Advisory Committee on the Marine Environment

ICES CM1996/Env:3

REPORT OF THE

JOINT MEETING OF THE WORKING GROUP ON MARINE SEDIMENTS IN RELATION TO POLLUTION AND THE WORKING GROUP ON BIOLOGICAL EFFECTS OF CONTAMINANTS

Ostend, Belgium

29 February 1996

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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1 OPENING OF THE MEETING

The joint meeting of the Working Group on Marine Sediments in Relation to Pollution and the Working Group on Biological Effects of Contaminants (JMSBEC) was opened at 10.00 hrs on 29 February 1996 by the Chairman, Dr I.M. Davies. Dr Ir. W. Vyncke welcomed the participants on behalf of the Rijksstation voor Zeevisserij in Ostend, Belgium.

2 ADOPTION OF THE AGENDA

The terms of reference (C.Res.1995/2:14:5) for the meeting are listed below:

A Joint Meeting of the Working Group on Marine Sediments in Relation to Pollution and the Working Group on Biological Effects of Contaminants will be held under the chairmanship of Dr I. Davies (UK) from 29 February to 1 March 1996 in Ostend, Belgium to:

a) review and report on new information and hypotheses concerning factors controlling the availability to marine organisms of contaminants in sediments;

b) review and report on factors controlling the release of contaminants from sediment, and the implications of this for assessment of the relative importance of different exposure routes to marine organisms;

c) begin the development of guidelines for monitoring based on the strategy outlined in the 1994 report of the joint meeting of WGBEC and WGMS;

d) prepare recommendations on the integration of biological and chemical measurements on sediments in relation to the objectives of AMAP;

e) review progress with the research proposal included as Annex 5 of the 1994 Joint Meeting Report (ICES, CM 1994/Env:2) concerned with investigating linkages between contaminants in sediments, bioaccumulation and biological effects, and receive and comment on other proposals for collaborative activities in similar and related subject areas;

f) review and comment on reports of new initiatives and results of field and experimental investigations linking sediment chemistry and biological effects studies;

g) review and comment on further progress in the development of chronic and sublethal sediment bioassays.

The draft agenda was accepted without amendment, and is appended as Annex 1. The list of participants is attached as Annex 2. The list of meeting papers is attached as Annex 3.

3 ARRANGEMENTS FOR THE PREPARATION OF THE REPORT

The Chairman reminded the meeting that it was now ICES policy for meetings to complete and approve their reports before the end of the meeting. This is necessary to meet the tight series of deadlines leading up to the ACME meeting in the early summer. Word processing, typing, and copying facilities were available for the participants to assist in the completion of the report.

4 REPORTS OF ACTIVITIES IN OTHER FORA OF INTEREST TO THE JOINT MEETING

4.1 SIME 1996

Ron Stagg presented a report on the OSPAR/ICES Workshop on Biological Effects Monitoring Techniques (Aberdeen, 2–6 October 1995) and the subsequent adoption by the 1996 meeting of the OSPAR Working Group on Concentrations, Trends and Effects of Substances in the Marine Environment (SIME 1996) (Oslo, 22–26 January 1996) of this report. The programme addressed issues of analytical quality control and statistical design of the programmes, for both a general biological effects programme and also for contaminant-specific programmes for PAHs, heavy metals, and TBT. A central theme of the programme was the adoption of biological effects techniques and chemical analysis forming a series of interconnecting measurements linking different biological hierarchies and severity of effect. The essential elements of the programmes are given in the table below.

Progr	Biological Effects	Chemistry
PAHs	P4501A Bile PAH metabolites DNA Adducts Liver Pathology	Sediment PAHs
Metals	Metallothionein (Cu, Cd, Zn) ALA - D inhibition (Pb) Antioxidant enzymes	Sediment metals Liver Cu, Cd, Zn Blood Pb
TBT	Inter/Imposex in gastropods Shell thickening in *Crassosterea*	Tissue for TBT
General	Bioassays - whole sediment - pore water - water column Biomarker - P4501A Lysosomal stability Liver pathology Liver nodules Population/Community responses External fish diseases Fish reproductive success Macrobenthic fauna	Not specified but will include chemical tracing of cause

At SIME 1996 it was decided that the work would be progressed by:

- developing the quality assurance (QA) programme;
- incorporating guidelines into the OSPAR Joint Assessment and Monitoring Programme (JAMP) manual;
- integrating the chemical and biological guidelines.

It was agreed that QA was to be developed under the auspices of ICES and that the next meeting of the OSPAR Ad Hoc Working Group on Monitoring would address the guidelines issues.

4.2 Ad Hoc MON Meeting 1995

T. Nunes gave a brief summary of the outcome of the last meeting of the OSPAR Ad Hoc Working Group on Monitoring (MON 1995). The meeting took place at ICES Headquarters in Copenhagen from 13–17 November 1995 under the chairmanship of the Vice-Chairman of SIME, Mr Norman Green, from Norway. The purpose was to revise the current Joint Monitoring Programme (JMP) monitoring guidelines, with regard to the principles and methodology of the new JAMP.

MON 1995 prepared and agreed on draft monitoring guidelines for most of the topics in the terms of reference, including, in many cases, detailed guidance in draft Technical Annexes. The short time available at meeting did not allow for them to be finalized.

MON 1995 considered that further harmonization work was required, with regard in particular to the final structure, and to statistical and quality assurance aspects. In order to do this, MON 1995 considered it essential to propose a follow-up MON meeting in the end of 1996, and to carry out intersessional work prior to this meeting. Contracting Parties of OSPAR were asked to send their comments and suggestions to the main contact points under the scheme already used to prepare MON 1995. The proposal, together with the terms of reference for the meeting and the schedule for intersessional work, was forwarded to SIME 1996, which in turn made a recommendation to the 1996 meeting of the OSPAR Environmental Assessment and Monitoring Committee (ASMO 1996), scheduled to meet in the beginning of March, for such a meeting to take place according to the proposed terms.

MON 1995 also considered the development of statistical objectives to be a matter of priority in the guidelines. Pending the adoption of these objectives and, in consequence, the finalization of the new guidelines, Contracting Parties of OSPAR will continue with their existing monitoring activities. It was emphasized that monitoring activities under JAMP, and the new guidelines, are not intended to be used for the Quality Status Report (QSR) 2000 only (there will be no time for their implementation!), but further beyond it.

Included in this finalization process, MON 1995 made a proposal to SIME 1996, who forwarded it to ASMO 1996, that some items be included in the Draft ICES Work Programme for 1997, namely matters related to temporal trend studies in sediments and normalization techniques, as well as relevant QA activities in relation to biological affects monitoring.

Other matters included in the MON 1996 proposed terms of reference were to develop further the general biological effects monitoring programme, in the light of the outcome of the OSPAR/ICES Workshop on Biological Effects Monitoring Techniques held in Aberdeen in October 1995, and to prepare guidelines for the proposed integrated chemical and biological monitoring programme.

In relation to TBT, MON 1995 made a proposal to SIME 1996, which was endorsed, that the methods for chemical analysis for TBTs in biota and sediments should be decided upon by the ICES Marine Chemistry Working Group (MCWG) (that already had this item on its agenda for the 1996 meeting), and that all the analyses should be carried out by laboratories capable of doing them to appropriate quality standards.

Finally, an informal working group met during MON 1995 to discuss the possibility for coordinating the testing of different sampling strategies among Contracting Parties of OSPAR. Several points were raised, among others, that:

1) more cost-effective sampling strategies were needed;

2) it would be good to obtain more information to further improve or decide on a change of guidelines;

3) new sampling strategies need to be tested, and it would be advisable to run them in parallel to the existing ones for 3–4 years (this would prevent "breaking points" in the data sets);

4) if an internationally coordinated effort to develop consensus on this is agreed, the work should start now.

A plan called Voluntary International Contaminant Monitoring (VIC) was proposed to take place under the coordination of the Chairman of MON. It was agreed that it should be undertaken on a voluntary basis, be simple, accommodate different sampling strategies that may vary from site to site, deal with biota and/or sediments, and have a commitment by the participants to follow the selected guidelines consistently, and to follow the selected strategy for at least three years.

Several countries were willing to participate, and the proposal was forwarded to SIME 1996, who endorsed it, agreeing that the results of VIC should be taken into account in the future development of JAMP. It also agreed that the programme plans and its results should be reviewed by ICES, and that the Chairman of MON will report to SIME 1997 on VIC progress.

5 AVAILABILITY OF CONTAMINANTS IN SEDIMENTS TO MARINE ORGANISMS

a) Influence of rainfall on inputs of contaminants

Åke Granmo reported that flood events in central Europe in January 1995 were followed by an investigation along the Swedish west coast in order to detect pesticides or herbicides which may have been transported by the flood water. Water samples, plankton, sediment trap material and common mussels were analysed. However, none of these compounds were detected. A system with passive samplers (SPMDs) was used in parallel with traditional sampling techniques and showed elevated concentrations of DDTs and PCBs at the first part of the total exposure time (March–June). This finding coincided with the distribution of flood water, as seen on satellite photographs.

