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WORKING GROUP ON BIOLOGICAL EFFECTS OF CONTAMINANTS

Copenhagen, 5 - 8 April 1988

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REPORT OF THE WORKING GROUP ON THE BIOLOGICAL EFFECTS OF CONTAMINANTS

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1 OPENING OF THE MEETING

The Chairman, Dr F. Thurberg, opened the meeting at 9.15 hrs on 5 April 1988 and welcomed the participants. The list of participants is attached as Annex 2.

After an introduction of the members of the Working Group, the minutes of the 1987 meeting were accepted without change.

2 ADOPTION OF AGENDA

The draft agenda for the meeting was accepted with some minor changes in the order of the discussion, and is attached as Annex 1. Dr Boon, Dr Stebbing, Dr McDowell Capuzzo and Prof. Dundas agreed to share the responsibility of recording the minutes of the meeting.

3 COUNCIL RESOLUTIONS

Relevant resolutions from the 1987 Council Meeting were noted, including C.Res.1987/2:36 on the meeting of the Benthos Ecology Working Group at Texel, 10-13 May 1988, and C.Res.1987/3:4 on the Intercalibration of Analyses of PCB Congeners in Seal Blubber.

In C.Res.1987/3:5, the Council authorised in principle the conduct of a sea-going biological effects monitoring techniques workshop, as proposed by the Working Group in 1987. This resolution established a Planning Group for the Workshop and requested that the detailed plans be presented at the 1988 Council Meeting.

4 IOC/ICES COOPERATION

It was noted that, in order to optimize the cooperation between the ICES Working Group on the Biological Effects of Contaminants (WGBEC) and the IOC Group of Experts on the Effects of Pollutants (GEEP), IOC has appointed Dr J. McDowell Capuzzo as its official representative on the WGBEC.

Dr McDowell Capuzzo presented a brief outline of the history of the work of IOC/GEEP. GEEP, like the chemically orientated GEMSI (Group of Experts on Methods, Standards and Intercalibration), has been established as a working group under the IOC GIPME (Working Committee for the Global Investigation of Pollution in the Marine Environment). The main task of GEEP is to assist in the development of biological effects monitoring in IOC regional programmes.

As a first practical task, GEEP organized a workshop in Norway utilizing a contaminant gradient in the Frierfjord and a mesocosm experiment located at the laboratory in Solbergstrand. This workshop was concerned with the further evaluation of many of the

techniques that were proposed at the 1979 ICES Beaufort Workshop on the Biological Effects of Marine Pollution and the Problems of Monitoring (McIntyre and Pearce, 1980, RPV No.179).

The main emphasis of the GEEP Oslo workshop was to evaluate techniques for monitoring biological effects. Essentially two types of biological effects studies were chosen for evaluation:

1) those based on the individual organism at different levels of biological organization (i.e., biochemical and physiological techniques), and

2) community structure analyses.

In the Frierfjord, a field gradient of contaminants was studied, while in the mesocosms at Solbergstrand, a benthic soft-bottom community and macrofauna were exposed to diesel oil and copper.

A total of 32 scientists cooperated in this very successful practical workshop, which was able to integrate different approaches to biological effects measurements. Among the more important results of the workshop were:

- The promising results of measurements of certain aspects of mixed function oxygenase (MFO) induction in flounder collected from the field. In particular, the measurements of the ethoxyresorufin-O-de-ethylase (EROD) assay proved successful. MFO induction was not obvious in fish held in the mesocosms, but the diesel fuel contained low amounts of high molecular weight PAHs.

- For the mussel Mytilus edulis a logical agreement was seen between the results of biological effects measurements at different integration levels (biochemical, physiological, histological and histopathological).

- The benthic community analyses showed the strength of different statistical analyses in discriminating between stations and the robustness of many of the data analyses to the aggregation of species to higher taxonomic units. This could lead to approaches to benthic monitoring that are less labour-intensive.

The main task of IOC/GEEP in the near future will be the development and application of biological effects monitoring techniques in UNEP and IOC regional programmes (mainly in tropical or subtropical regions). Training is an important aspect.

The main task of the WGBEC is the development and implementation of biological effects monitoring techniques for ICES coordinated monitoring programmes and those coordinated by regulatory commissions to which ICES provides scientific advice.

Both groups will work in close cooperation on the development and validation of biological effects monitoring techniques under the terms of the cooperative agreement between ICES and IOC.

The Working Group discussed the Oslo workshop results at some length and noted that the very successful execution of that activity will provide an excellent basis for the ICES/IOC R/V

"Meteor" Workshop. The Working Group also noted that the Oslo workshop has clearly demonstrated that no single technique will be sufficient in the near future to evaluate effects of contaminants. A suite of complementary techniques is needed. This important observation must be borne in mind when planning biological effects monitoring studies.

It was noted that the Oslo workshop results will be published as a special volume in the Marine Ecology Progress series and is in press at this time. The Chairman will inform the members of the Working Group about the availability of this special volume. IOC is attempting to procure a number of extra copies from the publisher.

Dr McDowell Capuzzo then presented a list of planned future GEEP activities:

a) Cartageña Workshop, Columbia, April 1988. (CCPS, UNEP, IOC, FAO, SIDA). This is a training course given in Spanish (lectures and practical) on acute toxicity and bioassay techniques. GEEP is serving as advisor to this Workshop.

b) Piran Workshop, Yugoslavia, June 1988. (FAO, IOC, UNEP). This is a training workshop in "Statistical treatment and interpretation of marine community data" for 15-20 people from the Mediterranean region. This will be led by Bob Clarke and John Gray.

c) Bermuda Workshop, 10 September-1 October 1988. This research/-practical workshop will re-examine some techniques from the Oslo Workshop and some bioassay techniques using sub-tropical species. It has been proposed as an IOC/GEEP venture. Dr Addison is convener (a link with the WGBEC will occur by participation of Dr Stebbing).

d) ICES/IOC R/V "Meteor" Workshop, North Sea, September 1989. This workshop will examine selected techniques from the Oslo workshop and other techniques concentrating on fish and shorter-term bioassays. Planning Group members are Drs. Stebbing, Dethlefsen, Thurberg and Capuzzo. This will be discussed in depth under agenda item 5.

e) Xiamen Chinese Workshop, 1990. Invitations have been received from Prof. Zhang of the 3rd Institute of Oceanography to conduct workshops (research and training) as follows: (1) Workshop on biological effects techniques, and (2) Statistical workshop on community analysis.

f) Workshops in the Philippines and the Caribbean, 1991. Practical training workshops are being planned.

5 IOC OSLO WORKSHOP

In discussing the results of the GEEP workshop in Oslo, a number of points were made, as summarized below.

Regarding the bioassays on the toxicity of compounds accumulated in the surface microlayer, Dr Føyn questioned whether this layer is coherent under quite turbulent circumstances. Cod eggs are mixed down in the water column fairly easily by wind and exposure to the microlayer would be for a very brief time. Thus, he questioned whether the testing of toxic effects on eggs by compounds in the surface microlayer is relevant. Dr McDowell Capuzzo responded that, in at least in the Northwestern Atlantic, larvae are present in the surface microlayer in large amounts.

It was stated that, in general, the magnitude of toxic effects in situ is often less well understood than that in artificial circumstances, such as bioassays under laboratory conditions. So, an increased knowledge of the field relevance of bioassays should be encouraged.

Mr Lloyd noted that the results of some Oslo workshop techniques did not show the precision required for monitoring purposes. The requirement was that the test could show more than could be obtained by chemical analysis. He mentioned MFO activity as an example.

Dr Boon stated that MFO induction may be caused by a mixture of organic chemicals that might not have been measured. Also, a threshold value for contaminant concentrations might exist, below which MFO induction does not occur at all. This might be of great value for regulatory purposes.

Mr Lloyd stressed the value of "scope for growth" measurements for these purposes. Tests with mussels performed well at the Oslo workshop and gave good resolution between sites. There are some general problems of interpretation of scope for growth data, however, which Mr Lloyd summarised in a short paper distributed to the Working Group (WGBEC 1988/4).

A discussion followed concerning the availability of specialized scientists to participate in workshops. Dr McDowell Capuzzo responded that many people have been trained in the relevant techniques, so that a good number of trained people are now available.

6 BIOLOGICAL EFFECTS TECHNIQUES METEOR WORKSHOP - GENERAL DISCUSSION

Dr Stebbing introduced this topic by reviewing the discussion at the last WGBEC meeting, at which the conduct of a practical international workshop was suggested. Dr Dethlefsen had offered to explore the possibilities of the use of the R/V "Meteor" in September 1989 as a platform for the biological effects monitoring workshop. 25-30 scientists can be accommodated on the vessel.

The workshop is intended to be a joint ICES/IOC venture. Therefore, in addition to the ICES members of the Planning Group for the workshop, namely Dr Thurberg, Dr Stebbing and Dr Dethlefsen, Dr McDowell Capuzzo has been included as an IOC representative on the Planning Group. Profs. Dundas and McIntyre have kindly agreed to act as advisors for this planning effort. Dr McDowell Capuzzo will also serve as the IOC representative on the Working Group.

Dr Stebbing provided two draft proposals (papers WGBEC 1988/4 and 6) for the workshop.

Important steps in the planning process include:

1) The selection of techniques/people; two approaches are possible:

   a) to ask specific people to apply their techniques at the workshop, or

   b) to use a broader front approach by addressing selected institutions who can then decide for themselves who will participate.

   Dr C. Heip, Chairman of the Benthos Ecology Working Group, has written to the Planning Group (Dr Stebbing) expressing the wish to repeat the Oslo exercise on benthic community studies over a stronger contamination gradient. He also offered to arrange for one of the Dutch research vessels to be available in case the necessity would arise.

   Dr Stebbing suggested that Dr Heip be added to the Planning Group to develop the benthic biology part of the workshop.

2) Funding: Dr Dethlefsen has offered to explore the possibility of the use of the ship and support of the scientists on board. The transport of people and scientific equipment, and the cost of the necessary chemicals need to be accounted for.

3) Choice of contamination gradients: After considerable discussion, the following sampling areas were included in a possible cruise track:

Area	Some dominant contaminants
Elbe Estuary plume	Complex mixture
Incineration site	(Halogenated) hydrocarbons, HCl
Dogger Bank	Cd, Pb, HCB, DDE
Rhine-Meuse Estuary plume	Complex mixture (PCBs)
Firth of Forth	Sewage sludge
Oil drilling rig	Petroleum hydrocarbons from oil-based drilling muds

   Four of these sites will be the primary sites where the major activities will be focused. Two will be optional stations where measurements are encouraged if time allows.

