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Report of the Fourth ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open Sea Areas (WKIMON IV)

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1 Executive summary

The development of assessment criteria for biological effects techniques was initiated at WKIMON III. Progress over the past year was reviewed. The development of the Fish Disease Index had progressed very well; some changes were made to the response ranges for reproduction in eelpout and for bioassays. An immediate requirement for further work related to the establishment of assessment criteria for EROD, and WKIMON recommended that an *ad hoc* workshop be held for 3 days in Oostende, Belgium, in late September/early October 2008. The OSPAR Background Documents for some of the biological effects technique (fish disease, bile metabolites, EROD) require updating because the present ones are either incomplete or contain outdated information.

The methods used to establish the response ranges needed as assessment criteria of the biological effects techniques differ considerably and some were regarded as either inadequate or as supported by too few data. In addition, it was felt that general guidelines for deriving assessment criteria should be developed to ensure there was some degree of consistency and harmony to the process. In particular, there is a need to define the boundaries between the three levels of "Background Response Range", the "Elevated Response Range" and the "High Effect Range"; these may be regarded as the EAC equivalent boundaries for contaminants i.e. below, between and above. In this respect one important task for each biological effect will be to define what constitutes a "significant high level of response" or "harm" to the organism. This is a longer-term goal and should be continued by WKIMON or its equivalent in the future. In this respect a number of tasks were identified to address the above issues and to progress further development of assessment criteria.

The compilation of Background Documents on Biological Effects Techniques for Monitoring was provided by the OSPAR secretariat for the WKIMON IV meeting. The current status of each document was reviewed concurrently with the review on progress with the development of assessment criteria. It was recommended that a new Background Document on intersex in fish is needed and for completeness the compilation of Background Documents should include TBT-induced imposex in whelks. The compiled Background Document was thought to be thorough and complete. Some slight updating of references to take account of the most recent publications is needed but not urgent. Specific comment was given on each of the background documents:-reproduction in eelpout, EROD, PAH bile metabolites, DNA adducts, bioassays, lysosomal stability, fish disease index and scope for growth. It was noted that a Technical Annex for oestrogenic effects was being progressed.

Current methods for integrated assessments that the group were aware of were discussed, for example the FullMonti, REGNS and US EPA approaches. After discussion a new approach was suggested along the lines discussed by MON for integrating groups of chemical contaminants. The new approach used different endpoints of biological effects in fish, mussels and gastropods into a Generic Assessment Framework, which can be used as a "Traffic light" for environmental assessments. It was concluded that further refinement and development was required and in addition, the integration of the generic assessment framework with contaminants needed further discussion and development.

A draft technical annex to support the integrated chemical-biological effect approach was considered. This included preliminary considerations on survey and statistical design but further work was needed on this. Information was collated for use as a

basis for further development of a technical annex and this included: an overview of selected methods for integrated fish monitoring; overview of selected methods for integrated bivalve monitoring; overview of methods and species for integrated gastropod/organotin monitoring; environmental parameters for inclusion in monitoring programmes (water); environmental parameters for inclusion in monitoring programmes (sediment); draft integrated guidelines for mussels: and sampling and analysis strategies for integrated fish and bivalve monitoring.

A draft technical annex on recommended packages of chemical and biological methods for monitoring on a determinant basis was prepared and included packages on metals, PCBs, polychlorinated dibenzodioxins and furans, PAH and alkylated PAH, organotins, BFRs and PFOS.

A practical workshop (ICON) was proposed at WKIMON III with the aim of demonstrating the core components of the WKIMON integrated chemical biological effect methods and guidelines. Much of the programme was initially being funded from the Norwegian oil industry and Research Council but this support was no longer available. As a result a revised programme was suggested with a reduced number of stations and species than were included in the original proposal for ICON. In addition, the revised ICON should also look at existing good data sets, and select a data set for analysis as a case study that might be suitable for inclusion as box text in QSR2010.

The status of WKIMON (convened as a workshop) is no longer tenable within the OSPAR or ICES system. It was agreed that the work being undertaken by the WKIMON group would take up to three years to complete. It was proposed that a new group be formed within the ICES/OSPAR setup, perhaps a joint Study Group, to complete the outstanding work. Tasks for the group were identified and recommendation put forward for consideration by ICES/OSPAR.

2 Opening of the meeting and adoption of the agenda

The meeting was opened at ICES Headquarters at 09:30 hr on Tuesday 5th February 2008 by Helle Gjeding Jorgensen who welcomed the delegates on behalf of the ICES Secretariat and provided information on the ICES computer network and various domestic matters.

The Chair then invited the participants to introduce themselves and their affiliations and describe their area of interest and field of expertise. Twenty participants from eight countries attended the meeting. See **Annex 1** for participants list.

An agenda had been circulated prior to the meeting (**Annex 2**). This was adopted by the meeting with Agenda items 5, 6 and 7 timetabled for day one of the meeting; these three items were worked on simultaneously by three sub-groups.

3 Review and note the Terms of Reference

The Chair invited the participants to examine the Terms of Reference (ToR) and explained the background to the agenda items. The Terms of Reference (**Annex 3**) as provided by both OSPAR and ICES were reviewed and it was agreed that they were reflected in the agenda.

4 Assessment criteria: Review the progress with the Fish Disease Index and progress with the development of assessment criteria for biological effects methods. Where possible develop further and complete and/or develop a timetable for completion as appropriate TOR a

4.1 Review of progress

The development of assessment criteria for biological effects techniques had progressed well at WKIMON III. The purpose of this agenda item was to look at progress over the past year and to identify further work to complete the development of assessment criteria for all of the biological effect techniques within the integrated chemical biological effect strategy.

Three presentations were given on progress made regarding the development of assessment criteria for biological effects techniques and these were as follows:

- Assessment criteria for the Fish Disease Index (W. Wosniok)
- Links between EROD and environmental and physiological parameters (K. Cooreman)
- Assessment criteria for bioassays (R. Beiras)

4.1.1 Assessment criteria for the Fish Disease Index (FDI) (W. Wosniok)

The Fish Disease Index summarizes the various disease conditions of a single fish. In its present form, 9 externally visible diseases, 2 macroscopically visible liver neoplasms and 5 liver histopathology conditions are involved. Partial versions of the index can be computed, e.g. for the externally visible conditions only.

The assessment of the fish disease status in a defined area (proposal: ICES statistical rectangle) and for a defined time period (proposal: 6 years backwards from the time point for which the assessment shall be valid) comprises the following steps:

a) calculate the FDI population means in the area for each available time point,

b) determine P33 and P67 (the 33rd and the 67th percentile) of the FDI means,

c) count the number of FDI means in each of the ranges (< P33, [P33–P67], > P67),

d) assign weights to these counts ('-1' for the lower third, '0' for the middle third, '1' for the upper third) and calculate their weighted sum,

e) calculate the Mann-Kendall trend test statistic for the FDI means (high values indicate an increasing trend, low (negative) values indicate a decreasing trend),

f) add the location statistic from step 4 and the trend test statistic from step 5 to obtain the FDI assessment statistic. High values of the assessment statistic indicate high FDI levels with increasing trend, low (negative) values indicate low FDI levels with decreasing trend,

g) determine the statistical distribution of the FDI assessment statistic under the null hypothesis of no trend (Monte Carlo Simulation).

h) If the observed assessment statistic is smaller than the 2.5% quantile of the simulated distribution, then it is assessed as a desirable FDI level and trend ('green smiling face', if a map is produced), i) if the observed assessment statistic is larger than the 97.5% quantile of the simulated distribution, then it is assessed as an undesirable FDI level and trend (‘red frowning face’),

j) in all other cases the assessment statistic is considered to give an indifferent signal (‘yellow neutral face’).

In the discussion the following issues were pointed out:

- the allocation of disease-specific weighting factors is a critical issue and was, therefore, done based on a common structured methodology applied for combining expert judgements (Bradley Terry approach), in this case involving 6 specialists;
- the assessment of the FDI calculated for different geographical areas is not based on a comparison to a 'universal' natural background FDI level but instead on an area-wise assessment of FDI levels and temporal trends:
- the smiley approach enables a regional comparison of recent FDI trends.

WKIMON noted the excellent progress with the FDI and noted how well the components had been integrated together and included a weighting procedure to take account of the “health significance” of the disease and in addition, allows the use of partial data sets. It was noted that further refinement and validation will be undertaken by ICES WGDPMO.

4.1.2 Links between EROD and environmental and physiological parameters (K. Cooreman)

Kris Cooreman presented a case study on the seasonal patterns of a number of parameters in dab liver from the Belgian Continental Shelf and the relationship to the climatologically changing seasons, mainly the bottom water temperature changes. The determinants covered were the fat, contaminant concentrations (6 PPCDs, 7 PCDFs, PAHs and PCBs, including the planar, mono-ortho and 10 of the ‘classical’ PCBs) and hepatic EROD activities. All measured contaminants have similar hydrophobic and persistent properties, accumulate in fatty tissue and most of them are well known inducers of EROD.

