This report not to be quoted without prior reference to the Council*

International Council for the
Exploration of the Sea

C.M.1989/E:19

Session T

REPORT OF THE WORKING GROUP ON THE BIOLOGICAL EFFECTS OF CONTAMINANTS

Aberdeen, Scotland, 9-12 May 1989

This document is a report of a Working Group of the International Council for the Exploration of the Sea and does not necessarily represent the views of the Council. Therefore, it should not be quoted without consultation with the General Secretary.

*General Secretary
ICES
Palægade 2-4
DK-1261 Copenhagen K
DENMARK

TABLE OF CONTENTS

---ooOoo---

REPORT OF THE WORKING GROUP ON THE BIOLOGICAL EFFECTS OF CONTAMINANTS

Aberdeen, 9 - 12 May 1989

1 OPENING OF THE MEETING

The Chairman, Dr F. Thurberg, opened the meeting at 9.30 hrs on 9 May 1989 and welcomed the participants. The list of participants is attached as Annex 2.

Dr John Davies welcomed the participants to Aberdeen on behalf of the Marine Laboratory Aberdeen, Department of Agriculture and Fisheries for Scotland.

After an introduction of the members of the Working Group, the minutes of the 1988 meeting were accepted without change.

2 ADOPTION OF AGENDA

The draft agenda for the meeting was accepted and several additional items were tabled for inclusion. The final agenda is attached as Annex 1.

3 COUNCIL RESOLUTIONS

Relevant resolutions from the 1987 and 1988 Council Meetings were noted including: C.Res.1987/3:5 and C.Res.1988/2:30a authorizing the establishment of a Planning Group to organize the Bremerhaven Workshop on Biological Effects Measurements; C.Res.1988/1:7, Report of the Second Sea-going Workshop on Fish Disease Surveys; C.Res.1988/2:31, Meeting of the Working Group on Marine Sediments in Relation to Pollution, Savannah, Georgia, USA, 20-23 February 1989; C.Res.1988/2:36, Meeting of the Benthos Ecology Working Group, La Coruña, Spain, 18-21 April 1989; C.Res.1988/2:26, Meeting of the Marine Chemistry Working Group, Savannah, Georgia, 13-17 February 1989.

4 FINAL PLANS FOR THE BREMERHAVEN WORKSHOP

Dr Stebbing introduced the status of preparations for the Workshop by reviewing a Draft Workshop Plan, which was prepared initially for the IOC GEEP V meeting at IMO, London (17-21 April 1989) and for the first meeting of the Planning Group for the Workshop held during the same week. The version tabled (Annex 3) incorporates modifications arising from the discussion at GEEP V.

Dr Dethlefsen introduced a document proposing a cruise track north-west from Bremerhaven, across the German Bight, indicating the track crossed a number of well documented contamination gradients (Annex 3). While there was some discussion on the definition of the gradients from the considerable amount of evidence presented, as no clearer gradient was identified, the proposal was accepted.

The availability of vessels was also reviewed and Dr Dethlefsen confirmed that the VICTOR HENSEN will now be available and we also learned that the Dutch have committed the AURELIA, as well as the HOLLANDIA.

The options for different oil platform gradients were discussed, including the possible use of platforms off Aberdeen (Shell), off Norway (Phillips Petroleum), and off Holland. It was agreed that access to a platform close to Bremerhaven for which there is a comprehensive and available data base was the most important requirement. The Dutch platform best fulfilled this requirement in that comprehensive studies had been published by NIOZ and this platform is closest to German territorial waters. As the oil platform gradient is one of the two gradients to be studied by the Benthic Group which will be conducted primarily from Dutch vessels starting out from the Netherlands, a Dutch platform is a natural choice.

The proposal to deploy techniques to detect contamination in the surface microlayer in the incineration area was examined critically, and the opinion was expressed by Dr Davies and Mr Lloyd that contamination of the microlayer was of little significance to marine ecosystems. While it was agreed that toxic contaminants do accumulate in the microlayer and that water quality bioassays may respond to the elevated concentrations, the overall relevance remains unclear. Dr Stebbing said he felt that studies reported in the recent special number of Marine Environmental Research on the biological significance of surface microlayer contamination provided sufficient grounds to examine whether or not there is a problem that available techniques could be used to monitor.

The overall budget for the Workshop was discussed at some length. Thus, far the additional costs amount to $55K, over and above those that the participants themselves might be able to cover. Some additional proposals are still expected that have significant associated costs. Various avenues for additional funding are now being explored. These include applications to:

1) IOC to support the fish pathology;

2) IMO to support the participation of two experts;

3) Industry, through IMO and LDC, to support work on the oil platform gradient and the incineration area;

4) NSF to support US participation;

5) DOE to support UK participation and analytical costs;

6) Canadian Government (Canadian Oil and Gas Administration) to support Canadian participation.

It was recognized that the contribution by the Federal Republic of Germany as the host country and the cost in research vessel time is very substantial, as is the commitment of two research vessels by the Netherlands. No further support will be sought from either country, although it was suggested that a request be put to the our hosts, the Alfred Wegener Institute in Bremerhaven, to accept the responsibility of administering the budget.

The Workshop Plan will be submitted to Marine Environmental Quality Committee and to ACMP for approval before presentation at the 1989 ICES Statutory Meeting in The Hague in October. At the same time, the plan will be submitted to IOC through the Chairman of GEEP, Dr B.L. Bayne, requesting that they provide financial support for the more research oriented element of the Workshop; more specifically fish studies and the objective identified in the Plan to integrate the various aspects of fish pathology, particularly gross pathology with sensitive indicators of biochemical and cellular damage.

One of the major reasons for the Working Group meeting in Aberdeen was to have the benefit of advice from Dr G. Topping and his colleagues, Dr D. Wells and Dr I. Davies, in identifying the analytical chemistry requirements for the Workshop. In prefacing these discussions, it was once again recognized that ecotoxicologists need to break with the tendency to expect chemistry to be provided as a service to biologists working on pollution problems. Neither chemical nor biological data alone can be expected to establish causal relationships between contaminants and their toxic effects, so we advocate, and hope for in planning the Workshop, to identify chemists as collaborators and co-authors who recognize that it is not merely the presence of contaminants in the environment, but their biological effects that are of ultimate concern.

The format of the discussion to consider the analytical chemistry for the Workshop was to first identify the kind of chemical input that would be ideally required to interpret the biological data, than to relate that to what proposals and collaboration have been proposed at this stage. Finally to identify ways of involving more chemists interested in the overall objectives of the Workshop, who would help provide an adequate chemical data base.

Considerable effort and discussion were devoted to drawing up the analytical and hydrographic requirements identified in Annex 3, representing the minimal chemical data set that would be expected to enable interpretation of the biological effects data. When related to the chemical proposals and analytical effort that has already been offered to the Workshop, it was clear that there is a considerable shortfall.

Dr Topping could identify a limited number of laboratories capable of providing analytical collaboration and agreed to make inquiries. The main constraints are that they are not only willing to participate, but that they have been involved in ICES intercalibration exercises run by the Marine Chemistry Working Group and that one laboratory could accommodate all the analyses of one group of compounds to eliminate inter-laboratory errors.

One possible way of involving a greater chemical input will be by asking participants to identify potential chemical collaborators within their own laboratories. Mr Lloyd indicated that although he could not commit his own laboratory, the possibility might be explored of seeking DOE funding for one or more period appointments at MAFF Burnham to assist with any chemical contribution.

It was recognized that the coordination of the chemistry associated with the Workshop is in itself a major undertaking and Dr Topping suggested that the Chairman of a Sub-group within the Marine Chemistry Working Group might be prepared to undertake this task. Dr Topping will make this inquiry.

5 PUBLICATION OF WORKSHOP PROCEEDINGS

A number of options were considered by the Working Group as possible publication outlets for the results of the Bremerhaven Workshop. It was noted that the first responsibility was to prepare a comprehensive report to the two sponsoring agencies, ICES and IOC. It was concluded that such a report would take the form of an executive summary followed by an abstract of each biological effects technique tested, and then a summary of conclusions and recommendations. This would be very similar to the report prepared by IOC for the Oslo Workshop. The chemistry analyses will take the longest to complete and it is hoped that the report could be finished within fifteen months following the Workshop. An article describing the Workshop results will also be prepared for publication in Marine Pollution Bulletin.

An excellent outlet for these papers would be an ICES-sponsored symposium where the papers would be formally presented as well as topic summaries presented by the section coordinators. This would also provide an appropriate forum for recommendations of those techniques proven useful at the three IOC and ICES/IOC workshops. This would provide ICES with a clear and tangible product after the long wait since the 1978 Beaufort Biological Effects Workshop.

If a Symposium were held, participants in the Workshop would be required to have papers submitted for publication at the time of the Symposium and these papers would be reviewed, edited and submitted for publication as a special volume of an appropriate marine journal. Among the journals under consideration are Aquatic Toxicology, Marine Ecology Progress Series, the proposed journal for the European division of SETAC, and the Journal of Experimental Marine Biology and Ecology.

6 REVIEW OF IOC/GEEP ACTIVITIES IN 1988 AND 1989

Dr Stebbing briefly reviewed the results of the IOC Bermuda Workshop, drawing out points that were relevant to the planning of the Bremerhaven Workshop. The Workshop was held at the Bermuda Biological Station from 10 September to 2 October 1988 for 10 invited experts and 6 scientists from the IOC Regional Programmes, with 6 other technician/scientists participating at their own expense. Some techniques were adopted from those used at the Oslo Workshop while others, such as the bioassay techniques, were new to the Workshop series. The main categories of techniques included biochemical techniques (such as those involving the MFO system and metallothioneins in fish), whole organism responses (scope for growth in bivalves and ciliate population growth), and benthic macrofaunal studies. A comprehensive chemical data set was provided, partly from among the participation of GEMSI.

One objective of the Workshop was to determine to what extent techniques developed and validated in temperate waters could be used with tropical species on unknown gradients over a relatively short period. Two supposed pollution gradients were studied: Hamilton Harbour (hydrocarbons and TBT) and Castle Harbour (dump of metalliferous rubbish used as infill at the waters edge). The contamination gradient in Hamilton Harbor correlated with biological indices such as scope for growth in Arca deployed in cages along the gradient, as well as some other indices of effect.

While the results of this Workshop will not be published until late 1989 (J. Exp. Mar. Biol. Ecol.), preliminary data indicate the value of coupling closely the different kinds of biological indices with one another and with chemical data.

Two additional workshops are under consideration by IOC at this time. A workshop in Xiamin, China and the North Sea Workshop out of Bremerhaven cosponsored by this ICES Working Group. The China workshop will have a very large training component and will test some techniques in a very new environment. This will be of limited interest to ICES as far as developing techniques that are appropriate to the North Atlantic area and their incorporation into a comprehensive ICES Monitoring Program. The Bremerhaven Workshop covered in detail above is a major IOC concern and is very high on the IOC list of priorities for 1989-1990.

7 RECOMMENDATIONS TO ACMP FOLLOWING OSLO, BERMUDA, AND BREMERHAVEN

Following a series of practical workshops to test and compare techniques that could be incorporated in international monitoring programmes, the Benthos Ecology Working Group is moving towards a position where an evaluation of techniques and specific recommendations can be made to ACMP. The IOC Oslo and Bermuda Workshops have provided a context to evaluate the predominately nearshore techniques in temperate and tropical waters, and the ICES/IOC Bremerhaven Workshop has made it possible to evaluate sea-going monitoring techniques for offshore waters.

In particular, it is intended not only to identify appropriate and relevant techniques, but to set them into an appropriate strategy initially identified by GESAMP and further developed by the ICES Study Group on the Biological Effects of Contaminants in Hirtshals 1985.

It is becoming increasingly clear to the Working Group that any strategy to monitor environmental contamination inevitably involves both biological and chemical techniques, deployed in a manner that recognizes their interdependence. Each disciplinary approach alone can at best only provide a partial interpretation of a polluting event. Monitoring programmes should, therefore, not only include biological and chemical elements, but it is a necessity that there is collaboration between both disciplines by scientists equally motivated to interpret what is happening at the interface between contaminant and organism. Good examples of investigative ecotoxicology typically involve biologists and chemists in a genuine collaboration. Furthermore, the workshop series has demonstrated that the more closely the biology and chemistry are coupled, the more readily one can interpret one data set from the other.

The Role of Biological Effects Monitoring Techniques

Biological effects of contaminants in the marine environment are more important than the mere presence of contaminants; chemical data can only be interpreted in terms of toxicological threshold concentrations. The criteria for marine environmental quality are ultimately biological, so it is logical to use biological systems by which to measure its quality. However, there are a number of other practical reasons for using biological techniques:

1) Detection of new pollutants.

   To rely on chemical methods of analysis alone pre-supposes that the potentially important contaminants are known and are monitored. Examples such as TBT demonstrate that this is not always the case and that biological systems provide the means of detecting the presence of new or unsuspected contaminants.

2) Bioavailability.

   Often chemical data do not reflect the bioavailability of contaminants due, for example, to speciation of organic compounds or the binding state of metals. By definition, biological systems can only respond to what is bioavailable and so organisms provide the most appropriate indication of bioavailable concentrations of contaminants exceeding toxic thresholds.

