Report of the Working Group on Biological Effects of Contaminants (WGBEC)

11-15 January 2010
Dublin, Ireland
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Executive Summary

The Working Group on the Biological Effects of Contaminants [WGBEC], chaired by John Thain, UK, met at Trinity College, Dublin, from 11–15 January 2010. There were 21 attendees representing 13 countries.

A summary of the key outcomes in respect of the Terms of Reference is described below.

WGBEC includes in its membership scientists from national government institutes, academia, industry and management. The group also has a diverse membership of expertise, ranging from chemists, biologists, biochemists and environmental scientists. This is beneficial as requests in the past, particularly from OSPAR have been wide-ranging. This year there were thirteen items on the agenda, including three items from ICES and one from OSPAR. Priority was given to the latter items. Presentations and discussions took place in plenary, with the exception of a sub-group working on specific requests from the ICES data centre. All items on the agenda (covering all ToR) were completed and are reported.

Progress with national and international monitoring activities. Presentations were received from Spain, Ireland, Sweden, Italy (MEDPOL), and the Baltic Sea. These included new monitoring activities such as in Ireland to well established programmes in other countries. Of particular importance for WGBEC to note were the developments on integrated assessment being pursued in the Baltic (BEAST), North Sea (ICON) and MEDPOL and the QC intercalibrations involving WGBEC members being facilitated by Italian and Spanish group members. The WGBEC considered the progress made by the Marine Strategy Directive (MDS) descriptor 8 Task Group. The WG fully supported the recommendations made by the Task Group which placed a strong emphasis on biological effect monitoring as developed by the WGBEC in the past decades and currently used by OSPAR.

Review the methodology used by the OSPAR workshop on the development of Chapter 11 of the QSR 2010 (Utrecht workshop). The WG considered the OSPAR report and concluded that there were serious shortcomings to the methodology of the workshop and its conclusions. There were a number of concerns in relation to the poor scientific basis underpinning several of the conclusions reached. It was noted that the workshop considered that this type of broader ecosystem assessment could be a useful contribution to an Initial Assessment for the Marine Strategy Framework Directive (MSFD) in 2012. WGBEC would be concerned if this were the case without further underpinning science and engagement with experts in the specific scientific fields.

Report to SSGHIE on potential and current contributions of your EG to the Strategic Initiative on Coastal and Marine Spatial Planning (SICMSP). The WG briefly reviewed the SICMSP and suggest three specific examples in respect to contaminants that should be considered that are directly relevant to area-based management: these are point sources of contaminants (i.e. industry, offshore platforms, and rivers), diffuse sources (e.g. harbours, urban areas) and contaminants in sediments.

Report to SSGHIE on your plans to promote cooperation between EGs covering similar scientific issues. After intensive discussions on the scope and future activities of the WG, and taken consideration of the various ICES vision and strategy documents, WGBEC identified 9 areas of its core business and 8 areas for future directions. While some of these activities are considered as “true” core business, others clearly
attend to broaden the scope of WGBEC, towards more ecology and wider ecosystem effects. WGBEC also identified how and where it fitted in to the ICES Science Plan and its potential to collaborate with other expert groups.

**Review ICES WGBEC list of recommended biological effects methods for monitoring purposes and define how this fits in for both OSPAR and EU MSFD purposes.** The WGBEC discussed and amended the ‘promising’ and ‘recommended’ monitoring techniques that had been last updated in 2007.

**Cooperation with ICES / OSPAR SGIMC conduct intersessional work for review at 2010 meeting based on the outcome of the SGIMC Aberdeen Workshop, October 2009.** The outcome of the SGIMC report was reviewed and noted and tasks deferred to WGBEC were considered. These included a review of draft background document on the Comet assay and amendments to background document on DNA adducts.

**Review progress with publication and electronic dissemination of biological effects techniques in the ICES TIMES series.** The group reviewed the status of publications that were in preparation or had been commissioned. Considering the number of manuscripts commissioned by the group, still in preparation (9) all with draft resolutions, it was decided to focus WGBEC efforts on delivery of these, rather than commission any new manuscripts.

**Answer queries / requests from the ICES Data Centre.** WGBEC responded to several requests from the ICES data centre relating to: legacy data, data quality issues, parameter codes and also to queries from WGBEC data submitters. In order to assist in data submission to ICES, WGBEC recommended that WGBEC / ICES data centre develop a live ‘working document’ to be added to at future WGBEC meetings to explain how biological effect data should be entered into the database and keep track of WGBEC advice on database issues.

**Review of emerging and novel contaminants.** Reviews were received and discussed on nanoparticles, marine litter and plastics, and contaminants in eels and associated biological effects.
1 **Opening of the meeting**

The Chair, John Thain (UK), opened the meeting at 09.30 on Monday, 11 January 2010, and thanked Michelle Giltrap (IE), for hosting the meeting at the Zoology Department, Trinity College, Dublin and for organising the meeting arrangements and hotel accommodation, etc. The Chair then invited the participants to introduce themselves and their affiliations and describe their area of interest and field of expertise. The list of attendees is given in Annex 1.

2 **Adoption of the agenda**

The Chair then invited participants to examine the Terms of Reference (ToR) and went through the agenda explaining the priority and background to the agenda items and in particular those requests from ICES and OSPAR. The ToR for the meeting can be found in Annex 2. A draft agenda was adopted by the meeting and a tentative timetable agreed, Annex 3 and 4 respectively. It was noted that Agenda Items 6 was a request from OSPAR and Agenda Item 7 and 8 was a recent request from ICES.

One item was agreed under any other business, the election of a new Chair person(s).

3 **Appointment of rapporteurs**

Principle rapporteurs were appointed for the agenda items and are given in Annex 4.

4 **Review progress with national/international monitoring activities; to include/ integrated assessment/ and application of biological effect techniques within OSPAR/ MEDPOL/ WFD/ HELCOM/ EU MSD + any other; (ToR c).**

4.1 **Spain**

Concepción Martínez-Gomes (ES) gave a presentation on progress with the national programme for monitoring marine pollution in Spain and details can be found in Annex 5. In summary, two major biomonitoring programmes, along the Northern Iberian coast and along the Iberian Mediterranean coast have been conducted through several research projects over the past decade. However, between 2010–2012 a new biomonitoring programme will be instigated to meet the obligations of both the OSPAR and Barcelona conventions and if possible to contribute to the GES assessment for the Marine Strategy Framework Directive.

The new programme along the Northern Iberian coast includes a chemical and biological effect integrated approach in order to establish clear relationships between results of chemical monitoring of pollution and the pollutant concentrations that may cause ecological damage. This will include (i) biological effect studies on sediment elutriates, using the sea urchin embryo-larval bioassay; (ii) conducting sediment toxicity assessments using the amphipod survival bioassay; and (iii) biological effects studies using molecular responses in mussels (GST and AChE).

In addition, to fulfil obligations for biological effect monitoring in MEDPOL Phase IV (2006–2013), the Contracting Parties to the Barcelona Convention adopted the strategy for the development of Mediterranean Marine Pollution Indicators (MPIs). This strategy will be adopted on the Spanish Mediterranean coast as well as extending this to include Spanish monitoring research activities with more biomarker measurements.
in mussels and fish as well as contaminant concentrations in surface sediments and fish.

In both programmes, measurements are performed yearly (excepting temporal trends in sediments that are conducted biannually, in the case of the Mediterranean program) and the application of both chemical and biological effect techniques (biomarkers/bioassays) is included. A summary of the sampling strategy and timescale is given in Table 4.1.1.

Table 4.1.1. Sampling strategy, parameters, timescales and matrices included in the Spanish monitoring programme.

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<th>BIOMONITORING IEO</th>
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<th>SPANISH MEDITERRANEAN MONITORING</th>
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<tr>
<td>Sediment (S)</td>
<td>Yearly</td>
<td>Autumn (Sept-Oct)</td>
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<td>Fish (MB/MM)</td>
<td>Autumn</td>
<td>Autumn (Sept-Oct)</td>
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<td>Excellent</td>
<td>Pre-spawning</td>
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<td>Mussels (MG)</td>
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<td>Sampling NR/NL</td>
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<td>TBTs</td>
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<td>Sea urchin Embryotoxicity assay</td>
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<td>Amphipod bioassay</td>
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<td>CI/CF</td>
<td>MG/MM</td>
<td>MG/MB</td>
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<td>GSI</td>
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MG: *Mytilus galloprovincialis*
MB: *Mullus barbatus*
MM: *Merluccius merluccius*
NL: *Nucella lapillus*
NR: *Nassarius reticulatus*
S: *Surficial sediments*
*pilot study*
More details of the sampling procedures, geographical spread of the sampling sites, testing methodology and proposed method for conducting the integrated assessment are given in Annex 5.

WGBEC were in full support of the activities being conducted in the Spanish monitoring programme, both in the approach and the MEDPOL, OSPAR and MSFD harmonization aspects. WGBEC look forward to seeing data from this programme in 2011 and 2012.

4.2 Marine monitoring in Ireland

Michelle Giltrap (IR) presented an update on the project entitled “Biological Effects and Chemical Measurements for the Assessment of Pollution in Irish Marine Waters”. The structure of the project is outlined in WGBEC report 2009. Tier I site sampling was completed at 8 sites around the coast of Ireland in 2009. For Tier I analysis samples were taken for clearance rate, stress on stress, condition index, sediment toxicity testing and chemical analysis. Sediment toxicity testing included whole sediment tests with *Corophium volutator* and *Arenicola marina*, porewater and elutriate testing with *Tisbe battagliai* and *Skeletonema costatum* and the microtox test with *Vibrio fischeri*. Results from Tier I analysis will direct analysis to 3 sites for full biological, ecotoxicological and chemical assessment. Tier II site analysis will take place in 2010 and involves analysis with a battery of biomarkers in mussels and fish, chemical analysis, fish and mussel histopathology, benthic monitoring, sediment bioassays and imposex/intersex analysis. The battery of bioassays for mussels include scope for growth, stress on stress, condition index (CI), lysosomal membrane stability (LMS), metallothionein (MT), acetylcholinesterase (AChE), alkali labile phosphate (ALP) and comet assay. For fish, the battery of biomarkers include CI, EROD, bile metabolites, vitellogenin induction, AChE, MT and comet assay. Natural reproductive cycles will be investigated for mussels from a control location on the west coast of Ireland with the use of the adipogranular scoring index as reported by Bignell et al. 2008 (REF). Flounder will be sampled quarterly in 2010 from 2-3 estuaries for histology/VTG in blood plasma and total protein to investigate reproductive cycles before commencement of more in depth fish studies. Sampling for benthic monitoring and imposex analysis in snails will commence in February 2010. Development of chemical methodology for natural and synthetic steroid estrogens in water and biota is underway at the Marine Institute, Galway. As well as Tier I/II site analysis, various caging study case studies are being conducted for the investigation of sewage related effects/chemical analysis in mussels. These sites include Kinvarra Bay and Mutton Island (Co. Galway), the North Bank Lighthouse in Dublin Bay. Potential sites for future caging studies include Haulbowline Island (Cork Harbour) and others yet to be confirmed. Passive sampling and stable isotope analysis is being conducted at MI for various case study sites also. An in-vivo exposure system is now set up in the Shannon Aquatic Toxicity Laboratory. A pilot study has been performed with mussels and the exposure of ethinylestradiol. This study was performed to demonstrate a positive control for alkali labile phosphate biomarker and also intertidal and submerged uptake of this contaminant will be investigated with chemical analysis. Further studies with flounder/dab exposures and alkylphenols will be investigated in 2010/2011. Collaborations with the Galway and Mayo Institute of Technology (EPA funded), Athlone Institute of Technology and MI (STRIVE EPA funded) shall allow for testing of pharmaceuticals (gemfibrozil and diclofenac) using proteomics, yeast estrogen screening assay and norovirus respectively.
References

4.3 Sweden
Halldóra Skarphéðinsdóttir (SE) from Stockholm University gave a presentation on recent studies on DNA adducts in blue mussel, fish and herring gulls and included field and experimental data.

DNA adducts are formed when a compound or its metabolite binds covalently to DNA and is commonly used as a biomarker of PAH exposure and effects. Adduct formation may occur as an integrative response to multiple factors such as uptake, metabolism, detoxification, and DNA repair. It may persist for weeks or months and can ultimately lead to physiological consequences if the DNA is not functioning right, can cause cancer and mutations if in germ cells resulting in effects on genetic diversity.

Mussels
In a study conducted in Iceland DNA adduct levels were measured in mussels at different sites with suspected contamination. Mussels were transplanted in the subtidal and tidal zone for a period of six weeks in summer and winter. The results showed: that DNA adduct levels were elevated in mussels at sites with suspected contamination; highest DNA adduct levels were found in gills of mussels in Reykjavik harbour; DNA adduct levels in mussels transplanted in Reykjavik harbour for six weeks were not similar to those of native mussels in the same area; there is a possibility that seasonal variation in adduct level occurs. This work is fully reported in Ericson et al 2002.

Following on from this some laboratory experiments were conducted to better understand the formation of DNA adducts in blue mussels, and to improve the interpretation of field results. Blue mussels were exposed to the genotoxic compound, benzoapyrene, and DNA adduct formation studied. The results showed that: BaP uptake at the end of the 4 day exposure was linear with dose in all the studied tissues; highest tissue concentration was found in the digestive gland; the uptake was linear with dose in all tissues; adducts were only significantly formed in the gills, no increase in DNA adduct levels in the digestive gland; there was only dose response up to 50ug BaP/l; with no difference in adduct levels between mussels exposed to 50 and 100 ug BaP/l; *BaP uptake during the 6 days exposure was rapid and linear over time (*Maximum levels were 107ug BaP/g dw 1 day after the end of exposure); DNA adducts were persistent in gills for at least 2 weeks, but BaP tissue levels decreased fast. This work is fully reported in Skarphéðinsdóttir et al 2003.

In a further study, seasonal variation in DNA adduct levels in blue mussels (Mytilus edulis), was investigated along with the impact of intertidal exposure on the DNA adduct levels, i.e. to explore if DNA adduct levels in mussels in the intertidal zone differ from those in the subtidal zone. Blue mussels were deployed separately in the intertidal and subtidal zone at a contaminated and a reference site in Iceland, and sampled regularly during one year. Gill DNA adduct levels were found to be higher in mussels in the intertidal zone compared to the subtidal zone at the contaminated site, the difference being largest in winter. Total PAH tissue levels were also higher in mussels in the intertidal zone. Seasonal variation was observed in both DNA adduct and PAH tissue levels in mussels at the contaminated site, with lower levels from the
time of transplantation in summer to autumn, maximum levels in winter, which decreased to lower levels again in spring and summer the following year. DNA adducts and PAH levels were low or below the detection limits in mussels at the reference site at all times, both in the intertidal and sub tidal zone. Concluding that intertidal differences and seasonal differences need to be taken account when using DNA adducts measurements in biomonitoring programmes. This work is fully reported Skarphedinsdottir et al. 2005.

In a field monitoring programme DNA adducts in gills and digestive gland, as well as polycyclic aromatic hydrocarbon (PAH) tissue levels were analysed in blue mussels (*Mytilus* spp.) from Nordic coastal areas (Iceland, Norway and Sweden) with diffuse or point sources of PAHs of various origins. Both DNA adduct and PAH tissue levels were generally low, indicating low PAH exposure to the mussels in the areas studied. DNA adducts were found to be higher in gills than in digestive gland of the mussels at all sites studied. Elevated DNA adduct levels in gills were found at 6 sites out of 18 compared to reference sites in respective coastal zones. Adduct levels ranged from 0.5 to 10 nmol adducts/mol normal nucleotides, being highest in mussels from Reykjavík harbour, Iceland (intertidal mussels), and from Fiskaatangen, Norway (sub tidal mussels). Total PAH tissue levels in the mussels ranged between 40 and 11,670 ng/g dry wt., and were significantly correlated with DNA adduct levels ($r^2 = 0.73$, $p < 0.001$). PAH ratio values indicated that the PAHs were in most cases of pyrolytic origin. Thos work is fully reported in Skarphedinsdottir et al 2007.

**Fish**

DNA adducts have been analysed in several fish species at ITM, Stockholm University, Sweden and these include; perch, pike, cod, haddock, saithe, halibut, greenland halibut, long rough dab, and more. In many studies these have been related to Norwegian oil platforms or produced water: lab experiments, field experiments, monitoring. In one study (Aas et al 2003), 11 species from pristine areas were analysed for liver DNA adducts in order to study “natural background” levels. Adduct values above 1 nmol add/mol normal nucleotides can be considered an effect, but values below that are too low to be considered an effect. In a laboratory study Atlantic cod were exposed for up to 44 weeks to environmentally relevant concentrations (resembled North Sea produced water) of low-molecular weight PAHs (2-4 ring), and short chained APs. Three treatments were used: - low (0.54ug PAH/L +1.14ug APs); high (5.4ug PAH/L +11.4ug APs); Pulsed (high dose and control exposures alt. at 2 weeks interval). DNA adducts were analysed after 0, 16 and 44 weeks. Few adducts were formed after 16 weeks so the period was extended and 44 weeks exposure was needed for formation of DNA adducts in female cod. No adducts were measured in the pulsed treatment: this might indicate that tissue contaminant loads were reduced during control exposure periods, even though bile PAH metabolite levels were maintained. A possible explanation may be that during such periods of control exposure, a continued metabolism and excretion of tissue contaminants could allow the rate of DNA repair to exceed adduct formation. This work is reported fully in Holth et al 2009.

**Birds**

A survey in both Sweden and Iceland has shown that adult herring gulls (*Larus argentatus*) are exposed to genotoxic chemicals as seen in elevated DNA adducts, analysed with the $^{32}$P postlabelling method. DNA adducts were highest in the liver, with levels ranging up to 72.6 nmol adducts/mol normal nucleotides, thereafter in kidneys, intestinal mucosa, and lowest in blood. Gulls from the urban site Skåne (Malmö harbour)
had significantly higher liver DNA adduct levels than Iceland, the control (P = 0.01), while the rural sites Blekinge and Södermanland did not (P > 0.05). Liver DNA adducts were detected in Swedish pulli, but not in pulli from Iceland. Frequency of micronucleated erythrocytes in adult birds was similar in all the regions studied, ranging from 0.18–0.28‰. Neither liver DNA adducts nor erythrocyte micronuclei levels were associated with observed hepatomegaly, poor condition or paralytic symptoms observed in the Swedish birds (P > 0.05), the DNA adduct levels are however suspected to reflect different pollution load of the respective regions. This work is full reported in Skarphedinsdottir et al (submitted 2010).

References

Aas et al. 2003. DNA adduct levels in fish from pristine areas are not detectable or low when analysed using the nuclease P1 version of the 32P postlabelling technique. Biomarkers, 8(6), 445–460.


4.4 Italy

MEDPOL – PHASE IV MONITORING ACTIVITIES

Prof. Aldo Viarengo (IT) presented progress with the MEDPOL – Phase IV monitoring activities.

The main purpose of the programme is to evaluate the biological effects of pollutants on marine organisms along the Mediterranean coasts. The programme highlights three important aspects. Firstly the choice of test species which is Mytilus spp. because of it is widely distributed and easily collected. Secondly, the use of biomarkers to evaluate the level of the stress syndrome induced by pollutants in the selected organisms; these are schematically categorized into two classes, biomarkers of stress and biomarker of exposure. A biomarker of stress reveals the stress syndrome by integrating the effects of a wide range of environmental pollutants and include the techniques; lysosomal membrane stability, micronuclei frequency, neutral lipid accumulation, lipofuscin accumulation. Biomarkers of exposure reflect the response of the organisms to a specific class of chemicals and include the techniques; metal-
lothionein content, exposure to heavy metals (Cd, Hg, Cu, Zn, etc.), stress on stress. The third aspect is the provision of a QA (Quality Assurance) Program, and this was achieved by a) the distribution of a “UNEP/MAP manual” for biomarker utilization, b) distribution of produced by RAMOGE in collaboration with UNEP/ MAP showing biomarker methodologies, c) organization of Training Courses to prepare the researchers to participate in the biomonitoring programs, and d) organization of an “Intercalibration Program”: the first one that has ever been realised to achieve a standardization of biomonitoring data.

