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D/O

REPORT OF THE
WORKING GROUP ON BIOLOGICAL EFFECTS OF CONTAMINANTS

Ostend, Belgium

4–7 March 1996

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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1 OPENING OF THE MEETING

The meeting of the Working Group on Biological Effects of Contaminants (WGBEC) was opened at 10.00 hrs on 4 March 1996 by the Chairman, Dr Ron Stagg. Dr Kris Cooreman welcomed the participants on behalf of the Rijksstation voor Zeevisserij in Ostend, Belgium. Dr Mike Moore and Dr Aldo Viarengo attended the meeting as representatives of UNESCO IOC and MEDPOL, respectively.

2 ADOPTION OF THE AGENDA

The terms of reference for the meeting (C.Res. 1995/2:14:4) are listed below:

The Working Group on Biological Effects of Contaminants (Chairman: Dr R Stagg, UK) will meet from 4–7 March 1996 in Ostend, Belgium, to:

a) review and report on existing biological effects techniques recommended by the group, including clearance rate and scope for growth (SFG) measurements, and oestrogenic contaminants;

b) elaborate guidelines for the use of recommended techniques for biological effects monitoring and interpretation of results, and identify information on possible new techniques [OSPAR 1.3];

c) prepare draft advice on the use of biological effects techniques for identifying the extent to which PCBs in marine mammals generate effects at the species and/or population level (with SGSEAL) [OSPAR 1.2];

d) provide information on methods to determine the biological effects of contaminants on reproduction, immunology, and metabolism of marine organisms, mainly fish [HELCOM 9];

e) review research proposals on:

- linkages between effects of contaminants on individuals and communities;
- particle transport in a maritime area influenced by upwelling of lipid droplets and the deposition of atmospheric material; and
- study of the risks of harmful effects on benthic communities in marine sediments;

f) review the progress on the results of intercomparison exercises for biological effects techniques including: scope for growth, acetylcholinesterase, and lyso-somal stability;

g) review information provided by WGPDMO on the usefulness of externally visible fish diseases and liver pathology for the monitoring of biological effects of contaminants and on methodologies for fish disease surveys;

h) review descriptions of techniques to be included in the *ICES Techniques in Marine Environmental Sciences* series;

i) discuss and report on the current state of QA procedures in biological effects monitoring;

j) assess biological effects measurements (e.g., imposex in molluscs) that can reflect responses to organotin compound exposure and, if appropriate, recommend methods with due regard for response sensitivity to other stimuli that might give false indications of organotin exposure;

k) develop guidelines on the statistical design of biological effects monitoring programmes;

l) consider the effects of UVB radiation on the marine environment.

The draft agenda was accepted without amendment and is appended as Annex 1. The list of participants is attached as Annex 2. The list of documents considered is contained in Annex 3.

3 APPOINTMENT OF RAPPORTEURS

It was agreed that the work of writing the report would be shared by all members of the group.

4 STATISTICAL DESIGN OF BIOLOGICAL EFFECTS PROGRAMMES

At the OSPAR/ICES Workshop on Biological Effects Monitoring Techniques (Aberdeen, October 1995) statistical guidelines were developed for use in biological effects programmes. Briefly these relate to the definition of three types of objectives:

Exploratory sampling. This is concerned with the estimation of levels and the description of the normal range of values of a parameter (i.e., a particular biological effect measurement) and spatial variability in a subregion or area. For example, to:

1) map the level (of a parameter of interest) at all points in an area with a specified precision;

2) estimate a parameter or parameters (e.g., mean, median, 95 percentile) to describe a population of interest within an area with a specified precision;

3) estimate a gradient in a parameter from a point source with a specified precision.

Areas of concern. This is complicated by the definition of an 'area of concern', which would need to be defined prior to any monitoring but would be concerned with location of 'areas of concern' or the measurement of the extent of an 'area of concern. Examples of such objectives might be to:

1) locate all 'areas of concern' of a certain size in an area with a specified probability of success;

2) estimate the extent of a known 'area of concern' with a specified precision.

Detection of change. Monitoring to detect either temporal or spatial changes over an area. For example, to:

1) estimate the change in a parameter over a specified time with a specified precision;

2) estimate the change in the spatial extent of an area of concern over a specified time with a specified precision.

Other work within ICES has been mainly concerned with defining the statistical objectives for temporal trend monitoring of contaminants (WGBEC96/4/1). However, although there are many similarities between chemical and biological monitoring, there is a fundamental difference. Contaminant monitoring currently focuses on individual measurements that may be compared with some absolute value or criterion. In contrast, the interpretation of biological effects monitoring requires an evaluation of the 'health status' of the ecosystem which will inevitably require the collective interpretation of a number of measurements. Ketil Hylland presented a discussion paper (WGBEC96/4/2) which identified some additional requirements for the development of a statistical framework. These relate to the fact that the goal of biological effects monitoring is to determine the severity of damage or effects on the health of the ecosystem, and the interpretation of such effects will be confounded by other environmental, ecological and biological covariables.

Following this, there was a discussion on what the output of a biological effects monitoring programme should be in the context of the overall scientific objective and the requirements of environmental managers. Managers often demand that observed biological effects measurements should be able to be interpreted in relation to effects on the ecosystem (e.g., at the population level) and are often seeking single parameter approaches to such assessments. The group regarded this as a flawed approach in that ecosystems are complex and that the outcome of perturbations is often difficult to predict. Although there is a need to link effects at the individual level to effects on the population, community or ecosystem levels, this was often difficult even for closely related systems. Although it may be possible to link biomarker responses to population measures, such as intrinsic population growth, there are cases when even closely related parameters do not behave predictably in this way. For example, it was reported in one study that scope for growth in individuals is a poor predictor of population growth rates. It was also noted that fisheries scientists had spent many years attempting to predict changes in fish populations but that such predictions were still difficult even in cases where fishing pressure is considerable. There was therefore a need to educate managers in new ways of approaching this problem and that this hinges on providing data from a sufficient set of techniques available to enable an integrated evaluation of effects, similar to medical diagnosis in human patients. The following approaches were suggested from members of the Group:

1) **Weight of evidence.** This requires the use of a set of techniques to make a holistic evaluation of biological effects in a given area; the significance of a response if more than one parameter is affected is much greater than if a single parameter deviates from normality.

2) **Deviation from physiological range.** Biological effects measurements will usually vary within a known or established physiological or biochemical range, i.e., within the normal homeostatic range, of response. If responses within individuals are measured which are outside this range, then deleterious population responses would be a likely consequence.

3) **Establishment of links across organizational levels.** If biological effects are used which have some causal link between different levels of biological organization or if there is a known progression of cause and effect, then the case for a link to a population effect is stronger.

4) **Additional work.** If one or a subset of deployed biological effects techniques gives results which are different from those of a reference group or site, this would indicate that further studies need to be done, e.g., the application of additional techniques.

There was a discussion of whether modelling of processes could be a way forward, but there was general agreement that such models could only be used as a research tool (e.g., to optimize the solutions to problems or to develop scientific understanding), and would not form a suitable basis for a managerial tool to be used by non-specialists.

The recommendations of the group were as follows:

a) Interpretation of the significance of biological effects measurements requires a holistic approach and it must be emphasized that biological effects techniques are not methods that will yield single numbers that may be compared to some criterion or level;

b) WGSAEM is requested to consider statistical techniques applicable to biological effects data in conjunction with members of WGBEC, particularly in the context of using multiple measures to assess health status;

c) Future assessments of data from biological effects programmes will require the further development of these concepts.

5 QUALITY ASSURANCE IN BIOLOGICAL EFFECTS MEASUREMENTS

WGBEC discussed paper WGBEC96/5/1 on quality assurance and biological effects monitoring, which had originally been presented to the 1996 meeting of the OSPAR Working Group on Concentrations, Trends, and Effects of Substances in the Marine Environment (SIME 1996). WGBEC also considered details of the QA/QC procedures developed by the MEDPOL Biomonitoring Programme which are based on measurements in both wild and caged organisms. This programme has, *inter alia*, emphasized the importance of using similar (but not necessarily the same) species from across the entire Mediterranean area, organizing training courses in the relevant techniques, utilizing standardized and protocolized methodologies and identical reagents, and ensuring that all laboratories participate in inter-comparison exercises (which include the exchange of blind samples). These efforts have borne fruit because interlaboratory variation has been shown to be acceptably small (for example, see Figure 5.1, below). The use of biomarker measurements in caged organisms has also proved to be worthwhile because variability in measurements which may fluctuate in individuals in the wild due to other environmental influences is minimized, enabling a clearer interpretation of responses.

The need for implementing biological effects monitoring techniques with full QA/QC procedures in the new OSPAR Joint Assessment and Monitoring Programme (JAMP) was reaffirmed, but it was recognized that support from Contracting Parties to the monitoring programme was required and also that other sources of funding should be explored.

5.1 Funding for a QA Programme

It was suggested that the European Commission (EC) may be receptive to requests for funding to set up an international QA/QC system for biological effects monitoring. At SIME 1996 Ron Stagg had agreed to approach the EC to investigate setting up a similar programme to the QUASIMEME programme for biological effects. This was possibly a longer-term option and Ian Davies presented to the group an outline of the EC COST programme, which may be a faster way of initiating funding for a QA system. The COST programme is a structure which exists to provide funding for the coordination of existing research activities in a particular area of science. The activities to be coordinated are funded nationally, but the COST system can support coordination activities to maximize the benefits to the participating countries. The COST programme was presented in relation to the needs for the development of standard analytical procedures and quality assurance procedures for methods to be used in integrated chemical and biological monitoring programmes.

A preliminary draft of a COST Memorandum of Understanding had been drawn up for discussion (Annex 4). The proposed activities under the COST initiative had been structured to take place through a series of contaminant-based Working Groups. There was considerable discussion of this form of organization, and it was agreed that a more effective alternative might be to structure the Working Groups around either defined analytical methods (e.g., molecular biomarkers or histopathology), or toxicological processes (e.g., effects on reproduction or cancerogenicity). Expressions of support for the further development of the proposal were received from scientists from Sweden, the UK, Norway, France, Italy, Germany, Belgium and the Netherlands. Ian Davies undertook to redraft parts of the proposal, in consultation with interested scientists both within the WGBEC and outside, with a view to presenting a more considered draft to the UK COST Secretariat in May 1996 for their comments.

5.2 Quality Assurance Requirements for International Monitoring

The outcome of the OSPAR/ICES Workshop as regards QA was presented by Ron Stagg. Briefly, the minimum essential elements of such a programme were defined as:

a) the adoption of only those methods which are referenced and have both a standard operating procedure (SOP) and associated analytical quality control (AQC);

b) staff trained to an agreed level of competence to conduct the test;

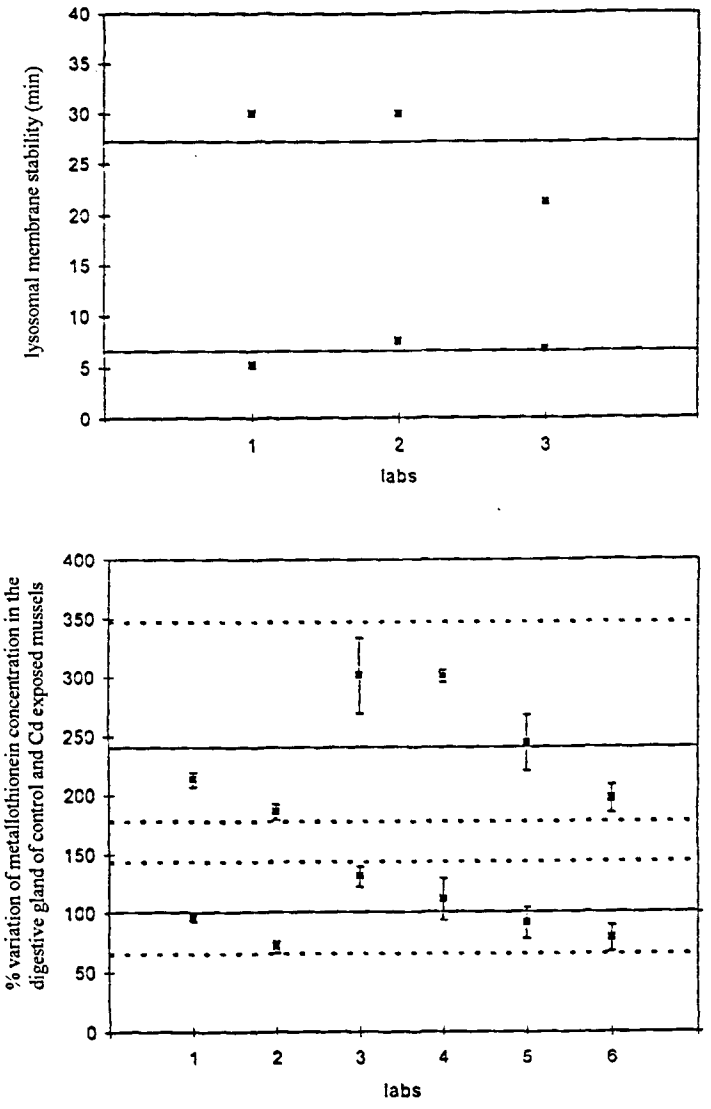
c) regular internal (within-laboratory) calibration, including, where possible, the introduction of blind

samples during normal analysis, and strict adherence to AQC procedures for each test method;

d) interlaboratory performance assessment with the periodic circulation of samples for analysis by participating laboratories;

e) an action plan to respond to breaches of acceptable limits (limits established in AQC procedures or agreed for interlaboratory performance assessment).

Figure 5.1. Results of the intercomparison exercise for metallothionein and lysosomal stability measurements in the MEDPOL programme.



At SIME 1996 it was recommended that lead laboratories be established to coordinate the QA programme for each technique. The Working Group endorsed this approach and identified the following general procedures relevant to all methods. It was recommended that these form generic terms of reference for the work to be carried out by each lead laboratory:

1) to specify the recommended method or methods to be used including: the species to be sampled; the specification for the standards and reagents required; the sampling requirements; suitable sample collection, preparation and preservation procedures; and the training requirements. It was also noted that the development of any one particular method should not be to the exclusion of all others: the acceptability of alternative methods would depend on the results from interlaboratory comparisons;

2) to develop an intercomparison programme and distribute reference material and, where applicable, standards to each laboratory; to define and agree with participants on the required intercomparison standards and reference materials;

3) to repeat intercomparison exercises at regular intervals;

4) to agree acceptable performance limits, the action to be taken if limits are breached, and organize a methods workshop if intercomparison exercise(s) indicate substantial variability (e.g., >20 %).

The Working Group agreed that the specific QA requirements for each of the methods recommended at the OSPAR/ICES Workshop on Biological Effects Monitoring Techniques should be considered under Agenda Item 7.

6 METHODS FOR REVIEWING BIOLOGICAL EFFECTS TECHNIQUES

The Chairman outlined the reasons for the inclusion of this agenda item. Briefly, this arose from a previous WGBEC meeting where Lennart Balk had suggested that the Working Group develop a procedure for assessing the effectiveness of biological effects measurements by comparison with established lists of hazardous substances. A guide to these chemical substance lists was presented to the meeting in the form of a report on the Sunset Project for the Swedish National Chemicals Inspectorate (WGBEC96/6/2). Following discussion, the WGBEC decided that it was inappropriate to use such lists for this purpose for the following reasons:

- the number of chemical contaminants is counted in terms of tens of thousands and, therefore, the comparison of biological effects measurements with these lists, which contain only a few hazardous substances, will not be a good indicator of their performance in the field;

- contaminants in the marine environment have complex and interactive effects (synergism, additivity, antagonism) and comparison of performance against single substances is not appropriate for judging the value of any particular biological effects measurements;

- one of the purposes of biological effects measurements is to measure integrated responses from animals exposed over long periods of time to low levels of contaminants;

- lists of chemicals generated using limited toxicological and physico-chemical data for ranking purposes are unlikely to be suitable for evaluating such measurements.

WGBEC then reviewed a paper entitled 'Evaluation of the SIME monitoring programme' (WGBEC96/6/1) which originated from SIME 1996. The purpose of this document was to develop a method for reviewing the applicability, feasibility, suitability, and cost of the SIME monitoring programme, including the biological effects components adopted following the Aberdeen Workshop. The WGBEC noted that this paper is not internally consistent. It recommends an integrated matrix of chemical and biological measurements for monitoring purposes which has been the approach consistently advocated by the WGBEC. However, the proposed biological measurements are subsequently presented in the paper in terms of a 'shopping list' with the implication that techniques which do not score well will be dropped from the programme. Such an approach would not provide a coherent interrelated suite of diagnostic/prognostic 'clinical type' tests, which is required if the problem is to be addressed effectively. Furthermore, there is a requirement for consistent parallel suites of tests for use in fish and invertebrate sentinel animals. The WGBEC also noted that the proposal to sum the scores in each column to provide a total score was wholly inappropriate. Some techniques would score highly for some criteria and low for others. Summing such scores would not give a satisfactory assessment of the contribution from any particular technique.

A letter for discussion by L. Balk (Sweden) was presented by Å. Granmo (WGBEC96/6/3). In this letter, the increasing use of biomarkers as the major tool in many monitoring programmes was questioned, particularly when biomarkers are the only strategy that is used. The reasons for this are related to problems with the interpretation of the results of such studies because:

- an increase in variance is often observed for parameters measured at polluted sites;

- inhibition of biomarker responses may occur at grossly polluted sites;

- the interaction of environmental factors can complicate interpretation, e.g., temperature, season; and

- the monitored species may be very mobile.

The letter suggested that in addition to biomarker studies, other strategies should also be used. The use of 'passive abiotic samplers' such as semi-permeable membrane devices (SPMDs) was suggested for concentrating contaminants, subsequent extraction and toxicity testing of the samples in the laboratory. The use of fractionation and bioassay could increase the possibility of identifying biological effects independent of the effects of environmental variation. Some Working Group members agreed that such a technique was valuable, particularly if biological effects have been found or if it was desired to combine the use of concentrated samples with bioassays. It was also thought that such methods were of value for chemical tracing of observed biological effects, particularly in combination with chemical fractionation methods. However, there was also a strong concern regarding the use of this type of technique in the context that such methods do not reflect bioavailability to organisms, as the chemical and physical environment is changed by such processing. Other possibilities suggested were the use of bioaccumulating organisms, caging animals in the environment, and the use of transgenic organisms with reporter genes in bioassays.

7 OSPAR/ICES WORKSHOP ON BIOLOGICAL EFFECTS TECHNIQUES

The report of the OSPAR/ICES Workshop on Biological Effects Monitoring Techniques was presented to the meeting (WGBEC96/7/1). This report proposed a contaminant-specific programme for PAHs, TBT and heavy metals (a so-called bottom-up programme) and a general (top-down) programme where the contaminants are unknown or unspecified. The Workshop had completed its work on the contaminant-specific programmes but had only partly completed the work on the general biological effects programme. The Workshop report had been welcomed by SIME 1996, which had adopted it. A meeting of the OSPAR *Ad Hoc* Working Group on Monitoring (Terms of Reference in WGBEC96/7/2) has been charged with taking the programme forward and incorporating guidelines for the programme into the monitoring manual for the new OSPAR Joint Assessment and Monitoring Programme (JAMP). The Chairman explained that the WGBEC could assist in taking the process forward by reconsidering the general biological effects programme particularly in the context of the objectives, the methods to be used and the implementation of the programme and by developing a QA programme for each of the techniques to be used.

7.1 Development of a General Programme (Top-Down)

The purpose of this programme is to establish where contaminants cause deleterious biological effects (JAMP issue 1.17). Two generic objectives were identified:

i) to monitor the general quality status of an area so that environmental impacts by contaminants can be identified;

ii) to identify biological effects in areas with known or suspected elevated levels of contaminants, e.g., areas receiving major point-source inputs, estuaries receiving significant contaminant inputs.

It was also agreed that a number of techniques should be used. These are summarized in Table 7.1.1, below.

Table 7.1.1. Summary of recommended methods for general monitoring of the biological effects of contaminants in the OSPAR area.

	Status	Specificity of contaminant response
Bioassays		
Whole sediment	B	General toxicity
Pore-water	B	General toxicity
Water-column	B	General toxicity
Biomarkers		
P4501A (EROD)	B	Planar molecules, PAHs, PCBs
Lysosomal stability	B	Organic contaminants
Liver pathology	B	General (but can be diagnostic)
Liver nodules	A	Cancer-forming chemicals
Population/ community responses		
External fish diseases	A	Not specific to contaminants
Fish reproductive success	B	Not specific to contaminants
Macrobenthic fauna	A	Not specific to contaminants

A: suitable for immediate application
B: suitable for application as soon as QA is in place

Neither the Workshop nor the subsequent discussions at SIME 1996 could resolve a mechanism for the implementation of such a programme, and therefore the Working Group considered the question of the objectives, generic principles and the implementation of a general programme designed to identify the effects of unknown or unspecified contaminants as described in the report of the OSPAR/ICES Workshop. It was also noted that this issue would be tackled at a special meeting of the OSPAR *Ad Hoc* Working Group on Monitoring (MON) in the autumn of 1996. The WGBEC considered that it could start this process, and some general principles emerged from the discussion, namely:

- in such a programme multiple measures of effect are essential;
- measurements used should include those indicative of both exposure and pathology;
- the combined suite of measurements should integrate responses across organizational levels;
- the combined suite of measurements should be interpretable in terms of cause and effect; and
- where possible, both invertebrate animals and fish should be used as environmental sentinels.