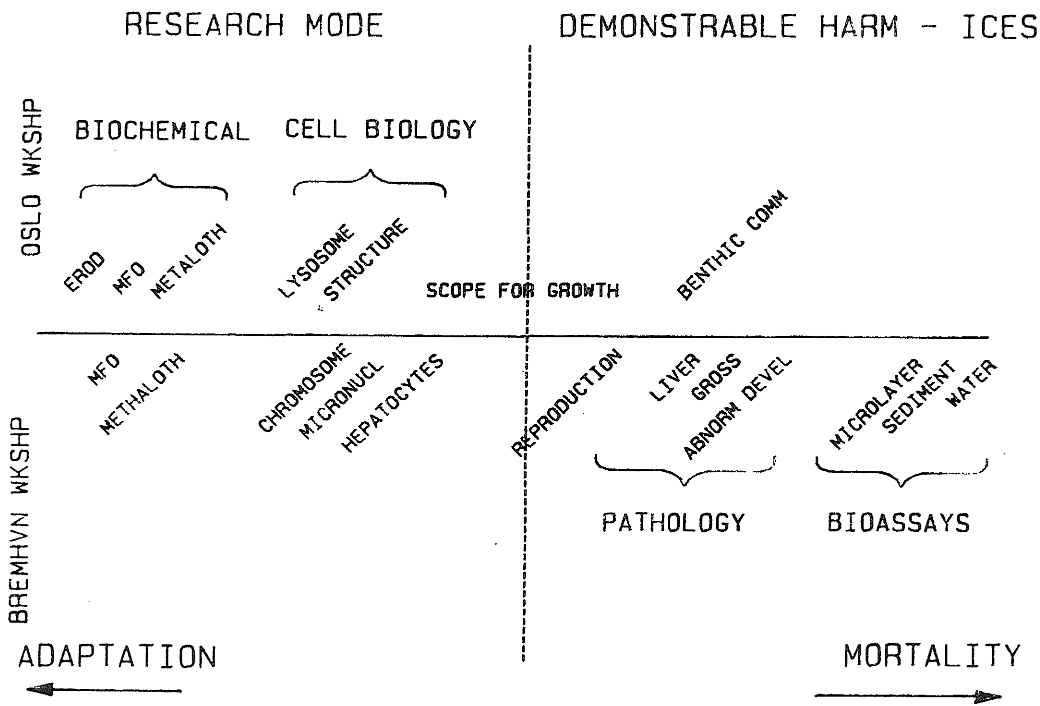
3) Integration of toxic effects.

   Typically toxic contaminants do not occur alone and it is their combined effect that determines environmental quality. Biological systems respond to the totality of the contaminated milieu, providing an integrated response to the totality of contaminants present and their interactions between one another.

4) Integration with time.

   Variations in concentrations of contaminants, particularly in nearshore and estuarine environments may vary by as much as an order of magnitude within a tidal cycle, which invalidates any single water sample as representative. It is the effect of tidal contaminants over time that is of concern, and biological systems ("sentinel organisms") and their responses provide a time-integrated response in a way that chemical data typically do not.

FIGURE 1


RESEARCH MODE
DEMONSTRABLE HARM - ICES
OSLO WKSHP
BIOCHEMICAL
CELL BIOLOGY
EROD
MFO
METALOTH
LYSOSOME
STRUCTURE
SCOPE FOR GROWTH
BENTHIC COMM
MFO
METHALOTH
CHROMOSOME
MICRONUCL
HEPATOCYTES
REPRODUCTION
LIVER
GROSS
ABNORM DEVEL
MICROLAYER
SEDIMENT
WATER
PATHOLOGY
BIOASSAYS
BREMHVN WKSHP
ADAPTATION
MORTALITY

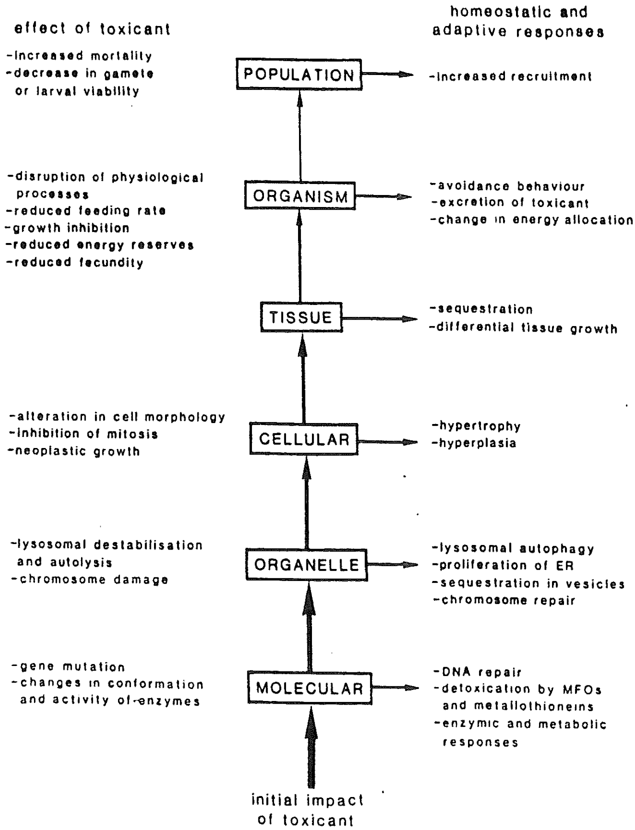
5) Cost.

The number of new environmental contaminants is growing annually and with it the number of toxic compounds to be monitored. As the lists of contaminants routinely monitored grows, so does the cost of sustaining chemical monitoring programmes. At the same time, the case for using biological techniques as reconnaissance systems is strengthened, because they could ensure that chemical analytical effort is only utilized when and where there are demonstrable biological problems.

Figure 1 illustrates the biological effects techniques employed at Oslo (above the line) and those proposed for Bremerhaven (below the line). To the right of the vertical dotted line are those techniques that have been of traditional interest to ICES - those that show an obvious demonstrable effect including bioassays, and pathological changes. These clearly result in near-term mortality. Those to the far left represent those that demonstrate an adaptive response to pollutants. These have biochemical or cell-level changes that occur here. If the contaminant stress continues, these adaptive mechanisms become overloaded and the stress is then manifested in the next higher level of organization (for example, when the cell level is overloaded, the tissue or organ level starts to become affected). This left side of the figure represents the sub-lethal area that has traditionally been recognized as a research area and has been largely ignored by ICES in the context of monitoring. This thinking is changing rapidly as the value of these measurements are demonstrated and refined. Reproduction, scope-for-growth and immunology probably fall in the middle area - they are rapidly gaining acceptance. The Bremerhaven Workshop will concentrate its efforts on the right side - the area not covered at Oslo. These are of special interest to ICES. It will also retest some of the Oslo techniques, especially the biochemical ones. These will have strong IOC support. The result will be a suite of measures that will have been carefully tested (some also at Bermuda) and from this suite this Working Group will be able to recommend those tests that provide a balance of measures from adaptive to mortality suitable for a monitoring program. We envision this set of tests will have options attached to it, so each monitoring program will have a choice of test modes so that the specific needs of a monitoring program can be met. As one can see we are very close to a well-tested and well-thought-out plan for a biological effects monitoring program.

The second figure (modified after Bayne et al., 1985) illustrates the importance of measuring the sub-lethal, adaptive end of the scale. In simple terms, it shows how each step in the biological process of contaminant accumulation copes with a pollutant. At the bottom the biochemical machinery of the cell changes to adapt to a certain level of contaminant. When the level exceeds that capacity, the next level (in this case the cellular level) is affected. The process repeats until the next level is affected. Finally, the whole organism is affected and mortality occurs. This can be extended to the population or even ecosystem level. It points out the adaptive capacity at each level; if, however, the stress (contaminant) continues then the effect progresses up the chain. It is important for managers to evaluate at what level

FIGURE 2



A hypothetical scheme suggesting how the impact of a toxicant upon an organism might pass through, and be dissipated by, its hierarchical organisation. Some effects of toxicants that we have considered, and adaptive responses to counteract those effects, are listed.

they wish to detect pollutant impact, and then to structure the monitoring program accordingly. If only mortality is important then techniques closer to the MORTALITY end should be selected; if sub-lethal, long-term exposure is important then more techniques toward the sub-lethal ADAPTIVE end of Figure 1 should be selected. Keep in mind that the ADAPTIVE END is easier to interpret in terms of cause and effect. Biochemical measures tend to be more contaminant specific; the mortality end is not as specific and mortality could be the result of any number of factors.

The Working Group feels that a review of biological effects techniques - their strengths and weaknesses, the state of refinement, their relative position on our Adaption to Mortality Array and a level of recommendation - is in order. Such a paper describing their application and interpretation would be valuable to many groups including ACMP, IOC, IMO, and member nations initiating monitoring programs. The Working Group will prepare this document and update it as necessary.

8 BIOLOGICAL CONCERNS OF CONTAMINANTS IN SEDIMENTS

According to the deliberations of the Working Group on Marine Sediments in Relation to Pollution, the question of bioavailability and bioaccumulation of contaminants from sediments can not be addressed by chemical methods alone and has asked this Working Group for information on using biological methods to quantify these phenomena. The Working Group discussed this situation at length and noted that a multidisciplinary approach is required to evaluate the role of sediments for holding contaminants, the flux back to the water, the bioavailabilty and the uptake by organisms. Sediment chemists, tissue chemists, community (benthic) biologists, specialists dealing with sediment bioassays and water extract bioassays, as well as toxicologists and perhaps physiologists and biochemists, are all required to get a full picture of the cycling of contaminant from sediments through living organisms.

A number of factors must be considered. Water tends to have a lot of contaminant availability but low concentration levels while the opposite is true of sediments. Where in the food chain does the bioaccumulation start to mean something to the survival and reproduction of a species? The top few centimeters of sediment is often covered or replaced very fast - recolonization is rapid and the contaminated material below is often quickly "biologically unavailable". These observations point to the complexity of the problem of finding an answer to an apparently simple question. The Bremmerhaven Workshop will approach a part of this problem when sediment, and sediment-extract bioassays are conducted side by side with community composition analyses while at the same time analytical chemical measurements are made. The Working Group feels that these multi-faceted studies are the only way to link the physical content of sediments to the biological considerations of availability, accumulation and effect and it will recommend this approach to the Working Group on Marine Sediments in Relation to Pollution.

As part of this discussion, Dr Ian Davies of the Aberdeen Marine Laboratory provided the Working Group with extensive advice on the sediment chemistry protocols that will be required at the Bremerhaven Workshop.

The Working Group agreed to prepare a review on biological aspects of this subject that will serve as advice to the Working Group on Sediments in Relation to Pollution and also serve as background material for the Bremerhaven Workshop.

9 AFFINITY OF LIPOPHILIC CONTAMINANTS FOR THE AH RECEPTOR

Dr Galgani led the discussion of the Aryl Hydrocarbon Hydroxylase (AHH) system and its role in biological monitoring.

For many years, evaluations of the effects of pollutants at a molecular level have been performed by the use of cytochrome P450 measurements. It has been proven recently that only specific isoforms of cytochrome P450 are involved in the metabolism of xenobiotics. Therefore, the catalytic activity of these isoforms, measured by the deethylation of ethoxy resorufin (resorufin-o-deethylase, EROD) and the hydroxylation of aryl hydrocarbon (aryl hydrocarbon hydroxylase, AHH) have been proven to be powerful techniques for monitoring the effect of pollution. Much of the basic research work has been performed by Canadian teams (Payne, Penrose, Addison). Improvement of methodology (Stegeman, USA; Coksoyr and Follin, Sweden; Galgani, France) field validation (ICES and IOC Workshops) and evaluation of natural variability are now in progress. At the present, seasonal and regional variations are being tested by several laboratories including the sensitivity to common variables such as temperature, feeding and food selection.

Invertebrate studies have shown that the MFO system is present in a number of species, but the lack of basic knowledge concerning its mechanism limits the possibility of using these organisms as models in monitoring experiments; however, the MFO system in fish does offer good opportunities for monitoring the effect of pollutants. The future of this work will be focused on improving the methodology and incorporating the method in a monitoring network.

The Working Group noted that much of the current work in this area is being conducted by Dr Payne (a member of this Working Group who could not attend this meeting) and by Dr Addison (a member of IOC who will act as a Biochemistry Coordinator at the Bremerhaven Workshop), and by Dr Stegeman at Woods Hole. A collegue of Dr Payne's, Dr W.R. Penrose prepared a review on this subject and recommended that the AHH system be used routinely as a biological indicator for monitoring oil contamination. The Working Group will consult with the three experts noted above to ascertain that this recommendation is still the current state of thinking among biochemists. In the meantime the Working Group recommends that ICES considers the well prepared paper by Penrose (Marine Pollution Bull. 9:231-234) as an interim source of information. The Working Group will also issue a recommendation in this area of effects monitoring following the Bremerhaven Workshop. (See Agenda Item 7.)