4) Choice of biological effects techniques:

   Dr McDowell Capuzzo suggested that the experience of the Oslo workshop should be built upon. She initially suggested the following:

a) Benthic community studies;

b) Studies on a flatfish species that shows only limited migratory movements. This may include fish embryo work;

c) Chemical concentration measurements on sediments, water and biological tissues.

Dr Boon suggested that benthic invertebrates, that are sessile and serve as food for the fish, be included in the contaminant measurements. This should account for a more rapid metabolism of certain organic contaminants (especially PAHs) in fish compared to their food.

It was further considered that, although the dab (Limanda limanda) should be the main fish species studied, investigations of other fish species or even other phyla should be encouraged wherever possible.

7 BIOLOGICAL EFFECTS TECHNIQUES WORKSHOP - SPECIFIC PLANS

7.1 Consideration of Techniques to employ

The Working Group summarised the biological effects techniques and related chemical techniques that it would like to have deployed on the R/V "Meteor", as shown in Table 1. The Working Group also noted the following points:

1) Contaminants in the sea tend to accumulate to higher levels at the interface with the bottom and at the interface at the surface, so effects may be expected to occur in these areas. The benthic zone and the surface layers are, therefore, the regions where most effort will be deployed, providing the best chance of encountering concentrations of contaminants to which the various techniques can respond.

2) The ICES Benthos Ecology Working Group will be invited to take responsibility for benthic community analysis, as its Chairman, Dr C. Heip, has expressed an interest in extending the work he and his colleagues carried out at the Oslo Workshop.

3) While the Working Group was committed to utilizing the experience gained in the Oslo workshop, it was important not to test techniques that would be difficult to deploy at sea, and which would, therefore, be unlikely to be incorporated into sea-going biological effects monitoring programmes. It was, therefore, decided not to use caged mussels for scope for growth and other tests. The requirement for a considerable period of exposure would necessitate that the cages of mussels be put out in advance of the cruise itself.

TABLE 1: GENERAL REQUIREMENTS FOR THE WORKSHOP

	Specialist areas	Specialists to invite	Essential chemistry/physics	Specialist chemists
Hydrography	- nutrients - productivity - oxygen - T/S profiles	German scientists ICES groups		
	Modelling			
Statistical design/ Sampling design	- sampling protocol - replicates Uniform analysis of raw data	K.R. Clarke		
Other techniques				
Molecular biology	DNA probes			
Immunology	Immunocompetence			
Genotoxicology	SCE, chromosomal aberrations			
Behaviour				

TABLE 2: SPECIFIC REQUIREMENTS FOR THE WORKSHOP

	Specialist areas	Specialists to invite	Essential chemistry/physics	Specialist chemists
Benthic "flatfish"	Pathology Immunology Histopathology Biochemistry	Bucke, Vethaak McVickar, Pulsford Moore, Lowe Stegeman, Goksøyr McElroy, Galgani, Overnell	Tissue chemistry	Boon (coordination)
Benthos	Ecology (abundance)		Sediment chemistry/	
- infauna	Benthic WG Sediment bioassay	Heip Schwartz	sedimentologist Chemical analyses	Rowlatt ICES/IOC
- epifauna	Crustaceans Molluscs	Davis, McHenery Anderson	of infauna food hydrography modelling	Chemical WG
Microlayer/ Neuston/ Plankton				
- neuston (observational)	Ecology - community - abundance Occurrence - eggs, larvae fish, and invertebrates	?	Microlayer chemistry	Hardy Hühnerfuss
		Longwell von Westernhagen		
- bioassays (experimental)	Eggs Larvae Ciliates Hydroids	Dixon Thain Johnson, Karbæ		
- microbiology	Methods Resistance Ecology	Kjelleberg		

4) It was appreciated that there are new and powerful techniques from immunology, molecular biology, and genotoxicology that are now being developed for use in marine organisms. It was further recognized that it is important to design the programme with sufficient flexibility to allow for the testing of these types of techniques at the Workshop, if they have not been made part of the benthic or surface layer work.

5) It was recognised that the testing of biological effects techniques is of limited relevance in the absence of appropriate chemical measurements. While the ICES Marine Chemistry Working Group and IOC GEMSI should be approached for their assistance in planning the chemical measurements to be made, it was considered important in certain areas to identify scientists whose physical-chemical interests relate most closely to the surface microlayer and sediment ecology.

6) The success of the Oslo Workshop depended to a considerable extent on the input provided by Dr R. Clarke who, acting primarily as a statistician, provided advice on the experimental design, carried out all statistical testing of data, and organised the sampling protocol. It was requested that he, or someone he might be prepared to nominate, could provide a similar service for the R/V "Meteor" Workshop.

7) It was appreciated that hydrographic data (salinity, temperature, dissolved oxygen, pH) must be collected so that the effects of water masses, fronts, and stratification can be taken into account in the analysis of the biological data.

8) Simulation model predictions were recognised as an important means of focussing where the effort should be directed geographically in the North Sea.

7.2 Invitations to potential Participants in the Workshop

After some discussion, it was agreed that letters of invitation should be sent to major marine laboratories and to individual experts identified by the Working Group. A copy of the letter and the proposal is attached as Annex 3. Dr Pawlak will ensure that invitations will be distributed as appropriate to the ICES Delegates and members of relevant Committees and working groups. Professor Kullenberg would distribute invitations to appropriate IOC contacts.

The scientists who wish to be considered for the workshop should send their proposals to Dr Stebbing by 31 July 1988.

7.3 Funding

It was hoped that the major cost of the workshop will be covered by the provision of the R/V "Meteor" by the Federal Republic of Germany, in response to requests on behalf of the Working Group by Dr Dethlefsen. Nevertheless, there are residual costs that will be incurred:

1) travel expenses of participants,

2) costs of transporting equipment, and

3) costs of consumables, including chemicals.

These costs were estimated to amount to $20,000-$30,000 USD. Administrative costs will be covered partly by the ICES Secretariat, who may also provide a secretary aboard the R/V "Meteor" for the Workshop itself.

Noting that the costs of chemical analyses for the Oslo Workshop had constituted a major item of expenditure ($10,000 USD), after some discussion, it was agreed that Dr McHenery would approach Dr G. Topping, Chairman of the Marine Chemistry Working Group and also a member of GEMSI, with a letter from WGBEC requesting the collaboration of these groups in obtaining advice on the chemical measurements required and the laboratories who could conduct these measurements for the R/V "Meteor" Workshop (see Annex 4). From within the Working Group, tentative commitments to undertake analyses, or agreements to make requests for analyses, were given as follows:

Dr Boon	-	PCBs	ca. 50 samples of tissues and sediments
Dr Marquenie	-	Metals	
Dr Føyn	-	PAHs	
Dr Galgani	-	Metals	
Dr Stebbing	-	Metals and total hydrocarbons in microlayer and sub-surface water samples	

Additional funding for the R/V "Meteor" Workshop will be pursued through an application to the Chairman of GEEP and thence to IOC. Dr Stebbing agreed to write to the Chairman of GEEP to initiate requests for funds to cover some of the expenses of the Workshop.

7.4 R/V "Meteor" Facilities

Dr Dethlefsen briefly outlined the capabilities of the R/V "Meteor" in terms of the Workshop, suggesting that a leaflet about the vessel could be sent out with the invitations.

7.5 Discussion on general Invitation Letter for R/V "Meteor" Workshop

The Working Group discussed the format and content of the general letter of invitation for participation in the R/V "Meteor" Workshop. It was decided that:

a) As for the Oslo Workshop, it will be assumed that participants will seek funding for travel, subsistence, and transportation of equipment from their own sources.

b) The letter should indicate that no charge will be made for room and board on the research vessel and that limited funding for transport may become available.

c) Dr. Dethlefsen's letter will be sent out immediately after ship time has been approved, hopefully on 25 May 1988.

d) A major grant proposal type of response is not required of prospective participants.

e) The criteria for choosing techniques are that the method is useful for monitoring, that it may be deployed from a research vessel, and that it can be directed to studies of benthic fauna (including flatfish) or to studies of neuston, although a few special studies outside of this scope may be included.

f) For workshop details, the complete proposal for the workshop will be included with the letter.

g) Prospective participants should consider their need for a common statistical framework for integrating their results and the relevance of their methods to important marine resources.

h) The proposals should be sent to Dr Stebbing of the Planning Group and the telephone numbers of each Planning Group member should be made available in the proposals in case prospective participants need additional information.

7.6 Draft of the Proposal for the Workshop

The Working Group held a general discussion on the media that should be studied in the Workshop. During this discussion, questions were raised as to:

i) the likelihood of finding eggs and larvae in September in the areas to be visited;

ii) the importance of the surface microlayer for pollution impact on fish eggs and larvae; and

iii) whether chemical analysis of the water column is necessary or desirable.

The consensus was that the chances of finding eggs and larvae in the North Sea in September might be small.

Dr Galgani pointed out that one may expect large variability in environmental conditions in September. The Chairman instructed the Group to seek expert advice on what species, eggs, or larvae are most likely to be present in September and cautioned that they should be prepared for the possibility of obtaining only very small samples.

Dr Stebbing noted that the biological assay work on the microlayer would be conducted using cultured eggs/embryoes. Nonetheless, Mr Føyn felt that the impact of pollution on eggs and larvae in the microlayer would be small because microlayers are broken down in quite turbulent conditions and eggs were mixed downward by moderate wind speeds.

Dr Boon questioned the need for extensive water column chemical analyses, but Dr Stebbing stated that sub-surface water analyses were necessary as a reference for microlayer work. Dr Dethlefsen offered to contact the German Hydrographic Institute for possible assistance concerning water column chemical analyses. Dr G. LeFevre-Lehoërff proposed contacting Dr A. Abarnou at IFREMER, Brest, regarding PCB analysis.

The Working Group agreed that chemical analysis of the biota was the first priority and that chemical analysis of the water column should be carried out only as specifically necessary.

Mr Føyn, Dr Marquenie and Dr Boon agreed to write a letter to the Chairman of the Marine Chemistry Working Group, Dr Graham Topping, to elicit his collaboration and that of the Marine Chemistry Working Group in structuring the chemical aspects of the Workshop (Annex 4).

The Working Group agreed that none of the stations to be studied would be properly estuarine, but some would be in the plumes of estuaries.

In discussing and editing the proposal for the Biological Effects Techniques Workshop, it was noted that in choosing a site in the vicinity of an oil production facility, the focus should be on those sites where sufficient background information on the impacts of drilling/production activities exist. Dr Marquenie and Dr McHenery will provide Dr Stebbing with recommendations for sites off the Dutch coast and the Scottish coast, respectively.

The two groups of prospective workshop participants, (a) those specially invited, and (b) those responding to a general invitation, should present their proposals by 31 July 1988 to Dr Stebbing. This time table is necessary in order to formulate a full proposal in time for the 1988 Council Meeting.

