

Workshop on Combined Effects in the Marine Environment

Report of the workshop, Copenhagen, 16-17 November 1998

Organized by:

Arctic Monitoring and Assessment Programme (AMAP)
European Environment Agency (EEA)
International Council for the Exploration of the Sea (ICES)



European Environment Agency



Contents

Preamble	3
1. Opening of the Workshop	4
2. Plenary presentations	5
2.1 Introductory presentations	5
2.2 Presentations of relevant activities under other organizations	6
2.3 Summary	7
3. Working group sessions	7
3.1 Organization of working groups	7
3.2 Working group reports: Summary conclusions and recommendations	8
3.2.1 Working Group 1 - Reproduction and Population Studies	8
3.2.2 Working Group 2 - Cellular and Biochemical Methods, including Enzymatic Activities	9
3.2.3 Working Group 3 - Effects on plankton, pelagic eggs, pelagic fish larvae. Effects in whole organism bioassays	11
3.2.4 Working Group 4 - Pathology and Diseases	12
4. Workshop Conclusions and Recommendations	12
<i>Annex 1: List of Participants</i>	15
<i>Annex 2: Draft Agenda and Programme</i>	22
<i>Annex 3: List of Documents</i>	28
<i>Annex 4: Introductory Presentation 1 - Challenges in the development of combined effects methods for use in ecological risk assessment. Valery E. Forbes</i>	31
<i>Annex 5: Introductory Presentation 2 - The Use Of Biological Effect Methods: Past, Present And Future Possibilities. M.B. Jones and M.H. Depledge</i>	32
<i>Annex 6: Introductory Presentation 3 - Effects Of Mixtures And Combined Effects. Cynthia de Wit</i>	33
<i>Annex 7: Introductory Presentation 4 - Assessing combined effects of pollutants against the backdrop of climatic variability and global change. Hein Rune Skjoldal</i>	35
<i>Annex 8: Introductory Presentation - Ongoing activities 1 - Draft Paper Submitted to QUASIMEME Bulletin - Biological Effects Quality Assurance In Monitoring Programmes (Bequalm). Peter Matthiessen</i>	37
<i>Annex 9: Introductory Presentation - Ongoing activities 2 - ICES activities in relation to biological effects monitoring. Janet Pawlak</i>	40
<i>Annex 10: Tables presenting the ICES review of the status of biological effects techniques relative to their potential application in monitoring programmes (modified after: ICES. 1997. Report of the ICES Advisory Committee on the Marine Environment, 1997. ICES Cooperative Research Research Report, No. 222: 12–20.).</i>	42
<i>Annex 11: Report of Working Group 1 - Reproduction and Population Studies.</i>	51
<i>Annex 12: Report of Working Group 2 - Cellular and Biochemical Methods, including Enzymatic Activities.</i>	56
<i>Annex 13: Report of Working Group 3 - Effects on plankton, pelagic eggs, pelagic fish larvae. Effects in whole organism bioassays.</i>	69
<i>Annex 14: Report of Working Group 4 - Pathology and Diseases (16. November).</i>	75

Preamble

At the second Inter-Regional Forum (IRF) meeting in Rome, November 1997, the participating organizations supported a proposal concerning a mutual need to address the issue of combined effects of pollutants and other environmental stressors. The Secretariat of the Arctic Monitoring and Assessment Programme (AMAP) agreed to take a lead on this issue and to organize a workshop on biological effects methods that may be used to detect effects due to multiple stressors in the marine environment. This workshop was arranged in close cooperation with the European Environment Agency (EEA) and the International Council for the Exploration of the Sea (ICES), who jointly offered meeting rooms and secretariat support for the workshop. The EEA and AMAP provided some financial support the participation of experts from countries in transition, and the workshop was also sponsored by the Danish Environmental Protection Agency. The organizers would like to express their thanks to these organizations for their support.

The workshop was organised in the form of an initial plenary session, with presentations from invited experts. These included presentations of relevant work ongoing within ICES, through their WG on biological effects of contaminants, and of the recently established EC sponsored BEQUALM project on quality assurance of biological effects methods. This opening session was followed by parallel working groups sessions to consider different aspects of the subject and prepare recommendations from the workshop that could be put forward to the various sponsoring agencies.

Workshop organizing committee:

Lars Otto Reiersen (AMAP)

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Hanne Petersen (AMAP)

Evangelos Papathanasiou (EEA)

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1. Opening of the Workshop

The workshop was opened at 09:00 Monday 16 November 1998 by Evangelos Papathanasiou of the European Environment Agency (EEA) who welcomed participants and provided a short presentation of the background to the workshop.

The workshop was a direct result of a recommendation arising from the Inter-Regional Forum (IRF) meeting in Rome, November 1997. At that meeting, the IRF highlighted four areas where activities should be initiated to stimulate activities and identify research needs, one of these areas being 'Combined effects of pollutants, and the application of biological effects methods in the marine environment'. Through the organization of the workshop it was hoped to further develop harmonised activities that could be adopted or supported by organizations and agencies involved in the respective regional programmes of the IRF. For this reason, participants were encouraged to take a wide ranging view in their deliberations, and especially to consider whether methods applicable in one (geographical) area could also be employed under the (e.g. climatic) conditions prevailing in other areas.

The participants were also informed that the workshop had been arranged back-to-back with a meeting to take place on Wednesday, 18 November, where the aim was to develop a common project proposals on research needs to support the application of biological effects methods, for submission to research funding agencies including the EU 5th Framework Programme. The workshop and its recommendations were considered to provide a key input to that meeting.

A [list of participants](#) is attached as Annex 1.

Lars-Otto Reiersen (AMAP) presented the [draft agenda for the meeting](#) (Annex 2) and elaborated on the aims and objectives of the workshop:

1. To exchange information and review the state-of-the-art of the topic under consideration, including:
 - Evaluation of biological methods in use;
 - Examining whether these methods can be used to study combined effects;
 - Considering which methods provide early warning and which reveal manifested negative trends;
 - Considering whether the methods can reveal cause-effect relationships.
2. To examine and propose areas for future cooperation between the Convention areas and/or regional programmes, in relation to:
 - Gaps in knowledge
 - Research needs
 - Field workshops

In particular, Lars-Otto Reiersen introduced proposals concerning the work to be accomplished within the four proposed working groups. The themes for the working group discussions on Monday and Tuesday, and the scenarios for combined effects that should be the focus of the working group considerations were described (see Annex 2) and accepted by the workshop as the starting point for work within the individual working groups.

Simon Wilson informed the meeting of arrangements concerning documentation. Participants had provided a large number of reports and reprints from the scientific literature to the meeting. Due to practical constraints on copying, only unpublished

documents specifically prepared for the workshop would be copied and distributed during the meeting. A complete [document list](#) would, however, be prepared (Annex 3) to enable participants to identify documents presented or referred to during the workshop, including the necessary information to allow published material to be obtained through normal channels, etc.

2. Plenary presentations

2.1 Introductory presentations

In the first of four plenary presentations intended to set the scene for the workshop, Valery Forbes (Denmark) introduced her presentation 'Challenges in the development of combined effects methods for use in ecological risk assessment'. The abstract and selected overheads to this talk are included at [Annex 4](#). In presenting risk assessment as one approach to providing a sound basis for environmental management, a series of examples were described to illustrate some of the effects methods employed, and their advantages and disadvantages. In particular, points relating to unexpected (non-intuitive) relationships in interactions between environmental contaminants/stressors, the strong dependency on timing of the studies, and the magnification or extrapolation of effects from the individual organism to population level were raised. The conclusion of the presentation was that effects measurements can be more cost effective than contaminants measurements, but that it is vital to ensure that the effects methods are telling us both what we want to know, and what we think they are telling us. The methods employed should also be relevant in an ecological sense and decisions were required on what to apply and how. A step-wise approach to aid the decision-making process was proposed.

The second introductory presentation, by Malcolm Jones (United Kingdom), entitled 'The use of biological effect methods: Past, present and future possibilities', ([Annex 5](#)) focussed on the potential of biomarkers as early warning signals. His presentation also stressed the need for 'integration', in terms of geographical/regional integration, integration of chemical and biological effects monitoring, and integration of approaches employed to measure biological effects (as an 'integrated response'). The term 'biomarkers' was defined as *"a biochemical, physiological or behavioural response that can be measured in tissues, body fluids or at the level of the whole organism that provides evidence of exposure to, and/or adverse effects of, chemical pollution"*. Two examples of a hierarchical approach in the use of biological effects techniques for early warning purposes were presented, one from the Black Sea employing the neutral red (lysosomal stability) bioassay, and the second from the Tees estuary (United Kingdom) where statistical methods were employed to integrate results from several tests. New techniques and methods, such as those described were considered to greatly increase the potential for the wider application of biological effects methods in environmental monitoring programmes, both in terms of increased sensitivity and in relation to practical considerations such as ease of use and comparability of results.

In the third introductory presentation, 'Effects of mixtures and combined effects' ([Annex 6](#)), Cynthia de Wit (Sweden) presented examples from experiences gained whilst working on the AMAP Assessment of the State of the Arctic Environment. During this assessment, attempts were made to evaluate results in terms of combined effects of contaminants and other environmental stressors. This included effects of mixtures of chemicals both within a particular group of substances (such as persistent organic pollutants) and between groups (e.g. POPs and metals). In a number of cases, chemical monitoring approaches had revealed contaminant levels in various biotic and abiotic media that were approaching effects levels. The potential for effects from single contaminants clearly also raised questions in relation to combined effects. A major problem encountered during AMAP's

consideration of this issue was the considerable gaps in knowledge that exist. Examples were presented of studies looking at combined effects from several substances within the POPs group, including studies that had revealed antagonistic, additive and synergistic effects. A number of biological responses (immunosuppression, neurotoxicological, hormone disruption, mutagenicity/carcinogenicity, etc.) were also considered in relation to the potential influence of different types of contaminant or other environmental stressor. The workshop was reminded of the need to consider practical issues during their evaluation of different biological effects techniques, especially in relation to the harsh conditions prevailing in regions such as the Arctic which limit both the techniques that can be applied and the extent and periods during which they can be deployed.

The final general introductory presentation, by Hein Rune Skjoldal (Norway), 'Assessing combined effects of pollutants against the backdrop of climatic variability and global change' ([Annex 7](#)), advocated an ecosystem approach, from definition of objectives, through monitoring to advice and management, in addressing combined effects in relation to fisheries. In addition to contaminants, environmental conditions, in particular climate, are important factors. Climate change and increased UV radiation due to ozone depletion at northern latitudes are therefore subjects that need to be addressed through biological effects studies. The need to link chemical and biological effects monitoring was stressed. The presentation also referred to the four steps in the ICES/GESAMP strategy in relation to biological effects studies, comprising: identification; quantification (on a relative scale); causation; and evaluation. In particular, the presentation focussed on climate as a factor in affecting fish populations, and the need to recognize limitations in our ability to predict responses to natural changes. Human influences impact the same ecosystem components (both directly and indirectly) and, in a management context, there is therefore a need to separate both natural and anthropogenic variability and the effects of different human activities. Climate was identified as the primary driving force for ecosystem development, influencing food webs and keystone species in predator/prey relationships and with consequences for not only stock dynamics, but also pollutant pathways. Consequently, any strategy for combined effects assessment involved pollution monitoring, controlled laboratory experiments, bioassays and fish reproduction studies, etc.

2.2 Presentations of relevant activities under other organizations

On behalf of P. Matthiessen, Michael Waldock (United Kingdom) presented the BEQUALM (Biological Effects in Marine Monitoring Programmes) programme ([Annex 8](#)). He noted that most existing effects monitoring had a background in reporting on hazardous substances to regulatory Commissions, etc. In relation to chemical monitoring programmes, it is no longer feasible to go on adding to the lists of chemicals to be monitored, particularly in view of the numerous organic chemicals that are currently in use but for which analytical capability is limited or non-existent; thus there is an increasing emphasis on biological effects monitoring. Associated with this development, there is an urgent need for QA programmes for the methods that are in use or will be introduced, and this is the main purpose of BEQUALM. To meet the objectives of the programme (see [Annex 8](#)) a steering group had been established and responsibilities for different parts of the programme divided between lead institutes covering the four subject areas of pathology, community studies, biochemistry, and bioassays and physiology. This organization and structure represented a network of participating laboratories. The programme is established as a three-year system supported by EU funding, after which it will need to be self-financing. The first workshops/intercalibrations will be arranged in 1999. The programme is directed at participants from countries of the EU (and those countries with which the EU has relevant cooperative agreements; it is also open to laboratories from other countries (e.g. Canada, USA) however they must cover their own costs of participation. Strong links exist between the BEQUALM programme and the QUASIMEME (Quality Assurance in Marine Monitoring)

programme established under a similar arrangement to cover the chemical monitoring programmes.

Janet Pawlak (ICES) presented a brief review of the background and involvement of the International Council for the Exploration of the Sea in work concerning biological effects of contaminants in the marine environment ([Annex 9](#)). This work has largely been conducted through their WG on Biological Effects of Contaminants, and ICES had prepared a comprehensive review in 1997 of biological effects techniques in use and those with potential for application in marine monitoring programmes. A modified version of this is included as [Annex 10](#). It was stressed that the work within ICES is not concerned with research into methods, but rather with their practical application, and in particular, QA of methods accepted for use in marine monitoring programmes.

2.3 Summary

Discussion of the introductory presentations highlighted the three basic philosophies that had been covered, namely:

- effects at a high level of biological organization should be the focus of concern in a management context;
- quick and pragmatic (cellular/biochemical) techniques should be used in the field to indicate responses (ideally linked to responses at higher levels), that could direct e.g. chemical monitoring effort;
- suites of techniques should be employed covering the entire spectrum of effects levels, between the biomarker/molecular level and population scale effects, and preferably integrated with chemical monitoring.

3. Working group sessions

3.1 Organization of working groups

Lars-Otto Reiersen reviewed and elaborated on the proposed arrangements for the remainder of the workshop, for which most of the time participants would divide and work in separate working groups. Four working groups were established to cover different aspects of the subject, as follows:

- Reproduction and population studies;
- Cellular and biochemical methods, including enzymatic activities;
- Egg/larval/fetal disturbances and mortality;
- Pathology and diseases.

During the first part of the discussion within the working groups, the groups were requested to consider 'The strength and weakness of the your methods', by introducing and critically reviewing the different techniques available in relation to their application (which organisms, contaminants, etc. were addressed), the positive and negative aspects of the method (practical application under different conditions, QA considerations, etc.), and recommendation for their application in routine monitoring programmes, or as research methods, etc.

The results of these working group discussions (see individual working group reports, Annexes 11-14) were presented in plenary.

Following the presentations by the different groups, the working groups reconvened to consider a second theme of discussions, 'The priority scenarios ... Which methods might be useful and/or have potential?'. For this part of the work, the groups were asked to consider a series of scenarios involving different groups of contaminants and/or other stress factors (see [Annex 2](#)), to evaluate how the different methods would respond to situations where one or a combination of these 'scenario components' was present. In combination with the results of their initial discussions, the groups were then requested to evaluate the different biological effects methods in terms of:

- their potential for application within (Pan-European) marine monitoring programmes;
- identification of gaps in knowledge that could be addressed through targeted research activities, and
- where possible, development of harmonised research proposals for input to the related meeting due to take place on Wednesday 18 November.

For this second working group session, Working group 4 (Pathology and diseases) was disbanded and their participants joined the other groups where their input was incorporated.

3.2 Working group reports: Summary conclusions and recommendations

The full reports of working groups 1 to 4 are presented in Annexes 11 - 14, respectively.

The following sections highlight some of the main conclusions and recommendations from the working group reports, focussing on their discussions regarding evaluation of available methods, strategies for effects monitoring, field studies, and research needs/gaps in knowledge.

3.2.1 Working Group 1 - Reproduction and Population Studies

- Discussing populations and communities, the group concluded that analysis of changes in population and community structure (i.e., population density, species composition/diversity) are most appropriate for relatively short-lived invertebrates or algal species. The benthic community was identified as being of key importance with methods for assessing changes in benthic community structure well established and having a long history of application.

Monitoring the ratio of oiled bird carcasses relative to the total number of carcasses censused was also identified as a technique with high potential for routine application, albeit specifically in relation to assessing the impacts of oil contamination rather than as a general combined effects measure.

- Considering endocrine/hormone disruptions, the group found that methods such as those involving measuring blood levels of hormones, immunological parameters, and other reproductive effects appear to be most useful for long-lived, homeothermic vertebrates; but that different species will need to be used in different geographical regions.
- Scope for growth was considered to be the most widely and well-developed of the physiological effects measures. Metabolic rate measures may also be sensitive indicators of physiological stress and could be included in a scope-for-growth assessment.
- Considering application of 'Combined Effects Measures', the group found that most of the effects measures that they had discussed would respond to many stresses. This was seen as advantageous for screening purposes, but makes assigning cause-effect

relationships difficult. The group therefore identified the need for a stepwise or hierarchical approach that also integrates chemical analyses with biological effects measures.