It was clear from the discussions that there was a need to refine the objectives and implementation plan for this programme. Following a brainstorming session under the chairmanship of Mike Moore, the Working Group developed a strategy for implementing a general biological effects programme. This incorporates all of the above-mentioned points and places them within the context of an environmental management plan which integrates scientific interpretation and management decision-making. A key aspect of this programme (illustrated in Figure 7.2.1) is the use of a variety of low-cost techniques which have a high signal-to-noise ratio and which in combination are indicative of both exposure and pathology. These techniques would be used to screen for indications of effects and a positive response(s) would then trigger a management plan to establish the significance of the observed effects, to identify the problem pollutants, and to stimulate management action. Thus the essence of the programme is to identify the 'real problem' rather than the 'perceived problem' and to test the outcome at essential points of the process in order to ensure that the problem has been defined appropriately, and, if so, whether it has been solved. This strategy can incorporate biological effects measurements at all organizational levels and is sufficiently flexible to accommodate new diagnostic tests for both pathology and exposure as they become available. The Working Group considered that this strategy should be considered by MON 1996.

7.2 Contaminant-Specific Programmes for PAHs, TBT and Heavy Metals

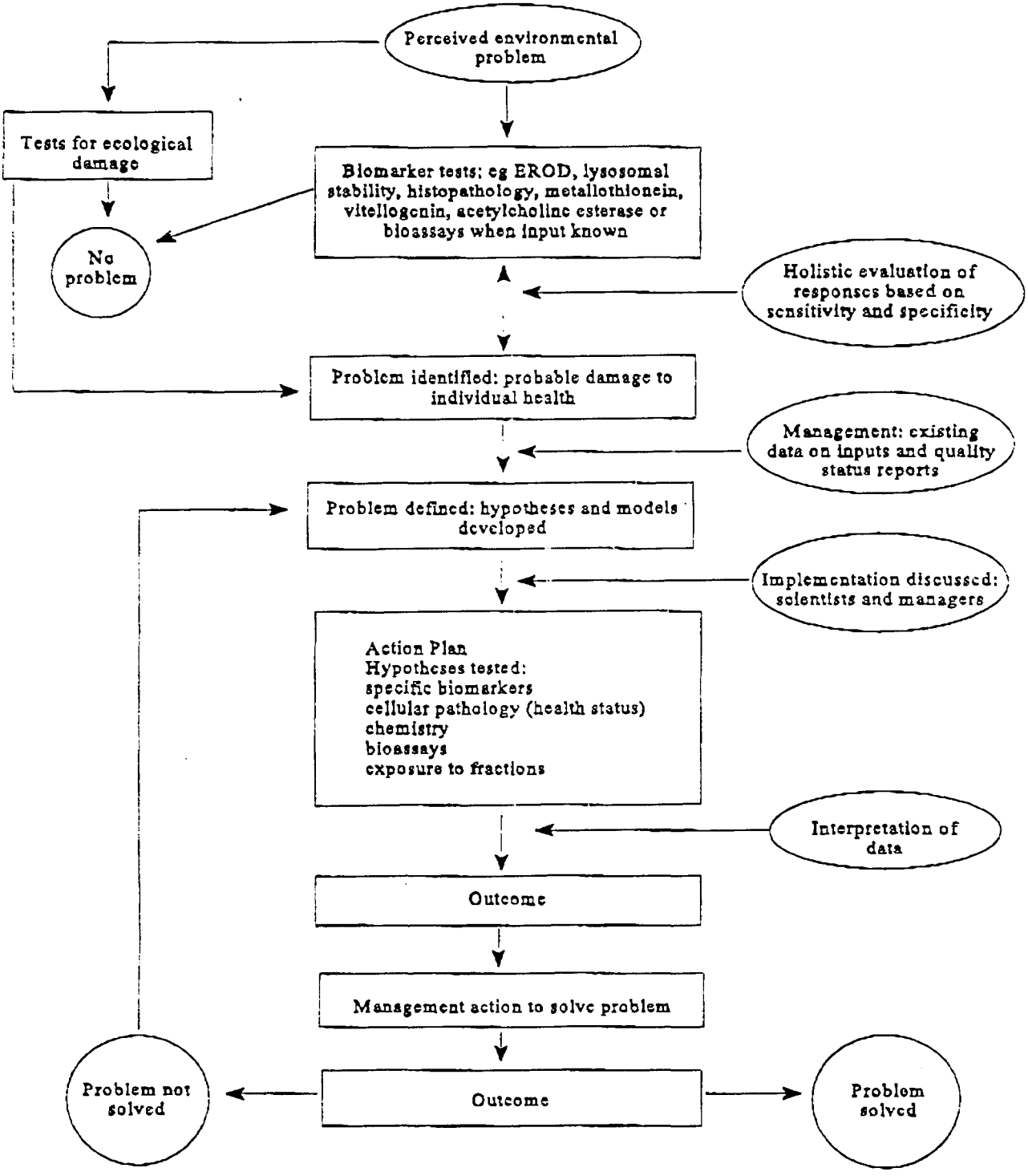
Ron Stagg summarized the outcome of the contaminant-specific programmes (designed to identify the biological effects of PAHs, TBT and heavy metal contamination) developed at the OSPAR/ICES Workshop on Biological Effects Monitoring Techniques. The background to the development of this programme was described, namely, it arose from a specific request from SIME who wish to have information on the significance of these specific contaminants, which have been monitored for many years in the OSPAR Convention Area. The elements of these programmes are as follows:

PAHs: P4501A, PAH metabolites in bile, DNA adducts, and histopathology in liver of fish;

TBT: Imposex and intersex in gastropods, shell thicken-ing in *Crassostrea;*

Heavy metals: Metallothionein (Cu, Cd and Zn), ALA-D (Pb), antioxidant enzymes (to include malone dialdehyde).

Table 7.2.1. Programme for general biological effects monitoring.



7.3 Quality Assurance Requirements of the Recommended Techniques

The Working Group decided that it would be appropriate to break into subgroups to deal with the requirements for the methods in the following groups:

Subgroup 1	Pathological biomarkers (lysosomal stability, pathology, imposex/intersex)
Subgroup 2	Biochemical biomarkers (metallothionein, P4501A, ALA-D, DNA adducts, bile metabolites, oxidative enzymes including malone dialdehyde)
Subgroup 3	Bioassays (sediment, pore-water and water column)

The participants in each subgroup are listed in Annex 5 and the terms of reference were to develop the following components of the QA programme for each method:

- the recommended methods;
- the necessary elements of an intercalibration programme;
- the intercalibration standards required;
- the standard reagents needed;
- the sampling requirements;
- suitable sample collection, preparation and preservation procedures;
- training requirements;
- acceptable performance limits;
- action to be taken if limits are breached;
- the requirements of good laboratory practice;
- appropriate species to be used.

7.3.1 Bioassays—whole sediment tests

Methods to be used:

There are several published methods available for whole sediment bioassays. The bioassay subgroup identified the following as being suitable for the JAMP, and it emphasizd that these tests should be used in conjunction with each other to maximize the chances of detecting toxicity:

Corophium volutator: 10-day acute lethal test. After ring-testing, this test was adopted by PARCOM for offshore chemicals testing. It is well described in a published protocol (Oslo and Paris Commissions, 1995), and will shortly be described in an ICES TIMES paper (Roddie and Thain, in prep.).

Arenicola marina: 10-day acute lethal and sublethal (casting rate) test. It has been ring-tested by PARCOM in 1993 and will soon be described in an ICES TIMES paper (Thain and Bifield, in prep.).

Other tests were also discussed, for example, a method using *Echinocardium cordatum* (sea urchin). This test has been used in an intercalibration exercise within PARCOM and was found to be sensitive (Bowmer, 1993). However, availability problems may occur in several countries, so this test was not considered further. The use of a test with the bivalve *Abra alba* (Stromgren *et al.*, 1993) was also rejected as this species is more or less restricted to fine-grained sediments.

Intercalibration:

The chosen lead laboratory will send spiked sediments and a 'clean' reference sediment to each participant. Organisms (*Corophium* and *Arenicola*) will also be sent out from a single supplier. Lindane will be the chosen test substance and spiking is to be performed according to the procedures described by PARCOM for offshore chemicals (Oslo and Paris Commissions, 1995). The lead laboratory will produce and send out (by courier or airfreight) five different concentrations of lindane-spiked sediments. Three of these concentrations should be within the effect range 10–90 %, one above this limit and one below the limit, plus a clean reference as a control. There must be analytical verification of test concentrations. Each laboratory will then measure toxicity of the samples (blind) and send the results to the lead laboratory for evaluation and calculation of a dose-response curve and the L(E)C50. Participants will not be admitted to the QA/QC scheme until their measured L(E)C50 lies within a specified deviation of the correct value as determined by the lead laboratory.

Standards:

Reagent-grade lindane.

Sampling requirements:

When sampling subtidal sediment, a box-corer should be used to avoid the loss of pore water. For intertidal sediments, a smaller corer like a Ponar grab should be used. After the removal of surface water, the upper 1 cm of sediment is collected if recently deposited contaminants are to be investigated. Alternatively, the upper 5 cm of sediment is sampled if older contaminants are of interest.

Sample preservation/preparation:

Sediment is passed through a 2-mm sieve to remove benthic macro invertebrates and then homogenized according to the relevant protocols. The sieved samples may be stored for a maximum of two weeks at 4 °C in the dark.

Training needs:

The lead laboratory will arrange for the production of training videos which will then be used to prepare participants for an initial training workshop. Subsequent training can then take place at any laboratory accredited to the QA/QC scheme.

Species availability:

Organisms from clean local sites should be used. If problems of supply occur, organisms can be obtained from other areas by airfreight or courier.

Definition of limits:

The reference chemical (lindane) should be used as a reference with every test. One concentration of lindane should be tested (five replicates) on the steep part of the dose-response curve, giving a 50 % response (a specified variance as determined by the lead laboratory). Mortality in controls should be a maximum of 10 % for *Arenicola* and 15–20 % for *Corophium*. All measurements of temperature, salinity, ammonia, dissolved oxygen, etc., should be within the limits specified in protocols.

Action requirements when limits are exceeded:

In cases where quality limits are exceeded, the test results must be rejected. Persistent failures will necessitate retraining of relevant personnel.

Good laboratory practice:

No formal good laboratory practice (GLP) will be needed provided that all tests are carried out by trained staff to defined protocols. Full data, including quality control measurements, will be provided to ICES.

7.3.2 Pore-water tests

Methods to be used:

This type of testing is restricted to small species because only small volumes of pore water can usually be sampled. The recommended procedures are as follows:

The *Tisbe battagliai* 48-hour acute bioassay (Williams, 1992). These harpacticoid copepods are widely used, sensitive, easily cultured and are also amenable to chronic testing (reproductive endpoint).

The *Nitocra spinipes* 48-hour acute bioassay (Dave *et al.*, 1993) is recommended for brackish water (1–35‰) as an alternative to *Tisbe*.

The *Crassostrea gigas* embryo 24-hour acute bioassay (Thain, 1991) is recommended to be used in parallel with *Tisbe*. This test is widely used and sensitive, and conditioned oysters for the production of gametes are easily available commercially.

Sampling requirements:

The sediments should be sampled either by box coring (subtidal) or by Ponar grabbing (intertidal sediments). In difficult situations (e.g., estuaries where access by large vessels is problematic), alternative subtidal sampling methods (e.g., diving) may have to be considered. Pore water is extracted from the sediment (surface, 1 cm or 5 cm, see above) by centrifugation (2,000 x g for 20 min.) or by suction sampling. Suction sampling can also be used with intertidal sediments *in situ*. Production of pore water by filtration or squeezing under pressure is not recommended because organic material (biotic) may be altered, which causes problems during subsequent testing. It is recommended that an international protocol for extraction of pore water be prepared.

Other requirements for pore-water tests are identical with those set out below for water-column tests.

7.3.3 Water-column bioassays

Methods to be used:

It is recommended that the *Tisbe* and *Crassostrea* bioassays described above should again be used in parallel, but an acute fish bioassay should also be used. The most appropriate protocol is the turbot (*Scophthalmus maximus*) juvenile 96-hour acute test (Oslo and Paris Commissions, 1995), with the stickleback (*Gasterosteus aculeatus*) 96-hour acute test (OECD, 1984) as an alternate for brackish waters. It is also possible to conduct early-life stage tests (OECD, 1992) with stickleback. Turbot juveniles are easily obtained from commercial suppliers, while stickleback can be cultured in the laboratory.

Intercalibration:

Reference, 'clean' natural sea water from a variety of sources, which is filtered through a sand or 0.45 mm filter, can be used by the different participating laboratories. The lead laboratory will send out the hydrophilic test chemical (sodium dichromate) to allow the participating laboratories to make up their own test solutions. Test organisms should come from the same stock for purposes of intercalibration. The organisms should be sent out by the lead laboratory, or purchased from a single recommended supplier. L(E)C50s must be determined using five test concentrations, plus a clean reference as a control. Quality criteria specified in the protocols concerning O_2, pH, salinity, H_2S, ammonia, etc., should be complied with and reported. There must be analytical verification of test concentrations. Each laboratory will then measure a

dose-response curve and send the results to the lead laboratory for evaluation and calculation of the L(E)C50. Participants will not be admitted to the QA/QC scheme until their measured L(E)C50 lies within a specified deviation from the correct value as determined by the lead laboratory.

Standards:

Reagent grade sodium dichromate.

Sampling requirements:

Water should be sampled at approximately 2 m depth, in glass or teflon containers. The containers should be pre-rinsed with the water to be sampled. At least twice the volume of water that is anticipated to be needed should be sampled.

Sample preservation/preparation:

Water should be filtered as soon as possible through a sand or a 0.45 mm filter. The maximum permitted storage time after sampling is one week, at 4 °C in the dark.

Training needs:

The lead laboratory will arrange for the production of training videos which will then be used to prepare participants for an initial training workshop. Subsequent training can then take place at any laboratory accredited to the QA/QC scheme.

Species availability:

As the organisms can be sent out by lead laboratories, purchased directly from suppliers, or cultured, availability should not be a problem.

Definition of limits:

Quality criteria concerning O_2, pH, salinity, H_2S, ammonia, etc., should be met according to the protocols and reported. Each time a new batch of tests is begun, or after significant changes are made in the testing procedure, L(E)C50 values for sodium dichromate should be measu-red. In parallel with each test, one concentration of sodium dichromate at the anticipated 50%-effect level should be tested as a positive control, together with a reference 'clean' sea water as a negative control. The L(E)C50 and positive control values should be within the range of variance specified by the lead laboratory. Mortality/abnormality in the negative control should be less than 10 %, with the exception of *Crassostrea* where the results are reported as percent net response, and percent abnormality in controls of up to 40 % is permissible.

Action requirements when limits are exceeded:

In cases where quality limits are exceeded, the test results must be rejected. Persistent failures will necessitate retraining of relevant personnel.

Good laboratory practice:

No formal GLP will be needed provided that all tests are carried out by trained staff to defined protocols. Full data, including quality control measurements, will be provided to ICES.

7.3.4 Lysosomal membrane stability

Methods to be used:

- *Histochemical measurement* of lysosomal membrane fragility;
- *Cellular dye retention technique* based on lysosomal uptake of Neutral Red in isolated cells (digestive gland of mussels, liver of fish) or blood cells (as a non-destructive technique in mussels); this can be used as an alternative to the histochemical method. However, this method, although very simple and easy to learn, requires more widespread use in other laboratories in order to be able to fully assess its utility;
- *Biochemical techniques* are also available for measurement of membrane-linked latency of lysosomal enzymes, but these are not in general use in environmental monitoring

Intercalibration standards:

For the intercalibration of the histochemical lysosomal stability test, frozen (quenched) tissues are prepared (Laboratory Reference Materials (LRMs)). The test is performed in the lead laboratory and the frozen tissues are sent to the participating laboratories in order for them also to perform the test. All samples should be coded and the test performed and assessed as a double blind exercise. This would involve the results being returned to a second laboratory for the compilation of the data.

A limited intercalibration exercise has been carried out in the MEDPOL programme using the histochemical technique. The results of this operation indicated that the technique could be used in the participating laboratories in an effective manner with low inter-laboratory variability.

The standards used in this intercalibration involved digestive glands from marine mussels prepared at the University of Genova (Italy). Comparisons of the histochemical and the neutral red cellular dye retention techniques have been performed in fish liver (Moore,

1990) and in mussels experimentally exposed to PAHs (Lowe *et al.*, 1995).

For intercalibration of the neutral red cellular dye retention test, which is performed on live cells *in vitro*, it will be necessary to hold an intercalibration workshop for the participating laboratories at a single site, since samples cannot be exchanged between laboratories.

Standards and reagents:

Histochemical method. The standards and reagents for the histochemical method are given in a TIMES publication (in prep.) and in Moore (1988).

Equipment: High quality motorized cryostat microtome (e.g., Bright Instrument Company or Microm M 500 OM); good quality water bath (preferably shaking) up to 40 °C; cleaned Hellendahl histological staining jars; good quality cleaned, but untreated, microscope slides with frosted glass writing area; good quality bright field binocular microscope with x10, x25 and x40 objectives; optional use of a 580 nm green filter to enhance contrast of the purple-red reaction product.

Reagents: Naphthol AS-BI-N-acetyl-ß-glucosaminide (Sigma); fast Violet B; collagen-derived polypeptide (Polypep, P5115, Sigma); citrate buffer 0.1 M, pH 4.5, containing 2.5 % sodium chloride (W/V); phosphate buffer 0.1 M, pH 7.4; aqueous mounting medium (Difco, Kaiser's glycerol-gelatine, Sigma or other).

The cellular dye retention test. Details of the method are described in Lowe *et al.* (1995).

Equipment: Good quality bright field binocular microscope with x10, x25 and/or x40 objectives; optional use of a 580 nm green filter to enhance contrast of the red dye; humidity chamber for incubation of the cells with neutral red.

Reagents: Neutral red (Sigma, general purpose grade).

Sampling requirements:

Mussels:

- Samples should contain a minimum of 10 animals which should be from a standardized size class in the area to be monitored, preferably the smallest available size class;
- Sampling should be avoided during the main spawning season;
- Mussels should be sampled from the sublittoral part of the population, since this will minimize fluctuations due to air exposure at low tide;
- Transport to the laboratory should avoid rough handling and mussels should be packed in an insulated container containing tissue paper soaked in sea water;
- For transportation times of more than four hours, ice packs should be placed in the bottom of the insulated box.

Fish:

- Flatfish are caught by 30-minute hauls with a technique appropriate for the species and are directly transferred into aerated flow-through seawater tanks in order to minimize catching and handling stress;
- The fish should be measured for total length, dissected and the sex determined;
- The livers of a maximum of 25 fish of a single sex (males or females used according to the requirements of the monitoring programme) should be removed;
- The length of the fish selected is dependent on the specific objectives of the monitoring programmes (early effects or liver cancer).

Sample preservation:

Histochemical method

Mussels:

- Digestive glands from mussels should be cut transversely into three approximately equal portions and the mid-portion used for cytochemistry;
- The remaining portions are available for histopathology.

Fish:

- Fish livers are cut into pieces of 5 mm x 5 mm x 5 mm immediately after dissection, put on a labelled, cooled, coded chuck at refrigerator temperature;
- The tissue and chucks are then quenched (supercooled) in n-hexane cooled to –70 °C and stored as described in Köhler (in prep.), Köhler (1991) and Köhler *et al.* (1992).

Cellular dye retention method

This method does not need any preservation because it is performed on live cells.

Training needs:

Training material includes documents, micrographs, videos and laboratory reference material (LRM). Training workshops are also recommended and can be readily coupled with those for other biological effects methods (e.g., pathology, metallothionein, or EROD). Inter-laboratory comparison exercises could be organized through a QUASIMEME-type scheme.

Species availability:

The techniques can be applied to a broad range of bivalve and gastropod molluscs as well as teleost fish. The currently preferred species are mussels, dab, flounder, dragonet, and grey mullet.

Definition of limits:
It should be possible to establish standard Shewart control charts for measurement of lysosomal stability using LRMs.

Action requirements when limits are exceeded:

Repeated measurements from LRMs produced by a lead laboratory will be used to control differences of interpretation between analysts.

Good laboratory practice:

All tests and determinations should be carried out by trained staff working to defined protocols. Any deviations from the protocols should be recorded and assessed by the laboratory manager for their potential to influence the results.