Dr Dethlefsen noted that a follow-up was needed for coordinating the presentation of the results of the Workshop. Dr McDowell Capuzzo suggested that the Oslo Workshop model be followed, by which a few groups of authors were formed, each with one leader responsible for submitting the manuscripts on time.

An ICES Special Meeting on Biological Effects Monitoring was suggested for the autumn of 1990 in association with the Statutory Meeting. A recommendation to that effect will be prepared for the 1988 Statutory Meeting.

Dr Dethlefsen suggested that the Planning Group would need to meet prior to the Statutory Meeting. It was suggested that a meeting in connection with the GEEP meeting on Bermuda in September would perhaps be the most economical, because three of the four Planning Group members will be in attendance at the GEEP meeting.

8 JMG PAPER ON "BIOLOGICAL EFFECTS MONITORING"

Dr McHenery introduced and summarized a paper (WGBEC 1988/2), prepared by Dr A. McIntyre, Dr J. Davies and himself for the

Joint Monitoring Group of the Oslo and Paris Commissions, that reviewed biological effects monitoring activities conducted by member countries of the Oslo and Paris Commissions. The document contained a number of specific recommendations and conclusions that were discussed by the Working Group.

These recommendations and conclusions were largely accepted by the Working Group, which noted that the plans for the R/V "Meteor" Workshop were compatible with the recommendations of the paper.

9 FISH DISEASE WORKSHOP

Dr Dethlefsen summarized the plans for the ICES Second Sea-Going Workshop on Studies of Fish Diseases and Parasites in relation to Pollution, to be held the following week on the Swedish research vessel "Argos". The aim of this workshop is to obtain agreement on the methods to be used on fish disease sampling surveys. Four areas will be discussed:

- the grading of infectious diseases;

- the use of liver abnormalities;

- the use of healed external lesions; and

- standards for ulcerations (cod).

There will be no discussion of pollution effects because the workshop will emphasize methods. A preliminary report on the results of this workshop will be available for the ICES Statutory Meeting in 1988.

10 LEAFLET SERIES "TECHNIQUES IN MARINE ENVIRONMENTAL - SCIENCES"

The Working Group recalled that at last year's meeting it had considered the possibility that detailed descriptions of the methods employed in certain biological effects studies should be prepared for possible publication in the new ICES series "Techniques in Marine Environmental Sciences" ("TIMES").

In this connection, Dr Serigstad outlined some of his work on the effects of hydrocarbons on fish eggs and larvae (cod), particularly on oxygen consumption and cytochrome P-450. Yolk sac larvae were most sensitive to pollutants; no recovery was noted upon transfer to unpolluted water for oxygen consumption effects, while P-450 induction recovery was evident. This indicates the need for more than one assay method for evaluating the effects of pollutants.

The Chairman noted that the Working Group should avoid as far as possible the preparation of leaflets on methods for which manuals are being produced or planned by IOC or FAO. Good communication is essential at an early stage to avoid this. Dr McDowell Cappuzzo reported that IOC/UNEP training manuals on scope for growth (mussels and other bivalves) and MFO are being planned, and manuals on other topics are being discussed. IOC handbooks

are largely targeted at training in developing countries, while ICES leaflets are aimed at providing detailed descriptions of the best methods available.

Dr Dethlefsen will discuss the development of a handbook on the use of fish eggs and larvae in biological effects monitoring with colleagues in the Federal Republic of Germany.

Dr Stebbing emphasized that the methods that show promise in the R/V "Meteor" Workshop would be good candidates for a detailed description in the TIMES series.

Dr Pawlak also noted that a video tape format has been tested and that this approach is also possible for methods descriptions in the TIMES series.

11 GEMSI MUSSEL WATCH QUESTIONNAIRE

Dr McDowell Capuzzo introduced this item, outlining the response to the GEMSI questionnaire on the IOC Mussel Watch programme and the planned IOC/UNEP Global Mussel Watch experiment. She reported that in 1984 the IOC Working Committee for GIPME had recommended that a critical review be undertaken of mussel watch activities within IOC and UNEP regional marine pollution monitoring programmes. Two objectives had been identified:

1) the preparation of an inventory of national and international mussel watch-type programmes, and

2) the assessment of the quality of data from these programmes, with particular reference to the degree to which the quality of reported data may meet the aims of each group.

A questionnaire was sent out during 1986 to laboratories within IOC and UNEP regional programmes and to additional laboratories in North America and Europe identified through the ICES Marine Chemistry Working Group. Questions on biological effects monitoring techniques were included in the questionnaire. Of the 25 responses, 15 indicated interest and/or participation in biological effects studies as part of Mussel Watch activities. Three indicated a high level involvement with a comprehensive programme; four indicated medium involvement using simple techniques; and eight indicated a desire to use biological effects techniques but had no programme at present. A definite interest in training was expressed by 12 respondents.

Global Mussel Watch Programme

IOC and UNEP are planning a global mussel watch experiment to assess the input of organochlorine biocides in tropical and Southern Hemisphere regions. The purpose of the programme will be to evaluate present levels of organochlorine biocide contamination, and relate these patterns and levels to those measured during the 1960s and 1970s, when elevated levels and serious environmental effects were observed in industrialized countries of the Northern Hemisphere.

12 REPORTS ON BIOLOGICAL MONITORING ACTIVITY IN ICES MEMBER COUNTRIES

Presentations were made of national programmes on biological effects monitoring within the ICES area, as given in the following paragraphs.

United Kingdom

Mr Lloyd discussed activities in England for the hazard assessment of contaminants in the marine environment. He presented a chart summarizing these activities and a review of ecotoxicological testing (Lloyd, 1984, Annex 5). One of the most difficult problems is the assessment of complex mixtures and identifying the presence of unknown contaminants that have the potential for environmental damage. There is a need for biological effects techniques that are initially fairly broad in their response that may be used to identify problems associated with unknown contaminants. After many years of experience, it is difficult to find a single bioassay species suitable for all analyses. Modifications of the sediment community recolonization bioassays developed by Tagety (U.S. Environmental Protection Agency) have been used for evaluation of a wide range of sediment contamination problems.

Dr McHenery reported on studies conducted in Scotland that focused on:

1) examination of EROD in fishes exposed to contaminants in experimental systems;

2) imposex of dogwhelks in response to contaminants;

3) reproductive success of fishes in response to contaminants - egg viability, egg and larval bioassays in response to the water-soluble fraction of oil;

4) studies of immunocompetence;

5) studies of effects of oil on fish fecundity;

6) expansion of studies to invertebrates, particularly Nephrops, and assessing changes in immunological response and reproduction, comparing with field observations in the vicinity of sewage sludge dumpsite;

7) beginning evaluations of the effects of chemicals from mariculture operations on the marine environment.

Dr Dundas asked whether any studies on pathogenic organisms in mariculture operations were being conducted. Dr McHenery reported that, at present, this is limited in scope. Of particular concern are chemicals proposed for use in de-lousing salmon.

France

Drs Lefevre and Galgani presented a summary of biological monitoring programmes that have been developed by IFREMER during the past decade (Summary, Annex 6).

Studies have focused on: (1) the effects of thermal discharges from nuclear power plants on planktonic and benthic communities; studies included effects on primary production, zooplankton, and benthic communities; (2) monitoring of contaminants in sea water and biota (mussels, fish, shrimp); (3) studies of nutrient flux and eutrophication in coastal waters, including surveys of algal blooms and red tides (e.g., Dinophysis).

New efforts are underway to link biological effects with levels of chemical contaminants in biota, with particular emphasis on physiological and biochemical methods. Major activities focus on (a) responses of different trophic levels, (b) comparison of responses of the same species at contaminated and uncontaminated sites, and (c) studies of contaminant transport and transformations. Studies are currently directed at Ostrea and Pleuronectes as well as other species. Physiological responses are examined in response to both short-term and long-term exposures.

Bioassay approaches using larval/embryonic mollusc tests (Ostrea, Mytilus) are currently being developed. Other studies are directed at linking the condition of mucus cells of Pleuronectes in response to contaminants in the sediments.

Biochemical studies are focused on: (1) the development of automated methods for the analysis of cytochrome P-450 and metallothioneins; (2) field assessments of induction of cytochrome P-450 and metallothionein at heavily contaminated sites, such as the Bay of Seine and the Gironde; and (3) a survey of contaminant levels in mussels and fish collected along the coast of France.

The Netherlands

Dr Marquenie presented the results of a Dutch study on the effects of dredged material in model tidal flat ecosystems (Marquenie and Zevenboom, 1988; Annex 7). A mesocosm study SEDEX (sediment experimental) was initiated during 1987 to examine the effects (and interacting processes controlling effects) of contaminated dredged material on the structure and function of tidal flat ecosystems. The relationship of this approach to standard bioassay approaches for evaluating dredged materials was discussed.

During the 1987 SEDEX, four experimental systems were used: (a) indirect loading of dredged materials via the water phase, (b) direct loading of dredged materials via the sediments, (c) a combination of (a) and (b), and (d) a reference tank using sand as a control. Results indicate enhancement of algal growth and a shift to small-sized algae in tanks receiving contaminated sediments in comparison to the control. Eutrophication effects on benthic communities were also observed and appeared to override effects of the contaminants. Studies planned for 1988 will attempt to separate the effects of contaminants from those of eutrophication.

Dr Marquenie also discussed the hydrography and flow characteristics of the Rhine River and Meuse River that will be important in choosing sites off the Dutch coast for evaluation during the Biological Effects Techniques Workshop aboard the R/V "Meteor". He recommended that a summary of these environmental conditions be prepared for presentation at the next meeting of WGBEC, and

that other groups prepare similar reports for other areas in order that appropriate sites may be chosen for evaluation during the workshop aboard the R/V "Meteor".

Dr. Marquenie also presented the results of mussel watch studies evaluating the concentrations of PCBs, copper and cadmium in mussels deployed in cages along the Dutch coast. The relationship between measured concentrations in mussels and expected effects on predators such as seals and ducks were discussed. Environmental factors are important in controlling bioaccumulation of contaminants in mussels and seasonal variations have been noted.