For general screening purposes, tests representing four different types of approach were recommended (similar tests have been recommended by OSPAR and are included on the ICES list of reviewed effects indicators), as follows:

I. Bioassay:

Whole sediment bioassay

Porewater bioassay

Water bioassay

Simple behaviour test (invertebrates)

Early life stage tests (invertebrates, fish)

II. Biomarker:

Reproductive success (fish, birds)

Clinical variables/hormones (birds, mammals)

Endocrine disruption (e.g., vitellogenin, imposex)

III. Population/community:

Fish disease prevalence (gross pathology)

Benthic community structure

Seabird/mammal counting (e.g., oiled bird ratios)

IV. Chemical monitoring:

Focussing primarily on sediment and tissues of key trophic groups

- In relation to field tests, the group concluded that future field trials should include carefully designed laboratory experiments using a multisystem approach in combination with measurements made directly in the field. Several new methods were considered appropriate for inclusion in an experimental field trial, including in particular tests using caged fish systems, the vitellogenin assay, and chemosensory response tests.
- The group identified several areas related to combined effects that could benefit from further research, including (a) information on endocrine systems in invertebrates and the links between endocrine disruption and effects at the population level; (b) evolutionary adaptations (reproductive, behavioural) to chemical contaminants; (c) statistical pattern analyses using multivariate methods, and (d) DNA markers for population identification.

3.2.2 Working Group 2 - Cellular and Biochemical Methods, including Enzymatic Activities

- Following an evaluation of available methods, the group concluded that a suite of methods were suitable for inclusion in monitoring programmes. The methods identified were lysosomal stability, AchE inhibition, metallothionein, EROD, and PAH bile metabolites (although only for a limited number of laboratories). Of these, lysosomal stability and AchE inhibition could be applied in routine monitoring programmes provided practicalities involved in sampling could be accommodated, and EROD/P4501A induction should be included in the suite to interpret results.

The group also considered that the measurement of DNA damage and/or the alkali elution methods could soon be available for routine use.

The group agreed that any monitoring programme utilising these methods would be greatly strengthened by the inclusion of analytical chemical support.

- In considering how this suite of methods might best be combined within a monitoring strategy, lysosomal stability (by the assessment of neutral red leakage) was recognized as a technique known to respond to a wide range of contaminants, and to be relatively rapid and inexpensive. The measurement of lysosomal stability in mussels and a fish species was therefore considered suitable as a first tier screening procedure. At the same time, the group recognized that a screening strategy using only one test may run the risk of failing to detect toxicants at significant levels.
- In discussing temperature effects, the group considered that temperature changes would affect the biomarker responses, but it was not clear what the effects would be or how large.

Temperature changes as a consequence of global climate change over the coming decades (likely to be of the order of 0.5 - 1 degree C) imply that the temperature signal related to climate is much 'slower' and smaller than that arising from seasonal factors.

- Concerning the influence of changes in UV radiation, the group agreed that increased UV-B irradiation would tend to activate the toxicants concerned (e.g. PAH) and consequently there might well be an impact on the biomarker responses. However, although a mechanism had been identified it was not possible to make any assessment of its likely importance.
- Considering biomarkers of temperature and UV-B exposure, the group, therefore, considered that there were no biomarkers available that could be used unambiguously to reflect the effects of the rates of temperature rise that have been predicted to arise from climate change, and that other areas of environmental science were much more likely to provide appropriate indicators. However, considering the documented effects of UV-B on plankton and macro-algae it was possible that community-scale markers could be developed.
- In relation to the question of the possible effects that may arise as a consequence of the presence of combinations of contaminants (synergism, antagonism, additive effects, etc.) the group noted reported instances of marked synergistic effects of combinations of contaminants. Specifically, mixtures of cadmium and carbofuran showing toxicities 5 to 10 times greater than would be predicted from calculations based on additive toxicities, and mixtures of dichlorvos and malathion around 100 times more toxic than predicted. It was indicated that similar effects were observed in relation to AchE activity.
- Following discussions of the desirability and opportunities for a field programme the group proposed the objectives for such a programme as follows:
 1. To test the response of a battery of biomarkers in different situations with respect to pollution and temperature.
 2. To test the proposed standard approach in different areas of Europe.
 3. To evaluate whether the approach works, i.e. whether the approach can detect and distinguish between different contaminant groups and their effects.
 4. To evaluate whether the approach can adequately describe the combined effects of different groups of contaminants.

The group further suggested criteria to be used to select study areas, including presence of clear pollution gradients; adequate logistical support, including access to

laboratory space, sampling boats, etc.; and choice of several sites to cover different geographical regions and different environmental settings. Based on these criteria, some possible study areas were suggested, together with organizational recommendations concerning project coordination, coordinating laboratory responsibilities and arrangements for supporting chemical analyses and QA/QC, etc..

- Research and development needs identified by the group were mainly those aimed at bringing methods including PAH metabolites in bile, DNA adducts with PAH, and an assay of DNA damage (probably the alkali elution method) into routine monitoring programmes. Assays of the MDR system (multi xenobiotic resistance in fish and mussel), hormonal shift in fish and molluscs, vitellogenin in fish and sperm motility in molluscs were methods for which a greater effort will probably be required.

3.2.3 Working Group 3 - Effects on plankton, pelagic eggs, pelagic fish larvae. Effects in whole organism bioassays

- In their evaluation of available methods, the group agreed from the outset that it would be vital for the success of contaminant-related monitoring to include both biological and chemical methods.

Various studies have indicated possible links between contaminants and effects on fish larvae, and there are also reports of possible changes in sex ratios in fish from the North Sea.

Bioassays can be performed with standard species (e.g. *Skeletonema*, *Acartia*) or species collected in the field.

- Considering contamination scenarios, the group identified fish eggs and larvae as the most sensitive and relevant trophic level for studying effects due to organohalogenes. Parental transfer was thought to be the main source of exposure, although it was recognised that eggs may be exposed through water. For other organic contaminants, it was noted that molluscs appear to be sensitive, whereas other benthic organisms (polychaetes, crustaceans) are less sensitive. In relation to metals, methyl-mercury (MeHg) may have profound effects on behaviour and development in fish. UV radiation in itself may affect health and survival in juvenile and adult fish, and would be expected to increase the rates of breakdown of organic contaminants, PAHs in particular.

The following gaps in knowledge were identified:

1. Knowledge concerning the ability of eggs, embryos or larvae to metabolise xenobiotics.
2. Information on how it may be possible to link effects at the individual level and population effects.
3. Knowledge on whether there are effects of contaminants on eggs and larvae of invertebrates.
4. The lack of biomarker techniques for small organisms or small amounts of tissue (for both histological techniques and biochemical/cellular techniques).
5. Combined effects in pelagic systems: eutrophication – contaminants, oil – contaminants, UV – contaminants.

The most urgent research need was considered to be the development of methods to assess the individual health of zooplankton and fish larvae, mainly to establish causality between effects and environmental factors.

3.2.4 Working Group 4 - Pathology and Diseases

- There was general consensus within the group that investigations should include both monitoring/research using methods of early warning and “end-point” effects. Early warning indicators could be behavioural responses, imposex/intersex or lysosomal changes. Examples of “end points” might: be disease (such as carcinogenesis, necrosis, as an end point of cellular interference or cytochrome P450 changes) and some population changes.
- In their evaluation of available methods, the group noted that neoplastic changes have been observed in areas with suspected contamination (as evidenced from histology of bivalves from the Baltic Sea), however, no direct links have been established. In relation to methods studying fish disease, the lysosomal stability test was considered a good generic indicator for a multiple set of contaminants, but can also indicate the onset of liver pathologies.

4. Workshop Conclusions and Recommendations

Based on the discussions in the plenary sessions and working groups, a general strategy for the application of combined effects assessment is proposed (Figure 1). It was recognized that an effects assessment may be initiated under various starting conditions that differ primarily in the amount of knowledge about the sources and types of contamination. The amount of information available prior to the start of the effects assessment will have an important influence on the types of effects measured and on the extent to which general screening tests will be required. In some situations, an effects assessment may be initiated with contaminant(s) and/or contaminant source(s) already known/identified. In such a scenario, the question of ‘are there ecological effects’ may not be an issue. Rather, the objective of the effects assessment may be to quantify (in space and time) the severity of the effects.

As Annex 10 indicates, there is a wide variety of effects measures available from which an appropriate combination of tests may be selected depending on whether a general screening or more focussed testing (in terms of contaminant, species, or level of organization) is desirable. Although different tests may be appropriate for specific scenarios under consideration, it is possible to identify general attributes of effects measures that should be sought at the screening stage and to perform a more focussed effects assessment.

Screening tests should:

1. Be practical for routine use, using well-established methods that are efficient in time and cost and have good quality control
2. Be able to detect a wide variety of effects
3. Have high statistical power
4. Include all potentially relevant compartments (i.e., water, sediment, food)

Focussed tests should:

1. Provide confirmation of screening results
2. Establish the spatial/temporal scale of effects
3. Enable quantification of the severity of effects in terms of likely risks to ecological systems or human health

4. Identify species or populations most likely to be at risk
5. Identify or confirm causal agents

Once the magnitude of effects has been quantified, the next step in the effects assessment will often be to identify the causes of the observed effects where these are not known. This may initially involve reducing the list of possible contaminants based on knowledge of local activities or environmental contaminant levels. An approach similar to the one shown in Box 1 (modified from Calow & Forbes 1998) may aid in eliminating unlikely agents.

Box 1. Identifying possible causes of pollution and prioritizing them for combined effects assessment.

1. Search international lists of hazardous substances.
2. Identify contenders for a regional or subregional priority list by considering if any substance from § 1 is likely to arise from industrial activities in and around the region or subregion. Most will be rejected as low or zero priority.
3. Are those from § 2 recorded within the region or subregion?

If yes: compare environmental concentrations with likely effects levels

If no: is this because there have been no attempts to monitor?

If no: discard as low or zero priority.

If yes: is the substance likely to be persistent?

If no: discard as low priority.

If yes: proceed with focussed effects measures.

If effects not detected: discard as low or zero priority.

If effects detected: perform refined effects assessment.

Various chemical fractionation techniques (e.g., toxicity identification evaluation (TIE); U.S. EPA 1991) can be used to isolate and characterize the physical-chemical nature of toxicants in complex mixtures based on a knowledge of how factors, such as pH, modify toxicity.

A final step in the combined effects assessment will be to identify the source(s) of the causal agent(s). This could involve the application of various types of transport models and/or chemical fingerprinting techniques.

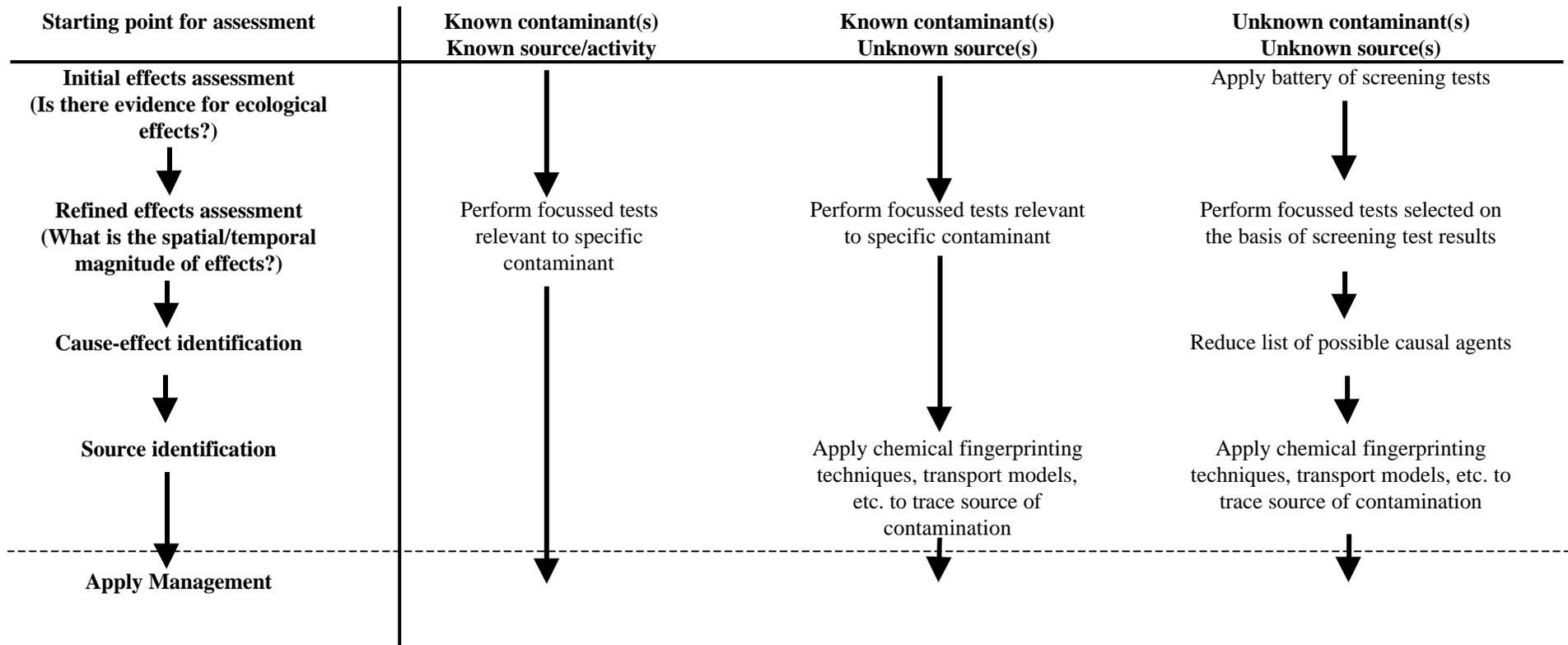
The results of the effects assessment are used to develop management strategies. These may often involve continued monitoring, using a selection of screening tests or more focussed measures, to provide feedback on the effectiveness of the management efforts and facilitate adjustments in management actions as necessary.

Literature Cited:

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U.S. EPA 1991. Sediment toxicity identification evaluation: Phase I (Characterization), Phase II (Identification), and Phase III (Confirmation) Modifications of Effluent Procedures, edited by G.T. Ankley; M.K. Schubauer-Berigan; J.R. Dierkes; and M. T. Lukasewycz. EPA-600/6-91/007.

Figure 1. Strategy for Combined Effects Assessment



Annex 1: List of Participants

To: Workshop on Biological Effects Methods to be applied to detect “Combined Effects” in Marine Ecosystems, Copenhagen, 16–18 November 1998

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Annex 2: Draft Agenda and Programme

For: Workshop on Biological Effects Methods to be applied to detect “Combined Effects” in Marine Ecosystems, Copenhagen, 16–18 November 1998

Location: (1) EEA - Kongens Nytorv 6 (2) ICES - Palægade 2-4

Monday 16	
0800 – 0900	Registration at the EEA office.
0900 – 1130	Plenary session at the EEA Conference Room. (Chair: Evangelos Papathanasiou EEA)
	Opening statements. (Evangelos Papathanassiou, EEA)
	The Objectives of the Workshop. (Lars-Otto Reiersen, AMAP)
	The use of biological effect methods: "Past, Present and Possibilities" Special focus on combined effect studies and what we know today. (Presentations by: Valerie Forbes, DK; Malcolm Jones, UK; Cynthia de Wit, Sweden)
	Effects of Climate and UV on the fate of pollutants and biological tests? (Hein Rune Skjoldal, Norway)
	BEQUALM - Presentation of the work initiated. (Mike Waldock, UK)
	ICES review of biological effects methods. (Janet Pawlak, ICES)
	Questions and Discussion related to the workshop objectives and the presentations.
	Arrangement for 3-4 working groups will be discussed and decided at this point (according to your scientific background, and the questions to be discussed). (Lars-Otto Reiersen, AMAP)
	The theme for the working group discussions on Monday: "The strength and weakness of the your methods"
The theme for the working group discussions on Tuesday: "The priority scenarios, Appendix 2. Which methods might be useful and/or have potential?"	
1130 – 1200	Coffee and reorganisation

1200 – 1800	<p>Parallel Working Group sessions at the EEA and ICES.</p> <p>1200 - 1430: Presentations by participants regarding personal experiences by applying different biological test methods that might be of interest for combined effect studies, possibilities, limitations, gaps in knowledge, research needs, etc.</p> <p>1430 – 1800: Discussion and preparation of a table and a short documentation paper on the applicability of the presented effect methods under different marine conditions – climatic, salinities (Arctic, North Sea, Baltic, Mediterranean, etc), gaps in knowledge and research needed.</p>
	Draft agenda for the Working Groups on Monday afternoon:
	<ol style="list-style-type: none"> 1. Short presentation of all members in the group. 2. Short intro by the Working groups Chair. 3. Presentation of methods used by participants, 10 min. per participant if wanted: <ul style="list-style-type: none"> • Applications and results. • Possibilities and limitations. • Gaps in knowledge. • Research needs. • Documentation of methods and results. • Future cooperation. 4. Discussion of the presented methods and their usefulness, including a discussion on the mechanisms or functions measured by the specific methods. 5. Systematic arrangements of the methods, applicability, research etc. 6. Working groups Chair's summing up.
Tuesday 17	
0900 – 1030	Plenary session at the EEA office (Chair: Janet Pawlak, ICES). Presentation of working groups work and a short round the table

	comments.
1045 – 1630	Working groups continue their work, arranged according to scenarios.
1630 – 1830	<p>Plenary session: Summing-up of the discussions and preparation of final recommendations (Chair: Lars-Otto Reiersen, AMAP):</p> <ul style="list-style-type: none"> • Methods that can be applied today in general, or under specific conditions. • Gaps in knowledge. • Priorities for research need to develop better methods. • Further actions, including possibilities for a field experiment.
	Draft agenda for the Working Groups on Tuesday:
	<ol style="list-style-type: none"> 1. Short presentation of all members in the group 2. Presentation of scenarios (intro by Chair) 3. Applicability of the methods in the scenarios (by participants): <ul style="list-style-type: none"> • What can be expected of problems and results? • Possibilities and limitations. • Gaps in knowledge. • Research needs. • Documentation of methods and results. 4. Discussion of the scenarios versus the methods, including a discussion on combination of different methods and which methods would best mesh together, i.e. whether they could be applied sequentially or simultaneously to determine synergistic/additive/antagonism? 5. Systematic arrangement of the methods; applicabilities, gaps, research needs etc. 6. Field experiments at 1-2 locations in 2000-2001. <ul style="list-style-type: none"> • Possibilities for practical field experiments where several methods could be tested at one or two locations during 2000 – 2001 should also be discussed. This could be arranged as the GEEP workshops were done

	<p>10 years ago. Selection of locations could be based on variability in temperature, salinity, pollution, UV radiation etc. A good infrastructure (laboratory etc) is needed in the vicinity of the field test area. One location could be under Arctic conditions e.g. at Svalbard, and the other under more temperate conditions e.g. in the Mediterranean.</p> <ul style="list-style-type: none"> • Can such a fieldwork provide valid information? • Is it feasible, practical, time, place? • Cost implications and financial possibilities? <p>7. Chair summing up.</p>
Wednesday 18	On Wednesday the Meeting to develop a common proposal to EU funding will take place at EEA office, (see separate agenda) while the Workshop on Biological Effects Methods to be applied to detect ‘Combined Effects’ in Marine Ecosystems, will continue at the ICES office.
0900 – 0930	Presentation of the workshops result to the EEA meeting, joint meeting.
0945 - 1500	Preparation of the Workshop report (draft Minutes) including recommendation for actions.
1500 – 1600	Summing-up of this workshop exercise and Future meetings.
1600	End of the workshop.