7.3.5 Histopathology of fish liver

Methods to be used:

Diagnosis and quantification of pathomorphological changes and metabolic alterations during early liver injury, degeneration, and carcinogenesis. The pathomorphological diagnosis can be conducted in plastic-embedded material in order to obtain an optimal optical resolution. The use of cryofixed material offers the advantage of being able to apply histopathological diagnoses simultaneously to histochemistry of NADPH-generating enzymes (G6PDH) as tumour markers and the lysosomal membrane stability test.

Intercalibration standards:

Tissue sections of specific types of lesions and their degree of severity should be prepared as laboratory reference materials (LRMs) by a lead and/or advising laboratory.

For the intercalibration of the histochemistry of enzyme tumour markers, frozen (quenched) tissues should be prepared (LRMs). The test will be performed in the lead laboratory and the frozen tissues sent to the participating laboratories in order for them also to perform the test. All samples should be coded and the test performed and assessed as a double blind exercise. This would involve the results being returned to a second laboratory for the compilation of the data. If equipment for image analysis is available, it should be used to facilitate the quantification of enzyme markers and histopathology, where appropriate.

A limited intercalibration exercise has been carried out with respect to enzyme tumour markers in the EERO-laboratory network for environmental toxicology and pathology. The results of this exercise indicated that a strict procedure for the calibration of the image analysis systems has to be performed, as described by Chieco *et al.* (1994).

Standards and reagents:

The standards and reagents are given in a TIMES publication (Köhler, in prep.) and in Köhler *et al.* (1992) and Moore (1988). Laboratories should have the standard equipment needed for histopathology and obtain their histological stains and histochemical reagents from suppliers whose products have been assessed by the lead laboratory.

Equipment: High quality rotary microtome; tissue processor; high quality motorized cryostat microtome (e.g., Bright Instrument Company or Microm M 500 OM); good quality cleaned, but untreated, microscope slides with frosted glass writing area; good quality bright field binocular photomicroscope with x10, x40 and x60 or x100 oil objectives; optional use of a 580 nm green filter to enhance contrast of the histochemical reaction product for enzymic tumour markers; micrometer eyepiece for the estimation of the size of the lesion or stereological analysis

Sampling requirements:

Fish should be caught by the appropriate technique for the species and transferred directly into aerated flow-through seawater tanks. The length (age) of the fish selected is dependent on the specific objectives of the monitoring programmes (early effects or liver cancer). The fish should be screened for grossly visible lesions, and parasitic infestations at the body surface, measured for total length, dissected and the internal organs inspected for macroscopic lesions, and the sex determined. The livers of a maximum of 25 fish of a single sex (males or females used according to the requirements of the monitoring programme) should be removed.

Sample preservation:

The livers are divided into three sectors for the simultaneous application of analytical chemistry, biochemistry and pathology. For pathology, a portion of liver derived from the original central region should be used for enzyme histochemistry; it should be cut into pieces of 5 mm x 5 mm x 5 mm immediately after dissection, and placed on a cooled, coded chuck at refrigerator temperature. The tissue and chucks are then quenched (supercooled) in n-hexane cooled to –70 °C and stored as described in Köhler (in prep.) and Köhler *et al.* (1992). For conventional histopathology

of embedded tissues, the liver pieces are fixed in Baker's formol.

Training needs:

Training material includes documentation, micrographs, videos and laboratory reference material (LRM). Training workshops are also recommended and can be readily coupled with those for other biological effects methods (e.g., lysosomal stability, metallothionein or EROD). Interlaboratory comparison exercises could be organized through a QUASIMEME-type scheme.

Species availability:

The techniques can be applied to a broad range of teleost fish. The currently preferred species are dab, flounder, turbot, dragonet, and grey mullet.

Definition of limits:

In cases of disagreement in diagnosis, the tissue sections should be returned to three different pathologists in order to assess the source of the problem. For enzyme tumour markers, it should be possible to establish standard Shewart control charts for measurement of LRMs.

Action requirements when limits are exceeded:

Repeated diagnoses and quantification from LRMs produced by a lead laboratory will be used to control differences of interpretation between analysts.

Good laboratory practice:

All diagnoses and determinations should be carried out by trained staff working to defined protocols. In case of the use of image analysis, consistent procedures for the intercalibration of the systems have to be applied. Any deviations from the protocols should be recorded and assessed by the laboratory manager for their potential to influence the results.

Workshop on liver pathology:

The WGBEC noted the invitation from the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) to attend a workshop on liver pathology to be held in Weymouth, UK, in October 1996 with S.W. Feist (UK) and T. Lang (Germany) as Conveners (ICES C. Res. 1995/2:31) and that there was a requirement to coordinate the work and advice from the two Working Groups. This was especially so since the WGBEC recommended these methods for biological effects measurements and has already a draft TIMES document (see Agenda Item 11) in progress. The WGBEC has recommended that Angela Köhler attend the Workshop and act as a liaison between WGPDMO and WGBEC, and the Chairman agreed to inform the organizers of this decision.

7.3.6 Techniques for monitoring effects of TBT compounds

An outline of a sampling programme, analytical procedures, and data handling is available in the report of the OSPAR/ICES Workshop held in Aberdeen in October 1995 (WGBEC96/7/1). The effects measurements are based on imposex and intersex measurements in gastropods, and shell thickening in *Crassostrea*. The subgroup agreed with the proposals in that Workshop report, and therefore will not repeat the same material here, but will confine their comments to expansions or clarifications of the OSPAR/ICES Workshop report.

The OSPAR/ICES Workshop report contained a section on Quality Assurance matters, which should be read in conjunction with the following text:

Methods to be used:

These are as specified in the OSPAR/ICES Workshop report. A TIMES document is in preparation describing the determination of imposex in *Nucella*. Methods for the examination of *Buccinum* and *Littorina* are outlined in the OSPAR/ICES Workshop report, but there would be value in preparing TIMES documents for these methods, and for the determination of shell thickness in *Crassostrea*.

Supporting chemistry should concentrate upon determination of TBT. There are several methods available and advice should be requested from MCWG on the most appropriate procedures. It is likely that intercomparison exercises will be required, and these might possibly be organized through QUASIMEME.

Intercalibration standards:

Intercomparison standards for effects measurements should be prepared as preserved material and used as reference standards by analytical laboratories during their routine work. Control charts can be prepared from repeated measurements of penis length on single preserved specimens.

MCWG should be asked to comment on the availability of suitable certified reference materials (CRMs) for TBT in biota.

Standards and reagents to be used:

TBT compounds can be purchased from chemical suppliers for the preparation of standard solutions. Reagents for the chemical analysis should be of appropriate quality to meet the needs of the determination.

Sampling requirements:

Sampling requirements are described in the OSPAR/ICES Workshop report.

Sample preservation:

It is recommended that gastropods be examined as live specimens wherever possible. The examination of preserved specimens is not recommended, although this will be necessary in the case of reference samples.

Training needs:

The need for training material (documents, videos) and workshops is described in the OSPAR/ICES Workshop report. The need for training in chemical analysis is not yet clear, but could be assessed after a preliminary interlaboratory comparison exercise, such as might be organized through QUASIMEME.

Species availability:

This was addressed at the OSPAR/ICES Workshop.

Definition of limits:

It should be possible to establish standard chemical Shewart control charts for the chemical analysis of biota in support of the biological effects measurements, either from CRMs or from LRMs.

The repeated measurement of penis length or prostate length in preserved specimens could be used to control biological measurements. This will control for differences in interpretation between analysts, and for gross errors in microscope calibration.

Good laboratory practice:

All determinations, both chemical and biological, should be carried out by trained staff working to defined protocols. Any deviations from the protocols should be recorded and assessed by the laboratory manager for their potential to influence the results.

Normal care should be taken during chemical analysis to minimize contamination or loss of analytes and interference from other substances, and to ensure accurate calibration of instruments. The necessary performance characteristics of the chemical methods are given in the OSPAR/ICES Workshop report.

7.3.7 Biochemical biomarkers

The following biomarkers were considered: metallothionein, cytochrome P450 (EROD), antioxidant enzymes (catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase) and malone dialdehyde, DNA-adducts, aminolevulinic acid dehydratase (ALA-D) and bile metabolites; all are measured in fish tissues.

Guidelines for field sampling:

- A random sample of at least ten apparently healthy fish (excluding those with grossly visible external lesions and parasitic infections) should be used;
- Fish should be sampled outside the spawning season and in temporal trend work at the same time of year (within two weeks); liver-somatic index (LSI) and gonadosomatic index (GSI) need to be recorded;
- Fish need to be of comparable size (different for each species), but of sufficient size to allow three samples of approximately one gram (or more) each;
- Either male or female fish should be used—the same sex should be chosen for all areas that are to be compared;
- Hydrographical data at the site (bottom) at the time of collection should be recorded, including temperature and salinity;
- The fish should be measured for total length, dissected, and macroscopic lesions on the internal organs recorded;
- Trisection of liver: the same part of the liver should be used for the same analysis;
- PAH-related markers: prior to sectioning the liver, it should be examined externally—any part with a nodule should be used for pathology:
  one part for DNA adducts,
  one part for cytochrome P4501A (EROD),
  one part for pathology;
- Metal-related markers:
  one part for metallothionein,
  one part for antioxidant enzymes,
  one part for analysis of Cd, Cu, Zn.

Protocols and reference materials for intercalibration:

Metallothionein

Protocols are as proposed in the OSPAR/ICES Workshop report (WGBEC96/7/1).

Reference material (3 x 1 g) from common pools of minced liver containing high levels (induced by injection of Cd) and low levels of metallothionein for selected species* should be distributed to participating laboratories. The material should be frozen in liquid nitrogen in a small volume of buffer (with protease inhibitors and reducing agent) and shipped to participating laboratories on dry ice. Analyses should be performed within 3–4 weeks.

Metallothionein standards for the selected species* should be distributed to participating laboratories.

Oxidative enzymes (catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase) and malonedialdehyde

Protocols are as proposed in the OSPAR/ICES Workshop report (WGBEC96/7/1).
Reference material (3 x 1 g) from common pools of minced liver for selected species* should be distributed to participating laboratories. Material should be frozen in liquid nitrogen in a small volume of distilled water or buffer and shipped to participating laboratories on dry ice. Analyses should be performed within 3–4 weeks.

d-Amino levulinic acid dehydratase (ALA-D)

Protocols are as proposed in the OSPAR/ICES Workshop report (WGBEC96/7/1).

Reference material (3 x 1 ml) from common pools of heparinized whole blood with high and low (injected with Pb) levels of ALA-D activity for selected species* should be distributed to participating laboratories. Material should be frozen in liquid nitrogen and shipped to participating laboratories on dry ice. Analyses should be performed within 3–4 weeks.

Cytochrome P4501A (EROD)

The protocol is from Stagg and McIntosh (in press).

Reference material (3 x 1 g) from common pools of minced liver with high (naphthoflavone-induced) and low activities of EROD for selected species** should be distributed to participating laboratories. Material should be frozen in liquid nitrogen in a small volume of distilled water or buffer and shipped to participating laboratories on dry ice. Analyses should be performed within 3–4 weeks.

Resorufin standards should be distributed to participating laboratories.

DNA-adducts

The protocol is from Reichert *et al.* (In press).

Reference material (3 x 1 g) from common pools of minced liver containing high levels (induced with PAH-contaminated sediment extract) and low levels of DNA adducts for selected species** should be distributed to participating laboratories. Material should be frozen in liquid nitrogen in a small volume of distilled water or buffer and shipped to participating laboratories on dry ice. Analyses should be performed within 4–6 weeks.

Standards for measuring efficiencies of DNA hydrolysis and sample blanks ('clean' commercially available DNA) should be distributed to participating laboratories.

It is critical that an external standard such as BAPDE-dG (available from NCI) is used.

Bile metabolites

Protocols are as proposed in the OSPAR/ICES Workshop report (WGBEC96/7/1).

Reference material (3 x 1 ml) of common pools of bile containing high levels (induced with PAH-contaminated sediment extract) and low levels of PAH metabolites for selected species** should be distributed to participating laboratories. Material should be frozen in liquid nitrogen and shipped to participating laboratories on dry ice. Analyses should be performed within 3–4 weeks.

Standards (mixture of naphthalene and phenanthrene) should be distributed to participating laboratories.

* Species proposed at the OSPAR/ICES Workshop were cod, dab, and flounder.
** Species proposed at the OSPAR/ICES Workshop were dab, flounder, and dragonet.

7.3.8 Externally visible fish diseases

The WGBEC took note of a paper prepared by Thomas Lang (attached as Annex 6) from the WGPDMO on the development of a quality assurance programme for the investigation of externally visible fish diseases. Unlike some of the other methods, a number of international intercomparison exercises have already been carried out and reports and training guides are either available or in their final stages of publication. It was felt that the paper by T. Lang comprehensively covers the aspects, but the topics clearly falls into the expertise of the WGPDMO. The paper is therefore forwarded to WGPDMO for further consideration and the reviewed version will then be put forward to relevant bodies. The statement in the paper by T. Lang that external fish diseases are a more integrative indicator for complex changes typically occurring under field conditions as compared to biomarkers for subtle early changes on the subcellular or the cellular level was not accepted by the WGBEC. The WGBEC noted that there is no conceptual basis for the hypothesis that externally visible fish diseases are a more appropriate integrative indicator of toxic chemical stress than biomarkers of cellular and subcellular change; indeed, no clear co-incidence or correlations have been found between external fish disease and contamination. The WGBEC endorses the value of monitoring and assessment studies for external fish diseases. However, visible diseases can be considered to be relatively *unspecific* indicators of stress. Visible fish diseases are a manifestation of a type of biological impairment but are not, for instance, adequate for assessing other aspects of

chemical toxicity such as pathologies associated with various organs or genetic, reproductive, and endocrine toxicity. The overall aim of biomarker studies is to focus on how various types of toxicity relate to pathology and its causation.

The WGBEC concluded by recommending that ICES recommend that the quality assurance programme here developed should be considered by MON in 1996.

8 BIOLOGICAL EFFECTS TECHNIQUES FOR DETERMINING EFFECTS OF PCBs ON MARINE MAMMALS

The WGBEC noted the report of the Study Group on Seals and Small Cetaceans in European Seas (SGSEAL) (WGBEC96/8/2, ICES CM 1996/N:1) and the report of the 1995 International Whaling Commission (IWC) Workshop on Chemical Pollution and Cetaceans (WGBEC96/8/3).

The IWC Workshop was held to carry out a full multi-disciplinary assessment of the significance of chemical contamination for cetaceans. The report noted that the purpose of existing monitoring was to determine whether levels of contaminants exist in marine mammals at levels at which adverse effects occur and whether these have implications for the health of individuals or populations. The IWC Workshop noted that most monitoring merely measured levels of contaminants and went on to recommend that monitoring of chemical contaminants be accompanied by biological effects measurements including appropriate biomarkers, pathological examination and indices of reproductive impairment. Furthermore, the Workshop also recommended that priority be given to well designed cause-effect studies between contaminant burdens and animal health.

The WGBEC then considered the report from SGSEAL (WGBEC96/8/2) (especially Section 7.2 on the applicability of biological effects techniques) and endorsed the general approach suggested in that report, which included the direction of effort towards:

- biological effects techniques measurable at the cellular level, termed 'toxicokinetic markers';
- effects on reproduction;
- effects on immune parameters; and
- induction of cancer formation or mutagenesis.

The text contains a number of erroneous and inaccurate statements, however, which should not form a basis for future work. Specifically, the text within each of the four categories listed above is either too general or misleading. The remaining contents of the section also indicate that one of the most important points from WGBEC's earlier reports and the OSPAR/ICES Workshop, namely, that an evaluation of biological effects is a holistic process entailing the use of more than one technique, was not taken on board by SGSEAL.

In discussion, the following points emerged:

- There were often opportunities for comparing biological responses in sea mammal populations with known elevated levels of contaminants, but often managerial difficulties confounded the conduct of such studies. Managers often did not appreciate the potential of studies which sought to compare the epidemiological and biological responses of contaminated and non-contaminated populations, and the geographical scale required was large resulting in additional problems because the scope of such studies therefore involved comparisons across national boundaries;

- It was felt that greater advantage could be gained from samples collected during the course of hunts by native peoples or official national culling programmes and that sampling at such events could be more directed towards biological sampling;

- There was a need for more experimental facilities to carry out properly controlled experiments with adequate replication;

- Non-destructive sampling (e.g., blood sampling) and the measurement of biological effects with biomarkers in such samples was a way forward;

- More opportunity should be given to carry out detailed histopathological studies specifically directed at understanding the effects of contaminants.

The WGBEC made the following recommendations in response to the request:

- Efforts should be made to make material available from comparable populations exposed to different levels of contaminants (e.g., seals in the BalticSea compared to seals in Canada or the Barents Sea) to a wider scientific community; materials for biological effects measurements need to be conserved according to specific guidelines (see OSPAR/ICES Workshop report);

- Experimental work with sea mammals should be encouraged, preferably utilizng non-destructive techniques (e.g., blood samples);

- Future studies should include histopathological methods.

It was also noted that ICES would have received better overall advice if this term of reference had been addressed by a joint session between members of

SGSEAL and members of WGBEC rather than discussion by way of agenda items in separate working groups.

9 BIOLOGICAL TECHNIQUES FOR DETERMINING EFFECTS OF CONTAMINANTS ON REPRODUCTION IMMUNOLOGY AND METABOLISM OF MARINE ORGANISMS

9.1 Immunology

Effects of contaminants on the immune system of fish are complex and no clear pattern of responses has yet emerged which could be used in environmental monitoring. Reactions of the immune system are still very much at the research stage in fish. It was also noted that there were often compensatory homeostatic responses within the immune system e.g., inhibition of a specific Ig response will also be associated with an increase in the non-specific responses (see Secombes ref.). However, a clearer pattern is emerging from studies of the much simpler immune system of molluscs which is largely dependent on phagocytosis of foreign micro- organisms by amoebocytic blood cells (haemocytes). Here, damage to the cellular compartment for killing and digesting phogocytosed microorganisms (i.e., lysosomes) has been linked with a reduced capacity for cellular ingestion, of foreign material (see Grundy *et al.*, 1996). Since the immune systems of most invertebrates involve phagocytic ingestion it is likely that this type of reaction to contaminants is generic.

A review on immunological responses to contaminants was requested from Prof. C.J. Bayne (Corvallis, USA) and Prof. M. Moore (Plymouth, UK).

9.2 Metabolism

Holistic methods for assessing metabolic status were discussed. Those included Adenylate Energy Charge, measured by nuclear magnetic resanance (NMR), nitrogen excretion and determination of liver water content, also by NMR. A new procedure for proximate analysis/cellular energy allocation in *Daphnia* was described by Dr W.M. De Coen (Gent), This new methodology, termed the determination of the 'Cellular Energy Allocation' (CEA), has been evaluated in *Daphnia magna* exposed to the xenobiotics cadmium and 2,4-dichlorophenoxy acetic acid. The concept is based on a biochemical assessment, using colorimetric methods, of the organism's energy consumption (E_c) and energy reserves available for metabolism (E_a), with E_a–E_c representing the energy available for growth and reproduction. Comparison between the new sub-organismal (CEA) and supra-organismal endpoints such as survival and reproduction shows that the biochemical assessment of energy allocation could be a rapid and cost-effective method for detecting long-terms effects which emerge at higher levels of organization.

The WGBEC noted that the method was mainly an indicator of acute effects (short laboratory exposures) but represented a good laboratory technique for measuring overall growth. It was recommended that this test should be compared with scope for growth (SFG) in order to assess its utility for field application and whether it can provide a simpler alternative to the complex measurements required in SFG.

In discussion, it was also noted that biomarkers for the effects of contaminants on metabolic status is an area where a lot of experimental work is needed and the following approaches were considered appropriate:

- the use of caged fish, thereby reducing the variability in responses (which is a major problem when studying metabolism), and the validation of methods developed in laboratory studies;
- analytical methods with potential are: ^{31}P-NMRS to study *in vivo* adenylate energy charge coupled with intracellular pH; NMR imaging may be used to measure water content, and water fluxes and study the overall water budget; measurement of aerobic and anaerobic metabolic (end) products;
- exposure of caged animals to a secondary 'metabolic stressor', e.g., hypoxia or forced activity with subsequent measurement of metabolic indicators and complemented with measurements of changes in critical oxygen concentration or the critical N point.

9.3 Reproduction

The background to this request was not fully understood by the Working Group but it was thought to relate either to the occurrence of the M-74 syndrome in the Baltic or to the general level of concern over contaminants directly implicated in reproductive disorders. In general, the WGBEC noted that biomarkers of exposure and pathology (e.g., EROD, DNA-damage and cellular pathology) could be more extensively used in measurements of effects on reproductive success. For example, the OSPAR/ICES Workshop on Biological Effects Monitoring Techniques recommended that the viviparous blenny could be used for reproductive studies. The viviparous blenny is very local in its movements and, hence, may provide a robust model for field sampling and caged exposure. This fish is ideal for tests for larval survival and coupling with biomarker responses. It has been used in studies of the impact of pulp-mill effluents, and gradients for hepatic EROD have been described in blennies from the Firth of Forth (UK).