The results of the study show that the seasonally changing bottom water temperatures, with coldest and warmest periods in, respectively, February/March and August/September, have a profound influence on the hepatic fat content. More than twofold differences were measured between the lowest and highest levels in, respectively, March and August/September. Recurrently on a yearly basis, the data show that more than half of the fat reserves decrease in the period between August/September and March and restore in the remaining period. Actually the steep decrease in the winter period starts in December and persists until approx. March. It is most likely that these fat reserves are being used to maintain the organism’s metabolism in the coldest period of the year for two reasons: food supplies are scarce in those periods, and, the fish are not allowed to feed much, even when enough food is available. The latter argument relates to the peristaltic action of the fish intestines which is passive and needs body movements to process the food and eliminate excrements. Body movements in water temperatures at approx. 5°C are restricted to necessities and inactivate the peristaltic responses with consequent risks of constipation and even death.

The liver fat fluxes have major impacts on all determined contaminant levels. In summary, all contaminant profiles fully parallel the liver fat profile on liver ww-basis with similar twofold concentration differences between lowest and highest in, respectively, March and August/September. In other words, more than half of the highest liver contaminant burdens, attained in August/September, are released and redistributed in the winter months until about March and are accumulated again during the subsequent spring and summer.

In terms of contaminant monitoring perspectives, August/September may be advised as the ideal sampling period, based on the highest liver contaminant levels. This is disputable because e.g. EROD-activities in that period (summer and the rest of the year) are close to background values in that area, indicating that the CYP1A1-receptor was not exposed during the influx and accumulation of its potential inducers. It can be concluded that these chemicals have not been able to reach the receptors, despite their high affinity. On the other hand, very high EROD-activity is measured recurrently in early spring; the period that major quantities of these contaminants are being mobilised as fat reserves are metabolised. The question rises whether these contaminant releases are involved in the induction process and, again, this is disputable. The main reason for doubt is that EROD-background levels are unknown and cannot be scientifically determined because of the lack of suitable reference sites. Two arguments supporting 'early spring' inductions by potential inducers are, according to the authors, the release of the contaminants during fat metabolism in the vicinity of the hepatic receptors and, secondly, research has shown that maturation results in an inhibitory effect in both males and females, with highest EROD activities being found in juveniles.

The assumption that these persistent contaminants are more or less immobilized in the fat is correct in the case of a static situation, but the process of seasonal water temperature changes and subsequent fat changes cause the release and redistribution of contaminants that may become available for exposure. These fluxes can actually be seen as active doses of exposure and can be quantified and distinguished from the bulk of contaminants in the fat. Depending on the availability of a suitable set of all necessary data, it might be possible to model the mechanistic role of contaminants kinetically. This could form the basis for a correct interpretation of biological effects of contaminants in the most vulnerable periods of the year.

The above information will need to be taken into account when interpreting and developing assessment criteria for EROD; see action point below.

4.1.3 Assessment criteria for biological effects methods: Bioassays (R. Beiras, University of Vigo)

WKIMON III reviewed the issue of sediment bioassays in a background document reflected in Chapters 8, 9 and 10 from OSPAR Background Document on Biological Effects Monitoring Techniques, (2007), now updated.

The assessment criteria for bioassays compiled at WKIMON III has been revised (see Table below) with the 10 year dataset of sediment, elutriates and seawater bioassays conducted with samples from the Galician Rias (NW Spain, the University of Vigo and I.E.O.-Vigo), as well as with data from the UK's FullMonti approach.

BIOASSAY	END-POINT	ORGANISMS	ASSESSMENT CRITERIA		
			Background	Elevated	High concern
Solid phase Bioassays	% mortality	*Corophium*	0–30	> 30–< 60	> 60
	% mortality	*Arenicola*	0–10	> 10–< 50	> 50
Liquid phase Bioassays	% abnormal	Bivalve embryo	0–20	> 20–< 50	> 50
	% mortality	Copepod	0–10	> 10–< 50	> 50
	% abnormal	Sea-urchin embryo	0–10	> 10–< 50	> 50
	% growth	Sea-urchin embryo	0–20	> 20–< 50	> 50

Some additional progress has been made for the sea-urchin test by a compilation of data sets obtained from the coastal monitoring in the Galician Rias from 1997 to date, and by a comprehensive review of data from published literature, and it can be summarized in the following points:

a) Sea-urchin embryo bioassay and bivalve embryo bioassay yield comparable sensitivity (Bivalve EC_{50}=1.01 Seaurchin EC_{50}, r^2=0.87, n=22 reference toxicants).

b) The acceptability criteria for quality assurance are 75% normal larvae for bivalves and 90% normal larvae for sea-urchin.

c) Assessment criteria to separate background from elevated response sites (or good from moderate sites following the EU-WFD terms) can be obtained by,

   i) correcting the response by a concurrently-sampled reference site to obtain a net response (PNR)

   ii) compiling the PNR datasets and calculating the 95% percentile (18% growth inhibition, approximated to 20% for simplicity).

d) A 50% effect level has been adopted as criteria to divide elevated response from high concern response (moderate from poor following the terms of the EU-WFD)

e) When results are expressed in terms of toxic units, the assessment criteria are the following: 0 to 0.32 TU: background, 0.32 to 1 TU elevated, >1 TU high concern.

4.1.4 Background responses and Assessment criteria for Scope for Growth (SFG)

Following the assessment criteria used by Widdows *et al.* (1995) it can be considered that a SFG between 15 and 20 J/h/g DW (75 to 100% of the maximum SFG) implies a healthy status. When SFG varies between 5 and 15 (25 to 75% of the maximum) the system has a moderate stress. When the SFG decreases to –5 (25 to –25% of the maximum) there is a high stress.

A survey was carried out in Spain during November and December 2007. Twenty sites selected from the North-Atlantic and Cantabric coast Spanish Monitoring Program for CEMP, were sampled for both chemical and physiological measurements.

Preliminary results obtained for SFG of mussels from the Spanish Monitoring Program showed important differences between sites (data presented at the meeting). Three levels of stress could be established according to SFG data, which are concordant with the Widdows criteria:

a) mussels with SFG between 20–30 J/h/g DW (75% of the maximum SFG) are considered healthy,

b) mussels with SFG between 8-20 J/h/ g DW (until 25 % of maximum) could be considered as moderately stressed,

c) mussels with a reduction of 70% of the maximum SFG, less than 8 J/h/ g DW could be considered highly stressed.

The assessment criteria derived last year require adjustment to take account of this new data.

4.2 Discussion on assessment criteria

The group discussed the current assessment criteria developed at WKIMON III and, based on new information available, some changes were made to the response ranges for reproduction in eelpout and for bioassays (Table 4.1).

It was emphasised that the methods used to establish the response ranges needed as assessment criteria of the biological effects techniques differ considerably. Some of the methods were regarded as either inadequate or as supported by too few data and it was thus decided to go through the assessment criteria in more detail by extracting relevant information from the existing OSPAR Background Documents into a separate table providing information on the current status (Table 4.2). The meeting felt that general guidelines for deriving assessment criteria should be developed to ensure there was some degree of consistency and harmony to the process. In particular, there is a need to define the boundaries between the three levels of "Background Response Range", the "Elevated Response Range" and the "High Effect Range"; these may be regarded as the EAC equivalent boundaries for contaminants i.e. below, between and above. In this respect one important task for each biological effect will be to define what constitutes a "significant high level of response" or "harm" to the organism. This is a longer-term goal and should be continued by WKIMON or its equivalent in the future.

An immediate requirement for further work related to the establishment of assessment criteria for EROD was identified, and WKIMON recommended that an *ad hoc* workshop be held for three days in Oostende, Belgium, in late September/early October 2008. The workshop will be co-chaired by P. Roose and K. Cooreman and will bring together EROD specialists and modellers. The aim of the workshop is to analyse existing data sets (that have to be readily available at the start of the workshop) and to identify suitable models that are able to adjust the data for effects of confounding factors in order to better define assessment criteria for EROD data.

A need was seen to amend the OSPAR Background Documents for some of the biological effects technique (fish disease, bile metabolites, EROD; see Table 4.2), because the present ones are either incomplete or contain outdated information.

4.3 Tasks identified to further develop assessment criteria

a) Development of guidelines for deriving assessment criteria; essential, to be taken forward by WKIMON or equivalent group in the autumn of 2008.

b) Development of EAC equivalents for biological effect responses; essential, to be taken forward by WKIMON or equivalent group in the autumn of 2008.

c) Further develop assessment criteria for EROD, and amend background document; to be taken forward by specialist group who will meet in September/October 2008 in Ostend.

d) VTG in flounder, further development of assessment criteria; Ian Davies to collate data to aid this process and report back to WKIMON in autumn 2008.

e) PAH Bile metabolites, background document requires considerable amendments and assessment criteria across all three levels needs developing; NOR and NL preparing a document for SIME 2008 the contents of which need to be taken onboard by WKIMON in autumn 2008.

f) DNA-adducts, requires amendment to background document and further development of assessment criteria; Brett Lyons agreed to take this forward and report back to WKIMON in the autumn.