13 ACTION LIST

The Working Group agreed to the following list of intersessional activities:

a) The Chairman will send information to all WGBEC members on the availability of the proceedings of the IOC/GEEP Oslo Workshop to be published in a special issue of the Marine Ecology Progress series.

b) All WGBEC members should consider recommendations for participants in the Biological Effects Techniques Workshop aboard the R/V "Meteor", and send their recommendations to Dr Stebbing.

c) Dr McHenery will contact Dr Topping concerning cooperation and collaboration of the Marine Chemistry Working Group in the workshop.

d) d) Dr Stebbing will contact Dr Heip concerning cooperation and collaboration of the Benthos Ecology Working Group in the workshop.

e) Dr Dethlefsen will present plans for the workshop to the scientific committee of the R/V "Meteor" and contact the Chairman of WGBEC on the decision.

f) The ICES Secretariat will send out letters requesting proposals for work to be conducted during the Meteor workshop.

g) Dr Dethlefsen will provide members of WGBEC with a brochure describing the facilities of the R/V "Meteor".

h) The ICES Environment Officer will contact selected members of the Working Group on Marine Sediments in Relation to Pollution concerning the workshop.

i) Dr Lefevre will contact Dr Abarnou on the possibility of IFREMER, Brest, conducting chemical analyses in support of the workshop.

j) Dr Føyn will explore the possibility of having chemical analyses for the workshop conducted at the Institute of Marine Research in Bergen, Norway.

k) Drs Boon, Føyn and Marquenie will coordinate, with Dr Topping, details on chemical analyses for the workshop.

l) The Planning Group will meet before the 1988 ICES Statutory Meeting to prepare the paper detailing workshop plans to be presented at the Statutory Meeting. At the same time, the Planning Group will review proposals.

m) The Planning Group will solicit background papers on environmental conditions (including timing of specific events, such as dumping or incineration) at sites proposed for evaluation during the workshop.

n) The Shelf Seas Hydrography Working Group will be consulted for advice on the selection of appropriate hydrographic measurements to be made during the workshop.

o) All WGBEC members should consider making suggestions to individuals who could prepare techniques manuals. Mr Lloyd will explore the possibility of developing a techniques manual on the larval oyster bioassay. Dr Dethlefsen will contact specialists to explore the possibility of developing a techniques manual on methods using fish eggs and larvae.

14 RECOMMENDATIONS

The Working Group recommended that the next meeting be convened for four days during the spring of 1989 at a venue to be chosen. Possible venues include Cuxhaven, Federal Republic of Germany; Aberdeen, Scotland (to meet with Dr Topping of the Marine Chemistry Working Group); and the Hague, the Netherlands.

The WGBEC agreed to the following agenda items for the next meeting:

1) to complete the final stages of planning for the Bremerhaven Workshop;

2) to develop plans for publication of the workshop results and proceedings;

3) to develop plans for a Special Meeting in association with the 1990 ICES Statutory Meeting;

4) to review draft leaflets on methods submitted during the year;

5) to discuss the best way to integrate biological effects techniques, tested at the IOC/GEEP Oslo Workshop and to be tested at the ICES/IOC workshop, into international monitoring programmes.

6) to review biological effects approaches for evaluating the bioavailability and bioaccumulation of contaminants from contaminated sediments;

7) to review the results of IOC/GEEP Workshop activities conducted during 1988 and early 1989;

8) to discuss the occurrence and effects of planar organic molecules on marine organisms, with particular emphasis on the affinity of lipophilic contaminants for the aryl hydrocarbon receptor.

This recommendation is attached as Annex 8.

As all business was complete, the Chairman thanked the participants for their contributions and adjourned the meeting at 13.15 hrs on 8 April 1988.

NOTE: At the end of May 1988, the Federal Republic of Germany changed its offer from the use of the sea-going vessel "Meteor" in September 1989 to the use of laboratory facilities at the Alfred Wegener Polar and Marine Research Institute in Bremerhaven plus the use of two smaller research vessels in late March 1990. No change could be made in the report to reflect this new situation, Annex 3, which contains the proposal for the workshop and the letter to prospective participants, has been changed accordingly.

ANNEX 1

WORKING GROUP ON BIOLOGICAL EFFECTS OF CONTAMINANTS

Copenhagen, 5 - 8 April 1988

Agenda

1. Opening of the Meeting
2. Adoption of Agenda
3. Council Business related to the Working Group
4. Review of the Oslo Workshop Biological Monitoring Techniques
5. The North Sea Workshop on the METEOR
6. Interactions with the Benthos Working Group
7. Interactions with IOC/GEEP
8. Pathology Working Group Workshop
9. Contributions to the ICES Series "Techniques in Marine Environmental Research"
10. Reports on Biological Monitoring Activity in Member Countries: Unified ICES Activities
11. IOC Mussel Watch and Biological Effects Monitoring
12. Other Business
13. Future Activities and Assignments
14. Approval of an Action List and Recommendations
15. Closure of the Meeting

ANNEX 2

WORKING GROUP ON BIOLOGICAL EFFECTS OF CONTAMINANTS

Copenhagen, 5 - 8 April 1988

List of Participants

Dr. J.P. Boon	Netherlands Institute for Sea Research (NIOZ) P.O. Box 59 1790 AB Den Burg, Texel THE NETHERLANDS
Dr. V. Dethlefsen	Bundesforschungsanstalt für Fischerei Institut für Küsten- und Binnenfischerei Aussenstelle Cuxhaven Niedersachsenstrasse 2190 Cuxhaven FEDERAL REPUBLIC OF GERMANY
Prof. I. Dundas	University of Bergen Department of Microbiology and Plant Physiology N-5000 Bergen NORWAY
Mr. L. Føyn	Institute of Marine Research P.O. Box 1870 N-5024 Bergen NORWAY
Dr. F. Galgani	IFREMER Centre de Nantes B.P. 1049 44037 Nantes FRANCE
Dr. G. LeFevre-Lehöerff	IFREMER Centre de Brest B.P. 70 29263 Plouzané FRANCE
Mr. R. Lloyd	Fisheries Laboratory Ministry of Agriculture, Fisheries and Food Remembrance Avenue Burnham-on-Crouch Essex CMO 8HA UNITED KINGDOM
Dr. J.M. Marquenie	Ministry of Transport and Public Works Tidal Waters Division P.O. Box 20904 2500 EX The Hague THE NETHERLANDS

Dr. J. McDowell Capuzzo	Woods Hole Oceanographic Institution Biology Department Woods Hole Mass. 02543 USA
Dr. J.G. McHenery	DAFS, Marine Laboratory P.O. Box 101, Victoria Road Aberdeen AB9 8DB UNITED KINGDOM
Dr. J. Pawlak	ICES Palægade 2-4 DK-1261 Copenhagen K DENMARK
Dr. B. Serigstad	Institute of Marine Research P.O. Box 1870 N-5024 Bergen NORWAY
Dr. T. Stebbing	Plymouth Marine Laboratory Prospect Place The Hoe Plymouth, Devon UNITED KINGDOM
Dr. F. Thurberg (Chairman)	Milford Laboratory NEFC/NMFS 212 Rogers Avenue Milford CT 06460 USA

ANNEX 3

ICES/IOC BIOLOGICAL EFFECTS TECHNIQUES WORKSHOP, BREMERHAVEN, FEDERAL REPUBLIC OF GERMANY, MARCH 1990

A. Rationale

The ICES (International Council for the Exploration of the Sea) Workshop in Beaufort, N.C. (USA) (McIntyre and Pearce, 1980) attempted first to identify a suite of biological effects techniques that could be incorporated in monitoring programmes. However, there is still no international biological effects monitoring programme in the ICES area, despite strong recommendations by the Joint Monitoring Group of the Oslo and Paris Commissions.

It has, therefore, been proposed to hold a Workshop in the North Sea during March 1990 on "ecotoxicological" monitoring techniques, in order to test a number of methods which have shown promise for incorporation in international monitoring programmes.

The term "ecotoxicological" is used in this context as a reflection of the fact that neither biological nor chemical data can be used alone to identify any hazardous effects of toxic contaminants. The necessity to have appropriate chemical data is reflected in the emphasis given in this proposal to the analysis of contaminants in water, sediments and tissues that will be related to biological effects data.

It is the hope of those planning the Workshop that as many scientists as possible with an interest in this field will wish to participate, both in the biological studies and in the associated chemical analyses. Such a venture cannot succeed without strong support from within the ICES community of scientists.

It is intended that the format of the Workshop will accommodate two parallel components. The first will be practical application and testing of short-term experimental bioassay techniques. The second component will involve techniques specialising in detecting changes in the plankton, benthos and fish at the individual and population levels, related to contaminant gradients. The two groups will be provided with opportunities to interact in a way that may give rise to novel approaches.

B. Collaboration

A Workshop with these objectives was proposed by the ICES Working Group on Biological Effects of Contaminants at its meeting in Copenhagen in May 1987, and it was considered advantageous that the Workshop be mounted in collaboration with IOC/GEEP (Intergovernmental Oceanographic Commission/Group of Experts on the Effects of Pollution). This is in recognition of their similar objectives and to benefit from GEEP's experience in conducting a Workshop at the University of Oslo in August 1986 (Bayne *et al.*, 1988). ICES has approved in principle the conduct of this Workshop (C.Res.1987/3:5) and established a Planning Group, including an IOC representative, to proceed with making the necessary arrangements for the Workshop. The responsibilities of the Group are:

- to invite and select prospective participants,
- to outline operating procedures, and
- to prepare a cruise timetable and programme.

C. Scientific Topics

There is a growing awareness that, as contaminants tend to accumulate at interfaces in the marine environment (air/sea, sediment/sea, halocline, thermocline), toxic threshold concentrations may be exceeded at these levels in the water column and effects on the biota are most likely to occur there first. While we would welcome suggestions for any alternative focus, we propose to give emphasis to the deployment of biological techniques to detect toxic effects by means of:

1) benthic studies, including analysis of benthic community structure, sediment bioassays, and assessment of a suite of biological effects techniques using flatfish and epifaunal invertebrates; and

2) studies on neuston, plankton and the surface microlayer, including both observational and experimental (bioassay) approaches.

Proposed support studies should include:

1) chemical analysis of macroinvertebrates, fish tissues and sediments; water samples should be analysed whenever the scientific need arises;

2) hydrographic studies, including profiles of temperature, salinity, dissolved oxygen, nutrients, and primary productivity;

3) surface microlayer characterisation, including chemical and microbiological analyses; and

4) statistical support for the analysis of data.

Additional studies may include new and innovative techniques (such as molecular biological and immunological studies) that can be integrated with other studies.

Benthic Infauna Community Studies

Building on the results of the Oslo Workshop, benthic studies will be conducted along defined contaminant gradients with various size groups (micro-, meio-, and macrofauna) being analysed. Results will be subjected to multivariate statistical analyses and examined for differences related to sediment chemistry. The Benthos Ecology Working Group of ICES, chaired by Dr C. Heip, will be asked to take responsibility for the design of this phase of the Workshop.

Sediment bioassays utilising infaunal species (such as amphipods) will be deployed in conjunction with benthic community studies and sediment chemistry studies to assess their utility in predicting benthic impacts.