Prof. Aldo Viarengo then presented the results from the UNEP – MAP MEDPOL programme from the last three years. Seventeen laboratories from different Mediterranean countries being involved to the UNEP MAP activity and they have contributed to the Mediterranean coast biomonitoring: these were Italy, Greece, Slovenia, Croatia, Tunisia, Monaco, France, Spain, Algerie, Morocco, Syria, Israel, Turkey, Albania, Malta, Egypt, Libanon, Palestine. Standardised protocols were agreed and used for, animal collection, transport of animals, storage of biological samples, biomarker choice and application and this included a training course at the MedPol Reference Centre in Alessandria and the equipping of laboratories in Egypt, Syria and Morocco. The biomarkers data was collected by following standard procedures and all results were sent to UNEP MAP by using a standard data transmission protocol. The results for lysosomal membrane stability, lysosomal lipofuscin content, and lysosomal neutral lipid accumulation showed that all the laboratories involved were able to identify the blind samples obtained from control and exposed mussels. The results for the metallothionein content intercalibration exercise showed that two labs were not able to correctly determine the metallothionein content. This indicates the need of yearly organized intercalibration activity and the importance of the training courses and of periods of training for the researcher involved in the program.

The current intercalibration exercise (2009–2010) is the first intercalibration exercise putting together Med Pol and ICES laboratories. It commenced in October 2009 and results from the sixteen laboratories are expected to be available by mid 2010. It is hoped that this programme will help to develop AQC harmonisation, including organising regular training courses, guarantee the quality of data, validate data collected throughout the year by laboratories and improve knowledge exchange between laboratories.

At previous meetings of WGBEC the “2-tier approach” used in MEDPOL to assess levels of pollution-induced distress syndrome in sentinel organisms had been described. At this meeting Prof Aldo Viarengo gave an update on these strategies and how they may be used in MEDPOL IV. These strategies have been developed and improved during the last decade, supported by funds from national and international programmes BEEP (EC), RAMOGE, NOMIRACLE (EC) and MED-POL (UNEP). The important use of this strategy is for biomonitoring/ecological risk assessment.

The 2-tier approach strategy is described as

Tier 1 – one biomarker: lysosomal stability + Stress on Stress + mortality

Tier 2 – full biomarker battery: 6 biomarkers – lipofuscin, neutral lipids, micronuclei test, metallothionein, acetylcholineesterase, lysosome/cytoplasm ratio and stress on stress

As result of the TIER I analyses: a) no effects on lysosomal membrane stability → clean sites → no other analysis necessary (biological or chemical); b) increased mortality → direct chemical analysis to identify pollutants that induce biological effects.
Apply TIER 2 analysis in sites where there are alterations in lysosomal membrane stability and use battery of biomarkers to quantify the stress syndrome.

This approach has been found to be cost effective. In order to assist the interpretation of results mussels are caged and sampled after 30 days exposure at the site of interest. This is important for the following reasons:

- using the same stock means the animals have similar genetic and physiological characteristics; and similar minimal content of toxic chemicals.
- variations occurring in the polluted sites allow comparison with chemical data obtained for organisms sampled in control/clean sites. This involves an accumulation of chemicals directly related to the month of mussel exposure in the polluted sites.
- it is not easy to correlate organism health status data to pollutant content in wild mussels that accumulate chemicals for years and detoxify them in order to survive in polluted areas.
- in wild mussels, it is possible to observe variations in biological parameters (such as gonad maturation) which can lead to problems with data analysis and interpretation. Caged mussels (3–4 weeks) maintain similar gonad maturation level.
- the use of caged mussels permits geographical referencing of the sampling sites.

It is well known that there are many difficulties in integrating biological effect and chemical data and to present the data objectively and meaningfully to decision makers. With this in mind Prof Aldo Viarengo then described an approach using an expert system using data from laboratory and field studies. A five-fold classification scheme has been derived (Stress Syndrome Level) from A (no stress) to E (pathologic stress) considering the alteration each biomarker on the basis of its stress response profile and the level of biological organisation (cell, tissue or organism). Further details on this approach can be found in Dondero et al., 2006.

Prof Aldo Viarengo also gave a short presentation on ecological risk assessment using modifications to the “Triad approach” using a case study on the Bormida river with fresh water and soil data.

References


Recommendation

WGBEC fully supported the proposed intercalibration exercise (Sept 2010) on lysosomal stability (NRR method) to be held in Alexandria in Italy; the first intercalibration exercise putting together MEDPOL and ICES laboratories. This is an important step forward for harmonisation between OSPAR, MEDPOL and HELCOM biomonitoring activities. WGBEC would recommend that ICES supports this initiative and recommends further support and uptake from organisations and laboratories within these communities.
4.5 Baltic Sea Issues: Report on ICES SGEH activities (Kari Lehtonen, by correspondence)

The ICES Study Group for the Development of Integrated Monitoring and Assessment of Ecosystem Health in the Baltic Sea (SGEH) will meet in Gdynia (PL), 1–5 March 2010. The group is chaired by Kari Lehtonen (FI), a WGBEC member. In short, the SGEH focuses its main activities on matters related to biological effects of contaminants in marine organisms in the Baltic Sea, a field with a significantly lesser research emphasis in this geographical area compared e.g. to eutrophication, biodiversity and fisheries, and high and urgent needs for development. Information on the effects of contaminants on biodiversity is also closely followed.

To achieve the target of developing assessments of Ecosystem Health in the Baltic Sea links with groups dealing with fisheries and eutrophication impacts will be established with expected participation of experts having data and information relevant to SGEH. Important aspects are identification of links between SGEH work related to HELCOM, OSPAR, EU (with a special reference to the Marine Strategy Framework Directive [MSFD]) and other ICES EGs, especially WGBEC, SGIMC and WGIAB. In regard to the MSFD, suggested criteria and methodological standards for the descriptors will be discussed in this group in the 2010 meeting. Since OSPAR is working on the Quality Status Report for 2010, SGEH will follow the outcome of this report, and it will also be discussed in the 2010 meeting.

Progress made through the BONUS+ programme BEAST project (Biological Effects of Anthropogenic Chemical Stress: Tools for the Assessment of Ecosystem Health [2009–2011]) and other similar activities in and outside the Baltic Sea will be reviewed at the 2010 SGEH meeting, with discussions on development of especially the parts of the project related to development of integrated monitoring (WP 2) and assessment of ecosystem health (WP 3) to serve the goals of the SGEH and Baltic Sea Action Plan (BSAP).

4.6 MSD

The WGBEC considered the progress made by the Marine Strategy Directive (MDS) descriptor 8 Task Group. No report was yet available but Dick Vethaak (NL) presented the executive summary (Annex 6). The WG fully supported the recommendations made by the Task Group which placed a strong emphasis on biological effect monitoring as developed by the WGBEC in the past decades and currently used by OSPAR. Given the recommendations made by the Task Group, the WGBEC anticipates that further work on biological effects monitoring and harmonisation of methods may increase in the next years. This is generally well in line with the WG’s activities in this area.

5  Review progress with the ICON (NSHEALTH) and Baltic BEAST programme; (ToR h).

5.1 Review progress within the BONUS+ Programme BEAST project (Kari Lehtonen, by correspondence)

The BEAST project (Biological Effects of Anthropogenic Chemical Stress: Tools for the Assessment of Ecosystem Health) was launched under the Baltic Sea BONUS+ Programme (2009–2011). The BEAST consists of 16 partners from all nine Baltic Sea countries. Detailed information on the BEAST project is available in the WGBEC Report 2009, at the BONUS+ website (http://www.bonusportal.org/research_projects), and at the BEAST project website (www.bonusportal.org/research_projects/...projects/beast/).
In short, the BEAST project consists of three thematic Work Packages (WP), see Figure 5.1.1:

WP1: Field studies and experiments in selected sub-regions of the Baltic Sea
- basic research: testing and validation of biomarkers in Baltic Sea species and environmental conditions

WP2: Application and validation of methods in monitoring and assessment in the Baltic Sea
- recommendations and practical guidelines for the integration of chemical-biological monitoring of hazardous substances in Baltic Sea monitoring programmes (mainly HELCOM)

WP3: Developing tools for ecosystem health assessment in the Baltic Sea
- testing and developing approaches (e.g. indices) for the assessment of Ecosystem Health in different sub-regions of the Baltic Sea

Research activities in the three WPs are organised under five sub-regional Tasks, i.e. field and experimental studies in the Gulf of Bothnia, G. of Finland, G. of Riga, G. of Gdansk and the Belt Sea (Fig. 5.1.2). In addition to WP leaders also each sub-regional Task has a responsible leader.

BEAST sampling campaigns started in April 2009 in the Gulf of Riga and they were continued at all target areas (except for the Gulf of Bothnia). The largest research activity in 2009 was the GOF-IA (Integrated Multidisciplinary Assessment of the Ecosystem Health of the Gulf of Finland) joint 2-week research cruise of r/v Aranda (FI) and r/v Walther Herwig III (DE) in August-September. Unfortunately, no permission to sample in Russian waters could be obtained and the original sampling plan had to be adjusted. Sampling was carried out at 20 point stations (Aranda) and 9 fishing areas (WHIII) in different parts of the Gulf of Finland within the Finnish and Estonian EEZ. The research performed consists of measurements of several biological and chemical parameters with emphasis on selected biomarkers (Table 5.1.1). The main aim is to use the data (plus additional existing data sets) for an integrated assessment of ecosystem health in the different sub-regions of the Gulf of Finland by using methods tested and developed under WP3. The BEAST sampling campaigns will continue in 2010 but no sampling is planned for the last year of the project, 2011.

In regard to WP2, a draft of a handbook with guidelines and standard operating procedures (SOPs) has been produced based on a document produced during the EU funded BEEP project (2001–2004). Harmonisation of the guidelines and SOPs with those under preparation for OSPAR is a further development plan.

An Excel-based project database has been developed in WP3 and is ready to receive data. The intention is also feed in data from the BEEP project and other available and relevant data from the Baltic Sea. These will be used for the testing and development of integrated indices and sub-regional assessments.

Collaboration with another BONUS+ project dealing with biological effects, BALCOFISH, has been established and aimed to be strengthened during 2011. In addition to practical collaboration activities (e.g. sampling, workshops) the aim is to start preparations for the coming call for projects for the Joint Baltic Sea Research Programme (BONUS-169). The objective of BONUS-169 is to enhance the Baltic Sea region research capacity to ensure a more sustainable development of the region. The Commission proposes to contribute € 50 million to a joint research investment with
eight EU Baltic Sea Member States. The € 100 million programme will provide a framework for the coordination of their environmental research.

Figure 5.1.1. Outline of the BEAST project.
Figure 5.1.2. Study areas of the BEAST project.
Table 5.1.1. Sampling scheme in the Gulf of Finland during the GOF-IA cruises with r/v Aranda and r/v Walther Herwig III in August-September 2009. The sampling was carried out at 20 point stations and 9 fishing areas around the G. of Finland in Finnish and Estonian EEZ.

<table>
<thead>
<tr>
<th>Sampling type</th>
<th>Sampling device</th>
<th>Parameter</th>
<th>Specific parameter</th>
<th>Species</th>
<th>Function or process</th>
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<td>Background data</td>
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<td>Water sampler</td>
<td>Community structure</td>
<td>Indicators &amp; indices</td>
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<td>Disturbed structure</td>
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<td>Nodularia spumigens</td>
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</table>
5.2 Progress with the ICON (Integrated Assessment of Contaminant Impacts on the North Sea): an international workshop

Ketil Hylland (NO) provided an overview and brief update on progress with the ICON (Integrated Assessment of Contaminant Impacts on the North Sea) project. The steering group for the project is Ketil Hylland (Chair)[Norway], Thomas Lang [Germany], Alistair McIntosh and Matt Gubbins [Scotland], Dick Vethaak [Netherlands], John Thain [England], Jörundur Svavarsson [Iceland].

The main objective of ICON, a practical workshop, is to provide a demonstration programme for the framework developed through the OSPAR/ICES WKIMON process (integrated chemical and biological monitoring). In addition the programme will allow the assessment of effects of contaminants over a range of North Sea, Icelandic and Mediterranean habitats and provide the opportunity to develop research topics and improve the underpinning science. The project was initiated by a kick-off meeting spring 2007: subsequently, samples have been collected during cruises and sampling campaigns in 2008 (all offshore locations, some inshore) and 2009 (additional inshore locations, including Iceland and UK). Locations to be included cover both coastal and offshore areas in the North Sea, Iceland and Mediterranean (figure 5.2.1).

Figure 5.2.1. Overview of locations to be included in ICON (stars).
Samples collected in 2008 have been distributed to participating laboratories across Europe for processing and analysis. These include dab samples from 10 locations, haddock from 4 locations and flounder from six locations. Further samples of flounder and mussels were taken from Iceland and the UK in 2009. The samples taken in Iceland were taken to determine “background responses”; Iceland is regarded as a pristine environment.

Coordination of the data and its assessment is crucial to the programme and in order to facilitate this process a central database has been established by the steering group at the University of Oslo (contact Ketil Hylland email: k.d.e.hylland@bio.uio.no).

Analysis of samples is ongoing and is due to be completed by August 2010. Each expert laboratory will assess its own data. Once all data has been submitted to the University of Oslo database the integrated assessment of the data will commence along the lines suggested by the OSPAR integrated approach or as deemed appropriate by the steering group.

It is anticipated that the integrated assessment will be completed in early autumn to enable a wrap-up conference to be held in November 2010 and subsequently to publish the outcomes in the open literature in 2011. ICON will also communicate the results of the programme to OSPAR and ICES in early 2011 in order to fulfil its obligation of running a demonstration programme on integrated chemical-biological effects as requested by OSPAR SIME in 2007.

In 2009 a poster was presented on the ICON programme at SETAC and a one page contribution was included in the OSPAR QSR 2010. In addition, a presentation will be made at the ICES ASC, Nantes, in the theme session (F) on monitoring of biological effects and contaminants.

6 Extending marine assessment and monitoring framework used in Chapter 10 of the QSR 2010 (OSPAR request 2010/1) - To review the methodology used by the OSPAR workshop on the development of Chapter 11 of the QSR 2010 (Utrecht workshop); (ToR j).

The working group considered the OSPAR report on the “biodiversity assessment workshop for the QSR 2010 (“Chapter 11” regional assessments)”. The workshop, held in Utrecht in February 2009, comprised 66 experts in different disciplines of marine science. The work during the workshop was divided into 8 groups, each with a Chair and Rapporteur, i.e. seabirds, cetaceans, seals, fish, rock and biogenic reef habitats (0–200 m depth), shallow sediment habitats (0–50 m depth), shelf sediment habitats (50–200 m depth), deep-sea habitats (>200 m depth). The aims for the workshop were very ambitious, i.e. (i) assess the quality status of the marine environment in each OSPAR Region, as represented by selected ecosystem components, (ii) assess trends since the QSR 2000 and provide an outlook on likely future trends (next 20 years), (iii) rank the pressures from human activities, based on their impact on the marine environment, (iv) identify priorities for future assessment, monitoring and management measures, recognizing the need for indicator development under the MSFD for the GES descriptors and any limitations in the data available. Although one would accept a large degree of uncertainty in any such assessment, this was clearly a process in which it would be equally important to indicate lack of knowledge as definite conclusions.

The outcome of the workshop will necessarily reflect the areas of expertise represented by its participants. This is would particularly be the case when the main
methodology is expert judgement, as in the present case. WGBEC was surprised that the workshop organisers had not seen it useful to include experts that are active in ICES and OSPAR working groups on effects of contaminants in marine ecosystems. The output from the workshop appeared to reflect a lack of sufficient scientific basis in marine ecotoxicology. Potential risks associated with the presence of contaminants in the selected compartments was generally evaluated as being low by the workshop, with worst case scenarios for hazardous substances limited to the effect of TBT on gastropods, PCB contamination in seals and effects on seabirds following the Prestige oil spill.

There were serious shortcomings to the methodology of the workshop and its conclusions. In addition to issues relating to a lack of ecotoxicological competence, the workshop decided not to include pelagic ecosystems and processes, thereby excluding the main marine primary producers of the oceans and the organisms that form the basis for most marine food webs (phyto- and zooplankton).

The workshop concluded that there is low risk from contaminants in cetaceans, seabirds and seals. The working group cannot see that this conclusion can be supported by current knowledge in any way. It is well known that populations of seabirds, toothed whales and seals in many of the regions included in the report have sufficiently high concentrations of a range of contaminants for there to be health impacts and such effects have indeed been shown (see e.g. Bustnes, 2006; De Swart et al., 1994; Hall et al., 2006; Reijnders et al., 1986; Ross et al., 1996).

The workshop identified harbour sediments as a worst case situation for shallow water sediments due to effects from TBT on gastropods. This is probably the case although the issue with TBT could probably be extended to include larger parts of coastal areas and some estuaries and fjords. In addition, there are a range of other contaminants in many harbours that could be expected to cause effects on sediment-dwelling organisms, e.g. PAHs, chlorinated POPs and metals.

It appears that the participants themselves were not entirely comfortable with the outcome and that there was a pressure to “produce” data even though the required information may have been lacking.

It was noted that the workshop considered that this type of broader ecosystem assessment could be a useful contribution to an Initial Assessment for the Marine Strategy Framework Directive (MSFD) in 2012. WGBEC would be concerned if this were the case without further underpinning science and engagement with experts in the specific scientific fields.

To conclude, the working group found the report to reflect an insufficient insight into levels and effects of contaminants in marine compartments and its conclusions should not in any way be used in future processes.

References
De Swart et al., 1994; Environ Health Perspect, 104: 823–828.
Hall et al., 2006; Environ Health Persp., 114: 704-711.
7 Report to SSGHIE on potential and current contributions of your EG to the Strategic Initiative on Coastal and Marine Spatial Planning (SICMSP); (ToR k)

The WG considered the draft strategy document on “Area-based science and management of marine ecosystems: from the coast to the high seas”. The activity links up to the ICES mission statement “To advance the scientific capacity to give advice on human activities affecting, and affected by, marine ecosystems”. This is an ongoing process and a comprehensive review of the draft document would not be appropriate, but the WG would like to emphasise that contaminant inputs and their effects in marine ecosystems are highly relevant to the ICES mission statement and a necessary component of marine ecosystem management. There are specific challenges associated with contaminants in terms of area-based management since contaminants and their effects may be associated with large areas. Three specific examples directly relevant to area-based management are point sources of contaminants (i.e. industry, offshore platforms, and rivers), diffuse sources (e.g. harbours, urban areas) and contaminants in sediments.

8 Report to SSGHIE on your plans to promote cooperation between EGs covering similar scientific issues; n (ToR l)

As an introduction the Chair, John Thain gave a presentation on the new ICES structure, the ICES Vision, Science Plan, Strategic Initiative and the role of EGs within the new SCICOM Steering Groups. WGBEC sits within the Steering Group on Human Interactions on Ecosystems (SGHIE), Chair: Erik Olsen (NO)

8.1 WGBEC core activities and future directions

After intensive discussions on the scope and future activities of the WG, and taken consideration of the various ICES vision and strategy documents, WGBEC identified the core business and 8 areas for future directions. While some of these activities are considered as core business, others clearly attend to broaden the scope of WGBEC, towards more ecology and wider ecosystem effects.

8.1.1 WGBEC core business

1) Development of strategies for biological effects in integrated monitoring and assessment and provide advice on appropriate methods for monitoring;
2) The role of biological effects techniques in environmental risk assessment;
3) Increase fundamental understanding of ecotoxicological processes;
4) Provide advice on effects of novel / emerging compounds;
5) Facilitate harmonisation and AQC concerning biological effects methods;
6) Improve understanding on how and whether contaminants in the marine environment interacts with other environmental factors and processes;
7) Improve ecosystem-oriented understanding of how contaminants affect marine systems and processes;
8) Initiate transnational cooperative research and monitoring (e.g. BECPELAG, ICON, BEQUALM);
9) Provide guidance to international organisations / conventions as required and agreed by ICES (OSPAR, HELCOM, AMAP).
Research needs required for the implementation of MSFD are not yet available but may be relevant. International cooperative research / monitoring areas research proposals should be initiated. This has successfully been done in the past, e.g. BECPELAG, ICON, BONUS+, BEAST and MEDPOL.

8.1.2 New future directions identified for WGBEC

8.1.2.1 Impacts of contaminants on food webs and ecosystem function / processes:
Continued attention should be given to top predators such as marine mammals, but also sea birds. Special emphasis should be placed on lower levels of the trophic food web, such as the impact of contaminants on benthic, pelagic algae and microbial populations and communities and their potential impact on carrying capacity of marine and coastal waters. Over the long term, knowledge of lower food web population and community effects can also result in new indicators to be included as additional components for integrated monitoring and assessment. It was pointed out that this type of research is very challenging due to complexity / diversity of plankton and that it will require experimental work. There are also clear interactions with eutrophication. This type of research needs modelling and energy budgeting.

8.1.2.2 Development of bioassays and/or biomarkers for detecting and determining the effects of contaminants on the immunocompetence and fitness of organisms
This seems particularly relevant to clarify the contributing role of contaminants in the recently observed epizootics in marine mammals and fish.

8.1.2.3 Ecogenetics.
There is increasing knowledge on the effects of contaminants on population genetics and for example antibacterial resistance development. So far WGBEC only considered this research field rarely, but this will deserve more attention in the future.