Recommendation

An *ad hoc* workshop on background values of EROD and appropriate assessment criteria to be held for three days in Oostende, Belgium, in late September/early October 2008. The workshop will be co-chaired by P. Roose and K. Cooreman and will bring together EROD specialists and modellers. The aim of the workshop is to analyse existing data sets (that have to be readily available at the start of the workshop) and to identify suitable models that are able to adjust the data for effects of confounding factors in order to better define assessment criteria for EROD data.

Justification

Assessment criteria for EROD need further development because to date only background response criteria are available; the Background Response Range (BRR) require validation and refinement and the Elevated Response Range (ERR) and High Response Range (HRR) need development.

ACTION

P Roose and K Cooreman to organise EROD assessment criteria workshop for autumn 2008. Invitations to be sent to specialists in this field in liaison with ICES WGBEC.

Several tasks were identified for action in Section 4.3 above. These will be taken forward by WKIMON or its equivalent, however this develops.

Table 4.1 Summary of current assessment criteria from WKIMON III (revised at WKIMON IV).

Biological Effect	Qualifying comments	Background Response Range	Elevated Response Range	High and Cause for Concern Response
VTG in plasma; µg/l	Cod	LOD to 2		
	Flounder	LOD to 2		
Reproduction in eelpout; mean frequency (%)	Malformed larvae	0–1	> 1–2	> 2
	Late dead larvae	0–2	> 2–3	> 3
	Growth/retarded larvae	0–4	> 4–6	> 6
EROD; pmol/mg protein	Cod	≤ 80		
	Dab	≤ 40		
	Flounder	≤ 10		
Bile metabolites; 1-OH pyrene (µg/ml; 341/383 nm fluorescence)	Dab	≤ 220		
	Cod	≤ 0.95		
DNA adducts; nm adducts / mol DNA	Dab	≤ 7.86		
	Haddock	≤ 6.84		
	Saithe	≤ 7.90		
Bioassays; % mortality	Sediment, *Corophium*	0–30	> 30–< 60	> 60
	Sediment, *Arenicola*	0–10	> 10–< 50	> 50
	Water, copepod	0–10	> 10–< 50	> 50
Bioassays; % abnormality	Water, bivalve embryo	0–20	> 20–< 50	> 50
	Water, sea urchin embryo	0–10	> 10–< 50	> 50
Bioassay; % growth	Water, sea urchin embryo	0–20	> 20–< 50	> 50
Lysosomal stability; minutes	Cytochemical; all species	> 20	≤ 20–≥ 10	< 10
	Neutral Red Retention: all species	> 120	≤ 120–≥ 50	< 50
Fish Disease Index	Cutpoints of the FDI assessment statistic are the 2.5% and the 97.5% quantiles of the assessment statistic. Their numerical values depend on the amount of data and are determined by simulation. Values below the 2.5% quantile are considered as "desirable", values between and including the quantiles are considered as "indifferent", values above the 97.5% quantile are considered as "undesirable" (raising concern).	< 2.5% quantile	2.5–97.5 % quantiles	> 97.5% quantile

Table 4.2 Summary of current position with OSPAR Background Documents and assessment criteria WKIMON IV. *EAC equivalents, see text above.

Biological Effect	Qualifying comments	Background document	Method of determination / expression of assessment criteria	Do BAC need to be developed	Assessment criteria?	Arrangements for development
VTG in plasma; µg/l	Cod	Thorough and complete	90 percentile from reference area / needs to be amended to zero	Needs to be developed but limited data available	Nothing on EAC equivalent*	Collation of existing data ID - FRS UK
	Flounder					
Reproduction in eelpout; mean frequency (%)	Malformed larvae	Thorough and complete	95 percentile of ref sites / control	Already in place	Already in place	Further refinement as more data becomes available
	Late dead larvae					
	Growth / retarded larvae					
EROD; pmol/mg protein	Cod	Incomplete	Range of methods used	Needs to be developed	Further development required. Nothing EAC equivalent*	No system available / further development of a model with existing data set. Amend OSPAR Background Document from p. 143 of last report. Workshop arranged
	Dab					
	Flounder					
Bile metabolites; 1-OH pyrene (µg/ml; 341/383 nm fluorescence)	Dab	Short and incomplete / need info on methodology etc	Reported as ranges from one data set	Further development	Further dev.- nothing on EAC equivalents*	NOR and NL preparing doc for SIME 2008 Background document needs updating
	Cod					
DNA adducts; nm adducts / mol DNA	Dab	Complete but needs slight amendment for haddock and saithe	90 percentile	Limited data requires further validation / development	Further dev – nothing on EAC equivalents*	BL-UK to update/amend
	Haddock					
	Saithe					

Biological Effect	Qualifying comments	Background document	Method of determination / expression of assessment criteria	Do BAC need to be developed	Assessment criteria?	Arrangements for development
Bioassays; % mortality	Sediment *Corophium*	Thorough and complete	95 percentile of ref sites / control	Already in place	Already in place	Further validation as more data becomes available
	Sediment *Arenicola*					
	Water bivalve embryo					
	Water copepod					
	Water echinoderm					
Lysosomal stability; minutes	Cytochemical; all species	Thorough and complete	Best professional judgment from extensive literature and laboratory studies	Already in place	Already in place	Further validation as more data becomes available. Currently based on limited data set from one country.
	Neutral Red Retention: all species	Thorough and complete	Best professional judgment from extensive literature and laboratory studies	Already in place	Already in place	No further development
Fish Disease Index	Combination of externally visible diseases, macroscopic liver neoplasms and liver histopathology	Details in Annex 11 in WGPMO 2007 report; A summary needs to be included in the OSPAR Background Document.	Cutpoints of the FDI assessment statistic: 2.5% and 97.5% quantiles of the assessment statistic. < P 2.5%: desirable, P 2.5%-P 97.5%: indifferent, > P 97.5%: raising concern	Already in place	Already in place	No further development required. May be refined by ICES WGDPMO as appropriate
Scope For Growth	Mussels	Thorough and complete	Best professional judgment from extensive literature and laboratory studies	Already in place	Already in place	Further validation as more data becomes available

5 Review the current status of background documents for biological effects techniques TORb

5.1 Review of background documents

The Background Document on Biological Effects Techniques for Monitoring was provided by the OSPAR secretariat for the WKIMON IV meeting. This consists of an updated compilation of the background documents produced for WKIMON III in 2007 (OSPAR 2007).

The meeting reviewed the status of each document concurrently with the review on progress with the development of assessment criteria, Agenda item 3, above. A summary of the current position is given in Table 4.2 above and in addition the following comments were made:

It was suggested to produce a new Background Document on intersex in fish (task: Stephen Feist, Cefas Weymouth, UK).

For completeness, the compilation of Background Documents should include TBT-induced imposex in whelks. This could be achieved with 1–2 pages of text with good cross referencing to existing OPSAR guidelines and assessment criteria. Matt Gubbins, FRS, UK agreed to undertake this task.

5.2 Comments on vtg background document and assessment criteria

The background document is thorough and complete. Some slight updating of references to take account of the most recent publications would be possible, but is not urgent. A Technical Annex for oestrogenic effects is being prepared.

The BCs proposed in the Background Document of 0.13 ug/ml for male flounder and 0.22 ug/ml for cod are based on 90th percentiles of data from reference areas in the UK or the North Sea. It would be better to express this as median values of the data sets. Discussion at WKIMON III revealed that the detection limit for determination of vtg in these studies was approximately 0.2 ug/ml. The medians may therefore be less than the detection limit, in which case the BC should effectively be zero, rather than the detection limit as proposed by WKIMON III. The text on assessment criteria in the Background document should be amended.

For comparison with field data, it will be necessary to develop BAC-equivalents for monitoring species. Data are available on within-population variability, and some limited data are available on inter-annual variability that could be used for this purpose. The Background document gives no guidance on possible EAC-equivalents for vtg.

Ian Davies to collate data sets from CEFAS (mainly, possibly some useful data elsewhere) and arrange derivation of BACs by Rob Fryer.

5.3 Comments on reproduction in eel pout

The background document is thorough and complete and based upon well documented procedures in the TIMES guidelines. The assessment criteria are founded on good field data.

