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REPORT OF THE

WORKING GROUP ON BIOLOGICAL EFFECTS OF CONTAMINANTS

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1 OPENING OF THE MEETING

The meeting of the Working Group on Biological Effects of Contaminants (WGBEC) was opened at 09.15 hrs on Monday 12 April 1999. WGBEC members (see Annex 1) were welcomed by Dr Dik Tromp, the Director of RIKZ, who wished them well in their deliberations. Dr Peter Matthiessen, Chair of WGBEC, thanked Dr Tromp for the hospitality and support of RIKZ.

2 ADOPTION OF THE AGENDA

The Agenda (see Annex 2) was adopted unchanged, although a few items were added under 'Any Other Business'.

3 APPOINTMENT OF RAPPORTEURS

It was agreed that the job of recording WGBEC sessions would be split up fairly among the participants.

4 CONSIDERATION OF POSSIBLE ARTIFICIAL INTELLIGENCE SYSTEMS FOR EVALUATION OF MONITORING DATA

Sean Nicholson (UK) briefed the full WGBEC on progress made on this subject at the preceding joint meeting of WGBEC and WGSAEM. The joint meeting of 1998 had identified the possible use of expert systems to help manage the large and complex range of data currently being gathered in monitoring programmes. The joint 1999 WGBEC/WGSAEM meeting in the week previous to the WGBEC 1999 meeting discussed these issues, taking in artificial intelligence (AI) systems in general. The draft section of the report from this joint meeting was handed out to WGBEC attendees. People were taken through the report, highlighting the options, methods and conclusions.

Expert systems (ES) work from a knowledge base of rules and facts. They have the ability to prove a hypothesis or predict possible outcomes using an inferencing engine. Such an ES requires people who are experts in order to build them. No data are required to build them. The expert may have deep knowledge, such that they cannot explain their expertise. In such circumstances a knowledge engineer can use techniques to help elucidate the expert's knowledge. It was felt that our current understanding of the mechanisms of action of contaminants was not sufficient to allow the building of such an expert system. Once our knowledge of these mechanisms has improved the issue should be revisited.

Neural networks provide the ability to model an output function given a series of inputs. They require large data sets (thousands) of examples to train them. Examples of inputs could be PAH concentrations in sediments, EROD levels, site temperature, etc. Number of liver neoplasms, for example, could be an output variable. If such a network was successfully trained, it could work operationally but would be unable to explain how it reached its conclusion. This was viewed as a major drawback.

A fuzzy logic-based model trained by a neural network is referred to as a NeuroFuzzy system. Fuzzy logic is the use of fuzzy sets to define membership values for a statement. Thus for example, the statement 'John is tall' might have a 0.75 membership of the 'tall' fuzzy set, given a certain height. Using Boolean logic gives a crisp cut-off point, e.g., he is either tall or not tall (Zadeh, 1965). These sets may then be logically combined (e.g., with an 'AND'), resulting in new fuzzy sets that may finally be reduced down to a crisp output value.

The fuzzy logic-based model uses fuzzy sets and rules to take a series of input variables and give an output variable(s). A data set is used to train the model in what confidence levels to use on individual rules. Thus a rule such as 'If PAH concentration in sediment is High and EROD is High then the occurrence of liver neoplasms will be Medium' might have a confidence level of 45 % after training. Rules can be generated by experts, statistical methods, e.g., classification trees, or just randomly.

Such neurofuzzy systems have the benefit of being able to learn where knowledge is poor, but can give insight into how conclusions are reached. There are a range of such techniques that can combine an explanatory model with the ability to learn from data. Such techniques are already used in industry and medicine, e.g., for stock market prediction, credit judgement, disease diagnosis. Three possible uses of such systems were suggested at the Joint Meeting.

- A reduced number of monitoring variables could be used to identify which sites may be most at risk. This would help make efficient use of resources. Secondly, it could indicate which further tests would be most useful to help clarify the future work required, e.g., where to sample and what measurements to take.

- The ability to use measurements that may give an early warning for effects that take years to appear. Thus after a contaminant spill some indicator of what level of future problems is to be expected would be useful. Getting training data for such a system might be a problem. If smaller data sets such as from mesocosm studies were combined with increases in our knowledge, then such systems would have greater chances of success.

- An indicator of ecosystem 'health' may be possible. By combining a variety of such systems, higher level judgements concerning populations and ecosystems would be possible. This requires some consensus from the experts on a definition of ecosystem health.

The ability to take a neurofuzzy system trained up for one scenario and use it as a framework for another scenario was discussed, e.g., would a system for predicting liver neoplasms in Puget Sound flatfish require fewer data/resources to get it working for North Sea flatfish. Whilst it may be possible, our current poor understanding of such systems made any sort of confidence in this low.

The Joint WGBEC/WGSAEM meeting proposed a joint workshop with PICES and SETAC in 2001 to evaluate the utility of artificial intelligence for the assessment of complex biological effects monitoring data sets. Further details are available in the 1999 WGBEC/WGSAEM joint meeting report (ICES, 1999).

WGBEC agreed with this proposal. Various members of the WGBEC made references to colleagues in the field of medicine and freshwater ecotoxicology/bioassay who already had experience in utilising such AI techniques. It was suggested that such people be invited to part of the next WGBEC meeting in 2000 to share experience and provide advice. Angela Köhler (Germany) suggested Gunther Valet from Munich and Pascal Cheieco from Bologna. Angela Köhler also mentioned a large data set (approx. 10,000) held by the Bundesanstalt für Schiffahrt und Hydrographie (BSH) in Germany that may be useful. Angela Köhler, Peter Matthiessen (UK), and Rob Fryer (UK) will follow up this lead. Some intersessional work on possible workshop data sets was also agreed to be vital as an indicator of possible success of the workshop.

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5 DEVELOPMENT OF SUITES OF BIOLOGICAL MONITORING METHODS FOR USE IN BRACKISH WATER

Brackish water systems, where salinity levels fluctuate, include tidal zones, estuaries and waters of more constant low salinity. At the last WGBEC meeting in Mont-Joli, Canada, it was suggested that the biological effects may be more severe under these conditions than in purely marine systems. The resultant toxicity is not solely related to changes in the bioavailability of a contaminant, but is due (at least in part) to the greater physiological stress experienced by organisms living in zones of fluctuating salinity.

Dick Vethaak presented a thesis by Wies Vonck (formerly of the RIKZ, NL), entitled 'Effects of estuarine conditions on cadmium toxicity and osmoregulatory performance in fish'. Laboratory experiments were conducted involving exposures of flounder (*Platichthys flesus*) to Cd under conditions of high (sea water), low (fresh water), and fluctuating salinities. Flounder are good models for osmoregulatory and toxicological studies in an estuarine environment and efficient osmo- and ionoregulators, which can easily adapt to different and fluctuating water salinities. Important findings were:

1) cadmium disrupts the hydromineral regulation in flounder—a Cd dose-dependent increase of the opercular chloride cell density was observed in freshwater-exposed fish, but not in seawater-exposed fish;

2) the effects of (environmentally realistic concentrations of) Cd administered intragastrically are influenced by the ambient water salinity. Intragastric Cd exposure hardly influences the chloride cell density in seawater-exposed fish. However when salinity was reduced from sea water to fresh water within 42 hours after administering cadmium in sea water, cell density of chloride cells increased by a factor of 2.6. Therefore Cd seems to be considerably more toxic to flounder under conditions of fluctuating salinity, a new finding for fish. The thesis also recommended that opercular cell density of flounder is a sensitive parameter for environmental factors such as

salinity changes and cadmium exposure. The chloride cell density therefore could be a useful biomarker for environmental monitoring.

Several participants mentioned the problem of extrapolating results of biomarker/bioassay studies from marine environments to low-salinity environments such as the Baltic Sea.

John Thain (UK) mentioned that the CEFAS laboratory makes use of species that tolerate low salinities, such as *Leptocheirus* (for sediments), in bioassays when testing samples from (low salinity) estuaries. Other species may be suitable for testing in low salinities (e.g., *Nitocra*) as suggested in the relevant ISO protocol (Dave *et al.*, 1993; ISO/CD 14669 96-01; ASTM, 1988, 1989).

Robert Roy (Canada) noted that in fresh water, laboratory studies have indicated that fluctuating concentrations of metals (Cu, Al) are more toxic than what would be suggested by calculating the average exposure concentration (Al: Mount *et al.*, 1990; Cleveland *et al.*, 1991; Cu: Seim *et al.*, 1984). Models of fluctuating exposure conditions have been described for Al toxicity (DeWalle *et al.*, 1995). These models, or similar approaches, may be useful for experiments involving fluctuations in salinity.

WGBEC suggested that the responses of biomarkers and bioassay organisms to contaminants in fluctuating or low salinity conditions should be investigated. However, the experiments must be designed with care to control for changes in the bioavailable fraction of a contaminant (be it the free ion of a metal or the dissolved phase of an organic contaminant) during changing conditions of salinity.

The above findings are of great importance for the use of fish biomarkers in monitoring biological effects in low-salinity, estuarine or specific salinity-stressed environments (e.g., outlets of freshwater discharges in the marine environment). They clearly illustrate that the sensitivity of aquatic organisms to contaminants is closely related to the disruption of water and ion regulation in these animals. It is therefore recommended that each of the biomarkers used in fish or invertebrate species should be validated for the influence of changing salinity, so that, if necessary, correction factors can be derived to account for this effect.

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6 EVALUATION OF THE USE OF BIOMARKERS AND BIOASSAYS IN THE RISK ASSESSMENT OF CHEMICALS IN EFFLUENTS AND RECEIVING WATERS

John Thain (UK) introduced this item by explaining the developments of Direct Toxicity Assessment (DTA) in the UK. Historically, the risk assessment of chemicals in effluents and receiving waters has been based on chemical analysis schemes for marine and fresh waters and biological assessment schemes for fresh waters based on benthic macroinvertebrate surveys. The purpose of DTA is to introduce ecotoxicological assessment of whole effluent samples to help control and monitor the release of toxic wastes. The specific aims of whole-sample toxicity-based control is to reduce acute lethal aquatic toxicity from point source discharges, and ensure acceptance of toxicity-based criteria as part of effluent control procedures. In addition, it is important to ensure that stakeholders (regulators, regulated, public, etc.) become familiar with the methodology, terminology and protocols, and to ensure wide acceptance of any future strategy by regulators, industry, academia and commercial testing laboratories.

A demonstration programme is currently under way in the UK to validate the monitoring and control methods; this is being carried out on two freshwater catchments, the Aire and Esk, and two estuaries, the Tees and Spey. The main areas of focus have been the selection and development of methods; the associated quality assurance (QA) and quality control (QC) procedures; data/information reporting procedures; use of DTA for receiving water risk assessment and effluent control; development of a laboratory approval scheme (Register of Approved Laboratories (RAL)); and review of method sensitivity and relevance in terms of predicting ecological impacts.

The table below shows methods used in the UK programme:

Trophic Level	Fresh water	Marine/estuarine
1 - Algae	*Selenastrum* sp. *Scenedesmus* sp.	*Skeletonema* sp. *Phaedactylum* sp.
2 - Invertebrates	*Daphnia magna*	Oyster embryo bioassay
3 - Fish	Rainbow trout Carp	Turbot Plaice

Other methods associated with the programme include Microtox, innovative rapid methods (e.g., ECLOX, AQUANOX), *Gammarus in situ* and Toxicity Identification Evaluation (TIE) and Toxicity Reduction Evaluation (TRE) procedures. Work has progressed well on the Esk and Tees. There have been differences in sensitivity of the tests and variability in discrete sampling of water and effluent. Currently no biomarkers are used.

Apart from the UK experience it appears that bioassays and biomarkers are not used in other countries in Europe to evaluate the toxicity of effluents entering the marine environment. In the Netherlands and Germany, direct toxicity assessment schemes are used widely for freshwaters (UBA, 1994; LAWA, 1998). At a three-day SETAC-Europe conference on direct toxicity assessment of effluents held in Edinburgh in March 1999, there were no presentations (apart from the UK) on, or examples of, 'marine' testing of effluents.

There is considerable experience in the USA in the use of marine toxicity tests for the testing of effluents. Protocols for several tests have been described (e.g., ASTM, 1988; Weber *et al.*, 1994) and two US EPA marine tests are used in Canada (see below).

6.1 Summary of Canadian Experience with Effluent Bioassays

In Canada (as described by Robert Roy), federal regulations cover discharges of several important sectors, including pulp and paper mills, mines and petroleum refineries. In general, regulations governing acute toxicity testing only specify tests involving freshwater organisms (rainbow trout, *Daphnia magna*). Toxicity tests are also required under Environment Effects Monitoring (EEM) programmes, which are designed to measure effects of 'chronic' exposures. The

toxicity tests recommended under EEM involve a fish, an invertebrate, and a 'plant' (usually an alga) species. The marine EEM bioassays include the following tests:

- reproduction of the macroalga *Champia parvula*;
- growth and survival of the inland silverside *Menidia beryllina*;
- reproduction of an echinoid (sea urchin or sand dollar).