In spite of the lack of detectable levels of pesticides and herbicides in water, plankton, mussels and sediment trap material, the SPMDs indicated the presence of different pollutants in sea water along the Swedish coast. The time series of samples analysed give a clear indication of increased concentrations of pollutants in the water masses during the first part of the exposure period (March–April 1995). The coincidence between raised levels of pollutants and the arrival of a water mass identified as diluted flood water, indicates that the flood event in Europe has contributed to the pollution of Skagerrak.

b) Interaction of eutrophication and contamination

Dr Jonsson presented some new results from the Baseline Study of Contaminants in Baltic Sea Sediments 1993. Since the 1970s, there has been a substantial decrease of PCB concentrations in biota, which has been interpreted as reflecting a decreased load, due to the ban on PCB use in the Baltic Sea area. The sediments, however, show a contradictory trend with increasing concentrations towards the sediment surface, suggesting an increased load of PCBs in the Baltic Sea. This is in agreement with the hypotheses of the recently launched EUCON project (Interactions between eutrophication and contaminants), that increased eutrophication may alter the distribution of organochlorine compounds in the ecosystem. Increased primary production will increase the sedimentation and may subsequently lead to a scavenging of chlorinated compounds from the water mass to the sediment. These, so far, preliminary results emphasize:

1) that trends in contaminants should not be interpreted without considering changes in the eutrophication situation;

2) the importance of monitoring contaminant concentrations not only in biota but also in sediments.

c) Influence of speciation of contaminants in aquatic systems on bioavailability

Dr R. Blust presented an account of his studies of the factors affecting the availability of contaminants in aquatic systems to biota. His work was based upon reliably calculating the speciation of contaminants in water (using cadmium as an example), taking account of the effects of changes in temperature, salinity, pH, major ion concentrations, etc., in aquatic systems from fresh water to fully marine. He had been able to account for over 90% of the variability of uptake of cadmium by shrimps through the thorough description of the speciation of the element.

There was some discussion of whether the approach he described could be adapted to address the bioavailability and accumulation of contaminants from sediments, rather than from the dissolved phase. It was concluded that theoretically there was no reason for not extending the process to contaminants in sediment. However, it would be necessary to have good data on the equilibrium (and kinetic) constants governing the exchange of contaminants between dissolved and solid phases, and of the interactions between contaminants and pore waters, which may show considerable differences (e.g., in sulphide, dissolved organic material, pH, etc.) from typical seawater conditions. As in many cases the values of these constants are not well known, the meeting concluded that whilst the approach showed considerable potential for assessing the availability of contaminants in sediments, it would be unlikely to provide a readily applicable procedure in the near future.

d) Influence of organisms on availability of metals in sediment

Lysosomal sequestration and granule formation in invertebrates: implications for bioavailabilty and toxicity of metals and organic chemicals in sediments.

Many invertebrate animals, such as molluscs and crustaceans, accumulate toxic metals and organic chemicals (xenobiotics) in intracellular granules (see figures in Annex 4 and reviews by Moore, 1990, and Nott, 1993). This process can be considered to be a detoxification mechanism since the granules can be excreted or else incorporated into faecal pellets. Production of these granules often involves the intracellular system of membranous vesicles known as the endocytic-lysosomal system. Metals are sequestered in mineralized granules as sulphides or phosphates, or else are bound to lipofuscin in tertiary lysosomes (residual bodies). If eaten by an invertebrate predator, these granules can apparently pass unchanged through their digestive system (Nott and Nicolaidou, 1994); this may not necessarily be the case with predatory fish. The granules are highly refractory and once shed in faeces or excreta will form a stable component of the sediment geochemistry (Nott, 1993).

Lipofuscin formation is frequently an important part of the sequestration and detoxification process. This pigment is also known as "stress pigment" or "ageing pigment", and is an end-product of protein and lipoprotein membrane breakdown by the process of autophagy (self-eating), which is the major pathway for the degradative part of protein turnover. Autophagy is generally enhanced as a response to environmental stress. Since lipofuscin is a peroxidative end-product of degradation of autophagocytosed cellular membranes, it probably has many ligand-binding sites, not only for metals but also for organic chemicals. The potential for binding these latter has not been properly assessed. However, lysosomes (including residual bodies) are noted for their ability to accumulate many different classes of organic xenobiotics, including PAHs, organohalogens, heterocyclics, nitroaromatics, sulphonated aromatics and azo-dyes (see review by Moore, 1990). This is, therefore, an important pathway for detoxification of organic contaminants, since many of these latter are not effectively metabolized by invertebrates. It is not known whether the xenobiotics remain bound to lipofuscin in the form of refractory granules, as with the metals, or are released into the pore water once they are shed from the animals.

These considerations may play an important role in determining the bioavailability and subsequent toxicity of both metals and organic xenobiotics in the sediment, since excreted and faecal granules will form a significant part of many estuarine and marine sediments. It is recommended that chemical analyses of sediment should take account of this factor as it will be important in determining the speciation of metals, as well as the physical-chemical speciation of organic xenobiotic contaminants.

Finally, the human health risks of consuming shellfish, particularly molluscs and crustaceans, that contain high concentrations of metals and/or xenobiotics within intracellular granules and vesicles have not been assessed. The effects of the high acidity of the human stomach (gastric) fluid on the chemistry of the granules from the ingested tissues of the shellfish may be an important factor here.

Moore, M.N. 1990. Lysosomal cytochemistry in marine environmental monitoring. Histochem. J., 22: 187–191.

Nott, J.A. 1993. X-ray microanalysis in pollution studies. *In*: X-ray microanalysis in biology: experimental techniques and applications. (Eds. D.C. Sigee, A.J. Morgan, A.T. Summer and A. Warley), pp. 257–281. Cambridge University Press, Cambridge.

e) Influence of organic enrichment on metal availability

Ketil Hylland presented a paper on the "Influence of organic enrichment and low oxygen levels on partitioning, bioaccumulation and effects of sediment-bound Cd and Hg" on behalf of himself and Morten Schaanning, John Arthur Berge, Dag Ø. Eriksen, Tone D. Selnæs, Jonas Gunnarsson, Mattias Sköld and Jens Skei.

Sediments in coastal areas commonly receive elevated inputs of both organic material and contaminants. The objective of this study was to clarify to what extent eutrophication effects will affect the partitioning and bioaccumulation of Cd and Hg.

An experimental system was established using glass aquaria (285 mm x 465 mm) with a continuous water supply. The experiment included three treatments: addition of organic material (40 g C/m^2 *vs.* none), addition of the contaminants Cd, Hg and benzo(*a*)pyrene (respectively, 7, 5 and 1 mg/kg dry sediment *vs.* no addition) and oxygen in water (2–3 mg/l *vs.* normal), resulting in eight different treatments. Tracers were used to simplify analyses and lower detection limits (^{109}Cd, ^{203}Hg and ^{14}C-B(*a*)P). There were three replicate aquaria for each treatment. Into each aquarium, individuals of three sediment-dwelling organisms were added: the brittle star *Amphiura filiformis*, the polychaete *Nereis diversicolor* and the bivalve *Abra alba*. In addition, blue mussels were kept in the outflowing water from each aquarium. The experiment lasted for 90 days with sediment samples being taken at the start and end, and biological samples at the end. Pore water was extracted by centrifugation immediately following sampling. There was a continuous logging of sulphide in the sediment and overlying water, daily measurements of oxygen and flow of incoming water, and weekly measurements of pE.

Although substantial amounts of organic matter were added there were only minor increases in TOC, TN or TC between treatments (10–15%). Also, there were no obvious changes in either pS or pE in any of the treatments. There were low mortalities of sediment-dwelling organisms and none of the treatments appeared to affect their behaviour. Sediments sampled at the end of the experiment were not toxic in a 96-hour toxicity test (*Nitocra*). The two metals behaved differently in the sediment: there were significantly higher concentrations of Cd in the pore water of the most "oligotrophic" treatment (low organic, high oxygen) than in the other treatments. Cd concentrations were lowest in the most "eutrophic" treatment (high organic, low oxygen). Sediment levels of Cd in the top layers supported a larger loss of Cd from the most "oligotrophic" treatment. In contrast, Hg was not detectable in pore water and there were no differences between treatments with regard to sediment levels.

The surface-sediment feeding bivalve *Abra alba* accumulated one order of magnitude more of both metals in treatments with organic material added. A similar pattern was observed for *Nereis diversicolor*, but with less marked increases. There were no apparent differences between any treatment regarding the accumulation of Cd or Hg in *Amphiura filiformis*. Concentrations of Cd were significantly increased in blue mussels kept downstream of the most "oligotrophic" treatment compared to the other treatments, corresponding to elevated levels in the pore water of those aquaria. The observation that Cd in mussels could derive from water-borne metal was supported by the fact that levels of Cd in pseudofeces from the mussels were below the detection limit.

Growth in *Abra* and *Amphiura* was only affected by the addition of organic matter; low oxygen levels or the presence of contaminants did not appear to have any effect. A similar result was found for arm-regeneration in *Amphiura*. Biomass change in *Nereis* was however also found to be affected by low oxygen levels and contaminants in addition to organic material. Biomarkers (glutathione S-transferase, metallothionein) in the three sediment-dwelling species did not appear to reflect the presence of contaminants, but to some extent related to growth.

6 FACTORS CONTROLLING THE RELEASE OF CONTAMINANTS FROM SEDIMENTS

This agenda item was amalgamated with agenda item 5, discussed above.

7 DEVELOPMENT OF MONITORING GUIDELINES

To begin the development of guidelines for monitoring based on the strategy outlined in the 1994 report of the joint meeting of WGBEC and WGMS, a subgroup was formed consisting of Ron Stagg, Mike Moore and Teresa Nunes.

The subgroup considered the three monitoring programmes developed at last year's meeting and summarized in Tables 1, 2, and 3, respectively, in last year's report (reproduced in Annex 5). It was decided that the question of guidelines for the biological components in these programmes was best considered in the meeting of the Working Group on Biological Effects of Contaminants (WGBEC) and that for most of the techniques this task was already included on the work programme of WGBEC. It was noted that the programmes were similar to those proposed by the Aberdeen workshop on biological effects, where methods and guidelines have already been specified. It was therefore decided to focus on the question of the unification of the guidelines for monitoring contaminants with the requirements for a combined contaminant/biological effects programme. Two types of integrated (chemical and biological) programmes exist–those which are targeted towards identifying the impacts of specific chemicals (the bottom-up approach) and the converse, i.e., those aimed at identifying the chemical cause of observed effects (the top-down approach). The guidelines requirements of these two approaches are different.

