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ICES/IOC Workshop on the Biological Effects of Contaminants,

Bremerhaven, Federal Republic of Germany

12-30 March 1990

by

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ICES/IOC WORKSHOP ON BIOLOGICAL EFFECTS OF CONTAMINANTS

BREMERHAVEN, FEDERAL REPUBLIC OF 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 (WG BEC) held at ICES Copenhagen in May 1987 (CM 1987/E:23). The decision to proceed was made at the next meeting of the WG BEC in April 1988 (CM 1988/E:26), and a Planning Group was formed consisting of Drs Stebbing, Dethlefsen, Thurberg and Heip. 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 and to evaluate new kinds of indices of toxic 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, as well as the effects of contaminants in the sea surface microlayer in relation to 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 which 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 on 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 finalising 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 Organisation (IMO) in London on 20-21 April 1989, and in the Alfred Wegener Institute on 19 September 1989 to consider the plans and proposals in detail. 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 OF THE WORKSHOP

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

2. To test primarily those techniques that can be deployed at sea, to

complement the emphasis given at the IOC Oslo Workshop to techniques suited to nearshore applications.

3. To test the techniques on known contamination 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 (eg fish) or habitat (eg benthos), by deploying them simultaneously on the same gradient.

PROPOSALS

Proposals have been received and grouped under the following topics with a coordinator for each (ANNEX 1).

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

2. Bioassays (L. Karbe)

3. Benthos
   - -sediment bioassays (P. Chapman and R. Swartz)
   - -benthic studies (C. Heip)

4. Chemistry (W. Cofino)

5. Statistics (M. Carr)

RATIONALE FOR DIFFERENT CONTAMINATION GRADIENTS

1. Offshore gradients on a transect NW out of Bremerhaven: A cruise track has been proposed (Figure and Table 1) which traverses contamination gradients that attempts to minimize the effects of natural and unrelated factors. Numerous requirements were considered important when this track was proposed, which include uniform depth, known differences in contaminant concentrations in sediments, uniform sediment type, a variety of contaminants (OCs, metals, PAHs, petroleum hydrocarbons) and proximity to Bremerhaven.

The benthos group will use inshore stations of this transect to detect the effect on the benthos of the R Elbe plume, involving 4 stations on a transect. Plans for this element were consolidated after a preliminary investigation of the plume area by Dr Rachor 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 will also provide fish samples for the biochemists. A Dutch oil

platform has been selected.

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 focussing effort on the surface microlayer, to see whether water quality in the microlayer is depressed and to identify the best techniques to monitor change. 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 and prevalence of disease in terms of gross and cell pathology. In recent times the relationship between distributions and concentrations of toxic contaminants and fish disease has created considerable interest. While some of these data provide correlations, the question whether or not a causal relationship exists remains unanswered. 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 proposals accepted cover a range of test species including ciliates, copepods and microalgae. 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.

3. 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 commnunities along pollution gradients. The Sediment Triad approach (chemistry, bioassay and community analysis) has not previously been used in European waters. At the workshop there is a good opportunity to test this approach (under the guidance of Dr P Chapman), as a number of 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. Sediment bioassays will be used 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 samples for macrofauna and meiofauna community analysis, sediment bioassays and sediment chemistry.

SAMPLING STRATEGY

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

2. Elbe Plume. 4 stations for benthic studies, including samples for macrofauna, meiofauna, sediment bioassay and sediment chemistry.

3. Oil Platform gradient: 2 stations for sampling fish and 5 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 this 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 co-ordinated 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 carry out tissue residue analyses. An ongoing programme of the German Hydrographic Institute covers 4 of these stations and their data on sediment chemistry (metals, OCs, PAHs) will be made available to the Workshop.

The plan for the sampling strategy and chemical requirements for the workshop was drawn up at an ICES BEC WG with advice from members of the Marine Chemistry and Sediments WGs. The listing of chemical requirements and sampling strategy (Annex 2) is the result of that meeting.

If for any reason the chemical analyses identified in Annex 2 can not be carried out, it has been decided that the total number of station worked will be reduced, rather than limit the range of contaminants analysed or the replication. It is 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 standardisation 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 responsible for data archiving and providing guidance on the treatment and presentation of data.

COORDINATORS

For each study area coordinators have been 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 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.