The fish and algal tests, which involve southern temperate Atlantic species, are conducted according to the US EPA protocol (Weber *et al.*, 1994). The echinoderm protocol is an Environment Canada test method (Environment Canada, 1992) and is usually conducted with sea urchins (temperate Atlantic or Pacific species). In previous EEM studies, difficulties were experienced with some of the tests (invalid tests due to insufficient responses in the controls), in particular, assays involving *Champia parvula*.

EEM studies must be conducted every few years, depending on the industry. The pulp and paper sector is in the midst of a second EEM cycle. There are approximately 25 mills that discharge into marine environments. Therefore, most of the Canadian experience with marine toxicity testing involves samples of pulp and paper mill effluents. The present regulations specify that two toxicity tests per year be conducted (for a total of twelve during the six-year programme). Effluent samples, in particular discharges from pulp mills, are highly variable. This variability, coupled with the frequency of testing, can result in difficulties in interpreting the toxicity test results. However, with the installation of secondary treatment systems at nearly all Canadian pulp and paper mills, the quality of pulp and paper effluents has generally improved.

6.2 Salinity Adjustment of 'Marine' Effluent Samples

There was some discussion on the use of marine tests on freshwater effluents. This involves adjusting the salinity of the effluent prior to testing. However there are problems with this approach insofar that the addition of salt can change the toxicity of the effluent and there are difficulties in reconstituting sea water (i.e., how this should be carried out and the degree of ageing required; refer to ASTM, 1998).

In Canada, the salinity of effluent samples is adjusted to 30 ± 2 ppt prior to testing. A recent Environment Canada guideline (Environment Canada, 1997) specifies that a concentrated natural brine solution (90 ppt) must be used for the adjustment of salinity. Thus the highest effluent concentration that can be tested is on average 66 %.

6.3 Sample Variability

It was noted also that there are difficulties with sample collection in respect of the temporal heterogeneity of the effluent; this may be overcome by continuous or integrated effluent sampling regimes. In Canada, pulp and paper effluent samples are collected either as an instantaneous grab or as a 24-hour composite. The regulations permit a delay of 72 hours from sample collection to test initiation.

Clearly there has been relatively little experience with testing end-of-pipe effluents in a marine context. It was recognised that for risk assessment purposes it may be more appropriate to deploy test procedures (both bioassay and biomarker) in the receiving water. Such testing would permit the deployment of chronic and *in situ* techniques. This subject area is in its infancy and has particular relevance not only for effluent discharges into estuarine and coastal areas but also for the North Sea offshore chemical industry. There is a need to develop specific *in situ* techniques for this purpose and there have already been some initiatives in this area.

6.4 Caged Fish Studies

In the UK, adult flounder (*Platichthys flesus*) have been held successfully in 1.5 m × 0.5 m × 0.5 m metal cages for three weeks in effluent discharges and at several locations in estuaries. Flounder were fed in one trial and not fed in a second trial; this did not affect survival. However, in preliminary trials it was observed that the fish became very stressed in high tidal flows and in situations where the cage is not placed on soft mud such that the fish cannot 'bed' into the sediment (its natural habitat). Also, survival rates in small fish (< 20 cm) were poor. This study is using caged flounder to identify the presence and degree of oestrogenic activity in effluent discharges. Oestrogenic activity is measured in the blood plasma using a radioimmunoassay for flounder vitellogenin (Matthiessen *et al.*, 1998).

Bjørn Serigstad (Norway) reported that caged fish studies have been carried out successfully in Norwegian waters and gave a video presentation of the experimental system. The fish cages measure up to approximately 4 m high and 3 m in diameter and are constructed of an aluminium lantern covered in mesh of an appropriate size, and have been deployed at water depths of up to 350 m. Each fish cage has a light source that attracts zooplankton and small fish which act as food for the caged fish. By using this method, the fish are exposed to the water as well as to potential contamination entering the fish through the food chain in the particular area where the cage is placed. The experimental system has been tested with three different species (cod, saithe, and pollock). The fish have been kept in the cages for up to twelve months with no additional feeding, and mortality rates have been less than 5 %. Each cage is equipped with a camera, temperature and salinity sensors, and a current meter; the logged data may be transmitted by cable or a radio transmitter.

Three different monitoring projects have used cod with a size of approximately 1 kg.

i) A four-month caging experiment at the discharge site from the oil refinery at Mongstad near Bergen.

ii) An experiment monitoring the effluents from the Troll B oil production platform in the North Sea, with a sea depth of 330 m. Samples were taken and batteries for the light were changed every six weeks. Nets with blue mussels and boxes with SPMDs (semi-permeable membrane devices) filled with triolein were attached to the cages and sampled at the same intervals as the fish.

iii) The method has also been tested in different locations along a transect from the inner part of a fjord to a location about 45 miles offshore.

Other studies in Norway include the use of cod in net cages to monitor for the presence of oestrogenic substances and the use of caged flounder in fjords receiving industrial discharges (Beyer *et al.*, 1996). Ketil Hylland (Norway) reported a study using 5 m × 5 m × 1 m net cages with open contact to the sediment. In this study, five flounder were introduced into each cage (three at each of two sites) by divers. The flounder were also sampled using divers.

In the Netherlands, flounder have been used successfully in caging experiments at estuarine and offshore locations. Flounder were exposed for up to three weeks and biomarker analysis (plasma vitellogenin, PAH metabolites in bile) carried out. The cages, with a size of 2 m × 2 m × 2 m, were partly buried in the sediments and were constructed of a steel framework with nylon net. The fish received no additional food. Occasionally cages were lost due to strong currents or damaged by trawling and boating activities. The cages were installed and regularly checked by divers.

Overall, WGBEC concluded that the use of biomarkers and bioassays for the evaluation and risk assessment of marine discharges has a big future, and developments in this area were to be encouraged.

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7 REVIEW OF NEW METHODS IN MOLECULAR BIOLOGY WHICH COULD BE APPLIED TO MARINE MONITORING

An extract from the 1995 WGBEC report was presented by the Chair (WGBEC 1999/7.1). Ketil Hylland (Norway) presented a discussion paper (WGBEC 1999/7.2) on molecular methods in monitoring. There was some discussion within the group as to the definition of the term 'molecular methods', and subsequently, which methods should be included.

7.1 Definition and Selection of Methods

For the purposes of marine monitoring programmes, molecular methods are defined as, 'methods that use the detection and/or quantification of single biological molecules through binding of external reagents'. Such methods are used with RNA and DNA by employing primers or probes, proteins using antibodies, and any cellular component using specific dye probes, and in the case of bioassays, cell lines transfected with receptor and reporter genes.

7.2 General Observations

WGBEC noted that the same limitations are relevant to molecular methods as for other biological effect methods. Such limitations include the prerequisite that molecular methods should be used in concert, not singly. Similarly, it was emphasised that confounding factors must be taken into account. Considering the disadvantages indicated in 1995, WGBEC identified areas in which progress has been made and areas in which there has been little improvement over the past years.

Considerable progress has been made in the following areas:

- analysis of immunochemical data (image analysis tools);
- use of immunoassays (RIA, ELISA) in monitoring (e.g., MT, VTG);
- use and understanding of membrane transporters in fish (MXR, MDR).

Little progress of relevance to monitoring methods has been made in the following areas:

- the use of PCR-based techniques;
- the use of *in situ* hybridisation techniques.

WGBEC observed that the use of oncogene expression (mRNA, protein) as markers of carcinogenic potential had not developed as was expected, either due to methodological or biological reasons.

7.3 Organisms with Inserted Genes

In the last few years, a number of cell-lines and yeasts with inserted genes have been used in the detection and monitoring of contaminants. The most well-known examples include the oestrogen-sensitive yeast assay (YES) and the dioxin- and oestrogen-sensitive mammalian cell-lines (DR- and ER-CALUX, respectively).

These methods have been used for water samples and extracts from water, sediment and biota. Note was taken that a transgenic zebra fish has been produced in the Netherlands, incorporating a luciferase gene with an oestrogen-sensitive promoter (ref. 31). WGBEC considered that transgenic fish could prove a powerful model as they would integrate processes from whole organism down to the cellular level. Cellular methods are further discussed in Section 8.

7.4 Status of Existing Methods

The major methods currently available are listed in Table 7.4.1.

Table 7.4.1. Existing molecular methods that could have potential in marine monitoring.

Method	Biomarker	Status	Explanation	References
Immunoassay (RIA, ELISA, western blot)	CYP1A MT VTG	In use	quantification, identification (protein)	9 6, 10, 26 see Table 8.1
Immunoassay (RIA, ELISA, western blot)	zona radiata protein spiggin DNA adducts MDR/MXR *ras*, *myc* oncogenes	Research phase		1 - see Table 8.2 11 23
Immuno-cytochemistry	CYP1A MDR/MXR G6PDH GST-A	In use	quantification, verification (protein)	15 7, 15–16 15 15
Northern blot, slot-blot	CYP1A MT VTG MDR/MXR *ras*, *myc* oncogenes	Used for explaining modes of action	quantification (mRNA)	4, 9, 17 3, 13 19 8, 22 2, 21
Subtractive hybridisation	-	Research phase	identify sequence differences (DNA)	28
In situ hybridisation	-	Used for explaining modes of action	quantification, verification (mRNA)	5
Liquid hybridisation	-	Research phase	quantification (mRNA)	29
Competitive PCR	-	Research phase	quantification (mRNA)	27
Probes	lipofuschin intracellular Ca membrane transport	Used for explaining modes of action	quantification, verification	20 24 12, 18
Receptor binding	oestradiol androgen cortisol	Used for explaining modes of action	affinity	14 14 14

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7.5 Future Work

WGBEC recommended the inclusion of relevant molecular techniques in marine monitoring. There was an understanding that PCR-based (polymerase chain reaction) techniques could have some potential, especially for small amounts of tissue.

8 UPDATE OF THE LISTS OF RECOMMENDED AND PROMISING BIOLOGICAL EFFECTS MONITORING TECHNIQUES

WGBEC revisited the tables of biological effects monitoring techniques which it had updated last in 1997, and brought them up to date, adding about 50 new references. Some methods were 'promoted' to the revised list of techniques recommended for monitoring programmes (Table 8.1), and some new ones were added to the revised list of promising techniques requiring further research (Table 8.2). The criteria used for including methods in Table 8.1 remained the same as in 1997, i.e., they should produce a concentration- or dose-response, should be sensitive to contaminants, and should be repeatable and reproducible.

Table 8.1. Recommended techniques for biological monitoring programmes at the national or international level.

Method	Organism	Refs.	Currently used in monitoring programmes[‡]	Quality control	Issues addressed	Biological significance
Bulky DNA adduct formation	Fish[1] Bivalve molluscs	1–6, 157–159	F, NL, S, USA	B[2]	PAHs Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)	Measures genotoxic effects. Possible predictor of pathology through mechanistic links. Sensitive indicator of past and present exposure.
Acetyl-cholinesterase (AChE) inhibition *	Fish[1], crustacea, bivalve molluscs	12–16, 114, 116, 118	F		Organophosphates and carbamates or similar molecules Possibly algal toxins	Measures exposure.
Metallothionein induction	Fish[1]	17–22	MEDPOL, N	B[2]	Measures induction of metallothionein protein by certain metals (e.g., Zn, Cu, Cd, Hg)	Measures exposure and disturbance of copper and zinc metabolism.
Ethoxyresorufin-*o*-deethylase (EROD) or cytochrome P4501A induction*	Fish[1]	46–51, 99, 115	D, F, NL, UK, B, MEDPOL, N	B[2]	Measures induction of enzymes which detoxify planar organic contaminants (e.g., PAHs, planar PCBs, dioxins)	Possible predictor of pathology through mechanistic links. Sensitive indicator of present exposure.
δ-amino levulinic acid (ALA-D) inhibition	Fish[1]	74–75	N	B[2]	Lead	Index of exposure.
Oxidative stress indicators	Fish[1]	76–78			Not contaminant specific, will respond to a wide range of environmental contaminants	Measures the presence of free radicals.
Fluorescent bile metabolites	Fish	79–80	N, NL, UK		PAHs	Measures exposure to and metabolism of PAHs.
Lysosomal stability	Fish[1] *Mytilus* spp.	23–25	D, MEDPOL	B[2]	Not contaminant specific, but will respond to a wide variety of xenobiotic contaminants and metals	Measures cellular damage and is a good predictor of pathology. Provides a link between exposure and pathological endpoints. Possibly, a tool for immuno-suppression studies in white blood cells.
Early toxicopathic lesions, pre-neoplastic and neoplastic liver histopathology	Fish[1]	7–11, 108, 110, 119–130, 164–167	D, NL, UK, USA		PAHs Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)	Measures pathological changes associated with exposure to genotoxic and non-genotoxic carcinogens.