1. Guidelines for identifying the biological effects associated with specific contaminants

Heavy metals and organic contaminants in sediments

Information on sediment levels of contaminants is required for the identification of sites where biological effects measurements will take place and for the interpretation of the chemical causes of observed effects. To identify areas where effects measurements will be carried out, criteria need to be established for choosing stations or sites for investigation. This would necessitate identifying gradients or areas of high or low levels of contamination.

Four approaches for this were identified:

1) Using local knowledge or expert judgement, e.g., in relation to inputs, known areas of contaminant or sediment deposition, hydrography, etc.

2) The pragmatic approach–simply selecting a proportion of all sites for which chemical data exist, e.g., carry out biological effects measurements at the highest 5% of sites.

3) Comparison with the ecotoxicological reference criteria.

4) Comparison with the background concentrations.

Several points were made:

- Different areas have unique features which may require different sampling designs.

- Work is ongoing in OSPAR fora in relation to the development of both background levels and ecotoxicological assessment criteria. The development of ecotoxicological assessment criteria is at too early a stage to be used sensibly. Background concentrations can be used in certain areas but international agreement is difficult.

- Although monitoring guidelines under the OSPAR Joint Monitoring Programme (JMP) were well established and the guidelines for JAMP are in the process of being finalized under MON there are some activities under the auspices of several ICES and OSPAR groups (MCWG action on sediments, VIC, etc.) which seek to improve the guidelines so that there is more cost-effective use of resources and so that guidelines can be better focused to the testing of hypotheses and the statistical aspects of programme design.

- QA matters are very important for both the chemical and the biological effects techniques. QA for some of the biological effects measurements is at an early stage of development. Surprisingly, for the chemical programmes in relation to organic determinands, between-laboratory coefficients of variation when all monitoring laboratories are considered are still extremely high, particularly for measurements in sediments (e.g., for PAHs, CBs, pesticides, and planar CBs). QA for metals is satisfactory.

- Normalization of contaminant levels in sediments. For various chemical programmes there is a continuing debate on the question of normalization of sediments for monitoring purposes. In the case of the relationship to biological effects, the total sediment load of contaminants should be determined. The use of normalization in relation to effects (for example, as a measure of the most available fraction) is considered to be a research priority, but is not a requirement for monitoring at present because the significance of such normalization is not understood.

- Factors relating to the nature of the sediment, such as total organic carbon and particle size distribution, are important for interpreting both chemical and biological data.

Taking all this into account, the subgroup decided that the total fraction of the sediment should be analysed for biological effects purposes and that additional information such as particle size distribution and organic carbon content should be reported for interpretation of the data.

Heavy metals and organic contaminants in biota

Some basic principles were developed, as follows:

- Wherever possible, the chemical determination should be performed on the same tissue compartment as the effects measurement. For example, the measurement of biomarkers or pathology in liver should be accompanied by chemical measurements in liver.

- Measurements in other body compartments may be made, but the relationship to contamination in the tissues used for the biological effects measurements should be demonstrated.

- Total tissue levels of contaminants should be determined without normalization to factors such as total lipid.

- Factors which influence the variability and levels of both the contaminants and the biological measurements should be reported. These would include variables such as lipid content, sex, degree of sexual development, stage in the reproductive cycle (gonadosomatic index (GSI)), age, season, temper-rature, etc.

- Particularly in the case of biological effects which may have latent periods (e.g., tumour formation and carcinogenesis), fish of different ages may need to be compared.

- In biomarker studies, analyses are carried out on individual organisms and this requires that chemical measurements are also carried out in the same way when the detection limits allow. Thus, for non-planar CBs, heavy metals, PAH metabolites in bile, and organochlorine pesticides it should be possible to conduct individual analyses, but for planar CBs and dioxins this would not be possible. The advantage of individual analysis is that it allows chemical interpretation of biological effects (e.g., by the establishment of concentration-response relationships) and will also allow better interpretation of chemical data (real population variance can be measured). The disadvantage is the extra cost and time taken for analysis.

TBT

For biological effects associated with TBT contamination, analyses of the levels of TBT in pooled homogenized soft parts of biota (gastropods or *Crassostrea*) have been recommended at the Aberdeen workshop on biological effects monitoring. This is consistent with the guidelines developed by MON and MCWG for chemical monitoring.

2. <u>Guidelines for identifying the chemical cause(s) of observed effects</u>

Chemical measurements for such monitoring are complicated by the fact that the contaminants responsible for the observed effects may not be known. Nevertheless, some broad principles were identified:

- Identical components should be used for the chemical and the biological effects measurements. For example, in whole sediment bioassays, the levels of contaminants in the total sediment should be determined.

- Chemical tracing techniques may be required which might involve fractionation techniques.

- No standard suite of contaminants will necessarily be appropriate.

- Methods to measure the diversity of chemicals and the complex interactions between chemical species need to be developed.

8 INTEGRATION OF BIOLOGICAL AND CHEMICAL MEASUREMENTS FOR AMAP

A subgroup was formed, consisting of Ketil Hylland, Doug Loring and Ian Davies, to address the question of the integration of biological and chemical measurements on sediments within the Arctic Monitoring and Assessment Programme (AMAP). The subgroup examined the 1995 modification of the AMAP Monitoring Programme, and the report from the 1993 Audit of the programme (AMAP 93:5).

It appeared to the subgroup that the AMAP programme tended to be a compilation of a wide range of projects directed at various aspects of the Arctic environment, but that there could be considerable benefits to the participating countries through the greater integration of chemical and biological effects measurements in the marine component of AMAP. The conceptual framework for integrated monitoring developed by JMSBEC at its meeting in 1995 applies equally well to the marine component of AMAP and to the JAMP. Coordination of chemical and biological approaches can add greatly to the ability of the programme to address the biological significance of the chemical measurements, and conversely to interpret biological effects measurements in terms of underlying chemical causes. However, to be effective, such a programme needs to be directed at agreed issues of concern. These should be expressed in terms of concern over contamination levels or concern over observed effects on biota.

The documents available to the subgroup stated that "there are many methodological difficulties associated with biological effects monitoring", but encouraged the use of a limited range of techniques in support of chemical monitoring activities. Recent developments within ICES and the OSPAR systems have indicated that a range of biological effects techniques are now available for application within coordinated international monitoring programmes. It would therefore be appropriate for AMAP to include such methods in their programme, particularly where they are relevant to recognized issues of concern.

The AMAP documents did not specifically state the important issues of concern in the Arctic from a contamination point of view. However, the subgroup took the lists of essential determinands as indicating the main concerns. This list included Cd, Hg, Se, and Pb as inorganic contaminants, and PAHs, DDTs, PCBs (not planar), HCB, and HCH as organic contaminants. These substances should be determined in sediments and in a wide range of species, including four essential species (blue mussel, polar cod, ringed seal, glaucous gull).

The subgroup considered that the identification of priority contaminants and priority species offered considerable potential for the effective integration of chemical and biological effects measurements. Within the ICES/OSPAR system, the process leading from the conceptual framework of integration, through a hierarchical approach to monitoring of chemicals in key components of the environment, to molecular level responses of organisms, and subsequently effects at higher levels of organization have been described in the reports from JMSBEC 1994 and 1995, and from the OSPAR/ICES Workshop held in Aberdeen in October 1995. These documents provide a model on which the AMAP could develop a structured approach to obtaining well-targeted data addressing primary causes of concern. It was noted that MT and EROD were included in the programme, but were not used in conjunction with other biological effects measurements in an integrated way.

JMSBEC is primarily concerned with the interactions of contaminants in sediments and biota. None of the essential monitoring organisms within AMAP are strongly associated with sediment. This is in contrast to OSPAR programmes which include flatfish (e.g., dab, flounder) as organisms which may respond to conditions in the sediment. It is therefore unlikely that biological effects or concentrations of contaminants in the essential species will be clearly relatable to contaminant levels in bottom sediments. Loring *et al.* (1995, Marine Geology, 128: 153–167) recently reported on sediment chemistry from the Pechora Sea, in relation to sediments from very large areas of the Arctic Seas. He concluded that the concentrations of most metals (for example, mercury, lead and cadmium) in these sediments are not elevated above background levels. Furthermore, he reports that the concentrations of PAHs, PCBs, and DDTs are also not elevated above those found in the Barents Sea and the northern North Sea. It therefore seems unlikely that transfer of these contaminants from sediments is a significant cause for concern in the Arctic.

However, Loring *et al.* did report elevated concentrations of radionuclides derived from weapons testing, and associated with these there were high concentrations of arsenic. The chemical form and biological activity of this arsenic is not known, but is worthy of investigation.

Loring *et al.* also reported elevated concentrations of lindane (HCH isomer) over wide areas of the Arctic sediments. The degree to which this may be transferred to organisms is not clear.

JMSBEC was led to believe that there is concern over the concentrations of both inorganic and organic contaminants at high trophic levels (birds, seals and other mammals). In contrast, there are no clearly identified ecological concerns over these contaminants in blue mussels or fish (polar cod). In order to directly address the primary biological issues of concern, JMSBEC recommends that the structured hierarchical approach to integrated monitoring described in the documents indicated above should be applied to the ringed seal and glaucous gull. These documents are primarily concerned with work in fish, however, it was considered that the approach can be transferred to gulls and seals, bearing in mind inter-species differences in the strategies for storing, detoxifying and eliminating contaminants.

In preparing the plan for such a programme, it will be necessary to ensure that adequate chemical and biological effects measurement expertise is available.

9 REVIEW OF RESEARCH PROPOSALS

A number of plenary sessions and other meetings considered the way forward with the research proposals. EQAMAS had been graded B by the EU, and had not been funded. It was agreed to develop a proposal based around the significance of PAHs in sediment, and an outline is given in Annex 6.