8.1.2.4 Mixture of toxicity and interactions with natural factors should receive increasing attention
This is a very challenging field of research, but essential to clarify the role of contaminants in cumulative stress impact assessments.

8.1.2.5 More focus on modelling fate of contaminants and effects
Most models are lacking an effect module on top of fate modelling. WGBEC could play a contributing role here. This should also include increased effort on expert system modelling for biomarkers based on data collected all around Europe. Such an approach was done in the late 90's but failed due to shortage of suitable data. Hence it will be particularly worthwhile to revisit the expert system approach.

8.1.2.6 Genomics / proteomics / metabolomics
Already regularly on the WGBEC agenda, this area will require increasing attention and effort. In the future the technology will make this easier and there will be much work in applying this technology in monitoring and assessment approaches.

8.1.2.7 Climate change including ocean acidification
The WG already conducted some work on the effects of climate change on ecotoxicological processes and environmental quality issues. Future work should also include the changes of PH on the bioavailability, uptake and other ecotoxicological processes.
8.1.2.8 Plastic particles - (addressed here under agenda item 14)

The WG envisaged this increasing environmental problem as a particular urgent area for future direction, given its potential impact on food chain energetics, food web transfer of contaminants, and increased risk for contaminant exposure and effects. The influence of plastic particle presence in sediments and their confounding effects on chemical and bioassay analysis results should be assessed.

A possible mechanism to widen the WG science basis is to yearly invite non-member experts that can add value and broaden scope. There is also a clear need to collaborate with WGs in other areas (see Table 8.3.1 below).

8.2 WGBEC activities relevant to ICES Science plan

WGBEC viewed the Science Plan and noted that sixteen research topics have been identified as being of strategic importance to the advisory needs of ICES Member Countries and clients in the coming decade. These topics have been organized in three thematic areas.

1) Understanding Ecosystem Functioning
2) Understanding Interactions of Human Activities with Ecosystems
3) Development of options for sustainable use of ecosystems

The sixteen research topics were discussed and activities relating to WGBEC activities were noted as follows:

8.2.1 Understanding ecosystem functioning

The WG has provided advice on possible effects of climate change, an activity which led to the production of a paper (Schiedek et al. 2007). The group has planned activities on ocean acidification. Members of the group are involved in studies on impacts modelling based on contaminant loading and climate change models.

There are links between contaminant-related responses and marine biodiversity. This is an area WGBEC wishes to invest more time for in the years to come. Some contaminant-related methods will be relevant to predict local population declines or even extinction.

Contaminant effects are highly relevant for top predators of marine ecosystems and need to be included. There is evidence of contaminant effects on marine birds and mammals (see e.g. Bustnes, 2006; De Swart et al., 1994; Hall et al., 2006; Reijnders et al., 1986; Ross et al., 1996).

The WG has ongoing work with sensitive areas and ecosystems, e.g. contaminants in the Arctic.

8.2.2 Understanding interactions of human activities with ecosystems

This thematic area is at the basis of WGBECs activities. Trawling will increase resuspension resulting in increased contaminant availability in areas with elevated concentration of contaminants in sediments. WGBEC members are involved in projects addressing contaminant inputs from aquaculture, which is relevant to mariculture carrying capacity. WGBEC has a range of activities relevant to understanding contaminant impacts on populations and communities. This theme is within the core activity of the working group.
8.2.3 Development of options for sustainable use of ecosystems

There is no direct link between the work of the group and living resource management tools, but WGBEC is interested and has appropriate connections to modelling work in this area. Impacts of oil spills may benefit from modelling toxic effects.

There is a potential impact of development activities (as a result of MSP) on contaminant loading and there is a need to look at socioeconomic impacts of contaminant effects in future.

8.3 Collaboration with other expert groups

The WG has a history of collaboration with a range of other ICES EGs as well as MEDPOL and HELCOM (Table 8.3.1).

Table 8.3.1. Overview of EGs with which WGBEC has had collaboration of with which WGBEC would envisage possible future interactions.

<table>
<thead>
<tr>
<th>EXPERT GROUPS</th>
<th>WORKED BEFORE?</th>
<th>INTERESTED IN JOINT ACTIVITY?</th>
<th>JOINT MEETING?</th>
</tr>
</thead>
<tbody>
<tr>
<td>WGPDMO</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>MCWG</td>
<td>Yes</td>
<td>Yes</td>
<td>Potential</td>
</tr>
<tr>
<td>MSWG</td>
<td>Yes</td>
<td>Yes</td>
<td>Potential</td>
</tr>
<tr>
<td>ICZM</td>
<td>No</td>
<td>Potential</td>
<td>No</td>
</tr>
<tr>
<td>SGONS</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>WGMASC</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>WGEIM</td>
<td>No</td>
<td>Yes</td>
<td>Potential</td>
</tr>
<tr>
<td>WGHABD</td>
<td>No</td>
<td>Potential</td>
<td>No</td>
</tr>
<tr>
<td>WGEXT</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>WGFCCIFS</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>WAGFDM</td>
<td>Yes</td>
<td>Yes</td>
<td>Potential</td>
</tr>
<tr>
<td>WGEEL</td>
<td>No</td>
<td>Yes</td>
<td>Potential</td>
</tr>
<tr>
<td>WGMME</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>SGIMC</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>SGEH</td>
<td>No</td>
<td>Yes</td>
<td>Potential</td>
</tr>
<tr>
<td>MEDPOL</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Working Group on Pathology and Diseases of Marine Organisms (WGPDMO)

Many common activities, e.g. interaction between contaminants and disease. Joint efforts to develop integrated monitoring and assessment. Scope for future development of immunotoxicological end points.

Working Group on Marine Sediments in Relation to Pollution (MCWG)

Several areas of common interest, e.g. passive sampling and TIE.

Working Group on Marine Sediments in Relation to Pollution (WGMS)

Have worked together in the past on developing concepts for sediment bioavailability and there is a current need to develop common projects on passive sampling. There is a large overlap concerning the integrated monitoring strategy.
Working Group for Marine Planning and Coastal Zone Management (WGMPCZM)
Contaminants are important in ICZM, but there has been little direct contact until now.

Working Group on Environmental Interactions of Mariculture (WGEIM)
Common ground with contaminant discharges from finfish farms and environmental interactions.

ICES - IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD)
Some overlap on toxicological effects and interactions of HAB toxins on toxicological endpoints.

Working Group on Application of Genetics in Fisheries and Mariculture (WGAGFM)
Interest from WGBEC on methods they use and potential applicability to field of toxicogenomics / population genetic effects from contaminants.

Joint EIFAC/ICES Working Group on Eels (WGEEL)
Interested in contaminant effects in eels and future joint activity.

Study Group in Integrated Monitoring of Contaminants and Biological Effects (SGIMC)
Already closely working in this area. WGBEC output feeding into SGIMC in Jan 2010.

Study Group for the Development of Integrated Monitoring and Assessment of Ecosystem Health in the Baltic Sea (SGEH)
Reports from SGEH members received by WGBEC at past meetings. Much overlap, need to coordinate work between SG and WG more.

MEDPOL monitoring group
Not ICES, but important to harmonise activities here. Common ground on AQC and integrated strategies. Joint workshops already planned.

Working Group on Marine Habitat Mapping (WGMME)
WGBEC believes contaminants in marine mammals play an important role and there is potential interaction in this field between the groups. The Chair will report back to SSGHIE for comment and investigate how some of these activities may be taken forward.

References
9 Review ICES WGBEC list of recommended biological effects methods for monitoring purposes and define how this fits in for both OSPAR and EU MSFD purposes; (ToR f)

The WGBEC discussed the ‘promising’ and ‘recommended’ monitoring techniques that had been last updated in 2007.

The objective of preparing tables 9.1 – 9.4 is to provide information on the status of methods to assess contaminant effects in marine ecosystems and which national programmes are currently using them. Methods should be used as part of an integrated package c.f. SGIMC / WKIMON integrated framework. (See SGIMC 2010 report, (annex 16 and 17) www.ices.dk)

During the 2010 meeting the WGBEC confirmed that recommended methods for monitoring programmes should conform to the following criteria:

1) A recommended method needs to be an established technique that is available as a published method, preferably in the TIMES series.

2) A recommended method (or combination of methods) should have been shown to respond to contaminant exposure in the field.

3) A recommended method (or combination of methods) should be able to differentiate the effects of contaminant from natural background variability.

The WGBEC also confirmed that updated descriptions of recommended methods should be published in the TIMES. Tables 9.1 and 9.2 have been edited to include a direct reference to the ICES Techniques in Marine Environmental Sciences publication (where available) and also included information relating to those countries where specific techniques are currently in use and those international monitoring programmes where their use is proposed.

Changes to the tables

Induction/inhibition of multidrug/multixenobiotic resistance (MDR/MXR) in Mytilus species was removed from Table 9.1b (Recommended techniques for biological monitoring programmes at the national or international level - methods for invertebrates) and grouped with MDR/MXR detection methods in fish and invertebrates in Table 9.2. (Promising biological effects monitoring methods that require further research before they can be recommended for monitoring both fish and invertebrates). This was due to the fact that the method specific to Mytilus lacked an ICES TIMES Series document, was only used in a limited number of laboratories had no current AQC programme in place.

Where appropriate references supporting recommended and promising techniques have been updated to reflect current literature.

Methods for consideration at the next meeting

The working group decided that review documents for the comet assay and micronucleus assay will be developed intersessionally and updates presented at the 2011 meeting to allow an assessment of their suitability for recommendation to be made.

Other points arising

Issues were raised concerning the current status of some of the ICES Times documents. For example, certain biomarkers including CYP1A analysis and Metal-
lothionein induction are now routinely measured using qPCR techniques. The working group discussed producing a general set of recommended guidelines for using molecular techniques in the determination of gene expression levels.
<table>
<thead>
<tr>
<th>METHOD</th>
<th>ORGANISM</th>
<th>AQC</th>
<th>ICES TIMES</th>
<th>ISSUES ADDRESSED</th>
<th>BIOLOGICAL SIGNIFICANCE</th>
<th>CURRENT NATIONAL ACTIVE USE</th>
<th>INTERNATIONAL PROGRAMME</th>
<th>REF.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulky DNA adduct formation</td>
<td>Fish</td>
<td>No current AQC programme.</td>
<td>No. 25</td>
<td>PAHs; other synthetic organics, e.g., nitro-organics, amino triazine pesticides</td>
<td>Measures genotoxic effects. Possible predictor of pathology through mechanistic links.</td>
<td>N, SE, UK*</td>
<td>WKIMON</td>
<td>1-6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(triazines)</td>
<td>Sensitive indicator of past and present exposure.</td>
<td>*(ad hoc only)</td>
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<tr>
<td>AChE inhibition</td>
<td>Fish</td>
<td>No current AQC programme.</td>
<td>No. 22</td>
<td>Organophosphates and carbamates or similar molecules</td>
<td>Measures exposure.</td>
<td>F, N. UK, E, IRE</td>
<td>BEAST</td>
<td>7-10</td>
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<tr>
<td>Metallothionein induction</td>
<td>Fish</td>
<td>No current AQC programme.</td>
<td>No. 26</td>
<td>Measures induction of metallothionein protein by certain metals (e.g., Zn, Cu, Cd, Hg)</td>
<td>Measures exposure and disturbance of copper and zinc metabolism.</td>
<td>UK, N, S, E, IRE</td>
<td>MEDPOL</td>
<td>11-15</td>
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<tr>
<td>EROD or P4501A induction</td>
<td>Fish</td>
<td>B (last run 2009)</td>
<td>No. 23 and No. 14</td>
<td>Measures induction of enzymes which metabolize planar organic contaminants (e.g., PAHs, planar PCBs, dioxins)</td>
<td>Possible predictor of pathology through mechanistic links. Sensitive indicator of past and present exposure.</td>
<td>UK, B, N, S, F, E, IRE</td>
<td>MEDPOL, WKIMON</td>
<td>16-23</td>
</tr>
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<tr>
<td>ALA-D inhibition</td>
<td>Fish</td>
<td>No current AQC programme.</td>
<td>No. 34</td>
<td>Lead</td>
<td>Index of exposure.</td>
<td>N, E*</td>
<td>*(ad hoc only)</td>
<td>24-25</td>
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<tr>
<td>PAH bile metabolites</td>
<td>Fish</td>
<td>No current AQC programme.</td>
<td>No. 39</td>
<td>PAHs</td>
<td>Measures exposure to and metabolism of PAHs.</td>
<td>IRE, UK, N, NE, B, D</td>
<td>WKIMON, BEAST</td>
<td>26-27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Lysosomal stability using histochemical</td>
<td>Fish</td>
<td>IMARE workshop</td>
<td></td>
<td>Not contaminant-specific but responds to a wide variety of xenobiotic contaminants and metals</td>
<td>Measures cellular damage and is a good predictor of pathology. Provides a link between exposure and pathological endpoints. Possibly, a tool for immunosuppression studies in white blood cells.</td>
<td>D, more expected to take it up after 2008 IMARE workshops</td>
<td>WKIMON, BEAST</td>
<td>28-31</td>
</tr>
<tr>
<td>detection</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>METHOD</td>
<td>ORGANISM</td>
<td>AQC</td>
<td>ICES TIMES</td>
<td>ISSUES ADDRESSED</td>
<td>BIOLOGICAL SIGNIFICANCE</td>
<td>CURRENT NATIONAL ACTIVE USE</td>
<td>INTERNATIONAL PROGRAMME</td>
<td>REF.</td>
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</tr>
<tr>
<td>Early toxicopathic lesions, pre-neoplastic and neoplastic liver lesions by and histopathology</td>
<td>Fish</td>
<td>B (last run 2009)</td>
<td>No. 38</td>
<td>PAHs, other synthetic organics, e.g., nitro-organics, amino triazine pesticides (triazines)</td>
<td>Diagnosis of pathological changes and enzymatic markers of carcinogenesis associated with exposure to genotoxic and non-genotoxic carcinogens.</td>
<td>UK, D, NE, IRE</td>
<td>WKIMON, BEAST</td>
<td>32 - 42</td>
</tr>
<tr>
<td>External visible lesions and parasites</td>
<td>Limanda limanda, Platichthys flesus, Gadus morhua</td>
<td>B (last run 2009)</td>
<td>No. 19</td>
<td>Responds to a wide variety of environmental contaminants and non-specific stressors</td>
<td>Integrative response; measures general fish health; elevated prevalence may indicate exposure to contaminants.</td>
<td>UK, NE, GER, F</td>
<td>WKIMON, BEAST</td>
<td>43-44</td>
</tr>
<tr>
<td>Vitellogenin induction</td>
<td>Male and juvenile fish</td>
<td>No current AQC programme.</td>
<td>No. 31</td>
<td>Oestrogenic substances</td>
<td>Measures feminization of male fish and reproductive impairment.</td>
<td>IRE, UK, NO, D* *(Ad hoc)</td>
<td>WKIMON</td>
<td>45-48</td>
</tr>
<tr>
<td>Intersex</td>
<td>Male flounder, eelpout, dab</td>
<td>No current AQC programme.</td>
<td>In prep.</td>
<td>Oestrogenic substances</td>
<td>Measures feminization of male fish and reproductive impairment.</td>
<td>IRE, D, UK</td>
<td>BEAST, WKIMON</td>
<td>49-50</td>
</tr>
<tr>
<td>Reproductive success in Zoarces viviparus</td>
<td>Zoarces viviparous</td>
<td>No current AQC programme.</td>
<td></td>
<td></td>
<td>Measures reproductive output and survival of eggs and fry in relation to contaminants. Restricted to period when young are carried by female viviparous fish.</td>
<td>SE, D, DE</td>
<td>BEAST</td>
<td>51</td>
</tr>
<tr>
<td>Alkylphenol-bile metabolites</td>
<td>Fish (cod)</td>
<td>No current AQC programme</td>
<td>In press</td>
<td>Alkyl phenols</td>
<td>Measures exposure to and metabolism of Alkylated phenols</td>
<td>NO</td>
<td>WKIMON</td>
<td>Awaiting publications</td>
</tr>
</tbody>
</table>

Table 9.1b. Recommended techniques for biological monitoring programmes at the national or international level - methods for invertebrates.

<table>
<thead>
<tr>
<th>METHOD</th>
<th>ORGANISM</th>
<th>QA</th>
<th>ICES TIMES</th>
<th>ISSUE ADDRESSED</th>
<th>BIOLOGICAL SIGNIFICANCE</th>
<th>NATIONAL</th>
<th>INTERNATIONAL</th>
<th>REF</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChE inhibition</td>
<td>Molluscs and crustaceans</td>
<td>No current AQC programme.</td>
<td>No. 22</td>
<td>Organophosphates and carbamates or similar molecules Possibly algal toxins</td>
<td>Measures exposure to a wide range of compounds and a marker of stress.</td>
<td>E, F, IRE, UK*</td>
<td>(ad hoc)</td>
<td>52-53</td>
</tr>
<tr>
<td>Metallothionein induction</td>
<td>Mytilus</td>
<td>Programme run under MEDPOL in 2009.</td>
<td>No. 36</td>
<td>Measures induction of metallothionein protein by certain metals (e.g., Zn, Cu, Cd, Hg)</td>
<td>Measures exposure and disturbance of copper and zinc metabolism.</td>
<td>E, UK, IRE</td>
<td>MEDPOL</td>
<td>54-55</td>
</tr>
<tr>
<td>Lysosomal stability (including NRR)</td>
<td>Mytilus, Oyster</td>
<td>MEDPOL training workshop 2010 ring trial 2011.</td>
<td>No. 36</td>
<td>Not contaminant-specific, but responds to a wide variety of xenobiotic contaminants and metals</td>
<td>Measures cellular damage and is a good predictor of pathology. Provides a link between exposure and pathological endpoints. Possibly, a tool for immunosuppression studies in white blood cells.</td>
<td>IT, IRE, UK, N, NE, IS</td>
<td>MEDPOL, WKIMON, BEAST</td>
<td>56-70</td>
</tr>
<tr>
<td>Scope for growth</td>
<td>Bivalve molluscs, e.g., Mytilus spp. and oysters</td>
<td>No current AQC programme.</td>
<td>No. 40</td>
<td>Responds to a wide variety of contaminants</td>
<td>Integrative response, a sensitive sub-lethal measure of energy available for growth.</td>
<td>IRE *, E *, UK *, IS *</td>
<td>(Ad hoc only)</td>
<td>71-72, 148</td>
</tr>
<tr>
<td>Imposex</td>
<td>Neogastropod molluscs (Nucella lapillus, Buccinum undatum, Hinia reticulata, Neptunea antiqua)</td>
<td>Q</td>
<td>No. 24 (N. lapillus)</td>
<td>Specific to organotins</td>
<td>Reproductive interference Estuarine and coastal littoral waters (Nucella) and offshore waters (Buccinum).</td>
<td>IRE, E, FR, UK, IRE, HE, DK, N</td>
<td>73-82</td>
<td></td>
</tr>
<tr>
<td>Intersex</td>
<td>Littorina littorea</td>
<td>Q</td>
<td>No. 37</td>
<td>Specific to reproductive effects of organotins</td>
<td>Reproductive interference in coastal (littoral) waters.</td>
<td>NE, (Ad hoc as replacement for Nucella)</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td><strong>Histopathology</strong></td>
<td><strong>Blue mussels</strong></td>
<td><strong>Cefas run histopathology workshop (2010)</strong></td>
<td><strong>In prep.</strong></td>
<td><strong>Not contaminant-specific</strong></td>
<td><strong>General responses</strong></td>
<td><strong>UK, IRE, NO, D, E, FR, I</strong></td>
<td><strong>MEDPOL, WKIMON</strong></td>
<td><strong>87 - 89</strong></td>
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</tbody>
</table>

| **Embryo aberrations in field-collected amphipod crustaceans** | **Amphipods** | **No current AQC programme.** | **No. 41** | **Contaminant-specific** | **Measures frequency of different types of lethal embryo aberrations; allows for separating effects of contaminants and environmental climate variables** | **SE** | **BEAST** | **90 - 94** |

B: BEQUALM; Q: QUASIMEME. ICES TIMES: [http://www.ices.dk/products/techniques.asp](http://www.ices.dk/products/techniques.asp)

Table 9.1c. Recommended techniques for biological monitoring programmes at the national or international level - Bioassays and methods for specific matrices.