5.4 Comments on EROD

The document is very brief and mentions some general principles and relevant confounding environmental factors. The baseline criteria are only based on Norwegian data. The selection criteria for deriving the final proposed background response are not clear. The work done at WKIMON III (Annex 14) is not mentioned in this document. There a statistical approach is explained and background values were calculated on the basis of the large dataset that was collated at the meeting. The authors define ranges in their text for the different species and subsequently suggest a number that is approximately the upper limit for Atlantic cod, the lower limit for flounder and a value outside and below the range suggested for dab. In fact, the numbers are the same or approximately those of Annex 14 mentioned above. Checking this against the available data would suggest that the data would nearly all be above background for flounder and dab, and to a larger extent below background for Atlantic cod.

5.5 Comments on background document and assessment criteria for PAH bile metabolites

The text in the Background Document is short and needs to be clearer with respect to the methods that should be used for analysis, whether/how data should be normalised and reported and should refer to the TIMES recommended method document. The document should be updated with the developments made at WKIMON III. Reference is made in the background document to QA and the availability of reference materials. These are actually not available and external QA needs development.

Background/reference levels are reported as ranges for cod, flounder, plaice and dab covering the key fish species needed for OSPAR. Value ranges are given as normalised HPLC data only and appear to be summed across different metabolite species. Non-normalised values are also required and there needs to be more clarity on how these ranges were derived from the seven pages of data from experiments, reference and potentially polluted sites given in the annexed tables. Background ranges are needed for 1-OH pyrene equivalents measured by direct fluorescence methods. The outcome of discussions at WKIMON III needs to be included in the background document.

It is clear that background assessment criteria could be developed on a species basis using existing data. The development work needs to be done to allow assessments. Raw data on variability rather than just ranges of values will be needed to achieve this.

It will be very difficult to develop assessment criteria thresholds that could be considered as significant effects or related to higher order effects. The assessment should be made in relation to significantly above background and indicating exposure.

It should be noted that the above points need to be taken into account and the work of WKIMON III accounted for. Norway and the Netherlands have already undertaken to develop assessment criteria in time for SIME 2008. Norway will be asked to undertake this task and report back to WKIMON in the autumn of 2008.

5.6 Comments on DNA adducts

The background documents for DNA adducts is thorough and complete referring to standardised protocols available via ICES TIMES papers. However the actual data available for determining 'Background Response Ranges' is limited with only fish data (dab, flounder and saithe) available from UK and Norwegian partners. Extensive effort should now focus on reviewing data available in peer reviewed studies to expand the data sets available for evaluation, along with increasing the range of species (e.g. mussels) investigated. Only when this data becomes available will it be possible to assess and refine background values and develop assessment criteria for 'elevated responses' and 'high cause for concern response' ranges.

5.7 Comments on bioassays

The background document is thorough and complete and based upon well documented procedures in the ICES TIMES guidelines, for both water and whole sediment procedures. The "background response range" is based on the 95 percentile of control values. The assessment criteria for "elevated response range and high cause for concern response range" are based on best professional judgement from large laboratory and field data sets available from Spain and the UK. As more data becomes available there is scope to reassess and refine these values.

5.8 Comments on lysosomal stability

Lysosomes play a key role in uptake, accumulation and sequestration of various classes of contaminants (metals, PAHs, Organochlorines PCBs eg), based on the observations that

- Lysosomes are targets of multiple classes of contaminant
- Are proven sites of accumulation of contaminants
- Tissue concentration correlate with impaired lysosomal stability
- In numerous field studies exposure to contaminant effluents such as oilspills, mixed wastes from industry or sewage sludge, lysosomal stability from fish as well as from mussel (liver, hepatopancreas, blood) was a sensitive indicator for contaminant induced damage.
- Lysosomal stability is a rapid tool to interpret bell-shaped responses of biomarkers of exposure
- Higher level effects indicated by impaired lysosomal function such as liver histopathology, scope of growth and reproductive success have been demonstrated

These aspects are all well described in the background document.

The assessment criteria given for lysosomal stability comprising "Background Response Range, Elevated Response Range and High Cause for Concern Response Range" are based on extensive parallel studies of measuring lysosomal stability by two methods, the cytochemical approach and the NRT test in relationship to higher level responses, namely histopathology of the respective tissues of identical individuals of fish and mussel. Parallel analyses of the chemicals accumulated in the same tissues have confirmed the ranking of the assessment criteria as given in the Background document. The Background Document may therefore be considered as thorough and complete.

5.9 Comments of Fish Disease Index

Fish disease is a key endpoint in marine environmental monitoring. Their quality assured observation has a long tradition and has generated long time series of disease prevalence for various geographical areas in the North and the Baltic Sea. The FDI summarizes several diseases into one number, thus simplifying the originally multidimensional picture provided by the data. Expert's judgement on relative disease severity is used in this summary. It also removes seasonal cycle effects and sampling fluctuation in the size/ sex composition of the samples.

As fish diseases have been observed also before the advent of anthropogenic contamination, there is no natural zero background prevalence. Instead, background levels seem to depend on local conditions. The criteria for the FDI-based assessment of the environmental status and trend of a region take up the fact of a local background and exploit range and variation of the locally observed prevalence time series. The resulting assessment makes a joint statement on level and trend of disease prevalence in an area and can easily be represented by smiley symbols. This approach is universal, given the availability of a local prevalence time series.

For the temporal assessment of a newly monitored area it is necessary to either adopt extrapolated time series from adjacent areas or to generate a time series for the new area, where the latter would allow a first assessment only after a relatively long observation period.

A regional comparison can be done in two ways: first, the smiley symbols for trend plus level can directly be compared; second, the FDI values themselves can directly be compared. While the first way is unproblematic (smileys of identical colour indicate the same degree of (non-) concern), the second way needs a careful interpretation. Observed differences in FDI means from different areas may be due to generally different living conditions, but could alternatively be a consequence of anthropogenic impact. This way of using FDI values needs further investigation and very likely a link to potentially explaining factors. Another direction of further development is the application to further sets of diseases and further species.

5.10 Comments on Scope For Growth

The background document is thorough and complete and based upon well documented procedures in the ICES TIMES guidelines. The "background response range, elevated response range and high cause for concern response range" are based on best professional judgement. There is considerable laboratory and field data on which the assessment criteria are founded; this data is available from studies in Spain and the UK. As more data becomes available there is scope to refine these values.

ACTION

Several tasks have been identified for action under this agenda item. New background documents are required for intersex in fish and TBT-induced impose in whelks. Some of the background documents are incomplete and require modification as indicated. It is recommended that these tasks be taken forward by WKIMON or its equivalent, however this develops.

Reference

OSPAR, 2007. Background Document on Biological Effects Techniques for Monitoring. Assessment and Monitoring Series, Oslo Paris Commission, ISBN 978–1–905859–72–-6. Publication Number: 333/2007, p 1–122.

6 Undertake further development of a generic assessment framework and method for the integrated biological effect-chemical approach to monitoring

6.1 Review of current methods

The different current methods for integrated assessments that the group were aware of were discussed at the WKIMON meeting. Especially the FullMonti, REGNS and US EPA approaches got attention (see comments below).

6.1.1 Comments on FullMonti approach

Concerning the FullMonti approach (presented by UK), it finally derives a single score system for contaminants, biological effects and biology, which combined them into an overall assessment of the status/health assessment. The score of effect level is weighted from top and down, with the most significant effects (i.e. reproduction, growth, behaviour, survival) weighted with a value of 10. Subcellular biomarkers are weighted with lower values, for instance EROD gets a value of 3, and VTG gets a value of 4. In addition, the response level is scored according to suggested intervals in the assessment criteria (green, amber, red getting values of 1, 5 and 10). An overall score for biological effect is derived by mean value of ale total score multiplied with weighted values.

For contaminants, the scores for each pollutant are integrated into single scores for sediment, shellfish and fish tissue, which again can be integrated into one overall score for contaminants.

In the end a final integrated score can be extracted based on the overall scores for contaminants, biology and biological effects.

WKIMON raised some concerns about the FullMonti approach:

- The level of protection (i.e. the setting the values between green, amber and red) is of concern. It should be evaluated to what extent it is only highly significant effects levels (reproduction and survival/bioassays) that really matter to the final assessment score, although subcellular responses also can indicate such significant effects. Because of low weightings, subcellular responses may not contribute greatly to the overall score.
- The FullMonti approach is using a mean value of the overall score, which may hide important single factors. An alternative approach to mean value could for instance be a "one out, all out" system, mperhaps modified according to the biological significance of the endpoints.