10 PREPARATION OF BIOLOGICAL EFFECTS METHODS LEAFLETS

a) Mr Lloyd introduced the draft method for the Pacific oyster bioassay that had been circulated before the meeting. This was discussed in the context of the format of the presentation, as well as the method itself. The Working Group agreed that the format was acceptable and that future leaflets should follow this format (see Annex 6 for format). After some discussion the Working Group agreed that the draft was suitable for final preparation after minor revision including the addition of a section which refers to the U.S. ASTM method, an internationally recognized procedure. Both the ASTM and the proposed ICES methods will be used at the Bremerhaven Workshop and will provide an intercomparison of their utility. The results will be available at the next Working Group meeting and these results could be included in the final version of the leaflet. Working Group members will forward editorial comment to Mr. Lloyd who will incorporate them into the final version.

b) Dr. Dethlefsen presented a paper on the use of fish eggs and larvae as sensitive indicators of pollution. The techniques are relatively simple, biological criteria analyzed are sensitive to contaminants, and reproduction is recognized as a vital and sensitive period in the life cycle of fish. The Working Group noted these methods are useful in the context of long-term monitoring and recommended that the paper be prepared in the Methods Leaflet format and resubmitted to the Working Group for further consideration.

c) Dr. Dethlefson presented a review of the use of fish disease studies in pollution monitoring programmes. The Working Group took note of the growing body of information and the increasing numbers of correlations indicating a link between the levels of pollution and the incidence of fish disease. It also took note of a number of caveats included in the review, the most important among them seemed to be: "a number of studies have failed to find the relationship between the prevalence of diseases and the supposed contaminant status of the sampling sites. Even negative correlations have been detected". The Working Group endorsed the recommendations formulated in the report including those requesting the beginning of an international program for monitoring the incidence of disease in the waters of the ICES area. The Working Group recommended that it defers preparations of Biological Effects Techniques leaflets in the fish disease area to the Working Group on the Pathology and Diseases of Marine Organisms. It noted especially the "Report of the Second Sea-going Workshop on Methodology of Fish Disease Surveys" prepared by the Working Group on Pathology and Diseases of Marine Organisms. The Working Group has had close relations (including joint sessions) with the Pathology Working Group and will continue to watch with interest the progress in developing fish disease as a monitoring technique.

d) Dr Galgani described new biochemical techniques for acetylcholinesterase, EROD, and metalothioneins that are now being introduced into the French Biological Effects Monitoring Program. He has proposed that the new methodology employed here might provide useful material for a leaflet in the Biological Effects Techniques Series. He will develop a draft for circulation to the membership.

11 RELATIONS WITH ACMP

Dr Topping, a member of ACMP, provided the Working Group with some valuable insight into the workings of the ACMP and a description of the needs of that Advisory Group. In the past some confusion has existed within the Working Group as to the role that it might play in the responsibilities of ACMP. Certainly a new format for the minutes was in order and that change is reflected in the present report. Both Dr Topping and the Working Group feel this style will be more helpful with a shorter executive summary attached highlighting those areas of particular interest to ACMP. All attendees agreed that the progress made in developing the Bremerhaven Workshop as well as the evaluation of the Oslo and Bermuda Workshops (the report of the Oslo Workshop was not available to the Working Group last year - this led to some confusion as to why the Working Group appeared to have discussed it incompletely) will be most useful to the ACMP. A description and discussion of the role of biological effects techniques in a monitoring program was also deemed beneficial to ACMP and other user groups of the Working Group deliberations, and that discussion is included here. A clearer listing of Action items and Resolutions is also included. By the end of the meeting both the Working Group members and the invited observers felt that a much better link between the needs of ACMP and the deliberations of the Working Group had been forged.

12 NATIONAL PROGRAMMES

Several international programs dealing with contaminant monitoring were presented. Drs Galgani and LeFevre-LeHoerff described the new French program for evaluating biological effects of contaminants (Annex 5). The sites selected initially are the Bay of Seine, a very polluted area, and one or two areas west of Brittany with low levels of contaminants. Both physiological and biochemical methods are used including bioassays, flatfish fecundity, copepod fecundity, zooplankton lipid metabolism, mucous cell biology of flatfish, EROD biochemistry, metalothioneins, and lipid and organochlorine content of flatfish and molluscs. This important program has been initiated and the French Working Group members invited the Working Group to meet in Nantes next spring where this program will be discussed in considerably more detail. This is the second invitation for the Nantes venue and the Working Group will recommend accepting this initiation for the 1989 meeting.

Dr Granmo reviewed his recent visits to China and Mexico and described the contaminant problems and efforts being initiated in monitoring pollution in these countries. Few biological measures are planned at this time but Dr Granmo will report future progress in this area to the Working Group. The programs will be of interest to IOC/GEEP, however, and Dr Granmo will prepare a brief report for that group.

13 <u>OTHER BUSINESS</u>

Dr Dethlefsen presented a paper on new results from German scientists on effects of titanium dioxide waste in the Dutch dumping grounds (Annex 4). Among the observations reported are increased numbers of skin tumors in dab, increased concentrations of various heavy metals in sediments and dab tissues, and meiobenthos community changes. The Working Group noted these results with interest and felt that further information was required before general comments from the Working Group are possible.

14 <u>ACTION LIST</u>

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

a) Prepare a review paper on biological aspects of bioaccumulation and bioavailability of contaminants from sediments. Working Group members forward appropriate national papers to the Chairman.

b) Revise the Oyster Bioassay Methods Leaflet (Mr Lloyd). Working Group members forward comments to Mr Lloyd.

c) Edit, revise, and prepare the paper on the use of fish eggs and larvae as a biological monitoring method in the format of a Effects Method Leaflet and forward the revision to the Chairman for distribution to the Working Group as an intersessional activity. (Dr Dethlefsen).

d) Request that several experts review the several existing papers as well as the present status of the use of the aryl hydrocarbon receptor as an effective biological effects indicator (Chairman).

e) Prepare a review paper on biological effects techniques with emphasis on application and interpretation (all members; Drs Thurberg and Stebbing will take the lead).

f) Continue plans for the Bremerhaven Biological Effects Workshop; funding, logistics, coordination (Planning Group).

g) Conduct the Bremerhaven Biological Effects Workshop 12-30 March 1990 (Planning Group).

h) Prepare preliminary report on Bremerhaven Workshop for next Working Group meeting (Planning Group).

i) Contact potential journal sources for publication of Workshop results (Planning Group).

j) Prepare draft methods leaflet on new biochemical techniques used in the French Biological Effects Programme (Dr. Galgani).

k) Prepare a presentation on the French Biological Effects Monitoring Program for the next WG meeting (Drs Galgani and LeFevre-LeHoerff).

l) Contact the Chairman of the Sediment WG and describe the material the WG will provide on bioaccumulation/bioavailabilty (Chairman).

m) Contact the Chairman of the WG on Environmental Assessments and Monitoring Stategies to bring that Working Group up to date on our activities (Chairman).

n) Dr Granmo will prepare a brief report on contaminant monitoring in Mexico and China for IOC/GEEP.

o) Send final Bremerhaven Workshop proposal to IOC and IMO through the Chairman of IOC/GEEP (Stebbing).

15 RECOMMENDATIONS

The Working Group recommend that

- ACMP approve the final plan for the Bremerhaven Workshop;
- ACMP endorse the future possibility of sponsoring a Symposium following the Bremmerhaven Workshop as a formal forum for presenting the results and recommendations;
- ACMP consider the "Adaption - Mortality Array" as described in this report as a logical point of initiation for a program incorporating biological effects techniques;
- the Working Group on Marine Sediments in Relation to Pollution consider multidisciplinary approaches involving both chemists and biologists to assess the questions of bioaccumulation and biolavailability of contaminants from sediments;
- the Working Group meet for 4 days in late April or the first week in May (one month after the Bremerhaven Workshop) in Nantes, France. The Workshop Coordinators will be invited to attend this session. The following agenda items have been tabled for the next meeting:

  1) Report on the results of the Bremerhaven Workshop.

  2) Plan for the Publication of the Workshop Results.

  3) Prepare for Workshop Symposium.

  4) Review edited and draft biological methods leaflets; prepare first set for publication.

5) Review the French Biological Effects Monitoring Program.

6) Complete a review of biological effects techniques including a recommended suite(s) of techniques as tested at IOC and Bremerhaven Workshops. A written guide will be edited and completed.

7) Discuss sediment bioavailability/bioaccumulation review before forwarding to the Working Group on Marine Sediments in Relation to Pollution.

8) Review IOC/GEEP activities especially the cooperative effort with the Bremerhaven Workshop as well as the IOC China Workshop.

16 CLOSURE OF THE MEETING

As all business was complete, the Chairman thanked the participants for their contributions and adjourned the meeting at 1530 hrs on 12 May 1989.

ANNEX 1

WORKING GROUP ON BIOLOGICAL EFFECTS OF CONTAMINANTS

Aberdeen, 9 - 12 May 1989

Agenda

1. Opening of the Meeting
2. Adoption of Agenda
3. Council Business related to the Working Group
4. Finalize Plans for Bremerhaven Workshop on Biological Effects Measurements
5. Publication of Workshop Proceedings
6. Review of IOC/GEEP Workshop Activity in 1988 and 1989.
7. Integration of Biological Effects Techniques tested at OSLO and Bremerhaven Workshops into Monitoring Programme
8. Biological Approaches for Evaluation of Bioavailability and Bioaccumulation of Contaminants from Sediments
9. Affinity of Lipophilic Contaminants for the AH Receptor
10. ICES Biologic Effects Measurements Methods Leaflets
11. Role of the WGBEC in Meeting the Needs of ACMP
12. National Programmes Using Biological Effects Measurements
13. Other Business
14. Intersessional Activities and Assignments
15. Action List and Recommendations
16. Closure of the Meeting

ANNEX 2

ICES WORKING GROUP ON THE BIOLOGICAL EFFECTS OF CONTAMINANTS, ABERDEEN 9-12 MAY 1989

List of Participants

Name	Institution
Dr. I. Davies	DAFS Marine Laboratory PO Box 101 Victoria Road Aberdeen, U.K.
Dr. J. Davies	DAFS Marine Laboratory PO Box 101 Victoria Road Aberdeen, U.K.
Dr. V. Dethlefsen	Bundesforschungsanstalt fur Fisheries Niedersachsenstrasse 219 Cuxhaven FRG
Mr. L. Føyn	Institute of Marine Research PO Box 1870 5024 Bergen Norway
Dr. F. Galgani	IFREMER/Nantes Rue de I'le d'yeu 40039 Nantes, Cedex France
Dr. A. Granmo	Kristineberg Marine Biol Stn National Environment Protection Board P1 2130 S-450 34 Fishkbachskil Sweden
Dr. G. Lohoerff	IFREMER/Brest BP 70 29263 Plouzane France
Mr. R. Lloyd	MAFF Fisheries Laboratory Burnham-on-Couch Essex CMO 8HA U.K.

D.J.M. Marquenie	Tidal Waters Division RWS PO Box 20907 2500 EX the Hague The Netherlands
D.J. McHenery	DAFS Marine Laboratory PO Box 101 Victoria Road Aberdeen, U.K.
Dr. B. Serigstad	Institute of Marine Research PO Box 1870 5024 Bergen Norway
Dr. R. Stagg	DAFS Marine Laboratory PO Box 101 Victoria Road Aberdeen, U.K.
Dr. A.R.D. Stebbing	Plymouth Marine Lab Prospect Place West Hoe Plymouth PL1 3DH Devon, UK
Dr. F. Thurberg (Chairman)	U.S. Department of Commerce-NOAA National Marine Fisheries Service 212 Rogers Avenue Milford, CT 06460 USA
Dr. G. Topping	DAFS Marine Laboratory PO Box 101 Victoria Road Aberdeen, U.K.
Dr. D. Wells	DAFS Marine Laboratory PO Box 101 Victoria Road Aberdeen, U.K.

ANNEX 3

ICES/IOC WORKSHOP ON BIOLOGICAL EFFECTS OF CONTAMINANTS

Bremerhaven, FR Germany 12-30 March 1990

INTRODUCTION

The suggestion that a seagoing workshop be held was made at the first meeting of the Working Group on the Biological Effects of Contaminants (WGBEC) held at ICES Copenhagen in May 1987 (CM 1987/E:23). The decision to proceed was made at the next meeting of the WGBEC in April 1988 (CM 1988/E:26), and a Planning Group was formed consisting of Drs. Stebbing, Dethlefsen and Thurberg. A "Proposal for a seagoing workshop on biological effects monitoring techniques" (CM 1987/E:34) was submitted to the 76th Statutory Meeting of ICES in Bergen in October 1988. In accordance with a resolution of that meeting (C. Res. 1988/2:30a), members of the Working Group on the Biological Effects of Contaminants (WGBEC) are continuing to plan the Workshop on Biological Effects Measurements to be held in Bremerhaven 12-30 March 1990.

The major requirement for ICES is to identify relevant techniques that can be incorporated in monitoring programmes, while a major requirement for IOC is to widen the geographic scale of the Workshop programme, while evaluating new kinds of indices of stress. In this workshop IOC's priority is their interest in integrating the results of biochemical and cellular indices of contaminant-induced stress in fish in relation to the gross pathology of disease. Several proposals to the workshop are directed to questions raised by IMO, specifically those involving the biological quality of sediments and the bioavailability of the contaminants they contain, the effects of contaminants from the incineration of toxic wastes at sea and pollution gradients related to oil platforms.

From the outset it was proposed that this should be a joint workshop with a group of IOC (Group of Experts on the Effects of Pollution - GEEP) with whom we share the same objective of establishing the use of biological effects techniques in monitoring programmes. Dr. McDowell Capuzzo was appointed by GEEP as their representative of the Planning Group.

Letters of invitation, together with the Workshop Proposal, were sent out from ICES in Copenhagen and from IOC in Paris. At the time of finalizing this document nearly 50 proposals had been received or promised from nearly 70 scientists wishing to participate. The Planning Group met at the International Maritime Organization (IMO) in London from 20 to 21 April 1989 to consider the plans and proposals in detail. Later the ICES WG on the Biological Effects of Contaminants met at DAFS Aberdeen from 9 to 12 May 1989, in particular to consolidate the chemical elements of the Workshop Plan.

OBJECTIVES

1. To test and intercalibrate biological effects techniques designed to detect and measure the effects of contamination.

2. To test primarily those techniques that can be deployed at sea, to complement the emphasis given at the Oslo Workshop to techniques suited to nearshore use.

3. To test the techniques on known contaminant gradients of the kind that would be likely to be covered by a monitoring programme.

4. To relate results from biological indices to chemical data for the contamination gradients in such a way that causal relationships can be identified.

5. To make a comparison of the suitability of different kinds of techniques that consider the same organisms (for example, fish) or habitat (for example, benthos), by deploying them simultaneously on the same gradient.