Appendix 1: Practical information:

Some Secretariat support will be available for registration, copying of papers and necessary typing. Some PCs will be available for the working group members.

One person will chair each of the working groups, and a rapporteur will be responsible for making necessary notes during the discussions and, together with the group, preparing the written proposals.

The plenary sessions will take place at the EEA building.

Two working groups will work in the EEA building and two in the ICES building.

Coffee will be made available during the meetings. Coffee breaks will be arranged between

1100 – 1130 and 1500 – 1530.

Lunch will be available at the EEA cantina between 1230 – 1430. The groups are free to break up for lunch when they so wish.

The four working groups will be arranged based on the field of expertise for the participants, and the possible mixing of the experts.

Please provide a short written description or publication describing the methods and results you like to present to the workshop.

Appendix 2: Draft proposal for the arrangement of the workshop

The objectives/aims of the workshop

The general aims of the workshop were discussed at the IRF meeting in Rome, where it was decided that the aims should be:

1. to exchange information and review the state-of-art on the topic in question, and
2. to examine and propose areas for future co-operation between the Convention areas.

Under aim 1) we would like to include an evaluation of actual biological effect methods that are in use today, and to examine whether these methods can be applied for combined effect studies? Also whether the methods can identify the two main phases of a disturbed marine ecosystem (i.e. an early warning signal as opposed to a negative temporal trend in ecosystem health)? Are any of these methods applicable for specific cause-effect relationships, or are they broader scanning methods that can indicate that a pollutant or environmental factor is stressing the system, but not exactly which factor?

Under aim 2) we would like to specify gaps in knowledge and research needs that could be followed up through an application to the 5th Framework Programme and other relevant funding mechanisms or agencies.

Defining ‘combined effects’ could either be narrowed to include only the effects due to the occurrence of several stressors belonging to one group, as for instance the Persistent Organic Pollutants (POPs), and studying the combined effect of a mixture of these, e.g. PCB, DDT and Toxaphene, or it could have a wider definition and also include the effects due to the co-occurrence of several stressors belonging to different groups, e.g. a mixture of Mercury (heavy metal), PCB (POPs), PAH (hydrocarbons), etc.

For this workshop we would like to see an evaluation of the different methods applied in relation to both definitions, and we would like to ask the experts to assess the applicability of the different methods to detect the combined effects as defined above. In addition, we would like to have an assessment of the potential use of the same methods under conditions where there may be an additional change to the environment, due e.g. to changes in climate (temperature) and UV radiation, and the occurrence of TBT together with some of the other.

At the workshop we would like to prioritise the following stressors, and thus conduct the work

by arranging the following Scenarios:

- Persistent Organic Pollutants (POPs), including PCB, DDT, Toxaphene, Chlordane;
- Heavy Metals (HM) including Mercury, Lead, Cadmium and Selenium;
- Organic Material (OM) as oil and dissolved and particle organics (DOM & POM).

The following matrix illustrate the scenarios/assessments we kindly would like to perform:

	POP	HM	OM	and additional influence of	
				Temperature	UV
POP	+*	+	+	+	+
HM	-	+*	+	+	+
OM	-	-	+*	+	+

* Applying the narrow definition of combined effects only (combined effects within a group).

Annex 3: List of Documents

For: Workshop on Biological Effects Methods to be applied to detect “Combined Effects” in Marine Ecosystems, Copenhagen, 16–18 November 1998

<u>Submitted by</u>	<u>Title/Reference</u>
Plenary	
S. Tambutté	<ul style="list-style-type: none">• A Biomonitoring Program of the Coastal Area of the Principality of Monaco• The Ramoge Biomonitoring Program
J. Vacelet	<ul style="list-style-type: none">• Current Projects and Interests
C. de Wit	<ul style="list-style-type: none">• Effects of mixtures and combined effects
T. Konsoulova	<ul style="list-style-type: none">• Black Sea—An unique water basin
M. Jawed Hameedi	<ul style="list-style-type: none">• M. Jawed Hameedi. 1997. Strategy for monitoring the environment in the coastal zone. <i>In Coastal Zone Management Imperative for Maritime Developing Nations</i>, 111–142. Kluwer Academic Publishers, The Netherlands.• Long <i>et al.</i> 1996. Estimates of the spatial extent of sediment toxicity in major U.S. estuaries. <i>Environmental Science and Technology</i>, 30 (12): 3585–3592.
H. Rumohr	<ul style="list-style-type: none">• Matthiessen, P. Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM). Submitted to QUASIMEME Bulletin.
J. Pawlak	<ul style="list-style-type: none">• ICES. 1997. Biological effects monitoring, pp. 11–20. <i>In Report of the ICES Advisory Committee on the Marine Environment, 1997. ICES Cooperative Research Report No. 222.</i>
M.B. Jones	<ul style="list-style-type: none">• The use of biological effect methods: Past, present and future possibilities
E.-L. Poutanen	<ul style="list-style-type: none">• Experience in Finland related to biological effects methods
M.J. Hameedi	<ul style="list-style-type: none">• Assessment of biological effects in the coastal ocean: Sediment toxicity, biomarkers, and ecological indicators (overhead presentation)
WG1	
REPRODUCTION & POPULATION STUDIES	
V. Forbes	<ul style="list-style-type: none">• Calow <i>et al.</i> 1997. Risk assessment on the basis of simplified life-history scenarios. <i>Environmental Toxicology and Chemistry</i>, 16:1983–1989.• Calow, P., and Forbes, V. 1998. Review: How do physiological responses to stress translate into ecological and evolutionary processes? <i>Comparative Biochemistry and Physiology, A</i>, 120: 11–16.• Møller <i>et al.</i> 1994. Influence of acclimation and exposure temperature on the acute toxicity of cadmium to the freshwater snail <i>Potamopyrgus antipodarum</i> (Hydrobiidae). <i>Environmental Toxicology and Chemistry</i>, 16:1983–1989.• Foss, H.E., and Forbes, V.E. 1997. Effects of the polycyclic aromatic hydrocarbon fluoranthene on growth rate and nucleic acid composition of <i>Catitella</i> sp. I. <i>Marine Biology</i>, 129: 489–497.
M.B. Jones	<ul style="list-style-type: none">• Roast <i>et al.</i> 1998. The position maintenance behaviour of <i>Neomysis integer</i> (Peracarida: Mysidacea) in response to current velocity, substratum and salinity. <i>Journal of Experimental Marine Biology and Ecology</i>, 220: 25–45.• Hebel <i>et al.</i> 1997. Responses of crustaceans to contaminant exposure: a holistic approach. <i>Estuarine, Coastal and Shelf Scienc</i>, 44: 177–184.
B. Jenssen	<ul style="list-style-type: none">• Jenssen <i>et al.</i> 1994. Blood sampling as a non-destructive method for monitoring levels and effects of organochlorines (PCB and DDT) in seals. <i>Chemosphere</i>, 28: 3–10.• Jenssen, B.M. 1994. Review article: Effects of oil pollution, chemically treated oil, and cleaning on the thermal balance of birds. <i>Environmental Pollution</i>, 86: 207–215.• Jenssen <i>et al.</i> 1995. Biomarkers in blood to assess effects of polychlorinated biphenyls in free-living grey seal pups. <i>In Whales, seals, fish and man</i>. Ed. By A.S. Blix, L. Walløe, and Ø. Ulltang. Elsevier Science.• Jenssen, B.M. 1996. An overview of exposure to, and effects of, petroleum oil and organochlorine pollution in grey seals (<i>Halichoerus grypus</i>). <i>The Science of the Total Environment</i>, 186: 109–118.• Jenssen <i>et al.</i> 1996. Organochlorine compounds in blubber, liver and brain in neonatal grey seal pups. <i>Chemosphere</i>, 32 (11): 2115–2125.• Henriksen <i>et al.</i> 1998. Relationships between PCB levels, hepatic EROD activity and plasma retinol in glaucous gulls, <i>Larus hyperboreus</i>. <i>Marine Environmental Research</i>, 46: 45–49.

Submitted byTitle/Reference

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- Daan, R., and Mulder, M. 1996. Long-term effects of OBM cutting discharges at 12 locations on the Dutch continental shelf. NIOZ-Rapport 1996-6.36 pp.
- Daan, R., and Mulder, M. 1993. Long-term effects of OBM cutting discharges at a drilling site on the Dutch continental shelf. NIOZ-Rapport 1993-15. 27 pp.
- Camphuysen, K. 1998. Beached bird surveys indicate decline in chronic oil pollution in the North Sea. *Marine Pollution Bulletin*, 36(7): 519–526.
- Boon *et al.* 1997. Concentration-dependent changes of PCB patterns in fish-eating mammals: Structural evidence for induction of cytochrome P450. *Archives of Environmental Contamination and Toxicology*, 33: 298–311.
- De Boer *et al.* 1998. Scientific correspondence: Do flame retardants threaten ocean life? *Nature*, 394: 28–29.
- Boon *et al.* 1996. In vitro biotransformation of chlorinated bornanes (toxaphene) in hepatic microsomes of marine mammals and birds. Influences on bioaccumulation and mutagenicity. In *Organohalogen Compounds*, 28: 416–421. Proceedings of 'Dioxin96', the 16th Symposium on Chlorinated Dioxins and Related Compounds, 12–16 August 1996, Amsterdam, The Netherlands.

WG2**CELLULAR & BIOCHEMICAL METHODS**

K. Hylland

- K. Hylland *et al.* 1998. Natural modulation of hepatic metallothionein and cytochrome P4501A in flounder *Platichthys flesus* L. *Marine Environmental Research*, 46: 51–55.

J. Forget

- Forget *et al.* 1997. Joint action of combinations of pollutants on the mortality and on the acetylcholinesterase activity of *Tigriopus brevicornis* (Copepoda, harpacticoida). SETAC 97, Amsterdam.

G. Bocquené

- Bocquené *et al.* 1995. Joint action of combinations of pollutants on the acetylcholinesterase activity of several marine species. *Ecotoxicology*, 4: 266–279.

C. de Wit

- Balk *et al.* 1994. Effects of exhaust from two-stroke outboard engines on fish. *TemaNord* 1994:528. 66 pp.

A. Torreblanca

- A. Torreblanca. Metallothionein in invertebrates.

J. Boon

- Sleiderink, H., and Boon, J. 1996. Temporal induction pattern of hepatic cytochrome P450 1A in thermally acclimated dab (*Limanda limanda*) treated with 3,3',4,4'-tetrachlorobiphenyl (CB77). *Chemosphere*, 32 (12): 2335–2344.
- Sleiderink *et al.* 1995. Hepatic cytochrome P4501A induction in dab (*Limanda limanda*) after oral dosing with the polychlorinated biphenyl mixture Clophen A40. *Environmental Toxicology and Chemistry*, 14 (4): 679–687.
- Sleiderink *et al.* 1995. Influence of temperature and polyaromatic contaminants on CYP1A levels in North Sea dab (*Limanda limanda*). *Aquatic Toxicology*, 32: 189–209.
- Sleiderink *et al.* 1995. Sensitivity of cytochrome P450 1A induction in dab (*Limanda limanda*) of different age and sex as a biomarker for environmental contaminants in the southern North Sea. *Archives of Environmental Contamination and Toxicology*, 28: 423–430.
- Sleiderink, H., and Boon, J. 1995. Cytochrome P450 1A response in North Sea dab (*Limanda limanda*) from offshore and coastal sites. *Marine Pollution Bulletin*, 30 (10): 660–666.

A. Köhler[†]

- Köhler, A. 1989. Regeneration of contaminant-induced liver lesions in flounder—experimental studies towards the identification of cause-effect relationships. *Aquatic Toxicology*, 14: 203–232.
- Köhler, A. 1990. Cellular responses in fish liver as indicator for toxic effects of environmental pollution. *ICES CM* 1990/E:30. 10 pp.
- Köhler, A. 1991. Lysosomal perturbations in fish liver as indicators for toxic effects of environmental pollution. *Comparative Biochemistry and Physiology*, 100C (1/2): 123–127.
- Köhler, A., Deisemann, H., and Lauritzen, B. 1992. Histological and cytochemical indices of toxic injury in the liver of dab *Limanda limanda*. *Marine Ecology Progress Series*, 91: 141–153.
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Annex 4 : Introductory Presentation 1

Challenges in the development of combined effects methods for use in ecological risk assessment.

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Abstract: The purpose of ecological risk assessment is to provide a scientifically sound basis for environmental management. The process of ecological risk assessment involves relating measured or predicted exposure levels to relevant ecological effects. This process is complicated when a combination of potential stress factors is involved. A variety of biological effects methods have been developed to measure effects of contaminants and other stresses on individual organism performance (i.e. survival, growth, reproduction) or on suborganismal processes (e.g. biochemical, molecular, physiological responses). Although the assumption is that these provide early warning indicators of ecological damage, the extent to which such responses are linked to processes occurring at higher levels of biological organization remains an open question. Of central importance for identifying relevant effects measures for risk assessment is the need to distinguish homeostatic responses from functional impairment. For use as exposure indicators it is not necessary that suborganismal responses link to alterations in organism fitness. However, in order to be used as relevant effects measures, suborganismal endpoints should, at the very least, link tightly to survival, reproductive output and/or timing. Even when they do, recent work has shown that consequences in terms of population dynamics are not obvious. However, substantial insights into population-level impacts may be obtained by applying life-history models that explore the relationships between changes in survival, timing and reproductive output to changes in population dynamics for different life-cycle types. Effects of a combination of stresses that either operate on different components of the life cycle or that affect life-cycle components in conflicting directions can be explored experimentally or by simulation.

Given that no single measure is likely to fulfil, simultaneously, all of the requirements for a useful effects indicator, the primary challenge for the future is to decide when to use which measures so that a sustainable and cost-effective strategy for risk assessment and risk management can be developed. An approach that starts with simple, general effects measures and proceeds, stepwise, to more sophisticated and specific indicators, as necessary, is one way to approach this problem.

The Use Of Biological Effect Methods: Past, Present And Future Possibilities

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Summary

- 1) In the past, ecotoxicological assessments have relied upon establishing the toxicity of chemicals in the laboratory to a relatively limited range of organisms under standard conditions. Once threshold levels of toxic effects were established, these were used to ensure, through chemical monitoring of water, sediment and biota, that environmental contamination remained below levels that produced overt toxic effects.
- 2) More recently, approaches have been developed which attempt to measure directly the biological effects of anthropogenic contaminants *in situ*. Examples include ecological assessments which attempt to detect changes in patterns of community structure and, also, biomarker assays in which early-warning molecular, physiological and behavioural responses of organisms to pollutant exposure are determined.
- 3) Biomarker responses must be related to changes in the fitness of organisms (either through correlation's or mechanistically) if they are to provide evidence of potentially ecologically significant effects on populations and communities.
- 4) The conventional biomarker approach is to select a biomarker in relation to an anticipated contaminant, compare samples from diverse sites, and interpret in relation to residue concentrations and other ecotoxicological data. Often, this approach uses field deployments of experimental organisms. But, biomarkers can be selected that reflect the integrated response of the cell, tissue or whole organisms and, therefore, changes in the patterns of response (rather than the concentration of a single biomarker) are more sensitive and more relevant to detecting effects of chemical mixtures.
- 5) Future approaches must be pragmatic, 'do-able', inexpensive and easy to use.
- 6) Important that biomarkers (and other approaches) are 'generic' in the sense that they measure fundamental cellular, biochemical and physiological processes common to most organisms occupying a diverse range of ecosystems (Arctic-Mediterranean-tropics). But, these will have to be validated in each ecosystem. For example, in low-temperature ecosystems we don't know about rates of uptake, biotransformation, biomarker responses, etc.