RESEARCH VESSELS

	12.3.90	30.3 90	number of scientists
1. Valdivia (Univ. of Hamburg)	_____		12
2. Victor Hensen (AWI)	_____		9
3. Friedrich Heinke (Biol Anst Helg)	_____		5
4. Solea (Fed Res Bd Fish)	_____18.3		5
5. Walter Herwig (Fed Res Bd Fish)		23.3_____	12
6. Hollandia (Rijkswaterstaat)	_____		7
7. Aurelia (NIOZ)	_____		7
6. Gauss (German Hydr Inst)	----------		

FOUL WEATHER CONTINGENCY PLAN

A bad weather contingency plan 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. Participants 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 are:

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

2. Water quality bioassays. Intercomparison on techniques under workshop conditions using a specific toxicant. Assessment of toxicity of elutriates of large water samples passed through 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

will 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).

PREPARATORY WORK

In preparation for the Workshop a number of activities have already been initiated, or will be carried out prior to the Workshop, including:-

1. Preliminary investigations of indices of toxic effect on isolated flounder hepatocytes from the R Elbe and R Eider (Lowe, Moore and Kohler-Gunther).

2. In addition total pathology is being carried out on flounders from the same area for familiarisation with these populations (Simpson).

3. Sampling of macro and meiobenthos at regular intervals from the Workshop stations (Fig. 1, Table 1) (Rachor).

4. Monthly analyses of sediment contaminants from Workshop stations (Fig. 1, Table 1) (German Hydrographic Institute).

5. Sampling of dabs for gross pathology and contingency plan (Dethlefsen).

TIMETABLE

December 1989	Planning Group Meeting
12-30 March 1990	Workshop
early May 1990	Combined meeting of WG BEC and Workshop co-ordinators
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. Publication as special number of a journal (eg. MEPS, Proces Verbaux)- Workshop Symposium Volume.

ANNEXES

Annex 1 List of proposals, subdivided into groups under Coordinators

Annex 2 Proposed analytical chemistry

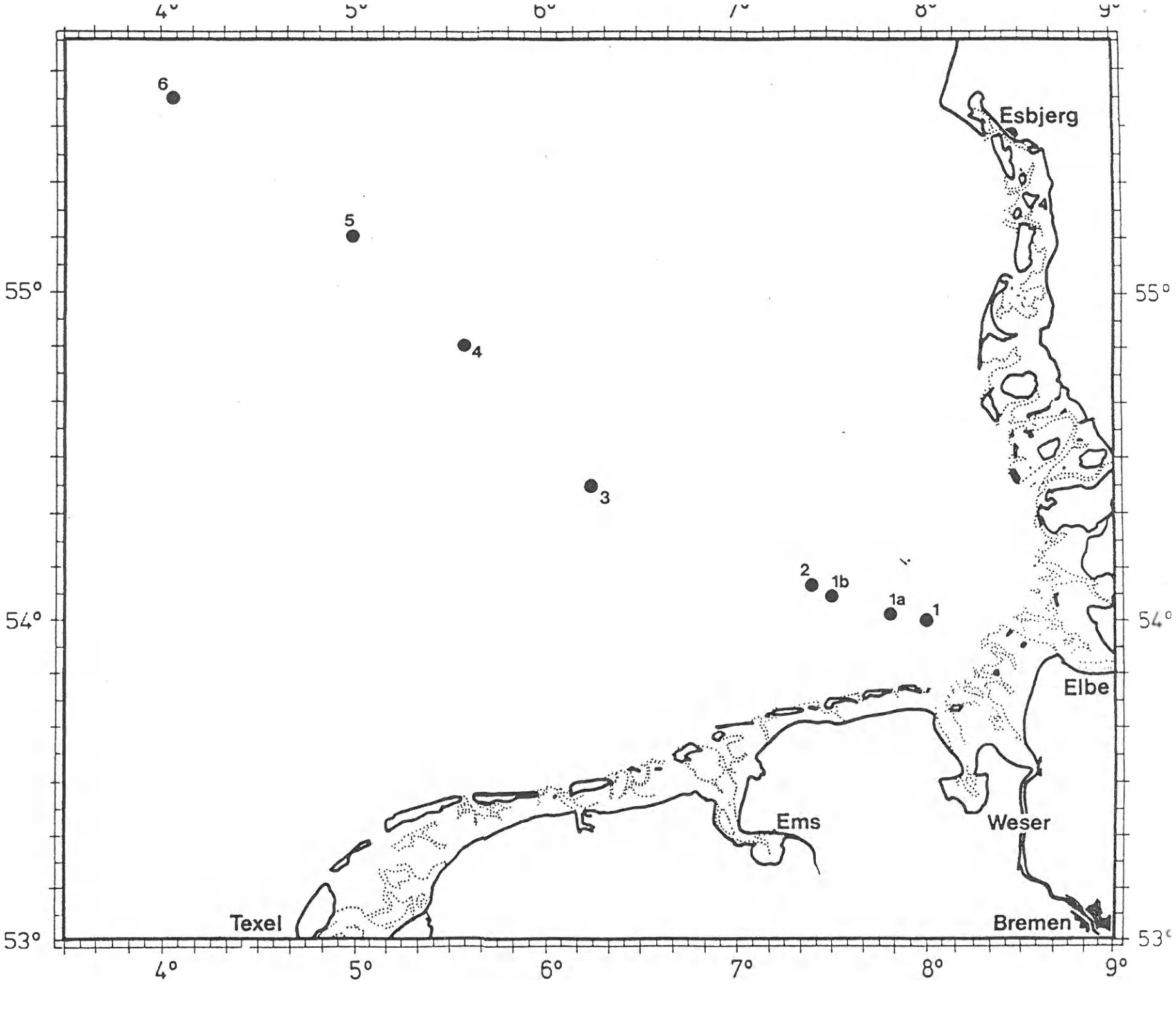
Table 1. Positions of German Bight transect stations plotted in Figure 1.