*Intercomparisons or quality control procedures complete for some methods (e.g., Refs. 31, 40, 99, 100).
[1] May also be applicable to mammals and birds.
[2]B=Quality control under BEQUALM.
[3]Q=Quality control under QUASIMEME.
[‡]B=Belgium; CAN=Canada; F=France; D=Germany; MEDPOL=Monitoring and Research Programme of the Mediterranean Action Plan; N=Norway; NL=Netherlands; S=Sweden

Table 8.1. Continued.

Method	Organism	Refs.	Currently used in monitoring programmes[‡]	Quality control	Issues addressed	Biological significance
Whole sediment bioassays*	• *Corophium* • *Echinocardium* • *Arenicola* • *Leptocheirus* • *Grandidierella* • *Rhepoxynius* • *Ampelisca*	31–35	NL, UK, USA, CAN	B[2]	Not contaminant specific, will respond to a wide range of environmental contaminants in sediments	Acute/lethal and acute/sublethal toxicity only at present. May enable retrospective interpretation of community changes.
Sediment pore water bioassays*	Any water column organism including: • *Dinophilus* • sea urchin • fertilization, etc. • bivalve embryo • Microtox	36–41	F, NL, USA		Will respond to a wide range of environmental contaminants	Acute and chronic toxicity, including genotoxicity, etc. Toxicity of hydrophobic contaminants might be underestimated in pore water assays.
Sediment sea water elutriates*	Any water column organism, as above	36–41	NL, UK		Will respond to a wide range of environmental contaminants in • dredge spoils • sediments liable to • resuspension	Acute/lethal and acute/sublethal toxicity, including genotoxicity, etc.
Water bioassays*	As for pore water and elutriates (see above)	36–41	NL, UK, USA, CAN	B[2]	Not contaminant specific, will respond to a wide range of environmental contaminants in inshore and estuarine waters	Acute/lethal and acute/sublethal toxicity, including genotoxicity, etc.
Scope for growth *	Bivalve molluscs, e.g., *Mytilus*	55–58			Responds to a wide variety of contaminants	Integrative response which is a sensitive and sublethal measure of energy available for growth.
Shell thickening	*Crassostrea gigas*	103	Portugal		Specific to organotins	Disruption to pattern of shell growth.
Vitellogenin induction	Male and juvenile fish	26–30	N, UK		Oestrogenic substances	Measures feminization of male fish and reproductive impairment.
Imposex	Neogastropod molluscs, e.g., dogwhelk (*Nucella lapillus*)	52–54	CAN, D, Ireland, Iceland, N, NL, S, UK	B[2] Q[3]	Specific to organotins	Reproductive interference. Estuarine and coastal littoral waters (*Nucella*) and offshore waters (*Buccinum*).
Intersex	Littorinids	101, 102	D, Ireland, N, UK	B[2]	Specific to reproductive effects of organotins	Reproductive interference in coastal (littoral) waters.

*Intercomparisons or quality control procedures complete for some methods (e.g., Refs. 31, 40, 99, 100).
[1] May also be applicable to mammals and birds.
[2]B=Quality control under BEQUALM.
[3]Q=Quality control under QUASIMEME.
[‡]B=Belgium; CAN=Canada; F=France; D=Germany; MEDPOL=Monitoring and Research Programme of the Mediterranean Action Plan; N=Norway; NL=Netherlands; S=Sweden

Table 8.1. Continued.

Method	Organism	Refs.	Currenty used in monitoring programmes[‡]	Quality control	Issues addressed	Biological significance
Protein or enzyme altered foci	Fish	92, 144–150			PAHs Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)	Indicates exposure to carcinogen(s).
Reproductive success in fish	• *Zoarces viviparus* • *Pseudopleuronectes americanus* • *Gadus morhua*	72 153, 160	D, S, USA, N	B[2]	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures reproductive output and survival of eggs and fry in relation to contaminants. In viviparous fish, restricted to period when young are carried by female.
Externally visible fish disease	Fish	104–108, 168–171	CAN, D, DK, UK, USA, NL, B		Measures the effects of non-specific stress by quantifying the presence of externally visible diseases, especially in dab (*Limanda limanda*)	These diseases are natural, but may be exacerbated by various stressors, including contaminants.
Benthic community analysis*	Macro-, meio-, and epibenthos	42–45, 100, 109	B, CAN, D, F, Ireland, N, UK, USA, NL	B[2]	Responds to a wide variety of contaminants, particularly those resulting in organic enrichment	Ecosystem level. Retrospective. Particularly useful for point sources. Most appropriate for deployment when other monitoring methods indicate a problem may exist.

*Intercomparisons or quality control procedures complete for some methods (e.g., Refs. 31, 40, 99, 100).
[1] May also be applicable to mammals and birds.
[2]B=Quality control under BEQUALM.
[3]Q=Quality control under QUASIMEME.
[‡]B=Belgium; CAN=Canada; F=France; D=Germany; MEDPOL=Monitoring and Research Programme of the Mediterranean Action Plan; N=Norway; NL=Netherlands; S=Sweden

Table 8.2. Promising biological effects monitoring methods which require further research before they can be recommended for monitoring.

Method	Organism	Refs.	Issues addressed	Biological significance
DNA strand breaks	Fish and mussels	113	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures genotoxic effects, but is also extremely sensitive to other environmental parameters.
Oncogenes	Fish	93–95	PAHs Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)	Activation of oncogenes (*ras*) or damage to tumour suppressor genes (p53). Measures genotoxic effects leading to carcinogenesis.
Cytochrome P4501A induction	Invertebrates	96	Induced enzyme response to PAHs, planar PCBs, dioxins and/or furans	Measures exposure to organic contaminants.
Glutathion-*S*-transferase(s) (GST)	Fish, mussels	97, 154	Predominantly organic xenobiotics	Measures exposure and the capacity of the major group of Phase II enzymes.
Multidrug/xenobiotic resistance (MDR/MXR)	Fish, invertebrates	85–92, 131–143	Organic xenobiotics	Measure of exposure.

Table 8.2. Continued.

Method	Organism	Refs.	Issues addressed	Biological significance
Various methods of measuring immunocompetence	Fish and invertebrates	73	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures factors which influence susceptibility to disease.
On-line monitoring	Mussels and crabs	98	Responds to metals and xenobiotics	Measures the effects of chemicals on heart rate using a simple and inexpensive remote biosensor. Gives an integrated response.
Degenerative gill and kidney histopathology	Fish (especially flatfish such as dab (*Limanda limanda*))	59–66	General toxicological response which will respond to a wide variety of contaminants	Measures degenerative change in tissues.
Abnormalities in wild fish embryos and larvae	Many fish, including demersal and pelagic species	70–71, 172	Not yet linked unequivocally to contaminants	Measures frequency of probably lethal abnormalities in fish larvae. Mutagenic, teratogenic.
Chronic whole sediment bioassays	Invertebrates	32	Responds to a wide range of contaminants	Measurements such as growth and reproduction, coupled to biomarker responses, which will give a measure of the bioavailability and chronic toxicity in whole sediments.
Pollution-induced community tolerance (PICT) water bioassay	Microalgae	67–69	Specific contaminants can be tested	Measure of degree of adaptation to specific pollutants. Not yet widely tested.
COMET assay (*in vitro* bioassay for sediments)	Cells exposed to extracts	111, 155, 156	Genotoxic compounds	Genotoxic potential of sediments.
Apoptosis	Fish	112	Responds to a wide range of contaminants	Research state.
Enzyme-linked immuno-sorbent assay (ELISA) for DNA adducts	Fish	161–163	Not contaminant specific	Genotoxic effects.
Dioxin-responsive chemical-activated luciferase gene assay (DR-CALUX)	Cells exposed to samples or extracts	151	Aryl hydrocarbon (Ah) receptor active compounds	Possible predictor of pathology.
Oestrogen-responsive chemical-activated luciferase gene assay (ER-CALUX)	Cells exposed to samples or extracts	152	Oestrogen receptor active compounds	Potential endocrine disruption.
Allometric response in the benthic community	Macro-, meio-, and epibenthos	81–84	Not contaminant specific, will respond to a wide range of environmental contaminants	Ecosystem level. Retrospective.

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9 DEVELOPMENT OF FORMATS TO BE USED TO REPORT DATA ARISING FROM OSPAR-JAMP CONTAMINANT-SPECIFIC OR GENERAL BIOLOGICAL EFFECTS MONITORING ACTIVITIES

9.1 Review of Biological Effects Data in the ICES Data Bank

9.1.1 ICES Environmental Data Bank

John Thain (UK) introduced this item. ICES set up the initial collection (on paper) of data on contaminants in fish and shellfish in 1972. A computerised system was developed in 1983 and since then it has expanded its services and now acts as a data collection centre for OSPAR, handling data collected under the Commission's Joint Monitoring Programmes. The ICES Environmental Data Centre now contains data on a large number of contaminants in fish, shellfish, sea water, sediments, birds, mammals and biological effects such as ethoxyresorufin-*O*-deethylase (EROD), oyster embryo bioassay (OEB) and fish disease prevalence. It is possible to extract an inventory of the records held on the database from the ICES web site.

With the exception of fish disease prevalence there are few biological effects records in the database, as shown below. An inventory of these data is available on the ICES web site:

1) contaminants in marine invertebrates, fish, birds and mammals (275,000 records)

2) contaminants in sea water (280,000 records)

3) contaminants in sediments (80,000 records)

4) fish disease prevalence (80,000 records)

5) EROD (3,800 records)

6) OEB (209 records)

7) Quality assurance (no information)

The ICES web site provides information on the parameter or contaminant only; no data are extractable. For example, OEB data are given in tabulated form as - station position (Lat./Long.) - date of sampling - laboratory code - matrix - determinand (PNR). In addition, an accompanying map indicating the station positions is also available. The same applies to the EROD data, but the information supplied is for EROD and protein independently.

Access to the actual data can be readily obtained by contacting the ICES data centre (Jørgen Nørrevang Jensen). The data can only be released with the permission of the owner. At present, the data centre operates a default system whereby the data may be released if no response or authorisation has been given within a set time period.

A summary of the OEB data held at the ICES Data Bank is given below:

OEB water data

Year	Number of Stations	Submitting Country
1990	31	UK
1991	71	UK

OEB sediment elutriate data

Year	Number of Stations	Submitting Country
1990	31	UK
1990	19	Netherlands
1991	38	UK
1993	18	Netherlands

The water OEB data that exist from the UK were generated for the North Sea Task Force (NSTF) Monitoring Master Plan (MMP). No other water data exist. However, the UK has been generating OEB data for a number of years as part of the UK National Monitoring Plan and they are stored in the UK NMP data bank. It was confirmed that OEB data also exist in France and the Netherlands.

The use of the OEB on sediment elutriate is no longer recommended as a whole sediment monitoring technique, but is of use for special applications (e.g., dredged material testing). OEB was used in the absence of suitable whole sediment tests; whole sediment tests have now been included in the JAMP recommended suite of techniques.

EROD data

Year	Number of Stations	Submitting Country
1990	7	Scotland
1991	14	Scotland
1991	8	England
1991	5	France
1991	5	Netherlands
1992	10	Netherlands
1993	3	Netherlands
1994	8	Netherlands
1995	10	Netherlands
1996	10	Netherlands

As with the OEB, much of the EROD data originated from the NSTF MMP. There are several EROD records generated from two sources in the UK and two in the Netherlands, but there is only one instance (data from Netherlands 1992–1996) where measurements were taken at the same station in a series of years, as shown below.

Table 9.1.1. Time series of data on EROD measurements in the ICES Data Bank.

Station	Position	1991	1992	1993	1994	1995	1996
N7	4° 44′ E 53° 41′ N	* (02)	X (03)			(*) (02)	
N6	3° 19′ E 52° 25′ N	* (02)	X (03)				
N9	3° 08′ E 55° 15′ N	* (02)	X (03)				
N8	4° 19′ E 54°09′ N	* (02)	X (03)				
N10	6° 45' E 53° 59' N	* (02)	X (03)				
N1	5° 02′ E 53° 03′ N		X (09)	X (09)	X (09)	* (09)	* (09)
N2	3° 31′ E 51° 25′ N		X (09)	X (09)	X (09)	* (09)	
N3	3° 52′ E 51° 40′ N		X (09)			* (09)	* (09)
N4	4° 00′ E 52° 25′ N		X (09)		X (08)	* (09)	* (09)
N5	6° 56′ E 53° 27′ N		X (09)	X (09)		* (09)	* (09)
N12	3° 24′ E 52° 49′ N				X (03)	* (03)	
N13	4° 59′ E 53° 41′ N				X (03)	* (03)	
N14	6° 37′ E 54° 19′ N				X (03)	* (03)	
N15	3° 50′ E 55° 11′ N				X (03)	* (03)	
N16	4° 18′ E 54° 06′ N				X (03)	* (03)	

* indicates that EROD is estimated as nmol resorufin/gram liver/min.
X indicates that EROD is estimated as pmol resorufin/mg protein/min.
The values in brackets indicate the month of the measurements.