The Working Group recommended that preparation of a TIMES paper be requested on the use of viviparous blenny in monitoring. It was also agreed that a review on reproductive effects should be invited from Dr H. von Westernhagen (Action: Dr V. Dethlefsen, Cuxhaven, Germany).

The WGBEC then discussed a review paper prepared by Peter Matthiessen on the observed effects of hormonal disruptors in the marine environment. Additional material was provided to the group from a Dutch Ph.D. thesis by Peter Janssen on reproduction in the flounder and from studies carried out in Norway by Ketil Hylland. The combined overview is given below.

Background:

The whole subject of endocrine disruptors in general, and environmental oestrogens in particular, has received considerable attention of late, perhaps because of somewhat controversial observations that men in several Western countries appear to be suffering from an increased incidence of a variety of sexual abnormalities (including reduced sperm counts) which could be linked to endocrine disrupting chemicals (e.g., Sharpe and Skakkebæk, 1993; Auger *et al.*, 1995). Recent experimental evidence from exposures of embryonic and juvenile rats to alkyl phenols and phthalates provides support for this potential link (Sharpe *et al.*, 1995), but much more research is required to provide a full understanding of the risks to humans.

Endocrine disruptors fall into five classes, being either oestrogens, anti-oestrogens, androgens, anti-androgens, or substances which affect the hormonal system indirectly. The most alarming characteristic of chemicals in each of the first four groups is that they act via specific receptors, and complex mixtures of substances at individually very low concentrations are therefore able to act additively. For example, 'true' oestrogenic hormones (such as oestradiol) act exclusively via an oestrogen receptor, but it is now known that the same receptor is also triggered (albeit with much lower potency) by a large range of apparently unrelated substances, including many organochlorine pesticides, polychlorinated biphenyls, alkyl phenols, styrenes and phthalates. Phyto-oestrogens, such as coumestrol and genistein derived from plants like soya and alfalfa are also able to trigger vertebrate (and some invertebrate) oestrogen receptors. A brief general survey of this field has been published by Theo Colborn of the Worldwide Fund for Nature (Colborn *et al.*, 1993), and more detailed recent reviews include IEH (1995), Toppari *et al.* (1995) and UBA (1996).

In contrast with the situation in humans, more is known about the potential risks to wildlife. For example, Guillette *et al.* (1994) have shown that serious feminizing abnormalities in alligators from Lake Apopka in Florida have been caused by a spill of dicofol contaminated with DDE, while female-female pairing in gulls (leading to supernormal clutches) has been attributed to DDT and PCBs (Fry and Toone, 1981), and TBT-induced imposex in dogwhelks is now known to be triggered by high levels of testosterone in females (Ochlmann *et al.*, 1991). Perhaps the best-known example of oestrogenic contamination in the aquatic environment is that discovered in England to be resulting from the discharge of treated sewage to rivers. These discoveries have depended on using the induction in male fish of a biomarker for exposure to exogenous oestrogens, namely the female yolk precursor protein vitellogenin. Studying this response in caged adult male rainbow trout (*Oncorhynchus mykiss*) which were placed in discharges for three weeks, Purdom *et al.* (1994) demonstrated that almost all sewage discharges are heavily contaminated with oestrogens or their mimics.

More recently, it has been shown that several English rivers receiving these discharges are also oestrogenic to rainbow trout, and in some cases this activity extends for several kilometres downstream (Harries *et al.*, 1995; in press; in prep.). In the more severe cases, vitellogenesis is accompanied by retarded testicular growth, and there is limited evidence that some wild male fish (roach *Rutilus rutilus*) are also producing vitellogenin (Harries *et al.*, 1995). Furthermore, in some cases there appears to be a low incidence of intersex roach in wild populations exposed to sewage effluent (UK National Rivers Authority, unpublished data).

Laboratory and field evidence (Jobling *et al.*, 1996; Blackburn and Waldock, 1995) has shown that the severe oestrogenic effects in one river (the Aire in Yorkshire) are almost exclusively caused by nonylphenol, a degradation product of nonylphenol ethoxylate detergents used in wool scouring. The fish population in the Aire is very impoverished, although this cannot be unequivocally attributed to oestrogens because many other polluting substances are also present. However, in the other rivers investigated, the effects seen are probably caused by several unrelated chemicals acting jointly. One of these may be the synthetic oestrogen ethynyl estradiol (Sheahan *et al.*, 1994) which is a component of the contraceptive pill, although its presence in sewage effluents has never been unequivocally confirmed. Life cycle and mesocosm experiments with fish are currently in progress with a number of environmental oestrogens in order to shed more light on the possible population-level effects of this contamination, and local regulatory action has been taken by the UK National Rivers Authority to reduce the discharge to rivers of alkyl phenol ethoxylates from textile plants.

Oestrogens in the marine environment

By comparison with the information available on fresh waters, very little is understood about oestrogenic effects in the marine environment. It is known that alkyl phenols are discharged to some UK estuaries (Blackburn and Waldock, 1995) at oestrogenically active concen-trations, and other oestrogen mimics such as a variety of organochlorines are well-known contaminants of the marine environment, albeit at low concentrations. Such contamination may enter the sea from sewage and industrial discharges, rivers and atmospheric sources, and it is known that elevated concentrations can occur in sedimentary sinks such as the Norwegian Trench. Evidence that these relatively low concentrations are causing reproductive abnormalities in marine organisms is very sparse, although Reijnders (1986) has shown that certain PCBs are largely responsible for poor reproduction in common seals from the Wadden Sea, and Moore and Stevenson (1991; 1994) have shown that sewage discharges in the Firth of Forth seem to be responsible for an intersex condition in several species of harpacticoid copepods (*Paramphiascella hyperborea, Stenhelia gibba* and *Halectinosoma similidistinctum*). Pereira *et al.* (1992) have demonstrated enhanced blood vitellogenin in female winter flounder (*Pleuronectes americanus*) from polluted estuarine environments, although this may be more the result of impaired ovarian uptake of vitellogenin rather than elevated vitellogenin production. Similarly, Janssen (1996) and co-workers have demonstrated that female flounder (*Platichthys flesus*) exposed to contaminated harbour dredgings in mesocosms exhibit premature vitellogenin production, but although this is mediated by enhanced plasma oestradiol, this enhancement is probably the result of decreased clearance and not enhanced ovarian production.

In the Dutch study (Janssen, 1996), the reproductive cycle of the male and female flounder was studied in fish that were exposed for three years in large (40 m x 40 m x 3 m) mesocosms to Wadden Sea sediment (mesocosm a), Rotterdam harbour dredged sediment contaminated with a variety of chemicals (mesocosm c), and an intermediate situation with clean sediment and polluted water (mesocosm b) in the Wadden Sea as a reference situation, and in wild flounder from the Wadden Sea. The Wadden Sea flounder were divided into several reproductive stages based on the histology of their gonads. For the females, reproductive stages were divided based on how far vitellogenin was incorporated into the gonads. In early winter spawning takes place, afterwards vitellogenin incorporation starts again. Although there is a clear pattern during the year, at one point in time organisms of various reproductive stages can be found. In the Wadden Sea flounder also, levels of 17b-oestradiol and vitellogenin in the plasma were determined and coupled to the reproductive stage of the animal. Vitellogenin and 17b-oestradiol were elevated in the females as the reproductive cycle was in a further stage.

For the fish that were exposed in the mesocosms, it was found that the relative frequencies of the phases of ovarian development were changed (premature development) in mesocosm c in May. In November, no clear changes were observed. Also, the plasma levels of 17b-oestradiol and vitellogenin were elevated in the females in mesocosm c in May. The elevated plasma level of vitellogenin could for the largest part be explained by the elevated 17b-oestradiol level. In males, no significant changes in the mesocosm animals were found with regard to testicular morphology and steroid levels in the blood compared to the reference animals from the Wadden Sea. Also, there was no vitellogenin present at detectable levels in the plasma, pointing to the fact that not many xeno-oestrogens had accumulated in the fish.

The elevated plasma levels of 17b-oestradiol in the fish from mesocosm c were not due to an altered ovarian steroidogenesis. From sub-sequent laboratory experiments there were indications that the metabolism of the various steroids (17b-oestradiol, testosterone and pregnonelon) was altered under the influence of contaminants. There appeared to be no direct competition between benzo(*a*)pyrene and the steroids, so different P450 isoenzymes are involved. However, after induction of P450 isoenzyme 1a due to pre-exposure to benzo(*a*)pyrene, the turnover of the steroids into their hydroxylated forms was decreased. This may explain the observed elevated 17b-oestradiol level in the plasma, which causes the elevated plasma vitellogenin levels. When vitellogenin in the female is used as a biomarker of exposure to contaminants that influence the endocrine system in various ways, the results can only be interpreted if there is also information on the reproductive stage of the animals. This means that an invasive technique should be used.

Information was provided on studies using marine fish species (cod, wrasse, salmon, flounder) in Norway. During autumn 1995, three studies on the effects of oestrogenic substances on marine fish were conducted by NIVA. Vitellogenin in plasma was used as a marker for responses and was measured using ELISA. The antisera was a gift from Carl Haux, University of Gothenburg. In the initial study, the four marine fish species cod (1+), wrasse (1+), salmon (0+) and flounder (adult) were injected intraperitoneally with either 4-nonylphenol (200 mg/kg) or 17b-oestradiol (0.5 mg/kg). A control group was injected with a carrier (peanut oil). The fish were marked individually and blood samples were taken on days 0, 6, and 10. In cod and salmon vitellogenin (VTG) increased 20–2,000 fold in both nonylphenol (NF) and oestradiol (OE2) treated groups. In NF-injected cod the response was highest on day 6 and decreased on day 10, whereas it remained elevated in NF-injected salmon. The second

study involved exposing cod and flounder to 20 % sewage effluent in aquaria with flow-through systems. Blood samples were taken on days 0, 7, 14 and 21. In cod held in 20 % sewage effluent, there was a significant increase in VTG levels after one week, after which they plateaued and remained constant after two and three weeks. There were no clear responses in flounder; however the interpretation was made more difficult by the fact that there were 80 % females in the experimental groups. The third study involved sampling cod (0+), wrasse (0+ and 1+), and flounder (adult) at six sites along the Norwegian Skagerrak coast and in the inner Oslofjord. At three of the sites there were significantly increased levels of VTG in juvenile cod compared to a reference group kept at NIVA's research facility for four weeks in clean sea water. There were no differences between groups of male flounder from the different sites. Vitellogenin in wrasse appeared to reflect size-dependent processes rather than differences between field sites. In conclusion, vitellogenin in marine fish could be quantified using ELISA. However, there were major species differences in plasma VTG following injections of nonyl-phenol or oestradiol. Vitellogenin in cod increased following exposure in 20 % sewage effluent. Vitellogenin in juvenile cod was sig-nificantly elevated at three out of six field sites.

Finally, Lang *et al.* (1995) have shown anomalies in the sex ratio of dab from the North Sea, with increased representation of females in some areas, and decreased representation in others. It is not yet clear, however, whether these fluctuations reflect exposure to endocrine disruptors.

In summary, little is known about the effects of environmental oestrogens (and other endocrine disruptors) in marine and estuarine waters, but that is mainly because there have been very few investigations of this potential problem. There is no reason to believe that marine organisms are likely to be less susceptible to endocrine disruption than their freshwater counterparts, although the greater dilution capacity of the sea is likely to mitigate potential effects to some extent. Research in polluted estuarine areas has now started at MAFF in the UK, RIKZ in The Netherlands and NIVA in Norway, but it will be at least one year before worthwhile results begin to be available.

Prospects for monitoring oestrogenic effects in the marine environment

While chemical monitoring is able to quantify concentrations of a few known oestrogen mimics, it is probable that the concentrations of many of these substances in marine matrices are below detection limits. Furthermore, it is likely that only a small fraction of oestrogen mimics have presently been identified, yet we know that (by definition) all these substances can act together in an additive fashion. For these reasons, it makes sense to deploy biological monitoring tools which can integrate the effects of all substances present in the matrix.

Vitellogenesis:

Relatively few approaches to biological monitoring of oestrogens in the marine environment are currently available. However, a diagnostic method for identifying oestrogen exposure is to measure vitellogenin in male fish. There is also scope for measuring vitellogenin in females (in combination with measurements of reproductive stage), and vitellogenesis in juveniles may be less susceptible to seasonal fluctuations. The most sensitive, widespread and reliable technique uses radioimmunoassay (RIA), but the immunological approach is fairly specific to the taxonomic group concerned. Thus, John Sumpter's team at Brunel University has developed a RIA for salmonids (Sumpter, 1985) and another for cyprinids, and MAFF is currently developing one for flounder and related species. It takes approximately nine months to develop a RIA for a new group of fish, but thereafter several hundred blood samples per month can be processed, using samples of only a few microlitres. Sampling is a simple matter of taking a small amount of blood (if necessary, without killing the fish), from which >10 ml plasma are stored frozen until the RIA is conducted. There are no internationally agreed procedures for conducting vitellogenin RIA, or for operating QA/QC procedures, but there is no reason why these cannot be rapidly developed. It should also be mentioned that ELISA techniques can be used with comparable sensitivity to RIA to measure vitellogenin, and they are faster to develop. Furthermore, there seems to be a reasonable prospect that immunocytochemical techniques could be developed for measuring vitellogenin in liver and gonad tissues, etc.

Bioassay of extracts of water, sediment and tissue:

This approach is being taken by MAFF and Brunel University to identify oestrogenic activity in sewage effluents. The work is proving to be successful, and although it is not yet published, in essence it involves making a series of solvent extracts which are then successively purified by a range of chemical techniques. The oestrogenic fractions are identified (using the Toxicity Identification Evaluation (TIE) strategy) by an oestrogen-specific bioassay based on yeast cells whose genome has been modified to include the human oestrogen receptor gene and a reporter gene which, in turn, produces a colour change in the medium (Routledge and Sumpter, in press). The yeast screen is rapid (24–48 hours) and sensitive, and in principle could also be used to study tissue and sediment extracts, although this has not yet been done. Such an approach could be used to screen large numbers of environmental samples, although it would be necessary to check that chemicals which cause a response in the yeast are also

able to cause *in vivo* oestrogenicity in fish and other organisms of interest. At least one other similar oestrogen bioassay is being used at a number of research centres, but the recombinant technology is not yet widely available. However, this situation will soon improve, and the technology has the advantage of simplicity, speed and low cost. Another *in vitro* assay for oestrogen which measures vitellogenin production in fish liver cell cultures is already available (Jobling and Sumpter, 1993), but it is relatively slow and difficult, and is probably not the best approach for this type of work.

Surveys of sexual abnormality in wild organisms:

These have the advantage that they employ simple and readily available techniques (anatomy, histology) to detect and quantify the occurrence of abnormality in natural populations. Thus, for example, it is a simple matter to look for intersex conditions in wild fish, or to quantify sex ratios and sperm viability, etc. It is also reasonably straightforward to monitor developmental abnormalities in wild fish embryos using the techniques developed by Patricia Cameron. The disadvantage of these approaches is that they are not necessarily oestrogen- or androgen-specific, and the conditions can also be caused by a variety of natural processes. They are therefore more appropriate for higher tiers of investigation, once the presence of oestrogens has been identified using the techniques described above. Clearly, if oestrogens in the environment are not found to be causing effects at the population level, this lessens concern about potential ecological impacts.

Recommendations:

The Working Group endorsed the following recommendations.

1) With little additional effort, radio-immunoassays or ELISAs (and possibly immunocytochemical techniques) for vitellogenin can be developed for a range of marine species, and applied in monitoring programmes. It would be desirable to organize an international intercomparison exercise for a RIA and/or ELISA applied to a common species such as flounder, and then to commission a paper for the TIMES series which describes the techniques in detail. However, before this expensive activity is begun, it would be sensible to wait for a few months until vitellogenesis in male marine fish has been established at a wider range of marine sites.

2) The use of simple *in vitro* oestrogen screens based on genetically engineered yeast (and other micro-organisms), in combination with TIE techniques, holds great promise for the identification of oestrogenic activity in a variety of marine matrices including animal tissues. It is hoped that collaborative research will soon be started at MAFF and RIKZ to explore this potential, and it is recommended that WGBEC maintains a watching brief in this area.

3) There is no reason why surveys of sexual abnormality in fish and other species (see e.g., Lang *et al.*, 1995) should not proceed immediately providing that it is recognized that such abnormalities are not necessarily diagnostic of endocrine disruption, or indeed of anthropogenic influence. In principle, however, such surveys are more appropriately used to follow up on initial measurements of oestrogenic activity.

4) Research should be encouraged into the possible effects of environmental oestrogens on invertebrates such as crustacea, and particularly on filter feeders such as mussels which are known to bioaccumulate compounds of oestrogenic interest and have the ability to respond to true oestrogens. Very little is known about possible mechanisms of endocrine disruption in invertebrates, but the very limited field evidence suggests that vertebrates are not the only group which is susceptible.

10 REVIEW OF OTHER BIOLOGICAL EFFECTS MONITORING TECHNIQUES

10.1 Scope for Growth

The joint paper by Widdows and Roddie (WGBEC 96/10/1) on a comparison of scope for growth (SFG) and clearance rate (CR) was discussed. The paper was considered to be useful, however, there still remain several points which were unclear or inconsistent. For instance, if SFG is independent of both reproductive cycle and temperative, why should measurements be confined to the summer period? And although it is accepted that SFG is more ecologically meaningful than CR, would a cost-benefit analysis in the context of monitoring point towards the extra effort and skill required for measurement of SFG?

The Working Group recommended that these points be considered by Widdows and Roddie for next year's meeting. In the meantime, integrated SFG and chemical monitoring will be carried out in the Irish Sea as part of the UK effort towards a Celtic Sea Quality Status Report (QSR).

10.2 Acetylcholinesterase

G. Bocquené presented an account of the programmes in progress in France on the use of cholinesterase as a biomarker of exposure to neurotoxic compounds. Work on the separation, purification, and characterization of cholinesterases in the gills of the common oyster *(Crassostrea gigas)* has been performed. The IFREMER Nantes laboratory has developed a

fundamental programme on the polymorphism of cholinesterases in bivalve molluscs because these species appear to have cholinesterases which are relatively insensitive to inhibitors when compared to enzymes from vertebrate species (e.g., fish muscle) or crustaceans (e.g., abdominal muscle of prawns).

Cholinesterases are a wide and complex family of esterases and several different cholinesterases may coexist in the same species with specific characteristics, particularly in terms of sensitivity to organophosphorus and carbamate compounds. The results obtained from the gills of oyster show the presence of two different cholinesterases in this tissue, each form showing distinct molecular and kinetic properties: a membrane-bound acetylcholinesterase (AChE) that is very sensitive to organophosphates (OPs) and carbamates and a soluble acetylcholinesterase that is insensitive to these compounds. Determination of the inhibition kinetics shows that the membrane-bound AChE is 40,000 times more sensitive to paraoxon (the product resulting from the metabolic activation of parathion) and 16,000 times more sensitive to the carbamate carbofuran than its soluble congener. This information makes it possible to improve the sensitivity of cholinesterase as a biomarker by separating the sensitive form from the insensitive form, for example, by using phase-partition separation in Triton X 114 or by using specific inhibitors.

There followed an account of the results of a monitoring cruise in La Martinique—a small French island in the Caribbean with an important agricultural industry producing principally bananas and pineapples. Intensive use of insecticides (mostly organophosphates and carbamate compounds) in this part of the world can result in extremely high application rates, up to one tonne km^{-2} yr^{-1} (compared to typical application rates in Europe of 6 to 10 kg km^{-2} yr^{-1}). Surgeon-fish (*Acanthurus bahianus)* sampled at different sites on the Atlantic coast of the island showed a clear and significant decrease in acetylcholinesterase activity when compared to animals from the control site. Further work was necessary to establish whether this inhibition is directly related to the presence of high concentrations of organophosphates and carbamates.

In the discussion that followed the presentation, several questions relating to the use of AChE inhibition as biomarkers were raised including:

- AChE can be used as an *in vitro* and *in vivo* biomarker but care must be taken with the former application as many OPs require biological activation to become strong cholinesterase inhibitors through metabolic transformation from the thio to the oxo form;
- Information on the half-life of the inhibited enzyme was required for interpretation of the time scale of the environmental effects;
- AChE inhibition measured in some urban rivers in a Canadian study to investigate the effects of pulp and paper mill effluents is probably not due to pesticides;
- Inhibition of AChE activity up to 20 % in mammals is associated with higher level effects such as behavioural changes;
- It is often assumed that insecticides such as organophosphates should not be a problem in the marine environment because of their degradation rate.

However, several members questioned whether the degradation tests used to regulate the licensing of chemicals gave information truly representative of degradation in the environment. Also, 50 % of organophosphate compounds are known to be transported by atmospheric processes and thus their occurrence at locations remote from sources may be expected. Chemical analyses of OP and carbamates in the Netherlands has revealed unexpectedly high concentrations of these compounds in the environment.