10 NEW FIELD TECHNIQUES LINKING SEDIMENT CHEMISTRY AND BIOLOGICAL EFFECTS STUDIES

This item was included in item 5, above.

11 CHRONIC AND SUB-LETHAL SEDIMENT BIOASSAYS

This matter was deferred to the separate WGBEC meeting, due to the absence of appropriate members of the joint meeting.

12 ANY OTHER BUSINESS

a) Genetic techniques

Workers at the University of Brussels reported the use of two genetic techniques to study the DNA breakage by mutagens which may be linked to genotoxic effects in fish (Brown trout): the classic micronucleus assay, and the alkaline (comet) single-cell gel assay (see Annex 7).

The micronucleus test has already been used in genotoxicology studies with fish (Hooftman and de Raat, 1982; Hooftman and Vink, 1981; Das and Nanda, 1986; Al Sabti, 1986a, 1986b, 1992, 1994; Metcalfe, 1988; De Flora *et al.*, 1993; Bahari *et al.*, 1994; Highes and Herbert, 1991).

The comet assay was first introduced by Singh and co-workers in 1988. This easy and sensitive technique is capable of detecting single-strand DNA breaks, any lesion capable of being transformed into a single-strand break at the alkaline pH used (i.e., alkali labile sites), DNA crosslinks (Tice, 1994) and incomplete excision repair events (Gedik *et al.*, 1992; Green *et al.*, 1992). This assay has already proven its usefulness in the area of genetic toxicology and environmental biomonitoring (for reviews see Fairbairn *et al.*, 1994, and Pandrangi *et al.*, 1995).

The technique has only been used intensively during the last five years and there is no quantification of the breaks yet possible. Single-stranded and double-stranded breaks can be identified by using the neutral and the alkaline protocols, respectively. So far the main parameters used to interpret the results of the comet assay are the tail length (measurement for the migration distance of the DNA resulting from a break), and the tail moment (= tail length x relative DNA content of the tail).

In Prague last summer the participants in the meeting of the Environmental Mutagen Society agreed to evaluate and standardize the technique. The results will be presented at their meeting in the United States in March 1996.

Many research groups realize the usefulness of this sensitive technique and are evaluating it for monitoring purposes (Pandrangi *et al.*, 1995).

In the laboratory at the University of Brussels, the technique has been applied on human lymphocytes, rat hepatocytes, fish erythrocytes, and also worm coelomocytes (*Nereis virens*).

So far results have been obtained on two mutagens, namely, a well-known mutagen ethylmethanesulfonate (EMS) and a planar CB (IUPAC number 77). EMS showed a clear time and dose-dependent response in the comet assay but no response at all in the micronucleus assay. CB 77 did not induce tails in the comet assay, nor micronuclei in the micronucleus test.

Discussion

1) Dr Moore drew attention to the problem of apoptosis when measuring DNA breakage. In particular, worm coelomocytes and invertebrate haemocytes play a very important role in the immune system, and phagocytosis of apoptotic fragments of cells could lead to an artifactual indication of DNA breakage. So we have to be very careful with the interpretation of the results of the comet assay.

2) A participant from Ghent agreed that genetic techniques can be very valuable as a component in a battery for monitoring.

3) Dr Moore mentioned that it would eventually be useful to use liver, instead of blood, because it is more likely to expect damage in liver than in blood, because the liver is metabolically very active. It would indeed be possible to use the liver in the comet assay; however, this is a destructive technique.

4) The participants agreed that the comet assay would be useful to screen for mutagenicity of sediments (e.g., by using polychaetes).

The abstract of a recent publication concerning the application of these techniques is given in Annex 6.

b) COST programme

Ian Davies reminded the meeting of the recent developments within the OSPAR system (i.e., the Aberdeen Workshop, SIME meeting, and MON 1995), all of which had considered aspects of the development of an integrated chemical/biological approach to monitoring activities to address particular issues of concern. Through these meetings, the general shape of the proposed programme had emerged, together with a clear recognition of the need for adequate dissemination of information concerning measurement techniques, training in these techniques, and development of necessary Quality Assurance procedures. Further progress was planned for the 1996 WGBEC and MON meetings.

The EC COST (Coordination of Science and Technology) programme offers an opportunity to obtain funding for the coordination of established research activities under way in several European Union (EU) countries. The research work is supported by national funds, but COST can provide support for coordination meetings, Workshops, study visits, etc. It therefore appeared that COST might be an appropriate vehicle for support of the Workshops and other activities required to progress the proposed new environmental assessment programmes. Ian Davies proposed to draft an initial version of a COST proposal over the next few days, and to bring it to the individual Working Groups for comment/endorsement. It would be necessary at some stage to formally obtain expressions of interest from relevant laboratories, and to create an interim Steering Group to finalize the proposal, and to undertake any revisions of the proposal that might be necessary.

Dr Moore enquired whether there could be a role for the Intergovernmental Oceanographic Commission (IOC) in this proposal. After discussion, it was agreed that there could well be benefits in IOC involvement, perhaps giving the group access to expertise in effects and chemical techniques from laboratories outside the EU and ICES areas. This could be of considerable assistance in some areas, for example, the development of QA procedures for some complex techniques. Dr Moore thought that IOC might well be interested in this form of kick-start support, and had already expressed agreement with the principle of closer integration of chemical and biological procedures. IOC might also be interested in using the structures which might be created under COST to provide training for scientists from countries outside the EU.

Dr Stagg reported that he had discussed with EC representatives at SIME the possibility of establishing a QUASIMEME-like project to address the Quality Assurance of biological effects measurements in the OSPAR monitoring programmes.

c) Definitions related to biological effects of contaminants

Both the Paris and the Helsinki Conventions contain a number of definitions making clear what exactly is meant. However, various terms are used in a very general way.

This is the case for most of the terms related to biological effects of contaminants, which are considered as having the potential to result in harm to living resources and/or marine ecosystems and which may have an impact on human health through the marine environment. The meeting considered that there may be an interest among Contracting Parties to have an agreement on how the effect-related terms are to be understood for the implementation of the provisions of the Conventions, including the technical appendices and annexes. For various aspects the definitions should probably be stated in a mechanistic and to be practicable, in an operational way, referring to existing protocols and guidelines.

The JMSBEC considered that it may be a task for ICES to give scientific advice making use of the specific expertise on this point available within the JMSBEC.

Dr Karbe had briefly examined the texts of the Paris and Helsinki Conventions, and provided the following examples of terms which were imprecise and open to different interpretations. It some cases, differences in interpretation could arise from different perspectives of the situations being considered, and in others the terms were likely to be best defined in technical terms, which will have changed through the increase in knowledge since the Conventions were agreed.

Examples of the former type of terms are as follows:

1992 Convention for the Protection of the Marine Environment of the North East Atlantic (OSPAR Convention)

Article 1(c) - appreciable increase in salinity

Article 1(d) - hazards to human health
- damage to amenities
- harm to living resources and marine ecosystems

Article 2, Clause 2(a) - reasonable grounds for concern

Examples of more technical terms, which in some cases may be operationally defined rather than have absolute meanings are:

1992 OSPAR Convention

Annex 1, Article 3(a) - toxic, persistent, liable to bioaccumulate

Annex 3, Article 10(b) - bioaccumulate

1992 Convention on the Protecton of the Marine Environment of the Baltic Sea (Helsinki Convention)

Annex 1, Part 1, paragraph 1.1 - carcinogenic, teratogenic, mutagenic properties in or through the marine environment

Annex VI, Regulation 13 - low toxicity

ACME is invited to consider whether it is appropriate for ICES to enter into discussions with OSPAR and HELCOM concerning the imprecision of some of the terms used in the Conventions and Annexes, with a view to ICES being asked by the Commissions to propose definitions of these terms.

d) Identification of contaminants of priority concern

The JMSBEC considered that approaches currently in use to fulfil the requirements to identify those chemicals that pose special risks to the environment and risks to human health through the marine environment are not comprehensive enough. This specifically holds for types of toxicity and modes of action that are not covered by the traditional approach of looking for acute toxicity, persistence and accumulation potential. Examples are new classes of substances of concern resulting, e.g., in neurological disorders, modulation of hormone interactions within the endocrine system or having negative effects on the immunocompetence of marine biota or humans via the marine environment. It may be a task of ICES to give scientific advice to OSPAR on how to handle this problem and to make proposals for an assessment scheme and technical procedures to be used in this scheme based on the expertise available in different institutions cooperating within the framework of ICES.

The 1992 report of WGEAMS contained a review of some of the procedures in use at that time for the identification of priority marine contaminants. WGEAMS concluded at that time that there was little more they could do on the subject unless and until significant new knowledge or data became available. The JMSBEC considered that the biological effects which have given rise to the recognition of the new classes of environmental contaminants indicated above are not adequately covered by the selection schemes described in the 1992 WGEAMS report. ACME is invited to consider whether it is therefore appropriate for this question to be reopened in discussions with the Commissions, with a view to the matter being referred for more detailed consideration by an appropriate Working Group.

e) Ecotoxicological assessment criteria

Dr Stagg, in the context of the need to accommodate newer types of toxicity in both the considerations of the Commissions and in the selection of priority marine contaminants, reported that he had attended an OSPAR Workshop on Ecotoxicological Assessment Criteria in Berlin recently. The purpose of the meeting had been to build upon earlier meetings to develop criteria for PAHs in sediment, and for heavy metals, PAHs and PCBs in biota. The principal approach adopted had been to mainly use aquatic toxicity data and, by the application of equilibrium partitioning constants, to derive reference values for concentrations in both sediment and biological tissue.

The JMSBEC meeting noted that this was only one approach out of the seven or eight currently under active discussion, and in some cases application, in the USA, as described in a recent publication by the Florida Department of Environmental Protection. These approaches were summarized as follows:

- Sediment background approach
- Spiked sediment bioassay approach
- Equilibrium partitioning approach
- Tissue residue approach
- Screening level concentration approach
- Sediment quality triad approach
- Apparent effects threshold approach
- Weight of evidence approach.