6.1.2 Comments on REGNS

The 2005 report from the ICES REGNS group reported that ecosystem status reports and integrated ecosystem assessments have been produced for the Eastern Scotian Shelf (DFO, 2003; Choi *et al.*, 2005). An essential, but difficult, part of such exercises is to extract some understanding of the functioning, changes and "health" of the eco-system without becoming swamped by information from the large number of variables (indicators) which are used to measure many aspects of the physical, chemical and biological state of the system. One of the methodologies, used by Canadian colleagues, involves rescaling and reducing dimensionality. Rescaling and reducing dimensionality can provide an effective way of presenting and simplifying multiple data sets and as such contribute to an integrated ecosystem assessment. The changes over time can then be presented on a common scale, which is useful for giving an overview and as a preliminary step in identifying coherent patterns of change. With their assistance, an analysis of North Sea data was carried out as part of the preliminary "proof of concept" approach adopted by the REGNS workshop.

Choi *et al*. (*op. cit.*) comment on the importance of data selection and give criteria for selection of data. For this preliminary analysis of North Sea data, the principal criterion has been data availability at appropriate spatial scales and duration. Values were averaged over three areas corresponding to ICES regions IVa, b and c. The data, which include biotic, abiotic and human variables, are listed in Table 6 of the report. They come from three sources: the Continuous Plankton Recorder (CPR) survey (SAHFOS), the ICES oceanography database and the ICES fisheries data-bases.

The variables (indicators) were compared with each other by presenting them in the order of the first eigenvector obtained from a multivariate ordination, so that the indicator sequence reflected the degree of similarity in their temporal dynamics. The way that suites of the variables changed over time could therefore be visualised.

The resultant analysis served to introduce the methodology, showed how the results can be presented and provided a rationale for assembling data sets. Even such a preliminary analysis identified some of the major changes in the physical and biological state of the North Sea that had occurred over the past three decades. In particular, it brings out a fairly abrupt change that took place in 1987–1988 and has been described as a "regime shift" (Reid *et al*., 2001; Beaugrand, 2004).

In review of the REGNS work, WKIMON noted that the core objective of the REGNS process, and of the underlying Canadian regional assessment work, was to detect and describe large scale historical changes (regime shifts) in the biological and physico-chemical character of ecosystems such as the North Sea. As part of the process, other ICES WGs had provided large data sets to the REGNS process, for example WGMS had provided information on sediment chemistry covering time series of up to perhaps 15–20 years at some stations. However, the REGNS analytical method had been unable to make use of these data, which were sparse in comparison to hydrographic data, fisheries data, CPR data etc. WKIMON therefore concluded that the diffuse data and generally short time series that were available for biological effects data would make the effects data unsuitable for analysis by a REGNS-type method.

6.1.3 Comments on US EPA approach

Ian Davies reported to the meeting on discussions that had been held at OSPAR MON in December 2007 on possible approaches to data assessment and reduction, particularly in the context of QSR 2010.

In particular, he mentioned the approach used in the US EPA assessment of the quality of estuarine waters (the National Estuary Program Coastal Condition.

Report, June 2007 available at http://www.epa.gov/owow/oceans/nepccr/index.html). This programme reports on multifactorial assessments of estuarine quality, covering water, sediment, and aspects of biota. The approached used generates a "traffic light" style assessment of each parameter in each estuary, based on assessment criteria, and then combines the assessments across determinands, estuaries and regions to produce national scale assessments. An important aspect of the presentation is that the underlying data are also available at different degrees of disaggregation to allow exploration of the reasons underlying particular assessments.

WKIMON noted that the questions underlying the JAMP are:

- What are the concentrations in the marine environment, and the effects, of the substances on the OSPAR List of Chemicals for Priority Action ("priority chemicals")? Are they at, or approaching, background levels for naturally occurring substances and close to zero for man made substances?
- Are there any problems emerging related to the presence of hazardous substances in the marine environment? In particular, are any unintended/unacceptable biological responses, or unintended/unacceptable levels of such responses, being caused by exposure to hazardous substances?

WKIMON considered that the first question was concerned with the relationship between observations and background (assessment) concentrations, and background responses in biological effects measurements. Background Concentrations (BCs) and Background Assessment Concentrations (BACs) are either available or being developed for priority contaminants in biota and sediment, and WKIMON3 proposed a range of background response levels for biological effects.

The second question was concerned primarily with unintended/unacceptable biological effects of contaminants. In the case of chemical measurements, this could be considered equivalent to the exceedance of an EAC. A similar form of assessment criterion could be envisaged for biological effects that could of themselves be unacceptable at high intensities, or which could be indicators of expected effects at higher levels of organisation (e.g. organ or whole organism).

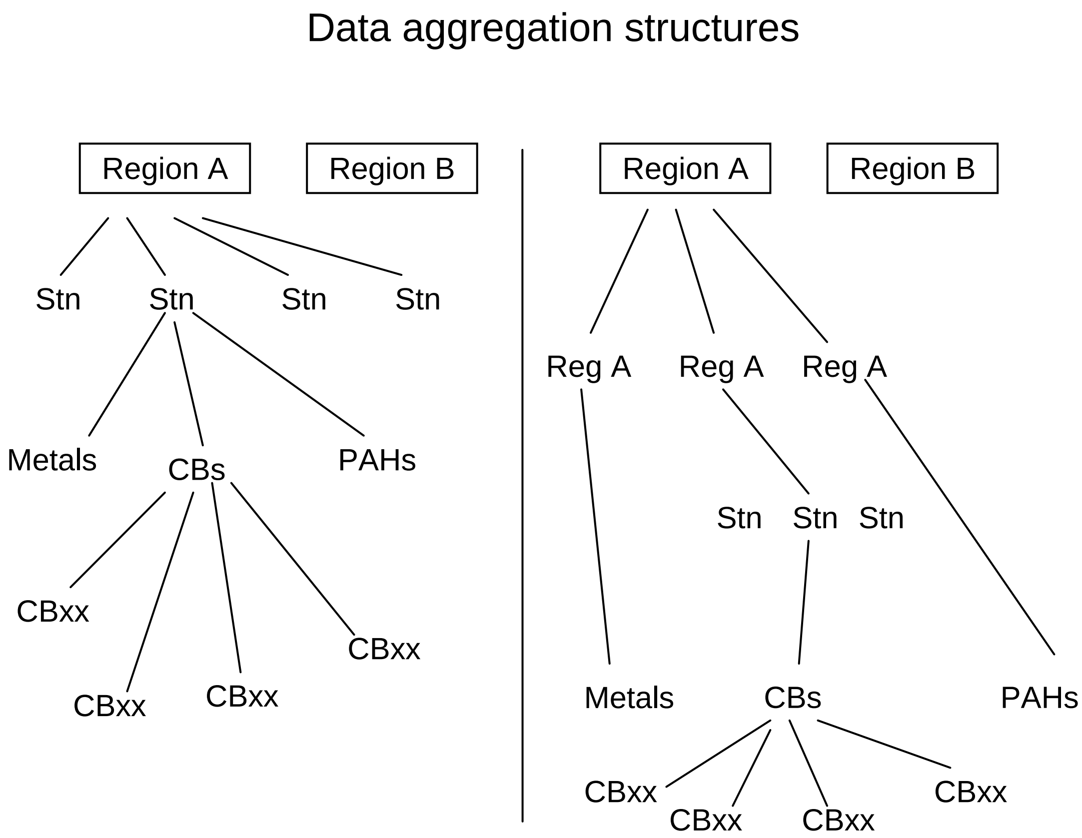
In developing their approach to integrated assessment of monitoring data for groups of contaminants (e.g. metals, CBs, or PAHs), MON considered that data for a group of contaminants, for example CBs, at a single station could be summarised as:

STATION XYZ	AT OR BELOW BAC	UNCERTAIN	ABOVE BAC
CBaaa	Yes		
CBbbb		Yes	
CBccc	Yes		
CBddd	Yes		
CBeee		Yes	
CBfff			Yes
Totals	3	2	1
Totals as %	50	33	17

MON then considered that a next step would be to create a set of rules that translated the bottom lines of these tables into a rather simplistic traffic light system which indicated whether the OSPAR objective was being met or not. MON noted that such tables would need to be able to take account of missing data, i.e. differing numbers of CBs (or PAHs). One approach, using percentages was suggested, as below:

	Description	If assessments for only 4 substances are available	If assessments for only 3 substances are available	If assessments for only 2 substances are available	If assessment for only 1 substance is available	Traffic light
>= 80% of concentrations at or below BAC	Objective fully achieved, or close to being achieved	3 or 4 concentrations at or below BAC	3 concentrations at or below BAC	N/A	N/A	Green
>50–80% of concentrations at or below BAC	Objective partially achieved	N/A	2 concentrations at or below BAC	2 concentrations at or below BAC	Concentration at or below BAC	Amber
<=50% of concentrations at or below BAC	Objective not generally being achieved	2 concentrations at or below BAC	Zero or 1 concentration at or below BAC	Zero or 1 concentrations at or below BAC	Concentration not at or below BAC	Red

The traffic light scheme on the right hand side could be used on a map of stations to show whether the OSPAR objective for concentrations of CBs (or metals, or PAHs) had been achieved. Assessments of the groups of compounds could be combined to produce assessments for contaminants at stations. MON developed possible structures for the combination of data as below:



MON went on to develop sets of rules for creating "traffic lights" for the concentrations of individual contaminants at a station (in relation to the BACs), for integrating to higher levels (e.g. for a group of contaminants within a station, and for several groups of contaminants within a station), and also for the combination of information on level with temporal trends. MON prepare maps summarising data at individual stations for single contaminants, groups of contaminants, and all groups of priority contaminants, and examples are given below:

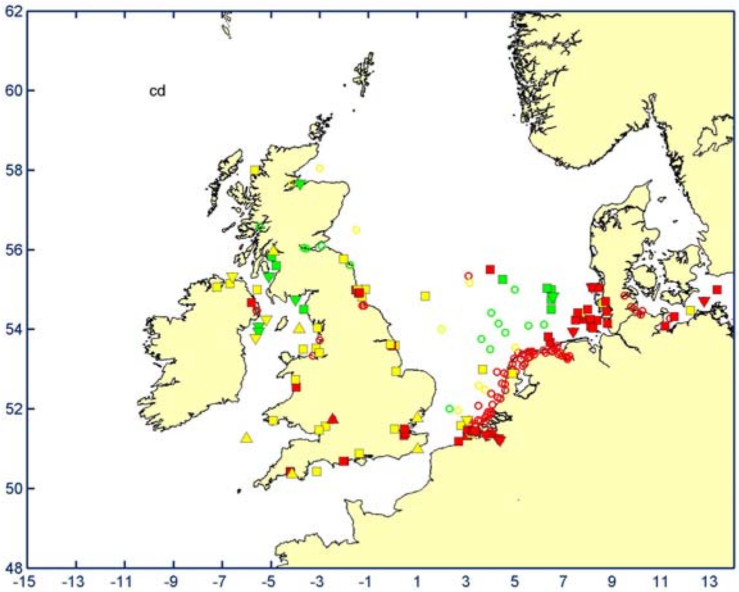


Figure 1 Example display of results for a single contaminant (cadmium) for all sediment stations.

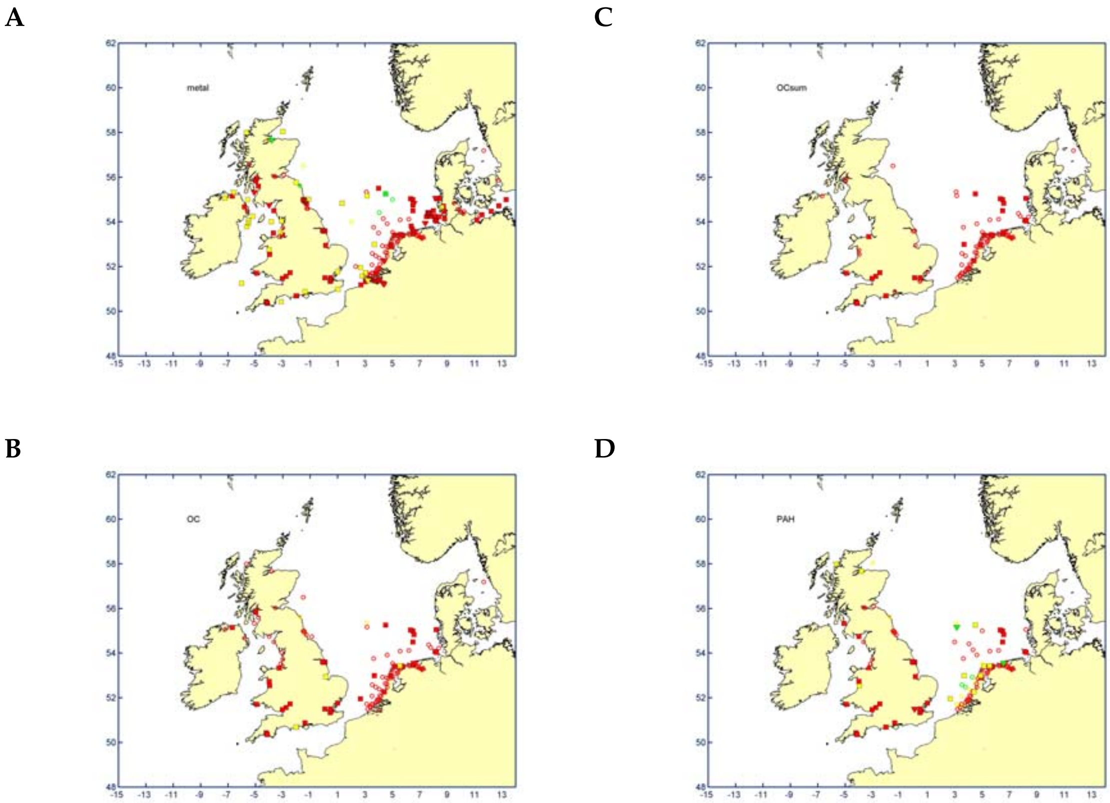


Figure 2 Example display of results for a group of contaminants; metals (A), PCBs (B), SumPCBs (C) and PAHs (D) for all sediment stations.

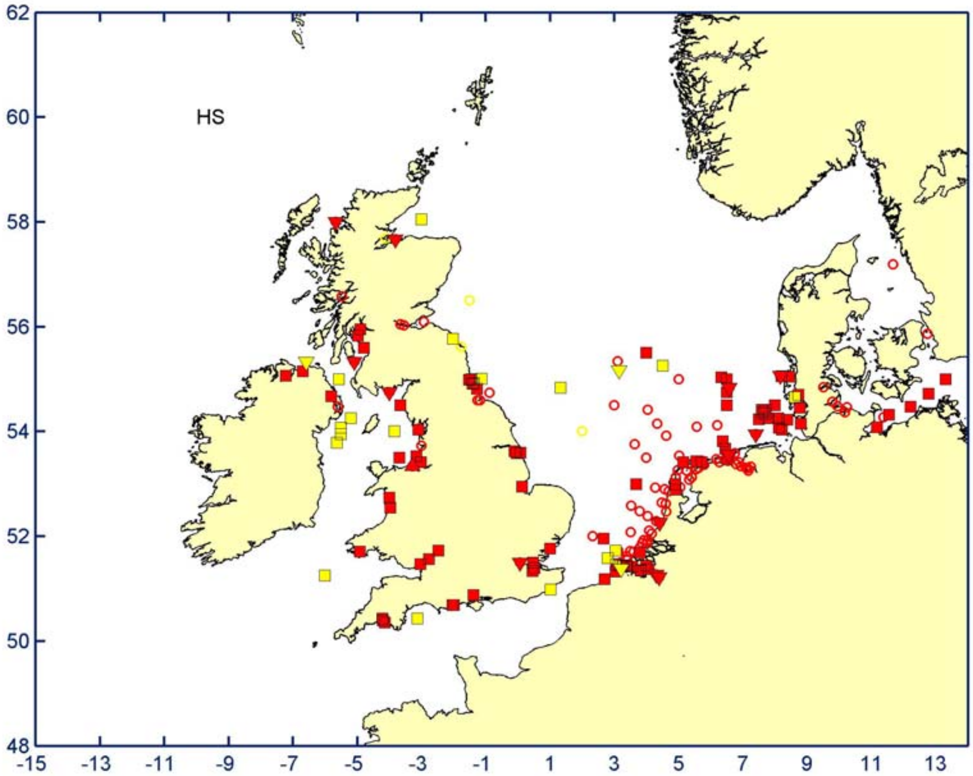


Figure 3 Example display of results for all groups of contaminants (metals, PCBs and PAHs) for all sediment stations.

WKIMON considered that a similar approach could potentially be used for the assessment of biological effects data, as required in the first of the JAMP questions. The approach could make use of comparisons with the draft background response levels developed at WKIMON III. They noted that this was probably an interim position until such time as BAC-equivalents can be developed for biological effects measurements.

WKIMON also considered that different biological effects were of different significance for the health of the organism. For example, indicators of exposure (e.g. EROD activity) were of lesser significance than effects at organ (e.g. liver neoplasms) or at whole organism level (e.g. effects on reproduction), and that therefore some form of weighting scheme could be advantageous to an assessment.

6.1.4 Integration of effects data with chemical data

The hierarchical data assessment and presentation system developed by OSPAR MON offers several different levels at which integration with effects data could occur. For example, imposex data and TBT concentrations in gastropods can be linked at the station level, as the effect and chemical are uniquely linked. In most others cases, effects measurements are rather less specific. For example, metallothionein reflects exposure to several metals, while EROD reflects exposure to a range of groups of contaminants that interact with the Ah receptor. The specificity of the links between effects and chemicals is generally increasingly weak at organ and whole organism levels, where effects measurements tend to reflect a wider range of chemical stresses. Generally, therefore, a biological effects monitoring programme that includes a range of biomarkers and indicators of effect at organ or organism level will reflect biological response to a range of groups of contaminants. WKIMON therefore concluded that assessments of effects should be integrated and combined with integrated chemical assessments at the station level.