Flatfish and Epifaunal Invertebrates

Flatfish and epifaunal invertebrates will be collected along defined contaminant gradients and examined for responses to contaminants. Techniques for evaluation will include the assessment and integration of observations on gross pathology, biochemistry (e.g., responses related to biotransformation systems - EROD, metallothionein, etc.), immunology, and histopathology. Animals collected will also be assayed for tissue concentrations of selected metals, PAHs, organochlorine pesticides and PCB congeners. Chemical analyses of sediments and benthic food sources will also be carried out.

Neuston/Plankton/Microlayer

Because of the potential for contaminants to accumulate in the surface microlayer (especially lipophilic contaminants), it is of interest to characterise the neustonic and planktonic communities that are found in surface films and define potential toxicity problems associated with elevated concentrations of contaminants in the microlayer. Studies will focus on observations of the types and abundance of organisms associated with surface films, with particular emphasis on the eggs and larvae that are available.

Shipboard bioassays will also be conducted using standard test species to evaluate the toxicity of water samples taken from the microlayer. Examination of genotoxic effects and chromosomal aberrations will be carried out using samples from both field collections and bioassays.

Contaminant analysis of microlayer samples, as well as other aspects of microlayer chemistry and microbiology will also be conducted.

Depending on the types of proposals received, studies may also be conducted in the water column, e.g., at the thermocline or halocline.

D. Sites

In order to deploy the ecotoxicological techniques on significant gradients of contamination in the North Sea, it is proposed to work transects at two sites. Possible candidate sites include:

1) the plume of the Elbe Estuary,

2) the plume of the Rhine-Meuse Estuary,

3) the sewage sludge dumpsite in the Firth of Forth,

4) the Dogger Bank,

5) the incineration site in the southern North Sea, and

6) the vicinity of an oil production facility.

E. Location and Timing

Present plans are to hold the Workshop in Bremerhaven at the Alfred Wegener Institute for Polar and Marine Research in the second half of March 1990. The Workshop will consist of a laboratory-based element to be held in the Alfred Wegener Institute and ship-based elements on two vessels (the "Victor Hensen" and another vessel provided by the German authorities).

F. Funding

The research vessels, accommodation and food for participating scientists living on board will be provided without charge. The living costs of scientists who are shore-based will have to be found from their own budgets. It is hoped that all participants will be able to cover their own travel costs and the expenses incurred transporting equipment to and from Bremerhaven. However, funds are being sought from a number of international bodies, and it is hoped to be able to provide limited support for participants unable to cover all their expenses.

G. Publication

The results of this workshop will form the basis for recommendations to ICES about the inclusion of biological effects techniques in monitoring programmes. The scientific results will be published.

H. References Cited

Bayne, B.L., Clarke, K.R. and Gray, J.S., 1988. Biological Effects of Contaminants. Marine Ecology Progress Series Vol.46 (1-3): 1-278.

McIntyre, A.D. and Pearce, J.B., 1980. Biological effects of marine pollution and the problems of monitoring. Rapp. P.V. Réun. Cons. int. Explor. Mer, 179: 1-346.

I. Planning Group Members

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INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA
CONSEIL INTERNATIONAL POUR L'EXPLORATION DE LA MER

To POTENTIAL PARTICIPANTS IN THE ICES/IOC
BIOLOGICAL EFFECTS TECHNIQUES WORKSHOP

Telefax: 01934215
Telemail: ICES.SECRETARIAT/OMNET

Our Ref C.8.p/JP/gp
Your Ref

15.08.1988

Dear Colleague,

ICES/IOC Workshop on Biological Effects Techniques - Bremerhaven March 1990

ICES and IOC are organizing a Workshop in Bremerhaven, Federal Republic of Germany, to test biological effects techniques and bioassays on selected gradients of anthropogenic contamination in the North Sea during the second half of March 1990. It is the intention to adopt the best ecotoxicological techniques available that can be deployed from a research vessel to detect and quantify those gradients. More specifically, we would like to test the techniques that are sensitive to small increments of anthropogenic inputs, which can reflect direct harm to organisms - or are precursors to such effects - and that are responsive to an important group(s) of contaminants. It is hoped by this means to identify the most appropriate and relevant techniques for incorporating in a biological effects monitoring programme in the ICES area. Our plan is to test techniques on stable gradients of contamination in the North Sea, considering in particular the sea surface and the sea bottom. A more detailed proposal is appended.

If you wish to be considered for participation, please send a proposal on one page, outlining the technique(s) you would like to deploy, appropriate sampling design, a rationale for the use and adoption of the technique, its relevance to biological resources, and citing some published account(s) of related applications. We also need to know what financial assistance would be required for your participation, and the major items of equipment you plan to bring with you. In addition, we need to know what additional items of equipment you would need to have provided. The use of the research vessels and the living expenses aboard will be provided at no cost to participants.

A small Planning Group is organizing the Workshop; the names and telephone numbers of Planning Group members are appended to the proposal should you have any queries. Please post your proposals to Dr A.R.D. Stebbing, Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH, England to arrive by the deadline of 31 October 1988.

Yours faithfully,

Janet L Pawlak

for Dr A.R.D. Stebbing

(on behalf of the Planning Group
for the Biological Effects Techniques Workshop)

ICES / CIEM | General Secretary: Dr Basil B. Parrish | Palægade 2–4, DK-1261 Copenhagen K, Denmark | Telephone: 01 15 42 25; 01 15 70 92 (General Secretary) | Telex: 22498 ices dk | Telegram: MEREXPLORATION, COPENHAGUE

ANNEX 4

INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA
CONSEIL INTERNATIONAL POUR L'EXPLORATION DE LA MER

Dr. G. Topping
Marine Laboratory
P.O.Box 101
Victoria Road
Aberdeen AB9 8DB
United Kingdom

Telefax: 01934215
Our Ref dl/C.8.p
Telemail: ICES.SECRETARIAT/OMNET
Your Ref
08.04.1988

Dear Graham,

We enclose a proposal for a sea-going Workshop in 1989 aboard R.V. Meteor to be organised by WGBEC. We have been asked by the Working Group to contact you to request the help and collaboration of the MCWG on sampling and analysis of contaminants in relation to the deployment of biological effects techniques. We are especially concerned that we adopted accepted ICES standards and protocols.

Some WGBEC members have offered the analysis of some groups of contaminants:

- Selected trace metals (Cu, Zn, Cd, Pb, Cr, Fe, Ba): Tidal waters division Rijkswaterstaat, the Netherlands. Contact persons: Dr. W. Cofino (MCWG) and J.M. Marquenie (WGBEC).

- PAHs (by GC-MS): Possibly the Chemistry Department of the Institute of Marine Research, Bergen, Norway. Contact persons; Dr. L. Føyn (MCWG and WGBEC) and Dr. J. Klungsøyr (MCWG).

- Organochlorines (HCB, HCHs, DDT-family, cyclodienes and PCB-congeners (Se-54 column). Netherlands Inst. for Sea Research, Texel, the Netherlands. Contact person; Dr. J.P. Boon (MCWG & WGBEC).

Compartments to be analysed for these contaminants include (i) Benthic macro-invertebrates, (ii) fish tissues (possibly liver and/or muscle) and (iii) a fine sediment fraction (<63 µm or <20 µm?)

When a gradient in a selected area is studied by means of biological effects measurements chemical analyses should be carried out at two stations where the contamination is expected to be at a maximum and at a minimum. It may be that further sampling for chemical analysis at a greater range of stations might be impossible for logistical reasons. Should more people be willing to analyze samples, then the number of samples to be analyzed could be increased and we welcome your suggestions for possible participants.

As the surface microlayer is an important component of our proposed work, we propose to consult with Dr. Hardy and Dr. Hühnerfuss. Therefore, some analyses of the surface microlayer and related subsurface waters might be required.

2 /.....

ICES / CIEM
General Secretary: Dr Basil B. Parrish
Palægade 2–4, DK-1261 Copenhagen K, Denmark
Telephone: 01 15 42 25; 01 15 70 92 (General Secretary)
Telex: 22498 ices dk
Telegram: MEREXPLORATION, COPENHAGUE

We would like to ask the advice of the MCWG specifically on the following matters:

- The selection of contaminants to be analyzed.
- The choice of environmental compartments for sampling.
- The sampling strategy.

We welcome any other comments you wish to make and look forward to your response. Please respond to Lars Føyn.

Yours sincerely,

Lars Føyn Joop Marquenie Ján Boon

Enclosure: Draft proposal for Biological Effects Techniques workshop on the RV Meteor in September 1989.

c.c. Dr. H. Albrecht
c.c. Dr. A.J. de Groot
c.c. Mr. S. Rowlatt

ECOTOXICOLOGICAL TESTING FOR THE MARINE ENVIRONMENT. 1984.
G. Persoone, E. Jaspers, and C. Claus (Eds). State Univ. Ghent and Inst. Mar. Scient. Res., Bredene, Belgium. Vol. 1. 798 p.

MARINE ECOTOXICOLOGICAL TESTING IN GREAT BRITAIN

R. LLOYD

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ABSTRACT

Within the past twenty years there has been a considerable development in marine ecotoxicology, reflecting the perceived need to protect the marine environment and to support regulatory action arising from legislation and conventions. Much of the research has been directed towards the effects of a few chemicals - cadmium, mercury, organochlorine pesticides - on various physiological or behavioural responses of a variety of marine organisms. The results of few of these studies have been useful in the derivation of water quality standards for these chemicals.

Standard toxicity tests with new products and effluents have been developed within the framework of regulatory requirements in parallel with these more research orientated projects. In general, these tests measure the mortality of "standard" test organisms within a short exposure period. Much of the controversy surrounding the relative merits of acute lethal and chronic sublethal tests derives from a lack of understanding of the way in which the results should be used.

Within a regulatory framework, standard toxicity test data provide an indication of the toxic properties of the products or waste, which, together with other relevant information and data on likely environmental concentrations, forms the data base for an initial hazard assessment.

Special tests may be required for products which present unusual problems or which are used in specific situations, of which alternative oil based drilling muds and oil spill dispersants are examples.

An integral part of the regulatory framework is subsequent environmental monitoring for biological effects. In both fresh and marine waters such monitoring has been confined mainly to benthic communities. Where harmful effects on organisms have been found, laboratory tests with the appropriate species can be designed to find the possible cause of the observed response. An example is the investigation of the cause of the shell deformation in oysters. There is also a need for a sensitive bioassay to test for harmful substances in the water column, and work has been carried out with hydroids, oyster embryos and mussels.

It has become abundantly clear that the nature and extent of the information required on the toxic properties of chemicals, products and effluents to fulfil different needs must be clearly defined at the outset, and tests should then be designed to provide the relevant data. Particular attention is given to the extent to which changes in the composition of marine communities are acceptable.

KEYWORDS

Marine ecotoxicology, Hazard assessment, Bioassays, Legislation, Great Britain, Review.