10.2.1 Announcement of Technical Workshop on Cholinesterase in Nantes

A technical Workshop on the measurement of acetylcholinesterase activity will be held in Nantes, France at the initiative of both WGBEC and IFREMER. This Workshop will take place from 4–6 June 1996 and will be devoted to the measurement of acetylcholinesterase specific activity in marine species in relation to inhibitory effects of contaminants following the reference method published in the TIMES series document (Bocquené and Galgani, 1996). Ten laboratories had already registered their attendance.

Persons interested in that workshop should contact Gilles Bocquené at the following address:

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10.3 Stress Proteins

Bart de Wachter presented a paper on stress proteins. These are a large family of proteins which are induced by a wide range of natural and anthropogenic stressors including temperature, UV-B, metals and xenobiotics. The main functions of stress proteins are to protect the cellular apparatus against protein denaturation by helping the correct (re)folding of proteins and to assist in the repair and transport of proteins. There exists a small amount of experimental data to show a

correlation between increased stress proteins and increased environmental stress. Due to their very conservative nature, their high sensitivity and induction by many different stressors, stress proteins are a potential candidate for a future biomarker. It is however important to realize that the knowledge of this response is still limited, and that extensive experimental work is needed, both in the laboratory and in the field.

11 REVIEW OF PROGRESS WITH ICES TIMES PAPERS

The current status of papers on biological effects methodologies for publication in the ICES TIMES series was reviewed by Dr Matthiessen, the WGBEC's editorial representative. The more pro-active stance with respect to the commissioning of new TIMES papers which was instigated two years ago is now bearing fruit, and a number of papers are close to publication. The present state of play is summarized below:

1) **Bocquené and Galgani - AChE inhibition**: This paper has been completed and was sent to the ICES Secretariat some months ago for publication. It will be in print shortly as TIMES No. 22.

2) **Gibbs - Imposex in dogwhelk**: This has been completed and is on the point of being sent to ICES for publication.

3) **Stagg and McIntosh - CYP 1A in dab**: Referees' suggestions have now been incorporated into a second draft, and the paper is on the point of being circulated to WGBEC members for final comments.

4) **Reichert, French and Stein - DNA adducts by ^{32}P-postlabelling**: This has just been submitted to Dr Matthiessen, and will shortly be despatched to referees.

5) **Thain and Bifield - Sediment bioassay with *Arenicola marina***: A draft is almost complete and is on the point of submission to Dr Matthiessen.

6) **Roddie and Thain - Sediment bioassay with *Corophium volutator***. A draft is almost complete and is on the point of submission.

7) **Moore and Köhler - Lysosomal stability**. A draft will shortly be submitted to Dr Matthiessen.

8) **Köhler - Fish liver histopathology**: A draft will shortly be submitted to Dr Matthiessen.

9) **Hylland - Metallothionein**: A draft will be submitted to Dr Matthiessen by the end of May 1996.

In view of the recent recommendations to JAMP by the OSPAR/ICES Workshop on Biological Effects Monitoring, it is urgent that items 5) to 8) above, be submitted without further delay, and Dr Matthiessen undertook to hound the authors unmercifully.

Early consultations have been held with John Widdows with regard to a paper describing the mussel scope-for-growth technique, and it is hoped that this will be written in time for the intercomparison trial this year. Finally, John Sumpter was approached a year ago to write a paper on the measurement of vitellogenin in (marine) fish. He felt that such a paper was not necessary until male vitellogenesis had been unequivocally demonstrated in a marine species. However, this has now been done, and it is now clear that there is considerable pressure to include the measurement of vitellogenin in marine monitoring programmes, so Dr Matthiessen intends to approach Prof. Sumpter again. This paper may need to include both radioimmunoassay and ELISA techniques.

The WGBEC agreed that the following new TIMES papers should, if possible, be commissioned in 1996 in order to provide a full suite of published procedures for use in the JAMP:

1) Sediment sampling, storage, and pore water extraction (author?).

2) Acute lethal toxicity to sticklebacks (only if requirements differ significantly from OECD Guideline 204).

3) Antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, malone di-aldehyde) (Livingstone or DiGiulio?).

4) PAH metabolites in bile (to include a normalization factor for the effects of feeding) (Krahn?).

5) ALA-D (Peter Hodson?).

6) The use of the viviparous blenny for monitoring the effects of contaminants on reproduction.

12 RESEARCH/DISCUSSION PROPOSALS

12.1 Biomarker Responses to Contaminants and Changes in Community Structure (M. Moore)

Biomarker responses can provide early warning of changes induced by anthropogenic stress. Structural changes in ecological communities require a longer time scale; however, it is these changes that are important in terms of the environmental impact of pollution. If the biomarker approach can be linked to

community-level change, then it will provide a very powerful predictive tool. In fact, it has proven to be easier to relate alterations at the level of the individual organism with those at the community level than it has with changes in populations.

In order to move forward in this area, a new approach to the use of biomarkers is required. Biomarker responses should be applied to **'relevant and sensitive' species** in communities. In the past, biomarker tests have tended to be used in eurytolerant animals, since these will frequently survive relatively severe environmental insults and, hence, continue to be available for monitoring purposes. However, the use of tolerant species may not be appropriate in assessing pollutant impact on the ecological processes which contribute to changes at the community level, because of this very resistance to environmental perturbation. It is proposed here that biomarker responses should be assessed in **sensitive species** that are known from ecological studies to be lost from the community at an early stage. Such an approach will have the advantage of combining the early warning property of biomarker tests with animals that are known to be critical to the processes contributing to the loss of diversity. Effective testing of this approach will require an integrated investigation of biomarker responses, closely coupled with ecological measurements in the same communities of organisms. It should also be clearly understood that biomarker responses will not be able to predict what changes will occur in communities, only that they will change in an adverse manner (i.e., reduced diversity).

In the discussion that followed, there was a debate on the most appropriate species to use for determining the effects of contaminants including:

- sensitive species in terms of their susceptibility to contaminants/toxicants and whether these were driven by different mechanisms;
- sensitive species in the sense of their pivotal role in a food web or community;
- sensitive species in terms of those which disappear from stressed communities at an early stage.

The WGBEC thought that interaction of ecological expertise with the biological effects expertise was needed within the group and recommended that a benthic ecologist such as John Gray should be invited to participate at the next Working Group meeting.

12.2 Use of *In Vivo* and *In Vitro* Bioassays in Environmental Monitoring of Biological Effects (A.-M. van Wezel)

Contaminants that are accumulated in organisms first cause effects at the molecular and cellular levels. If concentrations are sufficient, this may lead to adverse effects in the organism and possibly to an impact at the population level. Classically, a restricted set of contaminants is monitored in most programmes such as PCBs, PAHs and heavy metals. However, because estimates suggest that there are over 100,000 chemicals discharged to the environment, this approach is inadequate. Using biomarker responses, adverse effects caused by a much wider range of contaminants may be measured in organisms taken from the field. However, to obtain interpretable results, chemical gradients should be steep and there should be no other stressors or confounding factors (such as temperature or reproductive stage) that influence the biomarker response measured. *In vitro* and *in vivo* bioassays may be used to overcome some of these difficulties.

In vivo bioassays involve measurements of the effects of environmental extracts in animals maintained under controlled conditions in the laboratory, with the advantage that effects from other environmental stressors can be controlled. *In vitro* bioassays utilize effects at the molecular or cellular level on tissue culture or biochemical systems exposed to extracts from the field.

The extracts used can be obtained from a range of environmental compartments including water, sediment, pore water and biota taken from the field. The endpoints measured can be related to different toxicological mechanisms and thereby to different groups of chemicals. Toxicological mechanisms to be discerned are:

- polar and non-polar narcosis;
- reactive chemicals (parent compounds and biotransformation products) that may cause lipid peroxidation, protein disfunctioning, genotoxicity, mutagenicity and carcinogenicity;
- specifically acting chemicals that act by a specific interaction with proteins/receptors. Examples are xeno-oestrogens or -androgens, dioxin-like chemicals, chemicals that inhibit acetylcholine-esterase, etc.

Many of these toxicological mechanisms cannot be measured in acute *in vivo* tests, because it takes some time for the adverse effects to be expressed. However, endpoints at the molecular or cellular level can be measured soon after exposure. When a suite of *in vitro* bioassays is used related to the different toxicological mechanisms, insight is obtained into which of the toxicological mechanisms is important for the effects seen in the field. Because the toxicological mechanisms are often related to certain chemical structures, indications are also obtained of the relative importance of the different types of chemicals. Fractionation techniques (such as in hydrophobic fractions from reversed phase HPLC) may help to identify the chemicals that are responsible for the observed responses.

Some examples of this approach are given below:

1) *In vivo* testing of concentrated water samples. Hendriks *et al.* (1994) concentrated Rhine water on XAD resins and tested the extracts using acute and chronic *Daphnia magna* and Ames mutagenicity tests. The measure of toxicity in this study was obtained from the level of concentration required to produce an observed effect and the results showed clear patterns of response along the Dutch part of the River Rhine. In addition, approximately 160 chemicals were identified in the water extracts by GC-MS and, using toxicity databases and the toxic unit model, the toxicity of the identified compounds was summed. It appeared that the identified compounds could at most explain 11 % of the observed toxicity and in many cases less than 1 % of the observed toxicity.

2) A simple *in vitro* bioassay for narcotic chemicals. C18-empore disks are used to concentrate water samples, in the laboratory or *in situ* (Verhaar *et al.*, 1995). In this way, the biotic body burden of a mixture of pollutants is simulated. By means of vapour pressure osmometry the molar concentration in the disk is determined, which can be related directly to effects in living organisms (e.g., Van Wezel and Opperhuizen, 1995).

3) *In vitro* bioassays for specific-acting chemicals. Murk, Legler and Brouwer from the Agricultural University in Wageningen (in prep) tested recombinant receptor/reporter gene assays for dioxin-like chemicals and for (anti)-oestrogenic chemicals in polar and less polar sediment extracts of harbour sludge and pore water of various sediments from the Netherlands. Sediments that were contaminated with dioxin-like chemicals were discriminated from less polluted sites. The methods used were similar systems to those described in Zacharewski *et al.* (1995). Cell types of several organisms, including fish, can be used for this purpose. The systems give good dose-response curves and are very sensitive. Currently, in conjunction between RIKZ and the Agricultural University, more work is being done on the *in vivo* validation of the tests, with long time exposure studies.

In conclusion, it can be stated that developments in this area are rapid, and several techniques are very promising and are being used on a survey basis in several projects in the Netherlands.

12.3 Contaminant Transport in the Marine Area Affected by Upwelling of Lipid Compounds (L. Karbe)

Exploratory surveying of extended areas has revealed unusual contaminant levels and biological effects on marine organisms at sampling sites remote from expected point-source or diffuse inputs, e.g., the central northern North Sea, locations near the continental shelf edge, and in sub-Arctic regions.

To explain such phenomena, an hypothesis has been proposed—the Global Distillation and Fractionation hypothesis (Goldberg, 1975; Wania and Mackay, 1993)—which considers the role of long-range advective transport (in the atmosphere and in subsurface and deep waters) combined with water-to-air exchange processes. Important components of the net local and global fluxes are descending and ascending component fluxes within the water column. Biodeposition of compounds to particulate matter is of major importance for contaminants deposited in deep waters as the final sink. Upward fluxes of particulate organic matter within the deeper parts of the water column have also been observed on many occasions, although attempts to calculate the magnitude of these upward fluxes within deep waters show that they are small compared with the overall downward fluxes. However, under specific conditions, values as high as 66 % of the concurrently measured downward fluxes have been calculated (Smith *et al.*, 1989). Other work has focused on measurements of ascending and descending fluxes of lipid compounds in the abyssal North Atlantic and North Pacific waters (Grimalt *et al.*, 1990). Both buoyant lipid-rich particles as well as suspended lipids are considered to ascend from the deeper water to the surface. Lipophilic xenobiotics may follow this pathway and may be transported advectively, e.g., into the North Sea together with Atlantic inflows or may be re-emitted to the air for further long-range atmospheric transport. More effort is needed to understand the ecological relevance and the importance of such phenomena in order to better understand the fate of contaminants and associated risks in the wider North Sea and in adjacent sub-Arctic regions.

The WGBEC took note of this review but decided that more specific information was required on the nature and concentrations of contaminants in such areas. It was decided that the WGBEC should recommend that the following question be put to the Marine Chemistry Working Group:

'What are the levels of contaminants at fronts and along the shelf edge and what are the underlying processes responsible for these levels?'

12.4 Effects of UV Radiation on Marine Organisms and Interactions with Photosensitizing Organic Chemical Contaminants (M. Moore)

A joint report prepared by David Lowe, John Raven and Michael Moore was presented to the meeting. One of the main reasons for the widespread concern about depletion of the stratospheric ozone layer is the anticipated increase in the intensity of UV radiation

received at the surface of the Earth. There is little doubt that exposure to UV radiation is generally detrimental to biota. In the marine environment, UV-B has its greatest impact either at or immediately below the surface of the water, where primary production occurs and the eggs and early life stages of many ecologically and commercially important species of animals reside. Furthermore, tidal and intertidal zones of coastal and estuarine environments and fish farms are also potentially vulnerable to impact. However, information on change in UV radiation and its effects is limited, and the absence of an appropriate database, in conjunction with the absence of any understanding of the mechanisms of action, makes it extremely difficult to construct meaningful models of effect.

The presence of some anthropogenic contaminants in the water column may serve to attenuate UV radiation, however, the resulting derivatives of photochemical reactions may be more toxic than their parent compounds. In addition, the potency of UV can be enhanced through photosensitization of the biota by certain types of organic chemical pollutants, particularly those accumulating in intracellular vesicles known as lysosomes.

The report concluded that longer-term monitoring of changes in ozone and UV is a prime requirement, as is further research on the effects of UV radiation and contaminant photosensitizers on physiological and behavioural defence and adaptive responses. This leads on to a major requirement, which is the need for assessment of the extent and mechanisms of UV impact on ecological processes.

In discussion by the Working Group, it was pointed out that a QSAR approach had been applied to the process of photoxidation of PAHs (Machinni and Fyght) and that this was able to accurately predict which PAHs are sensitive to this process. Such studies had revealed that only a limited number of PAHs are affected by photochemical reactions and that this can lead to increased rates of degradation and solubilization.

12.5 Multidrug Resistance as a Biomarker of Exposure to Organic Chemical Pollutants (M. Moore)

EROD activity is now widely used as a biomarker of exposure to certain contaminant organic chemicals in fish. However, there is no EROD present in invertebrate animals, which severely limits the use of the technique in assessing risk of contaminant exposure in marine ecosystems. A comparable test for exposure is required for invertebrates and this may be provided by measuring induction of the multidrug resistance (MDR) system. The MDR system involves a transmembrane protein that functions as a pump or transporter for lipophilic xenobiotic contaminants. MDR has been shown to be induced in a range of marine invertebrates (worms, mussels and echinoderms) from polluted field sites (Minier and Galgani, 1995; Kurelec *et al.*, 1995; Toomey and Epel, 1995). MDR activity is also induced in fish (Kurelec *et al.*, 1995). Induction of MDR can be measured immunochemically as the MDR-protein or by the exclusion of various fluorescent dyes by living cells. This latter requires measuring dye uptake in the presence and absence of verapamil, which is an inhibitor of the MDR-transporter system. Further investigation of MDR is still required before it is recommended as a monitoring tool. However, this system appears to show considerable promise as a biomarker of exposure in the future.

12.6 EQAMAS: A Research Proposal to Establish the Effects of Contaminants in Marine Soils

The EC proposal on the chemical hazards in marine sediments prepared following the previous JMSBEC meeting in 1995 received a B-rating. The WGBEC spent some time revising and refocusing the proposal, and a draft outline is presented in Annex 7. It is expected that this will be submitted to the EC in the autumn.

13 FISHERIES QUESTION

The Working Group took note of the request for information on 'Fisheries and Fisheries Related Species and Habitats' from the Secretariat of the Fifth International Conference on the Protection of the North Sea, received via the ICES Secretariat. An extract of this questionnaire relevant to WGBEC is available (WGBEC96/13/1) and WGBEC was requested to address the following question:

'Indicate the activities involving hazardous substances that have been negatively affecting the mortality rate, the production of juveniles and the growth rate of marine species.'

The Working Group noted that the measurement of the impacts of contaminants was confined to the determination of effects at the individual level of organization. Measurement of changes in growth, fecundity, and mortality of populations caused by contaminants is not currently possible and ways of dealing with this from a management perspective have been dealt with elsewhere in this report (e.g., Section 4). However, the WGBEC also noted that there are areas of the convention waters where, for example, guidelines on the levels of contaminants in sediment have been exceeded. Although it should be emphasized that in many cases these guidelines are only provisional (due to lack of appropriate data), the science behind the determination of such guidelines (e.g., ecotoxicological reference values) was disputed and the data that do

exist are largely based on the measurement of toxicological response in individuals. The Chairman agreed to draft a reply to the ICES Fishery Secretary for members to comment, emphasizing the difficulty of obtaining population data but that measurements can be made at the individual level which can indicate the risk to the population.

14 ANY OTHER BUSINESS

14.1 Future Meeting Arrangements

It was agreed that the next meeting of the WGBEC would take place in Copenhagen in 1997 and that Dr Stagg should continue as Chairman for a further period of two years.

The list of intersessional activities was agreed and is attached as Annex 8.

Terms of reference for the 1997 WGBEC meeting were agreed. The recommendation for this meeting and other recommendations are contained in Annex 9.

14.2 Future of JMSBEC

In response to the decision made in the previous week by the JMSBEC, the future possible role of the JMSBEC was discussed jointly by WGMS and WGBEC. It was agreed that the JMSBEC had largely achieved its initial aim to develop conceptual frameworks for the integration of chemical measurements in sediments and biological effects measurements. The more specific recom-mendations of the JMSBEC for ways in which to integrate chemistry and biology (at various levels of organization) had been well received by ACME, and the principles had been adopted by OSPAR for application within the JAMP.

It was generally agreed that the JMSBEC was trying to address fundamental questions relating to the significance of contaminants in sediments, and the bioavailability of these contaminants. These were the underlying justification for including sediment chemistry in quality assessment programmes. The need to be able to assess whether a particular concentration of a contaminant presented a hazard to organisms was as vital as ever. However, the meeting agreed that there had been few new developments in this area since the 1995 JMSBEC meeting, and this had been reflected in the 1996 JMSBEC report.

The meeting discussed new areas in which the JMSBEC might be able to foster new cooperation between biological effects workers and sedimentologists. Several suggestions were made, including greater emphasis on the processes within sediments leading to solubilization and release of contaminants from sediments. The biogeochemistry of contaminants in sediments linked sediment geochemistry with the role of bacteria in sediments. Mobilization was also connected to physical and biological mixing processes, the role of bioturbation, etc. It was felt that the links between solid phase sediment chemistry and the water phase (overlying and pore water) required strengthening. It was suggested that the exchange of nutrients between sediment and water might warrant the invitation of a nutrient geochemist to a JMSBEC meeting, and that the inclusion of an organic physical chemist with environmental interests might also give rise to the recognition of new perspectives and activities.

There is continuing pressure to develop Sediment Quality Criteria—a process that inevitably requires the combination of chemical and biological expertise and measurements. There has been considerable investment in this area in North America, and regulatory authorities in Europe periodically indicate that reliable criteria would be useful to them. Currently, equilibrium partitioning theory is used in many countries to translate water quality criteria to sediment quality criteria. This should be validated by: descriptions of the kinetics, e.g., measurement of uptake kinetics from pore water and food, influence of aging, bioturbation (number, type and behaviour of the organisms), biodegradation of the chemical, sediment characteristics (organic carbon content, redox potential, etc.) and the type of chemical (K_{ow}, polarity, biodegradability, etc.).

It was concluded from the joint session that the two Working Groups would consider the development of new directions for investigating the links between sediment chemistry and biological processes intersessionally, and would propose to include items in this area in their draft terms of reference for 1997. In recognition that the process leading to well-considered new proposals for activities by the JMSBEC would need some time to yield substantive output, it was agreed to recommend that the JMSBEC should not meet in 1997, but that an opportunity for joint sessions of WGMS and WGBEC should be created within the framework of the 1997 meetings by requesting that the two Working Groups meet at ICES Headquarters during the same week, with a view to a JMSBEC meeting being proposed for 1998.

15 REFERENCES

Auger, J., Kuntsman, J.M., Czyglik, F., and Jouannet, P. 1995. Decline in semen quality among fertile men in Paris during the past 20 years. New England Journal of Medicine, 332: 281–285.

Blackburn, M.A., and Waldock, M.J. 1995. Concentrations of alkylphenols in rivers and

estuaries in England and Wales. Water Research, 29: 1623–1629.

Bocquené, G., and Galgani, F. 1996. Biological effects of contaminants: cholinesterase inhibition by organophosphorus and carbamate compounds. ICES Techniques in Marine Environmental Sciences No. 22.

Bowmer, C.T. 1993. Method for the assessment of acute toxicity of contaminated sediment using the burrowing sea urchin *Echinocardium cordatum*. Test guideline for PARCOM sediment reworker ring test. TNO-IMW, Delft, The Netherlands.