All data are from the Netherlands (DGWN and RIKZ). All other EROD measurements in the ICES Database cover only single years, although a few data sets from later than 1996 are pending as ICES Data Centre work.

A confounding factor in the EROD data set is that the measurement technique has changed over the data collection period and that even in the 'comprehensive' data set from the Netherlands the unit of EROD activity reported is different (i.e., nmole resorufin/g liver/min and pmole resorufin/mg protein/min).

As with the OEB, it is known that there are considerably more EROD data that should be in the database (in France, Germany, Norway, and the UK). Data also exist in non-governmental organisations.

9.2 Assistance to the Secratariat in Developing Formats to be used to Report Data

The current reporting format is extensive and is used by various international monitoring programmes - ICES, OSPAR, HELCOM, and AMAP.

Reporting formats currently exist for two biological effects techniques: EROD and oyster embryo bioassay (water and sediment elutriate); fish disease prevalence is considered independently. The formats are provided by ICES, an example of which was circulated at the meeting. Reporting is carried out in DOS editor or Microsoft Word; it comprises 120 columns wide and multiple rows with single digits or letters allocated to each 'box'. There is a screening program provided by ICES that checks that information is in the correct place: syntax for sequencing; internal value check for evaluating some fields against another field; external value check where values have to be in a defined range; and combination of fields check where one field may need to be filled in since it relates to another, e.g., standard deviation to mean.

An important consideration in relation to biological effects data is the ability to compare them with chemistry data in order to correlate the results. To achieve this, it is necessary to consider mechanisms for linking samples and subsamples analysed for biological effects with appropriate contaminants data.

Different levels of comparison within the database can be achieved:

1) same fish comparison;

2) same sample, but different fish;

3) no direct association between samples.

A number of issues concerning the reporting format and data bank were discussed:

a) There are very few data in the data bank but it was confirmed at the meeting that additional data sets exist for both OEB and EROD. The general consensus was that the reporting format is unwieldy (data entry is complicated/it is easy to make mistakes/it is time consuming) and is probably the main reason why Member Countries do not submit many EROD and OEB data. In some instances, the submitting organisations have invested considerable resources in developing software (a conversion tool) that converts data held in their national database to the ICES reporting format (e.g., DONAR in the Netherlands). The ICES reporting format was discussed at the recent meeting of SIME and the conclusions were circulated for information at this meeting. Members of WGBEC reinforced many of the comments from SIME and would strongly support the Secretariat in improving the reporting format, in particular, a more user-friendly front end. Jørgen Nørrevang Jensen (ICES Environmental Data Scientist) stated that it was the intention of ICES to develop an ACCESS database with an appropriate front end that would hopefully permit the transfer of data from programmes such as EXCEL. Peter Matthiessen suggested that it may be useful to contact Member Countries to ascertain how data are submitted and held in national data banks (e.g., the UK National Marine Monitoring Plan).

b) It is known that EROD and OEB data are generated in non-governmental laboratories. However, data can only be submitted to the ICES data bank through the nominated National Laboratory. Jørgen Nørrevang Jensen confirmed that data from any source can be submitted to the data bank as long as they go through a nominated National Laboratory, are generated according to the standard protocols, and fulfil all the requirements laid out for ICES reporting. In the future, this will also include QC requirements identified in the JAMP.

   It was concluded that ICES should encourage the submission of data from Member Countries by writing directly to the ICES Delegates to inform and remind them of the need for and importance of submitting biological effects data. This should include what the data are being used for, and that in the future the ICES data bank will be an important source of biological effects data.

c) As noted above, the existing EROD data held in the data bank were generated using varying methodology, and in some cases the reporting units were different. In view of this and the small data set, it was agreed that all old data should be re-submitted using the new format.

d) Review of OEB format:

   Insert one new field name on record 23—spawning natural/artificial.

   New fields will also be required for internal and external AQC.

e) Review of EROD format:

   Change one new field name from 'cofactor concentration' to 'NADPH concentration'.

   Insert new field names as follows:

   - length of tissue storage (codes required for length and condition of storage)
   - temperature of storage
   - whole liver/homogenate/S9 fraction/microsomal
   - protein microsomes/S9 fraction
   - substrate solvent type/concentration
   - detection limit for method, as implemented in practice

- use of protease inhibitor

New fields will also be required for internal and external AQC.

f) JAMP has recognised that an essential component of biological effects monitoring is that the recommended techniques have appropriate AQC procedures. Indeed, JAMP in the future may exclude data if they are not accompanied by internal and external AQC.

9.3 OEB QA/QC

General

Quality Assurance and Quality Control Programmes for ecotoxicity tests include day-to-day operation of the laboratory, control of the source and condition of test organisms, implementation of methods, replication, training of personnel, data analysis, reporting and reference toxicant testing. This may be achieved in two parts to demonstrate control over the repeatability of the test: firstly, the development of Shewart control charts to monitor test results against previous tests (internal quality control) and, secondly, to compare performance against externally imposed control limits (external quality control). The approach is similar for biomarker-type methods and bioassays. A scheme for bioassays is shown below.

Internal Quality Control

This involves the testing of an organic or inorganic reference chemical. In the initial stages, several test are carried out and a mean and standard deviation of the toxicity values are calculated (EC50 or LC50). A control chart is then constructed on which the normal or acceptable variation is defined (see Environment Canada, 1990). The US EPA requires that a minimum of five tests be conducted for a method before any control limits can be established (US EPA, 1994). Tests are carried out until the limits do not change, thereby reflecting the minimum variability. Up to 15–20 tests may be necessary to obtain a representative range (Dux, 1986). Warning limits on the control chart are defined as the values twice the standard deviation above and below the mean. For a large data set, these values represent the upper 95 % confidence limits. Action limits are derived as values three times the standard deviation, which represent the 99 % confidence limits. If a result is outside the action limit, or two consecutive values occur in the warning limits, the measurement is judged to be out of control. A consistent trend might also suggest that the measurement method is out of control.

External Quality Control

Internal control charts will stabilise the status quo; external control charts are required to control the variability with repeated testing. Reliance on internal control charts alone will result in different laboratories working to different acceptability thresholds of test methods and could simply reinforce bias. The limits of underlying repeatability are generated from the results of ring tests with external standards or reference toxicants. The results from different laboratories can then be analysed using Residual Maximum Likelihood (REML). As more external reference toxicant data are gathered, the quality control criteria are refined and narrowed until the limits representing the best practice with respect to repeatability are defined.

At the present time, the precise QA/QC procedures are being developed within BEQUALM (see Section 16) and will not be available until 2000.

9.4 Development of Formats for Biological Effects Techniques other than EROD, OEB, and Fish Disease Prevalence

In the terms of reference for the meeting, WGBEC was asked to support the development of the reporting formats for the JAMP techniques MT, ALA-D, oxidative stress, fluorescent bile metabolites, protein, imposex, DNA adducts, whole sediment, pore water, lysosomal stability, and fish reproductive success. The parameters and fields were discussed for all the above techniques and are summarised in Tables 9.2 and 9.3. Some discussion took place on the need to link data entries between samples, e.g., fish disease prevalence and biomarkers, ideally in the same fish.

Jørgen Nørrevang Jensen (ICES Environmental Data Scientist) provided a diagram (see below) which gives an overview of the data structure in selected parts of the ICES Environmental Data Bank. The number given indicates the record type-no. used in the ICES reporting format. This figure, e.g., visualizes the existing link between biomarkers like EROD

and contaminants in the same fish. It is suggested that links should be set up between fish disease and biomarkers in the same fish, although it is not clear where such links should be established.

Figure 9.4.1. Schematic diagram of selected parts of the present ICES Environmental database structure.

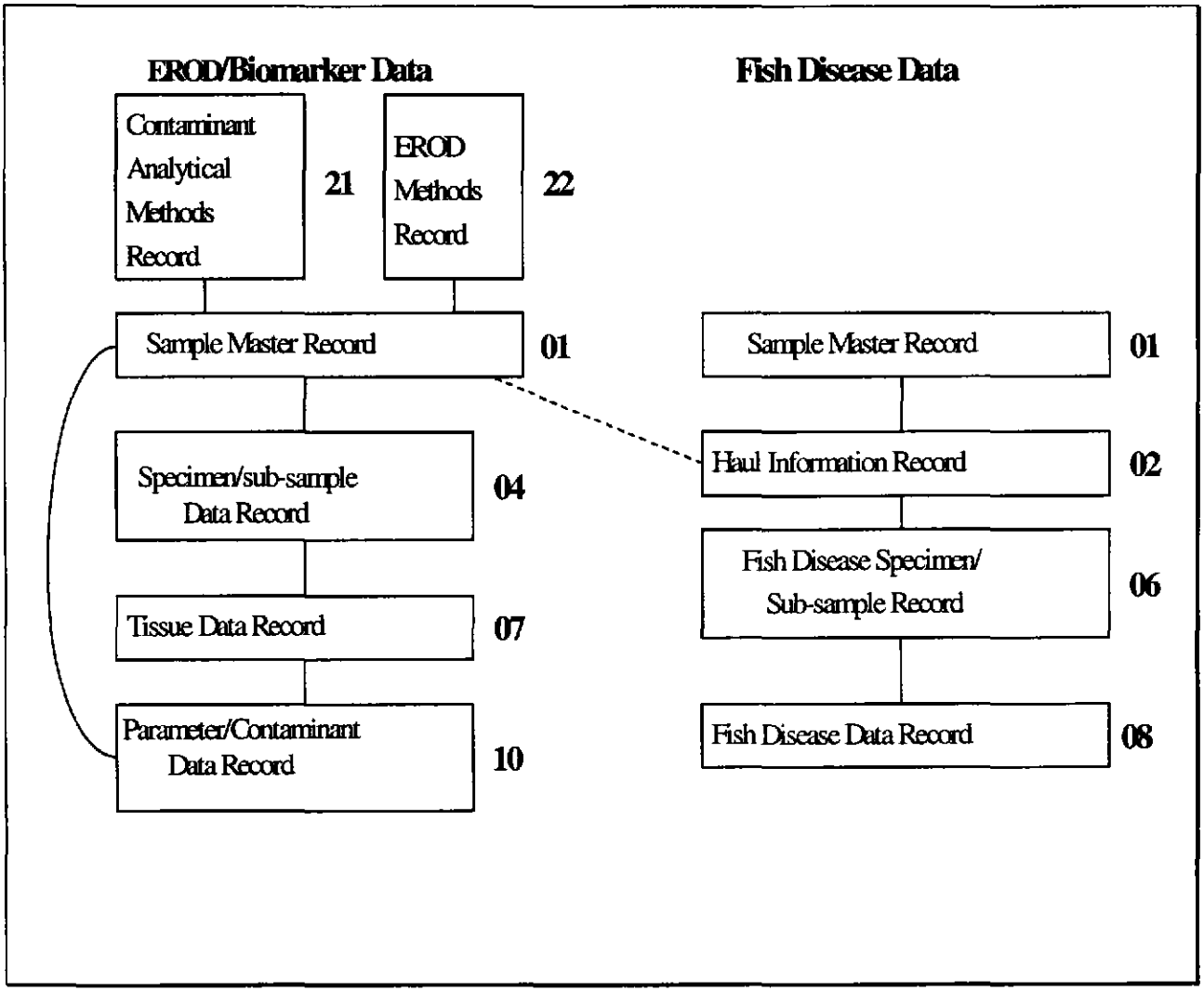


Table 9.4.1.1. Summary of the suggested parameters (10 and 22), methods (22) and other codes to be added to the existing fields in the ICES reporting format.