INTRODUCTION

Historically, the major concern for aquatic pollution has been confined to freshwater and estuaries, where there have been dramatic examples of devastated environments caused by industrial and domestic discharges. This problem has been recognised for over 100 years and a succession of legislative actions have been implemented in order to achieve some measure of control. In consequence there has been a substantial improvement in the state of UK inland waters and estuaries in recent decades. But very few, if any, of our rivers are in a "natural" condition ; abstractions,

discharges, land drainage schemes, and modified land use have all contributed towards changing the watercourses and the biota which they contain. Also, industrialisation around estuaries, with the construction of ports and docks, has substantially modified the physical environment and the associated aquatic biota. Generally, such changes in habitat and species have been accepted by the public, together with the main criterion of pollution which is whether populations of fish are reduced or absent. This criterion is embodied in all relevant UK freshwater pollution legislation. A similar criterion applies to the passage of migratory fish through estuaries. For this reason much of the freshwater toxicological information is for species of fish, and protection of invertebrates and plants is a subsidiary factor.

A different situation exists for coastal and offshore waters. In these areas there is no history of chronic pollution. Concern was raised in the 1960s about the threat to marine biota by the discharge of persistent substances, such as organochlorine pesticides, which were detectable by refined analytical techniques in ocean waters far from their source. However, in the absence of field evidence of an adverse impact of such substances on marine ecosystems, and because the ecological consequences of sensitive organisms being replaced by other more resistant species were largely unknown, the general consensus of opinion within the scientific community has been that all species should be protected. This is in contrast to freshwater experience where effects of chronic pollution (especially organic pollution) on biota have been measured and quantified, and where modifications of the biotype are generally considered acceptable so long as the main uses of the river (potable water supply, fisheries, etc) are not affected. It may also explain why only a minority of marine toxicological tests have been carried out with fish, despite the importance of these species as a resource.

This strong concern by the scientific community and the general public to protect all marine species has had two main consequences. Firstly, there has been a considerable expansion of research to measure a wide range of responses to persistent chemicals in a comprehensive selection of species of marine organisms. Secondly, this was accompanied by UK legislative activity derived from a number of Conventions (Oslo, Paris, London), and EC Directives leading to the Dumping at Sea Act 1974, and the implementation of Part II of the Control of Pollution Act 1974 which extends the control of discharges to all estuarine and coastal waters. These regulations cover two

main areas of pollution control strategy : the setting of water quality standards for specific chemicals which are considered to be either potentially harmful to marine biota or accumulated by species exploited for human consumption, and the control of discharges of complex wastes or products which, as in the case of oil spill dispersants, may represent a unique situation requiring a unique approach. This paper reviews the present position in these two areas of pollution control strategy and raises several issues for further consideration.

WATER QUALITY STANDARDS FOR PERSISTENT CHEMICALS

In retrospect, it was probably inevitable with the historical background outlined above that much of the early marine pollution research was biased towards the responses of various organisms rather than the critical concentrations of the chemical which, if exceeded, would threaten their survival. There were in existence a large number of research programmes concerned with the natural biochemical, physiological, behavioural and reproductive activities of a wide range of marine species, and the opportunity was seized to measure the effects of persistent chemicals on these processes. It is understandable that these experiments focussed more on the effects than on the concentrations of pollutants which caused them, and this is reflected in experimental shortcomings such as the lack of control of the concentration of chemical to which the organism was exposed, or the duration of exposure. Frequently, solutions containing very low ambient pollutant concentrations were not renewed, or concentrations in excess of the solubility were used so that in the absence of chemical analyses the actual exposure concentrations are not known. Also, there was little attempt to relate the response measured to the survival or well-being of the species in its natural environment. As a result the observed "effects" stand in isolation and generally their significance is unclear. Also, very often the concentrations of chemicals required to produce these effects were far in excess of those likely to be found in marine waters. As a result, much of the recorded information is of little value in the preparation of water quality standards for marine species.

One of the outcomes of this general research activity was the realisation that, if the persistent pollutants were having an effect on the marine environment at the concentrations found to be present from chemical surveys in the field, it must be through subtle effects which were not

apparent from field observation, nor from laboratory tests in which responses were found only at higher concentrations. This led to a search for sensitive responses by organisms which again became a goal in itself. The significance of the response in terms of survival of the species, or even whether the response was adaptive rather than deleterious, was rarely considered. For example, what is the ecological importance of a small but statistically significant reduction in the fecundity of a polychaete rarely found in UK waters ? Questions such as this are difficult to answer.

This broad critique of the marine pollution research programmes is, of course, a generalisation and should not be taken to imply that all the work has been of little value. But it is frustrating to those who, partly in response to legislation derived from the EC Directive on the Discharge of Dangerous Substances, have to derive valid water quality standards for the protection of marine organisms for a number of metals and persistent organic substances, and who find that much of the published literature is an inadequate contribution to the sound scientific data base from which such standards should be derived. This is a universal criticism and is not confined to tests carried out in the UK. It does, however, highlight some general principles. The value and limitations of the data obtained from the wide variety of ecotoxicological tests should be clearly understood. Within recent years attempts have been made to construct a conceptual framework of tiered or sequential tests designed to provide the ecotoxicological information necessary for the derivation of water quality criteria for specific chemicals (Dickson et al., 1979). This goes some way towards identifying the roles of acute and sublethal tests, and the extent to which the data can be used for the setting of water quality standards. Nevertheless, one basic question remains largely unanswered : what level of protection is required in the marine environment ?

As stated earlier, this question has been answered for inland waters, where the aims of environmental management (both physical and chemical) have been accepted and where there are many case histories of pollution and subsequent recovery. In these circumstances the following graded options for the level of environmental protection have been proposed (Lloyd, 1980) :

1. to protect communities of fish and shellfish of economic importance (recreational or commercial) and, for edible species, ensure their acceptability for human consumption ;

2. to protect the biota (including those species which depend on aquatic plants and animals) to the extent that any changes produced are acceptable on biological or amenity grounds ;
3. to ensure that the biota undergo a minimum of change such as would be acceptable in nature reserves or sites of special scientific interest.

It is understandable that, for the reasons given earlier, only the third option is usually chosen as the basis for marine water quality standards ; but, bearing in mind the experience in freshwaters, can such an extreme approach be justified for all locations, and are there areas such as estuaries where the second option may be, or has been, accepted ? The arguments for and against the use of coastal waters for the disposal of wastes are outside the scope of this paper, but clearly the option selected plays a major part in determining the type of information required from an ecotoxicological programme. Not the least of the problems encountered with the third option is that the amount of information required on the sensitivity of representative species from all the groups of organisms at risk is beyond the capability of any reasonable experimental programme. In the absence of such information there is a tendency to use toxicity data for the most sensitive of the limited number of species tested in setting water quality standards, usually with the incorporation of an additional "safety factor" to allow for the possible existence of organisms of even greater sensitivity. But what are the ecological implications of replacing a very sensitive species in a natural community with one of greater resistance ? Providing that the former is not of commercial importance, is the substitution acceptable ?

Despite the considerable amount of research which has been carried out, for example, on DDT, mercury and cadmium, the ecotoxicological available data base allows only a tentative derivation of water quality standards for the protection of all marine organisms. In the absence of field evidence of the effects of such substances on the biota, experimental mesocosms such as those used in the CEPEX programme in Scotland (Davies and Gamble, 1979), have been very useful in identifying significant primary and secondary effects of pollutants. However, there are economic limitations to the number of pollutants singly and in mixtures which can be investigated in this way. Consequently, there is pressure to resort to the alternative solution of pollution control by zero emission or the use of best available treatment of

the effluent. This is not so much a reflection of shortcomings in the ecotoxicological data available as of the near impossibility of meeting the third option.

USE OF ECOTOXICOLOGICAL TESTS IN THE CONTROL OF DISCHARGES OF COMPLEX WASTES AND PRODUCTS

During the past decade several potential or existing pollution problems have been identified, and the five examples which follow illustrate the use of specific ecotoxicological tests to provide information for their control.

DUMPING OF WASTES AT SEA

Under the UK Dumping at Sea Act 1974, application to dump wastes is made to the licensing authority, and that authority, in considering whether or not to grant a licence, is required to "have regard to the need to protect the marine environment and the living resources it supports from any adverse consequences of dumping.....". A similar expression of intent is contained within the Oslo and London Conventions.

In order to meet this requirement, simple acute toxicity tests with fish and shrimps are carried out on all liquid wastes for which a licence to dump at sea has been requested. These tests are not mandatory in that they are not specified in detail as a legal requirement. If necessary, the test protocols can be modified to meet particular problems posed by unusual wastes. Because details of the tests and interpretation of the data obtained have been published recently (Norton and Lloyd, 1981 ; Franklin, 1982), they will not be repeated here in full. Basically, the tests measure the 1, 24, 48, and 96 h LC50 of the waste, and these data, together with LT50s for each concentration tested, are used to construct a log concentration - log median response curve. If a licence is granted, samples of the waste discharged are taken at intervals by the licensing authority, and toxicity tests are repeated to ensure that this property of the waste has not increased. If a waste contains more than trace amounts of substances listed in Annex I of the Oslo Convention, tests requested under an agreed Prior Consultation Procedure have to be carried out and the results circulated to other contracting countries before a decision whether or not to grant a licence can be made. The procedures which are followed conform to the general

principles of hazard assessment developed for new chemicals. Chemical and biological monitoring of the dumping grounds are an essential integral part of the pollution control procedure in order to provide assurance that the controls are effective.

OIL SPILL DISPERSANTS

The Dumping at Sea Act is worded in such a way as to include oil spill dispersants, which present an almost unique problem in that they are used to combat pollution. Oil spilt at sea can be a threat to marine birds, and if the slick moves inshore it can threaten resources such as shellfish beds, amenity beaches, important littoral ecosystems and salt marshes. When a decision is made to disperse the oil chemically, the concentration of oil and dispersant in the water column depends, inter alia, on the thickness of the oil slick, its amenability to dispersant treatment, the mixing energy applied during operational spraying, the extent of natural mixing and dilution within the water column, and the drift direction of the oil slick in relation to the water column. Most oil spills occur over a short period of time, and there is a limited period of a few hours in which they can be chemically dispersed. It is not possible to predict in advance either the concentrations of oil and dispersant likely to be in the water column following spraying of an oil slick or the duration of exposure by marine organisms to the dispersion. Therefore, the normal expression of a water quality standard - that the concentration of a substance should not exceed a certain level - cannot be used. Consequently, the problem has to be looked at from a different angle.