The Florida authorities, after discussing the advantages and disadvantages of the various approaches, selected the weight-of-evidence approach for the development of their sediment quality assessment guidelines. Even so, the authors recognized limitations arising from doubts over the quality and compatibility of the data used to derive their values, together with the uncertain influence of synergistic and antagonistic effects.

The meeting noted that both the Working Group on Marine Sediments in Relation to Pollution (WGMS) and the WGBEC had expressed considerable reservations over the use of chemical criteria for sediment quality, which might subsequently be used retrospectively to assess the biological significance of chemical data, without reference to any measurements of biological effects. They felt that such values would work contrary to the established procedures of integration of chemical and biological techniques, and could lead to a rigid and unadaptable approach that would be unlikely to be able to accommodate new developments in technology and scientific understanding. The meeting agreed with the views expressed by the 1995 Working Group meetings, as summarized in the 1995 ACME report. The meeting considered that a flexible pragmatic approach is necessary, and that such an approach could more readily accommodate other factors such as the Precautionary Principle.

13 CONSIDERATION AND APPROVAL OF RECOMMENDATIONS

The recommendations contained in Annex 8 were agreed by participants in the JMSBEC meeting.

14 PROPOSALS FOR FURTHER MEETING

The meeting discussed at length the possible future role of the joint meeting. The participants recalled that the JMSBEC meetings were established to address the need for a conceptual framework for the closer collaboration and coordination between chemical aspects of sediment monitoring and the developing capabilities of biological effects measurements, to the benefit of coordinated international monitoring activities, primarily through the OSPAR system. The meeting noted that to a large extent the initial primary aim of the meetings had been achieved and adopted by OSPAR, as indicated by the results of the 1995 Aberdeen Workshop and the subsequent SIME meeting. There was to some extent a feeling that the current meeting had essentially acted as a pre-meeting for the WGBEC. This had been partially due to the interests of the members attending the joint meeting.

It was not clear to the joint meeting whether to recommend that JMSBEC should meet again in 1997. The meeting therefore agreed to ask the constituent Working Groups for their views on the Terms of Reference for a joint meeting in 1997.

The discussion also addressed briefly the broader problem of the relationship between the ICES Working Group structure and the types of problems/advice being requested. It was noted that there was an increasing trend for ACME to ask two or more Working Groups to address problems collaboratively, or in consultation. This suggested to the meeting that there was an increasing need for a multidisciplinary approach to be taken to problems and tasks, and that the current discipline-based Working Group structure was not ideally suited to such needs. Examples quoted were the current requests to review the AMAP by two Working Groups, and the requests for advice on biological effects measurements in seals, and contaminant accumulation models in sea birds.

The group noted that it is generally the experience in business that efficient organizational structures are able to adapt quickly to changing circumstances, and are flexible, project-oriented, and problem-solving. The character of government research institutes and international bodies means that this is not readily translated into an ICES context. However, the previous success of joint meetings such as JMSBEC and of WGEAMS with WGSAEM in limited, but well-defined areas suggests that such joint meetings can perform well on tasks where clear products have been defined, and where technical knowledge in the two or more disciplines concerned is sufficient, but needs to be integrated. The need for continuing review and comment on research developments may best be covered by the discipline-related Working Groups. Formal joint meetings may be used selectively to address particular problems, and would therefore be expected to have a limited lifetime, unless new appropriate questions arose.

The meeting recommended that:

a) ACME should review whether its current structures and procedures are the most appropriate way to develop the advice requested by the Commissions and other bodies, particularly in relation to questions requiring multidisciplinary approaches;

b) the two parent Working Groups of the joint meeting (WGMS and WGBEC) should meet together during the week following the joint meeting to consider the future role of the joint meeting.

15 ADOPTION OF THE JOINT MEETING REPORT

The meeting considered and approved the report of the Joint Meeting. The meeting closed at 18.00 hrs on 2 March.

ANNEX 1

AGENDA

Joint Meeting of the Working Group on Marine Sediments in Relation to Pollution
and the Working Group on Biological Effects of Contaminants

1) Opening of the meeting.

2) Adoption of the agenda.

3) Arrangements for the preparation of the report.

4) Reports of activities in other fora of interest to the Joint Meeting.

5) Review and report on new information and hypotheses concerning factors controlling the availability of contaminants in sediments to marine organisms.

6) Review and report on progress on factors controlling the release of contaminants from sediment, the implications of this for assessment of the relative importance of different exposure routes to marine organisms.

7) Begin the development of guidelines for monitoring based on the strategy outlined in the 1994 report of the joint meeting of WGBEC and WGMS.

8) Prepare recommendations on the integration of biological and chemical measurements on sediments in relation to the objectives of AMAP.

9) Review progress with the research proposal included as Annex 5 of the 1994 Joint Meeting Report (ICES CM 1994/ENV:2) concerned with investigating linkages between contaminants in sediments, bioaccumulation and biological effects, and receive and comment on other proposals for collaborative activities in similar and related subject areas.

10) Review and comment on reports of new initiatives and results of field and experimental investigations linking sediment chemistry and biological effects studies.

11) Review and comment on further progress in the development of chronic and sublethal sediment bioassays.

12) Any other business.

13) Consideration and approval of recommendations.

14) Proposals for a further meeting.

15) Consideration and approval of the Joint Meeting report.

ANNEX 2

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ANNEX 3

LIST OF MEETING PAPERS

JMSBEC 1996/2/1	Draft agenda
JMSBEC 1996/4/1	Report of the Aberdeen OSPAR/ICES Workshop on Biological Effects Monitoring Techniques (WGBEC96/7/1)
JMSBEC 1996/4/2	Report of the 1995 meeting of the OSPAR Ad Hoc Working Group on Monitoring (MON 1995), Copenhagen
JMSBEC 1996/4/3	Report of the 1996 meeting of the OSPAR Working Group on Concentrations, Trends, and Effects of Substances in the Marine Environment (SIME 1996), Oslo
JMSBEC 1996/5/1	Metal pathways in invertebrates. M. Moore
JMSBEC 1996/5/2	Effect of salinity on the uptake of cadmium by the brine shrimp *Artemia franciscana*. R. Blust *et al.*, 1992, Marine Ecology Progress Series, 84: 245–254.
JMSBEC 1996/7/1	ICES Monitoring Guidelines
JMSBEC 1996/7/2	JMSBEC Report 1995 (ICES CM 1995/Env:2)
JMSBEC 1996/7/3	ACME Report 1995 (*ICES Cooperative Research Report* No. 212)
JMSBEC 1996/7/4	Implementation of the JAMP – 1995. OSPAR
JMSBEC 1996/8/1	Summary of AMAP from LC/SG 18/7/2
JMSBEC 1996/8/2	AMAP Monitoring Programme
JMSBEC 1996/8/3	AMAP Audit Report 93.5
JMSBEC 1996/8/4	Arsenic, trace metals, and organic micro contaminants in sediments from the Pechora Sea, Russia. Loring *et al.*, Marine Geology, 128: 153–167
JMSBEC 1996/9/1	EQAMAS proposal 1995
JMSBEC 1996/10/1	Ecological monitoring of carcinogens. B.L. Rubenchik. TEN, 3: 14–19.
JMSBEC 1996/10/2	Alkaline single cell gel (Comet) Assay and genotoxicity monitoring using bullheads and carp. Pandrangi *et al.*, 1995, 26: 345–356.
JMSBEC 1996/10/3	Development of an *in vivo* genotoxicity assay using the marine worm *Platynereis dumerilii* (Polychaeta: Nereidae). A.N. Jha *et al.*, 1995. Mutation Research, 9074.
JMSBEC 1996/12/1	Normalisation models for evaluating metal contamination of coastal marine sediments.
JMSBEC 1996/12/2	1992 OSPAR Convention
JMSBEC 1996/12/3	1992 Helsinki Convention
JMSBEC 1996/12/4	COST Programme outline
JMSBEC 1996/12/5	Approach to the assessment of sediment quality in Florida coastal waters (4 volumes). Florida Department of Environmental Protection, 1994.

ANNEX 4

This Annex contains diagrams showing uptake, internal cycling, and excretion of metals in marine invertebrates.