Type of Biomarker	Parameters (PARAM)	Methods	Other Codes
Metallothionein	MT-S9 MT-cyt	differential pulse polarography (DPP) ELISA RIA sulfhydryl method Cd -saturation Hg -saturation Ag -saturation chromatography	Nature of buffer: tris phosphate
ALA-D	ALA-D		Sampling procedure: point pretreatment of syringe
Oxidative stress	MDA CAT GX SOD GR		
Fluorescent bile metabolites	PYR BAP...		
Protein	Microsomal cytosol S9 homogenate	Bradford Lowry	
Imposex and Intersex	VSDI RPSI RPLI ISI FPrL		
DNA adducts	DNA adducts		
Whole sediment	10 days survival		
Pore water	Mortality 48 h		
Lysosomal stability	Neutral red leakage in mins		
Fish reproductive success	Fecundity index Larval development index Larval mortality Atresia Hormone Hormone level		

Table 9.4.1.2. Summary of the suggested fields to be added to the present ICES reporting format divided on the basis of the type of biomarkers. *m* and *x* indicate mandatory and optional fields, respectively. *me* indicates that it is mandatory if using the ELISA method. * indicates that links to fish disease and related parameters in the same specimen are needed. # indicates that the field should be added both to the sediment sampling methods record (20) and to the (Oyster embryo) Bioassay record (23). As well as the link to fish disease, several other defined parameters have to be measured in conjunction with each method.

	Metallo thionein*	**ALA-D***	**Oxida-tive stress***	**Fluores-cent bile metabo-lites**	**Protein**	**Imposex/ Intersex**	**DNA adducts***	**Fish reproduc tive success***	**Lysosom al stability***	**Whole sediment**	**Pore water**
Sampling design						*x*					
Length of storage time	*x*	*m*	*m*	*m*		*m*	*m*			*m*	
Temperature of storage time	*me*	*m*	*m*	*m*			*m*		*x*	*m*	
Type of reducing agent	*me*										
Type of antibody	*me*										
Dil. of antiserum in assay	*me*										
Detection limit	*me*	*m*	*m*	*m*			*?*				
Use of protease inhibitor	*x*	*x*	*m*								
Standard used	*me*			*m*	*m*						
Sampling procedure		*m*	*x*								
DNA purification							*x*				
DNA digestion							*m*				
Extraction of adducts							*m*				
Labelling procedure							*m*				
Type of TLC-sheets							*m*				
Separation procedure							*m*				
Quantifica-tion method							*x*				
Liver weight								*m*			
State of reproductive cycle								*m*			
Date of assay								*m*	*m*	*m*	*m*
Incubation period									*m*		
Freezing mode									*m*		
Choice of substrate									*m*		

Table 9.4.1.2. Continued.

	Metallo thionein*	ALA-D*	Oxida-tive stress*	Fluores-cent bile metabo-lites	Protein	Imposex/ Intersex	DNA adducts*	Fish repro-ductive success*	Lysos-omal stability*	Whole sediment	Pore water
Substrate solvent									*m*		
Conc. of neutral red									*m*		
AVS										*x*	
Particle size										*m*	
Depth of sediment #										*m*	
No. of replicates #										*m*	*m*
No. of animals used										*m*	*m*
Origin of specimens										*m*	*m*
Size of animals										*m*	*m*
Sample manipula-tions										*m*	
Method of extraction											*m*
Volume extracted											*m*
Salinity adjustment											*m*
Volume of replicate										*m*	*m*
Single concentration											*m*
Dilution series											*m*

9.4.1 Recommendation

WGBEC recommends to ICES that when the revised data reporting fields for EROD and oyster embryo bioassay have been completed, Member Countries should be contacted and encouraged to re-submit data already in the ICES database, and also to submit existing EROD and oyster bioassay data that have not yet been placed in the database.

References

Dux, J.P. 1986. Handbook of Quality Assurance for the Analytical Chemistry Laboratory. Van Norstrand Rheinold Company, New York.

Environment Canada. 1990. Guidance document on control of toxicity test precision using reference toxicants. Environment Protection Series, Report EPS 1/RM/12. Environment Canada, Ottawa. 85 pp.

US EPA. 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. United States Environmental Protection Agency, Report EPS/600/4-89/001.

10 REVIEW OF THE AMAP/EEA/ICES WORKSHOP ON BIOLOGICAL EFFECTS METHODS APPLIED TO DETECT COMBINED EFFECTS IN MARINE ECOSYSTEMS

10.1 Introduction

WGBEC reviewed the report from the AMAP/EEA/ICES Workshop on Combined Effects in the Marine Environment (WKCEME) held in Copenhagen, 16–17 November 1998. The objective of WKCEME was to assess methods to detect combined effects of contaminants and either organic enrichment, UV-radiation and/or climate change.

WKCEME divided its work into four areas: (1) reproduction and population studies, (2) cellular and biochemical methods, (3) effects on plankton, pelagic eggs, pelagic fish larvae and whole organism bioassays, (4) pathology and diseases.

10.2 Resumé from the Workshop

The combined effects of pollutants is a very complex topic and one has to realise that most monitoring activities will be dealing with a matrix of a large number of factors. Various factors will differ in their impact on biological effects measures. Effects may be additive, antagonistic or synergistic. WKCEME did not recommend guidelines for how interactions could be assessed, other than pointing out the importance of keeping relevant factors in mind. In this way it was envisaged that required knowledge would accumulate. A major conclusion was that there is a need for more basic research. Many of the groups emphasised the importance of using methods that detect effects on physiological sensory processes and their links to behaviour.

The various techniques will be applied in different geographical areas with a broad range of environmental conditions. The most important environmental factors are temperature, salinity and light. These factors modulate the response of most biological effects techniques. The workshop emphasised the need for more knowledge on the use of selected biological methods under differing environmental conditions (e.g., the Baltic Sea in relation to the North Sea or the Mediterranean Sea).

10.3 Comments from WGBEC

WGBEC supports the main aim and results reported from WKCEME. WGBEC did not, however, have a clear view of WKCEME's conclusions and recommendations, which appeared generic and not relevant to the questions in hand. The recommendations from the four workgroups at WKCEME differed in their relevance. From the report of workgroup 1 (reproduction and population studies), WGBEC noted the recommendation that chemosensory tests should be used, which was thought an important component. The other recommendations from workgroups 1 and 2 are in line with the opinions expressed by WGBEC and others earlier. WGBEC noted the recommendations from workgroup 3 (plankton, eggs, and larvae) as being scientifically sound and agreed with the gaps in knowledge that were identified. There were few members in workgroup 4 (pathology and diseases) at WKCEME, but their recommendations, although not relating to combined effects, were sound.

10.4 Follow-up Activities

A pan-European joint research proposal will be submitted for the EU Fifth Framework Programme (Key Action 'Preserving the Ecosystem'—Sustainable Marine Ecosystem) in autumn 1999. The proposal will include the application of well-established biomarkers in field studies, development of promising recent methods, and an integrated study using mesocosms. The area under investigation comprises the western Mediterranean Sea, the Baltic Sea, and a sub-Arctic site (yet to be defined).

In connection with the research programme, a Training Course on selected biomarker techniques is planned, especially for participants from the Baltic Sea countries, in order to develop and encourage the use of biomarker approaches in marine ecosystems.

11 REVIEW OF THE IMPACTS OF CHEMICALS OF CONCERN

11.1 Report on the UK Project Endocrine Disruption in the Marine Environment (EDMAR)

WGBEC was presented with an oral report on the UK EDMAR programme by Peter Matthiessen (UK), and also received an information leaflet on the programme (WGBEC/99/16.1), and a CEFAS report on oestrogenic endocrine disruption in UK flounder (WGBEC/99/16.2) which in part triggered the present research. EDMAR started in June 1998 as a collaborative 4-year programme of research among several UK laboratories (CEFAS, FRS Aberdeen, Zeneca Brixham, Plymouth University, and Liverpool University—plus an allied project at Glasgow Caledonian University). It is primarily funded by the UK Government, but there is also an industrial component, and the total cost exceeds UK£1.4 million.

EDMAR's main objectives are to investigate the causes (substances and sources) of endocrine disruption in UK marine waters, to study their effects on the reproductive output of fish and crustacea, and to model the possible effects at the population level. Most work to date has focused on the development of improved biomarkers which can be used later in the project to survey UK waters. For example, the programme is successfully developing a biomarker of androgen exposure in female sticklebacks (*Gasterosteus aculeatus*) based on the induction of nest-glue protein (spiggin), and biomarkers of oestrogen exposure in male crustaceans (*Carcinus maenas* and *Crangon crangon*) based on vitellin induction.

Furthermore, some of these biomarkers are being adapted for detection by immunohistochemistry or gene probe techniques in small volumes of tissue. The project is investigating the reproductive success of fish (sand goby *Pomatoschistus minutus*, viviparous blenny *Zoarces viviparus* and migratory salmon *Salmo salar*) and crustaceans (*Chaetogammarus marinus*) in laboratory and/or contaminated field situations, and is also deploying caged sentinel organisms to trace major discharges of endocrine disrupters. There is a particular focus on species which spend the most sensitive parts of their life cycle in estuaries (where contamination is highest), and all observations of reproductive output will be accompanied by measurements of appropriate biomarkers. Identification of causative substances is being conducted primarily by means of Toxicity Identification and Evaluation (TIE) techniques employing chemical fractionation, and genetically modified yeast cell lines to bioassay the fractions for oestrogenic (and potentially androgenic) activity.

The EDMAR programme is an ambitious attempt to tackle the fundamental issues of biological significance and causality which surround the subject of endocrine disruption, although it is too early to comment on its likely degree of success. However, WGBEC endorsed the general approaches to the problem outlined above.

11.2 Dutch LOES Programme

Dick Vethaak (The Netherlands) presented the Dutch LOES programme on oestrogenic compounds in the aquatic environment. This is a large-scale baseline study entitled 'National Investigation into Oestrogenic Compounds in the Aquatic Environment' (Dutch acronym, LOES) which is being carried out in 1997–2000. The project is government funded with a total budget of 1.6 million ECU. The multidisciplinary LOES project involves various governmental bodies and universities. The Dutch part of the EU-funded programme COMPREHEND is integrated in LOES. The LOES project aims: (1) to investigate the occurrence and sources of various natural oestrogens and xeno-oestrogens in the Dutch aquatic environment including waste water, rain water, drinking water and (inland, estuarine and marine) surface waters; (2) to assess oestrogenic/reproductive impacts on sentinel fish species inhabiting this environment. As far as the marine environment is concerned, it will include estuarine as well as coastal and offshore sites (e.g., Dogger Bank) and two oil platforms situated on the Dutch continental shelf. Target chemicals include: natural hormones; ethinyl-oestradiol; alkylphenols; alkylphenol-ethoxylates (APE); bisphenol-A (BPA); phthalates; polybromobiphenyls (PBBs); and polybrominated diphenylethers (PBDEs). Furthermore, *in vitro* bioassay analyses (ER-binding, YES, and ER-CALUX assays), *in vivo* bioassays, bioassay-directed fractionation/TIE, diagnostic biomarkers in bream and flounder, and *in situ* caging experiments with rainbow trout and carp will be applied.

In 1997/1998 a pilot survey was carried out, the results of which have proven to be crucial in the final design and logistics of the main study, choice of parameters, and development of standardized sampling and analytical protocols (Belfroid *et al.*, 1999). In general, the findings showed that natural hormones, xeno-oestrogens and oestrogenic activity are present in Dutch waste waters and water systems including estuarine and marine waters. In surface water, hormones appear to be present, albeit irregularly and in very low concentrations below 5 ng/l, and below 1 ng/l in some estuaries. The most common hormones in surface waters were 17β-oestradiol and oestrone. Hormone glucuronides were not detected. Levels of BPA, phthalates, and APEs in surface waters were in the μg/l range, higher than levels of hormones. BPA and phthalates could be detected at all locations studied including marine waters. Of the phthalates, DEHP was

most common. APEs (in this case nonylphenol) were detected at only one estuarine site analysed for these compounds. Levels in sediment and particulate matter were below the limit of detection for BPA, while high concentrations were found for phthalates and APEs. Levels of these compounds were much higher in sediment and particulate matter (mg/kg range) than in surface water. In flounder liver samples and blue mussel samples, phthalates could also be detected, but levels of APEs stayed below the limit of detection.

Oestrogenic activity was measured both in surface water, with a maximum of 4 pmol Oestradiol Equivalents (EEQ) per litre, which is equal to 1 ng/l 17β-oestradiol, and in particulate matter. In both matrices, the oestrogenic activity was low.

In addition, this study showed that the applied analytical procedures and bioassays were mostly well suited to analyse these compounds in most matrices. However, for some matrices the analyses need to be improved. The data set also showed, however, that the measured oestrogenic activity cannot be completely explained by the measured compounds. The data set shows temporal variation in levels of compounds and activity that cannot be explained well. The results stress the need for good agreements and standardization on pretreatment of the sample and on what part of the sample is to be analysed. A major lesson learned is that logistics of this relatively simple pilot inventory, with more than one analysing laboratory and interdependencies of laboratories on each other, are already very complicated. The results of this pilot study, however, justify the large national baseline study with standardized techniques (LOES) which is currently under way.