<table>
<thead>
<tr>
<th><strong>METHOD</strong></th>
<th><strong>ORGANISM</strong></th>
<th><strong>ICES TIMES</strong></th>
<th><strong>QA</strong></th>
<th><strong>ISSUE ADDRESSED</strong></th>
<th><strong>BIOLOGICAL SIGNIFICANCE</strong></th>
<th><strong>NATIONAL PROGRAMMES</strong></th>
<th><strong>REFERENCES</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Benthic community analysis</td>
<td>Macro-, meio-, and epibenthos</td>
<td>B (low uptake outside UK)</td>
<td>Responds to a wide variety of contaminants, particularly those resulting in organic enrichment</td>
<td>Ecosystem level. Retrospective. Particularly useful for point sources. Most appropriate for deployment when other monitoring methods indicate that a problem may exist.</td>
<td>B, UK, N, NE, E, F, IRE</td>
<td>Coastal waters driven under WFD</td>
<td>95 – 100</td>
</tr>
<tr>
<td>Whole sediment bioassays</td>
<td>Corophium (problems with stocks i.e. look to get standards) Arenicola, Ampelisca brevicornis Other species may be used.</td>
<td>No. 29 (Arenicola) No. 28 (Corophium)</td>
<td>B</td>
<td>Not contaminant-specific, will respond to a wide range of environmental contaminants in sediments</td>
<td>Acute/lethal and acute/sub-lethal toxicity only at present. May enable retrospective interpretation of community changes</td>
<td>UK, E, NE, N, IRE, I</td>
<td>WKIMON MEDPOL, BEAST</td>
</tr>
</tbody>
</table>
Bioassays of sediment pore waters, sea water elutriates, sea water samples, extracts

Bivalve embryo Acartia, Sea urchin embryos, tisbe No. 11 (Oyster embryo), Sea urchin in prep. No current AQ programme. Will respond to a wide range of environmental contaminants, Useful for dredge spoils, sediments liable to re-suspension Acute and sub-lethal toxicity, including genotoxicity, etc. Toxicity of hydrophobic contaminants might be underestimated in pore water assays.

UK, NE, N, DE, D, F, E, I, IRE WKIMON, MEDPOL 103 – 104

CALUX Reporter gene assay MODELKEY Ah receptor-active compounds Predictor of dioxin like toxicity NE, UK, N, F* *(ad hoc) 105

YES Reporter gene assay (yeast) MODELKEY Oestrogen receptor-active compounds Potential endocrine disruption UK, N, NE, IRE 106 – 107

YAS Reporter gene assay (yeast) MODELKEY Androgen receptor-active compounds Potential endocrine disruption UK, N, NE 108 – 109

Table 9.2. Promising biological effects monitoring methods that require further research before they can be recommended for monitoring (both fish, and invertebrates).

<table>
<thead>
<tr>
<th>METHOD</th>
<th>ORGANISM</th>
<th>ISSUE ADDRESSED</th>
<th>BIOLOGICAL SIGNIFICANCE</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA strand breaks including Comet assay</td>
<td>Fish, mussels, cells</td>
<td>Not contaminant-specific, will respond to a wide range of environmental contaminants</td>
<td>Measures genotoxic effects, but is also extremely sensitive to other environmental parameters.</td>
<td>110 – 112</td>
</tr>
<tr>
<td>BaP Hydroxylase -like enzymes</td>
<td>Invertebrates</td>
<td>Induced enzyme response to PAHs, planar PCBs, dioxins and/or furans</td>
<td>Measures exposure to organic contaminants.</td>
<td>113 – 114</td>
</tr>
<tr>
<td>Induction/inhibition of Multidrug/multixenobiotic resistance (MDR/MXR)</td>
<td>Fish and invertebrates including Mytilus</td>
<td>Multiple contaminants (organics and metals)</td>
<td>Adaptation/inhibition in response to xenobiotic stress.</td>
<td>84 – 86, 115 – 119</td>
</tr>
<tr>
<td>Glutathion-S-transferase(s) (GST)</td>
<td>Fish, molluscs</td>
<td>Predominantly organic xenobiotics</td>
<td>Measures exposure and the capacity of the major group of phase II enzymes. Considered most promising for isoenzyme-specific measurements</td>
<td>120 – 122</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Fish, invertebrates</td>
<td>Not contaminant-specific, will respond to a wide range of environmental contaminants</td>
<td>Measures the presence of free radicals.</td>
<td>123 – 126</td>
</tr>
<tr>
<td>Method</td>
<td>Species</td>
<td>Description</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>Immunocompetence</td>
<td>Fish, invertebrates</td>
<td>Not contaminant-specific, will respond to a wide range of environmental contaminants</td>
<td>Measures factors that influence susceptibility to disease.</td>
<td>127</td>
</tr>
<tr>
<td>On-line monitoring</td>
<td>Mussels and crabs</td>
<td>Not contaminant-specific, will respond to a wide range of environmental contaminants</td>
<td>Measures the effects of chemicals on heart rate using a simple and inexpensive remote biosensor. Gives an integrated response.</td>
<td>128</td>
</tr>
<tr>
<td>Abnormalities in wild fish embryos and larvae</td>
<td>Fish, including demersal and pelagic species</td>
<td>Not linked unequivocally to contaminants</td>
<td>Measures frequency of probably lethal abnormalities in fish larvae. Mutagenic, teratogenic.</td>
<td>129 – 130</td>
</tr>
<tr>
<td>Bulky DNA adduct formation</td>
<td>Mussels, invertebrates</td>
<td>PAHs, other synthetic organics</td>
<td>Measures genotoxic effects</td>
<td>131 – 134</td>
</tr>
<tr>
<td>Gene arrays</td>
<td>Fish, mussels</td>
<td>Various</td>
<td>Combined responses from various biomarkers</td>
<td>135 – 137</td>
</tr>
<tr>
<td>Histopathology</td>
<td>Invertebrates (other than Mytilus)</td>
<td>Not contaminant-specific</td>
<td>General responses</td>
<td>Awaiting publications</td>
</tr>
<tr>
<td>Spiggin</td>
<td>Three-spined stickleback</td>
<td>Androgens</td>
<td>Measures environmental androgens</td>
<td>138</td>
</tr>
<tr>
<td>Micronuclei</td>
<td>Fish, bivalve molluscs</td>
<td>Not contaminant-specific</td>
<td>Exposure to aneugenic and clastogenic</td>
<td>139 - 141</td>
</tr>
<tr>
<td>Peroxisomal proliferation (enzyme assays)</td>
<td>Fish and invertebrates</td>
<td>Contaminant-specific</td>
<td>Potential alterations in lipid metabolism, non-genotoxic carcinogenesis</td>
<td>142 -144</td>
</tr>
<tr>
<td>Cellular Energy Allocation</td>
<td>Invertebrates and small fish</td>
<td>Wide range of stressors</td>
<td>Changes in metabolic turnover and specific allocations will be linked to effects at higher levels of ecological organization</td>
<td>145</td>
</tr>
</tbody>
</table>
Table 9.3. Promising biological effects monitoring methods that require further research before they can be recommended for monitoring - Bioassays and methods for specific matrices.

<table>
<thead>
<tr>
<th>METHOD</th>
<th>ORGANISM</th>
<th>ISSUE ADDRESSED</th>
<th>BIOLOGICAL SIGNIFICANCE</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALUX Reporter gene assay</td>
<td>Oestrogen receptor-active compounds</td>
<td>Potential endocrine disruption.</td>
<td>146</td>
<td></td>
</tr>
<tr>
<td>CALUX Reporter gene assay</td>
<td>Androgen receptor-active compounds</td>
<td>Potential endocrine disruption.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic whole sediment bioassays Invertebrates</td>
<td>Responds to a wide range of contaminants</td>
<td>Measurements such as growth and reproduction, coupled to biomarker responses, which will give a measure of the bioavailability and chronic toxicity in whole sediments.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pollution-induced community tolerance (PICT) water bioassay Microalgae, bacteria</td>
<td>Specific contaminants can be tested</td>
<td>Measure of degree of adaptation to specific pollutants. Not yet widely tested; retrospective.</td>
<td>147-148</td>
<td></td>
</tr>
</tbody>
</table>

Table 9.4. Biological effects methods that would require further development/application to be considered promising for use in the ICES area.

<table>
<thead>
<tr>
<th>METHOD</th>
<th>ORGANISM</th>
<th>ISSUE ADDRESSED</th>
<th>BIOLOGICAL SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncogenes</td>
<td>Fish</td>
<td>PAHs Other synthetic organics, e.g., nitro-organics, amino triazine pesticides (triazines)</td>
<td>Activation of oncogenes (ras) or damage to tumour-suppressor genes (p53). Measures genotoxic effects leading to carcinogenesis.</td>
</tr>
<tr>
<td>ELISA for DNA adducts</td>
<td>Fish</td>
<td>Not contaminant-specific</td>
<td>Genotoxic effects</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Fish cells</td>
<td>Responds to a wide range of contaminants</td>
<td>General response.</td>
</tr>
<tr>
<td>AChE inhibition</td>
<td>Other invertebrates</td>
<td>Organophosphates and carbamates or similar molecules. Possibly algal toxins</td>
<td>Measures exposure</td>
</tr>
<tr>
<td>Delayed reproduction/ gonadal maturation</td>
<td>Fish</td>
<td>Not contaminant-specific</td>
<td>Reproductive disruption</td>
</tr>
<tr>
<td>Aromatase</td>
<td>Fish</td>
<td></td>
<td>In assessing the potential ecological risk of CYP19 inhibitors, in particular in the context of relating alterations in subcellular indicators of endocrine function</td>
</tr>
</tbody>
</table>
References Agenda Item 9


Feist, S. W., Lang, T., Stentiford, G. D., and Koehler, A. 2004. Use of liver pathology of the European flatfish dab (Limanda limanda L.) and flounder (Platichthys flesus L.) for monitoring. ICES Techniques in Marine Environmental Sciences 38. 42pp


10 In close cooperation with ICES / OSPAR SGIMC conduct intersessional work for review at 2010 meeting based on the outcome of the SGIMC Aberdeen Workshop, October 2009.; (ToR e).

10.1 SGIMC work update

Dick Vethaak (NL) presented the outcome of the ICES/OSPAR Workshop on Assessment Criteria for Biological Effects Measurements (SKIMC) held in UK from 14 – 16 October 2009. The workshop focused on assessment criteria for PAH-related effect measurements and how they can be used in an integrated way. The following tasks were completed:

a) Review and updating of OSPAR Background Documents on a range of biological effects measurements.

b) Review and confirmation of assessment criteria for biological effects measurements, and development of new assessment criteria for a range of effects.

c) Elaboration of an integrated scheme for the assessment of biological effects and environmental chemistry data for use in environmental quality assessment.

d) Updating of the forward work programme for SGIMC, and for cooperation with WGBEC.

The output of the workshop will be reviewed by SGIMC 2010 and intermediate comments from WGBEC would be very welcomed. The work should result in proposals for adoption of assessment criteria formulated for adoption by OSPAR through ASMO. At this stage a complete draft report was not available, but 3 tables representing the progress made by the Workshop were presented and discussed, viz: Table A on progress on assessment criteria; Table B update of OSPAR Background Documents; and Table C proposed work programme for SGIMC from January 2009 to January 2011.

WGBEC appreciated the progress made in the workshop and emphasised the importance of this work in relation to the integrated monitoring approach by OSPAR and the implementation process of monitoring for the MSFD.

In relation to Table A the following remarks were made. For DNA adducts, there was limited amount of assessed data and some uncertainty about the chosen reference site. Halldóra Skarphéðinsdóttir (SE) will provide new data to SGIMC which can then be used for adjustment of the assessment criteria. Further it was noted that the range of biological effects measurements presented in Table A were not all included in the integrated approach. This particularly seems to be the case for the types of bioassays that have to be used for the sediment component in the integrated scheme. WGBEC suggested to ask SGIMC to recap the integrated approach to see if the appropriate biomarkers and bioassays have been taken into account. DV will take this action on board of WGIMC2010.

The OSPAR background document on biological effects techniques with proposed changes was made available to the WGBEC participants and Ricardo Beiras (ES) agreed to provide additional changes for bioassay chapters 8, 9, 10 for consideration by SGIMC later this month.

In relation to Table C; the status of actions to be delivered by WGBEC and to be fed back to SGIMC were:
• Extraction protocol for bioassays: Times series manuscript not yet finalized but a completed draft will be send to SGIMC2010 (action John Thain)
• VTG: no progress made, but state of the art will be send to SGIMC2010 (task John Thain/Matt Gubbins)
• VTG mRNA: this method will be discussed at WGBEC2011
• Acetylcholinesterase: Background document will be provided for SGIMC 2010, assessment criteria will follow in March 2010 (action Thierry Burgeot)
• Micronucleus assay and comet assay: Background document has been provided and reviewed by WGBEC and will be made available to SGIMC, assessment criteria are not yet available (action Brett Lyons)
• Bioassays: update of Background documents, see above.

In addition the background document on DNA adducts was reviewed by Halldóra Skarphéðinsdóttir(SE), an expert in this field and revised as appropriate. Halldóra Skarphéðinsdóttir is in the process of revising the assessment criteria for haddock based on background data from Iceland.

The working group further considered the strategy outlined for the integrated assessment of biological effects and concentrations of chemicals in the preliminary report from SKIMC in Aberdeen in October 2009. The suggested strategy would divide biological responses into those indicative of contaminant exposure and those indicative of effects. Each biological effect response and chemistry endpoint would be categorised into “background” (green), “exposed” (yellow) and “possibly deleteriously affected” (red). The division of responses between the three categories would then be summed up for each group of methods/determinands, resulting in a % score for green, yellow and red, respectively. Such scores were then averaged to provide a grand score for each location, thereby including biological effects and chemical determinands in one index.

The WG appreciated the concepts underlying the suggested assessment framework, but had a number of issues with the proposed framework and suggestions for how such an assessment framework may be designed.

Three main questions need to be resolved: (1) the level of aggregation for the range of components in the integrated programme, (2) the choice of quantification for the resulting components (each of which would comprise multiple responses/determinands) – e.g. averages, “one out – all out”, (3) how to resolve lacking responses/determinands.

In addition, the group discussed the possibility of selecting a subset of responses to address specific questions, e.g. PAH or oestrogen effects.

(1) Level of Aggregation

As was suggested by WKIMC, the group agreed that biological effects should be divided into early effect, sensitive methods (for which there would be only two categories – background and exposed) and methods that could indicate deleterious effects (for which there would be three categories (background, exposed and affected). With this in mind, the components available in the integrated framework would be the following: mussel – early effects, mussel – deleterious effects, mussel – chemical, fish
– early effects, fish – deleterious effects, fish – chemical, sediment – bioassay, sediment – chemical, gastropod – deleterious effects (only imposex/intersex). There was some discussion in the group as to which methods should be included as representing “deleterious effects” for each of the organism groups. Some members of the group were of the opinion that e.g. lysosomal stability should not be given the same weight as e.g. liver tumours.

The group discussed to which extent the components could be aggregated without losing essential information and ended up with the following suggestion for SGIMC:

A. mussel – early effects
B. mussel – deleterious effects
C. fish – early effects
D. fish – deleterious effects
E. gastropods – effects
F. sediment bioassays
G. levels of chemical determinands in mussel, fish or sediment

Except for E, all the above components would contain more than one measurement. There is therefore a requirement for a mechanism of quantification.

(2) Quantification

The procedure suggested by WGIMC involved a mechanism for averaging out responses within each of the components. WGBEC disagreed with this approach. Alternatives put forward were multivariate techniques, expert systems and “one out – all out” type quantification. The input to this analysis would be categorical, i.e. “green”, “yellow” or “red” (the latter only for components B, D, E, F, G) for each of the measurements within each component.

Component E will only have one component (whichever measure of imposex/intersex is used) and there is no need for further aggregation.

The group thought that the approach suggested by WGIMC, i.e. averaging out “greens” and “yellows”, could be appropriate for components A and C. If there would be more “yellows” than “greens” this would result in the component being “yellow”. Those two components will never become “red”, but will provide added information as to the type of chemical stress present.

WGBEC was of the opinion that “one out – all out” would be the most appropriate for components B, D, F & G, i.e. if one of the chemical determinands produced an EAC value producing a “red”, a sediment bioassay resulted in “red” or one of the deleterious effects measurements for either fish or mussel produced a “red”, this would cause the output to be “red” for this component. Similarly, one “yellow” would cause the index to become “yellow”.

There was some discussion of whether an EAC for a chemical or a group of chemicals (G; indicative of a level that may cause effect) should be given the same weight as measured effect (B, D, F), but as long as the components are kept separate this knowledge can be used in the subsequent assessment.
(3) Lacking responses or determinands

Although efforts should clearly be made to design monitoring programmes according to the proposed framework, it may happen that there are problems involved in sampling specific species or samples may be lost in analyses. There will need to be a requirement for a minimum number of methods to be included for B, D, F and G if the assessment is to be valid. Components A and C are more robust towards decreased number of methods included, but lacking methods will weaken the subsequent assessment.

Future development of the assessment framework should address the possibility of using a subset of methods for specific assessments (e.g. PAH, TBT, oestrogens).

In addition to a range of published strategies (MEDPOL: Viarengo, France: Narbonne, Spain: Bilbao, Germany: Broeg, UK: Galloway), HELCOM are currently developing an assessment strategy called CHASE.

Recommendation

WGBEC members have been involved with the ICES/OSPAR WKIMON / SGIMC process and there has been important consultation and support between the two groups over the past few years. WGBEC would recommend continued involvement with the work and strive to complete the revision of the integrated strategy as appropriate and where required by SGIMC for completion in 2011.

10.2 To receive Background Documents and draft assessment criteria from ICES WGBEC on: Acetyl cholinesterase, Mussel histopathology, Micronucleus and Comet assay, MT and ALA-D, and Intersex in fish

Background documents for acetyl cholinesterase, mussel histopathology and intersex in fish and micronucleus were in various stages of draft. Acetyl cholinesterase was almost complete and may be available for the SGIMC 2010 meeting. The MT and ALA-D background documents had been submitted to SGIMC but there was still some work to be done in agreeing assessment criteria. The background document on the COMET assay was presented at the meeting by Brett Lyons (UK).

10.2.1 Background document: Comet assay as a method for assessing DNA damage in aquatic organisms; Author: Brett Lyons (UK)

Background

The analysis of modified or damaged DNA has been shown to be a highly suitable method for assessing exposure to genotoxic contaminants in aquatic environments. In general, the methods developed are sensitive to a range of contaminant concentrations, applicable to a wide range of species and have the advantage of detecting and quantifying exposure to genotoxins without a detailed knowledge of the contaminants present. The Single Cell Gel Electrophoresis (SCGE) or comet assay was first applied to ecotoxicology over 15 years ago, and has since become one of the most widely used tests for detecting DNA strand breaks in aquatic animals.

The comet assay has many advantages over other methods commonly used to assess genotoxic exposure, including (1) genotoxic damage can be detected in most eukaryotic cell types at the single cell level; (2) only a small number of cells are required; (3) it is a rapid and sensitive technique; (3) Due to the nature of DNA strand break formation it provides an early warning response of genotoxic exposure.
As a consequence of the advantages listed above the comet assay has been used widely in both laboratory and field based studies to assess genotoxic exposure in many freshwater and marine organisms. However, unlike mammalian genotoxicology, where the focus is limited to a small number of model species, efforts in the aquatic field have generally lacked coordination and have used an extensive range of sentinel species\textsuperscript{1,3,5}. While guidelines relating to the use of the comet assay have been published for mammalian genotoxicology\textsuperscript{6,7}, no standard protocols currently exist for environmental studies. Consequently, the variations in protocols can lead to major differences in results and an inability to directly compare studies. Despite these obvious limitations the comet assay provides a well-researched tool for studying genotoxicity in aquatic species.

10.3 Confounding factors: Protocols, cell types and target organs

The majority of aquatic studies published to date have used circulating blood cells (either haemocytes or erythrocytes), as target cells for comet assay analysis. This is likely to be due to the practical advantage of processing tissues from a ready-made supply of nucleated cells in suspension. Solid tissues such as gill or fish hepatocytes require dissociation prior to analysis, with the potential of introducing damage through enzymatic or mechanical processes. Studies have also demonstrated that different cell types responded with different sensitivities to contaminant exposure. When comparing cells types it is usually reported that circulating cells are less sensitive than hepatocytes or gill cells\textsuperscript{8-13}. Blood and to a lesser extent the haemolymph of bivalve molluscs (e.g. mussels) are “buffered” tissues, in which contaminants arrive having crossed numerous biological barriers. Gill cells appeared to be the most sensitive following MNNG exposure, while liver and digestive gland were more sensitive to B(a)P\textsuperscript{12}, suggesting that uptake routes and bioaccumulation mechanisms need to be taken into account when designing experiment systems\textsuperscript{12}.