Chieco, P., Jonker, A., Melchiorri, C., Vanni, G., and Van Noorden, C.J.F. 1994. A user's guide for avoiding errors in absorbance image cytometry: a review with original experimental observations. Histochemical Journal, 1–19.

Colborn, T., vom Saal, F.S., and Soto, A.M. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environmental Health Perspectives, 101: 378–384.

Dave, G., Bjornestad, E., Efraimsen, H., and Tarkpea, M. 1993. Precision of the *Nitocra spinipes* acute toxicity test and the effect of salinity on toxicity of the reference toxicant potassium dichromate. Environmental Toxicology and Water Quality, 8: 271–277.

Fry, D.M., and Toone, C.K. 1981. DDT-induced feminization of gull embryos. Science, 213: 922–924.

Goldberg, E. 1975. Synthetic organohalides in the sea. Proc. R. Soc. Lond. B, 189: 277–289.

Grimalt, J.O., Simoneit, B.R.T., Gómez-Belinchón, J.I., Fischer, K., and Dymond, J. 1990. Ascending and descending fluxes of lipid compounds in North Atlantic and North Pacific abyssal water. Nature, 345: 147–150.

Guillette, L.J. Jr., Gross, T.S., Masson, G.R., Matter, J.M., Percival, H.J., and Woodward, A.R. 1994. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environmental Health Perspectives, 102: 680–688.

Harries, J.E., Jobling, S., Matthiessen, P., Sheahan, D.A., and Sumpter, J.P. 1995. Effects of Trace Organics on Fish—Phase 2. Report to the UK Department of the Environment, Foundation for Water Research, Marlow, Report No. FR/D 0022. 90 pp.

Harries, J.E., Sheahan, D.A., Jobling, S., Matthiessen, P., Neall, P., Routledge, E., Rycroft, R., Sumpter, J.P., and Taylor, T. In press. Survey of estrogenic activity in UK inland waters. Environmental Toxicology and Chemistry.

Harries, J.E., Sheahan, D.A., Jobling, S., Matthiessen, P., Neall, P., Sumpter, J.P., Taylor, T., and Zaman, N. In prep. Further evidence of estrogenic activity in UK rivers—vitellogenesis and testicular retardation in caged male fish.

Hendricks, A.J., Maas-Diepeveen, J.L., Noordsij, A., and van der Gaag, M.A. 1994. Monitoring response of XAD-concentrated water in the Rhine delta: a major part of the toxic compound remains unidentified. Water Research, 28: 581–598.

IEH. 1995. Environmental Oestrogens: Consequences to Human Health and Wildlife. Institute for Environment and Health, Leicester. 107 pp.

Janssen, P.A.H. 1996. Reproduction of the flounder, *Platichthys flesus* (L.), in relation to environmental pollution. Ph.D. Thesis, University of Utrecht. 174 pp.

Jobling, S., Sheahan, D., Osborne, J.A., Matthiessen, P., and Sumpter, J.P. 1996. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environmental Toxicology and Chemistry, 15: 194–202.

Jobling, S., and Sumpter, J.P. 1993. Detergent components in sewage effluent are weakly estrogenic to fish: an *in vitro* study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Aquatic Toxicology, 27: 361–372.

Köhler, A. 1991. Lysosomal disturbance in fish liver as indicators of the toxic effects of environmental pollution. Comparative Biochemistry and Physiology, 100C: 123–128.

Köhler, A. In prep. Fish liver histopathology. ICES Techniques in Marine Environmental Sciences.

Köhler, A., Deisemann, H. and Lauritzen, B. 1992. Histological and cytochemical indices of toxic injury in the liver of dab *Limanda limanda*. Marine Ecology Progress Series, 91: 141–150.

Kurelec, B., Piveevic, B., and Muller, W.E.G. 1995. Determination of pollutants with multi-xenobiotic-resistance inhibiting pro-perties. Marine Environmental Research, 39: 261–265.

Lang, T., Damm, U., and Dethlefsen, V. 1995. Changes in the sex ratio of North Sea dab (*Limanda limanda*) in the period 1981–1995. ICES CM 1995/G:25 (reprinted in UBA, 1996).

Lowe, D.M., Soverchia, C., Moore, M.N. 1995. Lysosomal membrane responses in the blood and digestive cells of mussels experimentally exposed to fluoranthene. Aquatic Toxicology, 33: 105–112.

Minier, C., and Galgani, F. 1995. Multi-xenobiotic resistance in *Mytilus edulis*. Marine Environmental Research, 39: 267–270.

Moore, C.G., and Stevenson, J.M. 1991. The occurrence of intersexuality in harpacticoid copepods and its relationship with pollution. Marine Pollution Bulletin, 22: 72–74.

Moore, C.G., and Stevenson, J.M. 1994. Intersexuality in benthic harpacticoid copepods in the Firth of Forth, Scotland. Journal of Natural Hisory, 28: 1213–1230.

Moore, M.N. 1988. Cytochemical responses of the lysosomal system and NADPH-ferri hemoprotein reductase in molluscan digestive cells after environmental and experimental exposure to xenobiotics. Marine Ecology Progress Series, 46: 81–89.

Moore, M.N. 1990. Lysosomal chemistry in marine environmental monitoring. Histochemistry Journal, 22: 187–191.

Moore, M.N. 1992. Molecular and cellular pathology: a summary. Marine Ecology Progress Series, 91: 117–119.

OECD. 1984. Guideline for testing of chemicals. Fish acute toxicity test OECD 203.

OECD. 1992. Guideline for testing of chemicals. Fish early-life stage toxicity test OECD 210.

Oehlmann, J., Storben, E., and Fiorini, P. 1991. The morphological expression of imposex in *Nucella lapillus* (Linnaeus) (Gastropoda: Muricidae). Journal of Molluscan Studies, 57: 375–390.

Oslo and Paris Commissions. 1995. PARCOM protocols on methods for the testing of chemicals used in the offshore industry. London. 35 pp.

Pereira, J.J., Ziskowski, J., Mercaldo-Allen, R., Kuropat, C., Luedke, D., and Gould, E. 1992. Vitellogenin in winter flounder (*Pleuronectes americanus*) from Long Island Sound and Boston Harbor. Estuaries, 15: 289–297.

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler, C.R., and Sumpter, J.P. 1994. Estrogenic effects of effluents from sewage treatment works. Chemistry and Ecology, 8: 275–285.

Reijnders, P.J.H. 1986. Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature, 324: 456–457.

Reichert, W.L., French, B.L., and Stein, J.E. In press. Protocols for assaying levels of large hydrophobic DNA adducts in fish by ^{32}P postlabelling. ICES Techniques in Marine Environmental Sciences.

Roddie, B.D., and Thain, J.E. In prep. Biological effects of sediment-bound contaminants: *Corophium* sp sediment bioassay and toxicity test. ICES Techniques in Marine Environmental Sciences.

Routledge, E.J. and Sumpter, J.P. In press. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environmental Toxicology and Chemistry.

Sharpe, R.M., Fisher, J.S., Millar, M.M., Jobling, S., and Sumpter, J.P. 1995. Gestational and lactational exposure of rats to xenoestrogens results in reduced testicular size and sperm production. Environmental Health Perspectives, 103: 1136–1143.

Sharpe, R.M., and Skakkebæk, N.E. 1993. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet, 341: 1392–1395.

Sheahan, D.A., Bucke, D., Matthiessen, P., Sumpter, J.P., Kirby, M.F., Neall, P., and Waldock, M. 1994. The effects of low levels of 17a-ethynylestradiol upon plasma vitellogenin levels in male and female rainbow trout, *Oncorhynchus mykiss* held at two acclimation temperatures. *In* Sublethal and Chronic Effects of Pollutants on Freshwater Fish. Ed. by R. Müller, and R. Lloyd, pp. 99–112. Fishing News Books, Blackwell Science Ltd., Oxford.

Smith, K.L., Williams, P.M., and Druffel, E.R.M. 1989. Upward fluxes of particulate organic matter in the deep North Pacific. Nature, 337: 724–726.

Stagg, R.M., and McIntosh, A.M. In press. Dilemination of CYPIA dependent monoxygenase activity in the liver of the dab (*Limanda limanda*) by fluorimetric measurement of 7-ethoxy resorufin-O-deethylase (EROD) activity. ICES Techniques in Marine Environmental Sciences.

Stromgren, T., Nielsen, M.V., and Reirson, L.O. 1993. The effect of hydrocarbons and drilling fluids on the faecal production of the deposit feeder *Abra alba*. Aquatic Toxicology, 24: 275–286.

Sumpter, J.P. 1985. The purification, radio-immunoassay and plasma levels of vitellogenin from the rainbow trout *Salmo gairdneri*. *In* Trends in Comparative Endocrinology, pp. 355–357. Ed. by B. Lofts and W.H. Holmes. Hong Kong University Press, Hong Kong.

Thain, J.E. 1991. Biological effects of contaminants: Oyster (*Crassostrea giga*) embryo bioassay. Techniques in Marine Environmental Sciences No. 11. 12 pp.

Thain, J.E., and Bifield, S. In prep. Biological effects of sediment-bound contaminants: *Arenicola marina* sediment bioassay and toxicity tests. ICES Techniques in Marine Environmental Sciences.

Toomey, B.H., and Epel, D. 1995. A multi-xenobiotic transporter in *Unechis caupo* embryos: protection from pesticides? Marine Environmental Research, 39: 299–302.

Toppari, J. *et al.* 1995. Male Reproductive Health and Environmental Chemicals with Estrogenic Effects. Miljøprojekt No. 290, Miljø- og Energiministeriet, Miljøstyrelsen, Copenhagen. 166 pp.

UBA. 1996. Endocrinically Active Chemicals in the Environment. Texte 3/96, Umweltbundesamt, Berlin. 151 pp.

Van Wezel, A.-M., and Oppenhuizen, A. 1995. Critical Reviews in Toxicology, 25: 255–279.

Verhaar, H.I.M., Busser, F.I.M., and Hermens, J.L.M. 1995. Surrogate parameter for the baseline toxicity content of contaminated water: simulating the bioconcentration of mixtures of pollutants and counting molecules. Environmental Science and Technology, 29: 726–734.

Wania, F., and Mackay, D. 1993. Global fractionation and cold condensation of low volatility organochlorine compounds in polar regions. Ambio, 22: 10–18.

Williams, T.D. 1992. Survival and development of copepod larvae of *Tisbe battagliai* in surface microlayer, water and sediment elutriates from the German Bight. Marine Ecology Progress Series, 91: 221–228.

Zacharewski, T.R., Berhave, K., and Gillesby, B.E. 1995. Detection of oestrogen- and dioxin-like activity in pulp and paper mill black liquor and effluent using *in vitro* recombinant receptor/reporter gene assays. Environmental Science and Technology, 29: 2140–2146.

ANNEX 1

AGENDA

1. Opening of the meeting

2. Appointment of rapporteur(s)

3. Adoption of the agenda

4. Statistical design of biological effects programmes

5. QA in biological effects measurements

6. Prepare methods for reviewing the effectiveness of existing biological effects techniques

7. Review the Report of the OSPAR/ICES Workshop on Biological Effects Monitoring Techniques and address the following issues that arise out of this:

   i) Develop the programme for general biological effects monitoring particularly as regards objectives and implementation.

   ii) Develop a quality assurance programme for each of the following methods:

   Bioassays
   Benthic community analysis
   Externally visible fish disease
   Liver pathology
   P4501A
   Lysosomal stability
   DNA adducts
   PAH metabolites in bile
   Oxidative stress
   Metallothionein
   Imposex
   Shell thickening in *Crassotrea*

   consisting of the following elements: approved methods; nomination of lead laboratories; terms of reference for a QA programme to be carried out by the lead laboratory; quality standards and level of intercomparison needed.

8. Advise on the use of biological effects for determining the effects of PCBs on sea mammals at the species or population level.

9. Advise on the use of biological effects of contaminants on reproduction, immunology, and metabolism of marine organisms, mainly fish.

10. Review of other biological effects monitoring techniques and recommendations for inclusion in monitoring - criteria for recommendation, methods, intercomparison,

    a) Scope for growth and clearance rate
    b) Oestrogenic contaminants
    c) Acetylcholinesterase
    d) DNA strand breaks
    e) Multi-drug resistance (MDR)
    f) Stress proteins

11. Review of the TIMES leaflets

12. Consider the following proposals/discussion papers:

- Linkages between effects of contaminants on individuals and communities
- Particle transport in the maritime area affected by upwelling
- A study of risks to benthic organisms
- The interaction between contaminants and UV-B exposure

13. Questionnaire on fisheries and fisheries-related species and habitats issues

14. Any other business

15. Recommendations and action list

16. Adoption of the report and closing of the meeting

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ANNEX 3

MEETING DOCUMENTS

WGBEC96/4/1	Extracts from 1995 ACME Report on statistical aspects of monitoring.
WGBEC96/4/2	Aspects of the statistical design of biological effects programmes (Ketil Hylland)
WGBEC96/5/1	Quality assurance and biological effects monitoring - the way forward
WGBEC96/6/1	Evaluation of the SIME Monitoring Programme
WGBEC96/6/2	Discussion paper from Lennart Balk and lists of hazardous substances.
WGBEC96/7/1	The report of the OSPAR/ICES Workshop on Biological Effects Monitoring Techniques
WGBEC96/7/2	Terms of Reference for the *Ad Hoc* Working Group on Monitoring in order to finalise the JAMP monitoring guidelines.
WGBEC96/7/3	Report on quality assurance for bile measurements: Alaska oil spill damage assessment programme.
WGBEC96/7/4	Quality assurance/quality control for ^{32}P-post labelling of DNA adducts
WGBEC96/7/5	QA Programmes for fish diseases
WGBEC96/7/6	*Ad Hoc* meeting on the use of liver pathology
WGBEC96/8/1	Proposal for requests from the Helsinki Commission to ICES for 1996
WGBEC96/8/2	Report of the Study Group on Seals and Small Cetaceans in European Seas (ICES CM 1996/N:1)
WGBEC96/8/3	Resolution on the Environment and Whale Stocks
WGBEC96/8/4	Levels of toxic organochlorines in seal blubber in the Riga Bay
WGBEC96/8/5	PCBs and OCs in Baltic herring and sprat
WGBEC96/8/6	Organic chemicals and heavy metals in eastern and central Europe.
WGBEC96/8/7	Blood sampling as a non-destructive method for monitoring levels and effects of OCs in seals.
WGBEC96/10/1	A comparison of SFG and clearance rate in bivalve mussels.
WGBEC96/12/1	Effects of UV radiation on marine organisms and interactions with photosensitising organic contamination.
WGBEC96/13/1	Reporting format on fisheries and fisheries related species and habitat issues.
WGBEC96/14/1	Draft terms of reference for OSPAR/ICES Workshop on Ecotoxicological Assessment Criteria.
WGBEC96/14/2	Draft terms of reference for OSPAR/ICES Workshop on Background Values
WGBEC96/14/3	Report of the OSPAR Workshop on Ecological Quality Objectives

ANNEX 4

DRAFT MEMORANDUM OF UNDERSTANDING - COST PROPOSAL

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Effects of Marine Pollution

The main objective of the Action is to increase knowledge of the biological effects of chemical contamination in the marine environment surrounding Europe, to enable the informed decisions to be made on regulatory action to support social and economic development, taking into account the continuing advances of understanding and techniques relating to the processes of toxicity and the impacts of toxic chemicals.

Proposal Part I

First draft technical annex

A. Background

The seas surrounding the Europe are a valuable and shared resource. Although the need to protect the living resources of these seas from adverse impact of toxic chemicals has been recognised for a number of years, there is increasing concern over the impact of chemicals on marine organisms at all levels of organisation from the molecular level to ecosystem and community level.

International regulation of the disposal of waste in European seas, and assessment of the impact of these wastes, is undertaken through the Oslo and Paris Conventions (OSPAR for NE Atlantic and North Sea) and the Helsinki Convention (HELCOM for Baltic Sea). Commissions under these Conventions administrate the activities of a series of technical and advisory groups established to meet the needs of environmental protection. Additional technical advice is also available through the International Council for the Exploration of the Sea (ICES). Commission activities include the agreement of targets for reductions in waste inputs, and environmental assessment and monitoring activities to provide comprehensive and balanced assessments of the quality status of the marine environment, and give guidance to subsequent international regulatory actions.

One of the recent outputs from OSPAR and ICES has been a Quality Status Report for the North Sea (1993), which contained a detailed review of the marine environment of the North Sea, and identified a series of international agreed particular causes for concern. These were mainly expressed in chemical terms, and it was noted that efforts to determine the biological significance of the concentrations of many chemicals measured in water, sediment, or organisms were in need of improvement.

Since 1993, there has been more widespread recognition that there was a need for much greater integration of chemical and biological monitoring programmes on an international scale to provide the necessary perspectives on contamination over wide geographical areas. Marine pollution is an international problem that does not respond to national boundaries. There are now research programmes underway in most countries, largely under national funding, to develop and improve methods to measure the effects of pollutants on marine organisms. The Commissions are developing coordinated programmes to address priority issues of biological impact of contaminants. The effects being studied range in scale from molecular effects (eg the induction of the formation of particular specialised proteins, of impacts on the activity of particular enzyme systems), cellular effects (eg stimulation of pre-cancerous conditions), organ-scale effects (eg development of carcinomas), to whole-organism effects (eg impacts on growth, survival, reproduction) and community effects (eg changes in the species composition of sea bed communities). The biological effects measurements are to be supported by targeted chemical analysis, and the whole programme is underpinned by continuing national research effort.

In order for these programmes to develop their full potential, and to ensure that all countries are able to participate in the Commission programmes and contribute to the cooperative knowledge base, it is necessary to instigate a coordinated programme of method development, validation, testing, standardisation, training, and quality assurance. Cooperation on a Europe-wide scale will ensure that the specialist technical and interpretive skills available in a limited number of centres quickly become available to all. It also raises opportunities for better integration and technology transfer between Europe and North America.

The substantial effort national resources already being expended in this area, both in research effort and pilot monitoring programmes must be made available as widely as possible, in the form of training in reliable and standardised methods of analysis and quality assurance. The product, a greater ability to understand the effects man is having on the marine environment through waste inputs, is a community benefit far more than a national benefit. The benefits, both social and economic, will be shared by all and the COST programme provides an ideal vehicle for the strengthening and acceleration of the technical base required for effective monitoring and assessment programmes.

B. Objectives and Benefits

It is important to increase knowledge in this area so that assessment of the contaminant status of different sea areas are made on comparable bases. New regulatory measures must address the most significant problems, to ensure that the environment is adequately protected for current and future generations, at the same time, ensure that investment by industry and public bodies is used as effectively as possible. The international implications of marine pollution, and the international approach taken through the Conventions to protect the environment provide the necessary base for European-wide cooperation in assessment of the biological consequences of waste disposal. In contrast to other frameworks, coordination through COST would enable participating countries to derive the maximum benefit from their own expenditure whilst contributing directly to the development of international marine pollution assessment, monitoring and control.

The main objective of the Action is to increase knowledge of the biological effects of chemical contamination in the marine environment surrounding Europe, to enable the informed decisions to be made on regulatory action to support social and economic development, taking into account the continuing advances of understanding and techniques relating to the processes of toxicity and the impacts of toxic chemicals.

Therefore, the goal of the COST action and its partners will be combine together to standardise, validate, test and develop quality assurance procedures for existing methodologies and research results in the field of marine toxicology with the aim of creating a coordinated and integrated scheme of procedures for assessing the quality status of European marine areas in relation to priority toxic chemicals.

The increased knowledge of both environmental processes and the biological impacts of chemicals that will result from the Action will enable the regulatory Commissions and national authorities to reach informed and reliable decisions on effective resource allocation in the field of pollution control. It will enable the significance of chemical contamination of the sea to be better assessed in relation to the impacts of other activities in the sea, such as fishing, aggregate extraction and marine engineering.

The rapidly increasing understanding that will result from the coordinated monitoring programmes being instigated by the Commissions will provide a valuable base for the formulation of research proposals to meet the developing needs of marine pollution control. These could for example:

1. lead to the development of procedures for the investigation of newly-recognised forms of toxicity in the sea, such as the actions of neurotoxic substances, substances which interfere with natural hormone systems, mutagenic and cancerogenic substances;

2. stimulate research into the molecular-scale impacts of toxic chemicals and of the interactions between impacts and sub-cellular and cellular levels;

3. develop understanding of the significance of molecular and cellular scale effects for whole organism processes such as growth, survival, and reproduction

Research on the integration of chemical and biological effects measurements in the marine environment is already underway in several European countries, and in North America. The results of the collaboration between the signatory states will show effects at national and international levels, particularly concerning technical missuses surrounding marine environmental quality assessment, and subsequently in to the social and economic benefits of good

management of marine resources through combined international and national actions. The networks served by this Action will increase the value of research and monitoring activity by the signatory states, and will:

a) serve as technically expert groupings of active scientists able to provide the Commissions with the best possible advice on the execution of integrated chemical and biological monitoring programmes

b) serve as vehicles for the dissemination of information on best analytical practice in the techniques required for the international coordinated monitoring programmes

c) target the priority issues of environmental contamination concern in European marine waters

d) provide fora for the validation and standardisation of analytical and interpretive procedures

e) provide fora for training in the execution of field and laboratory programmes, and quality assurance procedures

f) deliver an integrated series of reliable analytical techniques and associated quality assurance practices

g) deliver information on the impacts of toxic chemicals in the marine environment that can be interpreted in relation to causative agents and biological consequences

The main benefits that will accrue to participating countries are:

Improved networking and sharing of information and experience to assist in he development of reliable improved methods for the measurement of the effects of pollutants on marine organisms.