Reference

Belfroid, A.C., De Voogt, P., van der Velde, E.G., Rijs, G.B.J., Schäfer, A.J., and Vethaak. A.D. 1999. Hormoonontregelaars in water: een orienterende studie naar de aanwezigheid van oestrogeen-actieve stoffen en oestrogene activiteit in watersystemen en afval-water in Nederland. Pilot inventory on the occurrence of estrogenic substances in water systems and sewage water in The Netherlands. RIZA/RIKZ report.

12 CONTRIBUTION TO THE MARINE HABITAT COMMITTEE'S PART OF THE ICES STRATEGIC PLAN

WGBEC was asked to review and comment on the draft Five-Year Strategic Plan of the Marine Habitat Committee. This plan was drafted in September 1998 with considerable input from the membership of the WGBEC. As such, comments and revisions suggested here by WGBEC are limited. Of the six objectives proposed by the Marine Habitat Committee for its scientific work during the 1999–2004 period, the three relevant to WGBEC were addressed:

Objective 1—Development of a toolbox to assess marine habitat quality

WGBEC noted that this objective must integrate these 'tools' or activities into a holistic assessment of marine habitat quality and pointed to the activities of the Joint Meeting of WGBEC and WGSAEM, held earlier in the week, as an example of how this integration might be accomplished. An integration of biological measures of environmental variability together with analytical measures (e.g., biotic, abiotic and contaminant variables) and the use of artificial intelligence systems will be essential for summarising monitoring data and then making future predictions and inferences from the resultant data.

Objective 4—Development of knowledge on the effects of anthropogenic contaminants on habitat and dependent living resources

WGBEC noted that the timescales projected in this objective are probably underestimates of the effort required and many will spill over into the next Strategic Plan or beyond. This is not a critical observation but rather a recognition that these subjects will develop and evolve over time as we learn more about the effects that these anthropogenic contaminants have on living marine resources and their habitats.

An additional Generic Issue was suggested for inclusion in the Objective 4 table: Interaction between contaminants and eutrophication-related factors with a time scale of 5+ years. It is widely accepted that contaminant fluxes and effects are affected by the amount of organic material present. There is little quantitative knowledge on this subject. It is to be expected that responses in pelagic and benthic systems will be different and possibly opposite. For example, plant or animal growth may be higher in nutrient-rich eutrophic habitats and make contaminant-induced changes in growth difficult to detect when growth in non-eutrophic habitats is considered as a baseline.

A second additional block to the Generic Issues block suggested is: Effects of contaminants on the physiology and behaviour of fish and invertebrates with a time scale of 5+ years. There are a limited number of studies on the effects of contaminants upon bivalve physiology (e.g., scope-for-growth in mussels) and even fewer on effects of contaminants on migrating fish. Both areas need additional attention.

Block two of the Generic Issues table should include 'identification' in the sentence 'There will be an on-going need to look for the appearance and *identification* of novel contaminants, ...'

The last block of the Generic Issues table might include the phrase 'including protozoans' in the Subject block, to emphasise that the microbiological community includes these organisms as well as bacterial and viral components.

The last block of the Specific Issues table should include antibiotics used in the mariculture industry as one of the chemicals of concern in the list in the Comments block – 'Many highly toxic chemicals and wastes (e.g., *antibiotics,...*)'.

WGBEC felt unable to cost the endeavours covered by this objective, although most would clearly absorb many man-years of effort.

Objective 6—Enhancement of the knowledge on monitoring methodology in relation to the well-being of marine habitats

Agenda Item 8 for this meeting addressed the updating of the WGBEC list of promising biological monitoring methods which require further research. The following five additional methods from that list should be added to the activities table included under Objective 6 of the Strategic Plan:

1) P4501A induction in invertebrates. It would be valuable to have a biomarker of PAH and PCB exposure in invertebrates because they are often sessile and therefore reflect local conditions.

2) Degenerative gill and kidney histopathology in fish. (This will replace 6.1.9: Degenerative histopathology in fish)

3) ELISA for DNA adducts. Current methods for DNA adducts are very expensive and time consuming, but ELISA methods hold out promise of much cheaper and faster assays.

4) ER-CALUX. This is a promising new oestrogen receptor gene-mediated chemical-activated luciferase-gene expression assay in a T47-D human breast cancer cell line which responds to oestrogenic materials.

5) DR-CALUX. This is similar to ER-CALUX, but contains the Ah receptor and so responds to dioxin-like substances.

Development of most of the above methods to the stage where they can be used for marine monitoring will probably take about three years.

WGBEC felt unable to put costs on the development of these techniques.

13 EFFECTS OF CONTAMINANTS IN SEABIRDS

WGBEC made no progress on this item because Dr Bart Bosveld, who was to have led a discussion on this issue, was unfortunately unable to be present due to illness. However, WGBEC agreed that the issue of contaminant effects in seabirds was a potentially important one that they needed to consider with a view to its regular inclusion in WGBEC meetings. WGBEC agreed to defer discussion of this subject until 2000, and to arrange for several bird/contaminant experts to attend the WGBEC meeting at that time.

14 REVIEW OF PROGRESS WITH ICES TIMES PAPERS

Ketil Hylland (Norway) referred to the activities for the *ICES Techniques in Marine Environmental Sciences* (TIMES) in 1998/1999. Two manuscripts were published last autumn. At present, the status of TIMES submissions from WGBEC is as follows:

- manuscripts on DNA adducts (Reichert), imposex (Gibbs) and metallothionein (Hylland) have been cleared through referees and WGBEC; they await final preparation and publication;

- two additional manuscripts, the *Arenicola* sediment bioassay (Thain and Bifield) and the *Corophium* sediment bioassay (Roddie and Thain), have been cleared through referees and WGBEC; only minor questions remain before final preparation by ICES and publication;

- a manuscript on vitellogenin (Scott and Hylland) has been refereed and only needs comments from WGBEC before being submitted to ICES;

- a manuscript on fish histopathology (Feist *et al.*) is near completion and is expected to be submitted to the editor this summer;

- manuscripts which were commissioned but have not yet been submitted include the measurement of ALA-D (Hylland), lysosomal stability (Moore and Köhler), and intersex (Oehlmann).

In addition to the aforementioned reports, WGBEC has asked for manuscripts on PAH-metabolites and shell thickening in oysters. Manuscripts for those two methods have not been commissioned as yet.

The ICES TIMES is an important task for ICES and is an important input to the scientific community. As methods do change, manuscripts must be published within at most one year following submission to ICES. In the present situation, with new biological effects techniques being introduced into national and international (OSPAR) monitoring activities, the TIMES is more relevant than ever.

WGBEC strongly urges ICES to make sufficient resources available to ensure publication of TIMES leaflets within one year of submission to ICES, and notes with approval the ICES Publications Committee's comments on this issue. WGBEC further discussed the possibility of publishing method manuals (i.e., TIMES manuals) on the ICES web page. Two models were considered: (i) manuscripts could be made available through the web until a leaflet exists, and (ii) continuously updated methods could be published on the web. WGBEC asks the ICES Publication Committee to consider providing essential methods for the scientific community through the ICES web page. WGBEC also asks the Publication Committee to consider advertising campaigns to ensure that the scientific community is aware of published leaflets. Finally, the Publications Committee is asked to consider whether the relevant TIMES publications (revised) and other selected methods could be published in one volume, encompassing biological effects in marine monitoring, together with advice on monitoring strategy and practice.

In addition to the manuscripts already recommended, the WGBEC recommends the publication of the following manuscripts in 1999:

- a sediment bioassay using the polychaete *Arenicola marina* (Thain and Bifield);

- biological effects of sediment-bound contaminants on *Corophium volutator* (Roddie and Thain);

- radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) techniques for the measurement of marine fish vitellogenins (Scott and Hylland).

15 REVIEW OF PROGRESS WITH ORGANISATION OF THE SEA-GOING WORKSHOP TO STUDY THE PELAGIC EFFECTS OF CONTAMINANTS

Ketil Hylland (Norway) presented the framework for a planned workshop on the effects of contaminants on pelagic organisms ('Biological effects in pelagic ecosystems').

15.1 Background

There is a continuing development of methods to assess biological effects of pollution in marine ecosystems. In the past 15 years three practical workshops, in Oslo/Langesund (Bayne *et al.*, 1988), at Bermuda (Addison and Clarke, 1990) and in Bremerhaven (Stebbing and Dethlefsen, 1992), have been held to assess methods to determine biological effects of contaminants. The three workshops were held under the auspices of UN (GEEP, IOC) and/or ICES. These workshops

stimulated research into the use of biological effects methods to monitor pollution impacts in marine ecosystems and contributed towards a framework for general and contaminant-specific monitoring (OSPAR, 1998a, 1998b).

Whereas there has been progress in developing methods to assess pollution effects in benthic systems, there is still a lack of agreed methods to evaluate biological effects in pelagic systems. There are obvious reasons for this situation. Firstly, the quality of the water at any give site may vary substantially with time. Secondly, organisms in the pelagic zone either move with the water (plankton) or move through large volumes of water (nekton). For many of the methods in question, plankton represent very small samples and it is difficult to find sufficient material for replicate analyses, etc. The nekton will integrate over large areas and may not represent the water quality at any given site very well.

15.2 Scope and Aim of the Workshop

The proposed practical workshop is to concern itself with methods to detect and quantify biological effects of xenobiotics in pelagic ecosystems. The main aim of the workshop is to assess the ability of various methods to detect biological effects of xenobiotics in pelagic systems. In addition, the results from the workshop will be available as a basis to suggest methods for future monitoring of biological effects in pelagic systems.

15.3 Organisation and Time Schedule

The workshop will be organised through a steering group. Members of the steering group are: Peter Matthiessen (UK), Thomas Lang (D), Dick Vethaak (NL), Ståle Johnsen (N), Bjørn Serigstad (N) and Ketil Hylland (N) (Chair). A detailed description of the workshop programme will be prepared by the end of May 1999. The steering group will meet in Oslo in mid-June to finalise arrangements. A meeting for all workshop participants will be arranged in February 2000 and a reporting meeting in October 2000.

The workshop is planned for three weeks in March–May 2000. The period depends on the availability of vessels and needs to be timed with the presence of the preferred fish larvae (gadoids, herring, flatfish).

15.4 Selection of Methods and Areas

There will be a need for expertise in chemistry, hydrography/modelling, fisheries biology and statistics in addition to biological effects. Table 15.4.1 indicates the major parts of the workshop. The field study will comprise two studies: one study in the vicinity of a selected platform and one study in the vicinity of a land-based activity with a similar exposure situation. In addition, the field study will collect samples from a reference area, to be used in laboratory studies. Samples will be taken at two points in time—at a 3–4 week interval—coinciding with the deployment and retrieval of buoys with cages. Cages will be deployed at the selected study sites.

Table 15.4.1. Overview of workshop on pelagic effects.

Workshop item	Matrix/organism	Biological analyses	Chemical analyses
field study	water	bioassays	Y
	water/SPMD	bioassays	Y
	fish larvae	development, biomarkers	Y
	zooplankton	CEA, biomarkers	Y?
	fish	biomarkers, fecundity, etc	Y
cage exposure	mussels	SfG, biomarkers	Y
	fish larvae	development, biomarkers	Y
	fish	biomarkers	Y
laboratory study	mussels	as above	Y
	fish larvae		
	zooplankton		
	fish		

15.5 Recommendation

There is reason to believe that bringing together international expertise in this area in a practical workshop will bring the development of biological effects methods for pelagic ecosystems a large step forward.

WGBEC recommends that ICES support an international collaborative workshop on the effects of xenobiotics in pelagic ecosystems. The workshop will be a joint exercise between ICES, national monitoring organisations, and commercial interests.

References

Addison, R.F., and Clarke, K.R. 1990. Introduction: the IOC/GEEP Bermuda workshop. Journal of Experimental Marine Biology and Ecology, 138: 1–8.

Bayne, B.L., Addison, R.F., Capuzzo, J.M., Clarke, K.R., Gray, J.S., Moore, M.N., and Warwick, R.M. 1988. An overview of the GEEP workshop. Marine Ecology Progress Series, 46: 235–243.

OSPAR. 1998a. JAMP (Joint Assessment and Monitoring Programme) guidelines for general biological effects monitoring. OSPAR Commission, London, UK. 15 pp.

OSPAR. 1998b. JAMP (Joint Assessment and Monitoring Programme) guidelines for contaminant-specific biological effects monitoring. OSPAR Commission, London, UK. 38 pp.

Stebbing, A.R.D., and Dethlefsen, V., 1992. Introduction to the Bremerhaven Workshop on Biological Effects of Contaminants. Marine Ecology Progress Series, 91: 1–8.