Effects Of Mixtures And Combined Effects

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- Within POPs

Some people induce liver enzyme induction which can lead to more rapid metabolism and excretion of other POPs. This may also lead to a higher production of toxic metabolites. Some studies show antagonistic, additive and synergistic effects for different POPs. A combination of 2-4 ortho PCBs, mono-ortho PCBs and PCDFs given to mink had less effects on reproduction in mink than other combinations indicating antagonistic effects between the PCB fractions and the PCDFs (Kihlström *et al.*, 1992). All substances exerting their effects via the Ah receptor, i.e., having dioxin-like properties, have been shown to cause additive effects (NATO/CCMS, 1988; Ahlborg *et al.*, 1994). Two hydroxylated PCB congeners have been found to cause estrogenic effects synergistically (Crews *et al.*, 1995). A mixture of three PCB congeners was more potent in causing changes in brain function in non-human primates than a similar concentration of each PCB congener separately, indicating synergistic effects (Seegal and Schain, 1992).

- Between different substance groups

Some species may be at risk from current levels of both heavy metals such as Cd and POPs-glaucous gulls, identify other species when heavy metals chapter done. Cd leads to kidney damage. POPs to a range of other effects. These different effects may exacerbate each other.

- Other effects influencing combined effects

UV-B effects on humus breakdown in aquatic systems leading to changes in dissolved OC levels in water. Dissolved OC is the phase in water that POPs are found in and the phase in which they are most available for uptake by organisms. Higher organic carbon may thus cause increased bioavailability of POPs to aquatic organisms. leading to higher levels.

- Specific effects caused by many factors

Several animals in the Arctic undergo periods of starvation where they rely on stored fat to survive and to reproduce. As the fat is used up, the POPs concentrate in the remaining fat, leading to higher levels, which may exceed thresholds for some effect. So the combination of a subthreshold POPs level and a period of starvation can possibly lead to effects.

Starvation in and of itself can cause immunosuppression. High levels of a number of POPs, heavy metals and exposure to increased UV-B may also lead to immunosuppression, and the combination of these is probably also immunosuppressive.

Induction of liver enzyme production, particularly the cytochrome P450 mixed function oxidases (MFO) is caused by PAHs and most POPs. Increased MFO activity increased the rate of metabolism of endogenous substances including reproductive, immune system, thyroid hormones etc., which may lead to disturbances in hormone balance and subsequent effects.

PAHs and radionuclides are mutagenic, which is the first event in causing tumours. Most POPs are tumour promoters. Where organisms are exposed to mutagens and tumour promoters, there may be a risk for combined effects leading to increased tumour incidence.

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Annex 7 : Introductory Presentation 4

Assessing combined effects of pollutants against the backdrop of climatic variability and global change

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The issue of combined effects is not new but has been with us as an inherent difficulty and challenge in pollution monitoring and assessment over the last decades. The need for inclusion of biological effects techniques has been recognised as a necessary component along with chemical measurements in pollution monitoring. An earlier GESAMP strategy for pollution monitoring recognized four elements: 1) identification of pollution; 2) quantification of degree of pollution; 3) causation, linking contaminants with effects; and 4) evaluation of biological and ecological consequences. Different biological effects techniques can provide information relevant to each of these four elements.

Pollution can affect biological processes at different organizational levels, from the subcellular level, through individuals and populations, up to the large scale ecosystem level. Different biological effects techniques provide information relevant to assess effects at these different levels. Effects at the subcellular level can affect the mortality and reproductive capacity of individuals, thereby affecting their populations. The population level is of particular significance when it comes to assessing the biological and ecological consequences of pollution. If a population is significantly affected, there are likely to be indirect effects on other populations through trophic interactions at the ecosystem level.

Fish populations are particularly relevant for assessing combined effects of pollution. The large commercial stocks are economically important but also ecologically important due to their large size and prominent role in large marine ecosystems. Most fish species with large stocks produce very high numbers of small eggs which hatch into very high numbers of small fish larvae. These early life stages of fish, which are part of the free-drifting plankton, are delicate and sensitive to pollutants. Effects of contaminants on the reproductive capacity of fish or on the survival of eggs and larvae, can have effects on the population level.

Due to their reproductive mode with high numbers of eggs and larvae, fish stocks typically have large recruitment variability. This is related to the geographical life cycle closure, where spawning migration to spawning grounds and drift of larvae to nursery areas are located in relation to features of the ocean current systems. Climatic variability has been shown in many cases to be strongly linked to the recruitment variability of fishes. While this on one hand pose a problem in distinguishing effects of contaminants from natural variability, it offers on the other hand a sensitive biological system responsive with large amplitude to both climatic variability and

change, and to effects from contaminants and other stressors such as increased UV radiation. Fish stocks constitute an important part of the diet of higher trophic level organisms such as seabirds and sea mammals. Ecologically they serve as important links between the production at lower trophic levels and top predators in marine ecosystems.

A strategy for addressing combined effects in monitoring and assessment, should include the following elements:

1. Pollution monitoring, consisting of both chemical and biological effects measurements, should be integrated as a component in a larger, overall environmental monitoring and assessment program. Such a program must provide information on climatic, physical oceanographic, chemical, and biological conditions.
2. Controlled laboratory experiments are required as a basis for interpreting results from field monitoring programmes. Such experiments should provide information on dose-response and mechanisms of effects of contaminants. There is a need for experiments with exposure to multiple contaminants under different simulated environmental conditions.
3. Bioassays with controlled biological material should be used to obtain comparable information on contaminant uptake and body burdens and biological effects. Bioassays can bridge the gap between controlled laboratory experiments and studies of field populations.
4. Fish populations should be included as target organisms due to their ecological and economical importance. Biological effects techniques should include a range from indicators of general condition and health of individual fish to specific bioindicators. Fish populations offer a sensitive biological system with high relevance for assessment of effects of pollution and climate, as well as of fishing. The reproduction of fish allows studies of both long-term and acute effects on adult fish and their sensitive early life stages.

Draft Paper Submitted to QUASIMEME Bulletin

Biological Effects Quality Assurance In Monitoring Programmes (Bequalm)

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The use of biological effects techniques in marine contaminant monitoring programmes is something of a late starter, although two valuable (but non-contaminant-specific) approaches have been deployed for many years - benthic community analysis and fish disease assessment. The reasons for this are manifold, but suffice to say that monitoring organisations are partly techniques-driven, and chemical monitoring techniques happened to come along first. The balance is now being redressed, and the many advantages of monitoring with an integrated suite of chemical and biological methods are beginning to be realised. A previous article in QUASIMEME Bulletin (Stagg, 1998) described some of the more modern biological effects methods and their application by the Oslo and Paris Commission's Joint Assessment and Monitoring Programme - JAMP (JAMP, 1998a, 1998b). These new techniques allow one to detect the effects of complex mixtures of contaminants (the norm in the environment), many are diagnostic of causes, they provide information on the bioavailability of contaminants, and they therefore allow more accurate assessments to be made of potential ecological damage.

However, in their enthusiasm, biologists have tended to forget about the need for quality control of their favourite monitoring methods, and this has led to occasional problems. A case in point was a recent UK National Marine Monitoring Programme (NMMP) survey of estuarine water quality which employed the oyster embryo bioassay. If salinity is low, the method requires salinity correction by the addition of salt, and it was found that one laboratory was mistakenly using sodium chloride instead of sea-salt, resulting in anomalous data. The problem was caused by an insufficiently detailed protocol, but due to the lack of formal AQC, was only picked up at a late stage. In the UK, we have now set up a National Marine Ecotoxicological Analytical Quality Control (NMEAQC) group to tackle such issues before the event, but a much wider international initiative (BEQUALM) is now in progress.

The idea for BEQUALM originated in the ICES Working Group on Biological Effects of Contaminants which has been very active in developing and validating new biological monitoring techniques. It was recognised in 1996 that an international biological AQC system analogous to QUASIMEME was required to support the deployment of these methods, and monitoring organisations like JAMP and NMMP are now making implementation of AQC procedures a condition of such deployment. A funding bid to the European Commission Standards, Measurement and Testing (SMT) programme was therefore put together towards the end of 1997, and the 3-year BEQUALM project formally came into existence on 1 October 1998.

BEQUALM has three main objectives:

Objective 1: Development of appropriate reference materials or type collections

Objective 2: Development of an infrastructure for assessing the comparability of data from individual laboratories

Objective 3: Demonstration that biological effects analyses are under statistical control and are of known quality.

The intention is to construct an AQC infrastructure which will become self-funding after 3 years in a similar way to QUASIMEME. The project is being run by a Central Steering Group consisting of an expert from each Partner laboratory, plus a representative of QUASIMEME who will help us to avoid problems that have already been solved in chemical AQC programmes. Having said that, we anticipate that biological effects techniques will throw up their own unique set of problems - living organisms are not quite so cooperative as gas chromatographs.

Each Partner laboratory is expert in one or more of the biological techniques which come under the BEQUALM umbrella, and they will act as the leaders of each of the practical activities (workpackages in the programme. The Partners and techniques packages are listed in Table 1. The workpackages are modelled fairly closely on the requirements of JAMP, but are of course also largely applicable to other monitoring programmes run by organisations such as HELCOM and MEDPOL.

There is no space here to describe the work in detail, but in broad terms it will involve training workshops, inter- and intra-laboratory performance testing, and the construction of a compliance monitoring system. There are also likely to be joint ventures with other organisations such as QUASIMEME. All this will depend completely on the involvement of Participating Laboratories who will be expected to cover their own costs but who will in return be helped to maintain or upgrade their performance to acceptable standards. I will therefore end this article with an invitation for you to contact the relevant expert laboratories listed in Table 1 if you are interested in participating in the development of AQC for particular techniques and wish to obtain some more details of the BEQUALM programme. Your involvement will be very welcome.

Table 1. Partners and biological effects techniques in BEQUALM.

	Expert laboratory	Workpackages
Partner 1	Centre for Environment, Fisheries and Aquaculture Science (CEFAS) (Burnham-on-Crouch, UK) Contacts: John Thain and Peter Matthiessen	Workpackage 1 (Central coordination) and Workpackage 2 (Water and sediment bioassays)
Partner 2	Norwegian Institute for Water Research (NIVA) (Oslo, Norway) Contact: Ketil Hylland	Workpackage 3 (Metallothionein) Workpackage 4 (ALA-D activity)
Partner 3	Institute of Applied Environment Research (ITM) Contact: Lennart Balk (Stockholm)	Workpackage 5 (DNA adduct measurements)
Partner 4	FRS Marine Laboratory (Aberdeen, UK) Contact: Ian Davies	Workpackage 6 (P4501A induction) and Workpackage 7 (Imposex/intersex measurement)
Partner 5	NERC Plymouth Marine Laboratory (Plymouth, UK) Contact: David Lowe	Workpackage 8 (Lysosomal stability) In-vitro
Partner 6	Centre for Environment, Fisheries and Aquaculture Science (CEFAS) (Weymouth, UK) Contact: Steve Feist	Workpackage 9 (Liver histopathology, liver nodules and external fish disease measurement)
Partner 7	Institute of Coastal Research, Swedish National Board of Fisheries (SNBF), (Öregrund, Sweden) Contact: Olof Sandström	Workpackage 10 (Fish reproductive success)
Partner 8	Forschungs- und Technologie Zentrum Westküste, Christian-Albrechts-Universität zu Kiel (CAU) (Büsum, Germany) Contact: Franciscus Colijn	Workpackage 11 (Phytoplankton assemblage analysis and chlorophyll))
Partner 9	Institut für Meereskunde (IfM) (Kiel, Germany) Contact: Heye Rumohr	Workpackage 12 (Benthic community analysis)

Annex 9 : Introductory Presentation - Ongoing activities 2

ICES activities in relation to biological effects monitoring

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The International Council for the Exploration of the Sea (ICES) has been working on the development of methods to monitoring biological effects of contaminants in the marine environment for the past two decades. ICES is primarily concerned with developments in relation to biological effects methods that can be used for monitoring at the international level. ICES Working Groups (particularly the Working Group on Biological Effects of Contaminants) and Committees (particularly the Advisory Committee on the Marine Environment and the former Marine Environmental Quality Committee) have been concerned with: (1) method development and evaluation, (2) quality assurance, including the conduct of intercomparison exercises, and (3) publication of accepted methods, mainly via *the ICES Techniques in Marine Environmental Sciences* series.

In 1995, ICES adopted a strategy for biological effects monitoring in the marine environment based on closer integration of chemical and biological monitoring techniques (ICES, 1995). This strategy included a table listing techniques that ICES recommended for use in marine monitoring programmes at the national or international level, as well as a table containing methods that were promising but which required further research before they could be recommended for routine monitoring in the marine environment. These tables were reviewed and updated in 1997 (ICES, 1997) and are reproduced in [Annex 10](#), below, with annotations from this workshop.

The development of quality assurance procedures for biological effects techniques has also been an important aspect of the ICES work; this work has served as a basis for the new BEQUALM programme. Recently, ICES has begun work on the use of statistics in designing biological effects monitoring programmes. The 1998 report of the ICES Advisory Committee on the Marine Environment contains information on the design of effective sampling schemes for monitoring a single biological effects variable in terms of temporal trends at one site or determining differences between two sites. It also considers the design of sampling schemes for a suite of biological effects variables, including optimal sample sizes, minimizing variance components, and optimizing costs of the programme (ICES, in press).

Finally, ICES serves as the data centre for marine monitoring data for OSPAR, AMAP and HELCOM, traditionally handling data on contaminants in marine biota, sediments and sea water, but more recently covering also a growing number of biological effects techniques.

ICES. 1995. Report of the ICES Advisory Committee on the Marine Environment, 1995. ICES Cooperative Research Report, 212: 84–96.

ICES. 1997. Report of the ICES Advisory Committee on the Marine Environment, 1997. ICES Cooperative Research Report, 222: 12–20.

ICES. In press. Report of the ICES Advisory Committee on the Marine Environment, 1998. ICES Cooperative Research Report, in press.

Annex 10: Tables presenting the ICES review of the status of biological effects techniques relative to their potential application in monitoring programmes (modified after: ICES. 1997. Report of the ICES Advisory Committee on the Marine Environment, 1997. ICES Cooperative Research Report, No. 222: 12–20.)

Table a. Recommended techniques for biological monitoring programmes at the national or international level (revised version of Table 4.2.3.1 in ICES, 1997)

Method	Considered by working group number:	Organizational notes (I = ICES, O = OSPAR, B = BEQUALM)	Organism	Refs.	Issues addressed	Biological significance
Bulky DNA adduct formation	2	IOB	Fish ¹ Bivalve molluscs	1–6	PAHs Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)	Measures genotoxic effects.
AChE inhibition	2	IB	Fish ¹ , crustacea, bivalve molluscs	12–16	Organophosphates and carbamates or similar molecules Possibly algal toxins	Measures exposure.
Metallothionein induction	2	IB	Fish ¹	17–22	Measures induction of metallothionein protein by certain metals (e.g., Zn, Cu, Cd, Hg)	Measures exposure and disturbance of copper and zinc metabolism.
EROD or P4501A induction*	2	IOB	Fish ¹	46–51, 99	Measures induction of enzymes which detoxify planar organic contaminants (e.g., PAHs, planar PCBs, dioxins)	Possible predictor of pathology through mechanistic links. Sensitive indicator of exposure.
ALA-D inhibition	2	IOB	Fish ¹	74–75	Lead	Index of exposure.
Antioxidant enzymes	2	IB	Fish ¹	76–78	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures the presence of free radicals.
Fluorescent bile metabolites	2	IOB	Fish	79–80	PAHs	Measures exposure to and metabolism of PAHs.
Lysosomal stability	2	IOB	Fish ¹ <i>Mytilus</i> spp.	23–25	Not contaminant specific but responds to a wide variety of xenobiotic contaminants and metals	Measures cellular damage and is a good predictor of pathology. Provides a link between exposure and pathological endpoints. Possibly, a tool for immunosuppression studies in white blood cells.
Neoplastic and pre-neoplastic liver histopathology	2, 4	IOB	Fish ¹	7–11	PAHs Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)	Measures pathological changes associated with exposure to genotoxic and non-genotoxic carcinogens.

*Intercomparisons or quality control procedures complete for some methods (e.g., Refs. 31, 40, 99, 100).

¹ May also be applicable to mammals and birds.

Table b. Recommended techniques for biological monitoring programmes at the national or international level (continued).