PROPOSALS

Proposals have been received and grouped under the following topics with a proposed coordinator for each (Annex 2).

1. Fish
   - gross pathology (R. Vethaak)
   - biochemistry (R. Addison)
   - cell pathology (M. Moore)

2. Bioassays (L. Karbe)

3. Benthos
   - benthic community studies (C. Heip)
   - sediment bioassays (P. Chapman)

4. Chemistry (W. Cofino)

5. Statistics (M. Carr)

RATIONALE FOR DIFFERENT CONTAMINATION GRADIENTS

1. Offshore gradient on a transect NW out of Bremerhaven: A cruise track has been proposed (Annex 1) that traverses contamination gradients that attempts to minimize the effects of natural and unrelated factors. This transect is proposed particularly for those involved in fish studies, and to a lesser extent, those who intend to conduct water quality bioassays. Numerous criteria were considered when this track was proposed, which include uniform depth, a known difference in contaminant concentrations in sediments, uniform sediment type, a variety of contaminants (OCs, metals, PAHs, petroleum hydrocarbons, and

proximity to Bremerhaven.

The benthic group will use an inshore extension of this transect designed to detect the effect on the benthos of the R Elbe plume, perhaps involving 5 stations on an axial transect. Plans for this element will be consolidated after a preliminary investigation of the plume area by Dr. Rahor from the Alfred Wegener Institute.

2. Oil platform gradient: Techniques can sometimes best be tested on a gradient where a single toxic contaminant is dominant. To this end an oil platform gradient will be used to deploy the benthic techniques where oil and drilling mud are the main factors contributing to a biological impact. This gradient would also provide fish samples of interest to the biochemists. Several oil platforms are under consideration, including a Shell platform off Aberdeen, a Phillips Petroleum platform off Norway and a Dutch oil platform.

3. Incineration area: It is known that many contaminants accumulate at the sea surface, by association with buoyant material or by deposition from the atmosphere, resulting in concentrations orders of magnitude higher than the immediate subsurface. We therefore propose to consider the question that such data pose by focusing effort on the surface microlayer, to see whether water quality in the microlayer is depressed and to identify the best techniques to monitor changes.

The possibility of sampling with respect to time following an incineration event will be explored. If incineration has been suspended by March 1990, or if bad weather makes this element of the programme impractical, microlayer methods will be directed to a contaminated estuarine or harbour location.

RATIONALE FOR DIFFERENT TYPES OF BIOLOGICAL TECHNIQUES

1. Fish studies: For some time those involved in fish disease studies in the North Sea have been concerned primarily with the occurrence of disease in terms of gross and cell pathology, and its frequency. In recent times its possible relationship to the distributions and concentrations of toxic contaminants has created considerable interest in the role of contamination. While some of these data provide correlations with disease frequencies, the question of whether or not a causal relationship exists with contamination remains unanswered, and the problem was identified in the last Quality Status Report for the North Sea as one of some urgency.

A number of biochemical and cellular techniques have been proposed for use alongside the established approach to fish disease studies in the North Sea. With the range and number of submissions using fish, the Workshop will provide a good opportunity to integrate the results of these techniques, as well as a rigorous test of the value of different approaches for monitoring at sea.

2. Bioassay studies: The value in using biological systems to provide a rapid overall index of water quality is well established, and the proposals to use oyster larvae on water column samples, microlayer samples and sediment samples suggests some agreement as to the most useful. The workshop will provide practical case histories for addition to the ICES manual on the oyster larval bioassay technique. Few bioassay techniques are sensitive enough to detect variations in water quality offshore, but within the workshop the utility of assessing the effects of contaminants in the surface microlayer in the vicinity of an incineration area will be studied.

Benthic studies: The basis of the benthic component of the workshop will be the analysis of macrofauna and meiofauna community structure using multivariate techniques and other methods of differentiating communities along pollution gradients. The Sediment Triad approach (chemistry, bioassay and community analysis) has not previously been tested in European waters. The workshop is an opportunity to test the approach (under the guidance of Dr. P. Chapman), as a number benthic community, sediment bioassay and sediment chemistry proposals have been submitted. The Triad approach combines these three methods for the detection of contamination of sediments as a unified study. Sediment bioassays will be tested as a means of measuring sediment toxicity and an intercomparison made with benthic community data in the context of appropriate sediment chemistry. The coordination of these three elements will obviously require the use of the same box samples for macrofauna and meiofauna community analysis, sediment and elutriate bioassays and sediment chemistry.

SAMPLING STRATEGY

1. German Bight Transect, 5 stations on the transect for fish, water quality bioassays, sediment sampling and hydrography.

2. Elbe Plume (extension of German Bight transect), ca, 5 axial stations for benthic studies, including samples for macrofauna, meiofauna, sediment bioassay and sediment chemistry.

3. Oil Platform Gradient, 2 stations for sampling fish and stations for benthic studies as 2. above.

4. Incineration gradient, To include microlayer and subsurface samples for water chemistry and water quality bioassays.

CHEMISTRY

In any study of environmental contamination and its effects, it is self evident that both biological and chemical data are not merely required but are interdependent. In the workshop the requirement for an appropriate chemical data base and the collaboration of chemists to interpret their significance are indispensable.

Tissue, sediment and water chemistry will be coordinated by Dr. W. Cofino. Drs. Boon and Foyn who are members of both the ICES Working Groups for Marine Chemistry and the Biological Effects of Contaminants, will provide a significant analytical input of tissue analyses. The German Hydrographic Institute has offered to carry out much of the water and sediment chemistry, and the collection of hydrographic data.

The plan for the sampling strategy requirements for the Workshop was drawn up at an ICES WG meeting with Dr. G. Topping and Dr. D. Wells (Chairman and member of ICES Marine Chemistry WG respectively). Dr. I. Davies (ICES Sediment WG) advised on sampling and analysis of sediments. The listing of chemical requirements and sampling strategy (Annex 3) is the result of that meeting.

If for any reason the chemical analyses identified in Annex 3 can not be carried out, it has been decided that the total number of stations worked will be reduced rather than limit the range of contaminants analyzed or the replication of samples to be analyzed. It not intended to compromise the quality of the chemical data base considered necessary to interpret the biological data.

STATISTICS

Intercomparison of biological techniques to detect contamination gradients is only possible if there is standardization of methods for data presentation and use of the same statistical procedures and criteria to determine the significance of different responses. M. Carr (Plymouth Marine Laboratory) will be collaborating primarily with the benthic group and their own group of statisticians, but will also be responsible for data archiving and providing guidance on the treatment and presentation of data.

ROLE OF COORDINATORS

For each study area coordinators have identified and invited. We anticipate their role to be:

1. To coordinate the work of the participants in their group so as to make an intercomparison of the different techniques possible.

2. To coordinate the identification and provision of samples and equipment (for use in the lab and at sea) during the workshop.

3. To report the results on behalf of their group at the conclusion of the workshop and to prepare summary reports for their respective sections.

4. To carry out preliminary editing of reports and papers before publication, and to assist in the identification of appropriate reviewers for the papers.

(We anticipate assigning a student to each of the coordinators for the duration of the workshop to assist them.)

RESEARCH VESSELS

			numbers of scientists
1.	Victor Henson (AWI)	12 to 30 March	12
2.	Friedrich Heinke (Biol Anst Helg)	12 to 30 March	5
3.	Solea (Fed Res Bd Fish)	18 to 30 March	5
4.	Walter Herwig (Fed Res BD Fish)	23 to 30 March	12
5.	Gauss (German Hydr Inst)----------------------		
6.	Hollandis (Rijkswaterstaat)	12 to 30 March	7
7.	Aurelia (NIOZ)	12 to 30 March	7

FOUL WEATHER CONTINGENCY PLAN

It is likely that for at least part of the workshop, bad weather will prevent the research vessels from putting to sea, so it is important to develop a contingency plan. This will consist primarily of collecting samples on the identified gradients and stations in advance and preserving them in a form that can be used during the workshop. Once all the participants are familiar with the overall plan, they will be asked to identify the types and number of samples required and the methods of preservation to be used.

The outline plans for each topic area:

1. Fish studies. Collection of fish samples (dab and other flatfish) at intervals along the German Bight gradient (NW from Bremerhaven) and preservation.

2. Water quality bioassays. Intercomparison of techniques under workshop conditions using a specific toxicant (eg copper, TBT). Assessment of toxicity of elutriates of large water samples passed trough XAD ion exchange resin to concentrate organic contaminants (to be carried out under the direction of Dr. L. Karbe).

3. Benthic studies. Collection and preservation of macrofauna samples from one or more gradients, to include subsamples for meiofauna, for sediment bioassays and for chemical analysis. Preserved polluted harbour sediments could also be used for intercomparison of sediment bioassays. A paper exercise will be conducted with existing community, sediment bioassay and sediment chemistry data to examine the Triad approach using North Sea data (to be carried out under the direction of Dr. P. Chapman).

TIMETABLE

22 May 1989	Submission of Workshop Planning Documents to IOC
2 June 1989	Submission of Workshop Planning Documents to ICES
July 1989	Planning Group meet in Cuxhaven or Plymouth
October 1989	Planning Group meet in Bremerhaven
5 March 1990	Planning Group meet in Bremerhaven
12-30 March 1990	Workshop
early May 1990	Combined meeting of WG BEC and Workshop coordinators
1 July 1990	Report on Workshop to ICES and IOC
June 1991	ICES/IOC Workshop Symposium

FINANCE

Estimates of the total cost of the workshop at this stage, exceeding those covered by the participants, can only be approximate, but we can identify the major areas of expenditure.

Radiochemicals	$ 3K
Consumables (analytical standards, chemicals, etc)	$ 5K
Transport of equipment	$ 5K
Travel costs	$20K
Subsistence costs	$15K
Hire of students	$ 7K
	US $55K

PUBLICATIONS

1. Report to IOC (July 1990)
2. Report to ICES (July 1990)
3. Publications as special number of a journal - Workshop Symposium Volume.

ANNEXES

Annex 1 Proposed cruise track to study contamination across German Bight (V Dethlefsen).

Annex 2 List of proposals, subdivided into groups under Coordinators.

Annex 3 Proposed analytical chemistry.

ICES Working Group on Biological Effects of Contaminants Aberdeen
9 - 12 April 1989

Proposed Track for Studies during the Bremerhaven Workshop on Biological Effects Monitoring - March 1990

Volkert Dethlefsen
Bundesforschungsanstalt für Fischerei
Institut für Küsten- und Binnenfischerei
Aussenstelle Cuxhaven
Niedersachsenstraße, 2190 Cuxhaven, F.R.Germany

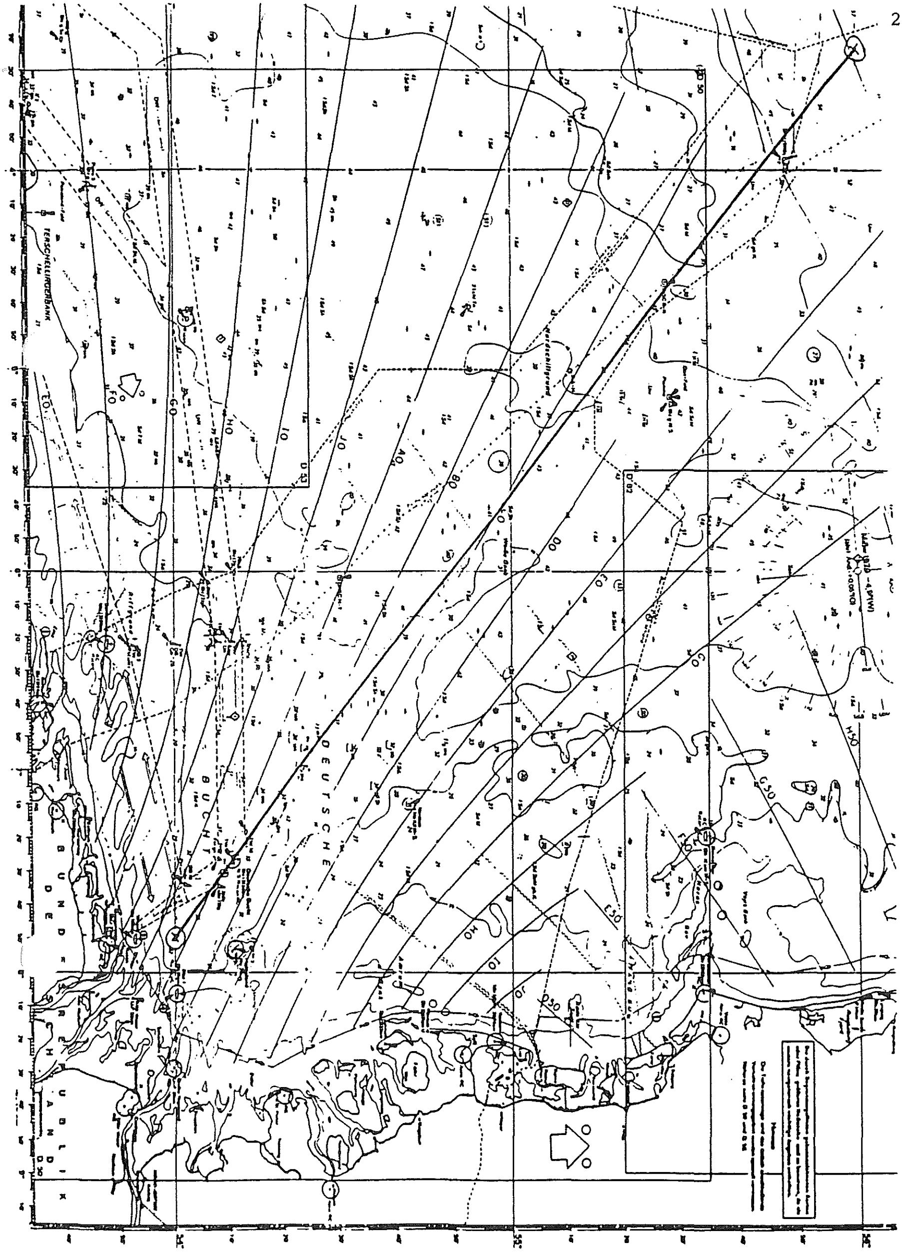
The proposed track on which all studies of the workshop could be performed is situated between 54°N/07°50'E and 56°N/03°24'E, its total length roughly 200 nm. It is entirely located on the German part of the continental shelf of the North Sea. This will have the advantage that the chemical support offered by the German Hydrographic Institution would be available for all possible stations on this proposed track. On the first 120 nm depths only vary between 32 and 45 m. The sediment structure is quite uniform, the fraction smaller than 63 µm is variable between 5 and 20 %. The track beyond the 120 nm to the end of the German part of the continental shelf covers the northeastern tip of the Dogger Bank which is known for its high fish disease rates and contamination. Enclosed information on contamination of various compartments should be used to discuss the final placement of the stations for the workshop.

