources of funding should be sought. He finally thanked the participants for their active participation, the bureau of the meeting for the excellent work they have done and the IcoD for hosting the meeting.

46. The Chairman, after the usual exchange of courtesies, closed the meeting at 15.00 hrs of 1st October 1997.

ANNEX I

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ANNEX II

AGENDA

1. Opening of the Meeting
2. Election of officers
3. Adoption of the Agenda and organisation of work
4. Background and scope
5. Review of the results of the 1996 pilot biomonitoring programme including evaluation of techniques
6. Data quality assurance
7. Review of manual on biomonitoring techniques
8. Recommendations for future action
9. Any other matter
10. Adoption of the report of the Meeting
11. Closure of the Meeting