16 ANY OTHER BUSINESS

16.1 Endocrine Disturbances from Domestic Disposal Sites

Lennart Balk reported that recent studies in Sweden have focused on the influence of dump sites as a possible source of endocrine disturbances in the aquatic environment (Noaksson *et al.*, 1999). Ongoing work was presented from a freshwater lake in Sweden (Lake Molnbyggen) which receives leakage water from an 18-year-old domestic waste (mainly) dump site. The reported findings included a very strongly reduced capacity among female fish to produce ripe gonads. These observations were valid for a number of teleost species (4–5) investigated in the area. Although the main effects observed were on female gonads, male fish in the area were also affected. These observations of reduced gonad weight were correlated with biomarkers of endocrine disruption, such as aromatase activity in the brain of females and reduced oestradiol and testosterone levels in blood plasma. In addition, several of the affected species showed an increased frequency of open wounds. It should be pointed out that the observed effects in this area are probably not due to 'classical' contaminants such as PAHs, PCBs, etc., since a number of other biomarkers indicate low general pollution in the area. Instead it was suggested that a possible influence of a rather specific nature due to endocrine disturbances is occurring. Furthermore, whether these phenomena are due to uncommon unknown compound(s) at the dump site, or are expressed due to the general low pollution in the area, is presently unknown. The general importance of these observations, and the potential impact to the aquatic environment, therefore remains to be investigated. Although these observations were made in a freshwater system, it is possible that similar effects could be occurring in some saline environments close to various types of waste disposal site.

Reference

Noaksson, E., de Poorte, J., Linderoth, M., and Balk, L. 1999. Reproductive disorders in fish from Lake Molnbyggen and an adjacent stream contaminated by leakage water from a refuse dump. Abstract from the Tenth International Symposium 'Pollutant Responses in Marine Organisms' (PRIMO 10), 25–29 April 1999, Williamsburg, VA, USA.

16.2 Report on Progress with the BEQUALM Programme

Peter Matthiessen, the BEQUALM programme project manager, reported on progress to the WGBEC. It will be recalled that BEQUALM, an EU-funded programme, was set up in late 1998 to initiate the development of a quality control (QC) scheme for all of the biological effects techniques in the OSPAR/JAMP (with the exception of measures of

oxidative stress). The intention is that BEQUALM will eventually become self-funding in a similar way to the QUASIMEME programme which provides QC for marine chemistry measurements.

BEQUALM has now been running for five months, and its practical activities are due to start imminently. The individual BEQUALM projects (each of which is being led by an expert laboratory) have been identifying potential participating laboratories over the last few months, and are now in the process of inviting their formal commitment to the programme. However, it is not too late for new participants to join up, and they are strongly encouraged to do so. Full contact details can be found on the BEQUALM website (http://www.cefas.co.uk/bequalm), and contact can also be made via the programme manager at CEFAS Burnham (p.matthiessen@cefas.co.uk).

The practical activities in the first full year of the programme (1999) vary considerably between the individual projects, but many involve practical workshops to agree on methodological details and in some cases to provide training, and all involve the development and implementation of intercalibration methods. This stage will be followed in 2000 by the establishment of a performance assessment system. At that point, laboratories compliant for a particular technique will be in a position to submit their marine biological effects monitoring data to the ICES database.

16.3 Proposal for Pilot Surveys on Cytochrome P450 Activities in relation to the Size, Age, and Maturity of Juvenile Dab (*Limanda limanda*) in the ICES Area

A proposal to conduct pilot surveys on this subject emerged from discussions in WGBEC following the presentation held on EROD at the JBSEAM meeting that preceded the separate meetings of WGSEAM and WGBEC. There is evidence that Cytochrome P4501A activity levels are most elevated in juvenile dab in the size range 5–9 cm. The EROD levels rapidly decrease in relation to increasing size of male and female dab (Figures 16.3.1 and 16.3.2) and, more precisely, to increasing maturity. The maturity level, measured as ovary size, has a major impact on the EROD level (Figure 16.3.3). The ovary size may be considered a good indicator of the reproductive state, and the biochemical reasons for reducing Cytochrome P4501A activities in relation to increasing maturity are well known. Unfortunately, a simple indicator of the level of the reproductive state of males could not be identified. The range of ovary sizes in grown females is wide (Figure 16.3.4). As a result, the signal-to-noise ratio of EROD in these dab is profoundly reduced (Figure 16.3.3). The arguments given support the idea that EROD measurements in juveniles might contribute to a more reliable pollution assessment. An important advantage is that the information derived from juvenile dab may be more reliable to identify areas of concern since juvenile dab are more sedentary than older specimens.

However, a prerequisite for the use of juvenile dab is that the relationships between the Cytochrome P450 activities and the size, age and maturity state of juvenile dab (*Limanda limanda*) should be similar in the different parts of the ICES area and that juvenile dab should be present in sufficient numbers in different periods of the year (Rijnsdorp *et al.*, 1992). In this respect, WGBEC felt that the conduct of pilot surveys in different parts of the ICES area should be held prior to considerations of the use of juveniles for monitoring purposes.

It should be stressed that WGBEC is not in general deviating from its principles that monitoring should be based on integrated measurements of multiple parameters. However, such integrated measurements cannot be performed in small dab for technical and biological reasons (e.g., they are too small for multiple sampling). Any future recommendation on the use of juvenile dab for routine monitoring purposes in the JAMP and elsewhere that might emerge from the current initiatives would therefore have to remain an optional extra.

Reference

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

Recommendation

WGBEC recommends that ICES ACME encourage pilot surveys on the relationships of Cytochrome P450 activities and the size, age and maturational state of juvenile dab (*Limanda limanda*) in the length range 5–9 cm in different parts of the ICES area.

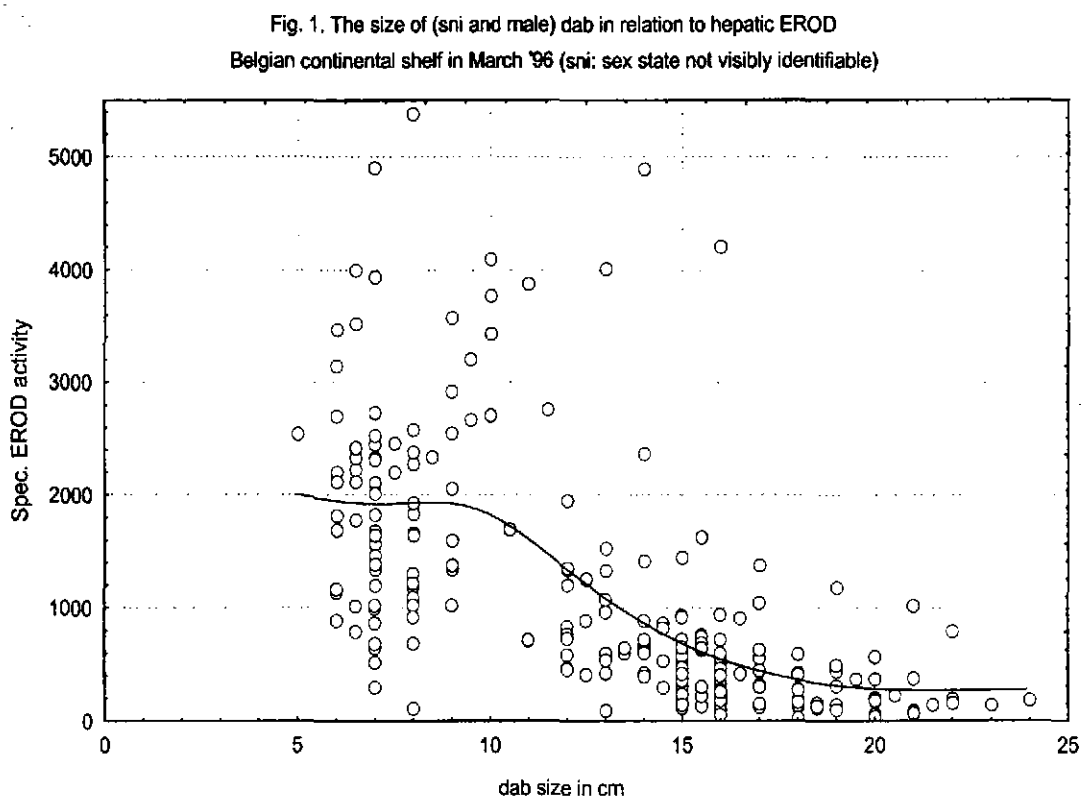
Justification

Juvenile dab possess the highest hepatic EROD level to noise ratio and are considered more sedentary compared to older specimens. For these reasons, juvenile dab may be useful for spatial and temporal trend monitoring purposes. The

proposed pilot studies are needed because WGBEC felt that it is necessary to investigate the relationships of the Cytochrome P450 activities with the size, age and maturational state of juvenile dab throughout the ICES area.

Figures 16.3.1–16.3.4. EROD measurements in Belgian dab (*Limanda limanda*).

Figure 16.3.1



Figures 16.3.2 – 16.3.4

Fig. 2. The size of (shi and female) dab in relation to hepatic EROD on the Belgian continental shelf in March '96 (sni: sex state not visibly identifiable)

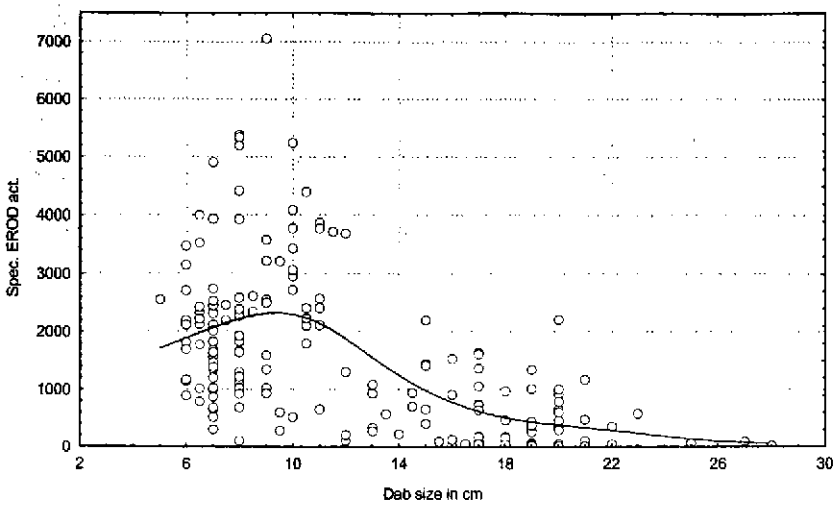


Fig. 3. The ovary size in relation to EROD in dab from the Belgian continental shelf in March '96

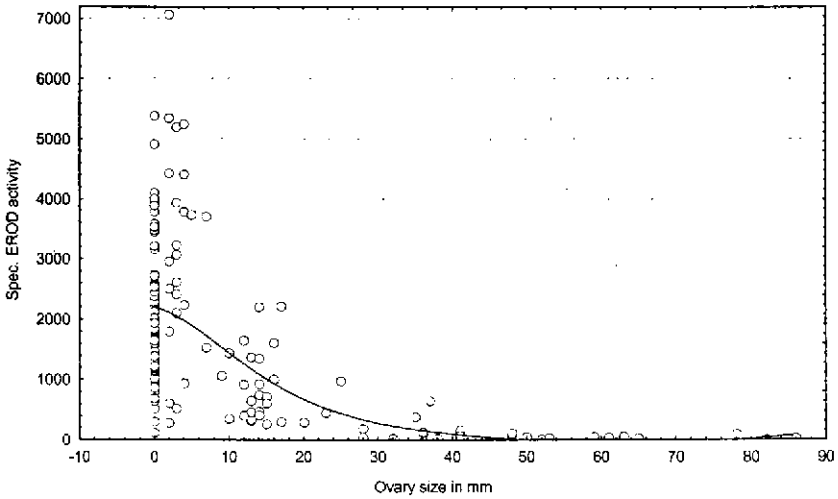
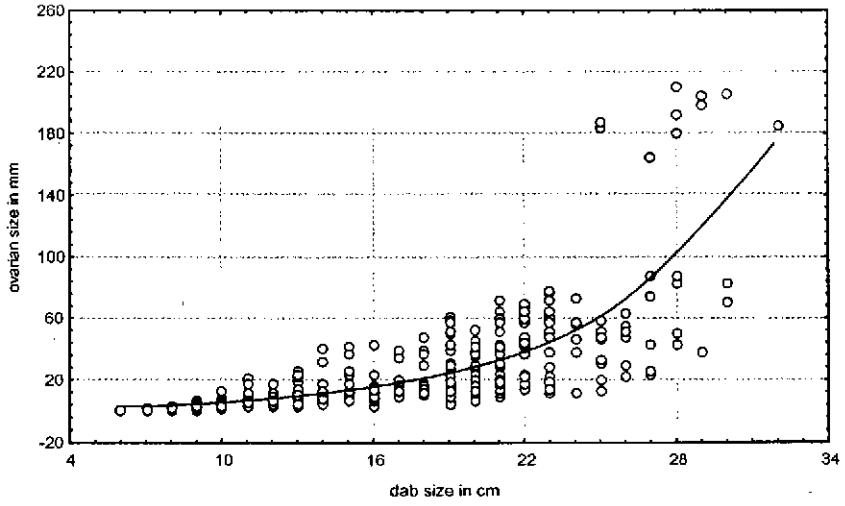


Fig. 4. Relation between animal size and ovarian size of female dab from the Belgian continental shelf



17 RECOMMENDATIONS AND ACTION LIST

17.1 Recommendations

WGBEC considered and adopted several recommendations to ICES; they are appended as Annex 4.