Method	Considered by working group number:	Organizational notes (I = ICES, O = OSPAR, B = BEQUALM)	Organism	Refs.	Issues addressed	Biological significance
Whole sediment bioassays*	1, 3	IOB	<ul style="list-style-type: none"> • <i>Corophium</i> • <i>Echinocardium</i> • <i>Arenicola</i> • <i>Leptocheirus</i> • <i>Grandidierella</i> • <i>Rhepoxynius</i> • <i>Ampelisca</i> 	31–35	Not contaminant specific, will respond to a wide range of environmental contaminants in sediments	Acute/lethal and acute/sublethal toxicity only at present. May enable retrospective interpretation of community changes.
Sediment pore water bioassays*	1, 3	IOB (BEQUALM for a different species)	Any water column organism including: <ul style="list-style-type: none"> • <i>Dinophilus</i> • sea urchin fertilization, etc. • bivalve embryo • Microtox 	36–41	Will respond to a wide range of environmental contaminants	Acute and chronic toxicity, including genotoxicity, etc. Toxicity of hydrophobic contaminants might be underestimated in pore water assays.
Sediment sea water elutriates*	1, 3	IO	Any water column organism, as above	36–41	Will respond to a wide range of environmental contaminants in <ul style="list-style-type: none"> • dredge spoils • sediments liable to resuspension 	Acute/lethal and acute/sublethal toxicity, including genotoxicity, etc.
Water bioassays*	1, 3	IOB	As for pore water and elutriates (see above)	36–41	Not contaminant specific, will respond to a wide range of environmental contaminants in inshore and estuarine waters	Acute/lethal and acute/sublethal toxicity, including genotoxicity, etc.
Scope for growth	1	IB	Bivalve molluscs, e.g., <i>Mytilus</i>	55–58	Responds to a wide variety of contaminants	Integrative response which is a sensitive and sublethal measure of energy available for growth.
Shell thickening	-	IB	<i>Crassostrea gigas</i>	103	Specific to organotins	Disruption to pattern of shell growth.
Vitellogenin induction	1, 2	IB	Male and juvenile fish	26–30	Oestrogenic substances	Measures feminization of male fish and reproductive impairment.
Imposex	1, 4	IOB	Neogastropod molluscs, e.g., dogwhelk (<i>Nucella lapillus</i>)	52–54	Specific to organotins	Reproductive interference. Estuarine and coastal littoral waters (<i>Nucella</i>) and offshore waters (<i>Buccinum</i>).
Intersex	4	IOB	Littorinids	101, 102	Specific to reproductive effects of organotins	Reproductive interference in coastal (littoral) waters.

Method	Considered by working group number:	Organizational notes (I = ICES, O = OSPAR, B = BEQUALM)	Organism	Refs.	Issues addressed	Biological significance
Reproductive success in fish	1, 3	IOB	<i>Zoarces viviparus</i>	72	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures reproductive output and survival of eggs and fry in relation to contaminants in a viviparous fish species. Restricted to period when young are carried by female.
Externally visible fish disease	1, 4	IOB	Fish	104–108	Measures the effects of non-specific stress by quantifying the presence of externally visible diseases, especially in dab (<i>Limanda limanda</i>)	These diseases are natural, but may be exacerbated by various stressors, including contaminants.
Benthic community analysis*	1	IOB	Macro-, meio-, and epibenthos	42–45, 100, 109	Responds to a wide variety of contaminants, particularly those resulting in organic enrichment	Ecosystem level. Retrospective. Particularly useful for point sources. Most appropriate for deployment when other monitoring methods indicate a problem may exist.
Phytoplankton assemblages	3	B				
Zooplankton assemblages	3	B				

*Intercomparisons or quality control procedures complete for some methods (e.g., Refs. 31, 40, 99, 100).

¹ May also be applicable to mammals and birds.

Table c. Promising biological effects monitoring methods which require further research before they can be recommended (revised version of Table 4.3.2.2 in ICES, 1997).

Method	Considered by working group number:	Organizational notes (I = ICES, O = OSPAR, B = BEQUALM)	Organism	Refs.	Issue addressed	Biological significance
DNA strand breaks	2		Fish and mussels	1–6	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures genotoxic effects, but is also extremely sensitive to other environmental parameters.
Oncogenes	2		Fish	93–95	PAHs Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)	Activation of oncogenes (<i>ras</i>) or damage to tumour suppressor genes (p53). Measures genotoxic effects leading to carcinogenesis.
P4501A induction	2		Invertebrates	96	Induced enzyme response to PAHs, planar PCBs, dioxins and/or furans	Measures exposure to organic contaminants.
Glutathion-S-transferase(s) (GST)	2		Fish	97	Predominantly organic xenobiotics	Measures exposure and the capacity of the major group of Phase II enzymes.
Multidrug/xenobiotic resistance (MDR/MXR)	2		Fish, invertebrates	85–92	Organic xenobiotics	Measure of exposure.
Protein or enzyme altered foci	2		Fish	92	PAHs Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)	Indicates exposure to carcinogen(s).
Various methods of measuring immunocompetence	1		Fish and invertebrates	73	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures factors which influence susceptibility to disease.
On-line monitoring	1		Mussels and crabs	98	Responds to metals and xenobiotics	Measures the effects of chemicals on heart rate using a simple and inexpensive remote biosensor. Gives an integrated response.
Degenerative liver, gill and kidney histopathology	1,4		Fish (especially flatfish such as dab (<i>Limanda limanda</i>))	59–66	General toxicological response which will respond to a wide variety of contaminants	Measures degenerative change in tissues.
Abnormalities in wild fish embryos and larvae	3		Many fish, including demersal and pelagic species	70–71	Not yet linked unequivocally to contaminants	Measures frequency of probably lethal abnormalities in fish larvae. Mutagenic, teratogenic.

Method	Considered by working group number:	Organizational notes (I = ICES, O = OSPAR, B = BEQUALM)	Organism	Refs.	Issue addressed	Biological significance
Chronic whole sediment bioassays	3		Invertebrates	32	Responds to a wide range of contaminants	Measurements such as growth and reproduction, coupled to biomarker responses, which will give a measure of the bioavailability and chronic toxicity in whole sediments.
Pollution-induced community tolerance (PICT) water bioassay	3		Microalgae	67–69	Specific contaminants can be tested	Measure of degree of adaptation to specific pollutants. Not yet widely tested.
Allometric response in the benthic community	1		Macro-, meio-, and epibenthos	81–84	Not contaminant specific, will respond to a wide range of environmental contaminants	Ecosystem level. Retrospective.

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Annex 11: Report of Working Group 1 - Reproduction and Population Studies

Chair: Valery Forbes

Rapporteur: Malcolm Jones

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Issues and effects methods covered

Group 1 was assigned the following categories of effects measures to address:

- Populations/communities (benthos, birds, mammals)
- Endocrine/hormone disruptions
- Physiology/scope for growth
- Quality Assurance/Quality Control of above

Below are described in more detail the types of tests that were discussed in each of the categories, together with the most important advantages, disadvantages and/or concerns that were noted by participants. Due to time limitations, the group did not discuss in detail issues related to quality assurance or quality control of specific tests, though it was recognized that such issues are a key part of the development of relevant effects measures and should play an important role in future field tests.

1. Populations/Communities

- *Benthic population dynamics and community structure*
- *Oiled bird ratios*

Analysis of changes in population and community structure (i.e., population density, species composition/diversity) are most appropriate for relatively short-lived invertebrates or algal species. Such communities respond relatively rapidly to environmental stress, and recovery is also measurable over reasonable time periods. Present lack of understanding of the linkages between suborganismal responses (e.g., biochemical, molecular, physiological) and individual fitness of lower organisms limits the usefulness of suborganismal effects measures for such groups. In addition, there may be substantial decoupling between individual survival and reproduction and population dynamics for short-lived, highly fecund species. Factors controlling these linkages need further study before contaminant effects on suborganismal or even individual organism performance can be interpreted with confidence. In contrast, for longer-lived, less fecund species there are likely to be tighter linkages between individual survival/reproductive output and population persistence, and impacts on population dynamics may be less easily reversible. For such species it is desirable to

employ suborganismal and preferably non-destructive effects measures that signal changes in individual organism health.

The benthic community is of key importance e.g., for its role in organic matter cycling and as an important food source for commercial and non-commercial fish. Hydrophobic chemicals that tend to be accumulated by biota also tend to concentrate in sediments, where they may persist for long periods of time. Sediment-associated contaminants may damage the structure and function of benthic communities and may also be released to the overlying water and thus act as a source of contaminant exposure to pelagic biota. Methods for assessing changes in benthic community structure are well established and have a long history of application. However, ensuring accurate taxonomic identification requires specific training for the local situation. Rare species are difficult to detect with the traditional methods. In species-poor areas, such as the Baltic, it may be more effective to look at changes in population dynamics of selected species. Finally, it may be difficult to distinguish natural from anthropogenic causes of community disturbance. The application of multivariate statistical analyses to benthic community data may greatly improve the power of such measures to detect effects of combined stresses.

In many areas bird carcasses are regularly censused. Examination of the ratio of oiled bird carcasses to total number of carcasses censused can be used to assess the effects of oil contamination on bird populations. The use of a ratio approach has the advantage of correcting for differences in sampling intensity in different regions. However, the group noted that this measure is specifically designed to assess the impacts of oil contamination and is less useful as a general combined effects measure.

II. Endocrine/hormone disruptions

- *Blood levels of hormones (e.g., steroid, thyroid) and immunological parameters (e.g., white blood cell counts)*
- *Other reproductive effects (e.g., morphological abnormalities, histological measures)*

These methods appear to be most useful for long-lived, homeothermic vertebrates. A number of specific examples was discussed, and the most well-developed of these are listed in Annex 10. These measures are generally indicative of the health of the individual organism. They have the advantage of often being non-destructive, a feature that is especially useful for rare, endangered or long-lived species. However, questions about reliability need to be addressed and links to reproductive performance and/or disease need strengthening in some cases. We noted that different species will need to be used in different geographical regions and among-species differences in dose-response patterns will have to be evaluated so that interpretations in different regions will lead to consistent conclusions.

III. Physiology

- *Scope for growth*
- *Metabolic rate indicators*
- *Heart rate monitoring*
- *Gill ultrastructure*

Due to time limitations we did not discuss these methods in any detail. Scope for growth is the most widely and well-developed of the physiological effects measures. Most experience with this method derives from studies with suspension-feeding bivalves. However, many other species have been examined in a research context, and efforts are underway to modify and standardize the technique for application with benthic crustaceans.

Metabolic rate measures (e.g., oxygen consumption, metabolic heat production) may be sensitive indicators of physiological stress and may be measured as part of a scope-for-growth assessment. Limitations of these methods, particularly for poikilothermic organisms include: 1) respiration may be strongly influenced by environmental factors such as temperature, oxygen availability, salinity; 2) the shape of the relationship between exposure and response may be non-monotonic (e.g., rising at low levels of stress and then reducing rapidly as physiological limits are exceeded); 3) the shape of the relationship between exposure and response may be contaminant-specific; and 4) many invertebrates can switch between aerobic and anaerobic respiratory pathways, and the factors controlling this are not fully understood.

Heart-rate monitoring, performed by attaching a computer-controlled infrared censor to an organism in situ, is a relatively new technique which is still in the research stage. It has the advantage of often being able to be employed in the field, but presently its application is limited to intertidal or shallow subtidal situations. Interpreting changes in heart-rate patterns may not be entirely straightforward.

Gill ultrastructure can be used to detect physical damage to gill tissue and is especially appropriate for bivalve molluscs and fish that are exposed to contaminants in the water phase.

IV. Behavioral effects

- *Swimming*
- *Predation success*
- *Other directed responses (e.g., responses of benthos to hypoxia)*

Alterations in swimming behavior were addressed by M. Jones in his introductory presentation and were not discussed in further detail by the group. Predation success was proposed as an effects measure that could be employed in studies of caged fish. Other so-called directed responses were briefly discussed. The most widely observed is a behavior exhibited by benthic invertebrates in which they raise themselves above the sediment-water interface in response to low oxygen in bottom water. This is a very clear and specific indication of hypoxia.

Application of Combined Effects Measures

Most of the above effects measures respond to many stresses. This is advantageous for screening purposes, but makes assigning cause-effect relationships difficult. The group discussed the need for a stepwise or hierarchical approach that integrates chemical analyses with biological effects measures. The biological effects considered

in making an assessment should include measures at a variety of levels of organization from the suborganismal to the community.

In addition to discussing the types of relevant effects measures, we considered the types of situations in which they might be applied.

Scenario 1: Site/activity specific scenario. This would include such activities as oil platforms and fish farms in which the cause(s) of the potential effects is(are) clearly defined and the scale of the sampling effort is relatively obvious.

Scenario 2: Routine national or international monitoring programmes. In this situation the causes of potential effects are unknown and may include a complex combination of natural and anthropogenic factors. In addition, the spatial and temporal scales at which sampling should be conducted are not obvious.

Scenario 3: Combined effluent or estuarine gradient monitoring. This scenario is intermediate between the first two in that point-sources of effects may be clearly definable, but the chemical complexity of the contamination may impede identification of causal agents.

The first question to be addressed in the assessment is, 'Is there existing evidence of any biological effect or reason to suspect the potential for effects?' This question could be answered by applying a battery of general screening tests or by the application of more contaminant- or effect-specific tests if the causal factors can be easily defined. If the results of one or more of the screening tests provide evidence for effects, further testing should be performed with the specific purposes of 1) confirming the results of the screening tests, 2) determining the magnitude and scale of effects, 3) determining whether the effects are increasing or decreasing, and 4) identifying the cause(s) of the effects.

For general screening purposes we discussed tests representing four different types of analyses. Similar tests have been recommended by OSPAR and are included on the ICES list of reviewed effects indicators. They are:

Bioassay:

- Whole sediment
- Porewater
- Water
- Simple behavior (invertebrates)
- Early life stage tests (invertebrates, fish)

Biomarker:

- Reproductive success (fish, birds)
- Clinical variables/hormones (birds, mammals)
- Endocrine disruption (e.g., vitellogenin, imposex)

Population/community:

- Fish disease prevalence (gross pathology)
- Benthic community structure
- Seabird/mammal counting (e.g., oiled bird ratios)

Chemical monitoring:

Should primarily focus on sediment and tissue of key trophic groups

We discussed the desirable features of species to be selected for combined effects assessment and agreed that rather than selecting one or few species appropriate to the entire region, a more sensible approach would be to select species relevant to the local system. We considered relevance to be characterized by the following:

- Sensitivity to contaminant stress
- Abundance (i.e., should be easy to collect or observe)
- Reflecting local situation (i.e., non-migratory)
- Representing different key environmental compartments (e.g., benthos, pelagic, intertidal, etc.)

Field tests

Our group devoted little time to discussing future field studies. However, it was suggested that a component of future field trials should include carefully designed laboratory experiments using a multisystem approach in combination with measurements made directly in the field. In addition, the importance of training was emphasized and could provide a component of future field trials. We also identified several new methods that could be examined in an experimental field trial. These included:

- 1) Caged fish systems in which a group of fish are placed in situ at selected monitoring stations and followed by videocamera. A variety of destructive and nondestructive effects indicators can be measured in the caged fish. The technique has several advantages over wild fish monitoring, in particular that the genetic composition and past history of the monitoring organisms are known and controlled. This approach may also be more cost-effective than traditional monitoring of fish populations due to greater control and reduced ship-time costs. However, it may not be appropriate for all environments as a result of problems with food availability and excessive fouling of cages.
- 2) Vitellogenin assay. This is a new technique used to indicate endocrine disruption that has primarily be employed in fish.
- 3) Chemosensory responses. Such measures could include a variety of techniques that examine food-searching, settling, or other behaviors that are dependent on effective chemosensory detection.

In addition to the above three types of effects measures, the group identified several areas related to combined effects that could benefit from further research. They were:

- Information on endocrine systems in invertebrates and the links between endocrine disruption and effects at the population level.
- Evolutionary adaptations (reproductive, behavioral) to chemical contaminants.
- Statistical pattern analyses using multivariate methods.
- DNA markers for population identification.

Annex 12: Report of Working Group 2 - Cellular and Biochemical Methods, including Enzymatic Activities

Chair: Dr Ian M Davies (UK)

Rapporteur: Dr Cynthia de Wit (Sweden)

Group members: Aldo Viarengo, Aminadav Yawetz, Amparo Torreblanca Tamarit, Angela Köhler, Biserka Raspor, Bjørn Eirnar Grøsvik, Christophe Minier, Cinta Porte Visa, David Lowe, E. Cotou, Evangelos Papathanassiou, Gabriel P. Gabrielides, Gilles Bocquene, Janet Pawlak, Jean Vacelet, Joëlle Forget, José Benedicto Albaladejo, Kari Lehtonen, Martin Eggens, Rasa Jokubauskaite, S. Tambutté, Thierry Perez, Thomas Forbes, Toomas Saat, Ulrike Kammann.

Method evaluation

The first task undertaken by the group was a review of those biomarker methods which fell within its scope. The objective of the review was to identify those methods which are sufficiently well established as to form the basis of a monitoring programme to investigate the combined effects of contaminants within the area of concern of the meeting. This encompassed the European Seas and the AMAP area and therefore included a wide range of environments and degrees of contamination. Members of the group made a series of presentation concerning their knowledge and experience of relevant methods. Recent reports from ICES (ACME 1997) were also taken into account.