Information is available on
- salinity gradients, suspended solids, areas of low dissolved oxygen;
- cadmium and lead in seawater;
- cadmium, lead and vanadium in particulate matter;
- contents of fines in the sediments of the North Sea;
- concentrations of lead, arsenic, mercury and cadmium in the fine fraction of sediments < 20 µm;
- cadmium, copper, lead and mercury in **Pagurus bernhardus**;
- cadmium, lead and mercury in livers of dab (**Limanda limanda**) of the German Bight;
- cadmium, lead and mercury in livers of dab (**Limanda limanda**) of the North Sea;
- for organochlorine material information is included on trichlorethen in seawater of the German Bight;
- on gamma-HCH and HCB in seawater;
  PCBs, HCB, ΣDDT in sediments;
  PCBs, HCB, DDE in zooplankton;
  PCBs, DDE and HCB in **Pagurus bernhardus**;
  PCBs, ΣDDT in livers of dab;
  petroleum hydrocarbons in the German Bight.

The last three figures contain information on diseases of dab and malformation rates of fish embryos.

From material included it can be taken that no uniform gradients for the substances of the various pollutant groups exist. Also there is no uniform trend with decreasing contamination with incrasing distance from the coast. For some of the substances in some of the compartments gradients can be found the other way round.

For the group of contaminants to be analyzed in the course the workshop, i.e. heavy metals, organochlorines, and petroleum hydrocarbons, it can be stated that based on the material enclosed significant differences in concentrations do exist on the proposed track. The members of the planning committee and the members of the ICES Working Group on Biological Effects Monitoring might wish to consider the suitability of the sampling area.


TERSCHELLINGERBANK
DEUTSCHE
BUCHT

APPENDIX 2

ICES/IOC WORKSHOP ON BIOLOGICAL EFFECTS TECHNIQUES - BREMERHAVEN

Provisional List of Included Participants and Proposed Topic Coordinators (agreed at Planning Group meeting at IMO 17-21 April 1989)

I. FISH

A. GROSS PATHOLOGY - Vethaak (Coordinator)

1. Gross pathology of livers of dab - Kranz
2. Characterize pathological changes in flatfish - Bucke
3. Gross disease and histopathology of flatfish - Vethaak
4. Gross pathology of fish - ap Rheinallt

B. CELLULAR PATHOLOGY - M. N. Moore (Coordinator)

1. In vivo cell injury and oxyradical damage in fish livers - Moore.
2. Erythrocyte micronuclear formation in fish - Roddie
3. Effects of contaminants on the functional integrity of fish hepatocytes - Lowe
4. Contaminant induced cellular responses of the fish liver - Kohler-Gunther
5. Unscheduled DNA synthesis as a measure of genetic damage in fish and P32 post-labelling method of genotoxin-DNA adducts - Chipman and Livingstone
6. Histopathological analysis of bivalve molluscs from selected gradients of anthropogenic contamination - Auffret

C. BIOCHEMISTRY - Addison (Coordinator)

1. Estimation of lysozyme activity in the blood of marine fish - Mock
2. Catalytic enzyme activity in crustaceans - McHenry
3. Catalytic activity and number of enzyme sites of Na/K ATPase in flatfish - Stagg
4. Sublethal effects of pollutants on fish - Forlin and Balk
5. Immunochemical analysis of cytochrome P-450 monooxygenase induction in larval and adult fish tissue - Goksoyr
6. Effects of xenobiotics on the MFOs of fish - Hansen, Addison and Renton
7. Tissue levels of metallothionein and heavy metals - Hogstrand and Haux
8. Biochemical composition of fish in response to stress - Leavittt and Capuzzo
9. Morphological and chromosomal aberrations during embryonic development in pelagic fish embryos - Cameron and von Westerhagen
10. Detection of effects of organophosphates and organic contaminants in fish tissue using automated biochemical method - Galgani and Suteau

II. BIOASSAYS - Karbe

1. Application of liquid/ solid extraction techniques for water quality bioassay studies - Karbe, Behning and Bloemeke
2. Oyster embryo bioassay - Thain
3. Copepod toxicity studies - Roddie
4. Water quality bioassays of surface microlayer in relation to contaminant concentrations - Stebbing and Cleary
5. Pollution induced community tolerance - Blanck, Tiselius and Molander
6. Sea surface microlayer bioassay with oyster larvae - van den Hurk
7. Availability of heavy metals along a pollution gradient and its effects upon the growth of marine phytoplankton - Rijstenbil
8. Sea-surface microlayer: contamination, ecotoxicology and monitoring - Hardy
9. Effects of a gradient of pollutants in water and sediment on the ecophsiology of benthic organisms - Absil and Hummel

III. BENTHOS

A. SEDIMENT BIOASSAYS - Chapman (Coordinator)

1. Sediment bioassays with amphipods, a polychaete and oyster larvae - Chapman and Swartz
2. Sediment quality triad - Chapman
3. Sediment bioassay with a clam and oyster larval bioassay - Phelps
4. Sea surface microlayer and sediment quality bioassay with oyster larvae and an amphipod - van den Hurk
5. Sediment toxicity and contaminant bioavailability - Roddie and Butler

B. BENTHIC STUDIES - Heip (Coordinator)

1. Meiofauna benthic community effects - Warwick, Platt, Vincx and Heip
2. * Macrofauna benthic community effects - Rachor and Kroncke
3. Epifauna benthic community effects - Duineveld and Rumohr
4. Imaging the upper sediment layers using REMOTS Sediment Profiling camera and the sediment surface with TV and Stills photography - Rumohr
5. * Mesocosm experiment - boxcosm - de Wilde, Duineveld, Berghuis and Smaal
6. Size structure of benthic communities - Schwinghamer

IV. CHEMISTRY - Cofino (Coordinator)

1. Analysis of organic microcontaminants in sediments - Abarnou and Bodennec
2. Selected metal analyses in fish organs related to disease - Protasowicki
3. OCs (HCB, HCHs, DDT family, cyclodienes and PCB congeners in tissues of fish, benthic macroinvertebrates and sediments - Boon

4. Selected metals (Cu, Zn, Cd, Pb, Cr, Fe, Ba) in tissues of fish benthic macroinvertebrates and sediments - Cofino and Marquenie
5. PAHs by GC-MS in tissues of fish, benthic macroinvertebrates and sediments - Foyn and Klungsoyr
* 6. Brockmann, University of Hamburg
* 7. Huhnerfuss, University of Hamburg
8. Oil, metals and OCs in water and sediment samples - German Hydrographic Institute

V. STATISTICS - Carr (Coordinator)

VI. VESSEL OPERATIONS - Dethlefsen (Coordinator)

(* - proposal not yet received)

APPENDIX 3

ANALYTICAL REQUIREMENTS FOR ICES/IOC NORTH SEA WORKSHOP - BREMERHAVEN 12-30 MARCH 1990

A. GERMAN BIGHT TRANSECT - Bremerhaven to NW across German Bight
-3-5 stations for fish studies and water quality bioassays
-3-5 stations for benthos studies in Elbe plume

1. Fish Samples
-dab (Limanda limanda) one sex only, otoliths to be taken for aging, size range 20-25 cms. 10-25 individual fish analyses per station.
-3 pooled samples of 25 fish each

-liver: polar and apolar lipids, metals (Pb, Hg, Cd, Cr), DDT, OCs, PAHs, THC, As, 10C list of chlorobiphenyl congeners (see Wells, 1988).
(The distribution of the liver tissue between participants to be organized by Dethlefsen)

-muscle: of secondary importance, chemical analyses to be a subset of those for liver.

2. Sediments samples
- 5 samples per station
subsamples of box core samples taken with small corer, top 2-3 cms extruded and sliced off, mixed and stored by appropriate methods for different contaminants
(As whole sediments will be used, normalization procedures will be needed to accommodate differences in grain size)
- Same suite of contaminants as for fish.
- granulometry, organic carbon content, redox, sulphide, ammonia
- sediment bioassays

3. Hermit crabs (Eupagurus bernhardus) 3*10 at each station (analysis of abdomen only)
-same suite of analyses as for fish

(possible collaborative link to be explored to assist with analytical load.)

4. Benthos
-pooled samples at each station of 5 representatives species.
-same suite of analyses as for fish.

5. Water samples
-single large volume pumped water samples from surface and bottom at each station
-analysis of both soluble and particulate phases.
-metals, OCs, PAHs, THC

-subsamples for water quality bioassays

6. Hydrographic data
   - salinity, temperature, dissolved oxygen, suspended particulate load, chlorophyl, nutrients (silicate, phosphate & nitrate).
   All except chlorophyl to be carried out by the German Hydrographic Institute.

B. OIL PLATFORM GRADIENT
   -off Dutch coast
   -2 stations for fish
   - 3-5 stations for benthos

1. Fish samples
   -preferably dab, one sex, otoliths for aging, size range 20-25 cms.
   -20 individual fish analyses per station
   - liver: THC (IR & GC), PAHs, barium, (distribution of liver samples to be organized by Dethlefsen.)

2. Sediment samples
   -5 box core samples at 3-5 stations
   -THC (IR & GC), PAHs, barium, carbonates, DOC,
   - redox and granulometry

3. Benthos
   - pooled representative samples of 5 species same suite or analyses as fish

C. INCINERATION AREA
   -samples for water quality bioassay from surface microlayer and 0.5m
   -analyses to include OCs (octachlorostyrene and hexachlorobenzene), TBT and metals (Cd, Cr, Zn).
   -sampling strategy to be worked out later, once it is established that incineration is still taking place in 1990

ANNEX 4

ICES Working Group on Biological Effects of Contaminants Aberdeen 9 - 12 April 1989

New Results of Investigations on the Effects of Dumping of Wastes from Titanium Dioxide Production in the Dutch Dumping Grounds

Volkert Dethlefsen
Bundesforschungsanstalt für Fischerei
Institut für Küsten- und Binnenfischerei
Aussenstelle Cuxhaven
Niedersachsenstraße, 2190 Cuxhaven, F.R.Germany

In 1980 Dethlefsen and Watermann first reported on increased prevalences of epidermal papilloma in North Sea dab from the German dumping area for wastes from titanium dioxide production (Dethlefsen and Watermann, 1980). These results have since then been controversely discussed within various bodies of the International Council for the Exploration of the Sea. The study led to an extensive programme to qualify and quantify diseaes of important species in the North Sea and initiated programmes in various ICES member countries.

For the latest session of SACSA (Standing Advisory Committee for Scientific Advice of the Oslo Commission) Dutch colleagues prepared a summary of results of the monitoring in the the Dutch titanium dioxide dumping grounds between 1986 and 1988 (Bos, 1989; copy attached).

The major results of the German studies can be summarized as follows (for a review of these results see Dethlefsen, 1986):

1. Increased prevalences of dab afflicted with epidermal papilloma (epidermal tumours) were regularly encountered in the dumping area as compared to surrounding station. Differences were especially marked at seasons of disease maxima, i.e. early spring, and less marked in August, when this type of disease occurred in lowest prevalences.

2. Increased concentrations of various heavy metals plus iron were encountered in sediments and the water column of the dumping area as compared to reference stations.

3. Increased concentrations on chromium were found to occur in external tissues of dab from the dumping area.

4. A correlation existed between the size, i.e. intensity of epidermal papilloma of dab and its contamination with chromium.

5. Increased prevalences of malformed pelagic fish embryos were found for spring spawning species in the dumping area as compared to reference stations (Dethlefsen et al., 1987).

6. Unpublished results on the composition of mobile macrobenthos indicate fewer species and lesser abundance in the dumping area as compared to reference stations (Reise, K., unpublished).

The Dutch results can be summarized as follows:

1. Increased prevalences of epidermal papilloma (skin tumours) and liver abnormalities in dab of the Dutch dumping area as compared to reference stations.

2. Increased concentrations of heavy metals and iron in sediments of the dumping area.

3. Increased concentrations of chromium in gills of dab of the dumping area.

4. Increased concentrations of heavy metals in hermit crab of the dumping area.

5. Shift in meiobenthos composition from copepods to nematodes in the dumping area (Bos, 1989).

In the results of the German studies regional coincidences of biological deviations from normal, and increased concentrations of wastes in the area were interpreted as circumstantial evidence for a cause effect relationship between biological effects and waste input. In order to further strengthen the hypothesis that the assumed cause and effects are related studies with the same techniques have to be done in areas which differ in as many aspects as possible from the first one with the exception of the trigger under question. The Dutch dumping area differs from the German in many aspects.