17.2 Intersessional Activities

1) Peter Matthiessen and Dick Vethaak are to collaborate over the acquisition of large data sets, the organisation of a preliminary trial of the Neuro-Fuzzy AI approach, and the arrangements for a collaborative workshop on the topic.
2) Peter Matthiessen is to arrange for the proposed data reporting fields to be commented on by BEQUALM, modified if necessary, and circulated to WGBEC for final approval.
3) Dick Vethaak will investigate possible travel funding for Bart Bosveld to attend the 2000 meeting in Nantes as an expert on bird issues.
4) Peter Matthiessen will contact the WGPDMO Chair (Stig Mellergaard) to confirm that he has no objections to WGBEC working on the relationship of contaminants to invertebrate histopathology. He will then contact Lillemor Svärdh (Tjärno Marine Biological Laboratory, Sweden); Miren Cajaraville (University of Bilbao); Esther Peters (TetraTech Inc., peteres@tetratech-ffx.com); Inke Sunila (Dept. of Agriculture, Bureau of Aquaculture, PO Box 97, Milford, Connecticut 06460); Tim Bowmer (TNO); and Michel Auffret (University of Brest) to invite one or more to attend the 2000 WGBEC meeting in Nantes.
5) Peter Matthiessen will contact Geir Gabrielsen (Norwegian Polar Institute, Tromsø), Robert Roy will contact R.J. Norstrom, K.D. Hughes, Craig Hebert or D.V. Weseloh of the Canadian Wildlife Service, and Thierry Burgeot will contact Florence Cauran (University of La Rochelle) to ask if they are prepared to attend the 2000 meeting in Nantes concerning the item on birds and contaminants. P. Matthiessen will also contact Mark Tasker (Chair of the ICES Working Group on Seabird Ecology) to avoid duplication of activities.
6) Dick Vethaak, Robert Roy, and Rolf Schneider will collaborate on the production of a review on salinity effects on biomarkers, etc.
7) John Thain will take the lead (with Joost Stronkhorst) in developing information for discussion on bioassay approaches to dredged material assessment.
8) Bjørn Serigstad will take the lead in developing information for discussion on in situ bioassays.

18 ADOPTION OF THE REPORT AND CLOSURE OF THE MEETING

The first draft of the report was read by WGBEC and amendments were made. The revised report was adopted, but was subsequently circulated by e-mail in order to add references, etc.

The meeting was brought to a close by the Chair at 12.30 hrs on Friday 16 April 1999. He thanked WGBEC members for all their hard work, with a special thanks to Dick Vethaak and RIKZ for their excellent assistance and hospitality during the week.

ANNEX 1

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ANNEX 2

AGENDA

1) Opening of the meeting

2) Appointment of rapporteurs

3) Adoption of the agenda

4) Consider possible expert systems for the evaluation of monitoring data (follow-on from the joint meeting of WGBEC and WGSAEM

5) Develop suites of biological monitoring methods for use in brackish water systems

6) Evaluate the use of biomarkers and bioassays in the risk assessment of chemicals in effluents and receiving waters

7) Review and report on new methods in molecular biology which could be applied to marine monitoring

8) Update the lists of recommended and promising biological effects monitoring techniques

9) Assist the Secretariat in the development of formats to be used to report data arising from the OSPAR JAMP contaminant-specific or general biological effects monitoring activities [OSPAR 1999/3.1]

10) Review the outcome of the AMAP/EEA/ICES Workshop on Combined Effects in the Marine Environment

11) Review and report on the impacts of specific contaminants of concern

    Report on the UK EDMAR programme

    Report on the Dutch LOES programme

12) Contribute to the ICES strategic plan by helping the Marine Habitat Committee with the following:

    i) formulate tactics to achieve the MHC's six objectives

    ii) suggest or develop activities and products to fulfil the objectives

    iii) estimate the resource required for each activity

13) Consider information on the effects of contaminants on seabirds

14) Review progress in the preparation of papers for the ICES TIMES series

15) Review organisation of a sea-going workshop to study pelagic effects of contaminants

16) Any other business

    Proposal to add measurement of EROD in juvenile dab to the list of measurements for inclusion in the OSPAR JAMP

    Report on the EC BEQUALM programme

17) Recommendations and action list

18) Adoption of the report and closure of the meeting

ANNEX 3

LIST OF MEETING PAPERS

WGBEC/99/1.1	List of Participants.
WGBEC/99/2.1	ICES guidelines for drafting recommendations.
WGBEC/99/3.1	Draft Agenda.
WGBEC/99/3.2	Draft Timetable.
WGBEC/99/4.1	Extract of WGBEC/WGSAEM joint report, concerning artificial intelligence systems.
WGBEC/99/5.1	Effects of estuarine conditions on cadmium toxicity and osmoregulatory performance in fish. Ph.D. Thesis by Wies Vonck, Nijmegen Catholic University, 1999. 96 pp.
WGBEC/99/7.1	Extract from 1995 WGBEC report, concerning molecular techniques.
WGBEC/99/7.2	Molecular methods in biological effects monitoring. Unpublished position paper by Ketil Hylland.
WGBEC/99/7.3	Figure illustrating some aspects of molecular interactions in cells—Ludwig Karbe.
WGBEC/99/8.1	Extract from the 1997 ACME report—the list of recommended and promising biological effects techniques.
WGBEC/99/8.2	OSPAR/SIME draft document on the Coordinated Environmental Monitoring Programme (CEMP).
WGBEC/99/8.3	Marine invertebrate histopathology – a tool to detect effects of pollution. Position paper by Lillemor Svärdh. Submitted by Åke Granmo.
WGBEC/99/9.1	National comments to OSPAR/SIME concerning ICES data reporting formats.
WGBEC/99/9.2	Position paper by John Thain on biological effects data in the ICES database.
WGBEC/99/10.1	Report of the AMAP/EEA/ICES Workshop on Combined Effects in the Marine Environment (excluding annexes).
WGBEC/99/11.1	Report by Kris Cooreman on the outcome of the 1999 meeting of OSPAR/SIME.
WGBEC/99/12.1	ICES Marine Habitat Committee draft Strategic Plan.
WGBEC/99/13.1	Camphuysen, C.J. (1998). Beached bird surveys indicate decline in chronic oil pollution in the North Sea. Marine Pollution Bulletin, 36: 519–526.
WGBEC/99/16.1	EDMAR—a programme of research (Issue 1). 1998. Information leaflet published by the UK Department of Environment, Transport and the Regions.
WGBEC/99/16.2	Matthiessen, P., *et al.* 1998. Oestrogenic endocrine disruption in flounder (*Platichthys flesus* L.) from United Kingdom estuarine and marine waters. Science Series Technical Report No. 107. Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, UK. 48 pp.

ANNEX 4

RECOMMENDATIONS

Category 1

Recommendation for the publication of ICES TIMES papers

In addition to manuscripts already recommended, WGBEC recommends the publication of the following manuscripts in 1999:

1) A sediment bioassay using the polychaete *Arenicola marina* (Thain and Bifield)

2) Biological effects of sediment-bound contaminants on *Corophium volutator* (Roddie and Thain)

3) Radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) techniques for the measurement of marine fish vitellogenins (Scott and Hylland)

Justification

These manuscripts will be ready within the next two months and are urgently needed for the OSPAR JAMP monitoring programme.

Category 2

The **Working Group on Biological Effects of Contaminants** [WGBEC] (Chair: Dr P. Matthiessen, UK) will meet at IFREMER, Nantes, France from 27–31 March 2000 **[provisional dates]** to:

a) hold a joint meeting with WGSAEM (during the preceding week) to consider data sets on biological, chemical, biomarker and biological endpoints, and to use them to explore the minimum differences that can be detected between stations (by univariate methods), to investigate statistical methods for modelling relationships between the variables at each station, and to consider ways of combining the variables into summary indices. [formal proposal can be found in the report of the 1999 joint meeting];

b) consider the outcome of the joint meeting with WGSAEM;

c) consider information on the effects of contaminants in seabirds and discuss this with experts in this field;

d) hear presentations, subject to the agreement of the WGPDMO Chair, on the effects of contaminants on invertebrate histopathology, and to consider whether there is sufficient knowledge on this subject to support a recommendation that invertebrate histopathology could be used for biological effects monitoring in the marine environment;

e) consider a review on the influence of fluctuating salinity on the biomarker and bioassay responses of organisms to contaminants;

f) discuss the biological assessment of dredged materials disposed of in the marine environment;

g) discuss the use of *in situ* bioassays for evaluating the effects of contaminants in the marine environment;

h) receive a final report on progress with the organisation of a sea-going workshop to evaluate methods used in monitoring biological effects in the marine environment;

i) receive a report on the progress of biological effects publications in the ICES TIMES series, and on progress with electronic dissemination of these documents;

j) review the new ICES data reporting formats on biological effects data.

Justifications:

a) There is an urgent need to establish whether the JAMP sampling strategy is adequate for distinguishing differences between biological effects at various sites, and to develop improved statistical procedures for handling such data.

b) WGBEC members not present at the joint meeting will wish to consider the draft report of that meeting.

c) This subject has been unavoidably deferred from 1999.

d) Histopathology of invertebrates may have considerable potential as a monitoring tool, partly because many invertebrates are sessile and therefore reflect the contamination of a particular locality.

e) WGBEC has seen evidence that salinity may act as a confounding factor in biomarker and/or bioassay responses to contaminants, and therefore needs to study this area more closely.

f) Dredged materials are now the only significant materials dumped in the sea, and there is a need to assess their potential impact by bioassay as well as by chemical analysis.

g) *In situ* bioassays have several advantages over those conducted in the laboratory, and experience in their use should be assessed and disseminated.

h) WGBEC will wish to see and comment on final plans for the sea-going workshop which is planned for the spring of 2000.

i) It is important for WGBEC to keep track of progress with the publications it has commissioned.

j) This is to satisfy an OSPAR request.

Category 4

Recommendation for a pilot survey on cytochrome P450

WGBEC recommends that ICES ACME encourage pilot surveys on the relationships of cytochrome P450 activities and the size, age and maturational state of juvenile dab (*Limanda limanda*) in the length range 5–9 cm in different parts of the ICES area.

Justification

Juvenile dab possess the highest hepatic EROD level to noise ratio and are considered more sedentary compared to older specimens. For these reasons, juvenile dab may be useful for spatial and temporal trend monitoring purposes. The proposed pilot studies are needed because WGBEC felt that it is necessary to investigate the relationships of the cytochrome P450 activities with the size, age and maturational state of juvenile dab throughout the ICES area.

Recommendation for a collaborative ICES sea-going workshop on pelagic biological effects methods

There is reason to believe that a practical workshop which brings together international expertise in this area will facilitate the development of biological effects methods for pelagic ecosystems.

WGBEC therefore recommends that an international sea-going Workshop on the Effects of Contaminants in Pelagic Ecosystems should be held for three weeks in spring 2000, to assess the ability of various methods to detect biological effects of contaminants in pelagic ecosystems. The Workshop will be organized by a Steering Group (Chair: Dr K. Hylland, Norway) and will be conducted according to plans developed in detail by the Steering Group. The Workshop will be a joint exercise between ICES, national monitoring organisations, and other interested parties.

Justification

Little is known about the impacts of contaminants on pelagic ecosystems. However, before such impacts can be understood, it will be necessary to develop and validate methods for studying them. This workshop aims to contribute towards that goal, using known gradients of pelagic contamination.

Recommendation for ICES to encourage Member Countries to submit biological effects data to the ICES database

WGBEC recommends to ICES that when the revised data reporting fields for EROD and oyster embryo bioassay measurements have been completed, Member Countries should be contacted and encouraged to re-submit data already in the ICES database, and also to submit existing EROD and oyster bioassay data that have not yet been submitted to the database.

Justification

There are considerable amounts of biological effects monitoring data held by ICES Member Countries which have not been submitted to the ICES database. These are required to allow assessment of temporal and spatial trends in biological effects. Existing data should be re-submitted in the new formats so that they can be compared with new data.