Particular attention was paid to the degree to which a standard analytical method existed, whether QA procedures had been established, the appropriate organisms to which the method could be applied, the contaminant groups which give rise to the observed effect, the mechanisms or functions measured by each method and points supporting and weakening the case for the inclusion of the method in a routine monitoring programme. Finally, brief recommendations were made regarding the current usefulness of each method in monitoring programmes.

The biological effects methods considered by the group were as follows:

- Acetylcholinesterase inhibition (AChE)
- Lysosomal stability
- Metallotionein induction (MT)
- Ethoxyresorufin de-ethylase (EROD)
- Bile metabolites of PAH
- Bulky DNA adducts with PAH
- DNA unwinding rate
- Micronuclei development
- Comet assay
- Antioxidant enzymes
- Vitellogenin
- Multiple drug resistance (MDR)
- ALA-D inhibition
- Ras* oncogenes

Glutathione S-transferase (GST) Immunocompetence

The outcome of the discussion is summarised in Table 1 below.

The group concluded that a suite of methods were suitable for inclusion in monitoring programmes. These were lysosomal stability, AchE, MT, EROD, PAH bile metabolites (although only available to a limited number of laboratories). In addition, the measurement adducts should become more widely available as a result of its inclusion in BEQUALM. The measurement of DNA damage was being approached through several methods. Micronuclei development was simple but very time-consuming. The alkali elution method for assessing the unwinding rate of DNA showed considerable promise but was not yet widely available. The group considered that one or more of these methods could soon be available for routine use.

It was emphasised that the power of any monitoring programme utilising these methods would be greatly strengthened by the inclusion of analytical chemical support and the measurement of appropriate background data, such as temperature, salinity, nutrient levels etc. It was also noted that the remit of the group had only included effects measurements at low levels of biological organisation (cellular and sub-cellular levels). Again, the power of a programme including these assays would be increased by the inclusion of measurements at higher levels of organisation, for example the whole organism level. In this way, links may be enabled between the observed effects on a molecular scale and the possible consequential impacts at population and community levels.

Specific research and development needs included bringing more of the methods listed in Table 1 into routine monitoring programmes. These included PAH metabolites in bile, DNA adducts with PAH, an assay of DNA damage (probably the alkali elution method). Other methods on which a greater effort is anticipated to be required are the development of assays of the MDR system (multi-xenobiotic resistance in fish and mussel), hormonal shift in fish and molluscs, vitellogenin in fish and sperm motility in molluscs.

Table 1. Evaluation of available methods.

Method name	Publication	Organisms	Contaminants	Positive experiences	Negative experiences	Recommendations
AchE inhibition Measure of neurotoxicity	ICES TIMES series (Nov 1998)	Crustaceans (including zooplankton), Fish (flatfish), worms, mussels, oysters etc.	Organophosphates, carbamates, (heavy metals). Temperature effects UV not expected to produce effects	Polymorphism well described Can be used to see synergistic effects of combinations Robust Broad applicability Stable enzyme Clear consequences for individuals Disadvantageous alterations of behavior QA being developed through BEQUALM	Low sensitivity and low activity in bivalves	Can be applied in routine monitoring programs provided practicalities of sampling can be accommodated
Lysosomal stability Generalized subcellular response to toxicants	Neutral red dye retention method. 6 publications	Fish, crustaceans, bivalves, polychaetes etc.	PAHs, heavy metals, POPs, UV-B Works within normal temperature and salinity ranges	Cheap Fast (4 hours) Requires minimum of equipment Already used in mussel watch in S. America, MedPol etc. Robust	Does not work during reproductive phases Animals have to be alive and in good condition Subjective - need skill and training	Can be applied in routine monitoring programs provided practicalities of sampling can be accommodated
Metallothionein Metal detoxification protein	Many analytical approaches available	Molluscs, crustaceans, fish	Metals (Cd, Cu, Zn, Hg, Ag) Temperature may affect UV probably has no effect in mussels	A new simplified, spectrophotometric method of analysis now available. Measure of exposure Beneficial to organism and probably has no significant impact on energy utilization	Marine reference materials not available Spawning can affect	Can be applied in routine monitoring programs

<p>EROD/P4501A induction</p> <p>Xenobiotic detoxification system</p>	<p>TIMES series</p> <p>3 analytical approaches available (Enzyme activity, protein level, mRNA)</p>	<p>Vertebrates (fish)</p> <p>Invertebrates - weak response</p>	<p>Planar organic compounds such as PAHs, dioxins, non-ortho-CBs etc.</p> <p>Temperature effects in plaice, dab; not in flounder. No salinity effects in flounder.</p>	<p>QA covered in BEQUALM and through MedPol</p> <p>P4501A protein content and mRNA techniques not inhibited by high concentrations of inducers.</p> <p>Widely used.</p>	<p>Unidentified variance factors limit the discriminatory power of the assay.</p> <p>Known to vary with season, sex, reproductive status</p> <p>EROD activity can be inhibited at high concentrations of inducers</p>	<p>Best response we have to planar organics but affected by many confounding variables</p> <p>Should be used in a suite to interpret results</p>
<p>PAH bile metabolites</p> <p>Exposure to and metabolism of PAHs</p>	<p>Standardized method and QA should be available soon</p>	<p>Fish</p>	<p>PAHs with 2,3,4 and 5 rings</p> <p>Not very temperature sensitive</p>	<p>Cheap</p> <p>Sensitive</p> <p>Not affected by P450 induction</p> <p>Low variance between individuals</p>	<p>Analytical standards are a problem</p> <p>No direct links to higher level effects</p> <p>Fish need to have fasted a few days before sampling</p>	<p>Has potential but currently not done by many laboratories</p>
<p>DNA adducts</p> <p>Covalent bonding of PAH with DNA</p>	<p>Antibody method</p> <p>P32 postlabelling method</p>	<p>Fish</p>	<p>PAHs</p>	<p>Effect of exposure</p> <p>QA covered in BEQUALM</p>	<p>Complicated</p> <p>Expensive</p>	<p>Research tool</p>
<p>DNA damage</p> <p>Rate of unwinding of DNA</p>	<p>Methods available</p> <p>(Alkali elution)</p>	<p>Molluscs, fish, mammals</p>	<p>Mutagenic compounds</p> <p>Cd, Cr, Pb, PAH, hydrocarbons</p> <p>No information on temperature effects</p> <p>UV will affect</p>			

Micronuclei development Measures genotoxicity	Methods available	Mussels (gills, hematocytes), fish (white blood cells, hematocytes), mammals	Genotoxic substances	Methods standardized	Very slow to carry out No QA available	Unsuitable for routine use?
Comet assay Measures genotoxicity	Method published	Fish and mollusc blood	Genotoxic substances		No QA Very few field studies	Research phase
Antioxidant enzymes Measuring consumption of free radicals		?	PAH, hydrocarbons, heavy metals	Laboratory results are encouraging	No information on kinetics Responses small Non-specific and not always consistent results	Research phase
Vitellogenin Measures response to environmental estrogens	Several published methods OECD developing a standard method	Male and juvenile fish	Estrogenic compounds Integrates effects of a range of estrogenic compounds	HPLC methods very sensitive Responses can be large Several field studies in progress	Methods not standardized Few field studies published for the marine environment	Await results of current research
Multiple drug resistance Resistance to a wide range of therapeutants and xenobiotics	Methods published	Many species	Many therapeutants and xenobiotics			Research phase

ALA-D inhibition Exposure to lead			Pb		Unlikely that Pb levels are high enough in most areas to cause significant inhibition	Limited applicability
<i>ras</i> oncogenes Measures carcinogenicity			Carcinogens		Difficult to apply in the field Low incidence of cancer in natural populations of marine organisms	Limited applicability
Glutathione S-transferase (MFO phase II) Measures activity of phase II enzymes		Fish	Xenobiotics		No data on kinetics No response in some species e.g. flounder Confounding variables e.g. algal concentrations	Limited applicability
Immunocompetence Measures effects on immune system function	Several approaches available	Fish, seabirds, mammals	Immunosuppressors such as organochlorines, some heavy metals, UVB	Non-invasive	High variability Requires measuring multiple variables to interpret data	Important variable but research phase

Monitoring strategy considerations

The group then considered how this suite of methods might best be combined within a monitoring strategy. One of the techniques (lysosomal stability by the assessment of neutral red leakage) is known to respond to a wide range of contaminants, and to be relatively rapid and inexpensive and to be suitable as a screening procedure. The other techniques were mostly more demanding on facilities and resources but also to be more specific with regard to the contaminants to which they responded. A strategy was therefore developed in which the measurement of lysosomal stability in the common mussel and a fish were used as a first tier screening tools. The more specific methods would then be employed as a second tier in response to positive results from the lysosomal stability test. The structure is given in more detail in Table 2 below.

Table 2. Biomarker strategy

Measurement	Mechanism of toxicity, or process assessed	Matrix	
		Mussel	Fish
Lysosomal membrane stability	Leakage of lysosomal membranes	Yes	Yes
AchE	Neurotoxicity	Yes	Yes
Metallothionein	Detoxification of metals	Yes	Yes
EROD	MFO Phase 1 enzymes		Yes
Bile PAH metabolites	Metabolism of PAH		Yes
DNA damage	Genotoxicity	Yes	Yes
Lipofuscin and neutral lipid accumulation		Yes	
Vitellogenin	Female reproductive hormone levels in males		Yes

The group noted that the recommended methods covered a range of mechanisms of toxicity and of adaptive responses, but that there were no routine assays available for the potentially important mechanisms of immunotoxicity or for the disruption of hormone systems other than the effects of environmental estrogens on sex hormone system.

The group discussed the possibility that using lysosomal stability as a screening procedure ran significant risk of failing to detect toxicants at significant levels. In general, it was agreed that positive results from this test were closely correlated with adverse effects in the test organism, and that therefore false positives were unlikely to be a common occurrence. The test seems to reflect a generalised response to toxin stress and is known to respond to a wide range of organic toxicants and also to various heavy metals. It was felt that there must be some possibility of false negatives, for example TBT at environmentally significant levels did not cause a response in an earlier lysosomal stability test. It was not known whether the current (neutral red

retention) test shared this feature. Further experience in its use should clarify the frequency and significance of false negatives.

Scenario assessment

The group then considered the application of the proposed strategy in three environmental scenarios. These were situations of contamination by organic matter (oil, OM), OM and heavy metals (HM) and OM, HM and persistent organic pollutants (POP). In each case, additional questions were asked regarding the possible influence of temperature change (in a global climate change sense) and increased irradiation by UV-B light on the biomarker results. The group was asked to assess the ability of the methods to assess the combined effects of the pollutant groups (OM, HM, POP) under conditions of the above markers of climate change.

The assessment made by the group is summarised in Table 3 below.

Table 3. Scenario 1 assessment

Scenario 1	OM	OM
Test method	Mussel	Fish
Lysosomal membrane stability	Yes	Yes
AchE		
Metallothionein		
EROD		Yes
Bile PAH metabolites		Yes
DNA adducts		Yes
DNA damage	Yes	Yes
Lipofuscin and neutral lipid accumulation		
Vitellogenin		
Notes:	May be possible to detect PAH metabolites or parent compound in mussel digestive gland	

The group considered that temperature changes would affect the biomarker responses, but it was not clear what the effects would be or how large (significant) they might be. Temperature differences tend to be viewed as confounding variables which arise during the sampling of organisms for biomarker analysis, rather than as a target for the interpretation of the results. There are many reports of correlation between biomarker responses and temperature, but commonly these temperature changes are associated with seasonal changes and therefore also include seasonality in physiological condition, breeding cycles, behavioural changes etc. Seasonal temperature changes are commonly around 10 degree C. The changes that are likely to arise from global climate change over the coming decades are likely to be of the

order of 0.5 - 1 degree C. The climatic signal on temperature is therefore much slower and smaller than that arising from seasonal factors.

It was agreed that increased UV-B irradiation would tend to activate the toxicants concerned (e.g. PAH) and that therefore there might well be an impact on the biomarker responses. However, although a mechanism had been identified it was not possible to make any assessment of its likely importance. Similarly, it is possible that UV-B might affect the residues found in the organisms, but how and to what extent could not be determined.

Table 4. Scenario 2 assessment

Scenario 2	OM + HM	OM + HM
Test method	Mussel	Fish
Lysosomal membrane stability	Yes	Yes
AchE	Yes Some metals are known to amplify the response to organic inhibitors	Yes Some metals are known to amplify the response to organic inhibitors
Metallothionein	Yes	Yes
EROD		Yes High concentrations of metals might inhibit this response
Bile PAH metabolites		Yes
DNA adducts		Yes
DNA damage	Yes	Yes
Lipofuscin and neutral lipid accumulation		
Vitellogenin		
Notes:	May be possible to detect PAH metabolites or parent compound in mussel digestive gland	

The group again concluded that temperature would have some effect on the biomarker response. Data were available that indicated that temperature changes of 10 Centigrade degrees from 13 - 23 degree C led to an increase in metallothionein of 100%. However, exposure to metals could increase MT by 10,000%. It was therefore unlikely that a change of less than one Centigrade degree would be readily detectable in the presence of the much more potent metallic inducers.

Exposure to UV-B was known to affect the redox balance in organisms. This could lead to the induction of both heat shock proteins and MT. It is therefore possible that UV-B could lead to MT induction in fish.

Table 5. Scenario 3 assessment

Scenario 3	OM + HM + POP	OM + HM + POP
Test method	Mussel	Fish
Lysosomal membrane stability	Yes	Yes
AchE	Yes Some metals are known to amplify the response to organic inhibitors	Yes Some metals are known to amplify the response to organic inhibitors
Metallothionein	Yes	Yes
EROD		Yes High concentrations of metals might inhibit this response
Bile PAH metabolites		Yes
DNA adducts		Yes
DNA damage	Yes	Yes
Lipofuscin and neutral lipid accumulation		
Vitellogenin		Might not, depending on the nature of the POP
Notes:	May be possible to detect PAH metabolites or parent compound in mussel digestive gland	

Note: The tables above include the determinations of 'Lipofuscin and neutral lipid accumulation'. These rather standard procedures that can be used as a molluscan equivalent of EROD in fish, i.e. markers of xenobiotic compounds, were not discussed by the working group in plenary, but were inserted into the tables by a subgroup which met to discuss the possible field.

It was suggested that bile might also be suitable as an analytical matrix for metals, as an indication of what is being metabolised by the animal.

The table above indicates that it is not possible to readily identify POPs in the presence of PAH and that some supporting information is necessary. Chemical analysis of tissues and other environmental matrices should assist and it might be possible to apply TIE-type (Toxicity Identification Evaluation) approaches to identify the toxicants responsible for the observed effects. Other possible approaches include the assessment of thyroid activity and vitamin A levels which are affected by organochlorine compounds rather than PAH. However, these variables may also be affected by metals and processes leading to the production of oxyradicals.

Emergent properties

The group noted that the above discussion had in part addressed the question of the effects that arise as a consequence of the presence of combinations of contaminants

(synergism, antagonism, additive effects etc). The group noted that in some cases the presence of additive effects was well recognised. These included the use of Toxic Equivalent Factors (TEFs) and the calculation of TEQ values from chemical analyses of PCBs, dioxins etc as a method of assessing the net toxicity of the presence of a number of compounds which give rise to similar biological effects (in the case the stimulation of the P450 system). However, the group were sceptical as to the practicality and reliability of attempting to partition any effects observed on EROD activity in field samples between different groups of contaminants. It was felt that the variability of EROD within populations was a major limitation to the potential power of this approach. In addition, it is possible to over stimulate the EROD system leading to a net decrease in activity.

Limited studies of the effects of combinations of diverse contaminants (eg copper and DMBA) on lysosomal stability suggested that effects were additive.

Giles Bocquenet reported some instances of marked synergistic effects of combinations of contaminants. Mixtures of cadmium and carbofuran showed toxicities 5 - 10 times greater than would be predicted from calculations based on additive toxicities. Mixtures of dichlorvos and malathion were around 100 times more toxic than predicted. It was indicated that similar effects were observed in relation to AchE activity.

It has already been noted that some metals can inhibit EROD activity. It was recognised that different groups of POPs could also elicit similar responses, but that it was not possible to reliably predict the nature or magnitude of the interactions (synergistic or antagonistic) between contaminants.

Biomarkers of temperature and UV-B exposure

The group noted that the preceding discussion had been structured around the detection of the effects of groups of contaminants and that the effects of temperature and UV-B (as indicators of global warming/ climate change) were considered secondarily. The group therefore addressed directly the question of the applicability of biomarkers to the assessment of the effects of temperature change and UV-B exposure.

The group considered that there were no biomarkers available that could be used unambiguously to reflect the effects of the rates of temperature rise that have been predicted to arise from climate change and that other areas of environmental science were much more likely to provide appropriate indicators.

David Lowe had recently undertaken a review of the effects of UV-B for the UK Government. He noted a very large literature on the effects of UV-B on plankton and macro-algae and that it was possible that community-scale markers could be developed.