Station number	Longitude	Latitude	
1	54°00'N	08°00'E	
1a	54°01'N	07°49'E	BEWG
1b	54°04'N	07°30'E	BEWG
2	54°6.5'N	07°24'E	GHI
3	54°25'N	06°15'E	GHI
4	54°50'N	05°35'E	
5	55°10'N	05°00'E	GHI
6(optional)	55°35'N	04°10'E	GHI

GHI - German Hydrographic Institute station

BEWG - ICES Benthic Ecology Working Group station.

Figure 1


4°
5°
6°
7°
8°
9°
55°
54°
53°
6
5
4
3
2
1b
1a
1
Esbjerg
Elbe
Weser
Ems
Texel
Bremen

ANNEX 1 - List of participants, proposals and invited coordinators.

I. FISH

A. GROSS PATHOLOGY - Vethaak (Coordinator)

* 1. Gross pathology of livers of dab - Kranz

2. Characterise 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. An in-depth pathology profile of flatfish - Simpson and Hutchinson.

7. Morphological and chromosomal aberrations during embryonic development in pelagic fish embryos - Cameron and Berg.

C. BIOCHEMISTRY - Addison (Coordinator)

1. Estimation of lysozyme activity in the blood of marine fish - Mock

2. Catalytic enzyme activity in crustaceans - McHenery

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 contaminant gradients - Leavitt and Capuzzo

9. Detection of effects of organophoshates 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, ecotoxicolgy and monitoring - Hardy

9. Effects of a gradient of pollutants in water and sediment on the ecophsiology of benthic organisms - Absil and Hummel

10. Water quality bioassay using a copepod - Williams.

III. BENTHOS

A. SEDIMENT BIOASSAYS - Chapman and Swartz (Coordinators)

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 photograhy - 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. Surface microlayer chemistry - Brockmann

7. 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)

ANNEX 2 - PROPOSED ANALYTICAL CHEMISTRY

A. GERMAN BIGHT TRANSECT - Bremerhaven to NW across German Bight

- 5 stations for fish studies and water quality bioassays

- 5 stations for benthos studies in Elbe plume

1. Fish samples

- dab (*Limanda limanda*) one sex only, otoliths to be taken for ageing, size range 20-25 cm. 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, IOC list of chlorophenyl congeners (see Wells, 1988).
  The distribution of the liver tissue between participants to be organised by Dethlefsen).

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

2. Sediment 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, normalisation 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 as each station of 5 representative 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, chlorophyll, nutrient (silicate, phosphate and nitrate).
All except chlorophyll 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 ageing, size range 20-25 cms.

20 individual fish analyses per station.

liver: THC (IR & GC), PAHs, barium.
(distribution of liver samples to be organised 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 of 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.