Mammalian studies have demonstrated that certain tissue types may have higher background levels of DNA damage due to presence of alkali sensitive sites in cells with highly condensed chromatin\textsuperscript{14}. Similar studies comparing basal levels of DNA migration in mussel gill cells, haemocytes and fish erythrocytes under both mild alkaline (pH 12.1) and alkaline versions (pH > 13) of comet assay have supported this assumption\textsuperscript{15,16}. Indicating that the mild alkaline version of the assay should be employed when dealing with certain cell types (e.g. fish erythrocytes), in order to prevent higher background levels of DNA strand breaks inhibiting data interpretation. Indeed, this problem has been highlighted in other studies using fish species where excessive DNA tail migration has inhibited the interpretation of results\textsuperscript{17}.

In addition to the variation in response depending on cell type, it is also apparent a range of comet assay protocols (differing in terms of agarose concentrations, lysing and electrophoresis parameters) have been used in studies with aquatic organisms\textsuperscript{1-5}. Therefore, effort is required to establish standardized protocols for the main species and cell type commonly used in environmental studies. The production of standard protocols, or the initiation of inter laboratory ring testing workshops focused on aquatic species are essential if the comet assay is to develop further as an environmental monitoring tool.
10.4 Ecological relevance:

Marine invertebrates

Marine invertebrates have been widely used as sentinel species in environmental monitoring programs. This is mainly due to their sessile nature, ability to bio-accumulate contaminants and general ease of capture\textsuperscript{18-20}. The majority of work has focused on coastal and estuarine environments. For example, Hartl \textit{et al.}, used the clam \textit{(Tapes semidecussatus)} as an indicator species for the presence of potentially genotoxic substances in estuarine environments, demonstrating an increase in DNA damage in haemocytes, gill and digestive gland cells of animals exposed to contaminated sediments\textsuperscript{8}. The study also highlighted the differences in sensitivity between cell types, with gill and digestive gland cells appearing to be the most sensitive target tissues for detecting genotoxic exposure. The Mediterranean mussel \textit{(Mytilus galloprovincialis)} has also been extensively deployed as a sentinel organism to assess the genotoxic effects of crude oil spills\textsuperscript{21-23}. Studies have demonstrated the sensitivity of mussels to oil exposure and laboratory studies have clearly linked the total polycyclic aromatic hydrocarbon (TPAHs) content of oils with the level of DNA damage observed\textsuperscript{21}. In Northern European studies the Blue mussels \textit{(M. edulis)} has also been used to differentiate sites receiving waste treatment effluent, with positive correlations detected between the presence of selected contaminants and the level of DNA damage\textsuperscript{24}.

Mussels have also been used extensively in the field as part of transplantation studies\textsuperscript{25-27}. The use of indigenous organisms is often hampered by the absence of a suitable sentinel species, or if present, the genotoxic responses obtained may be influenced by local physiological adaptations. Furthermore the use of transplanted organisms also offers advantages over indigenous species, such as ensuring genetic homogeneity, developmental/reproductive status and controlling the precise exposure window. Validation studies have been undertaken with the comet assay to assess the time course variations in DNA damage following field transplantation experiments\textsuperscript{25, 26}. It was observed that within the first 7 days following transplantation the level of DNA damage can fluctuate, which is likely to be caused by manipulation disturbance, then after 2 weeks the level reaches a plateau. Such data suggests that transplantation experiments lasting less that 2 weeks may give spurious results, with the levels of DNA damage detected attributable to artefacts associated with the sampling procedure rather than genotoxic exposure. Studies conducted in a coastal area of Denmark, impacted by a disused chemical site, have also highlighted that the levels of DNA damage in mussels can be affected by seasonal variations in baseline levels\textsuperscript{25}. Such results are likely to be influenced by the seasonal variations, which are known to exist for a range of physiological and reproductive processes in mussels\textsuperscript{28, 29}.

The sampling location has also been shown to influence the results of field-based surveys. For example, mussels \textit{(M. edulis)} sampled from the intertidal zone in Reykjavik harbour had higher levels of DNA damage when compared with mussels collected from the sub tidal zone at the same site\textsuperscript{30}. While the study supports the use of DNA strand breaks as a measure of environmental pollution it also highlights the high levels of intra site variability in DNA damage that can occur. As such the study further serves to underline the importance of validating experimental protocols and sampling procedures to ensure that non-contaminant related factors (e.g. physiological and biochemical responses to variations in oxygen availability and temperature stress) do not adversely affect biomarkers data.
Marine vertebrates

There are a limited number of comet assay studies utilizing marine fish species in comparison to those utilizing freshwater species (for detailed review see1, 4, 5). This is mainly due to the logistical problems associated with collecting fish at sea (e.g. need for a research vessel) and technical problems inherent within the assay, such as the difficulty of performing electrophoresis reproducibly at sea (e.g. dealing with adverse weather conditions). To date those studies undertaken have mainly focused on flatfish and bottom-feeding species, which due to their close association with sediment bound contaminants are widely used in marine monitoring programmes31, 32. In vivo studies have been undertaken to investigate oxidative stress in the European eel (*Anguilla anguilla*)33. The comet assay has also proven to be a useful tool for studying the genotoxic effects of non bio-accumulating contaminants in the marine environment. For example, the environmental effects of the known mutagen and potential carcinogen styrene has been studied in the mussel (*M. edulis*) and fish (*Symphodus melops*)34. Styrene hasn’t previously been considered to be harmful to marine fauna due to its high volatility and low capacity to bio-accumulate. However, it was shown to cause a statistically significant increase in DNA damage in blood cells, probably due to the formation of a radical styrene metabolite, which is thought to have potent oxidative capacity. Hatchery-reared turbot (*Scophthalmus maximus*) have been used successfully to investigate the genotoxic potential of PAH and heavy metal contaminated sediment from sites in Cork Harbour (Ireland)35. Eelpout (*Zoarces viviparus*) have been used in site-specific investigative monitoring following a bunker oil spill in Goteborg harbour, Sweden. The comet assay was deployed along site a battery of other bioassays and elevated levels of DNA damage were correlated to the presence of PAH metabolites in the bile of fish36. The marine flatfish dab (*Limanda limanda*) is a commonly used flatfish species in offshore monitoring programmes and it has been used in a number of studies investigating the impacts of genotoxic contaminants in coastal and estuarine waters37-39. Studies have shown that both sex and age of the fish have a significant effect on the presence of DNA strand breaks, which again highlights the influence other factors (i.e. reproductive status) may have on the extent of DNA damage.37, 38.

10.5 Quality assurance

No formal quality assurance programmes are currently run within the marine monitoring community. However, a series of comet assay workshops have taken place with the aim of drafting a common regulatory strategy for industrial genotoxicology screening6,7. Final guidelines drafted after the 4th International Workgroup on Genotoxicity testing: Results of the in vivo Comet assay workgroup7 provide a useful starting point for developing quality assurance programmes specifically focused on protocols employed in marine species. These include consideration of 1) cell isolation processes[if required]; 2) cryopreservation processes; 3) concurrent measures of cytotoxicity; 4) Image analysis and scoring method.

Currently data can be reported in a number of formats. % DNA in tail has been reported to be the most linearly related to exposure dose7. However there is no clear consensus of which measure of DNA migration should be used (% DNA in tail, Tail moment, Tail length). This difference in scoring criteria hinders our ability to develop a consensus background response and assessment criteria.
10.6 Background responses and assessment criteria

It is recognised that setting baseline/background response levels have an important role in integrating biological effect parameters into environmental impact assessments of the marine environment. The general philosophy is that an elevated level of a particular biomarker, when compared with a background response, indicates that a hazardous substance has caused an unintended or unacceptable level of biological effect. Therefore, in order to understand and apply the Comet Assay as a biomarker of genotoxic exposure it is of fundamental importance to gain information on the natural background levels in non-contaminated organisms. Table 1 summarises a number of studies that have utilised commonly deployed bioindicator species collected from reference locations (as supported by chemical and biomarker analyses) or kept under control conditions in the laboratory. While these studies provide a starting point for determining “background” levels of DNA damage they also serve to highlight the number of different tissues, protocols and endpoints currently reported.

Table 10.2.1. Assessment of “control DNA damage” by Comet assays after in vivo exposure to commonly used biomonitoring organisms.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>CELL TYPE</th>
<th>AGENT</th>
<th>EXPOSURE TIME</th>
<th>PARAMETER</th>
<th>CONTROL RESPONSE</th>
<th>REF.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. edulis</td>
<td>Haemocytes</td>
<td>MMS</td>
<td>0-4 days</td>
<td>Tail Moment</td>
<td>2.08 ± 3.43</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.96 ± 4.60</td>
<td></td>
</tr>
<tr>
<td>M. edulis</td>
<td>Haemocytes</td>
<td>Tritiated water</td>
<td>96 hrs</td>
<td>% DNA Tail</td>
<td>&lt;10</td>
<td>40</td>
</tr>
<tr>
<td>M. edulis</td>
<td>Haemocytes</td>
<td>TBT</td>
<td>7 days</td>
<td>% DNA Tail</td>
<td>5-10</td>
<td>41</td>
</tr>
<tr>
<td>M. edulis</td>
<td>Haemocytes</td>
<td>MMS</td>
<td>3-7 d</td>
<td>% DNA Tail</td>
<td>&lt;10</td>
<td>44</td>
</tr>
<tr>
<td>M. edulis</td>
<td>Gill cells</td>
<td>Cd</td>
<td>10 days</td>
<td>% DNA Tail</td>
<td>&lt;15</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cr</td>
<td>7 days injection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cr VI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. edulis</td>
<td>Gill cells</td>
<td>MMS</td>
<td>Tail Moment</td>
<td></td>
<td>1.87 ±2.23</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.60 ± 1.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.84 ± 3.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.22 ± 1.47</td>
<td></td>
</tr>
<tr>
<td>M. edulis</td>
<td>Gill cells</td>
<td>Field site</td>
<td>In situ</td>
<td>Tail Moment</td>
<td>&lt;1.5</td>
<td>45</td>
</tr>
<tr>
<td>M. edulis</td>
<td>Gill cells</td>
<td>Field site</td>
<td>In situ</td>
<td>Tail Moment</td>
<td>&lt;5</td>
<td>46</td>
</tr>
<tr>
<td>M. edulis</td>
<td>Digestive gland</td>
<td>H2O2, BaP</td>
<td>1hr</td>
<td>% DNA Tail</td>
<td>&lt; 10</td>
<td>43</td>
</tr>
<tr>
<td>Vertebrates</td>
<td>Erythrocytes</td>
<td>Field</td>
<td>In situ</td>
<td>Tail Moment</td>
<td>&lt;5</td>
<td>39</td>
</tr>
<tr>
<td>L. limanda</td>
<td>Erythrocytes</td>
<td>Field</td>
<td>In situ</td>
<td>% DNA Tail*</td>
<td>4-6</td>
<td>37</td>
</tr>
<tr>
<td>P. olivaceus</td>
<td>Erythrocytes</td>
<td>Field</td>
<td>In situ</td>
<td>Tail length</td>
<td>&lt;10</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(µm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z. viviparus</td>
<td>Erythrocytes</td>
<td>Field</td>
<td>In situ</td>
<td>% DNA Tail</td>
<td>&lt;15</td>
<td>36</td>
</tr>
</tbody>
</table>

10.7 *Mean square root of percent tail DNA measured

The requirement now is to establish a common set of protocols for those tissues/species routine used in biomonitoring programmes. Once established it will be
possible to define internationally accepted background levels of DNA damage and from their establish assessment criteria

Required steps:

- Consensus on standardized protocol from main species currently used in marine biomonitoring programmes (OSPAR, HELCOM, MEDPOL and MSFD).
- Establish minimum acceptable reporting criteria (cellular toxicity, +/- control etc)
- Agree data reporting format to allow cross study comparisons of data (Tail moment, % DNA in Tail, Tail moment).

References


4) A.N. Jha. Ecotoxicological applications and significance of the comet assay, Mutagenesis, 2008, 23(3): 207–221


34) E. Mamaca, R.K. Bechmann, S. Torgrimsen, E. Aas, A. Bjornstad, T. Baussant and S.L. Floch, The neutral red lysosomal retention assay and Comet assay on haemolymph cells from mussels (Mytilus edulis) and fish (Symphodus melops) exposed to styrene, Aquat. Toxicol. , 2005, 75, 191–201.


11 Review progress with publication and electronic dissemination of biological effects techniques in the ICES TIMES series; (ToR a)

The group reviewed the status of publications that were in preparation or were commissioned at last year’s meeting. Two draft manuscripts were received at or just prior to the meeting (Alkylphenol bile metabolites & sea urchin embryo bioassays). These were still pending external peer review, but were placed on the SharePoint for consideration by the group. WGBEC members are to respond to Matt Gubbins (WGBEC TIMES coordinator by the end of February with any comments).

Considering the number of manuscripts commissioned by the group, still in preparation (9) all with draft resolutions. It was decided to focus WGBEC efforts on delivery of these, rather than commission any new manuscripts.

During review of the table of WGBEC TIMES manuscripts it was noted that several documents were nearing completion and could be expected imminently (EROD, OEB, Extraction methods).

The current status of manuscripts is given in table 11.1 below:

<table>
<thead>
<tr>
<th>C. Res</th>
<th>Method</th>
<th>ICES Deadline</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006/1/MHC06</td>
<td>The Protocol for Extraction Methods for Bioassays. Hans Klamer and John Thain (UK)</td>
<td>31/12/09</td>
<td>Draft available from authors for SGIMC meeting at end of January 2010.</td>
</tr>
<tr>
<td>2006/1/MHC07</td>
<td>The protocol for conducting EROD determinations in flatfish By M. Gubbins</td>
<td>31/12/09</td>
<td>Reviewed at WGBEC 2009. Minor edits required. Peer review not required.</td>
</tr>
<tr>
<td>2007/1/MHC02</td>
<td>Blue Mussel Histopathology, John Bignell, Steve Feist &amp; Miren Cajaraville</td>
<td>01/03/10</td>
<td>David Lowe is no longer an author of this MS. Main author is awaiting input from co-authors on specific pathologies. In preparation.</td>
</tr>
</tbody>
</table>
In 2009, WGBEC noted that there were some restrictions on the availability of the CALUX method (2008/MHC13) and that this may limit the usefulness of pursuing a TIMES manuscript on this method. Dick Vethaak reported that an alternative source of cell lines was readily available for research purposes and that the manuscript should progress.

The role of TIMES coordinator for WGBEC was discussed. Matt Gubbins indicated that he would stand down in this role as he was taking on chairmanship duties. Ricardo Beiras was appointed to this role by the group.

12 **Assess the amount of biological effects data submitted to the ICES database and answer queries / requests from the ICES Data Centre; and to consider codes for techniques now in the integrated approach – scheme; (ToRb)**

A spreadsheet of biological effects data submissions to the ICES database was provided to the WG by an ICES data manager. WGBEC reviewed this and noted that there were some substantial additions to the database in 2009. These included the following parameter types:

- TBT effects / imposex / intersex: France, UK, Norway, Sweden, Netherlands
- PAH bile metabolites: Germany, Norway, and UK
New submissions of biological effects data and legacy data conversion activity over the last year has brought data quality issues to the attention of the data centre that now requires WGBEC advice. These were directed to the group by Marilynn Sorensen and addressed as indicated below:

The DATSU checks proposed intersessionally for imposex were approved by WGBEC:

- Values for VDS should be an integer from 0–6
- VDSI should be a variable from 0–6
- IS = INTS should be an integer from 0–4
- ISI = INTSI should be a variable from 0–4
- PCI should be a variable from 0–3.5
- IMP = IMPSI should be a variable from 0–6

Additional checks were suggested:

For all population level parameters for TBT effects (i.e., when MUNIT = ‘index’) a warning should be triggered if the condition “NOINP >39” is not met for parameters VDSI, INTSI, IMPSI and PCI.

For data on individuals, if the condition “NOINP = 1” is not met for parameters VDS, INTS, IMP, a non-critical error should be triggered. Critical errors are reserved for database requirements.

For parameters EROD and CYP1A, if the condition “NOINP > 19” is not met, a warning should be triggered.

The lists of DATSU checks and FINFL (factors influencing results) codes were also reviewed by a WGBEC sub-group. It was noted that there were very few data quality checks currently included for biological effects data and that it was up to WGBEC to provide these to the data centre. WGBEC members were asked to suggest appropriate data checks for parameters that they were particularly involved with. These will be developed intersessionally before the next meeting, but will focus initially on EROD.

For conversion of legacy data, standardisation of EROD units and matrices is required. WGBEC advises that the recommended unit for EROD is picomole/minute/milligram protein and all the varying units that appear in the submitted legacy data are either equivalent to this or can be converted to this (conversion factors were provided to the data centre for converting data submitted as nanomole/minute/milligram liver to picomole/minute/milligram protein where data are available on protein content of liver. Legacy data in the database as codes ERODS, ERODM, and ERODL were assumed by the WG to be S9, microsomal fraction and normalised to liver weight values respectively. All were converted to parameter EROD with matrices liver S9 (LIS9), liver microsomes (LIMIC) and liver (LI) respectively. For ERODL it is unclear whether measurements were done on S9s or microsomes so data should be assessed with caution (e.g. were not included in the derivation of BACs). Legacy data with codes CODLM, ECODL, RODLM, and PRODP should not be converted.

Norway has data on CYP1A measured by ELISA. The unit of measurement requested by a data submitter for use in the database (absorbance 450/milligram protein) was
not appropriate for this technique. WGBEC advised the data centre that
UNITS/milligram protein was the relevant unit for this technique as it is semi-
quantitative and the ELISA results are unit less.

WGBEC was asked to clarify WGBEC2009’s request for condition factor / index and
somatic indices data into the ICES database. The response to the data centre was for
fish to rely on existing parameter codes: length (LNMEA/LNMIN/LNMAX), weight
(WTMEA/WTMIN/WTMAX) and for some countries gutted weight (GUTWTMEA
etc.), and somatic weight. The data centre pointed out that data are being submitted
without length/weight etc. but the new OSPAR contaminant cofactor checks in DA-
TSU will check for some of these parameters. Parameter codes LISOI and GOSOI are
already available for hepato-somatic and gonado-somatic indices respectively. In
mussels a new parameter code for mussel condition index needs to be developed and
defined in the OSPAR background document on condition indices and supporting
parameters for mussel integrated monitoring.

A scope for growth parameter code (SFG) and MUNIT code were previously reco-
mended by WGBEC. Since there are various combinations of methods for scope for
growth, the data centre recommended that METOA codes be developed specifically
for SFG method variations to enable a quality check by DATSU at the time of submis-
son. It was decided that clearance rate measurements alone would not be acceptable
but that clearance rate / oxygen consumption and clearance rate / oxygen consump-
tion / excretion calculations should be accepted but recorded separately. Failure to
submit the method information will trigger a non-critical error.

Gametogenesis in mussels. WGBEC2009 recommended that a new parameter code
“Gonadal stage” was used for this information by reporting an alphanumeric value in
the range of 1–5 for pre-spawning and 1s-5s for post-spawning. The ICES database
requires that the field “VALUE” is a number and therefore suggested the use of the
existing “condition of specimen” field “CONES” in the database for recording this
information. Multiple options could be added for pre- and post-spawning. WGBEC
prefers that specific parameter codes are used to avoid confusion and it was therefore
agreed that two parameters would be added: pre-spawning gonadal stage and post-
spawning gonadal stage should be created and acceptable values for these should be
integers from 1-5 where MUNIT = stage. These parameters should be allowed for
individuals only, i.e. if the condition “NOINP=1” is not met, an error will be trig-
gered.

The Data Centre requested an update on specifications for the new Quality Assurance
database for storing biological effects intercalibration results. WGBEC advised that
the existing QUASIMEME z-score system already established within ICES data centre
was appropriate for TBT effects, but that for other methods a more flexible approach
would be required. This will require further development as QA schemes are devel-
oped by WGBEC in the future, but it was envisaged that WGBEC might pass data
files to ICES data centre containing lists of participating laboratories and pass/fails for
specific parameters by monitoring year.

WGBEC members also had questions for the ICES data centre. These were:

1) What procedures should be followed to extract data from the database?

The ICES data centre will supply WGs with data extractions as required for meetings.
This can be as a standing request for data in ToR or simply by emailing acces-
sions@ices.dk. Individual labs can check their own submitted data files by accessing
DOME.ices.dk, choosing “submitted files” and filtering on their lab code. Alterna-
tively, anyone can download data (up to 50 000 lines) from the EcoSystemData website ecosystemdata.ices.dk by going to the ‘inventory’ on the website, selecting a region on the interactive map and filtering by parameter. For > 50 000 lines of data you can email accessions@ices.dk. Downloads are in csv format and are simpler in structure than submitted files in V3.2 format but lack method and QA information.

2) What possibilities are there for training in data submissions?

One can always contact a data manager for help. In addition, the ICES data centre offered to host a training workshop in Copenhagen. A call for interest from the group did not identify much demand for this. It is suggested that expertise is enhanced by direct communication with ICES data centre and through the generation of guidance documents by WGBEC (see recommendation).