There was a much smaller literature on the effects of UV-B on fish which concentrated on "sun-burn" and possible eye damage. Effects included skin lesions and skin thinning which might be the basis of usable biomarkers. Similar effects had been reported in marine mammals and included eye damage and disease such as

glaucoma. It might be the case that exposure to UV-B led to reduced immuno-competence and possibilities might exist to develop simple markers of immuno-competence, particularly in marine mammals.

UV-B levels are rapidly attenuated with depth in sea water and so animals which are found in sub-surface waters are not expected to be particularly vulnerable to changes in UV-B radiation. Marine organisms which emerge onto the surface are predicted to be at much greater risk. Such organisms include marine mammals which may haul out onto beaches or ice, and intertidal organisms which are exposed to the atmosphere during low tide periods.

A number of other observations were reported which might lead to usable biomarkers. These included:

- a) The degree of pigmentation of 0-group fish appeared to affect their susceptibility to exposure to UV-B
- b) UV-B exposure could lead to dimerisation of DNA bases.
- c) It has been suggested that the incidence of blindness in farmed salmon might reflect UV-B exposure.
- d) It was possible that the level of abnormalities in fish larvae might respond to UV-B level.

Field study

The group discussed the desirability and opportunities for a field programme based around the structures and strategy outlines in the Tables above. They concluded that objectives for such a programme could include:

1. To test the response of a battery of biomarkers in different situations with respect to pollution and temperature.
2. To test the proposed standard approach in different areas of Europe.
3. Does the approach work, in the sense of can the approach detect and distinguish between different contaminant groups and their effects?
4. Can the approach adequately describe the combined effects of different groups of contaminants?

The Workgroup suggested that the following criteria could be used to select study areas:

- a) The areas should have clear pollution gradients.
- b) There should be adequate logistical support, including access to laboratory space, sampling boats, etc

c) More than one site should be selected to cover different geographical regions and different environmental settings.

Possible study areas included:

1. The area around Haugesund, Norway. This area is fully marine and within it is a disused copper mining area which has resulted in heavy contamination of marine sediments and overlying sea water, and also an area of more mixed pollution, within which the effects of PAH (from an aluminium smelter) and TBT (from shipping) have been identified.
2. The mouth of the Po river, Italy. This area is estuarine and contains a wide range of contaminants. It is the largest source of contaminants to the Adriatic Sea.
3. A selected area in the Baltic Sea. The Baltic Sea is a brackish water basin in which some areas are heavily contaminated.
4. A selected area within the Arctic. The purpose being to study the applicability of methods in cold climate areas. Although the marine environment in the Arctic is relatively 'clean' compared with most other regions identified, there are areas of elevated contamination which may be suitable, e.g. the area around Svalbard, where contaminants subjected to long-range transport (by ocean, ice and atmospheric pathways) appear to accumulate together with contaminants from local sources (e.g. mining).

Proposed outline project structure

The Project should have an overall coordinator, and a Regional coordinator for each of the two (or three) study areas. Within each Region, a laboratory should have the responsibility for each bioassay/biomarker. These groups of laboratories must cover all the core assays listed in the tables above. The main sampling should be carried out during a coordinated Workshop at each location, ie the participating laboratories should come together and meet during Workshops.

The biological measurements should be supported by appropriate chemical analysis. This would include PAH, PCB, trace metals, and organotin compounds (TBT). Quality assurance (laboratory performance studies) for chemical analyses is available through QUASIMEME. Quality assurance for the biological measurements will soon be available as a result of work currently underway through the BEQUALM project and that already established in the MEDPOL area.

Research needs:

Research activity was required to develop convenient and robust procedures to determine:

1. Multiple xenobiotic resistance
2. Immunocompetence
3. Changes in hormonal level/balance

Annex 13: Report of Working Group 3 - Effects on plankton, pelagic eggs, pelagic fish larvae. Effects in whole organism bioassays

Chair: Hein Rune Skjoldal
Rapporteur: Ketil Hylland

Group Members: Odd Ketil Andersen (first day), Åke Granmo, Jaweed Hameedi, Lars-Otto Reiersen, Bjørn Serigstad (for presentation), Rolf Schneider, Alla Tsyban, Michael Waldock.

Presentations

The group initially took note of the methods described in the JAMP guidelines (OSPAR, 1997). In that document, a distinction was made between “diagnostic” and “general” methods. All the methods relevant to this section are general methods.

Members of the group presented the following work: relevant activities at Rogaland Research (Andersen), tests with *Mytilus* larvae and the Skagerrak monitoring programme (Granmo), assessment of biological effects in the coastal ocean (Hameedi), conditions for a conflict between oil and marine organisms (Serigstad), body burdens of lipophilic xenobiotics and reproductive success in baltic cod (Schneider), combined effects in the marine environment (Tsyban) and aspects of the national monitoring programme in the UK (Waldock).

Assessment of methods

The group agreed to survey methods to detect effects on autotrophic organisms, heterotrophic organisms (bacteria and protozoa), zooplankton, fish eggs and larvae. In addition, bioassays employing the same groups of organisms, were also included where relevant.

Autotrophic organisms are mainly photosynthetic algae, but may also include bluegreen bacteria (algae). A distinction was made between the two main groups of heterotrophs – bacteria and protozoans. Zooplankton was taken to include both meroplankton and holoplankton as well as eggs of those.

The methods were evaluated in relation to ease of use, extent of previous use, apparent promise as biological effects method (response to contaminants), cf. Table 1.

The group agreed that it would be vital for the success of contaminant-related monitoring to include both biological and chemical methods.

Various studies have indicated possible links between contaminants and effects on fish larvae (Dethlefsen, von Westernhagen, et al. 1996; von Westernhagen, Dethlefsen, et al. 1988; von Westernhagen, Cameron, et al. 1989). There are also reports of possible changes in sex ratios in fish from the North Sea (Lang, Damm, et al. 1995). The frequency of malformations of fish larvae do appear to be affected by natural environmental factors such as temperature. The group noted that fish eggs are at least partly permeable to contaminants in water and that exposure of embryos to contaminants may therefore be through any of three routes: (i) through maternal

exposure (and accumulation in yolk), (ii) through exposure of the embryo while still in the egg, (iii) through post-hatch exposure. The group also noted that studies on endocrine disruption have shown that the period of exposure is vital and that little is known of such “critical periods” for malformation or other developmental endpoints.

There is only limited evidence for effects on algae or heterotrophs from xenobiotics. Results from studies in the Bering Sea were presented by A. Tsyban. The results reflected studies on specific bacteria and their ability to metabolise xenobiotics. The specialist skills required for these methods may make them unsuitable for general application in monitoring at the present time. The concept PICT (pollution-induced community tolerance) was introduced in the beginning of the 1980s. It entails incubating phytoplankton communities from different areas with dilution series of selected contaminants. In theory, communities that are more or less adapted to specific contaminants should then be able to keep up growth in the presence of that contaminant. It has shown some promise in relation to TBT and specific herbicides, but has not been widely applied.

There has been only limited development of QA/QC procedures for the suggested techniques. Work is in progress for some of the phytoplankton techniques in relation to eutrophication monitoring.

Table 1. Overview of trophic levels and relevant methods.

trophic level	parameter	evaluation	comment
autotrophs	community structure	-	spatial changes large natural variability
	indicator species	+	
	primary production	-	
	growth rate*	+	
	PICT*	+?	
heterotrophs (bacteria)	genetic composition	?	the merit of these methods is largely unknown
	growth rate*	?	
heterotrophs (protozoa)	growth rate*	?	not well established
zooplankton	community structure	-	large variability indicator for eutrophication promising method <i>in situ</i> (diel migration) and <i>in vitro</i> promising, but not widely studied
	indicator species	+	
	biomass	-	
	egg production/fecundity*	+	
	behaviour(*)	?	
	malformation	?	
hatching success*	?		
fish eggs	hatching success*	+	
	respiration*	-	
	malformation	+	
fish larvae	malformation	+	relate to hatching success affected by food-availability and temperature
	survival/viability	+	
	growth (otoliths)	+	
	behaviour*	+	

*requires incubation

In addition to *in situ* methods, the group also considered various bioassay techniques in which planktonic species are used. The endpoint in the bioassays are indicated in Table 2. Bioassays can be performed with standard species (e.g. *Skeletonema*, *Acartia*) or species collected in the field. In addition to these, natural phytoplankton or

zooplankton may also be used in such tests. The endpoint for most zooplankton test is mortality. Endpoint for most phytoplankton tests is cell division (growth).

Table 2. Overview of established bioassay methods relevant to pelagic organisms.

trophic level	parameter	comment
autotrophs	growth	various algae
zooplankton	mortality mortality mortality mortality fertilisation	oyster embryo mussel larvae sea urchin larvae <i>Acartia tonsa</i> sea urchin
fish eggs, larvae	development growth	

The group considered other methods that are available to assess effects on pelagic communities or to facilitate testing for such effects. Those included:

1. Techniques to concentrate water samples to increase sensitivity, e.g. extraction procedures or the use semi-permeable membrane devices (SPMD)
2. The use of genetically modified cells or bacteria in the screening of water or tissue samples for effects of specific contaminants (Ah-receptor based, E-receptor based).

There is an obvious lack of methods to identify effects of specific contaminants and the cellular/physiological basis for effects (e.g. biomarkers). At the present, such methods would be most valuable for zooplankton and fish eggs/larvae, although similar methods may well be applicable to protozoans.

Scenarios

The group decided to treat POPs in three sections: organohalogen (OHs), polycyclic aromatic hydrocarbons (PAHs) and others (organotin compounds, organophosphates, non-chlorinated herbicides). Organic material was divided into oil and eutrophication. Interactions or combined effects were discussed in relation to each of the factors eutrophication, oil, UV and climate change.

As was mentioned above, all methods considered here are general methods, i.e. they will integrate over the effects of various contaminants and other stressors in their response. Nevertheless, the group considered it useful to indicate the most appropriate method(s) for each group of contaminants and conditions.

Organohalogen

The group identified fish eggs and larvae as the most sensitive and relevant trophic level. Parental transfer was thought to be the main source of exposure, although it was recognised that eggs may be exposed through water. All endpoints in Table 1 for fish eggs and fish larvae were thought to be relevant. Organohalogen contaminants may exert their effect directly or through accumulation. The latter mechanism appears to be important in many cases and the group considered it relevant to focus on long-lived organisms or transfer to progeny. Development was identified as a sensitive process.

Bioassay: oyster or mussel development.

Polycyclic aromatic hydrocarbons (PAHs)

Effects of PAHs would be expected to affect all trophic levels. The group did not conclude on which groups would be more sensitive. All endpoints in Table 1 were thought to merit investigation. There are indications that some ciliates could be sensitive. Oil and produced water are important sources for the more water-soluble PAHs (petrogenic). Other important sources of PAH are combustion or smelter-derived (pyrogenic). The source of PAHs is important for composition, bioavailability and effects. The group noted that PAHs comprise a range of components, all of which have different chemical properties and biological effects.

Bioassay: any water assay.

Other organic contaminants

Other organic contaminants include organotins, mainly tributyltin (TBT), organophosphates (OPs) and various classes of non-halogenated herbicides, e.g. triazines. The group found that there exists no good method to assess short-term effects of TBT. It was noted that molluscs appear to be sensitive, whereas other benthic organisms (polychaetes, crustaceans) are less sensitive. Both adult and larval stages of zooplankton were thought to be sensitive to organophosphate exposure. Phytoplankton species are (naturally) sensitive to herbicides, including triazines.

Bioassay: zooplankton mortality (OPs), primary production (herbicides).

Metals

The most relevant metals include mercury (Hg), cadmium (Cd) and copper (Cu). The group was of the opinion that lead (Pb) does not comprise a major problem in most marine areas. There is evidence that methyl-mercury (MeHg) may have profound effects on behaviour and development in fish (Weis & Khan 1990) and that such effects may first become apparent in the adult (Fjeld, Haugen, et al. 1998). The group considered that Cd would predominantly be a problem in long-lived organisms or in organisms high in the food chain (birds, mammals). It is established that some planktonic algae are sensitive to Cu, and it has been thought that the concentrations of this element in surface waters may inhibit algal growth in specific areas in some periods of the year.

Bioassay: any water assay.

Eutrophication

The transport and exposure of all classes of contaminants will be strongly affected by changes in DOM and POM in the water column. There are well-established methods to assess eutrophication status. Changes in eutrophication status may affect the impact of contaminants in ecosystems through at least three mechanisms:

1. Through causing changes in species composition and thereby trophic nets.
2. Through affecting food availability and energetics of organisms at all levels.
3. Through changing bioavailability of contaminants.

In general, contaminants are supposed to be less bioavailable at higher concentrations of organic material. There is some evidence that the quality of the organic material is

of importance, at least for sediment-dwelling organisms. Low oxygen levels in bottom waters will decrease the bioavailability of metals through the association of metal with sulphide.

Oil

Oil is a heterogenous mixture of alkanes and other hydrocarbons. PAHs comprise the fraction of oil that will have the highest biological activity. In addition to toxic effects, oil may be a source for organic carbon and may also modulate the effects of other contaminants through narcotisation or through affecting the physical-chemical properties of substrate or contaminants. There is limited knowledge concerning how oil may interact with contaminants, but one study has indicated that oil in sediment may reduce the accumulation of contaminants (Cd, TBT) in sediment-dwelling invertebrates (Hylland & Følsvik, 1998).

Climate change

Changes in climate will lead to large changes in ecosystems and it is difficult to couple such changes to other environmental factors such as contaminants. Increased water temperature will increase growth rates of all species, but some more than others, thereby possibly changing species composition. Small changes in yearly temperature may also move the distribution limit of species.

In addition, temperature changes will affect degradation rates of some contaminants and their metabolism in poikilotherm species.

Large scale changes will affect weather, precipitation, storm frequency, etc, thereby affecting transport routes of contaminants.

Ultraviolet radiation (UV)

There are indications that UV in itself may affect health and survival in juvenile and adult fish. In addition, UV appears to potentiate toxic effects of PAHs in both algae and protozoans.

In addition to biological effects, increased UV radiation would be expected to increase the rates of breakdown of organic contaminants, PAHs in particular.

Table 3. The ability of the selected methods to include effects from eutrophication (E), oil, UV or climate change (T).

trophic level	parameter	interactions
autotrophs	community structure	E, T
	indicator species	E, T
	primary production	E, UV, T
	growth rate	-
heterotrophs (bacteria)	genetic composition	E, UV, T
	growth rate	-
heterotrophs (protozoa)	growth rate	E, UV, oil, T
zooplankton	community structure	E, UV?, oil T
	indicator species	E, UV?, oil, T
	biomass	E, T
	egg production/fecundity	?
	behaviour	E, UV, oil, T
	egg/early life stage	
fish eggs	hatching success	T
	respiration	E, oil, T
	embryo malformation	T
fish larvae	malformation	E?, T
	survival/viability	UV, oil, T
	growth (otoliths)	E, UV, oil, T
	behaviour	oil?

Other factors

The group recognised that other factors may have larger impacts on ecosystems than the ones discussed above. Such factors include fishing activities, introduction of alien species and habitat destruction. The additional effect of contaminants, UV and/or climate may aggravate damage by these ecosystem stressors.

Gaps in knowledge and research needs

The following gaps in knowledge were identified:

1. What is the ability of eggs, embryos or larvae to metabolise xenobiotics?
2. How is it possible to link effects at the individual level and population effects?
3. Are there effects of contaminants on eggs and larvae of invertebrates?
4. The lack of biomarker techniques for small organisms or small amounts of tissue. Such methods would include biochemical/cellular techniques as well as histological techniques.
5. Combined effects in pelagic systems: eutrophication – contaminants, oil – contaminants, UV – contaminants.

The most urgent research need is to develop methods to assess the individual health of zooplankton and fish larvae, mainly to establish causality between effects and environmental factors.

Annex 14 : Report of Working Group 4 - Pathology and Diseases (16. November)

Chair: Rune Dietz (AMAP, levels and trends of contaminants in biota)

Rapporteur: Norman Green (JAMP, levels and trends of contaminants in biota),

Group Members: Kari Lehtonen (animal physiology, benthic fauna, histology in bivalves), Angela Köhler (histopathology), Thomas Lang (fish diseases, ICES), Jörundur Svavarsson (effects of TBT on gastropods and fish larvae), Cato ten Hallers-Tjabbes (IMO, EPC; TBT and gastropods physiological and sensory behavioural effects), Hanne Petersen (AMAP)

Tasks and approach

The group was to focus on: TBT, fish diseases, visible diseases, parasites and associated QA/QC. There was some confusion as to the scope of the work expected considering the constellation of its members; only 7 of 15 invited were present. There were only a few whose field was specifically involved with points in question and these fields had little overlap. Furthermore their field of studies involved low/mid trophic levels (gastropods, fish). The group was aware of but felt not entirely competent to address other important aspects of the combined effect of contaminants. The group disbanded itself after one meeting considering that it would be more effective.

The group agreed that its purpose was to compile a rough synthesis of methods relating to the combined effects of contaminants based on the experiences and knowledge of those present. This consisted of TBT, histology in bivalves, fish diseases and potential use of eel pout as a combined effect indicator. The literature cited, selected notes and copies of overheads provided by the participants is included. An attempt was made to summarize the methods and related information presented at the group's meeting in Table 1 templated by the workshop organizers.