Modern oil spill dispersants are generally less toxic than oil. Efficient dispersants are generally able to disperse oil at an oil : dispersant ratio of 10 : 1 so that the additional toxicity caused by the dispersant will be less than 10 percent of that of the oil alone. (It should be noted that the dispersant is usually applied at a standard rate, so that where very thin slicks are sprayed the oil : dispersant ratio will be much less, or vice versa if the oil slick is thick.). The test protocol devised in the UK for licensing dispersants for use at sea (Blackman et al., 1977) uses the brown shrimp (Crangon crangon) as the test organism and identifies those dispersants which, at an oil : dispersant ratio of 10 : 1, do not make the oil significantly more toxic than a physical dispersion of the oil alone. This procedure takes into account the possibility of

"more-than-additive" toxic effects. It can be calculated that, under normal operational application rates of dispersant to oil, UK licensed dispersants would not increase the toxicity of dispersed oil to shrimps by more than about 3 % on the basis of concentration additive toxicity (Franklin and Lloyd, 1982). In view of this small increment in toxicity, and because the sensitivity of other marine organisms may have a different ranking from that of Crangon, there appeared to be little value in ranking dispersants in their order of toxicity. Therefore, all dispersants which pass this test are considered to be equally acceptable and as such they are licensed for use.

However, the granting of such a licence does not allow the dispersant to be used without any further controls. Protection of those biotic resources which, from published information on laboratory tests and field trials, have been shown to be particularly sensitive to dispersed oil is achieved by controlling the areas within which dispersant can be used. Thus, in waters less than 1 mile from the shoreline and less than 20 m deep, oil clearance officers must seek advice from the local officers of MAFF and the Nature Conservancy Council, as well as through a central coordinating body, before a decision is made either to spray an oil slick with dispersant, or not to spray and so allow it to disperse naturally at sea or to come ashore. The main pollution controls are placed, therefore, on the use of dispersants in sensitive areas (which may vary seasonally) ; in practice the choice may be the lesser of two evils, rather than what is safe or unsafe. In those areas where minor oil spills are not uncommon, such as in the vicinity of oil terminals and harbours, dispensation is given for the use of small quantities of dispersant in open water without prior authority being sought. In one well-monitored and well flushed area, Milford Haven, where this procedure has been used, there have been no dispersant- and oil-related effects found on the intertidal and subtidal biota despite frequent dispersant use (B. Dicks, Field Studies Council, pers. commun.).

A similar philosophy has been used for the licensing of dispersants for beach use, where early experience showed that some littoral organisms were less affected by spilt oil than by the dispersants used to clean the substrate. The criterion used in the MAFF beach test (Blackman et al., 1977) is that the dispersant should not be more toxic to limpets (Patella vulgata) than oil alone. Recent operational changes in oil spill clean-up, such as the use of aircraft to spray undiluted concentrate dispersant without subsequent physical mixing of the oil and dispersant, and technical

improvements in physical beach cleaning operations, may lead to technical changes in the test procedures but it is unlikely that the basic rationale of the tests will be modified.

OIL-BASED DRILLING MUDS

Surveys of discharges from North sea platforms showed that in some cases diesel oil was a major component. This was derived from residual diesel oil in drilling mud discharged together with the drill cuttings arising from exploitation wells. These cuttings are usually washed in diesel oil to remove the drilling mud for subsequent reuse. Typically, the cuttings have contained 10 - 15 % diesel oil on discharge, and chemical and biological surveys of the areas around the rigs have indicated that the hydrocarbons were affecting the biota. A reduction of the diesel oil content on the cuttings to 1 % was considered desirable, but this was technically difficult to achieve within the confined area of a drilling rig. Recently, alternative oils have been developed for use in drilling muds. These are refined cuts of crude oil and have a much reduced aromatic hydrocarbon content so that their acute toxicity is consequently reduced. Again, the problem of assessing the acceptability of these oils had to be solved. It was decided that oils whose toxicity to brown shrimps (Crangon crangon) was less than 10 % of that of diesel oil would be acceptable, as this was equivalent to reducing the diesel content of cuttings to < 1 % (Blackman et al., 1983). To have attempted to derive "safe" concentrations of these alternative oils to a wide range of benthic organisms would have been very time-consuming and extremely difficult. No attempt has been made to rank the toxicity of the oils, except in a very general way. Preliminary tests with microcosms have confirmed the general validity of this approach (Blackman et al., 1983). A cooperative programme is in progress with the Norwegian State Pollution Control Authority, in which oil-based drilling muds or their aqueous extracts are subjected to toxicity tests using several species of marine organisms.

In this example, both the input sites and the effects of existing discharges are known. Simple toxicity tests have identified substitute products of lower toxicity whose use should lead to a reduction in some of the observed environmental effects in the vicinity of oil exploitation drilling rigs.

CONTROL OF CHEMICALS USED AT OFFSHORE SITES

Following concern expressed at the rapid increase in the use of a wide variety of chemicals by the offshore oil and gas industry, a voluntary "Notification Scheme for the Selection of Chemicals for use Offshore" was set up by the UK Department of Energy. Under this scheme, materials to be used have to be notified to the Department, with information on their chemical properties, acute toxicity to aquatic organisms, and prospective scale of use (Blackman, 1982). Based on the toxicity data, chemicals are placed in one of six categories of decreasing toxicity, and the industry is required to notify the Department if the annual usage at each site exceeds an agreed tonnage, *e.g.* : category 1 (most toxic) - all uses to be notified; category 5 - use in excess of 1 000 tonnes per installation per year to be notified.

Although the ranking scheme is crude, it does identify those products which are potentially the most harmful and which can be substituted by less toxic substances. Also, the information is used as a basic input when assessments have to be made of the potential harmfulness of pipeline discharges, especially those used for hydraulic tests of the pipelines. No attempt has been made to formalise the toxicity test protocols. As with the UK Pesticides Safety Precautions Scheme, the data submitted by the notifier are assessed and, if found to be unsatisfactory, further data are requested and advice given on acceptable test protocols. At present, this scheme is still in the information gathering stage.

USE OF SENSITIVE TOXICITY TESTS AS BIOASSAYS IN MONITORING PROGRAMMES

Traditionally, the major components of monitoring programmes have been chemical analysis of the water and biota (including sentinel organisms such as the mussel, and also fish and shellfish for human consumption), and surveys of the spatial and temporal distribution of benthic organisms. More recently there have been a number of initiatives to develop the use of responses by sensitive organisms to bioassay the "biological" quality of the water column (Stebbing *et al.*, 1980). Within the UK, the responses range from lysosomal fragility (Moore, 1980) and scope for growth (Bayne *et al.*, 1979, 1981) in mussels, growth, gonozooid production and stolon curving of a colonial hydroid (Stebbing *et al.*, 1983), changes in glycolytic enzymes of a polychaete (Pearson and Blackstock, 1984), and embryonic development of the oyster (J.E. Thain, in preparation).

These sensitive responses are not specific to discharged chemical substances, as recent experience indicates that, at least for oyster embryo development, detrimental water quality can be caused by natural events. For example, water samples taken during a bloom of Gyrodinium aureolum off the south-west coast of England inhibited embryo development even after the algae were removed by filtration (J.E. Thain, in preparation).

It is important to recognise the limitations of such bioassays. A nil response indicates that there are no substances present in the water at concentrations which produce the response in the test organism. A positive response indicates that such substances are present, but their identity is usually unknown. They may be derived from either natural events or anthropogenic sources. An exception to this principle is when the bioassay is used at sites to detect changes in water quality which could be caused only by a known input. For example, the oyster embryo bioassay has been used to measure changes in water quality below an oil slick before and after aerial spraying with a dispersant (Cormack, 1983). In this case, useful information was obtained about the movement of the dispersant through the water column.

ECOTOXICOLOGICAL TESTS TO INVESTIGATE THE CAUSE OF AN OBSERVED ENVIRONMENTAL IMPACT

If reliance is to be placed on biological monitoring programmes to show whether or not marine communities are adversely affected, there should be evidence that the cause or causes of such impacts can be identified. An example of such an investigation has arisen from the poor performance of Pacific oysters (Crassostrea gigas) observed in east coast waters of the UK. The main symptom was excessive thickening and chambering of the shell and a much reduced growth rate of the flesh. Surveys in France indicated that tributyl tin antifouling compounds leaching from pleasure craft may be responsible for this phenomenon (Alzieu et al., 1980, 1982). Chemical surveys of UK estuaries showed that some contained significant concentrations of tributyl tin compounds, particularly in the vicinity of marinas, and that oysters which showed excessive shell thickening also contained elevated levels of tributyl tin (Waldock and Miller, 1983).

Laboratory tests in which young C. gigas were exposed to clean water, tributyl tin oxide, and sediment with and without tributyl tin, confirmed that concentrations of tributyl tin similar to those found in the survey water samples could accumulate in the oyster tissues to levels equivalent to those associated with excessive shell thickening in the field, and that similar thickening occurred in the laboratory oysters during the 8 week exposure period, especially when suspended solids were present (Waldock and Thain, 1983). Further field surveys with oysters of different species relaid in clean and contaminated estuaries, together with associated laboratory experiments, are in progress.

It is not surprising that the use of a pesticide in a paint formulation designed to leach or produce a renewable surface which kills the most resistant organism settling on it, could give rise to local environmental concentrations which are sufficient to produce a sublethal response in a sensitive organism. This example illustrates the paramount need for good chemical analytical data within a properly designed laboratory and field experimental programme, to confirm a causal relationship between an observed adverse effect and a pollutant. It is possible, however, that in this instance other estuarine organisms are of a similar, or greater, sensitivity to antifouling compounds (Thain, 1983) and that their responses have gone unnoticed.

CONCLUSIONS

In both freshwater and marine ecotoxicological research, much of the effort has been directed towards the design of tests with a wide range of species and which satisfy criteria such as statistical validity, precision and accuracy, as described elsewhere in this Symposium. There has been little attempt to define the degree of environmental protection required in such a way that these test data provide unequivocal information for pollution control purposes. The absence of such definitions can lead only to controversy on the extent of the ecotoxicological information required for environmental protection.

Of the three graded options for water quality objectives given earlier in this paper, the first, to protect the communities and quality of edible species, is the simplest in terms of the ecotoxicological information required : tests on the toxicity of the discharge to commercial fish and

shellfish species, and on bioaccumulation if necessary, are relatively easy to carry out, and subsequent monitoring can show whether the objective is being met. The second option, to protect biota to the extent that any changes are acceptable, presents increasing difficulties in terms of the number of species which have to be tested, either singly or in combination. The third option, to protect almost all species, may require an excessive amount of ecotoxicological data if total proof be required that a discharge would not affect the water quality objective.