3) How should data submitters record the information that fish in a single sample may have come from multiple trawls of an area?

To indicate that the fish came from multiple trawls, fill in the field NOAGG in the sample record with the number of hauls taken to comprise the sample. A single sample record is required when linking of individual fish measurements with pooled measurements from bulks is needed.

4) How best to record bottom water temperature as a supporting parameter for EROD?

Use a 92 record (site description) associated with each 91 (station) record. A new parameter for ‘bottom water temperature’ will need to be created. This type of parameter is usually kept separate from the hydrographic database.

5) Which member states use a central reporting system and what issues are associated with this?

UK, Denmark, Germany submit all their data via a centralised reporting institute. Advantages are that fewer people require training in ICES reporting formats. Disadvantages are that data submitters are then more removed from the data being submitted, which can make issues harder to resolve. Some WGBEC members expressed a desire to move towards such a system. Some submitters noted that some aspects of data submission were handled in different organisations. ICES pointed out that if samples are being analysed by multiple laboratories (biological effects at one laboratory, organics at another, and metals at a third), a single data file by reporting laboratory and monitoring year was required so all sample analyses can be linked and assessed together.

WGBEC would like information on how biological effects data submission is handled by HELCOM and MEDPOL to see if there are any possibilities for standardisation across systems.

**Recommendations**

That WGBEC members (and ICES member states) actively submit biological effects monitoring data onto the ICES database using the relevant (v3.2) reporting formats. To assist in this process WGBEC recommends that WGBEC / ICES data centre develop a live ‘working document’ to be added to at future WGBEC meetings to explain how biological effect data should be entered into the database and keep track of WGBEC advice on database issues.

**Actions:** Develop guidance document on biological effects data submissions to include example data files that comprise multiple effects parameters and BULKID
Review progress with AQC procedures for biological effect methods and include harmonisation activities within OSPAR, Baltic and MEDPOL maritime areas; (ToR d)

13.1 Harmonisation activities within OSPAR, Baltic and MEDPOL

SGIMC in 2009 proposed a ICES/OSPAR practical workshop on lysosomal membrane stability (LMS) by using the neutral red retention (NRR) assay to be organized by Spain (lead IEO/C. Martinez). This workshop was aimed at scientists who have certain experience and are familiar with the NRR assay, but need further training to progress with the harmonised interpretation criteria for this semiquantitative technique. C. Martínez-Gómez (IEO, Spain) informed WGBEC that a funding proposal will be send in January 2010 to the Coordinator for Training Programme in ICES, to support the organization of this practical training workshop. Apart from LMS, it was proposed to include also practical sessions concerning the biomarker Stress on Stress, also recommended as a biological effect method in the OSPAR integrated mussel component.

Prof. Aldo Viarengo (IT) informed WGBEC that a training course on the use of certain biomarkers including LMS and genotoxicity biomarkers in mussels) will be held in 13-17 September 2010 at the University of Piemonte Orientale, Alessandria (Italy). The course will be organized by Prof. Aldo Viarengo and supported by high-profile scientists and instructors (Dr. M.N. Moore and C. Bolognesi), as part of the QA work required in the framework of the MED POL Programme. Additional aims of this training course is to help build capacity in the MED POL biomonitoring programme and to provide special support to new scientists/Institutions involved in the monitoring activities of the riparian Mediterranean countries.

Since the aims and activities of both workshops largely overlap, WGBEC proposed to combine both workshops. Prof. Aldo Viarengo (IT) happily agreed to do this. It is expected that a combined training workshop will attract a higher number of participants and also facilitates harmonisation of biological effects methods within and between OSPAR and MEDPOL maritime areas. A draft proposal of the MEDPOL-ICES/OSPAR Training Workshop will be made available for SGIMC in January 2010 for further elaboration (Task CM and AV). See also 4.4 above.

13.2 Review of progress with AQC procedures.

Quality assurance is a necessity for any method to be used for national or international monitoring and it is important to be aware that this is a continuous process, not a one-off intercalibration or other exercise. Through the past decade there have been various rounds of training workshops and intercalibrations for biological effects methods within BEQUALM, QUASIMEME, MEDPOL and HELCOM, as well as through EU research projects such as BEEP and COMPREHEND. BEQUALM (http://www.bequalm.org) has been the only organisation to offer QA for a broad range of methods, including benthic community studies, fish histopathology, bioassays and biomarkers. The current linking of the UK NMMP with BEQUALM in offering QA for benthic community studies appear to be satisfactory and should be retained as it is. Likewise, BEQUALM QA for fish histopathology is an ongoing activity that appears to be providing the required services for the scientific and monitoring community.
There is, however, a need for a renewed strategy for bioassay and biomarker QA. Critical components of such a programme are regularity (annual or biannual) and cost. A requirement for submitting data to the ICES database is that there is a certified AQC scheme. In order to take this forward WGBEC felt it should review this process and assess whether it was feasible to coordinate from within the group.

Following discussions in the working group it was agreed to launch a low-cost programme for methods included in the integrated monitoring framework. A basic website will be launched (Cefas) to provide information to prospective users and WGBEC members will be the scientific basis and main users of the services provided by this activity. The results from intercalibration exercises will be evaluated by WGBEC members during a half-day meeting prior to the main meeting on an annual basis and a brief report produced. The activity will need a WGBEC member to co-ordinate sampling, shipment, communication with participants and registering of results. A steering committee (Ketil Hyland, Matt Gubbins, and John Thain) (in communication with MEDPOL and HELCOM) was formed to communicate bimonthly and coordinate necessary activity to deliver this programme.

The methods would be divided into three categories: (1) material could be prepared and distributed (most methods, e.g. AChE, EROD); (2) a workshop or similar would be required (LMS, Comet); (3) prepared toxic mixtures would be distributed (bioassays).

Liver, plasma, bile, blood cell and muscle samples would be collected at one polluted, one intermediate and one clean site during routine monitoring cruises organised by WGBEC members. Samples from 15-20 individual fish would be pooled, homogenised and aliquotted into cryovials before storage at -80. Protocols for sample preparation are available from BEQUALM and would involve homogenisation of tissues under liquid nitrogen (for non-liquid matrices). The only direct cost to participating laboratories would be shipment of samples. An agreement would have to be signed by participating laboratories to ensure timely reporting of results.

The methods required for the integrated approach (fish) would comprise DNA adducts, AChE inhibition, EROD/CYP1A activity, PAH metabolites, lysosomal membrane stability and vitellogenin concentration (Table 13.2.1). Optional methods for fish are Comet (workshop planned), metallothionein (no activity planned), ALA-D (no activity planned) and reproductive success in fish (activity will be clarified with HELCOM).

Table 13.2.1. Overview of methods for the integrated approach (fish) which require WGBEC QA.

<table>
<thead>
<tr>
<th>METHOD</th>
<th>STATUS</th>
<th>LIKELY UPTAKE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulky DNA adduct formation</td>
<td>None currently available; was done through BEQUALM; could be done by sending fish liver samples</td>
<td>limited, maybe 3 labs (France, Sweden, Italy)</td>
</tr>
<tr>
<td>AChE inhibition</td>
<td>BEEP; none currently active; could be done by sending fish muscle tissue</td>
<td>probably &gt;10 labs (Norway, UK, Spain, Italy, Netherlands, Portugal etc)</td>
</tr>
<tr>
<td>EROD or P4501A induction</td>
<td>BEQUALM; last intercalibration 2008; could be done by sending fish liver samples</td>
<td>&gt; 10 labs</td>
</tr>
<tr>
<td>PAH bile metabolites</td>
<td>Quasimeme; last intercalibration 2002; either as part of proposed AP intercalibration or from research cruises; methodology needs to be reported for each participant (FF, SS, HPLC, GC/MS)</td>
<td>probably &gt;10 labs</td>
</tr>
</tbody>
</table>
Lysosomal membrane stability  MEDPOL and ad hoc; need to be done as workshop; planned activity AWI (cytochemical methods)  >5 labs

Vitellogenin induction  COMPREHEND; none currently active; different species need specific antibodies and standards; antisera commercially available, but standards for most species not available  >5 labs

The methods required for the integrated approach (mussel) would comprise scope for growth, AChE inhibition, lysosomal membrane stability and micronucleus formation (Table 13.2.2). Histopathology should be handled by BEQUALM (will need to be confirmed). Optional methods for mussel are metallothionein induction (intercalibration by MEDPOL), Comet (workshop planned) and stress on stress (training and intercalibration can be done in parallel with LMS workshop). The group proposes to remove MXR from the framework.

Table 13.2.2. Overview of methods for the integrated approach (mussel) which require WGBEC QA.

<table>
<thead>
<tr>
<th>RECOMMENDED METHODS (INVERTEBRATES)</th>
<th>EXTERNAL QA COMMENTS</th>
<th>LIKELY UPTAKE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChE inhibition</td>
<td>No current activity on intercalibration. Possible to do by simple ring test distribution of material.</td>
<td>At least 3 labs: Spain, Finland (clams not mussels), France</td>
</tr>
<tr>
<td>Lysosomal stability</td>
<td>MEDPOL and BEQUALM; two methods (cytochemical and NRR); need to be done as workshop; planned activity MEDPOL/SGIMC</td>
<td>&gt;10 labs</td>
</tr>
<tr>
<td>Scope for growth</td>
<td>Originally run by PML (WGBEC initiated); needs workshop</td>
<td>Limited; 3-5 labs?</td>
</tr>
<tr>
<td>Micronucleus formation</td>
<td>None currently available; slides can be prepared and distributed</td>
<td>&gt;5 labs</td>
</tr>
</tbody>
</table>

Bioassay intercalibration will have to be done either by workshops or by distribution of toxic mixtures to be tested. WGBEC involvement with such intercalibration should be investigated intersessionally.

To take the AQC process forward WGBEC intends to form a small steering group (KH, JT, MG) to coordinate QA activities to deliver the above work plan.
14 Continue to review of emerging and novel contaminants as they arise and specifically nanoparticles; (ToR g).

14.1 Continuing review of emerging and novel contaminants including nanoparticles; presented by Jim Readman (UK)

As described at previous WGBEC meetings, urban and industrial sewage effluents contain important quantities of emerging pollutants (including pharmaceuticals, personal care products and endocrine disrupters). Many of these substances are emitted in substantial quantities and our lack of knowledge concerning their environmental behaviour and long-term ecotoxicological impacts need to be addressed if we are to understand the environmental, economic and human health implications. JR provided a presentation on recent research into this topic. He described a selection of emerging contaminants, commencing with pharmaceuticals, personal care products and phenolic endocrine disrupters. All three are amenable to a single analytical protocol to investigate their behaviour. Recent research regarding toxicological investigations of pharmaceuticals including paracetamol, a beta-blocker (propanolol) and Tamiflu were also described. For endocrine disrupters, challenges relating to the quantification, particularly of biologically active concentrations of female steroids, and most recent approaches, were summarised. Next, the approach to measure contaminants in biological fluids so as to evaluate the biologically available fraction (frequently at concentrated levels) was described, using the analysis of a fungicide in crab urine with a bacterial bioreporter, as an example. Environmental implications associated with ubiquitous synthetic musks (in particular galaxolide and tonalide) was then addressed.

Integration of biological effects assessments with chemical fingerprinting in the context of shipping accidents was then discussed. The case of the MCS Napoli was used as an example and demonstrates biological effects in limpets associated with spilled fuel oil from the ship.

Finally, research relating to nanoparticles was described. This included the uptake and biological effects of fullerenes and carbon nano-tubes on the marine mussel, the impact and effects of silver nanoparticles on bacterial communities and the subsequent potential for antibiotic resistance. Iron nanoparticles are becoming increasingly used as a food supplement, and results from preliminary toxicological research on these was summarised.


Emerging and novel arising contaminants: marine litter and plastics; presented by Thomas Maes (UK)

There have been many new emerging contamination problems in the last half-century, but one of the most instantly observable is the ubiquity and abundance of marine debris. It is a growing problem which will persist for centuries. From what started as an aesthetic problem of littering, the number of potentially harmful implications of debris that have been identified has escalated and include the accumulation and transport of persistent organic pollutants and carcinogenic, mutagenic or toxic for reproduction (POPs and CMRs; Mato et al. 2001), the release of toxic compounds, including medicines, the assistance of alien invasions (Barnes 2002), the distribution of algae associated with red tides (Masó et al. 2003), the entanglement in and ingestion of plastic by marine organisms with associated mortality (Katsanevakis 2008), alteration of the structure of benthic communities (Katsanevakis et al. 2007), and socioeconomic impacts such as the threat of floating debris to navigation, reduction of the recreational value of beaches and lost tourism, and damages to fishing gear.

Future policy drivers in relation with this emerging problem are the Marine Strategy Framework Directive (MSFD). The definition of marine litter and good environmental status (GES) for this descriptor are stated below:

Marine litter is any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment. Marine litter consists of items that have been made or used by people and deliberately discarded or unintentionally lost into the sea or coastline including such materials transported into...
the marine environment from land by rivers, drainage or sewage systems or wind. This definition does not include semi-solid remains of for example mineral and vegetable oils, paraffine and chemicals that sometime litter sea and shores.

Good environmental status is defined by the commission as “Properties and quantities of marine litter do not cause harm to the coastal and marine environment”.

“Harm” is subdivided in different matrices:

- Social (e.g. reduction in aesthetic value and public safety)
- Economic (e.g. cost to tourism, damage to vessels, fishing gear and facilities, losses to fishery operations, cleaning costs)
- Ecological (e.g. mortality or sublethal impacts to plants and animals through entanglements, physical damage and ingestion including uptake of microplastics including chemical pollutants, assist the invasion of alien species, alter the benthic biocommunity structure).

Definitions of the acceptable levels of harm in these categories and good environmental status must consider impacts as assessed by

- the amount of litter in different compartments of the marine environment (seabed, sea surface, water column, coastline)
- ecological effects of the litter (e.g. plastics ingested by marine organisms; entanglement rates)
- problems associated with degradation of litter (microplastics) as well as social and economic aspects.

An overriding objective for marine litter pollution will be a measurable decrease in the total load of litter in the environment by 2020.

Debris are progressively fragmenting in the environment (Colton et al. 1974; Thompson et al. 2004). In addition, the use of plastic granules as abrasives in skin cleaning products has increased considerably in recent years. The prevalence of small pieces and granules (<5mm in diameter) varies considerably among habitats. Quantities of plastic microparticles in excess of 100,000 items m\(^{-2}\) (Gregory 1978) or 1250 items 250g\(^{-1}\) of natural material (Zubris & Richards 2005) have been reported, while in intertidal habitats near Plymouth more than 10% small (<5mm) plastic pieces by weight have been reported (Browne et al. in review). As well as these small pieces, in 2004, Thompson et al. reported on the accumulation of microscopic plastic fragments (≥ 20µm diameter) on shorelines and in the water column around the UK (Thompson et al., 2004). Similar debris has been reported in India (Reddy et al., 2006) and Singapore (Ng & Obbard 2006) and a recently completed global survey confirmed that polyethylene, polyvinyl chloride and polypropylene fragments are now present on shorelines worldwide (Barnes et al., 2009). Production of plastic is increasing rapidly and since conventional plastics will not biodegrade it is inevitable that the abundance of small fragments like these will increase over the next few decades. Such fragments have a considerably larger surface area to volume ratio and hence a greater potential to transport and release contaminants than larger items. In addition, because of their size they are available to a wide range of organisms including deposit feeders, filter feeders and detritivores (Thompson et al., 2004). Ingestion of microplastic material therefore presents a likely route by which chemicals could pass from plastics to the food chain. Ryan et al. (1988) found a positive correlation between the mass of ingested plastics and PCB concentrations in the fat tissue of Great Shearwaters Puffinus
*gravis*, and presented the first indication that marine organisms can assimilate toxic chemicals from ingested plastics.

A range of potentially toxic chemicals, including flame retardants, plasticizers and antimicrobials are frequently added during the production of plastics. Because of the nature of the plastic surface, hydrophobic pollutants such as PCBs are accumulated on the pellets from the surrounding seawater with concentration factors of up to $10^6$. The high pollutant concentrations in the plastic pellets may also be due to the marine microlayer where hydrophobic contaminants are known to be enriched (e.g., Teuten et al., 2007; Teuten et al., 2009; Endo et al., 2005; Ogata et al., 2009). Maybe one of the only positive aspects of this is the utility of these particles as monitoring media for contaminants in coastal waters with low-cost of sampling and shipping as compared with conventional monitoring using water, sediment and biological samples.

**Recommendation**

WGBEC recognises that the field of microparticles is an important research area and would recommend that work in this field (nanoparticles, microplastics) is reviewed at its 2011 meeting. This review should include; studies that are undertaken to understand dose response relationship for microparticles; studies of biological effects from contaminants attached to particles and bioavailability / biomagnification of contaminants in microparticles; and strategies for monitoring microparticles in the marine environment.

**References**


15 Review current knowledge and research on contaminants in eel and associated biological effects; (ToR i).

Comments by WGBEC on recent publications and reports relating to the decline in eel populations during recent years.

Due to the complex life cycle of eels, geographic range and habitat requirements, this species has been difficult to manage and faces a broad range of threats. Threats and potential causes of decline in eels include: overharvesting, habitat loss/degradation, oceanographic conditions, parasites and contaminants. During the 2009 WGBEC meeting, the Report of the 2007 session of the Joint EIFAC/ICES Working Group on Eels was reviewed. It was concluded that a trend is clearly appearing indicating a reduction in the eel populations. Eels are unusual in that their fat content is an order of magnitude higher than that of other fish and that levels of lipophilic contaminants generally reflect the elevated fat content, being 5 to 10-fold higher than in other fish and invertebrates, depending on the contaminant and the species. The elevated lipid content is important as an energy reserve and is regulated through steroidal/endocrine systems, although these fat reserves appear to be declining overall. The relationships between lipid contents and environmental variables have been studied by analysing extensive contaminant datasets, and statistical modelling demonstrates that especially highly chlorinated PCBs, DDT (and related compounds) and Cd have a negative impact on the lipid content of eels (Belpaire and Goemans, 2006; Geeraerts et al., 2007). Other characteristics that render environmental evaluation of eels difficult include identification of gender and that eels only reproduce once in each generation. The latter impairs contaminant losses through gonadal releases. PCB-loaded females negatively influence the survival of larvae (Palstra et al., 2006). A negative correlation exists between embryo survival time and TEQ levels in the gonads implying TEQ-induced teratogenic effects. The disrupting effects were caused at levels below 4 pg TEQ/g wet weight gonad which are below the EU maximum consumption limit for dioxin in food (Palstra et al., 2006). Van Ginneken et al. (2009) also indicated that transoceanic spawning migration is altered by PCBs.

The European eel (Anguilla anguilla) is now included in the OSPAR list of threatened and/or declining species and habitats and an OSPAR background document on eel is currently being developed (led by France) and publication may be expected in the first half of 2010.

Whilst it was considered that the Report of the 2007 session of the Joint EIFAC/ICES Working Group on Eels thoroughly reviewed much of the available literature, WGBEC in 2009 believed that some areas would benefit from further scrutiny:

- contaminants studied should be diversified (and include emerging contaminants). Focus of biological effects should include skewing of the sex ratios, reduction in lipid content or disruption of endocrine systems, effects at different life stages in eels, potential influence on spawner quality.
- clarification is required on the historical changes in lipid contents and compositions. Are the analytical techniques comparable and quality assured?
- the potential impact of climate change to alter metabolism and affect lipid content and pathogenic/parasitic pressures needs to be assessed.
- the potential for contaminants that may affect the genetic pathway that regulates biochemical pathways in lipid metabolism should be evaluated.
• it would be useful to investigate how the eel decline maps against performance of other species (e.g. cod, dab, plaice and eel pout).

WGBEC 2009 recommended:
• Inclusion of biological effects measurements (e.g. Guimaraes et al., 2009) in the database.
• Attempt compatibility between databases holding pertinent information (both of contaminants, condition factors and biological effects).
• National Monitoring authorities should be encouraged to maintain existing chemical contaminant monitoring programmes for eels, or where they do not exist should consider initiating monitoring programmes. In addition the monitoring programme should include appropriate biological effect techniques.