There was general consensus that investigations should include both monitoring/research using methods of early warning and "end-point" effects. Early warning indicators could be behavioural responses, imposex/intersex or lysosomal changes. Examples of "end points" might: be disease (such as carcinogenesis, necrosis, as an end point of cellular interference or cytochrome P450 changes) and some population changes.

TBT

It was noted that there are OSPAR guidelines for monitoring the effects of TBT on *Nucella*, *Littorina* and *Buccinum*. QA has been assessed by QUASIMEME and will be considered by BEQUALM. Local and large scale surveys have carried out, some since 1986. Temporal trends have also been evaluated (e.g., in Ireland, UK and the North Sea). It was noted in plenary that several at least two studies described some functional influences *Nucella* affected by TBT might have on its habitat (exact references could not be immediately identified by concerned Steve Hawkings paper in the Porcupine newsletter (1996-97) and Hubert Rees's paper to ICES within the last two years). TBT is found in organisms from both shallow waters and the deep sea. Biomagnification has been demonstrated in some species. Evidence of effects are limited to a few marine organisms, for example some gastropods (noted by imposex/intersex) and mortality of fish eggs. Impact of TBT (imposex, disappearance of *Buccinum undatum*, levels of organotin) in offshore waters is correlated with shipping density. Toxic effects occur at low concentrations (1 ng/l). However, studies of neuro-endocrine effects

have indicated that there might also be physiological effects, chemoreceptory responses and enzymatic changes/inhibition. One might want to test TBT against estrogenic disturbing compounds (like PCBs, DDEs) in areas where these substances show elevated concentrations. Marine systems tend to enhance uptake of TBT because pH (higher in marine systems than freshwater) cause greater membrane permeability. Investigations indicate that *Gammarus* in low salinity areas indicate that this organism is more sensitive to TBT at low salinities may be more sensitive.

Histology of bivalves from the Baltic Sea

Bivalves *Mytilus edulis* and *Malcoma balthica* have been histopathologically investigated with emphasis on: neoplasms, kidney concretions, parasite infestations. Results from neoplastic studies indicated effect of impacted areas. Neoplastic changes have been observed in areas with suspected contamination, however, no direct link has been established. There is also a study on the combined effects of copper and phenanthrene on lysosomal compartment of cell (Moore *et al.* cited by Köhler but complete reference was not available.)

Fish disease

(See review by T. Lang, [Appendix 1](#)) Began in late 1970's by suspicion that there was link between diseases and impacted area. It was found to be a generic method for studying the impact of environmental factors though some contaminant specifics occur. International guidelines and a databank have been developed through ICES. There is a problem with relating infectious disease effects to impacted area because they have a complex multifactorial aetiology. Lysosomal stability test is a good generic indicator for a multiple sets of contaminants but can also indicate the onset of liver pathologies. In general the main manifestation of disease (especially liver cancer) is the result of a chronic process. Biomarkers have prognostic value to identify the steps during carcinogenesis ([Appendix 2](#)).

Eel pout

Angela Köhler reported of an Alfred Wegener Institute of Polar Research BAH programme "Combined effects of pollutants and temperature stress in marine fish (eel pout) - influence on the expression physiology and population dynamics". This species that has wide temperature tolerance, 40 years population dynamics data, reproduction viviparous, and is stationary. Eel pout occurs in the North Sea, Baltic Sea up to the White Sea. Other viviparous relative are present in the Mediterranean Sea. The main objective is to study the effect of TBT as androgenic compounds versus PAHs and related substances as estrogenic effect in relation to comparative stress. An experimental approach as well as field studies are planned over a large geographical scale using viviparous species. The study allows a detailed investigation of effects of endocrine disrupting compounds at the population level. A suite of biomarkers has been selected which are suspected to show response either by activation or inhibition, physiological changes affecting energy metabolism, ionic balance and signal transduction, and hormonal balance ([Appendix 3](#)).

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Table 1 (see text)

Method	Application: Organisms, pollutants, conditions	Positive experiences	Negative experiences	Recommendations (routine work (monitoring), gaps, research, no use)
TBT: concentrations and imposex (see Ten Hallers-Tjabbes literature)	Organisms: Neo-/meso-gastropods, Pollutants: TBT, TPT Conditions: Influence of shipping lanes and dumpsites	Easy to apply, non-intrusive (High correlation between disappearance of <i>Buccinum</i> and TBT and shipping intensity both in temperate and tropical areas)	Less practical compared to <i>Nucella</i> sampling (need vessel)	routine? yes gaps? Further understanding of ecological consequences on higher trophic levels. research? Wider geographic baseline and relation to other contaminants use? yes
TBT PAH?: other behavioural effects, combined (see Ten Hallers-Tjabbes literature)	Organisms: Neo-/meso-gastropods, Pollutants: TBT/PAH? Conditions: Influence of discharges	Indication that dredged materials effect chemoreception, in a potentially reversible way	Not sure which chemicals in dredged material is causal	routine? no gaps? Is TBT the reason or combined chemicals. research? Further testing materials and method development use?
TBT, method as described by OSPAR (general information related by Davies (UK))	(see literature)	(see literature)	(see literature)	routine? gaps? Effects where <i>Nucella</i> , <i>Littorina</i> and <i>Buccinum</i> is not found Cross-calibration of dose-response relationships between indicator species How do other endocrine disrupters interact with TBT research? needed use?
Stress on Sculpin using imposex <i>Nucella</i> as an indicator of TBT contamination (orientation by Svavarsson)	Organisms: Sculpin (Island) Pollutants: TBT Conditions: study in progress (multiple biomarkers)	Wide distribution, potentially good indicator	(study in progress)	routine? (study in progress) gaps? research? use?

Table 1 (cont. see text)

Method	Application: Organisms, pollutants, conditions	Positive experiences	Negative experiences	Recommendations (routine work (monitoring), gaps, research, no use)
Pathological/Histopathology (presented by Lehtonen, no literature cited)	Organisms: Bivalves Pollutants: uncertain Conditions: esp. neoplastic	Method for common species (<i>Mytilus</i> , <i>Macoma</i>)	Non specific, speculative link with impacted area.	routine? possibly gaps? need for experts. causal links research? repeated investigation and more experience needed in Baltic and other areas use? possibly
Fish disease (presented by Lang, see Appendix 1 and 2)	Organisms: Fish esp. dab, flounder, cod Pollutants: multifactoral influence (hydrography, behaviour, contaminants) Conditions:	Large ICES data set exists (420,000). Some contaminants show correlation with non-infectious diseases. Experience/methodology might be applicable for other spp. and areas	Difficult to establish clear link between contamination and prevalence of infectious diseases	routine? yes gaps? cause unknown /effect studies are needed, uncertainty of “reference values” research? statistical work correlating contaminants and hydrographical conditions study immune system, greater geographical coverage use? yes
Eel pout (programme in development by Germany presented by Köhler, see also Appendix 3)	Organisms: Fish esp. dab, flounder? Pollutants: multifactoral influence (hydrography, behaviour, contaminants) Conditions:	Potentially good indicator sp. because: wide distribution, viviparous, stationary, well investigated	Need for greater participation	routine? yes, biomarker established gaps? effects of TBT, temperature stress on population research? use? Prediction of endocrine disrupting effects at the population level

Appendix 1 to Annex 14 : Report of Working Group 4

Studies on diseases and pathology in marine fish species as contribution to biological effects monitoring

Thomas Lang: Bundesforschungsanstalt für Fischerei, Institut für Fischereiökologie, Cuxhaven, Germany

Introduction

Studies on the prevalence and spatial distribution of fish diseases have a long tradition in marine environmental research and monitoring programmes (Vethaak and ap Rheinallt 1992; Lang and Dethlefsen 1996), in particular in ICES Member Countries. Systematic investigations into the relationship between fish diseases and contaminants started in the late 1970's and focused in the beginning mainly on coastal and estuarine areas. In some regions, however, they subsequently have been extended to include also offshore stations. The North Sea certainly is the area for which the most data exist so far, followed by the Baltic Sea, Irish Sea and coastal zones in Atlantic and Pacific North America.

Although a considerable number of studies provide circumstantial evidence of a link between particular or groups of contaminants and certain fish diseases (Vethaak and ap Rheinallt 1992, Sindermann 1996), there is now a general consensus that most diseases have a multifactorial aetiology and that changes in the prevalence might be caused by a large variety of environmental factors, including anthropogenic and natural ones as well as their combinations. Therefore, changes should be considered as a non-specific indicator of environmental stress which can be used for a general rather than a contaminant-specific biological effects monitoring (ICES 1996; Lang and Dethlefsen 1996). However, for the development of certain types of non-infectious diseases such as liver tumours in bottom-dwelling fish species, there is sufficient evidence of a direct impact of carcinogenic environmental contaminants. Therefore, studies on liver nodules/tumour have been recommended repeatedly as technique for contaminant-specific (e.g., PAH, PCB) biological effects monitoring (ICES 1996a,b; 1997).

International activities

Mainly due to the long-term activities of the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO), standardised methodologies for fish disease surveys have been established (Dethlefsen *et al.* 1986; Anonymous 1989; Bucke *et al.* 1996; ICES 1996a) and are largely applied in ICES Member Countries. Recently, guidelines have also been developed for studies on flatfish liver pathology (ICES 1996a). Methodologies for gross disease examination have been intercalibrated and standardised during three sea-going ICES Workshops in the North Sea (Dethlefsen *et al.* 1986), the Kattegat (Anonymous 1989) and the Baltic Sea (together with the Baltic Marine Biologists, BMB) (Lang *et al.* 1995). Methodologies for liver histopathology diagnosis will be intercalibrated as part of the EU project 'Biological Effects Quality Assurance in Monitoring Programmes, BEQUALM'.

Disease data recorded by ICES Member Countries according to the defined standard methodologies are submitted to the ICES Environmental Data Centre on an annual basis by using the ICES Fish Disease Data Entry Program (FDE) or the ICES Fish Disease Data Reporting Formats. So far, the ICES fish disease data comprise information on diseases of more than 420.000 individual fish (dab, *Limanda limanda*, and flounder, *Platichthys flesus*, from the North Sea, Irish Sea, English Channel, Baltic Sea and adjacent waters) partly dating back until 1981. It is intended to extend the databank

to include information from other areas and other fish species. In addition to the disease data, the databank includes individual length and sex data as well as information on sampling characteristics (dates, locations, gear types etc.).

Statistical procedures for the analysis of the fish disease data have been elaborated by the ICES Sub-group on Statistical Analysis of Fish Disease Data in Marine Stocks (SGFDDS) and have been applied recently for an analysis of spatial and temporal trends in disease prevalence in dab (North Sea) and flounder (North Sea and Baltic Sea). A report providing information on the methodologies used and the results of this exercise is so far only available in a draft form. At present, plans are being developed by WGPDMO for a holistic data analysis combining disease data with other data available in the ICES Environmental Data Centre (e.g., contaminants), the ICES Oceanography Data Centre (e.g., salinity, temperature, nutrients) and the ICES Fisheries Databank (e.g., fishing effort, stock density).

With regard to international monitoring programmes, fish disease data have been used for the North Sea Quality Status Report 1993 (North Sea Task Force 1993) and the HELCOM Third Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1989-93 (Helsinki Commission 1996) and will be part of the biological effects component of the OSPAR Joint Assessment and Monitoring Programme (JAMP). The JAMP Monitoring Guidelines for studies on externally visible diseases (part of the general biological effects monitoring) and for liver nodules and liver histopathology (contaminant-specific monitoring) are based on standard methodologies developed by ICES.

The German fish disease monitoring programme

The German programme is carried out by the Bundesforschungsanstalt für Fischerei since the late 1970's on an annual basis and is focused on the North Sea and the south-western Baltic Sea. Other areas studied as reference areas have been the Irish Sea, Celtic Sea, English Channel and Icelandic waters.

Externally visible diseases and parasites are recorded for most abundant fish species, with dab and flounder as major flatfish target species and cod (*Gadus morhua*), whiting (*Merlangius merlangus*) and haddock (*Melanogrammus aeglefini*) as major representatives of the roundfish. In addition to the externally visible diseases, dab and flounder are examined macroscopically and histologically for the occurrence of liver neoplasms. All procedures applied are according to ICES standard methodologies (see above) and data are submitted to the ICES Environmental Data Centre.

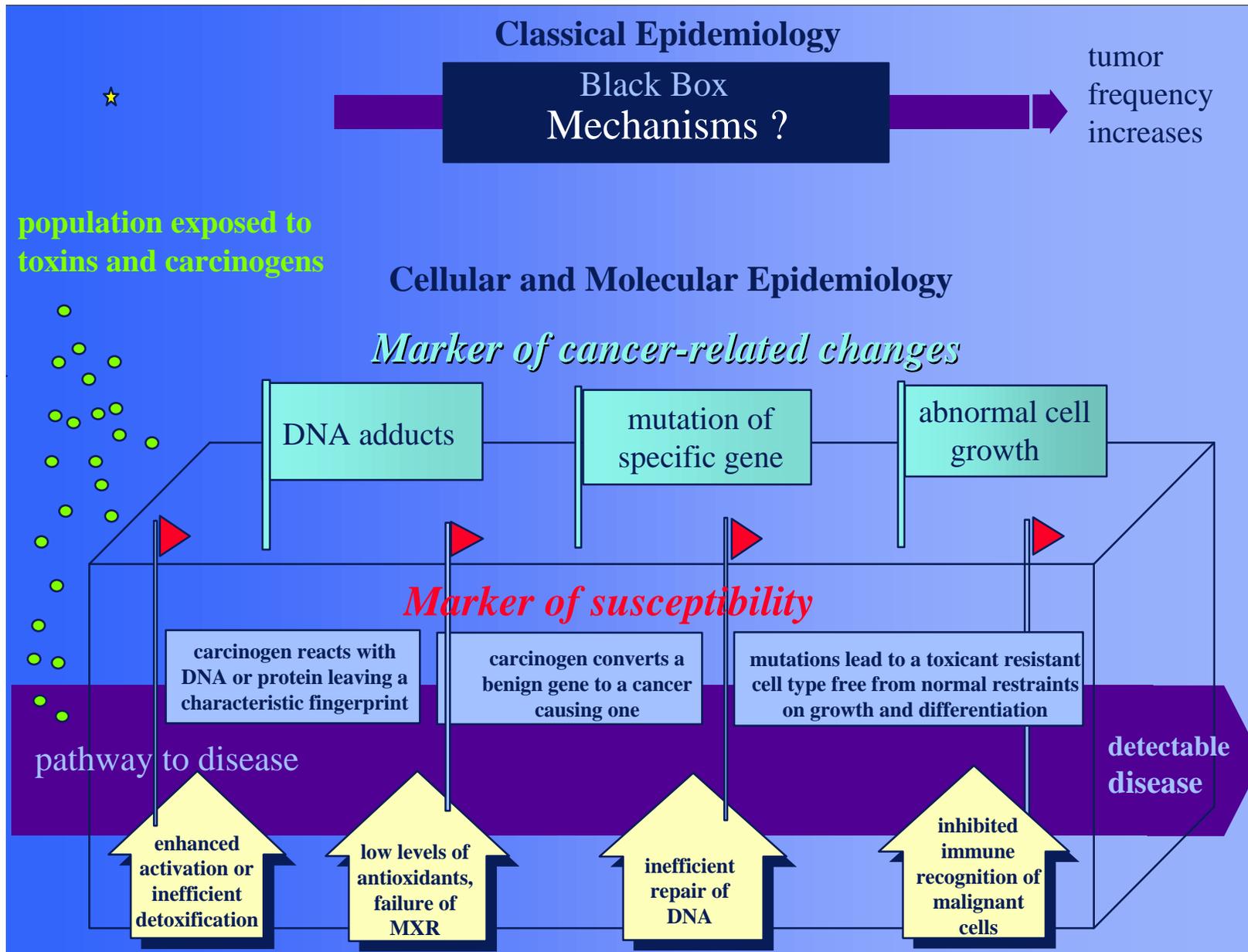
Parameters recorded in combination with the disease inspection are: hydrography (temperature, salinity, oxygen), catch per unit effort, age/length relationship, condition factor, organosomatic indices, contaminant levels in liver tissue (organochlorines) and EROD.

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Combined Effects of Contaminant Exposure and Temperature Stress

Cascade of Cytogenetic Responses

Cell Protection

heat shock proteins
transmembrane drug transporter (MXR)
P450 monooxygenase system
antioxidant enzymes and lysosomal ingestion
DNA repair

Cell injury

reactive oxygen species (ROS)
lipid peroxidation (MDA)
mitochondrial and lysosomal damage
DNA strand breaks (TUNEL, COMET)
inhibition of enzyme expression and activity

Metabolic stress signals

breakdown of ionic homeostasis, inhibition of ATP and NADPH producing pathways, of biotransformation enzymes (CYP 450) and drug transporter (MXR), inhibition of cell division, glycogen depletion, fat accumulation
shift in androgen-estrogen balance

Effects on populations

Reduction of growth and reproduction by reduced fertility, yolk production and survival of embryos.
Higher mortality (higher disease frequency in the population)

Adaptional gene shift in populations (4-6th year of project)

Fig. 1

A.Köhler-Günther, 1998