This problem associated with the third option can be overcome to some extent by carrying out a few well-chosen tests, extracting the maximum amount of useful information from them, and then making a crude estimate of the margin of safety - the difference between the predicted or measured environmental concentration of a substance and the minimum no effect concentration measured in the ecotoxicity tests. If the margin of safety is several orders of magnitude, and all other available information on the substance indicates that it has a low toxicity, then it may be assumed with reasonable confidence that its discharge will have little or no effect on marine organisms. But there is no consensus of opinion on the minimum size of the safety margin at which the risk of affecting marine biota becomes unacceptable for any of the three options. Therefore, decisions have to be made on the basis of professional judgement, and professional attitudes to waste disposal at sea can vary widely.

Even in those areas where limited environmental damage is accepted, such as in the so-called "mixing zones" close to discharge points, there are no criteria for biological changes that are considered unacceptable, or for the spatial limits of these zones of acceptable impact. Since any such effects would have to be seen in the context of the population variations arising from natural physical, chemical and biological fluctuations, the derivation of a set of basic rules poses considerable difficulties.

This leads to the second major problem which faces the marine ecotoxicologist concerned with pollution control. Should he carry out an extensive range of tests on a substance or waste, using sensitive but non-specific response, before making an assessment of the potential hazards, or is it feasible to carry out a much smaller number of acute toxicity tests, use generous margins of safety and then rely on biological monitoring programmes to reveal any unsuspected and unacceptable responses by the

marine biota ? In the UK the tendency has been to adopt the former approach for those substances for which water quality standards need to be set, and the latter approach for complex effluents and products.

Whichever approach is adopted, the difficulty in predicting potential environmental impact from a limited number of toxicity tests necessitates that waste disposal programmes should include physical, chemical, and biological monitoring as an integral part of the pollution prevention strategy. The results of biological monitoring programmes are usually assessed subjectively in order to determine whether observed changes are acceptable, but they are unlikely to demonstrate the presence of subtle effects which may be swamped in natural background variability. Consequently, as with mesocosm experiments, only dramatic effects are likely to be revealed. But in the absence of dramatic effects, are subtle effects important ? Again, the answer must depend on the degree of protection required for marine organisms. Until this question is resolved, the quest for sensitive species and sensitive responses in laboratory tests and field surveys will continue, and the pressure to set water quality standards or control discharges on the basis of the lowest concentration which has produced a response of any type will remain.

Sufficient experience has been gained over the past decade to make it feasible to solve these problems, using case histories of known inputs and observed effects in which ecotoxicological tests (sometimes specially designed) have played a well-defined role. Such a rationalisation would go a considerable way towards resolving the differences which exist between the various national pollution control strategies. It would also place the use of ecotoxicological tests within a better-defined framework.

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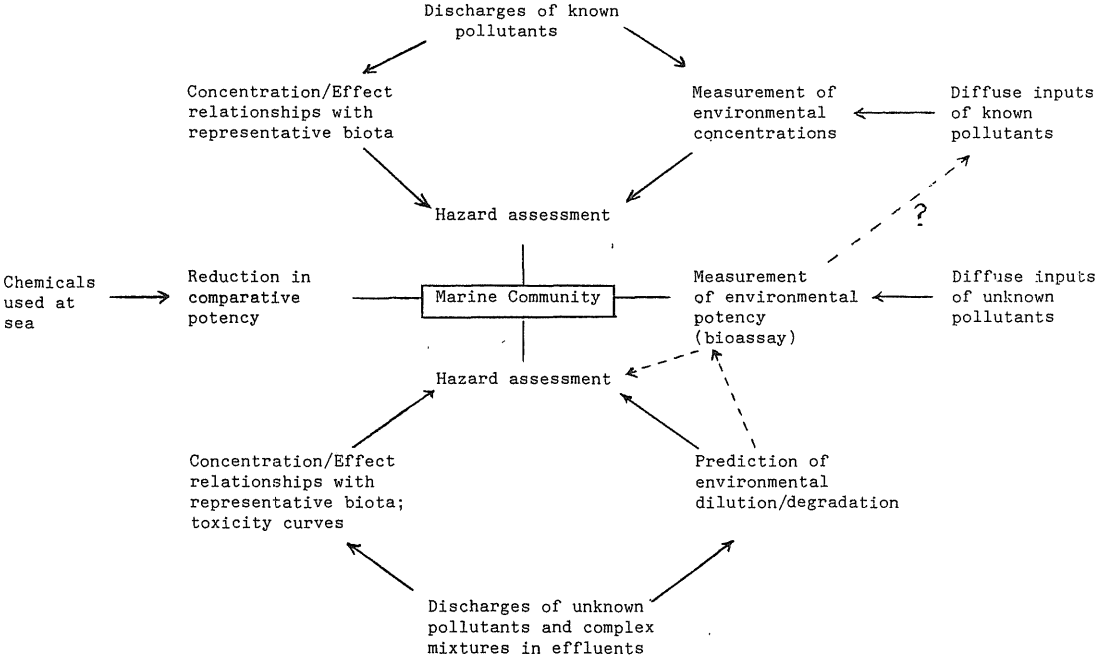

ANNEX 6

FRENCH MONITORING PROGRAMME

IFREMER surveillance

During recent years, biological monitoring programmes have been developed by IFREMER, France. These programmes are mainly concerned with the following topics:

- assessment of thermal effects on benthic and planktonic communities near nuclear power plants,
- the relationship between nutrient fluxes and eutrophication, and
- monitoring of contaminants in sea water and biota (mussels, fish, shrimps).

These programmes have been involved in evaluation of both short-term and long-term responses since 1975.

A new approach in monitoring activities is the surveillance of biological effects to link levels of contaminants with modifications of ecosystems, populations, and species.

The most important aspects of the French programme on biological effects monitoring cover:

1) the responses of different trophic levels,
2) comparison of responses for the same species in heavily contaminated areas and a reference area,
3) major pollution problems in France, and
4) studies of transport, chemical transformation, and bioaccumulation in organisms.

Studies of the responses of different trophic levels include the measurement of

- hydrological parameters (continuously recording stations),
- plankton (phytoplankton and zooplankton), and
- pelagic and benthic herbivores and carnivores.

In the first step, molluscs and flatfish are selected: Mytilus edulis, Ostrea, Pecten maximus, Limanda limanda, Pleuronectes platessa, Solea solea

The sites selected are the Bay of Seine which is a very polluted area, and one or two areas in the west of Brittany with low levels of contaminants.

The main contaminants studied are PCBs, PAHs, mercury, lead, cadmium, and copper.

Both physiological and biochemical monitoring techniques have been used.

In the physiological approach, the main study concerns the physiology of reproduction using the embryo development of molluscs with histological observations and bioassay tests.

For the group of flatfish, two methods are also used:

The first method is to establish a relationship between levels of con taminants with high solubility in lipids (e.g., PCBs) and the physiological state of the reproductive organ (R.G.S. Index). The fertility of females, and the concentration of contaminants in the lipid fraction of organs are being evaluated.

The second method is to link the levels of contaminants and the physiological state of mucus cells on the skin of flatfish with levels of contaminants in sediments.

The programme concerning the biochemical aspects of monitoring will be developed in five different stages:

1) The development of methodology. The aim of this work will be the automation of measurements of biochemical parameters (including P450 and metallothioneins);

2) The study of physiological variations (seasonal variations, growth and sexual maturation variations) of biochemical parameters;

3) In vitro contamination experiments. The aim of this work is to know the levels of contaminants that induce biochemical modifications;

4) Development of field monitoring experiments. The programme includes the measurements of P450 in the Bay of Seine fish and metallothioneins in mussels in the Gironde; and

5) Actual levels of contamination in mussels and fish along the coast of France.

The results obtained from the whole programme will lead to the development of a national monitoring programme of biochemical and physiological parameters.

ANNEX 7

SEDEX (Sediment Experimental)
A Dutch joint study on the effects of dredged material in
Model Tidal Flat Ecosystems (MOTIF)

Participating institutes in this joint project are:

Tidal Waters Division (DWG)
Netherlands Institute of Sea Research (NIOZ)
Institute for Nature Management, Estuarine Division (RIN)
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Summary

Predictions and/or assessments of possible effects of dumping materials on the receiving marine environment are often difficult to make on the basis of measurements in the field, where a number of other activities and complex interacting factors are present. Therefore, a mesocosm study SEDEX (Sediment Experimental) was initiated in 1987 in order to highlight the effects (and interacting processes) of contaminated dredged material on the structure and functioning of tidal flat ecosystems.

Pathways and effects of contaminants in combination with possible eutrophication effects are being studied in Model Tidal Flat (MOTIF) ecosystems.

Four experimental systems were used, each one comprising a MOTIF (the receiving system) and, in parallel, a MODUS (representing the Model Dumping Site). The experimental design was as follows: a) indirect loading via waterphase; b) direct loading via sediment; c) a combination of a) and b); and d) tidal flat sand as control.

Preliminary results of the 1987 study are summarized below:

1) Vigorous algal growth (eutrophication phenomenon), with a shift to small-sized algae, and increased bacterial numbers were observed in the loaded/contaminated systems in comparison with the control.

2) The contaminants seemed to be bound to the dredged material sediment phase and to suspended matter (algae). Mobilisation and transport (in solid form) were probably promoted by algal growth.

3) The concentrations of contaminants in the water phase and mussels (indicator of level of contamination in the water phase) were relatively low in the loaded systems and more or less comparable with those in the control system.

4) Zooplankton showed a species-specific behaviour per treatment, which may be related with the development of certain algal species.

5) Both meio- and macrobenthos showed different species-specific behaviour in the contaminated systems.

From the results it is hypothesized that eutrophication effects of dredged material overrule the effects of organic/inorganic micropollutants in the water phase. This hypothesis will be tested in 1988, using various dredged materials with different levels of (eutrophying) nutrients (N and P) and comparable concentrations of micropollutants.

ANNEX 8

RECOMMENDATIONS

Recommendation 1

The Working Group on the Biological Effects of Contaminants recommends that the next meeting of the Group be held for four days in spring 1989 at a venue to be chosen to carry out the following tasks:

1) to complete the final stages of planning for the Bremerhaven Workshop on Biological Effects Measurements;

2) to develop plans for publication of the workshop results and proceedings;

3) to develop plans for a Special Meeting in association with the 1990 ICES Statutory Meeting;

4) to review draft leaflets on methods submitted during the year;

5) to discuss the best way to integrate biological effects techniques, tested at the IOC/GEEP Oslo Workshop and to be tested at the ICES/IOC Bremerhaven workshop, into international monitoring programmes.

6) to review biological effects approaches for evaluating the bioavailability and bioaccumulation of contaminants from contaminated sediments;

7) to review the results of IOC/GEEP Workshop activities conducted during 1988 and early 1989; and

8) to discuss the occurrence and effects of planar organic molecules on marine organisms, with particular emphasis on the affinity of lipophilic contaminants for the aryl hydrocarbon receptor.