1. The water exchange is rapid.

2. There are no accumulative properties in the Dutch dumping area.

3. The sediment structure is different, course sediment prevail.

4. Population density of fishes and macrobenthos is lower in the Dutch dumping area as compared to the German Bight.

The factors coinciding are: both areas show little variability of salinity and other hydrographic factors, and both areas are receiving high quantities of diluted acids from titanium dioxide production. The new Dutch results therefore should be interpreted as further circumstantial evidence. A cause effect relationship between increased disease prevalences and other biological changes in the area and wastes from titanium dioxide production is more likely than it was before.

Literature

Bos, H. R., 1989. A summary on the results of the monitoring of the Dutch TiO_2-dumping grounds between 1986 und 1988. - SACSA 16/4/6-E.

Dethlefsen, V., 1986. Überblick über Auswirkungen der Verklappung von Abfällen aus der Titandioxidproduktion in der Deutschen Bucht. - Veröff. Inst. Küsten- und Binnenfisch. 95, 1-42.

Dethlefsen, V. & Watermann, B., 1980. Epidermal papilloma of North Sea dab (**Limanda limanda**); histology, epidemiology and relation to dumping of wastes from TiO_2 industry. - ICES Special Meeting on Diseases of Commercially Important Marine Fish and Shellfish. 8.

Dethlefsen, V., Cameron, P., Westernhagen, H. von & Janssen, D., 1987. Morphologische und chromosomale Untersuchungen an Fischembryonen der südlichen Nordsee in Zusammenhang mit der Organochlorkontamination der Elterntiere. - Veröff. Inst. Küsten- und Binnenfisch. 97, 1-57.

ANNEX 5

EVALUATION OF BIOLOGICAL EFFECTS OF CONTAMINANTS
- Monitoring programmes -

- ° - ° - ° -

Surveillance of biological effects to link levels of contaminants with modifications of ecosystems, populations and species is a new approach in monitoring activities at IFREMER - FRANCE.

The most important aspects of this programme on biological effects 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

4/ Studies of transport, chemical transformation and bioaccumulation in organisms.

A summary of the first programme aspects is described in report of Copenhagen meeting W.G.B.E.C. Copenhagen 1988, CM. 1988 IE : 26, Sess S Annex 6 // 47-48.

The sites selected are the Bay of Seine, a very polluted area, and one or two areas in the west of Brittany with low levels of contaminants. Both physiological and biochemical monitoring techniques are used.

Flatfishes molluscs hydrozoa and zooplankton are studied.

- Flatfishes : study of mucus cells of skin and fecundity (Louis QUINIOU),

- Invertebrates hydrozoa - bioessais (France TOULARASTEL),

  Invertebrates Molluscs Embryo - bioessais (Françoise QUINIOU),

- Zooplankton lipids metabolism - success of eggs laying of Copepoda (Geneviève LE FEVRE-LEHOËRFF).

Biochemical aspects.

Flatfishes and Molluscs are used.

- Development of methodology. P 450. EROD activity - Metallothionines (François GALGANI).

Chemical aspects.

Lipids : specialist Guy BODENNEC.

Organochlorinated contaminant : Alain ABARNOU.

Literature

Bos, H. R., 1989. A summary on the results of the monitoring of the Dutch TiO_2-dumping grounds between 1986 und 1988. - SACSA 16/4/6-E.

Dethlefsen, V., 1986. Überblick über Auswirkungen der Verklappung von Abfällen aus der Titandioxidproduktion in der Deutschen Bucht. - Veröff. Inst. Küsten- und Binnenfisch. 95, 1-42.

Dethlefsen, V. & Watermann, B., 1980. Epidermal papilloma of North Sea dab (**Limanda limanda**); histology, epidemiology and relation to dumping of wastes from TiO_2 industry. - ICES Special Meeting on Diseases of Commercially Important Marine Fish and Shellfish. 8.

Dethlefsen, V., Cameron, P., Westernhagen, H. von & Janssen, D., 1987. Morphologische und chromosomale Untersuchungen an Fischembryonen der südlichen Nordsee in Zusammenhang mit der Organochlorkontamination der Elterntiere. - Veröff. Inst. Küsten- und Binnenfisch. 97, 1-57.

ANNEX 5

EVALUATION OF BIOLOGICAL EFFECTS OF CONTAMINANTS
- Monitoring programmes -

\- ° - ° - ° -

Surveillance of biological effects to link levels of contaminants with modifications of ecosystems, populations and species is a new approach in monitoring activities at IFREMER - FRANCE.

The most important aspects of this programme on biological effects 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

4/ Studies of transport, chemical transformation and bioaccumulation in organisms.

A summary of the first programme aspects is described in report of Copenhagen meeting W.G.B.E.C. Copenhagen 1988, CM. 1988 IE : 26, Sess S Annex 6 // 47-48.

The sites selected are the Bay of Seine, a very polluted area, and one or two areas in the west of Brittany with low levels of contaminants. Both physiological and biochemical monitoring techniques are used.

Flatfishes molluscs hydrozoa and zooplankton are studied.

- Flatfishes : study of mucus cells of skin and fecundity (Louis QUINIOU),

- Invertebrates hydrozoa - bioessais (France TOULARASTEL),

  Invertebrates Molluscs Embryo - bioessais (Françoise QUINIOU),

- Zooplankton lipids metabolism - success of eggs laying of Copepoda (Geneviève LE FEVRE-LEHOËRFF).

Biochemical aspects.

Flatfishes and Molluscs are used.

- Development of methodology. P 450. EROD activity - Metallothionines (François GALGANI).

Chemical aspects.

Lipids : specialist Guy BODENNEC.

Organochlorinated contaminant : Alain ABARNOU.

SCIENTISTS PARTICIPATING IN THE PROGRAMME

ABARNOU Alain, BODENNEC Guy, LE FEVRE-LEHOËRFF Geneviève,
QUINIOU Françoise, TOULARASTEL France

IFREMER, Centre de Brest
B.P. 70 - 29263 Plouzané - FRANCE

GALGANI François

IFREMER, Centre de Nantes
rue de l'Ile d'Yeu
B.P. 1049
44037 NANTES Cedex 01 - FRANCE

QUINIOU Louis

Université de Bretagne Occidentale
Laboratoire de Biologie animale
6 avenue Le Gorgeu
29287 BREST Cedex

EVALUATION OF BIOLOGICAL EFFECTS OF CONTAMINANTS
- Monitoring programmes -

Physiological aspects : Flatfishes studies

by Louis QUINIOU

_ • _ • _ • _

Flatfishes living on the bottom of the sea are in contact with sediment and exposed to the contamination.

Flat fishes studied are Pleuronectes platessa, Solea vulgaris and Limanda limanda to establish a comparison of responses for the same species in heavily contaminated area and a reference area :

. Bay of Seine : contaminated area
. Bay of Douarnenez : reference area.

The modifications of epidermic mucus cells are studied by using of two methods :

- counting of mucus cells by Spot analyser
- structural histology of the skin.

On the same samples, lenght, weight, physiological state of organ, ovary, liver are studied.

Measurement of contaminants in the sediment of same geographic area is known (cf. A. ABARNOU paper).

EVALUATION OF BIOLOGICAL EFFECTS OF CONTAMINANTS

MONITORING PROGRAMMES

PHYSIOLOGICAL ASPECTS:

ASSESMENT OF BIOLOGICAL WATER QUALITY AND SEDIMENTS OR URBAN WASTES TOXICITY UPON BIVALVES EMBRYOS.

by Francoise QUINIOU

_A similar embryo-bioassay than Thain and Watts (1987) is used since 1987,with Mytilus edulis and Crassostrea gigas.
_The objective is to classify the sea-water of the French coasts whith the measure of its biological water quality;and so establish a gradient of the toxicity of a sediment or urban wastes at differents concentrations.
_These results are obtained by the ability of the bivalve embryo to develop normally and reach the "D" shaped larval stage.The differents test-water are compared with the results of control samples(with a reference sea-water)using the Percent Net Risq (PNR) of Thain and Watts (1987).
_With Mytilus edulis the "D" shaped larval stage is reached within 48h at a temperature of 20C;but only within 24h at 24C for Crassostrea gigas.
_It is interesting to use both species to test differents "polluted" sea-water because Mytilus edulis can be more sensitive than Crassostrea gigas (Martin and al.,1981)

_The results cannot be extended to predict the effect on all-over ecosystem but they can be used as "indicator" of biological water quality.

_ Some differences exist with Thain's method (january 1989) :

Mature bivalves come from hatcheries or the sea-shore.
Reference sea-water from Argenton (Brittany station of IFREMER) is used quickly after sampling.
All water samples for bioassays are filtered through a 0,2µ membrane (Garland and al.,1986;His and Robert,1986),and the salinity is rectified (28‰ for C.g.;30‰ for M.e.).
Only 1 female and 1 male are used and the mixing of gametes is made directly in test-water;some pollutants can have a direct harmful effect on the gametes (His and Robert,1980).
All the samples are placed at obscurity during the embryo development.

BIBLIOGRAPHY

GARLAND,C.D.,COOKE,S.L.,MACMEEKIN,T.A.,and VALENTINE,J.E.,1986 Effects of 0.2µm membrane-filtered seawater as a culture medium on fertilized eggs and larvae of the pacific oyster,Crassostrea gigas.Aust.J.Mar.Freshw.Res.,37:713-720.

HIS,E.and ROBERT,R.,1980 _ Action d'un sel organo-metallique l'acetate de tributyl-etain sur les oeufs et les larves D de Crassostrea gigas (Thunberg).C.E.I.M.,C.M. 1980/F:10p.

HIS,E. and ROBERT,R.,1986 _ Utilisation des elevages larvaires de Crassotrea gigas en ecotoxicologie marine. Haliotis,15:301-308.

MARTIN,M.,OSBORN,K.E.,BILLY,P. and GLICKSTEIN,N.,1981 _ Toxicities of ten metals to Crassostrea gigas and Mytilus edulis embryos and Cancer magiter larvae. Marine Pollution Bulletin ,12(9):305-308.

THAIN,J.E.and WATTS,J.,1987 _ The use of a bioassay to measure changes in water quality associated with a bloom of Gyrodinium aureolum Hulburt. Rapp. P.V. Reun.C.I.E.M.,187:103-107.

EVALUATION OF BIOLOGICAL EFFECTS OF CONTAMINANTS
- Monitoring programmes -

Physiological aspects : zooplankton level

by Geneviève LE FEVRE-LEHOËRFF

_ ° _ ° _ ° _

Some aspects of a new study in 1989 and 1990

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

Zooplankton is considered as an important trophic level in the pelagic and benthic ecosystem with the holoplankton and meroplankton component.

The specific composition, biomass (dry weight), organic carbon and nitrogen, lipids are measured. Composition of lipid fraction will be estimated for different areas and different seasons (cf. G. BODENNEC paper).

Reproduction and success of eggs of a very abundant species of Copepod Temora longicornis will be observed by bio-essais techniques in laboratory.

The lipophilic contaminants in the polluted area are estimated in another study (cf. A. ABARNOU paper).

This study is carried out with the aim to link levels of lipophilic contaminants and metabolism of lipids in zooplankton (physiological adaptation, utilization of lipids).

EVALUATION OF BIOLOGICAL EFFECTS OF CONTAMINANTS

Biochemical Aspects: F Galgani

A new methodology that enables large numbers of measurements of biochemical parameters in a short time has been developed.

Evaluation of different parameters have been performed. Acetylcholinesterase, metallothioneins and ethoxyresorufin-o-deoxylase (EROD) have been characterised in different species that could be used for the monitoring programmes along the coast of France. Natural variability including investigations of the effects of sex, maturation, growth and season are now in progress. Monitoring experiments are also in progress using measurement of EROD activity in plaice from the Bay of Seine and metallothioneins of oysters from the Bay of Gironde.

Evaluation of contaminants, methodologies and species distribution along the French coast lead us to restrict the monitoring program to some well defined areas. A monitoring network will start at the beginning of 1990, for the assessment of effects of organic pollutants in Bay of Seine by measuring EROD activity in plaice.

EVALUATION OF BIOLOGICAL EFFECTS OF CONTAMINANTS
- Monitoring programmes -

Evaluation of contaminants : PCB as markers of chemical pollution

by Alain ABARNOU

_ • _ • _ • _

Polychlorobiphenyls (PCB) are man-made chemicals. These organochlorinated compounds consist of 209 different congeners with various physico-chemical properties determined by the number and the position of chlorine atoms on the biphenyl sleleton. These contaminants are :

- ubiquist in the environment
- recalcitrant to degradation
- highly biomagnified through the food webs
- poorly soluble in water and readily adsorbed onto fined particles
- toxic.

Coastal waters, adjacent to urbanized and industrialized areas are heavily contaminated with PCB. The map (fig. 1) shows concentrations of CB n° 138 and CB n° 180 as major components of PCB. Concentrations in water reach ten nanograms per liter in estuaries of main european rivers whereas in coastal waters they are less than the nanogram per liter. Flatfish, and particularly flounder from contaminated areas, have bioaccumulated PCB : for some major compounds concentration are higher than fifty nanograms per gram (fresh weight).

A case study : the Seine estuary and the Bay of Seine

Since 1979, bivalves (mussels, oysters) have been sampled four times each year in about one hundred places along the french coastline. These samples were analysed for trace metals (Hg, Cu, Cd, Pb, Zn), hydrocarbons (PAH) and organochlorinated compounds (DDT, PCB). One important result is represented (fig. 2). Near the Seine estuary, PCB concentrations are in the range 4000-5000 µg/kg (dry weight). In other words, the contamination is one order of magnitude higher than the mean concentration for all sampled sites. High PCB concentration is also observed in estuaries and near industrialized area. These observations confirm than, broadly speaking, PCB come from urbanized and industrialized areas. These anthropogenic contaminants characterize chronic contaminants.