Considering the very recent reports reviewing available data (Geeraerts and Belpaire, 2009; EIFAC/ICES WGEEL report 2009) WGBEC 2010 supports the recommendations, given by Geeraerts and Belpaire (2009), and made the following comments:
• lipid content reductions occurring on large geographical scales should be examined.
• contaminant loads on the large geographical scales require mapping.
• older substances such as PCBs, heavy metals and some pesticides are relatively well studied. On the other hand more extensive research is needed to evaluate how ‘newer’ substances (e.g. BFRs, bisphenol A, VOCs, PFOS, alklyphenols, phthalates, TBT etc.) are detrimental to eel populations.
• as controlled reproduction of A. japonica is now possible on an experimental scale, new opportunities for experimental work on effects of contaminants on the different life stages of eel have become feasible.
• the development of adequate biomarkers with great sensitivity for both the concentration and length of exposure will be another challenge and would allow detection of the genetic variation underlying environmentally dependent fitness traits in eels.
• for the EU-eel recovery plan (European Commission 2007), whose efforts are concentrated on increasing the quantity of silver eels leaving continental waters, it is recommended to include quality aspect in the stock wide recovery plan. Quality targets should include contamination levels, biomarker responses, lipid content and condition. Generating a comprehensive overview of the quality of the silver eel population all over Europe seems to be an essential and urgent objective for the global eel management.

Recommendation
To take forward any future work on contaminants and their effects on eels it is recommended that WGBEC liaise with WGEEL and work intersessionally to progress, (review the most recent information on effects in eels) and report back on developments and research in this area.

References
Any other business

16.1 Election of Chairperson

John Thain (current Chair), had indicated at previous meetings the need to elect a new Chairperson but no volunteers had been found. After some discussion it was agreed that WGBEC should opt for two co-chairs for the 2011 meeting. Matt Gubbins was unanimously elected as one chairperson and in the absence of another candidate John Thain agreed to act as a supporting co-chair for the next meeting.

16.2 ICES ASC 2010 in Nantes

The Chairperson reminded WGBEC members of the ICES Annual Science Conference in Nantes, France in September 2010. In particular the theme session relating to biological effects and asked for the group to support this if possible.

Details of the session were outlined as described in the ICES call for submissions:-

Session F: Monitoring biological effects and contaminants in the marine environment: where do we go from here? Conveners: John Thain (UK), Catherine Couillard (Canada), and Dick Vethaak (The Netherlands).

Many countries within the ICES community have monitoring programmes to measure chemical contaminants and biological effects in coastal and offshore waters. The programmes are carried out to measure the “health status” of the marine environment and to meet national and international obligations e.g. OSPAR, HELCOM, EU WFD, LEM, U.S. Clean Water Act, Canadian Oil Pollution Act, etc. With the OSPAR QSR 2010 looming and the introduction of the EU Marine Strategy Directive (MSD) it is time to take stock and ask important questions.

Do our measurements tell us anything useful?

- Are they fit for purpose and cost effective?
- Can the data be assessed in an integrated manner?
Do we have good indicators of environmental “health”?
Are the indicators being applied for management purposes?
Are past and current measurements useful for the future and are they broadly applicable?

The emphasis of the theme session will be to address these issues and to this end contributions are invited on:

- Marine chemical contaminant data and their assessment
- Biological effect data and their assessment
- Development and implementation of chemical contaminant and biological effect indicators
- Chemical contaminant and biological effect integrated assessment
- Integrated assessment of chemical contaminant and other environmental stressors
- Use of chemical contaminant and biological effect data for risk assessment purposes
- Management applications.

Contributions as presentations / posters can include case studies, assessment of long-term data sets or just be scientifically stimulating, but must aim to address the issues stated above. In particular, presentations will be highly ranked if they provide good evidence and a strategy for monitoring chemical contaminants and their effects as part of ecosystem health assessment into the future.

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Dick Vethaak, The Netherlands [e-mail: dick.vethaak@deltares.nl]

17 Recommendations and action list

17.1 Recommendations

1 Recommendation (From agenda item 4.4):
WGBEC fully supported the proposed intercalibration exercise (Sept 2010) on lysosomal stability(NRR method) to be held in Alexandria in Italy; the first intercalibration exercise putting together MEDPOL and ICES laboratories. This is an important step forward for harmonisation between OSPAR, MEDPOL and HELCOM biomonitoring activities. WGBEC would recommend that ICES supports this initiative and recommends further support and uptake from organisations and laboratories within these communities.

2 (Recommendation From agenda item 7 & 8):

Recommendations for further WGBEC work areas
During discussion of the future role and scope of WGBEC under agenda items 7 & 8, several new work areas were raised as potential priority areas and of emerging interest to ICES and the group. These are considered in turn below together with consideration of how WGBEC might action these issues at future meetings. WGBEC would recommend these to SGHIE for comment and advice.
Effects on algae / primary production / eutrophication

Primary production by micro algae embodies the carrying capacity of marine and coastal ecosystems and has primarily been linked to nutrient availability in policy studies. However recently it is indicated that certain industrial chemicals (e.g. TBT, PAHs, irgarol, atrazine, azaarenes) may have a direct impact on coastal phytoplankton communities by (photo)toxicity and hence on the carrying capacity on estuarine and marine ecosystems. Recent results already show that Irgarol, a substitute for TBT compounds on ships to prevent anti fouling, already reduces the primary production at very low concentrations in a few days. The impact of toxicants on aquatic ecosystems has been recognized to interact with eutrophication. Also the frequency and intensity of algae blooms are increasing globally, resulting in increased levels of toxin prospected to affect coastal ecosystems. These different chemical stressors (toxicants and natural toxins) are hypothesised to disturb regulatory mechanisms with algae communities, modifying the competitive abilities of individual species and resulting in shifts from highly nutritious to unfavorable algal species that destabilize the food chain. The research on this issue will be of relevance for reaching a good ecological or environmental status. In the Netherlands, several research groups are working on this topic. Dick Vethaak agreed to report on the Dutch progress and provide a short review paper on this subject for WGBEC 2011. Other participants will be asked to provide relevant information on this topic.

Immunotoxicology

A gap in the current battery of recommended techniques for biological effects monitoring is a method for determining the level of immune system suppression potentially caused by toxicant exposure (immunotoxicity). This is a potential missing link in integrated monitoring frameworks between contaminant exposure and fish disease prevalence. A number of techniques are available such as measures of macrophage activity and for the non-specific immune system, but it is not clear how these could be employed in a monitoring context on wild fish rather than by examining effects over a controlled time course following pathogen challenge. WGBEC should review the range of available techniques at its next meeting and consider their applicability in an integrated monitoring context. Some experts in this field could be invited to present this issue to the group. One group member also has experience of using some of these techniques in the field (Andrea Johnson) and may be able to present some of the methodology at the 2011 meeting.

Benthic community structure and its relationship to contaminants

WGBEC would like to review the links between contaminant exposure and effects on benthic community structure. Although not a new area of interest to the group, WGBEC would like to resolve some outstanding areas of uncertainty. Benthic community analysis is a recommended technique for biological effects, however there has been little formal consideration by the group of the evidence for the causal links. WGBEC would like to ask BEWG to review this issue at their 2010 meeting so that it can be considered by the group. Some of the key issues are:

1) To what extent is benthic community structure affected by sediment contaminant levels compared to other environmental factors (sediment structure, organic material etc)?

2) What monitoring data is available from across contamination gradients in the field to support the use of this method as an indicator for contaminant
effects (taking confounding factors such as change in sediment composition across the same gradient into account).

3 ) To what extent are changes in benthic community structure in offshore environments influenced by lower levels of contaminant exposure?

4 ) What consideration of contaminant levels has been taken into account in assessment of national benthos monitoring data across the ICES area?

Recommendation: “WGBEC requests that BEWG review the evidence for benthic community structure as a suitable method for monitoring effects of contaminants, taking into account the considerations above and provide WGBEC with a report (or presentation) to consider at their meeting in March 2011”

Comparison of qPCR measurements of mRNA responses to traditional biomarkers

Current technology allows easy measurement of gene expression, also in cases where it may be challenging to determine the concentration of a biomarker protein (e.g. vitellogenin). Gene expression and protein measurements do however reflect different components of a temporal response to contaminant stress. There is a need for a review of relationships between “traditional” biomarker measurements and gene expression as well as suggestions for how to assess the latter in current frameworks and how the two may be combined in environmental assessment.

Species specific differences (including bioassay test animals)

It has been taken for granted that biomarker responses can be more or less directly transferred between different fish or mussel species. This is clearly not the case and there is a need for a review outlining such differences for the relevant biological effects methods.

Most toxicity tests use clonal cultures which may commonly be lab-specific. In contrast, sediment bioassays rely to a large extent on field-collected individuals which may have different nutritional status, reproductive status or contamination history. The advantage of using field-collected individuals is of course that results may be more directly applied to a natural situation, whereas it is less obvious that this will be the case for a more or less homozygous lab-population.

Ocean acidification

One of the likely marine problems associated with climate change is decreasing pH. Consequences for marine organisms could be dramatic. There is a need to consider the existing knowledge concerning ocean acidification, which organisms would be most likely to be most sensitive and possible biomarkers for such effects.

3 Recommendation (From agenda item 10.1):

WGBEC members have been involved with the ICES/OSPAR WKIMON / SGIMC process and there has been important consultation and support between the two groups over the past few years. WGBEC would recommend continued involvement with the work and strive to complete the revision of the integrated strategy as appropriate and where required by SGIMC for completion in 2011.

4 Recommendation (From agenda item 12):

That WGBEC members (and ICES member states) actively submit biological effects monitoring data onto the ICES database using the relevant (v3.2) reporting formats. To assist in this process WGBEC recommends that WGBEC / ICES data centre de-
velop a live ‘working document’ to be added to at future WGBEC meetings to explain how biological effect data should be entered into the database and keep track of WGBEC advice on database issues.

5 Recommendation (From agenda item 14.2):
WGBEC recognises that the field of microparticles is an important research area and would recommend that work in this field (nanoparticles, microplastics) is reviewed at its 2011 meeting. This review should include: studies that are undertaken to understand dose response relationship for microparticles; studies of biological effects from contaminants attached to particles and bioavailability / biomagnification of contaminants in microparticles; and strategies for monitoring microparticles in the marine environment.

6 Recommendation (From agenda item 15):
To take forward any future work on contaminants and their effects on eels it is recommended that WGBEC liaise with WGEEL and work intersessionally to progress, (review the most recent information on effects in eels) and report back on developments and research in this area.

17.2 Actions

1 (From agenda item 12)
Develop guidance document on biological effects data submissions to include example data files that comprise multiple effects parameters and BULKID codes to show how to handle data for pooled samples (UK), Supporting parameters for each method, DATSU and FINFL checks for BE data (All WGBEC members)

2 From agenda item 13.2)
WGBEC intends to form a small steering group (KH, JT, and MG) to coordinate QA activities to both develop and deliver a work plan.

18 Adoption of the report and closure of the meeting

Text for the report where available was edited and agreed at the meeting, in particular those items relating to ICES and OSPAR requests. Other editing to be conducted by correspondence.

The Chairperson thanked Michelle Giltrap again for hosting the meeting and the hospitality provided by Trinity College Dublin and finally thanked the group members for their contribution and closed the meeting at 15:00 hrs
### Annex 1: List of participants

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<tr>
<td>John Thain</td>
<td>CEFAS</td>
<td>TEL: +44 (0)1621787239</td>
<td><a href="mailto:j.e.thain@cefas.co.uk">j.e.thain@cefas.co.uk</a></td>
</tr>
<tr>
<td>(Chair)</td>
<td>Burham-on-Crouch Laboratory</td>
<td>FAX: +44 (0)1621784989</td>
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<tr>
<td>Dick Vethaak</td>
<td>Deltares, Unit Marine and Coastal Systems, Section</td>
<td>TEL: +31 15-2858659 / +31 651232412</td>
<td><a href="mailto:dick.vethaak@deltares.nl">dick.vethaak@deltares.nl</a></td>
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<tr>
<td></td>
<td>Ecosystem Analysis and Assessment (ESA)</td>
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<tr>
<td></td>
<td>Rotterdamseweg 185</td>
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<tr>
<td></td>
<td>2629 HD Delft</td>
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<tr>
<td></td>
<td>The Netherlands</td>
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<tr>
<td>Aldo Viarengo</td>
<td>University of Eastern Piedmont, Via Bellini, 25G</td>
<td>TEL: +39 0131 360 370</td>
<td><a href="mailto:viarengo@unipmn.it">viarengo@unipmn.it</a></td>
</tr>
<tr>
<td></td>
<td>I5100 Alessandria, Italy</td>
<td>FAX: +39 33 357182439</td>
<td></td>
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</table>
Annex 2: Terms of Reference for 2010

2009/2/SSGHIE01  The Working Group on Biological Effects of Contaminants (WGBEC), chaired by John Thain, CEFAS, UK, will meet in Dublin, Ireland, 10–15 January 2010:

a) Review progress with publication and electronic dissemination of biological effects techniques in the ICES TIMES series;

b) Assess the amount of biological effects data submitted to the ICES database and answer queries / requests from the ICES Data Centre; and to consider codes for techniques now in the integrated approach – scheme;

c) Review progress with national /international monitoring activities; to include / integrated assessment / and application of biological effect techniques within OSPAR / MEDPOL / WFD / HELCOM / EU MSD;

d) Review progress with AQC procedures for biological effect methods and include harmonisation activities within OSPAR, Baltic and MEDPOL maritime areas;

e) In close cooperation with ICES / OSPAR SGIMC conduct intersessional work for review at 2010 meeting based on the outcome of the SGIMC Aberdeen Workshop, October 2009.

f) Review ICES WGBEC list of recommended biological effects methods for monitoring purposes and define how this fits in for both OSPAR and EU MSFD purposes;

g) Continue to review of emerging and novel contaminants as they arise and specifically nanoparticles;

h) Review progress with the ICON (NSHEALTH) and Baltic BEAST programme;

i) Review current knowledge and research on contaminants in eel and associated biological effects;

j) Extending marine assessment and monitoring framework used in Chapter 10 of the QSR 2010 (OSPAR request 2010/1)

To review the methodology used by the OSPAR workshop on the development of Chapter 11 of the QSR 2010 (Utrecht workshop) and taking into account, inter alia, ICES work on integrated assessment, provide advice on the following aspects:

---

1 Although the workshop title referred to Chapter 11. the output has subsequently been reflected in Chapter 10 of the QSR.
i) improvements that could be made to the thresholds between different assessment classes, including any scientific basis for proposed thresholds;

ii) extending the methodology to support the assessment of plankton communities;

iii) improving the method for working at different scales, such as the level of an OSPAR Region, the level of sub-Regions such as the Irish Sea or the Channel or the level of an estuary or an MPA;

k) Report to SSGHIE on potential and current contributions of your EG to the Strategic Initiative on Coastal and Marine Spatial Planning (SICMSP).

l) Report to SSGHIE on your plans to promote cooperation between EGs covering similar scientific issues.

WGBEC will report by 15 February 2010 (via SSGHIE) for the attention of SCI-COM and ACOM.

Supporting Information

<table>
<thead>
<tr>
<th>Priority</th>
<th>The activities of this group will enable ICES to advise on issues relating to the design, implementation and execution of regional research and monitoring programmes pertaining to hazardous substances in the marine environment. To develop procedure for quality assurance of biological effects data and to improve assessments of data relating to the biological effects of contaminants in the marine environment.</th>
</tr>
</thead>
</table>
| Scientific justification | a) It is important for WGBEC to keep track of publication progress with biological effects methods it has sponsored. Protocols are needed for national and international programmes as well as the OSPAR programmes.  
b) Biological effects data is increasingly being entered into the ICES database and WGBEC is encouraging this and monitors this activity. In addition as more data is being submitted technical queries arise and WGBEC can assist with answering queries from the ICES Data Centre.  
c) WGBEC has found it of value to discuss, feedback and support national monitoring programmes across the maritime areas and this is a valuable opportunity to improve and harmonise programme designs and assessment of data (e.g. OSPAR / MEDPOL / WFD / HELCOM / EU FWM);  
d) AQC is vital to support, report and assess data, particularly for cross maritime areas and developments and harmonisation in this area need to be taken forward in a co-ordinated manner.  
e) ICES / OSPAR SGIMC have a heavy work programme and WGBEC have noted that this Study Group have already identified tasks for ICES WGBEC, both intersessionally and at the WGBEC meeting. These tasks are not insignificant and WGBEC are willing to provide the support and expertise for taking this important work forward;  
f) WGBEC last reviewed the list of biological effect recommended and promising monitoring techniques in 2007. There has been considerable developments over three years and WGBEC feels it is necessary to conduct a major review, including the rationale for recommending techniques and how they fit in with SGIMC and EUMSFD activities;  
g) As information on emerging contaminants becomes available it is important to be in a position to advise and assess their impact on biological systems and the environment and to advise on suitable monitoring techniques, and nanoparticles have been identified as a fast moving research area.  
h) The ICON demonstration programme and the Baltic Beast programme underpins the integrated chemical – biological effects approach advocated by OSPAR and in the Baltic. WGBEC needs to monitor and evaluate these activities.  
i) It has been identified (see ICES WGEEL reports) that contaminants and
associated biological effects may be contributing to the demise in eel populations across Europe and WGBEC will review what research there is available to support this suggestion.

j) This is an OSPAR request (2010/1)
k) This strategic initiative is currently being planned and suggestions from EGs on their engagement in the SICMSP are sought.
l) Collaboration across EGs is encouraged and may be facilitated by e.g. inviting EG chairs and/or key members to attend meetings of your EG, and to use teleconferencing and videoconferencing as means to engage participants remotely.

<table>
<thead>
<tr>
<th>Resource requirements</th>
<th>The main input to this group is from National experts. Each attendee is self-funded from their own / organisation / institute resources.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>The Group is normally attended by ca. 16 members and guests.</td>
</tr>
<tr>
<td>Secretariat facilities</td>
<td>None required.</td>
</tr>
<tr>
<td>Financial:</td>
<td>No financial implications.</td>
</tr>
<tr>
<td>Linkages to advisory committees</td>
<td>ACOM</td>
</tr>
<tr>
<td>Linkages to other committees or groups</td>
<td>There are linkages with WGSAEM, MCWG, WGMS and WGPDMO.</td>
</tr>
<tr>
<td>Linkages to other organizations</td>
<td>None identified.</td>
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</table>
Annex 3: Agenda

The Working Group on Biological Effects of Contaminants [WGBEC]

Dublin, from 11–15 January 2010

1) Opening of the meeting;
2) Adoption of the agenda;
3) Appointment of rapporteurs;
4) Review progress with national/international monitoring activities; to include/ integrated assessment/ and application of biological effect techniques within OSPAR/MEDPOL/WFD/HELCOM/EU MSD + any other; (ToR c).
5) Review progress with the ICON (NSHEALTH) and Baltic BEAST programme; (ToR h).
6) Extending marine assessment and monitoring framework used in Chapter 10 of the QSR 2010 (OSPAR request 2010/1) - To review the methodology used by the OSPAR workshop on the development of Chapter 11 of the QSR 2010 (Utrecht workshop); (ToR j).
7) Report to SSGHIE on potential and current contributions of your EG to the Strategic Initiative on Coastal and Marine Spatial Planning (SICMSP); (ToR k).
8) Report to SSGHIE on your plans to promote cooperation between EGs covering similar scientific issues; (ToR l).
9) Review ICES WGBEC list of recommended biological effects methods for monitoring purposes and define how this fits in for both OSPAR and EU MSFD purposes; (ToR f).
10) In close cooperation with ICES/OSPAR SGIMC conduct intersessional work for review at 2010 meeting based on the outcome of the SGIMC Aberdeen Workshop, October 2009; (ToR e) “to receive Background Documents and draft assessment criteria from ICES WGBEC on:
   • Acetyl cholinesterase
   • Mussel histopathology
   • Micronucleus and Comet assay
   • MT and ALA-D
   • Intersex in fish”
11) Review progress with publication and electronic dissemination of biological effects techniques in the ICES TIMES series; (ToR a).
12) Assess the amount of biological effects data submitted to the ICES database and answer queries/requests from the ICES Data Centre; and to consider codes for techniques now in the integrated approach – scheme; (ToR b).
13) Review progress with AQC procedures for biological effect methods and include harmonisation activities within OSPAR, Baltic and MEDPOL maritime areas; (ToR d).
14) Continue to review of emerging and novel contaminants as they arise and specifically nanoparticles; (ToR g).
15) Review current knowledge and research on contaminants in eel and associated biological effects; (ToR i).
16) Any other business;
17) Recommendations and action list;
18) Adoption of the report and closure of the meeting
19) WGBEC will report by 15 February 2010 (via SSGHIE) for the attention of SCICOM and ACOM
### Annex 4: Tentative timetable