Figure 1. Diagram of internal workings of bivalves

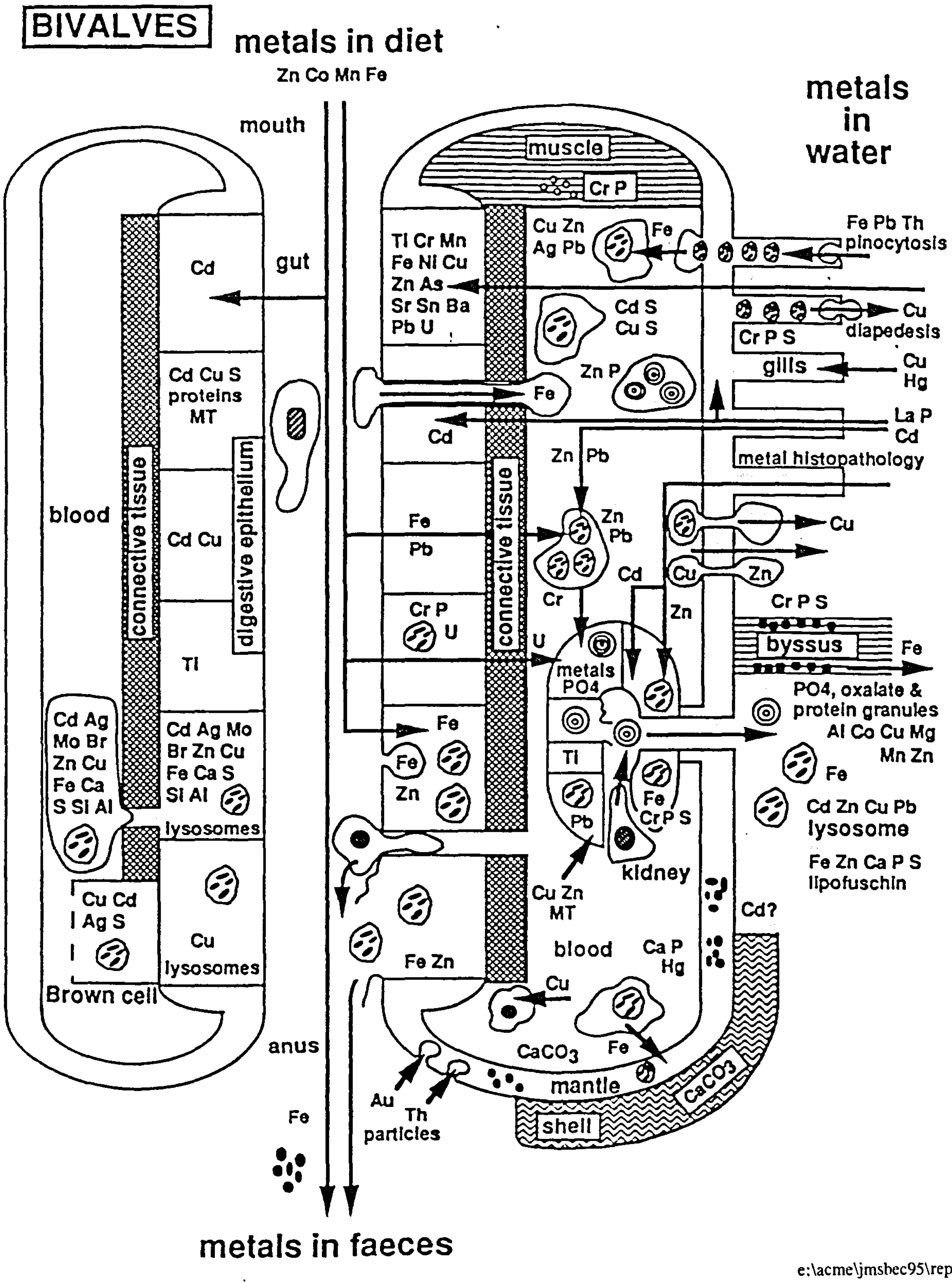

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Figure 2. Diagram of internal workings of crustacea