The french monitoring programs have also shown some links between PAH and PCB concentrations in bivalves. For both types of contaminants high concentrations are observed in estuaries ; in some cases higher hydrocarbons are explained by harbour activities (Le Havre, St Nazaire) or accidental pollution by oil (North Brittany) (MICHEL, in press).

The distribution and mechanisms transport of PCB in the Seine estuary and adjacent coastal waters were investigated (ABARNOU et al. 1987, ABARNOU, 1988). Main results appeared as follows :

- Amongst the distinct PCB congeners, highly chlorinated components were predominant. These undegradable chlorobiphenyls are scarcely soluble and adsorbed onto suspended particles ;

- The distribution of the contamination. The Bay of Seine clearly indicated the riverine origin of the contamination (fig. 4). Within the estuary, higher concentrations were measured upstream, and are related to the presence of the turbidity maximum. In the Bay of Seine concentrations are lower and can be unterestingly compared to other results obtained in the North Sea (GAUL et ZIEBARTH, 1983) ;

- During a tidal cycle at a fixed position situated off the entrance to the estuary, higher PCB concentrations were observed at low tide (fig. 5). PCB concentration follow SPM (suspended particular material). Moreover, during conditions of high river discharge, suspended particles appeared to be more contaminated. A decrease of the mean grain size of SPM can be partly responsible for higher contamination.

Conclusion

We feel that these results argue for the use of PCB as general marker of chemical contamination :

- The presence of PCB in coastal waters clearly indicate contamination from industrialized and urbanized area. This chronic chemical pollution is excessively complex to be analyzed. Therefore, in a first step, PCB can be used as chemical indicators to establish pollution gradients in relation with possible biological effects ;

- Analytical techniques are now available to measure these compounds with the require sensivity and specificity. Presently, it is not possible for many others chemicals. Therefore, as many others, we recommend quantification of PCB on individual components, that is to say, for few selected congeners. We also express the need for some intercomparison exercises between coworkers ;

- PCB and many other apolar compounds are mainly associated with fine solid particles and sediment. This compartment is the source for contamination for living material either directly or after resuspension of superficial sediment. For the evaluation of biological effects of contaminants superficial sediment appear as the most appropriate compartment to be analyzed ;

- In the case of interesting result in the biological aspect of the programm, it seems very attractive to perform in a further step evaluation of other contaminants like for instance : coplanar organochlorinated compounds, PAH ...

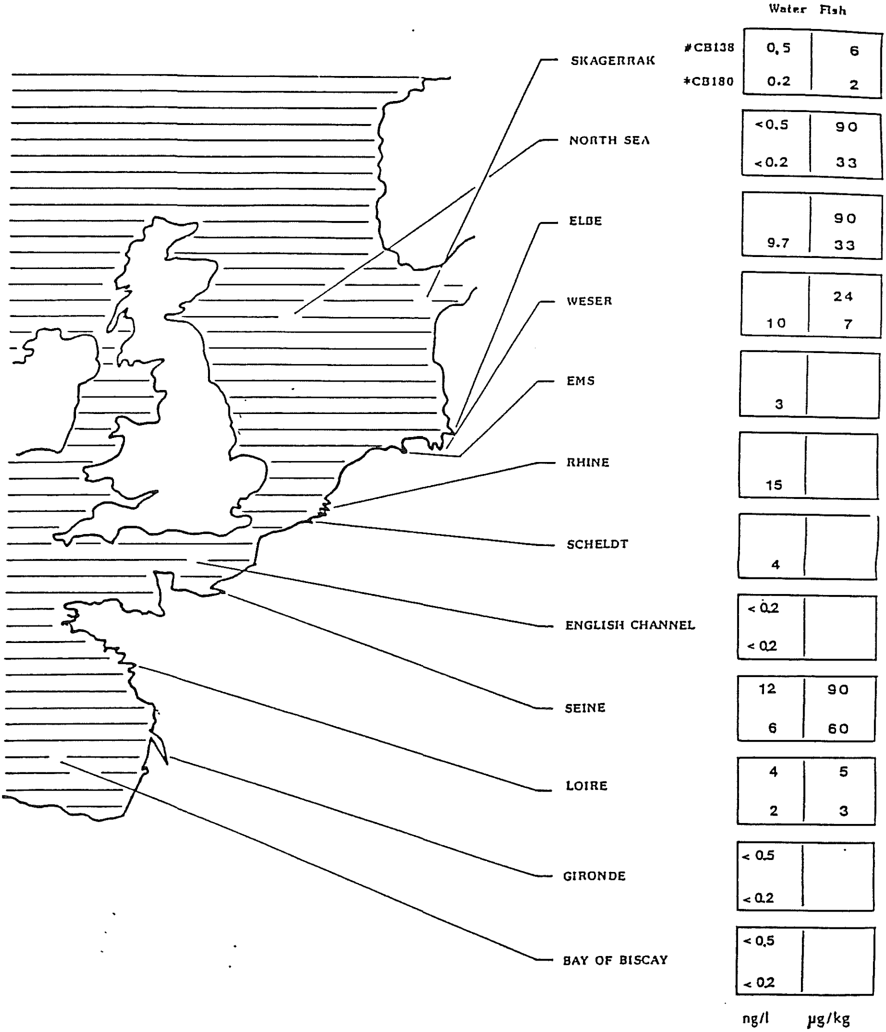


Fig 1 PCB CONCENTRATIONS IN EUROPEAN COASTAL MARINE ENVIRONMENT.

WATER AND FLAT FISH *Platichthys flesus*.

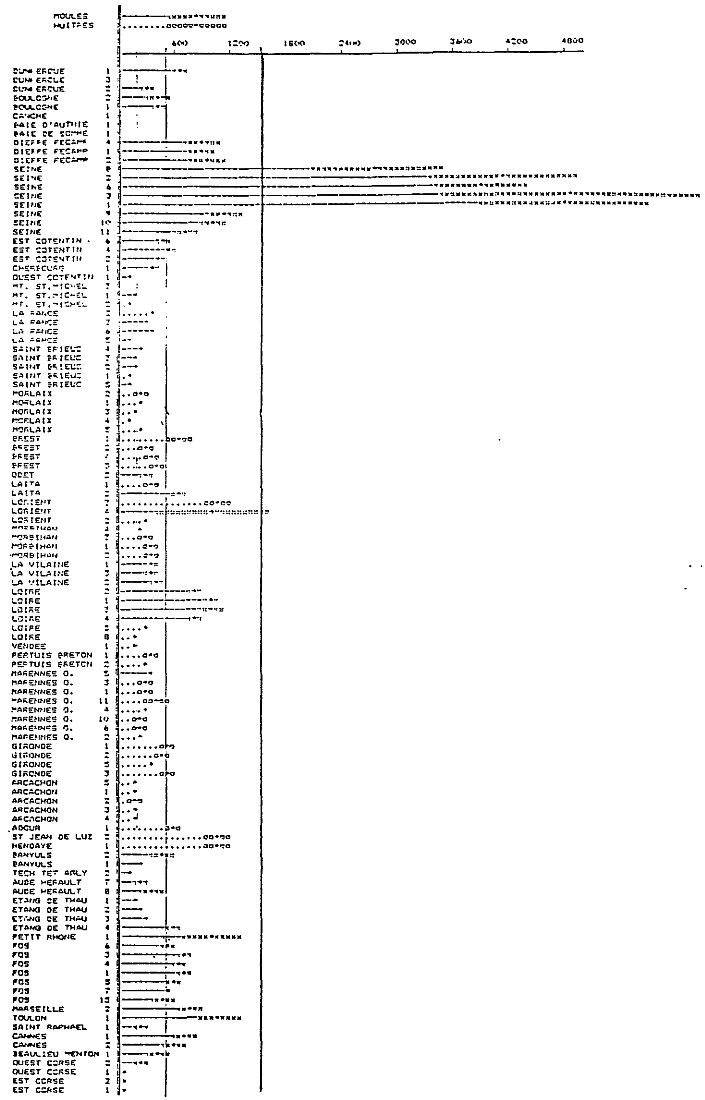


Figure 2 :

Concentration moyenne en µg/kg (poids sec) dans les moules et les huîtres du littoral français (RNO, 1979–1986).

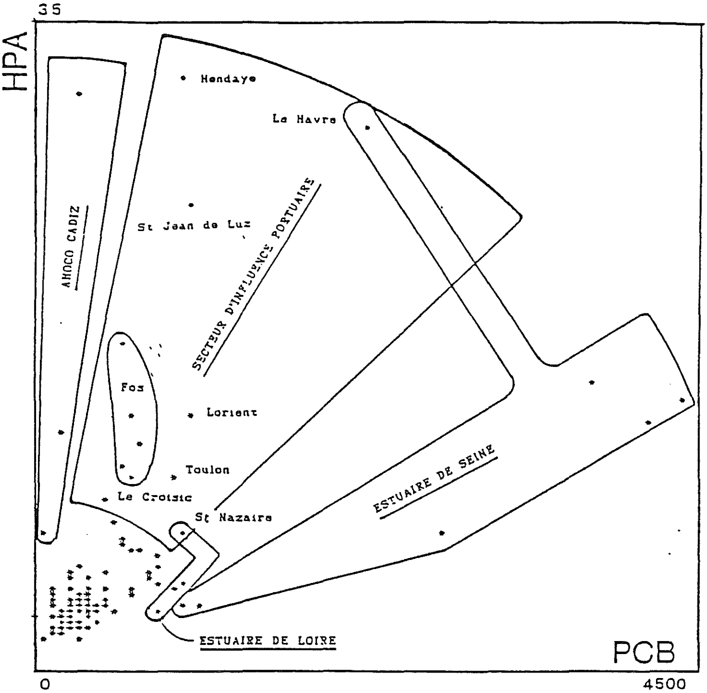
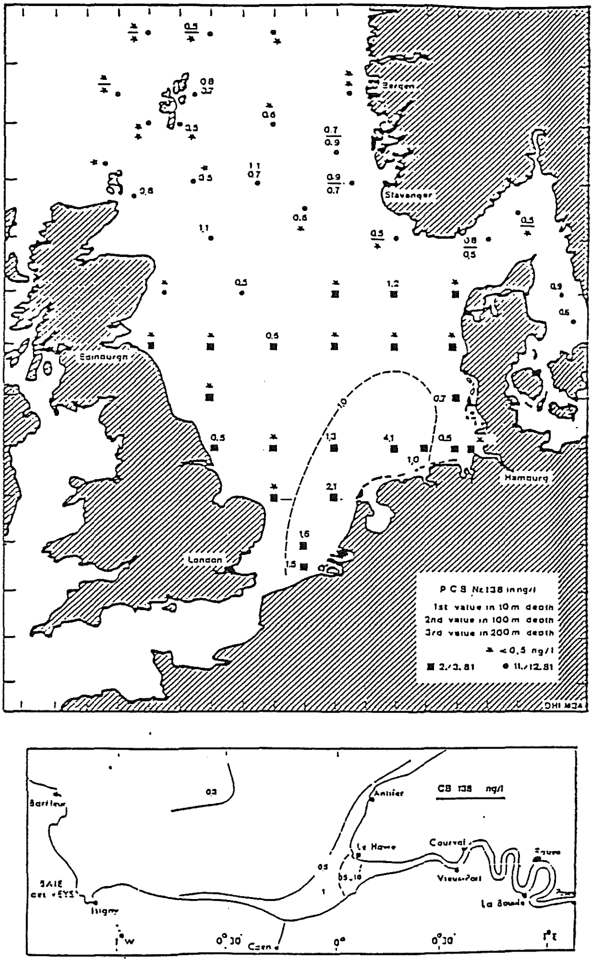


Fig 3 - Typologie des zones d'apport d'après les teneurs en PCB (ppb) et en HPA (ppm).



<u>Figure 4</u> :

Niveaux de contamination par les PCB dans l'eau.
Concentrations en hexachlorobiphényle (CB n° 138).
GAUL et ZIEBARTH (1983).

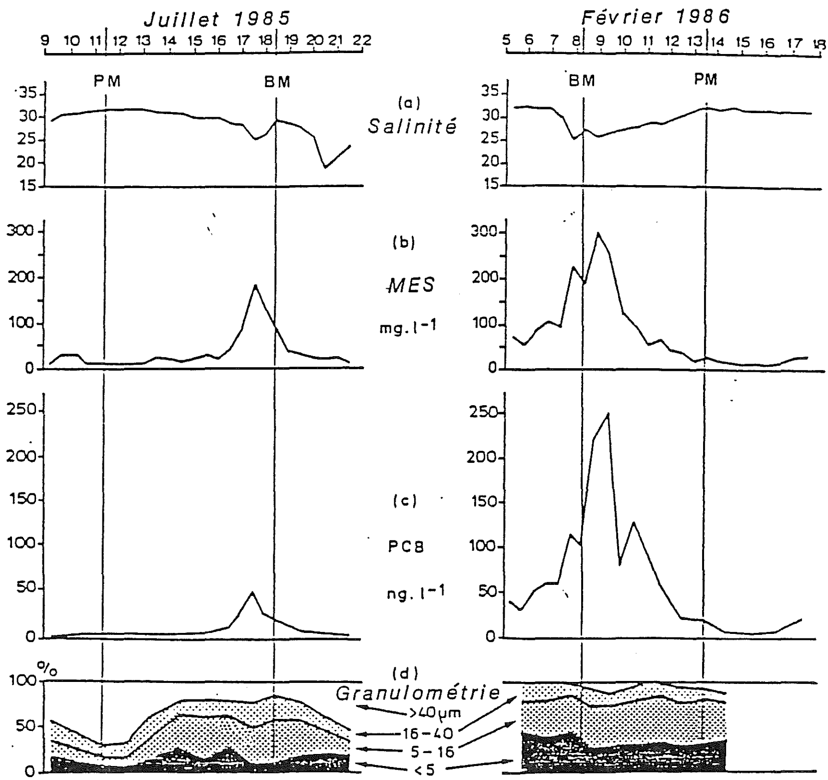


Figure 5 :

Variation de la contamination durant les cycles de marée.