<table>
<thead>
<tr>
<th>Date</th>
<th>Approx. Time</th>
<th>Agenda Item</th>
<th>Rapporteurs</th>
<th>Issue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday 11 January</td>
<td>09:30</td>
<td>1</td>
<td>JT</td>
<td>Introduction by Chairperson and Michelle Giltrap, housekeeping issues, tour de table.</td>
</tr>
<tr>
<td>10:00</td>
<td></td>
<td>2</td>
<td>JT</td>
<td>Adoption of agenda, tabling of documents</td>
</tr>
<tr>
<td>10:15</td>
<td></td>
<td>3</td>
<td>JT</td>
<td>Appointment of rapporteurs.</td>
</tr>
<tr>
<td>12:45</td>
<td></td>
<td></td>
<td></td>
<td>Lunch</td>
</tr>
<tr>
<td>13:30</td>
<td></td>
<td>8</td>
<td>DV + KH + JT</td>
<td>Report to SSCHIE on your plans to promote cooperation between EGs covering similar scientific issues.</td>
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<tr>
<td>17/18:00</td>
<td></td>
<td></td>
<td></td>
<td>Close of business</td>
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<tr>
<td>Tuesday 12 January</td>
<td>09:00</td>
<td>11</td>
<td>MG</td>
<td>Review progress with publication and electronic dissemination of biological effects techniques in the ICES TIMES series.</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td>JT</td>
<td>Proposal of new chairperson</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td>JT</td>
<td>Review ICES WGBEC list of recommended biological effects methods for monitoring purposes and define how this fits in for both OSPAR and EU MSFD purposes.</td>
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<tr>
<td>12:45</td>
<td></td>
<td></td>
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<td>Lunch</td>
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<tr>
<td>14:30</td>
<td></td>
<td>12</td>
<td>TM + MG + RB + TB + liaise ICES</td>
<td>Assess the amount of biological effects data submitted to the ICES database and answer queries / requests from the ICES Data Centre.</td>
</tr>
<tr>
<td>15:30</td>
<td></td>
<td>6</td>
<td>JT + KH + DV</td>
<td>. Extending marine assessment and monitoring framework used in Chapter 10 of the QSR 2010 (OSPAR request 2010/1).</td>
</tr>
<tr>
<td>16:15</td>
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<td>Close of business</td>
</tr>
<tr>
<td>Wednesday 13 January</td>
<td>09:00</td>
<td>10</td>
<td>DV</td>
<td>Review SGIMC meeting report and provide support as requested.</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>BL</td>
<td>Micronuclei + Comet</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HS</td>
<td>DNA adducts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4a</td>
<td>Review progress with national /international monitoring</td>
</tr>
<tr>
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<td>Lunch</td>
</tr>
<tr>
<td>13:30</td>
<td></td>
<td>5</td>
<td>KH + KL</td>
<td>Review progress with the ICON (NSHEALTH) and Baltic BEAST programme.</td>
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<tr>
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<td>Close of business</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>13</td>
<td>Review progress with AQC procedures for biological effect methods and include harmonisation activities within OSPAR, Baltic and MEDPOL maritime areas.</td>
</tr>
<tr>
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<td>Approx. Time</td>
<td>Agenda Item</td>
<td>Rapporteurs/Contributors</td>
<td>Issue</td>
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<tr>
<td>Thursday</td>
<td>09:15</td>
<td>4b</td>
<td>HS</td>
<td>Review progress with national/international monitoring activities; within OSPAR / MEDPOL / WFD / HELCOM / EU MSD + any other</td>
</tr>
<tr>
<td>14 January</td>
<td></td>
<td></td>
<td>MG</td>
<td>Sweeden DNA</td>
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<td>CM</td>
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<td>Spain</td>
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<td></td>
<td></td>
<td></td>
<td>DV already had</td>
<td>MEDPOL</td>
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<td>MSFD</td>
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<td>13:45</td>
<td>15</td>
<td>JR + ...???</td>
<td>Review current knowledge and research on contaminants in eel and associated biological effects.</td>
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<td>14</td>
<td>TM</td>
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<td>Continue to review of emerging and novel contaminants as they arise….micriplastics</td>
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<td>JR</td>
<td>.................nanoparticles and emerging contaminants</td>
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<td>16</td>
<td>JT</td>
<td>Any other business.</td>
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<tr>
<td></td>
<td>17/18:00</td>
<td></td>
<td>ICES ASC Nante</td>
<td>Close of business.</td>
</tr>
<tr>
<td>Friday</td>
<td>09:00</td>
<td>17</td>
<td>JT</td>
<td>Recommendations and action list.</td>
</tr>
<tr>
<td>15 January</td>
<td></td>
<td></td>
<td></td>
<td>Adoption of the report.</td>
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<tr>
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<td>Lunch</td>
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<tr>
<td></td>
<td>12:30</td>
<td></td>
<td></td>
<td>Closure of the meeting.</td>
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Annex 5: Progress in the national programme for monitoring marine pollution in Spain

From agenda item 4.

1. BACKGROUND

Two major biomonitoring programmes, along the Northern Iberian coast and along the Iberian Mediterranean coast have been conducted through several research projects since past decades until 2009, by the Instituto Español de Oceanografía (IEO). In January 2010, an agreement has been finally signed by the Instituto Español de Oceanografía (IEO) and the Ministerio de Medio Ambiente Rural y Marino (MARM) for 2010-2012 in order to conduct a biomonitoring program to meet the obligations of the both conventions (OSPAR and Barcelona), but also to potentially contribute to the GES assessment in the Marine Strategy Framework Directive.

Recent progress in the Atlantic Spanish marine pollution Monitoring Program, conducted by Instituto Español de Oceanografía (IEO) includes the adoption of an integrative approach that includes CEMP chemical methods and pre-CEMP biological methods. In order to establish clear relationships between results of chemical monitoring of pollution and the pollutant concentrations that may cause ecological damage, we are intending to carry out the following actions taking into account the general biological effects considered by the CEMP and PreCEMP: (i) To obtain data for conducting a study on the biological effects of sediment elutriates by using the sea urchin embryo-larval bioassay; (ii) To obtain data for conducting a study on the toxicity of sediments by using the amphipod survival bioassay; (iii) To conduct a study on the biological effects of chemical pollutants on molecular responses in mussels (GST and AChE).

For the new organisation of the biological effects monitoring in MEDPOL Phase IV (2006-2013), the Contracting Parties to the Barcelona Convention adopted the strategy for the development of Mediterranean Marine Pollution Indicators (MPIs). This strategy will be considered as the basis for the preparation of marine ecosystem health assessments in a manner which could facilitate the development and implementation of a policy for the protection and conservation of the Mediterranean Sea and coastal areas (UNEP, 2003). Therefore, Spanish monitoring research activities in Mediterranean waters were recently also extended with more biomarker measurements in mussels and fish as well as contaminant concentrations in surficial sediments and fish. In order to make an integrated assessment of the quality/health status of the marine ecosystem, chemical contaminant concentrations (mussels, fish and sediments) and biomarker responses (mussels and fish) are analysed in selected areas.

In both programmes, measurements are performed yearly (excepting temporal trends in sediments that are conducted biannually, in the case of the Mediterranean program) and the application of both chemical and biological effect techniques (biomarkers/bioassays) is included (Table 1).
<table>
<thead>
<tr>
<th>BIOMONITORING IEO</th>
<th>SPANISH ATLANTIC MONITORING</th>
<th>SPANISH MEDITERRANEAN MONITORING</th>
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<td>Yearly</td>
<td>Autumn (Sept-Oct)</td>
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<td>Fish (MB/MM)</td>
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<td>Autumn (Sept-Oct)</td>
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<td>Post-spawning</td>
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<td>Autumn (Oct-Nov)</td>
<td>Spring (May-June)</td>
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<td>MG*</td>
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<td>Sea urchin Embryotoxicity assay</td>
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<td>S*</td>
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</tr>
<tr>
<td>CI/CF</td>
<td>MG/MM</td>
<td>MG/MB</td>
</tr>
<tr>
<td>GSI</td>
<td>not</td>
<td>MB</td>
</tr>
</tbody>
</table>

MG: *Mytilus galloprovincialis*
MB: *Mullus barbatus*
MM: *Merluccius merluccius*
NL: *Nucella lapillus*
NR: *Nassarius reticulatus*
S: Surficial sediments
*pilot study

2. METHODOLOGY AND WORKING PLAN FOR THE ATLANTIC MONITORING

2.1. Sampling

Sediment samples will be taken with a box-corer dredge and the surface layer (2 cm) will be collected, placed into sealed polyethylene bags, carried to the laboratory and stored at 4°C in the dark. Organic matter content and percentage of fine particles (<63 microns) will be determined.
Sediment elutriates intended for embryo-larval bioassays will be obtained following Beiras (2002) by rotatory mixing of 100 g of sediment and 500 ml of control FSW at 60 rpm for 30 min in airtight polypropylene flasks with no head space. After overnight decantation at 20°C in the dark, the liquid phase (elutriate) is siphoned into a separate beaker and then aerated for 10 min to discard any potential toxicity caused by H2S.

Intertidal wild mussels (Mytilus galloprovincialis) will be collected by hand during the low tides, in the prespawning season at this area (October-November), in order to minimize seasonal variations in the enzymatic activity levels. Mussels will be transported in a portable ice-box to the laboratory.

2.2. Study of temporal trends

Data corresponding to temporal trend studies of the monitoring program in 2007 and 2008 will be analyzed in order to fill the data gaps that may exist.

The study of the temporal trends of the sediment toxicity will be carried out through annual sampling cruises in four areas (Vigo, Pontevedra, Gijón-Avilés and Gulf of Cádiz) from 2010 to 2012 (Figure 1).

The study of the temporal trends of the biomarker levels in mussels will be carried out through annual sampling cruises in seven areas of interest, including reference sites (Vigo, Pontevedra, Arousa, A Coruña, Avilés, Santander y Bilbao) from 2010 to 2012 (Figure 2).

2.3. Study of the spatial distribution

Data corresponding to the spatial distribution studies of the monitoring program in 2007 and 2008 will be analyzed in order to fill the data gaps that may exist.

The spatial distribution of the sediment toxicity will be carried out in 2010 on sampling sites along the coast including inner areas in Rías and estuaries (Figure 3).

During 2010 mussels will be collected at the sampling sites indicated in Figure 2 in order to study the spatial distribution of the biomarker levels.

2.4. Sea-urchin embryo-larval bioassay

PreCEMP COMPONENT: Effects of marine pollution in invertebrate embryos and larvae

OBJECTIVES: Biological monitoring of pollution. Implementation of an integrative monitoring in the Surveillance Programmes (recommended by OSPAR/ICES WIKIMON II).

MATRIX: Sediments

The toxicity of sediment elutriates will be measured by using the sea-urchin (Paracentrotus lividus) embryo-larval bioassay. The experimental basis of these bioassays consist in the exposure of fertilized eggs to the sediment elutriates and, after an incubation period in controlled conditions, an ecologically relevant biological response is registered. About 20-40 fertilized eggs will be delivered into 4 ml polypropylene vials with the elutriate dilutions. Experimental vials will be incubated for 48 h at 20°C in the dark, in culture chambers. After the incubation, samples will be fixed with a few drops of 40% formalin.

The toxicity study of approximately 50 sediment samples concurrently with chemical data from the sediments will be part of the integrative monitoring program.
2.3. Amphipod survival bioassay

PreCEMP COMPONENT: Amphipod survival study in sediment samples.

OBJECTIVES: Biological monitoring of pollution. Implementation of an integrative monitoring in the Surveillance Programmes (recommended by OSPAR/ICES WIKIMON II).

MATRIX: Sediments

The amphipod (Corophium sp.) survival bioassay will be used to evaluate the toxicity of sediments, as a complement of the sea-urchin embryo-larval bioassay. The biological response measured is the survival of amphipods during a 10 day exposure to sampled sediments at 20°C and 12:12 h day:light cycle. Organisms are placed in 1 L beakers with the sampled sediments with 3 replicates per site and 5 replicates in the control sediment treatment. During the experiment temperature, salinity, pH and dissolved oxygen will be controlled. After 10 days exposure, each beaker will be sieved through 2 mm and the number of individuals surviving will be recorded.

The toxicity study of approximately 25 sediment samples concurrently with the study of the associated biota and the chemical data from the sediments will be part of the integrative monitoring program.

2.4. Biomarkers in mussels

OBJECTIVES: Biological monitoring of pollution. Implementation of an integrative monitoring in the Surveillance Programmes (recommended by OSPAR/ICES WIKIMON II).

MATRIX: Biota

The GST enzymatic activity in mussel gills (Mytilus galloprovincialis) will be determined following the method of Habig et al. (1974) adapted to microplate. The enzymatic activity will be determined by measuring the increase in absorbance at 340 nm every 20 seconds for 5 minutes. AChE will be measured according to Bocquené and Galgani (1998) and adapted to microplate.

The toxicity study of approximately 50 mussel samples concurrently with the study of the chemical data in mussel tissues will be part of the integrative monitoring program.

3. METHODOLOGY AND WORKING PLAN FOR THE MEDITERRANEAN MONITORING

Current biomonitoring programme conducted in Spanish Mediterranean Waters comprises different partial monitoring programmes and the main objectives to achieve are:

1) The determination of spatial distribution and temporal trends of selected contaminants in mollusc, fish and sediments in coastal, hot spots and reference areas;

2) To seek evidence and assess over time the detrimental biological effects in mollusc and fish.

With such aims, IEO is conducting two field samplings yearly along the Iberian Mediterranean coast. The first field sampling is conducted between 15 May and 15 June (outside spawning period for Mytilus galloprovincialis) in order to collect native mussel samples for chemical and biological effects along the Iberian Mediterranean coastline (Figure 4). The second survey is conducted between 1 and 15 October (post-
spawning period for *Mullus barbatus*) in order to collect in coordinated way sediments and also fish samples for chemical and biological effects in selected areas of concern along the Iberian Mediterranean coast.

For chemical analysis and biomarkers in biota, the temporal monitoring programme comprises a number of locations that are sampled yearly, while the spatial monitoring programme comprises a larger number of locations that are sampled once every 5 years (Figure 4).

For chemical concentration in sediments, the temporal monitoring programme will be conducted at least once every 2 years, once the appropriated areas have been identified. At present, a pilot study (2006-2010) is being conducted to identify suitable sediment sampling areas along the Spanish Mediterranean coast with the best characteristics (undisturbed bottoms by anthropogenic activities with high sedimentation rate, percentage of fine fraction and content of organic matter, etc.).

### 3.1. Integrated assessment

The approach of an integrated assessment of the health status of the marine environment is being stressed also in the MEDPOL Programme and in order to progress on it and optimize the funding resources available, fish sampling is being carried out in a coordinated way with sediment sampling and catching fish from main fish grounds in the regional vicinity of sediment sampling areas. MCBE (IEO) started at 2006 the integrated assessment of the chemical contamination in some selected sites/areas chemically well characterized as hot-spots. In such areas, biomarkers and supporting parameters are measured in fish and/or mussels, and selected contaminants (PAHs, OCPs and trace metals) are measured both in biota and surficial sediments. Surficial sediments samples from the same box corer are being also sampled to perform sea urchin embryo toxicity bioassays. The final objective to achieve in 2012 is to identify the main areas of concern along the inner continental shelf (<70 m) to perform the integrated monitoring approach in fish underpinned with the data obtained from chemical trend monitoring in sediments.

### 3.2. Caged mussels and two-tier approach:

During last Workshop on the MEDPOL Biological Effects programme, the use of caged specimens was highly recommended (Alexandria, 2006). Developments in the biomonitoring programme conducted by IEO in the Mediterranean Spanish waters will continue using native mussels for study temporal trends on chemical concentration and the long-term effects on biological effects but also will use transplanting mussels to solve the problem of scarce natural mussel stocks in certain areas. To initiate and validate the use of caged mussels and two-tier approach recommended in MEDPOL Phase IV, the IEO initiated in 2008-2009 pilot field studies using caged mussels at selected locations along the SE Spanish coast. Results of these studies should help to make a final decision if the spatial biomonitoring with native mussels can be simplified using the two-tier approach and if in particular cases, the use of caged mussels is appropriate.

### 4. References


Figure 1. Sampling sites for the study of temporal trends of sediment toxicity in the Atlantic coast of Spain using sea-urchin and amphipod bioassays.
Figure 2. Sampling sites for the study of temporal trends of mussel (M. galloprovincialis) biomarkers (GST and AChE) in the Atlantic coast of Spain.
Figure 3. Sampling sites for the study of the spatial distribution of sediment toxicity in the Atlantic coast of Spain using sea-urchin and amphipod bioasays.
Fig. 4 Sampling sites corresponding to field samplings conducted since 2006 along the Spanish Mediterranean coast by the Instituto Español de Oceanografía (IEO): Mussel sampling stations (only contaminant concentrations in black circles; contaminants and biomarker responses in mussels in blue circles), contaminant content and biomarkers in fish and contaminant concentrations in sediments (yellow flags stations proposed to be sampled in the period 2010-2012).
Annex 6: From agenda item 4, MSD executive summary

Executive summary

We recommend that the assessment of achievement of GES under MSFD Descriptor 8 “Concentrations of contaminants are at levels not giving rise to pollution effects” should be based upon monitoring programmes covering the concentrations of chemical contaminants and also biological measurements relating to the effects of pollutants on marine organisms in each of the assessment Regions. The combination of conventional and newer, effect based, methodologies, with the assessment of environmental concentrations of contaminants provides a powerful and comprehensive approach. As the occurrence of adverse effects at various levels of organisation (organism, population, community, ecosystem) needs to be avoided, monitoring schemes should also indicate the approaching of critical values as early warning.

Therefore, for the purpose of implementing Descriptor 8 under the MSFD, three core elements of data assessment are recommended:

- Concentrations of contaminants in water, sediment and biota are below assessment thresholds identified on the basis of toxicological data.
- Levels of pollution effects are below assessment thresholds representing harm at organism, population, community and ecosystem levels.
- Concentrations of contaminants in water, sediment and biota, and the occurrence and severity of pollution effects, should not be increasing.

Monitoring programmes should include the assessment of concentrations of priority contaminants in environmental matrices, i.e. water, sediment, and the tissues of biota. Monitoring programmes should also include the quantification of biological effects of contaminants at different levels of biological organisation. The selection of priority contaminants, monitoring species and biological effects measurements should be made for each assessment Region by the Member States with responsibility for implementation of MSFD in each Region. Therefore, the priority monitoring matrices, and chemical and biological measurements made may vary between assessment Regions in response to Regional concerns and environmental conditions. However, monitoring and assessment should be harmonised to the greatest possible degree between assessment Regions.

Monitoring data should be interpreted against the objective described by Descriptor 8 through a series of assessment thresholds, expressed as concentrations of chemical contaminants, or levels of biological response. In particular, monitoring data should be interpreted against assessment thresholds that are designed to protect against the occurrence of pollution effects. Examples of suitable assessment thresholds include Environmental Quality Standards (EQS) derived under the WFD, Environmental Assessment Criteria (EACs) as defined within OSPAR for water, sediment and biota, and parallel assessment thresholds used by other Regional Conventions or Member States for the interpretation of monitoring data. Biological effects will be assessed against threshold levels of response that are indicative of significant harm to the organisms concerned. The aim is to prevent pollution effects occurring at the organism, population, community and ecosystem level.

In addition, monitoring data should be assessed against background concentrations of contaminants or levels of biological response to enable added-risk approaches to be used in the derivation of assessment thresholds, to enable greater use to be made
of monitoring data in interpreting the causative agents of pollution effects, and to give early warnings of potential developing problems.

Increasing contaminant concentrations increase the likelihood of pollution effects. In order to minimize the risk of deleterious effects, concentrations of contaminants in water, sediment and biota, and the occurrence and severity of pollution effects, should not be increasing. Regional Conventions have developed robust statistical approaches to the analysis of time series of monitoring data to detect significant trends over time. These should be applied to chemical and biological effects monitoring data.

The integration of the results of chemical monitoring programmes, and combination of data from chemical and biological effects monitoring, is an active area of science within the Regional Conventions (i.e. OSPAR, HELCOM, MEDPOL). Current experience indicates that integration is greatly facilitated by coherent and consistent sets of assessment thresholds (EQSs, EACS, etc). Further development work is necessary, through the EU, Regional Conventions or MS, to expand the range of assessment thresholds to include a greater number of contaminants and biological effects. Integrated monitoring programmes, data collation, interpretation and presentation schemes are being developed and applied by the Regional Conventions, and we recommend that this work continues and that Member States apply the best international advice applicable to MSFD Regions for which they have responsibility.

A core of both chemical analytical methods and biological effects methods exists which can be applied now. There are considerable benefits to be gained from the international experience in programme design, measurement methodology and data management and interpretation available from the Regional Convention programmes, and the EU (e.g. WFD). Detailed implementation of programmes for MSFD Descriptor 8 should build upon these, and upon existing data, to ensure that assessments against GES as robust as possible. However, marine monitoring science continues to develop, and the implementation strategy for MSFD should allow for programmes and procedures to evolve with time so as to maintain and improve the level of protection for marine ecosystems.