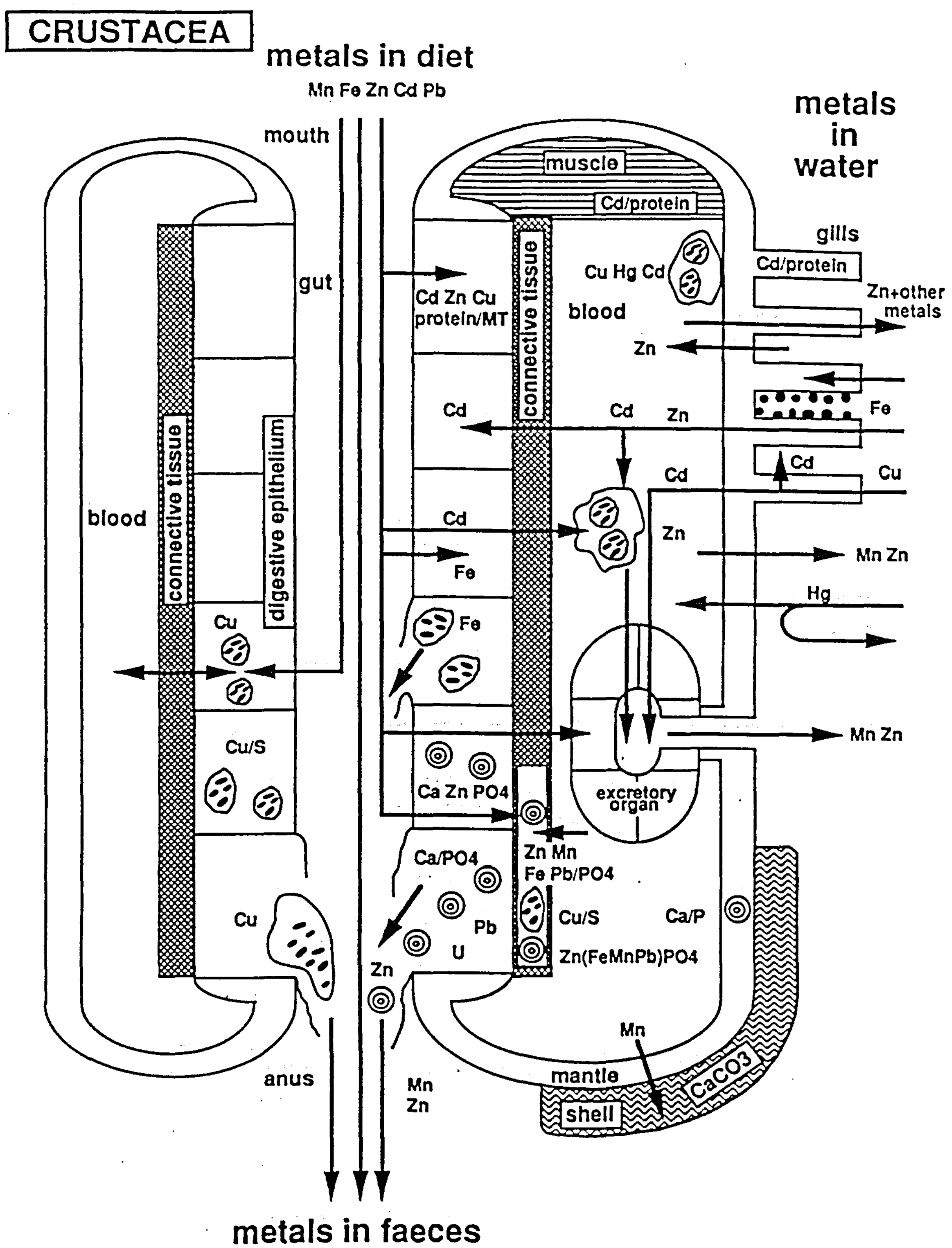


Figure 3. Diagram of internal workings of gastropods

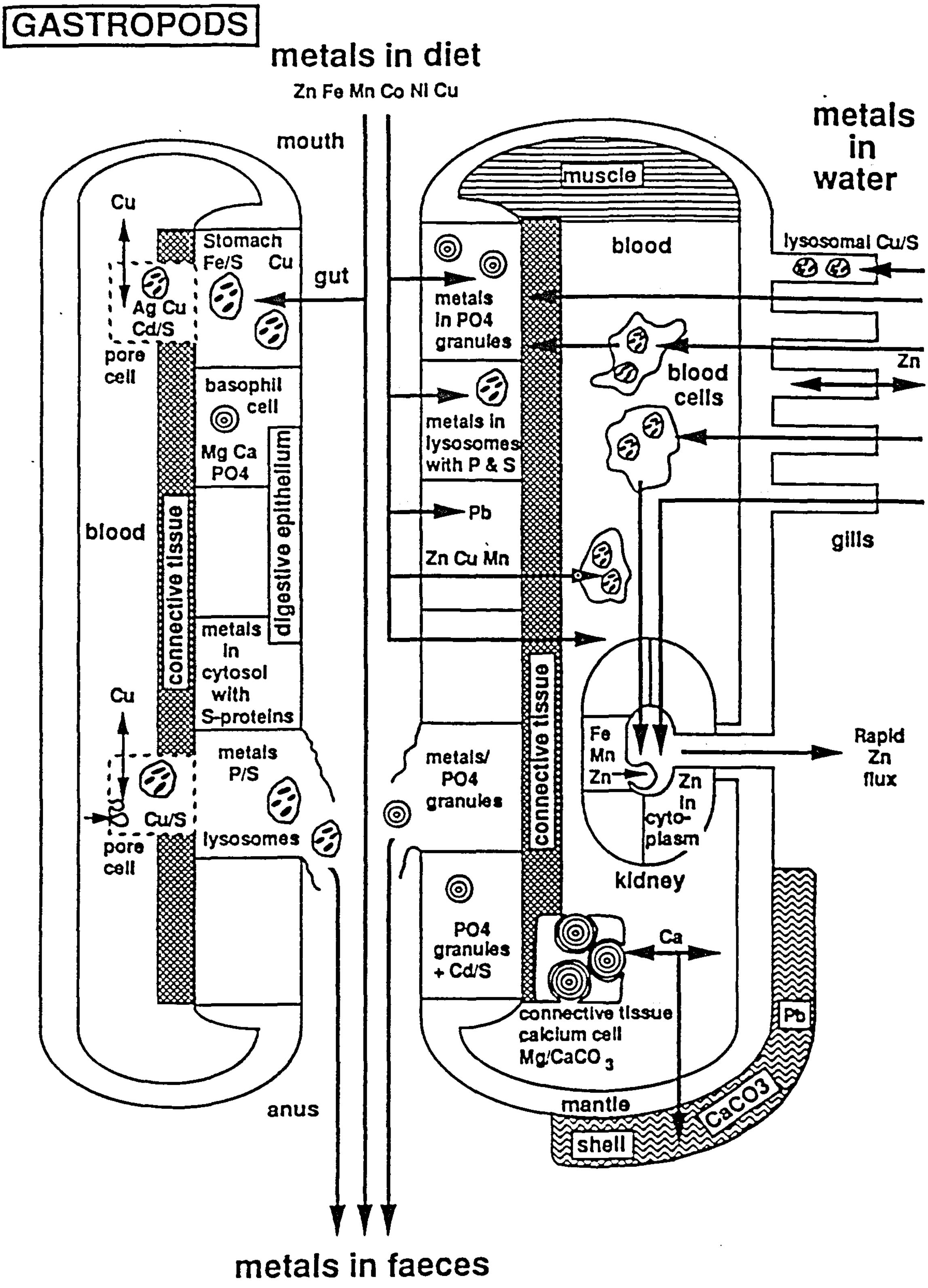
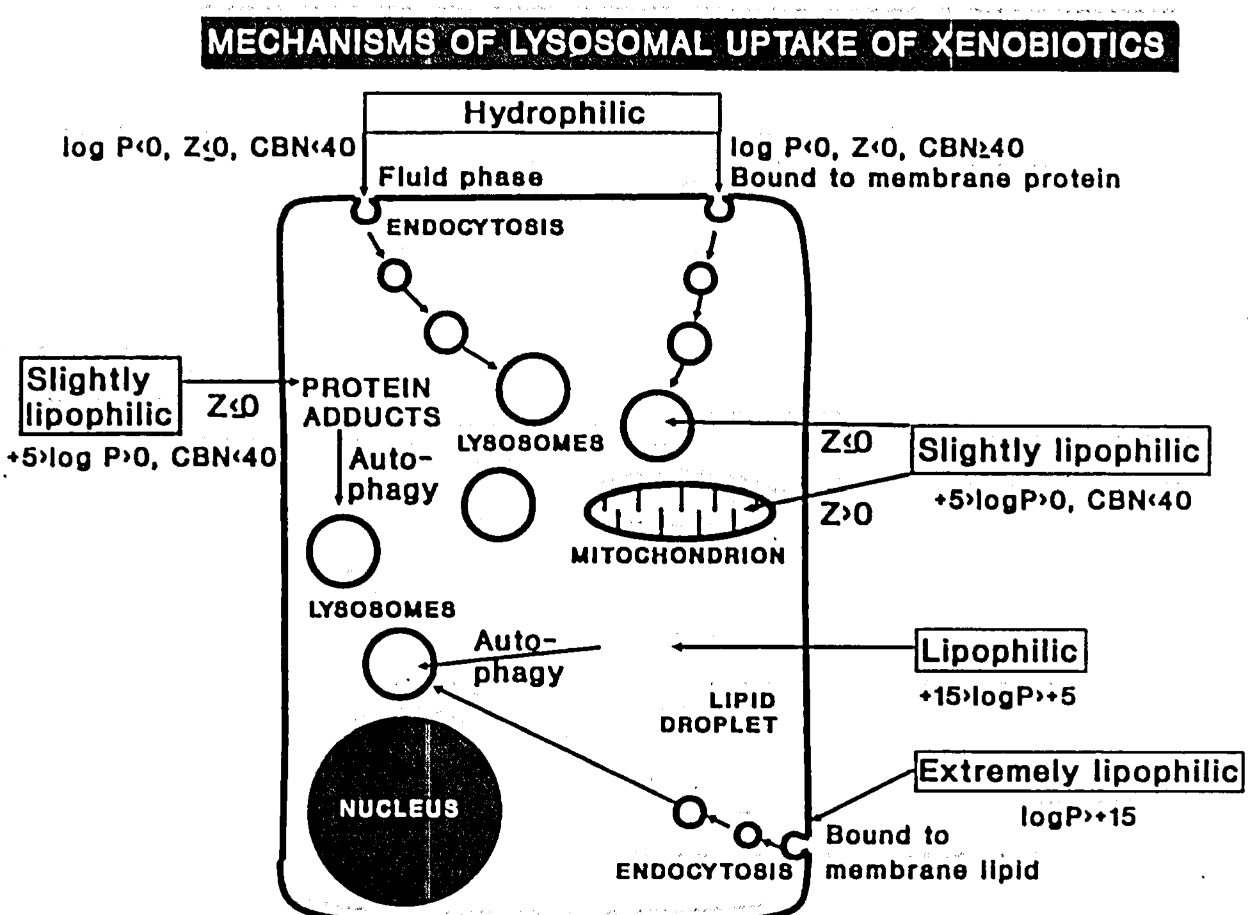


Figure 4. Mechanisms of lysosomal uptake of xenobiotics



Adapted from Rashid & Horobin (1990) and Rashid et al. (1991)

Michael Moore
PML (1993)

ANNEX 5

Table 6.6.1. Organic contaminants that can be monitored in biota and sediments on a routine basis. (From the Report of the ICES Advisory Committee on the Marine Environment, 1995. ICES Cooperative Research Report No. 212, p.40.)

Organic Contaminant	Recent I/C data available [1]	QC material available	Laboratory capability [8]
1. Chlorobiphenyls CBs 101, 118, 138, 153, 180	Yes (sediment, lean and fatty fish tissue, seal oil)[2]	CRMs (SRMs) and certified standards	sediment CBs 118, 138, 153 s_R[3] 15%, R 50% seal oil CBs 138, 153, 180 s_R 15%, R 50% fish oil CBs 101, 118, 138, 153, 180 s_R 15%, R 50% lean fish CBs 118, 138, 153, 180 s_R 50–70%, R 200–330%
2. Non-*ortho* CBs 77, 126, 169	Yes (fish oil)[4]	No	Some specialist laboratories (fish oil) s_R 20–50%, R 65-200%
3. Organochlorine pesticides HCHs, DDT, DDD, DDE, HCB, dieldrin, *trans*-nonachlor	Yes (sediment, fish oil)[5]	CRMs and certified standards	fish oil s_R 15–30%, R 50–100% sediment s_R 35–100%, R 130–800%
4. PAHs	No	CRMs and certified standards	Unknown
5. Chlorinated dioxins and furans	No	CRMs and certified standards	Some specialist laboratories
6. CHBs (toxaphene)	Yes [6]	No	Some specialist laboratories (fish oil) s_R ca. 50%, R 200%
7. Organotin (TBT, TPT)	No	CRM (TBT)	Some specialist laboratories
8. Methyl mercury	No [7]	No	Some specialist laboratories

Abbreviations

CHBs = chlorinated bornanes
HCB = hexachlorobenzene
OCP = organochlorine pesticides
TBT = tributyltin
CRM = certified reference material
HCHs = hexachlorocyclohexanes
PAHs = polycyclic aromatic hydrocarbons
TPT = triphenyltin
SRMs = standard reference materials
QC = quality control

[1] This column refers to intercomparison exercises (I/C) carried out with regard to marine environmental samples.

[2] References: ICES reports on the results of the ICES/IOC/OSPARCOM Intercomparison Programme on the Analysis of Chlorobiphenyls in Marine Media; QUASIMEME reports on CB intercomparison exercises (1993–1995).

[3] S_R: standard error; R: reproducibility

[4] References: Voogt, P. de, *et al.* 1994. Analytical Chemistry, 66: 1012–1016; Wells, D.E. 1994. Report on ICES intercomparison exercise on non-*ortho* CBs. Working Paper at the 1994 MCWG meeting.

[5] Reference: QUASIMEME reports on CB and OCP intercomparison exercises (1994–1995).

[6] Reference: Andrews, P.A. 1994. Interlaboratory study on the analysis of toxaphene. Proceedings of the 24th International Symposium on Environmental Chemistry. Ottawa, Canada. A second study is under way.

[7] Recently information from an intercomparison exercise on sediment became available from the European Commission. A CRM for sediment is in preparation.

[8] Where the between-laboratory coefficient of variation (R) exceeds 50%, it is reasonable to conclude that there is insufficient agreement for the group of laboratories, as a whole, to undertake these measurements. A selection of more experienced laboratories would be recommended.

ANNEX 5 (continued)

Figure A2.1. Effects on fish leading to liver tumours, caused by exposure to chemical contaminants.

Stage	Sequence of Processes	Monitoring Targets
1	Compounds in the environment	PCBs, PAHs, dioxins in sediment, suspended particulate matter, mussels
	↓	
2	Compounds in the organism	Same substances in fish liver
	↓	
3	Induction of MFO/EROD systems	MFO/EROD in fish liver
	↓	
4	Molecular and cellular processes of carcinogenesis	DNA adducts, oncogene activation; oxidative modification of DNA and chromosomes
	↓	
5	Pre-neoplastic changes in liver tissue	Fish liver histopathology
	↓	
6	Liver tumours	Presence of liver cancers

Figure A2.2. Effects on reproductive success of fish caused by the impact of chemical contaminants on hormonal systems.

Stage	Sequence of Processes	Monitoring Targets
1	Compounds in the environment ↓	PCBs, PAHs, dioxins in sediment, suspended particulate matter, mussels
2	Compounds in the organism ↓	Same substances in fish liver
3	Induction of MFO/EROD systems ↓	MFO/EROD in fish liver
4	Effects on hormonal systems ↓	Blood analysis: a) vitellogenin b) vitamin A c) hormones
5	Effects on reproductive success	Indices of reproductive success: a) Gonado-somatic index (GSI) b) Fecundity c) Sex ratios d) Egg viability, or quality e) Chromosome abnormalities (In some species, e.g., viviparous blenny, the growth and survival of embryos can be measured.)

The possible influences of environmental oestrogens are not shown in the above diagram. They are known to have effects directly at stage 4, without affecting the MFO system. There have been no investigations so far of the influence of these substances on marine fish. It is therefore premature to include them in a monitoring scheme, but they should be the subject of a research programme to determine whether they have significant influence in the sea, and therefore whether they should be the subject of a monitoring programme.

Figure A2.3. An approach to investigating the effects of contaminants on the health of the benthic infaunal community.