EVALUATION OF BIOLOGICAL EFFECTS OF CONTAMINANTS
- Monitoring programmes -

Chemical aspects : composition of lipids

by Guy BODENNEC

_ ° _ ° _ ° _

In marine organisms, contaminant concentrations increase with the lipid content of their tissues. Two different organisms : flat fishes and zooplankton will be studied.

In flat fishes, it is well known that lipophilic contaminants are mainly accumulated and stored in fatty tissue organs as liver or ovary. The lipidic composition of these tissues is roughly defined according to the natural variability (age, sexe, phases of vital cycle ...) of the species. Some anormal changes may be appeared under the effects of stresses or chemical pollution. In addition to biological studies about the effects of pollutants, analyses of lipids in ovary will be carried out on samples collected in polluted and unpolluted areas.

Chemical analyses

. Total lipids were extracted according Bligh and Dyer (1959) and analysed in chemical classes (triglycerides, fatty acids, phospholipids ...) by thin layer chromatography (TLC) using a flame ionisation detector (FID) technique (Iatroscan MK IV apparatus). The rapidity with which the Iatroscan TLC-FID system provides synoptic lipid class data from small amounts of samples suggested that it would be useful for screening a lot of samples prior to performing more detailed chromatographic analyses.

. Total aromatic hydrocarbons were determined by U.V. spectrofluorimetry (Perkin Elmer 3000) after chromatography on a florisil column.

. Organochlorinated compounds (PCBs, HCH, DDT ...) will be analysed by gas chromatography (E.C. detector) by A. ABARNOU (see corresponding paper).

Studied sites

Samples collected in two French geographical areas will be compared : west Brittany (low level of contaminants) and Bay of Seine (very polluted site). Sampling time and periodicity will be discussed.

Strategy of analysis (1989-1990)

. Qualitative and quantitative determination of lipid content in flat fish will be studied. The fishes collected in the two areas are Plemonectes platessa, Limanda limanda and Solea solea (cf. L. QUINIOU paper). This preliminary study was mainly focussed on the plaices collected at different periods of the annual cycle of reproduction (before and after reproduction). In the Bay of Seine, sampling stations are located according to the pollution gradient. Natural variability in each area will be studied by measurement of replicates.
For zooplankton analysis : lipid composition will be studied on total zooplankton and, if possible, on the dominant species, using the same methodology as for the plaices.

DRAFT

ICES WORKING GROUP ON BIOLOGICAL EFFECTS OF CONTAMINANTS

Draft method for "Techniques in Marine Environmental Sciences"

OYSTER EMBRYO BIOASSAY

Developed by: J E Thain
Fisheries Laboratory, MAFF
Remembrance Avenue
BURNHAM-ON-CROUCH
Essex CM0 8HA
UK

Submitted by: R Lloyd

January 1989

2. Water samples for bioassay: Sample volumes should be 200 ml; they are filtered through Whatman GFC filter paper to remove particulates and suspended solids which can affect embryo development (Davis and Hidu, 1969) and stored in sterilized bottles. They are bioassayed as soon as possible to avoid sample deterioration.

3. Organism: The test described in this paper uses the Pacific oyster (Crassostrea gigas), but the method can be readily adapated for those other species of bivalve mollusc whose eggs are fertilized in the water column. Adult oysters are conditioned for spawning in the laboratory by maintaining then at 22°C in flowing sea water, to which a mixed algal diet supplement of Isochrysis, Tetrasalmis and Skeletonema sp. is added. Maturation occurs within 1 week in summer and 8 weeks in winter.

4. Collection of oyster gametes: For each assay, mature oysters are opened and the gametes stripped from the first 2 males and 3 females, using clean Pasteur pipettes for each oyster. The gametes from each oyster are then transferred to separate 1 litre volumes of reference sea water at 24°C. Sperm are identified by their milky appearance and eggs by their granular appearance, once they are in the water. After filtration through a 90-um filter to remove tissue debris, the three batches of eggs are pooled and the egg density adjusted with reference sea water to give a density of between 1000 and 4000 (and ideally between 3000 and 4000) eggs per 1 ml. This density is then measured accurately using a Coulter Electronics particle counter. The two batches of sperm are also pooled but not filtered. All the glassware used is sterilized in an autoclave.

5. Fertilization: Mixing of gametes is made at a ratio of approximately 2 ml of sperm to 1 litre of egg suspension. After mixing, they are left for 1-2 hours, during which a sample is examined under a microscope to confirm that the early stages of cleavage are occurring. If cleavage is not observed within 2 hours, the eggs should be discarded and further oysters stripped for gametes.

6. Test sample: At least four 30 ml aliquots of each sample of filtered sea water from the survey area are placed in 50 ml polystyrene vials (stoppered or capped), and the temperature raised to 24°C.

7. Control sample: At least eight 30 ml aliquots of reference sea water at 24°C are placed in 50 ml polystyrene vials (stoppered or capped).

8. Start of exposure: Using a sterilised Gilson (or similar model) automatic dispensing pipette, 1500 eggs at the early stage of cleavage are added to each 30 ml sample; the added volume of water containing the eggs is between about 0.37 and 1.50 ml (preferably between 0.37 and 0.50 ml), depending on the density of the egg suspension. The samples are incubated at 24°C for 24 hours; no aeration is necessary unless the samples have a high oxidisable organic content. Measurements of dissolved oxygen and pH are necessary only if these are likely to deviate from natural values.

10. End of exposure: At the end of the 24 hour exposure period, a 2ml aliquot of sample is removed and 2 drops of 8% formalin added. This sample, which should have originally contained 100 eggs, is transferred to a gridded shallow dish or slide, and the number of normal 'D' shaped larvae counted. Where large numbers of samples have been taken, the contents of the vials can be preserved for future counting by the addition of 0.5 ml of buffered formalin.

10. Calculation of results

The number of "abnormal" embryos is calculated to be 100 minus the number of normal D-shaped larvae. 'Abnormal' includes those eggs which were not fertilized, and those which died at an early stage of development or became malformed. The additional abnormalities in the test samples, compared to those in the control, is expressed as the Percent Net Risk (PNR).

$$PNR = \frac{\%\ \text{test abnormality} - \%\ \text{control abnormality}}{100 - \%\ \text{control abnormality}} \times 100$$

C. Sources of error

1. Number of exposed embryos

The calculation of the results is based on the assumption that there were 100 embryos exposed in each 2 ml of test or control sample. This assumption may not be valid because of errors in

(i) the measurement of the original egg density

(ii) pipetting the aliquot containing the calculated 1500 eggs into each 30 ml in the sample vials

(iii) the extra dilution which this aliquot gives to the 30 ml sample

(iv) pipetting out the 2 ml aliquot for embryo examination.

Errors in (i) and (iii) should be constant between the test and control samples, and therefore lost to some extent in the calculation of the PNR. For example, if in practice only 95 eggs were present in each 2 ml sample, this would be equivalent to an extra 5 percent of non fertilized eggs. In one series of tests the mean number of eggs transferred to the sample vials was found to be 96 (n = 58, SD = 8.0, SE = 1.05); this slightly low value may reflect an error in the measurement of the original egg density and the variation between vials can be reduced by ensuring that the eggs are evenly distributed in the stock suspension. Errors in (ii) and (iv) are random, and the overall error can be reduced by the use of replicate samples.

While additional procedures could be introduced into the method which would help to achieve the nominal egg density, these would be time-consuming. Speed of operation is essential at the start of the exposure period, particularly when a large number of samples are being assayed, and the small errors which occur may be considered acceptable.

Appendix 1 shows the results of a typical experiment; it is clear that with experience in the techniques the variation between replicates can be small. With a precision of this order, a statistically significant reduction in biological water quality can be shown when the PNR exceeds 5.

2. Dilution of sample:

The procedure described above will lead to a dilution of the test sample by up to 5 percent. Such a dilution effect may not be important in field surveys, where a variation between PNRs is looked for in relation to a known or suspected source of pollution (Figure 1). However, if the toxicity of a chemical is being measured, it will be necessary to take this additional dilution into account when calculating the nominal exposure concentrations. If the volume of egg inoculum is below 0.5 ml, the error from this source is minimal.

3. Acceptable control abormality

In the original method of Woelke (1972), it is recommended that control abnormalities should not exceed 5 percent. However, these recorded abnormalities at the end of the exposure period do not include mortalities at the early stage of development, nor do they include non-fertilised eggs. It is common hatchery experience that with good management practice, at least 80-85 percent of oyster eggs should develop successfully to the 'D' shaped stage, although sometimes this falls to 50 percent (Loosanoff and Davis, 1963). Experience with the oyster embryo bioassay indicates that control "abnormalities" of up to 20 percent is normal, and that up to 30 or even 40 percent is acceptable. Higher percentages of abnormalities may be caused by contaminated reference sea water, or the use of immature gametes from oysters which have not reached sexual maturity, or gametes from oysters in poor condition.

D. Reproducability of the test

In recent years, tributyl tin has been used as a reference toxicant. Exposure of developing oyster embryos to a range of concentration showed that

1.65 ug TBT/l would give a PNR of 50, and this concentration has been used in conjunction with subsequent field surveys. PNRs recorded for the reference toxicant in 8 successive surveys have ranged from 37 to 71 with a mean value of 51; using data from the initial calibration test, this range of PNRs is equivalent to a concentration range of 1.25 to 1.95 ug TBT/l. Although the control abnormality in the separate tests ranged from 13 to 44 per cent, there was no apparent correlation between these levels and the calculated PNRs. This bioassay therefore has a reasonable reproducability.

E. Example of survey data

Appendix 1 gives details of the oyster embryo bioassay data obtained in 1984 from a survey of the sewage sludge disposal ground between Plymouth and the Eddystone lighthouse, Devon, UK. This shows the degree of reproducability between replicate analyses of single samples.

Analyses of the toxicity of the sewage sludge to oyster embryos, showed that the field PNRs correlated well with the concentration of sludge at the disposal ground as calculated from suspended solids concentrations. (Thain and Stebbing, in prep.).

Figure 1 shows the variation in water quality at a sewage sludge disposal ground when discharges were being made as an example of the type of information obtained.

The design of such surveys should follow the same pattern, and be conducted with the same rigour, as that used for chemical sampling in similar circumstances.

F. Interpretation of data

The results of this bioassay cannot be used to predict the effects of a small measured deterioration in biological water quality on oyster populations, and even less on the general aquatic biota. This is especially the case where the natural biota are exposed to poor biological water quality for only a short period of time, whereas in the bioassay the embryos are exposed to the water sample for 24 hours. The results can be used to measure the gradients and distribution of poor biological water quality in the vicinity of pollutant inputs; where a gradient is found, it can be assumed that the potential for harm to aquatic biota will increase with the degree of the bioassay response, but the nature and extent of the effect which may occur in practice cannot be predicted. The bioassay is sensitive to a wide range of chemicals; it is also sensitive to a deterioration in biological water quality caused by algal blooms (Thain and Watts, 1987).

G. References

Davis, H. C. and Hidu, H. (1969). Effects of turbidity producing substances in sea water on eggs and larvae of three genera of bivalve mollusks. Veliger, 11, 316-323.

Loosanoff, V. L. and Davis, H. C. (1963). Rearing of bivalve mollusks. Adv. Mar. Biol., 1, 1-136.

Thain, J. E. and Watts, J. (1987). The use of a bioassay to measure changes in water quality associated with a bloom of Gyrodinium aureolum Hulburt. Rapp. P.-v Reun. Cons. int. Explor. Mer, 187: 103-107.

Woelke, C. E. (1972). Development of a receiving water quality bioassay criterion based on the 48-hour Pacific oyster (Crassostrea gigas) embryo. Tech. Rep. Dept. Fish. Wash., 9, 1-93.

Appendix 1

Data from a survey of a transect between Plymouth and the Eddystone lighthouse. The sewage disposal ground is at stations 5 and 6. This information is given to show the variation obtained between replicates.

Sample	Number of D larvae in a 2ml sample	$\bar{x}$	SD	PNR
Before dumping				
Station 1: Plymouth	73, 75, 70, 74 77, 69, 69, 67	72	3.5	-4.3
4	69, 67, 66, 73 67, 74, 70, 70	69	2.9	0
6	67, 69, 72, 68 70, 70, 74, 68	70	2.3	-1.4
8 Eddystone	69, 70, 70, 72 76, 69, 65, 71	70	3.1	-1.4
Control	74, 68, 68, 70 65, 69, 68, 73 71, 66, 69, 66	69	2.7	-

Station 6 was then sampled at intervals during the disposal of sewage sludge.

6.1	22, 18, 24, 20 28, 29, 19, 21	23	4.1	67.0
6.2	35, 40, 40, 32 33, 34, 38, 35	36	3.1	48.0
6.3	0, 0, 0, 0, 0, 0, 0, 0	0	-	100.0
6.4	46, 49, 53, 42 42, 47, 42, 53	47	4.6	32.0
6.5	76, 70, 71, 73 65, 69, 72, 71	71	3.2	-2.9

Figure 1 Oyster embryo bioassay, Liverpool Bay, Aug 1981. Per cent net response for surface water samples Underlined values are significantly different from the control value.