6.2 Development of an integrated assessment approach for biological endpoints

Following on from the above discussion it was decided to suggest a new approach for integrating different endpoints of biological effects in fish (Table 6.1), mussels (Table 6.2) and gastropods (Table 6.3) into a Generic Assessment Framework, which can be used as "Traffic light" for environmental assessments.

First of all, assessment criteria should be derived for each endpoint of biological effects and include three levels; a "Background Response Range", a "Elevated Response Range" and a "High Effect Range" (see also Agenda item 3).

Next, the level of biological significance in the different recommended endpoints are taken into account, and the endpoints were grouped into "Exposure Indicators", "Significant Effect Indicators" or "Higher Effect Level Indicators", and rules were defined for how to integrate them into a Generic Assessment Framework.

Table 6.1 Rules for Generic assessment framework based on grouping of the various endpoints to biological significance in fish. Minimum 4 endpoints are required and at least 1 from each level of biological significance. Default to the worst case.

Endpoints for Fish	Biological significance	Green	Amber	Red
PAH metabolites EROD/CYP1A Metallothionin ALA-D AChE	Exposure Indicators	If 3 or more are within the background response range.	If 2 or more are in the elevated response range	If 1 or more are in the high response range
DNA adducts Comet assay Lysosomal Stability Vitellogenin	Significant Effects Indicators	If 2 or more are within the background response range.	If 2 or more are in the elevated response range	If 1 or more are in the high response range
Liver pathology neoplasia/hyperplasia Liver nodules External diseases Intersex Reproductive success	Higher Level Effects Indicators	If 1 or more are within the background response range.	If 1 or more are in the elevated response range	If 1 or more are in the high response range
CI, LSI, GSI	Non specific	Supporting parameters	Supporting parameters	Supporting parameters
Blue is at CEMP list				

Table 6.2 Rules for Generic assessment framework based on grouping of the various endpoints to biological significance in mussels. Minimum 4 endpoints are required and at least 1 from each level of biological significance. Default to the worst case.

Endpoints for mussel	Biological significance	Green	Amber	Red
Metallothionein	Exposure Indicators	If 3 or more are within the background response range.	If 2 or more are in the elevated response range	If 1 or more are in the high response range
AChE				
MXR				
GST				
Lysosomal Stability	Significant Effects indicators			
Comet assay				
Scope for Growth	Higher Level Effects Indicators	If 1 or more are within the background response range	If 1 or more are in the elevated response range	If 1 or more are in the high response range
Histopatology				
Gametogensis				
Growth	Non specific	Supporting parameters	Supporting parameters	Supporting parameters
Condition Index				

Table 6.3 TBT specific effects in gastropods. Based OSPAR assessment criteria for imposex and intersex in marine gastropods (6 classes).

Endpoints for Gastropods	Biological significance	Green	Amber	Red
Imposex/intersex	Higher Level Effects Indicator	Class A and B*	Class C*	Class D-F*

Table 6.4 Rules for Generic assessment framework for bioassays.

Endpoints for Bioassays	Biological significance	Green	Amber	Red
Sediment Corophium	Higher Level Effects Indicators	If 2 or more are within the background response range.	If 1 or more are in the elevated response range.	If 1 or more are in the high response range.
Sediment Arenicola				
Water bivalve embryo				
Water cocepod				
Water echinoderm				

It was agreed that the rule of thumb for the Generic Assessment Framework should be "Default to the worst case" when integrating the different level of biological significance, although a minimum number of endpoints will be required (Table 6.5).

6.2.1 What is the minimum required endpoints?

A minimum number of 4 for fish, 4 for mussels or 1 for gastropods for a specific site is suggested to make the assessment system operational. However, it is recommended that lysosomal stability, in both fish and mussel, is one of them as it 1) integrates responses to various classes of pollutants, 2) helps to interpret bell-shaped responses of biomarkers of exposure and 3) is a fast tool which reflects progression of liver histopathology termed as Higher Level Effects Indicators.

Table 6.5 The minimum required endpoints for integrated assessment criteria of biological effects in fish, mussels or gastropods.

	REQUIRED NUMBER OF ENDPOINTS
Fish	Minimum 4: at least 1 from each level, i.e. Exposure, Significant Effect Indicator and Higher Level Effect Indicator.
Mussels	Minimum 4: at least 1 from each level, i.e. Exposure, Significant Effect Indicator and Higher Level Effect Indicator.
Gastropods	Minimum 1: Mandatory, related to specific TBT effects.

6.2.2 Integration with Generic framework of contaminants

The above approach suggested for biological effects has merit insofar that it aligns itself with the approach suggested at MON. Clearly, at this stage further refinement and development is required and in addition, the integration of the generic assessment framework with contaminants needs further discussion and development.

ACTION

A follow-on group to WKIMON should take this approach forward to develop and validate the process with real data sets and data generated through the OSPAR ICON demonstration project.

References

Beaugrand, G. 2004. The North Sea regime shift: evidence, causes, mechanisms and consequences. Progress in Oceanography, 60: 245–262.

Choi, J. S., Frank, K. T., Petrie, B. D., and Leggett, W. C. 2005. Integrated ecosystem assessment of a large marine ecosystem: a case study of the devolution of the Eastern Scotian Shelf, Canada. Oceanography and Marine Biology: an Annual Review, 43: 47–67.

DFO. 2003. State of the Eastern Scotian Shelf Ecosystem. Canadian Science Advisory Secretariat Ecosystem Status Report, 2003/004.

Reid, P. C., Edwards, M., Beaugrand, G., Skogen, M., and Stevens, D. 2003. Periodic changes in the zooplankton of the North Sea during the twentieth century linked to oceanic inflow. Fisheries Oceanography, 12: 260–269.

7 In support of the integrated chemical-biological effect approach, develop an initial draft of a technical annex on sampling design and supporting parameters TOR c

7.1 This agenda item was first addressed by considering the follow up within OSPAR to the meeting of WKIMON III, which highlighted the need for 2 new technical annexes covering survey design and sampling design and supporting parameters

7.1.1 OSPAR reaction to WKIMON III

SIME noted that there were still a number of issues that needed to be addressed before the draft guidelines could be presented to ASMO and that further work was necessary to develop technical annexes before the draft guideline could be fully adopted and was operational. SIME concluded that four technical annexes should be developed to complement the draft guidelines as follows:

a) a draft technical Annex 1 on the survey design to be prepared on the basis of work by ICES WGSAEM. The purpose is to provide guidance on the selection of representative stations, taking into account requirements under the Water Framework Directive and the proposed Marine Strategy Directive. This work should build on work by WGSAEM 2007 relating to the spatial design of monitoring programmes and should take into account the approach taken by the UK in re-designing their station network;

b) a draft technical Annex 3 on sampling and analysis which would mainly be based on a compilation of references to existing JAMP Guidelines and could be prepared by the Secretariat. The need for this annex should be reviewed by SIME 2008 in the light of the overall compilation of CEMP related monitoring guidance in a CEMP Monitoring Manual and any gaps in the current suite of JAMP guidelines for example on sampling and analysis for supporting determinands such as salinity or temperature;

The following points were noted:

(i) Survey design had been discussed briefly by WGSEAM 2007, but it had concluded that it was not possible to take this item forward during the meeting. Consequently there was no work on which WKIMON IV could build.

(ii) Input from WGSAEM is required to develop a robust Technical Annex for survey design and clearly further work will be required to achieve this.

(iii) The group considered several products that might provide useful building blocks for the survey and sampling design annexes. These were further developed by the group below.

(iv) The existing JAMP Guidelines provide appropriate information for undertaking either chemical or biological effects sampling independently, but some revisions are required for integrated sampling. A Technical annex consisting purely of a compilation of references to existing JAMP Guidelines will not provide information consistent with that required for integrated monitoring. A mechanism is required to revise the JAMP Guidelines to accommodate integrated monitoring.

7.2 Draft Technical Annex on survey design

7.2.1 UK approach to redesign

The UK approach to redesigning its station network was discussed. This approach moves away from site specific monitoring of hazardous substances to a more regional approach and uses random stratified sediment sampling to inform on status and trends supplemented by a minimum of one fish sampling site per region (contained within one stratum) to inform on status and to provide supporting information for biological effects monitoring.

Regions and strata have been defined covering the UK continental Shelf. Figure 7.1 shows an example of this, for the Region defined as Humber/Wash. The region consists several strata, which include WFD water bodies in the 0–1 nm limit, an intermediate stratum 1–12 nm and two open sea strata, NE open sea and S open sea.