Support for the development of agreed procedures for assessing the significance, at whole organism, population and community levels, of the measured biological effects of contaminants.

The availability of a suite of protocols describing tried, tested and reliable methods for measuring the biological effects of recognised priority marine contaminants.

The main benefits that will accrue to the international pollution regulatory authorities will be:

- enhancement of the rate of development and documentation of robust methods for the measurement of the effects of contaminants on marine organisms, and of procedures for assessing the significance of these effects;
- enhanced opportunities for the dissemination of expertise in biological effects measurements to laboratories through the COST area;
- enhanced opportunity to address identified marine environmental problems through effective combinations of chemical and biological effects techniques;
- improved estimation of the effectiveness of proposed new measures to reduce the impact of pollutants on marine organisms through the regulation of waste disposal, or other activities which affect the sea.

The main benefits to European society will be:

- improved protection of marine ecosystems from the effects of chemical contaminants;
- more effective targeting to investment/expenditure at priority pollution control actions.

The Scientific Content of the Action

The strategic lead for the work covered by the Action is provided by the structures of the Paris and Helsinki Commissions, and their continuing need for improved environmental monitoring and assessment programmes on a coordinated international scale. The fundamental aims of these programmes may be summarised as:

Aims of JAMP and COMBINE

The role of the activities to be specifically supported by the COST Programme is to provide the necessary coordination at a technical level to assist in achieving the aims and objectives of the Commissions through integrated chemical and biological effects monitoring programmes. The priority causes for concern in the North Sea area (for example) have been identified by the Oslo and Paris Commission Environmental Assessment and Monitoring Committee (ASMO), and through this group and third tier groups under ASMO the details of the requirements for effective monitoring

programmes to address these priorities are being defined. The developing programme specifications emphasise the need for standardised and reliable data of defined quality to be available in all of the Convention waters. There is an urgent need for more rapid progress inn the definition of validated procedures, and their wide dissemination through the European Community. The main contribution to the Action to be provided under the COST Programme is the support of the definition, testing, and dissemination of the methods required to meet the current and foreseeable needs of the Commissions' programmes.

D. Organisation and Timetable

The supported activities under the COST Action area will be coordinated under XX Working Groups, under the guidance of a Steering Group. The definition of the monitoring and assessment programmes, and the preparation of subsequent Quality Assessment Reports are fully the responsibilities of established groups under the Commissions and their advisors, and will not be duplicated by new structures created under COST support. Equally, the national monitoring and research programmes contributing to the Action must remain under national control to ensure national priorities are met in addition to international commitments.

Each of the Working Groups will consist of members from the participating countries, while the Management Committee will consist of a single representative from each country (with additional support as necessary). The first meeting of the Management Committee will discuss and agree the structure and roles of the respective Working Groups, and establish terms of reference and target outputs and dates for them taking into account the requirements of the programmes established by the Commissions. The Working Groups will initially review the state of knowledge and availability of expertise in the analytical and quality assurance procedures required by the international coordinated programmes, and plan their work to ensure that the targets defined by the Management Committee are met.

In general, it is anticipated that the following activities will be taking place jointly within all the signatory states:

a) establishment of networks of scientists with expertise in appropriate chemical and biological effects measurements;

b) planning of resource allocation and timetables for the execution of the programmes defined by the Commissions;

c) participation in various Committees, Working Groups and Technical Groups under the Commissions;

d) research conferences, workshops and publications of proceedings;

e) publication of guidelines and protocols for measurements;

f) execution of monitoring programmes to address the priority targets established by the Commissions;

g) exchange of research and monitoring results and contribution of data to a central data bank, probably located at ICES Headquarters in Copenhagen;

h) exchange of researchers between cooperating institutes;

i) preparation of reports, publications, and Quality Status Reports for marine areas.

The Working Groups will be responsible for the following monitoring and research areas:

Working Group A: The effects of metallic contaminants on marine organisms
Working Group B: The effects of polychlorinated biphenyls on marine organisms
Working Group C: The effects of poly-aromatic hydrocarbons on marine organisms
Working Group D: The effects of tributyltin compounds on marine organisms
Working Group E: The effects of chlorinated dioxins and furans on marine organisms
Working Group F: The assessment of the significance of the effects of newly-recognised groups of contaminants, such as neurotoxins, mutagens, and compounds affecting hormone systems on marine organisms

The structure of the Working Groups therefore reflects the current expressions of areas of concern, significant issues of environmental chemical contamination, identified by the Commissions, and anticipate the development of their interests into new areas.

The objectives of each Working Group will include the establishment of a suite of standard analytical procedures, with defined quality assurance procedures, which when used in an integrated manner will provide a reliable data set appropriate to the assessment of a particular group of contaminants. The Groups will also establish appropriate mechanisms to ensure that the expertise to carry out these procedures is as widely available within the signatory countries as is necessary to provide the Commissions with data of a sufficiently comprehensive nature to allow a defensible assessment of the significance of a particular group of contaminants.

Although the area of work covered by the Action, namely the monitoring and assessment programmes of the Commissions are planned to last beyond the year 2000, it is proposed that the main work to be supported by COST needs to be completed relatively quickly to that the necessary techniques become available speedily. The project is therefore estimated to last four years, at the end of which it will be necessary to review the stage of development within each Working Group area to determine the current state of knowledge and the potential for new and more effective approaches to be taken. This will be particularly necessary in relation to newly-recognised contaminants, and the emergence of the recognition of new mechanisms of toxicity.

All signatories will have scientists in each individual Working Group, and a representative on the Management Committee. Each Working Group will be chaired by a Coordinator, who will be elected by the Management Committee. The Coordinators of the Working Groups will meet with the Management Committee once a year, which will serve as an annual joint meeting at which the Coordinators will present formal reports concerning the progress of each Working Group, and proposals for the forthcoming work of each Working Group. If progress with the Action or administrative reasons make it necessary, more frequent meetings will be held. It is anticipated that these may well arise where detailed liaison is needed between different Working Groups, for example of the same, or very similar techniques are relevant to more than one Group.

The final report of the COST Action will include:

a) descriptions of the work carried out within each Working Group;

b) copies of standard analytical and quality assurance protocols produced under the Action;

c) copies of training materials and manuals produced under the Action;

d) an account of the development of the application of the techniques covered by the Action in the programmes of the Commissions;

e) copies of other publications and reports which have been influenced by the COST Action.

The final report will also include an assessment of the need for further research activities to improve existing measurement methods or develop new approaches to existing measurements or develop new types of measurement.

E. Economic Dimension of the Action

The costs of the Action are based on the average salary costs for COST countries as follows:

THESE NEED TO BE UPDATED FROM 1993 FIGURES:

Senior scientist: Category A 60,000 ECU
Technician: Category B 40,000 ECU
Junior scientist: Category C 25,000 ECU

In some countries, there are significant costs associated with the use of research and charter vessels to undertake sampling programmes at sea in support of both monitoring programmes and underpinning research programmes. These have been estimated from costs and days at sea quoted by the countries indicated below.

The running/operational costs (eg consumable items and proportion capital equipment) of the staff involved have been estimated as 30% of the salary costs, and overheads of 50% have been added to salary and operational costs.

I am not yet clear what costs might be claimed, whether it just includes things like travel and subsistence costs or whether they will pay staff costs associated with coordination work

It is proposed to employ one (two ?) junior scientists to provide scientific administrative support for the coordination activities (? and to allow a certain amount of senior staff time for involvement in the Management Group and Coordinators of Working Groups and general project leadership).

Current Data Requirements

Data to be collected at this stage for each participating country: these do not have to be very precise.
Man years of effort in each country by staff category
Days at sea per year, and cost per day

Form of Text Suggested by UK DTI

The following COST countries have actively participated in the preparation of the Action, or otherwise indicated their interest.

List:
UK
Belgium
etc

On the basis of national estimates provided by the representatives of these countries and taking into account the coordination costs to be covered over the COST budget of the European Commission, the overall cost of the activities to be carried out under the Action has been estimated, in 1996 prices, at roughly ECU ...

This estimate is valid under the assumption that ball the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

We need to form a small group of interested scientists to estimate the coordination costs other than staff costs.

ANNEX 5

COMPOSITION OF SUBGROUPS TO DEVELOP QA PROCEDURES

Subgroup 1 - Pathological Biomarkers

Techniques to be considered: lysosomal stability, pathology, imposex/intersex.

Michael Moore (Chairman)
Angela Kohler
Ian Davies

Subgroup 2 - Biochemical Biomarkers

Techniques to be considered: metallothionein, P4501A, ALA-D, DNA adducts, bile metabolites, oxidative enzymes including malonedialdehyde.

Jerry Payne (Chairman)
Ketil Hylland
Aldo Viarengo
Gilles Boquené
Volkert Dethlefsen

Subgroup 3 - Bioassays

Techniques to be considered: sediment, pore-water and water column bioassays and also the particular assays to be used:

Peter Matthiessen (Chairman)
Åke Granmo
Anne-Marie van Wezel
Ludwig Karbe

ANNEX 6

Agenda Item 7

Review the Report of the OSPAR/ICES Workshop on Biological Effects Monitoring Techniques and address the following issues that arise out of this

ii) Develop a quality assurance programme for each of the following methods:
Externally visible fish diseases (Thomas Lang)

STATE-OF-THE-ART FISH DISEASE MONITORING

Epidemiological studies on diseases and parasites of wild marine fish have a long tradition in ICES. For example, regular intensive North Sea monitoring programmes focusing on externally visible diseases of the common dab (*Limanda limanda*) were already underway at the end of the 1970s. At the same time, the first systematic investigations were initiated in the Baltic Sea with cod (*Gadus morhua*) and flounder (*Platichthys flesus*) as major target species. At present, the majority of countries bordering the North Sea and Baltic Sea are carrying out regular fish disease surveys. However, some countries have reduced or even stopped their programmes which has been regretted repeatedly by ICES.

When results of the early studies were discussed within ICES bodies, it became apparent that there was a lack of intercalibration and standardization of the methodologies applied and, consequently, results reported did not seem to be comparable. In the beginning of the 1980s, the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) initiated the first attempts to solve this problem. Since that time, three sea-going workhops (1984 North Sea, 1988 Kattegat, 1994 Baltic Sea) have been held under the sponsorship of ICES in order to intercalibrate and standardize methodologies for fish disease surveys and to establish practical guidelines for an integrated international programme to determine long-term trends in fish disease prevalence levels. The resulting guidelines included recommendations on minimum sampling requirements, target fish species, types of diseases to be monitored and cut-off points thereof, sampling stations and areas, and additional measurements (Dethlefsen *et al.*, 1986; ICES, 1989; Lang *et al.*, 1995). Most of the existing regular fish disease monitoring programmes are designed according to these guidelines.

Since the inception of wild fish disease studies, ICES Member Countries have been requested to submit their results to ICES on an annual basis. At first, paper formats were used for this purpose; now a (recently revised) fish disease data reporting format as well as a specific data entry program are available, facilitating compatibility with the other environmental data (for example, contaminants in biota and sediments) stored in the ICES Environmental Databank. Some of the fish disease prevalence data submitted to ICES date as far back as 1981 and represent a unique set of long-term data on biological community/population responses of marine organisms to environmental changes. The statistical analysis of these data in conjunction with other ICES environmental data is a task addressed by the ICES Sub-Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks (SGFDD). At its forthcoming meeting (19–20 March 1996, Copenhagen), an evaluation of the revised disease data format and a preliminary analysis of the data are part of the agenda.

Other ICES activities coordinated by the WGPDMO related to externally visible fish diseases were the ICES/IOC Bremerhaven Workshop on Biological Effects of Contaminants in the North Sea (Vethaak *et al.*, 1992) and the publication of the "Training Guide for the Identification of Common Diseases and Parasites of Fish in the North Atlantic" (Bucke *et al.*, 1996).

Apart from these official ICES activities, scientists involved in fish disease monitoring programmes in the North Sea continue to work closely together and the studies carried out have been, to a large extent, coordinated efforts. Due to the political changes in the Baltic Sea area and the activities of the Baltic Marine Biologists (BMB) Working Group 25 on "Fish Diseases and Parasites in the Baltic Sea", the cooperation between scientists involved in fish disease studies in the Baltic Sea has also improved considerably during recent years.

In conclusion, it can be stated that methodologies used for studies on externally visible diseases of wild fish in the Oslo/Paris and Helsinki Convention areas are to a large extent coordinated and standardized on an international level. Standard operating procedures including sampling design, disease diagnosis, and standard protocols for recording and reporting fish disease data have been developed and applied successfully. All methodologies have now been tested

practically for a considerable period of time. Due to activities (e.g., sea-going workshops) organized by WGPDMO and BMB WG 25, interlaboratory performance testing exercises on disease diagnosis have been carried out successfully. Therefore, disease data submitted to the ICES Environmental Databank by institutes actively participating in the quality assurance procedures described above are regarded as having consistently high quality.

Objectives of fish disease monitoring

From the beginning of systematic surveys on the spatial distribution of diseases in wild marine fish, one of the main objectives of these studies has been to test whether changes in the prevalences of certain diseases beyond normal background levels could be used as a biological indicator for an impact of anthropogenic contaminants released into the marine environment.

Consequently, the first North Sea studies, mainly carried out by German and UK scientists, investigated the effects of dumping activities (sewage sludge, industrial wastes) on the health status of fish.

After reviewing the results of long-term studies, there is now a general consensus that in most cases a direct cause-effect relationship between exposure to contaminants and occurrence of elevated disease prevalences is very difficult to establish *in situ* due to the fact that most common diseases have a multifactorial instead of a mono-causal aetiology, involving the impact of anthropogenic and/or natural variations of host, pathogen, and environmental characteristics. However, in some cases an impact of anthropogenic contaminants on the prevalence of non-infectious externally visible diseases could be demonstrated (for example, effects of pulp mill effluents and other industrial discharges in the Baltic Sea) and there is increasing evidence that environmental changes such as oxygen deficiency may significantly affect the prevalence of infectious externally visible diseases.

Due to their responsiveness to environmental changes and their high ecological relevance (fish diseases may have an impact on growth, reproduction, and survival in affected fish populations), it has been recommended repeatedly to include externally visible fish diseases as bioindicators in monitoring programmes on biological effects of contaminants. Studies on spatial and temporal aspects can provide generic information on population/community responses to environmental stressors including exposure to contaminants.

Since the manifestation of externally visible fish diseases (as well as liver tumours, which are also included in most monitoring programmes) represents an endpoint of numerous biochemical and physiological changes affecting the homeostasis of affected fish, they are a useful bioindicator for chronic rather than acute environmental stress and may, therefore, be a more appropriate integrative indicator for complex changes typically occurring under field conditions as compared to biomarkers for subtle early changes at the subcellular or cellular level (EROD activity, lysosomal stability, etc.). The latter indicators can be considered of higher toxicological value than externally visible fish diseases and may, due to their rapid response, be more suitable for monitoring acute effects of point-source contamination by environmental chemicals known to affect the biomarkers.

Further advantages of studies on fish diseases as bioindicators are:

- target diseases are, with a certain degree of training, easy to recognize;
- a large number of fish can be screened within a short time, thus enabling sound statistical data analysis;
- it is a cost-effective means for monitoring, not requiring expensive and time-consuming laboratory analyses.

Ideally, for the monitoring of biological effects of contaminants on a larger scale, bioindicators measuring biological responses at different levels of organization (subcellular, cellular, tissue; individual, population/community) should be combined in order to obtain a more comprehensive overview of anthropogenic effects in the marine environment. In order to obtain information on possible cause-effect relationships, chemical monitoring should be applied at the same time, preferably using samples obtained from the same individuals, when contamination in biota is to be analysed.

QUALITY ASSURANCE PROGRAMME

Monitoring of externally visible fish diseases in the Oslo and Paris Commissions area should be carried out according to guidelines elaborated by the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) and the ICES Sub-Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks (SGFDD). For the Helsinki Commission area (Baltic Sea), deliberations by the Baltic Marine Biologists (BMB Working Group 25, "Fish Diseases and Parasites in the Baltic Sea") should be utilized in addition.

A Approved Methods

Methods applied in current monitoring programmes on externally visible fish diseases and liver nodules/tumours carried out in the European ICES area have been established for a long period and conform, to a large extent, to guidelines developed by ICES (Dethlefsen *et al.*, 1986; ICES, 1989; Bucke *et al.*, 1996). Since they have been standardized and intercalibrated repeatedly on an international level, they can be considered to be approved methods and standard operating procedures.

A summary of approved methods is given below. Some suggestions for modifications and additional standard procedures are included which are based solely on personal ideas of T. Lang and other individuals and need to be endorsed by the ICES WGPDMO before inclusion in any official programmes or publications. These suggestions occur in italics print type.

During 22–25 October 1996, an ICES *Ad Hoc* Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants will be held at the MAFF Fish Diseases Laboratory, Weymouth, UK. The primary aim of the workshop is to intercalibrate and standardize currently used methodologies and to provide guidelines for methods suitable for monitoring purposes. This meeting can be regarded as a continuation of the ICES activities for quality assurance of fish disease studies.

1 Fish Species

The fish species selected for monitoring purposes should be benthic, fairly static (outside the spawning period), abundant, and exhibit high prevalences of diseases which are easily recognizable.

For the shallow waters of the North Sea (< 100 m), the common dab (*Limanda limanda*) fulfills these criteria. For estuaries and coastal regions as well as the Baltic Sea, the flounder (*Platichthys flesus*) is suitable. The cod (*Gadus morhua*) was chosen as an additional species for disease monitoring. *However, this species is, at present, rare in the North Sea and therefore can only be recommended for spatial and temporal fish disease monitoring in the western Baltic Sea where it is more abundant. If disease monitoring in the North Sea includes studies on prevalences and intensities of parasitic infestation, the whiting (Merlangius merlangus) may be suitable due to its availability and the presence of conspicuous externally visible parasites.*

2 Sampling Strategy

2.1 Sampling gear

Sampling on a long-term basis should preferably be conducted using identical equipment (ship, gear, etc.) to minimize sampling variability. *The use of identical fishing gears is also recommended since the type of gear used may influence the prevalence of diseases in a sample due to selective catching because of differences in the behaviour and catchability of diseased and healthy fish. For the sampling of demersal fish in the open North Sea, the GOV-trawl (equipped with codend, mesh size 50 mm) is recommended, since it is a standard gear also used for internationally coordinated stock assessment programmes.*

2.2 Sampling sites

Selection of sampling sites should take into account fish species availability, disease occurrence, and knowledge of contaminant levels and fish stock movements (migration). In addition, information on size and age distribution in the populations of the target fish species should be available (age/length keys). Sampling sites in areas that mix different stocks of a particular species should be avoided, since these stocks may differ in ther genetic and behavioural characteristics and their susceptibility to environmental stressors. Changes in the proportion of each stock represented in the samples may, thus, affect the disease prevalence (Lang *et al.*, 1995).

Sampling should be accurately positioned on a nominated latitude and longitude with all repeat hauls being within clearly defined limits, e.g., within a radius of 2 to 4 nautical miles. Sampling on a station should be based on multiple hauls, even in the presence of large numbers of fish. This is necessary to reduce sampling variation, i.e., haul-to-haul variation, the problem of patchiness, etc. Therefore, at least two hauls, but preferably five hauls per station should be aimed for.

2.3 Sampling frequency and season

Sampling should be conducted on a long-term basis, once a year within the same narrow time window (two weeks to one month) or, if possible, at two periods to provide separate data for summer and winter. The non-spawning period is recommended, since spawning may be associated with considerable migration of the fish between their feeding and spawning grounds (for example, for North Sea dab (*Limanda limanda)* see Damm *et al.*, 1991; Rijnsdorp *et al.*, 1992). Sampling during spawning time may, thus, not reflect the spatial distribution patterns of the diseases typical for most of the year. *Furthermore, sampling during the spawning period should be avoided, since the adverse effects of spawning stress on the health status may exceed the effects of any other environmental stressors, the effects of which are to be monitored (Lang et al., 1995).*

2.4 Sample size and statistical analysis of fish disease prevalence data

The minimum sample size of fish to be examined for diseases should be based on the statistical requirements of the specific monitoring programme and might differ for spatial and temporal monitoring.

According to ICES guidelines, it is recommended that 250 specimens per haul be examined which allows the detection of a disease prevalence of at least 1.5 % with 9 % confidence limits. These specimens should be sorted out from total catches or subsamples and should be catagorized according to three size groupings, as given in the following table.