Stage	Sequence of Processes	Monitoring Targets
1	Causative contaminant	Many are possible
2	Contaminated sediment	Conduct bioassays using: a) Whole sediment b) Pore water c) Elutriates End points: a) Mortality – acute tests b) Behaviour – e.g., feeding rate c) Growth and reproduction d) Biomarkers and cytotoxicity
3	Impaired benthic faunal community	Benthic faunal community analysis

ANNEX 6

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

EQAMAS

Introduction

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

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

Objectives

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

1. Identification of the magnitude of the problem
    - estimate the loading of selected areas by point sources such as oil drilling rigs and land based sources) by PAHs
    - quantify the partitioning and speciation of PAHs in sediments, water and suspended particulate matter
    - quantify the transfer of PAHs between environment and biota
    - quantify the biological impact from PAHs in sediments and soils of both pyrogenic and petrogenic origin on benthic organisms by characterising a sequence of linked processes and specific monitoring targets, including targets of early detection of toxicity and targets of deleterious effects on populations and ecosystem.
    - evaluate the state of the selected areas

2. Solving the problem
    - quantify the degree of elimination by biodegradation, photodegradation? and dilution of the PAHs
    - estimate the use of the gained knowledge in a broader context (application to other areas, other pollution types, other?)
    - make recommendations for managerial purposes

Approach

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

To fill in the gaps that are left open by the current approaches the project is designed to provide an environmental quality assessment scheme to estimate the impact of PAH sources that is based on the interrelationships between PAH speciation in the water column, sediment and suspended particulate matter, bioaccumulation of PAHs, responses to PAH exposure in benthic organisms and elimination routes. The scheme will yield quantitative information concerning the nature of PAHs in soil/sediment, as well as bioavailability, bioaccumulation, toxicity, elimination and sublethal biological effects of PAHs associated with soils or sediments.

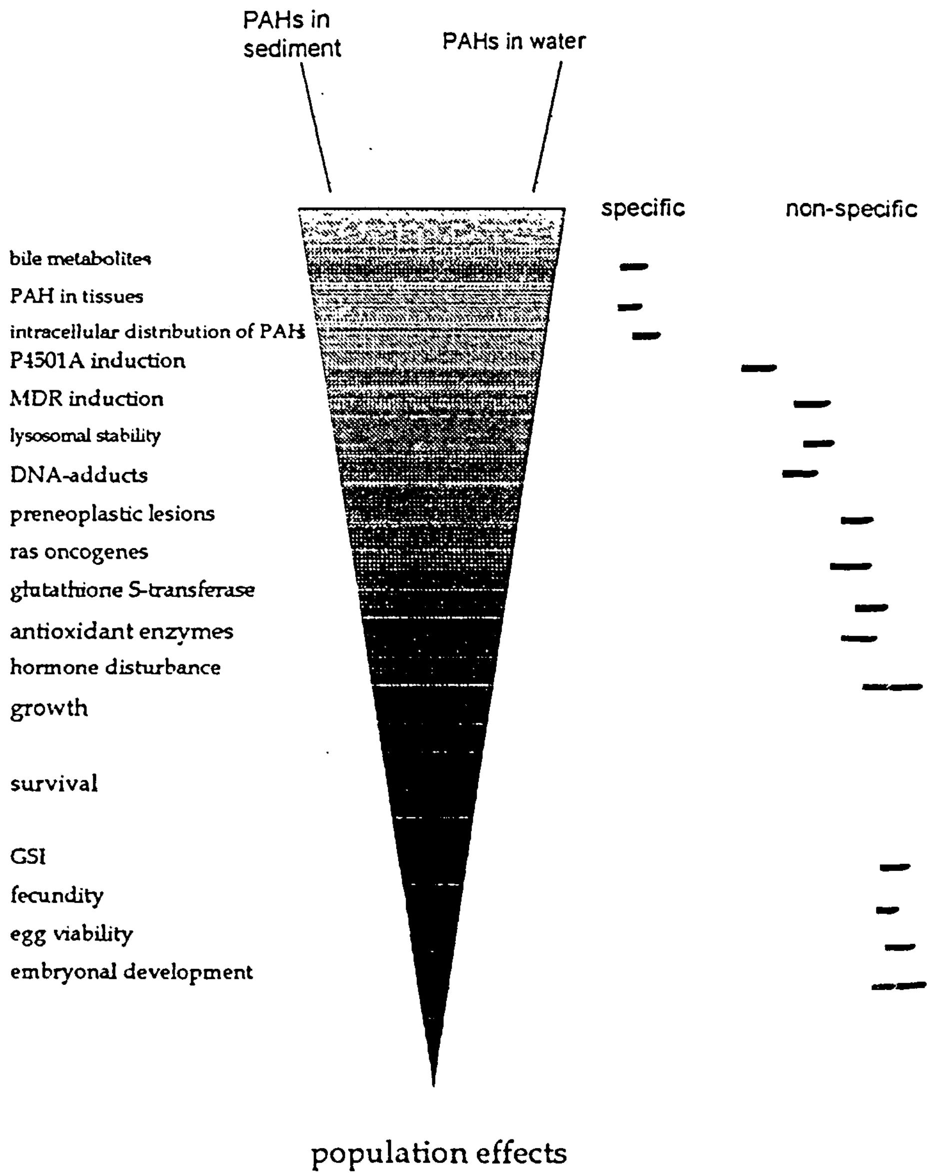


Figure 1. The relationship between techniques to characterise biological impact of PAHs, their specificity and relevance for effects on population level.

description

characteristics of inputs
concentrations, characteristics sediments
concentrations in water column, pore water
concentrations in suspended particulate matter

intracellular distribution of PAHs
cytochrome P450 (fish)
MDR (invertebrate)
lysosomal stability
DNA-adducts (HPLC)
glutathione S-transferase
antioxidant enzymes
ras oncogenes
bile metabolites (fish)
p53 ?
DNA strand breaks
histopathology
hormone effects
vit A
GSI
egg viability
chromosome aberrations
fecundity
bioassays
benthic community structure (meio, macro)

comment

PAH-profile, particle-size distribution
PAH-concentration, profile, speciation
PAH-concentration, profile
benthic fish

filter feeder (Mytilus)
sediment-dwelling/-feeding
novel animal model

ANNEX 7

Using the micronucleus test and the single cell gel (comet) assay to study clastogenic effects of PCB77 in fish

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PCBs (polychlorinated biphenyls) are stable pollutants found in almost every compartment of terrestrial and aquatic ecosystems. They are highly lipophilic and therefore have the potential of accumulating in the fat stores of animals. Aquatic species (mammals and fishes) in particular undergo health problems caused probably by the PCBs. The mechanisms by which PCBs exert their adverse effects are still unclear. It is known that PCBs induce some important biotransformation enzymes, but their mutagenic properties remain controversial.

Our aim was to determine the DNA breakage and clastogenic potential of a planar PCB (PCB 77) *in vivo* in fishes, using the single cell gel electrophoresis (SCGE) and the micronucleus (MN) assay on erythrocytes of brown trout exposed for 3, 9, and 14 days to 4 ng/ml PCB 77 in water. Blood was taken by a caudal puncture and the erythrocytes were either deposed in the agarose gel (0.6%) for the SCGE or smeared directly on slides for the MN assay. Five fishes were studied per treatment. Fifty and 2000 erythrocytes per concentration were analysed for SCGE and MN, respectively. EMS (ethylmethanesulphonate) at a concentration of 25 mg/l was used as positive results for PCBs were found.

In parallel PCB 77 was also tested on human lymphocytes. The lymphocytes were exposed for 30 minutes, 1 hour, 3 hours, and 48 hours to PCB 77 (0.01, 0.1, 10, 25, 100 µg/ml). In contradiction of a previous finding (Sargent *et al.*, 1989), no increase of either DNA SS breaks or MN was observed. The different results may be related to the fact that, in our experiment, the PCBs were tested at a final DMSO concentration of 0.5%, instead of 2% as for Sargent.

The data indicate that PCB 77 does not have clastogenic properties in human lymphocytes or fish erythrocytes.

ANNEX 8

RECOMMENDATIONS

To Council

The Joint Meeting of WGMS and WGBEC recommends that:

A joint meeting of WGMS and WGBEC should be held for two days in 1998 to address Terms of Reference to be defined at the 1997 ICES Annual Science Conference in response to recommendations to be prepared by the 1997 meetings of WGBEC and WGMS.

To ACME

The JMSBEC recommends that:

1) Monitoring guidelines for integrated chemical and biological monitoring programmes should take account of:

   a) the need to vary sampling designs to meet different objectives and unique features of sampling areas;

   b) the lack of availability of reliable ecotoxicological assessment criteria;

   c) the need for QA procedures for both biological effects measurements and associated chemical determinations;

   d) that for biological effects interpretation purposes, whole sediment (<2 mm) should be analysed for contaminants, together with supporting normalizing variables;

   e) that biomarker studies should be supported by chemical measurements in the same tissues.

2) The marine component of the AMAP programme does not adequately integrate chemical and biological effects measurements in sediment, and changes should be made to the programme along the lines indicated for sediment-related monitoring in the 1995 ACME report.

3) ACME be invited to express support for the development of a collaborative proposal for a research programme to assess the significance of PAHs in marine sediment.

4) ACME be invited to consider initiating discussions with the Commissions on the need for improved definitions of technical terms in the Convention documents, and on the need for review of the methods for the identification of priority marine chemical contaminants with a view to these tasks subsequently being allocated to appropriate Working Groups.

5) ACME be invited to review whether the current structures and procedures are the most appropriate way to develop the advice requested by the Commissions and other bodies, particularly in relation to questions requiring multidisciplinary approaches.

To the ICES Secretariat

The JMSBEC recommends that:

1) The format for Working Group reports provided this year by the Secretariat be simplified, avoiding relatively sophisticated automated numbering, etc., systems, and double column presentation. It would be of assistance to the Working Group to have an appropriate report structure provided in a simple text format, together with those sections of the report that can be drafted prior to the meeting, to ensure that all the necessary sections are included in the report. However, it is suggested that the final transfer to ICES standard text and page structure format is carried out by the Secretariat.