Species	Size Grouping	Number of Specimens
Dab *Limanda limanda*	15–19 cm 20–24 cm ≥ 25 cm	100 100 50
Flounder *Platichthys flesus*	20–24 cm 25–29 cm ≥ 30 cm	100 100 50
Cod *Gadus morhua*	< 29 cm 30–44 cm ≥ 45 cm	100 100 50

However, in many cases it will not be possible to sample a sufficient number of specimens per size grouping, either due to low catches or to a particular length-frequency distribution with small or large fish dominating. Additionally, due to size stratification, the prevalences recorded do not necessarily represent the prevalence in the population and, therefore, data have to be interpolated.

Another disadvantage of examinations based only on length stratification is that the age of the fish, which may have a profound effect on the presence or absence of disease, is not taken into account. Since the growth patterns of fish may vary considerably between sampling sites it might be that fish of the same size from different sites differ significantly in age and, therefore, the probability for the occurrence of a disease may be different. This may possibly lead to misinterpretation of disease prevalence data. For this reason, information on the age structure of fish species monitored is essential for the assessment of results on spatial trends of disease prevalences.
It is felt that, for spatial and temporal monitoring purposes, the ICES guidelines for sample design and sample size need to be reviewed and, if necessary, revised. This task should be addressed by the ICES Sub-Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks.

3 Diseases

3.1 Disease examination procedures

Disease examination for spatial and temporal monitoring purposes should be carried out by trained experts following a strict protocol (see below). If new staff have to be trained, this should be done only by experts and results should be intercalibrated internally.

After each haul, the fish species to be examined should be sorted, either from the total catch or from representative subsamples. The sample weight should be measured and the length-frequency distribution should be recorded (total length rounded to the nearest cm below the measurement), separately for females and males. Measured fish should either be completely examined for diseases, or be sorted according to length into different size categories prior to examination.

The fish species selected for examination should be examined whilst fresh, i.e., shortly after they have been landed on the ship or taken from nets (not frozen or refrigerated). An area for working should be cleared, preferably a bench or table at standing height, with good lighting and running water.

At least two people are needed for examining a large number of fish. One person makes the examination and the other records (in pencil) the data onto special paper forms (to be developed) or directly onto a computer keyboard if direct data entry programs are used. The positions should be interchangeable, so that both workers know how to take the measurements and how to transcribe the data. The following procedures should be used:

1) Take the fish, with bare hands (or wearing thin gloves), rinse it in clean water and, under a good light, examine it externally and, whether or not an anomaly is observed, determine the total length and sex of the fish. Externally visible diseases (including those affecting the gills) quantified for monitoring purposes should be recorded according to the guidelines on the type and severity of diseases provided below.

2) For internal examination of flatfish for liver nodules, place the fish underside downwards, make an incision on the upper side with a sharp blade from the pectoral fin to the outer edge of the abdominal cavity. With a finger, pull out the intestine, and the liver will be clearly visible. Carefully dissect with a blade around any adhesions and the liver will come free. Examine the liver on both sides. Any nodules > 2 mm in diameter should be recorded. It is advised that all nodules should be examined histologically in order to confirm macroscopic findings. For confirmation of liver nodules, the affected area including some normal, adjacent tissue should be carefully dissected (up to 5 mm thick pieces only) and placed in a jar of 10 % neutral buffered formalin or Bouin's fluid for preservation and subsequent histological examination.

Note 1: It is advised that the samples from the first haul are considered a practice run, and the results not counted on the final reporting form. This should sort out any problems which may arise, especially for persons not working at sea on a regular basis. Possibly an intercalibration of this sample could be conducted if more than one person is to be involved in disease diagnosis.

Note 2: It is advisable that the examination of each trawl haul is completed before the next haul is landed. With hauls coming in close together, or hauls of short duration, timing is critical. Additionally, most research ships work to strict timing, including meal breaks, therefore, planning between the scientists and the crew is necessary to maintain working harmony.

3.2 Diseases useful for monitoring purposes

Diseases used for spatial and temporal monitoring should be ones that:

- occur commonly in the selected fish species;
- are easy to recognize;
- have a possible response to surrounding environmental conditions;
- have a response that can be expressed in significant prevalence values.

For the host species identified in Section 2.4, the following diseases and minimum requirements are recommended for monitoring purposes on an international level:

Host Species	Disease	Minimum requirement for international reporting
Dab *Limanda limanda*	Lymphocystis Epidermal hyperplasia/papilloma Skin ulcer disease X-cell gill lesion Liver nodules	More than one surface nodule Lesions larger than 2 mm Open lesions (including acute and healing stages) One or more filaments affected Larger than 2 mm in diameter
Flounder *Platichthys flesus*	Lymphocystis Skin ulcer disease Liver nodules Skeletal deformities*	More than one surface nodule Open lesions (including acute and healing stages Larger than 2 mm in diameter Grossly visible
Cod *Gadus morhua*	Skin ulcer disease Skeletal deformities Pseudobranchial swelling (X-cell disease) *Cryptocotyle lingua* *Lernaeocera branchialis**	Open lesions (including acute and healing stages Grossly visible or by filleting Grossly visible One or more cysts in the skin One or more parasites in the gill cavity

*not included in previous ICES recommendations

4 Reporting and statistical analysis of disease data

Fish disease prevalence data obtained according to the ICES guidelines should be submitted to ICES on an annual basis using the ICES Reporting Format for Fish Disease Data (Version 2.2) which has recently been revised. The fish disease data are part of the ICES Environmental Databank which, at present, contains data on contaminants in sea water, sediments and biota, certain biological effects measurements, and fish disease prevalences. The structure of the environmental database facilitates compatibility of data and, therefore, joint statistical analysis.

For the submission of fish disease prevalence data, ICES provides on request the new ICES Fish Disease Data Entry Program (FDE 2.0) which allows the transformation of data into the ICES Reporting Format for Fish Disease Data.

Evaluation and statistical analysis of fish disease data submitted to the ICES Environmental Databank are carried out by the ICES Sub-Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks. For monitoring purposes, the Sub-Group should provide guidelines on appropriate methods of statistical analysis of spatial and temporal disease prevalence data as well as on factors with potential impact on disease prevalences which should be included in the data analysis (e.g., age, length, sex, etc.).

B Nomination of Lead Laboratories

There are a number of laboratories in the European ICES area with long-term experience in monitoring diseases and parasites in wild marine fish using methodologies elaborated by ICES. For the North Sea, these include:

- Federal Research Centre for Fisheries, Institute of Fish Ecology, Cuxhaven, Germany
- MAFF Fish Disease Laboratory, Weymouth, UK
- SOAEFD Marine Laboratory, Aberdeen, UK
- Danish Institute for Fisheries Research, Fish Disease Laboratory, Frederiksberg, Denmark
- National Institute for Coastal and Marine Management, Middelburg, The Netherlands

If possible, these institutes should be involved in disease monitoring programmes and in the event that international QA programmes are established.

C Interlaboratory Performance Assessment

Interlaboratory performance assessments of diagnoses of externally visible fish diseases have been carried out during several sea-going workshops (North Sea 1984, 1990; Kattegat 1988; Baltic Sea 1991, 1994) organized by ICES and other co-sponsors (IOC, BMB). Therefore, a new assessment would only be necessary if institutes which had not been involved in earlier activities will be involved in future monitoring programmes.

The diagnosis of pathological liver changes will be intercalibrated among laboratories during the ICES *Ad Hoc* Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants in October 1996.

D Conclusions

Except for liver pathology, methodologies used for fish disease surveys in the OSPAR Area are approved and have been intercalibrated among participating laboratories on a regular basis. Data derived from fish disease studies have already been reported to the ICES Environmental Databank for a considerable time using standardized reporting formats. Standardized methods for analysis of disease prevalence data are being developed by the ICES Sub-Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks.

Therefore, fish disease studies that follow the guidelines mentioned above are ready for application within the OSPAR Joint Assessment and Monitoring Programme (JAMP).

Literature cited

ICES. 1989. Methodology of fish disease studies—report of a sea-going workshop held on U/F "Argos" 16–23 April 1988. ICES Cooperative Research Report, No. 166. 43 pp.

Bucke, D., Vethaak, A.D., Lang, T., and Mellergaard, S. 1996. Common diseases and parasites of fish in the North Atlantic: Training guide for identification. ICES Techniques in Marine Environmental Sciences, No. 19.

Damm, U., Lang, T., and Rijnsdorp, A.D. 1991. Movements of dab (*Limanda limanda* L.) in the German Bight and Southern Bight—results of German and Dutch tagging experiments in 1988, 1989. ICES CM 1991/E:22. 18 pp.

Dethlefsen, V., Egidius, E., and McVicar, A.H. (Eds.) 1986. Methodology of fish disease surveys—Report of a sea-going workshop held on RV "Anton Dohrn" 3–12 January 1984. ICES Cooperative Research Report, No. 140. 33 pp.

Lang, T., Mellergaard, S., Bezgachina, T., Bogovski, S., Grygiel, W., Kadakas, V., Koie, M., Nagel, G., Neumann, K., Paukste, A., Tabolina, I., and Wiklund, T. 1995. BMB/ICES Sea-going Workshop "Fish Diseases and Parasites in the Baltic Sea"—a preliminary report. ICES CM 1995/F:11. 14 pp.

Rijnsdorp, A.D., Vethaak, A.D., and Leeuwen, P.I. 1992. Population biology of dab *Limanda limanda* in the southeastern North Sea. Marine Ecology Progress Series, 91: 19–35.

Vethaak, A.D., Bucke, D., Lang, T., Wester, P.W., Jol, J., and Carr, M. 1992. Fish disease monitoring along a pollution transect: a case study using dab *Limanda limanda* in the German Bight. Marine Ecology Progress Series, 91: 173–192.

ANNEX 7

FRAMEWORK FOR AN ENVIRONMENTAL QUALITY ASSESSMENT SCHEME FOR PAH IN MARINE SOILS

EQAMAS

Introduction

Two important objectives in the Enviroment and Climate Programme are the development of an improved ecological science base and practical methodologies in support of the current and envisaged EU Environmental policy regarding the hazard, risk assessment, management of chemicals, integrated environmental quality and functional assessment.
The research tasks that are needed to achieve these goals are :

- improvement of exposure assessment methods for hazards and risks to the environment from chemicals, such as PAHs, including the development of methods for prediction of impacts.
- Development of suitable effects assessment methodologies, including alternative approaches to the use of animals in testing, and considering ecological principles in an appropriate manner. In addition, improvement of the science base and holistic assessment approaches for delineating integrated ecological quality criteria for sediments and soils.

Objectives

The objectives for the proposed project can be divided into 2 main actions:

1. Identification of the magnitude of the problem
    - estimate the loading of selected areas by point sources such as oil drilling rigs and land based sources) by PAHs
    - quantify the partitioning and speciation of PAHs in sediments, water and suspended particulate matter
    - quantify the transfer of PAHs between environment and biota
    - quantify the biological impact from PAHs in sediments and soils of both pyrogenic and petrogenic origin on benthic organisms by characterising a sequence of linked processes and specific monitoring targets, including targets of early detection of toxicity and targets of deleterious effects on populations and ecosystem.
    - evaluate the state of the selected areas
2. Solving the problem
    - quantify the degree of elimination by biodegradation, photodegradation? and dilution of the PAHs
    - estimate the use of the gained knowledge in a broader context (application to other areas, other pollution types, other?)
    - make recommendations for managerial purposes

Approach

The project is an initiative of the scientists who regularly participate in the Working Group meetings on Marine Sediments in Relation to Pollution and Biological Effects of Contaminants of the International Council for the Exploration of the Sea (ICES) and resulted from discussions on reports of assessment approaches on the availability and effects of contaminants, specifically PAHs, in soils and sediments. Restrictions and problems of the currently used assessment approaches have been clearly defined and form the basis of this proposal.

To fill in the gaps that are left open by the current approaches the project is designed to provide an environmental quality assessment scheme to estimate the impact of PAH sources that is based on the

interrelationships between PAH speciation in the water column, sediment and suspended particulate matter, bioaccumulation of PAHs, responses to PAH exposure in benthic organisms and elimination routes. The scheme will yield quantitative information concerning the nature of PAHs in soil/sediment, as well as bioavailability, bioaccumulation, toxicity, elimination and sublethal biological effects of PAHs associated with soils or sediments.

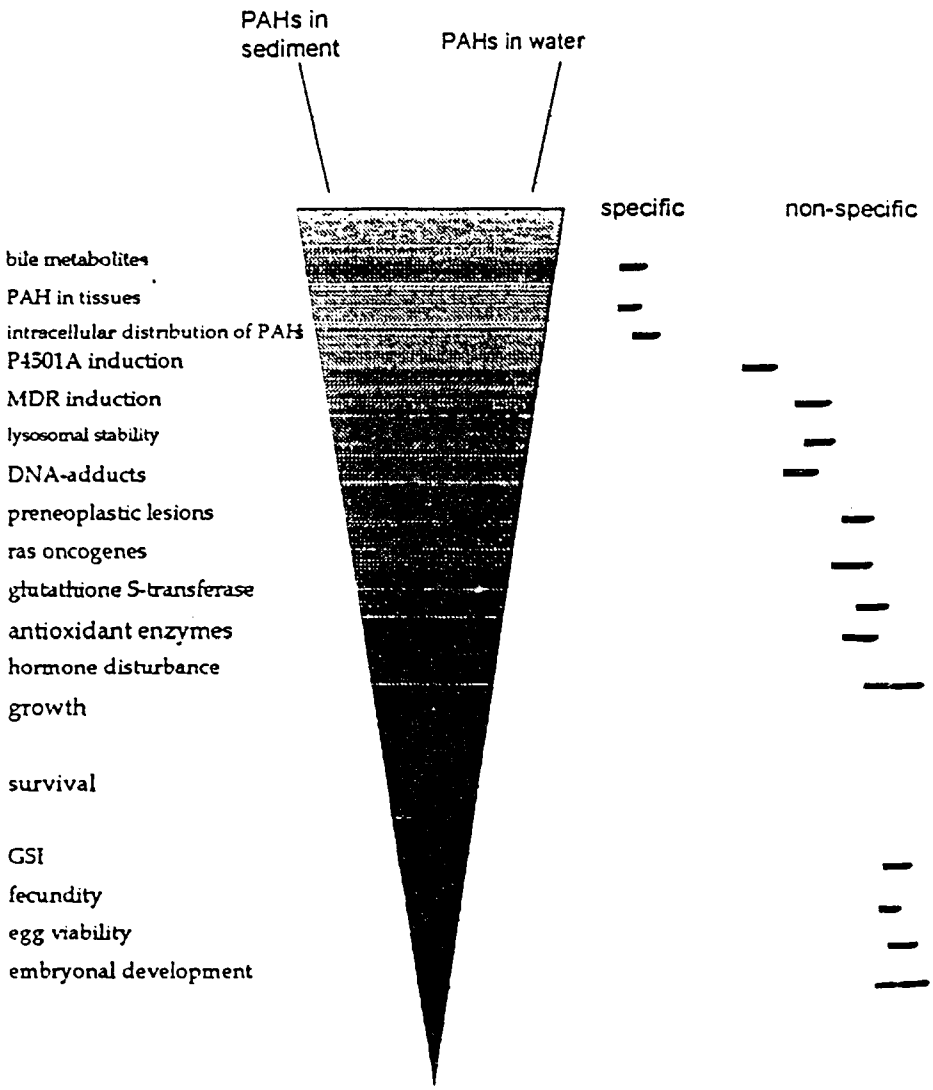

Figure 1. The relationship between techniques to characterise biological impact of PAHs, their specificity and relevance for effects on population level.

description	comment
characteristics of inputs	PAH-profile, particle-size distribution
concentrations, characteristics sediments	PAH-concentration, profile, speciation
concentrations in water column, pore water	PAH-concentration, profile
concentrations in suspended particulate matter	benthic fish
	filter feeder (Mytilus)
	sediment-dwelling/-feeding
	novel animal model
intracellular distribution of PAHs	
cytochrome P450 (fish)	
MDR (invertebrate)	
lysosomal stability	
DNA-adducts (HPLC)	
glutathione S-transferase	
antioxidant enzymes	
ras oncogenes	
bile metabolites (fish)	
p53 ?	
DNA strand breaks	
histopathology	
hormone effects	
vit A	
GSI	
egg viability	
chromosome aberrations	
fecundity	
bioassays	
benthic community structure (meio, macro)	

ANNEX 8

INTERSESSIONAL ACTIVITIES

Chairman: to approach the EU to investigate setting up a QA programme for biological effects.

Ian Davies: to prepare a COST proposal.

Angela Köhler: to attend the Workshop on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants.

Chairman: to solicit the following work for next year's meeting:

- A review on immunological responses to contaminants from Professor C.J. Bayne (Corvallis, USA) and Professor M. Moore (Plymouth, UK).
- A review on reproductive effects should be invited from Dr H. von Westernhagen (Action Dr V. Dethlefsen, Cuxhaven, Germany).
- A review on the interaction between biomarker responses in sensitive species and community responses from John Gray.

Peter Matthiessen (TIMES editor): to chase authors of TIMES publications as follows:

- Reichert, French and Stein - DNA adducts by ^{32}P-postlabelling
- Thain and Bifield - Sediment bioassay with *Arenicola marina*
- Roddie and Thain - Sediment bioassay with *Corophium volutator*
- Moore and Köhler - Lysosomal stability
- Köhler - Fish liver histopathology
- Hylland - Metallothionein

and to commission new papers in the following areas:

- A protocol for sediment sampling, storage and the preparation of a pore water extraction method.
- The use of the viviparous blenny for monitoring the effects of contaminants on reproduction (author?).
- Acute lethal toxicity to sticklebacks (only if requirements differ significantly from OECD Guideline 204 - to be checked by Anne-Marie).
- Antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, malone dialdehyde) (Livingston or DiGiulioi?).

ANNEX 9

RECOMMENDATIONS

To Council

The WGBEC recommends that a meeting of WGBEC should be held for four days in Copenhagen in 1997 to address terms of reference to be defined at the 1996 Statutory Meeting but to include the following:

a) In conjunction with WGSAEM, to consider the statistical techniques applicable to biological effects data particularly in the context of using multiple measures collectively to assess health status;

b) To develop assessment tools and procedures for the interpretation of biological effects monitoring programmes;

c) To seek ways in which cellular energy allocation could be compared with scope for growth in order to assess its utility for field application and whether it can provide a simpler alternative to SFG measurements;

d) To stimulate activity in the area of reproductive effects in the marine environment, particularly as regards intercomparison and method development for assays of vitellogenin in fish. The use of simple *in vitro* oestrogen screens based on genetically engineered organisms and research should be encouraged into the possible effects of environmental oestrogens on invertebrates such as crustacea, and particularly on filter feeders such as mussels which are known to bioaccumulate compounds of oestrogenic interest and have the ability to respond to true oestrogens;

e) In conjunction with WGMS, to consider the development of new directions for investigating the links between sediment chemistry and biological processes.

A joint meeting of the WGBEC and the WGMS should take place in 1998; the terms of reference for this joint meeting should be formulated at an informal meeting between WGBEC and WGMS in 1997.

To ACME

The WGBEC recommends that:

1) In the context of international monitoring programmes such as that being developed by OSPAR under JAMP, the following advice be considered:

   a) The strategy for a general biological effects monitoring programme outlined in Section 7.1 of this report should be forwarded for consideration by MON 1996 as a basis for the development of a general biological effects monitoring programme;

   b) The QA programme for biological effects monitoring developed in Section 7 of this report should be forwarded for consideration by MON 1996 as an essential component of the guidelines for biological effects monitoring under JAMP;

   c) The interpretation of the significance of biological effects measurements requires a holistic approach encompassing multiple measurements and it must be emphasized that biological effects techniques are not methods that will yield single numbers that may be compared to some criterion or level.

2) WGSAEM in conjunction with members of WGBEC should be requested to consider the statistical techniques applicable to biological effects data, particularly in the context of using multiple measures collectively to assess health status.

3) MCWG should be asked to consider 'What are the levels of contaminants at fronts and along the shelf edge and what are the underlying processes responsible for these levels?'.

4) If the Commissions wish to identify the extent to which PCBs in marine mammals generate effects, then the following actions need to be facilitated:

- efforts should be made to make material available from comparable populations exposed to different levels of contaminants (e.g., seals in the Baltic Sea compared to seals in Canada or the Barents Sea);
- experimental work with sea mammals should be encouraged, preferably utilizing non-destructive techniques (e.g., blood samples);
- future studies should include histopathological methods.

To ACME and the ICES Secretariat

Two requests have come to the Working Group for advice on the effects of contaminants on ecosystems or populations (terms of reference (c); North Sea Conference population effects of contaminants). The Working Group has spent many years developing biological effects programmes which indicate effects at the individual level and sometimes at the community level or organization. These measures will indicate the health status of individuals within a population but cannot be used to predict specific population changes and do not indicate the degree of detriment. The WGBEC recommends that ACME and the Secretariat only accept deliverable requests and that they begin a process of educating environmental managers in the sorts of questions that are answerable.

To the ICES Secretariat

To proceed with the publication of the following leaflets in the TIMES series:

AChE
Imposex
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