Collecting all samples at the same time and place may be considered to be the 'ideal' survey/sampling strategy for integrated monitoring, however this is usually not achievable in practice due to the seasonal limitations of some parameters, mobility of fish, unsuitable sediment types, etc. and such 'snap-shot sampling often fails to control local temporal and spatial variation in contaminant concentrations.

A regional approach generates more useful management information and can improve the power of the programme to detect trends by controlling local spatial variation.

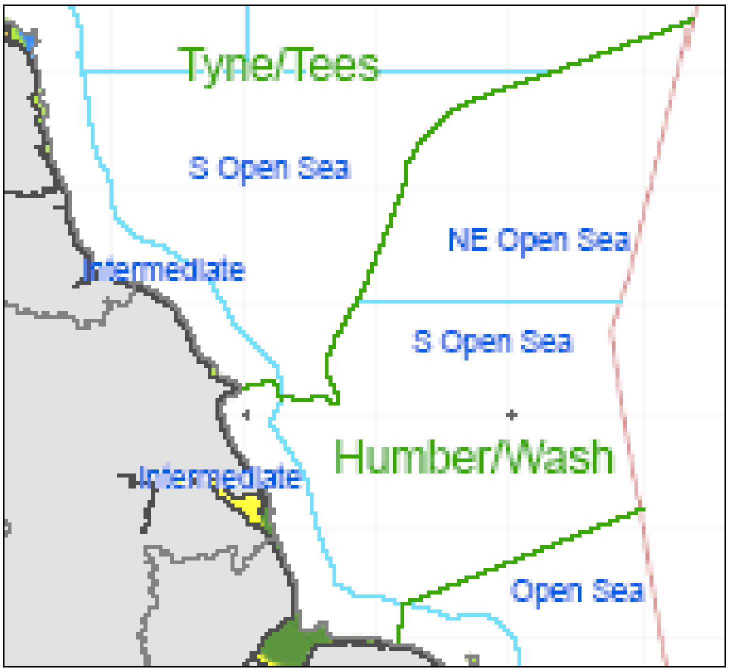


Figure 7.1 An example of the UK regional approach to redesigning the national monitoring station network.

7.2.2 Statistical considerations

7.2.2.1 Survey design: general (Werner Wosniok)

Survey design is driven by the objectives of the sampling, which are (WKIMON III report, p. 170).

- to assess status (existing level of marine contamination and its effect) and trends across the OSPAR maritime area;
- to assess the effectiveness of measures taken for the reduction of marine contamination;
- to assess harm (unintended/unacceptable biological responses) to living resources and marine life;
- to identify areas of serious concern/hotspots and elucidate their underlying causes;
- to identify unforeseen impacts and new areas of concern;
- to create the background to develop prediction of expected effects and the verification thereof (hindcasting); and
- to direct future monitoring programmes.

Of course, each choice of sampling points and sample sizes for a survey leads to some data about marine contamination and possible effects (as long as anything at all is measured). However, if the survey is expected to generate statements like:

- an assessment of an absolute level ("level at position A is below/above a critical value") or
- a spatial comparison ("level at position A is lower than / comparable to / higher than at position B) or
- a temporal comparison ("level at position A at time T1 was lower than / comparable to / higher than the level at this position at time T2)
- the level of a parameter has changed in part X of the OSPAR maritime area

with a defined precision, it is necessary to appropriately organize the survey with respect to sample sizes and sampling positions. The aim is to find a survey design, which is optimal in the sense that with a pre-specified effort the most precise map of the spatial parameter distribution is obtained or that a pre-specified precision is achieved with the smallest possible effort. To this end, various specifications are needed as input to the survey design, as given in Table 7.2.1 at the beginning of the next section. If the required specifications cannot be given, no *a priori* statement about the quality of the sampling can be made. In this case, a pragmatic way of designing the survey has to be followed as indicated in the last section. Then, however, an *a posteriori* determination of the power of the monitoring scheme should be performed to obtain a quantification of the monitoring quality. This should also be done if the optimal design were formally determined, but could not be followed in reality due to practical restrictions.

7.2.2.2 Survey design: the optimal version

Table 7.2.1 Specifications needed as input to the derivation of an optimal survey design

d, the change of biological interest	numerical specification of the change in parameter level that, if present, is to be detected with safety ß. Must be specified for each parameter.	no standard
ß, the power of test procedures	probability that an existing change at least as large as d is detected	90% or 95%
s_a, the analytical error of the biological/chemical analysis procedure	obtained from analytical experience, e. g. multiple measurements of the same sample	no standard
s_b, the biological variation	obtained from earlier investigation	no standard
D, the geographical area of interest		no standard
F, an initial guess of the spatial distribution of the parameter of interest	may be taken from pilot investigations or derived as educated guess.	if no other information, assume uniform spatial distribution.

An optimal survey design can only be developed in an iterative fashion. Prior to each campaign, an optimal design for that campaign is found by the procedure below. The results obtained from this campaign serve as input information for the optimization of the subsequent campaign.

Assuming that monitoring in a large area is intended, and that *a priori* information on the geographical distribution of the quantity under study is available, the following procedure can be used to derive an initial survey design (size and positions) for a monitoring according to the first part of the first bullet point.

Step 1: Define D, the geographical area of interest (for which the assessment shall be valid) (See 7.1 above).

Step 2: Determine the necessary number of replicates per sampling location (needs knowledge about the sampling variability (analytical + biological, e.g. s_a, s_b), precision requirement plus standard statistics).

Step 3: Take the existing information F about the parameter of interest in this area and generate a map of the parameter level over the area of interest (use a standard geostatistical technique). Subdivide the range of the parameter in "iso-concentration" ranges. Find the corresponding "iso-concentration" areas on the map. If an iso-concentration area is ring-shaped, subdivide the ring into at least 4 sections (e.g. according to compass directions). Ring sections and the non-ring iso-concentration areas define the "sampling cells" addressed below.

Step 4: Define samplings points that are of basic interest or required for formal reasons. These points will not be changed by the following steps.

Step 5: Define an initial number of sampling points (a guess), additional to those from Step 4.

Step 6: Allocate sampling points from Step 5 to initial positions, starting with the geographical means of the sampling cells from Step 3. Define a grid of further candidate positions.

Step 7: For all present sampling points (initially those from Steps 4 and 5), calculate the estimated parameter value from the map of Step 3.

Step 8: Compare the map predictions from Step 3 and Step 7, e.g. by computing the Integrated means square error to characterize the present survey design. Record the IMSE.

Step 9: If there still are unvisited candidate grid locations, change the geographic locations of the free sampling positions to the next grid position (one change per step) and continue with Step 7. Otherwise finish.

The optimal survey design will then be the design that produced the smallest IMSE, e.g. the predictions that best reproduce the initial information. If this IMSE is considered too large, the number of sampling positions has to be increased and Steps 4–9, possibly 3–9, are repeated until a satisfactory result is achieved.

7.2.2.3 Survey design: the Emergency Exit

The procedure above may, for various reasons, not be acceptable when designing a monitoring scheme. As an alternative, a simple rule is proposed below.

- Determine the necessary sampling size per sampling position according to precision requirements as above.
- Use at least three sampling positions. Select these such that they include an unimpacted, a heavily impacted and an intermediate situation.
- If more than three sample positions are used, their positions should again cover the whole range of parameter values, preferably along a gradient.

The rationale behind this proposal is that it is necessary to obtain information about the best and the worst situations. The extremes are more likely to exhibit changes in future monitoring campaigns than sampling positions with a mean level.

No attempt should be made to generalize the findings from as few as three sampling positions to a large map. The quality achieved by the chosen design should be investigated by an *a posteriori* power analysis.

7.3 Draft Technical Annex on sampling and analysis

7.3.1 Overview of selected methods for integrated monitoring

The following tables were considered to be useful as a basis for a technical annex on sampling design and supporting parameters. These were extracted from Annex 17 'Draft ICES/OSPAR Guidelines for Integrated Chemical and Biological Effects Monitoring in coastal and offshore areas' of the 2005 WKIMON Report (ICES CM 2005/ACME:01). Some revisions were made to ensure the information provided correctly covered requirements for integrating chemical and biological effects sampling. Some of the same information is contained in existing JAMP Guidelines, but is not consistent with these Tables and a mechanism is required to revise the JAMP Guidelines to accommodate integrated sampling.

Tables 7.2–7.4 cover methods to be used for integrated fish, bivalve and gastropod monitoring, Tables 6.5 and 6.6 cover methods for monitoring of water and sediments.

A new scheme for organotin-related integrated monitoring using marine gastropods is added Figure 7.2.

Table 7.3.1 Overview of selected methods for integrated fish monitoring (2005 WKIMON Report, revised).

SUBJECT	PARAMETER	COMMENT
Species	Primary species: dab, flounder, whiting Alternative species: plaice, cod, herring, eelpout, hake, dragonet or other	Alternative species may be used if primary species are not available.
Sex	females and/or males	For certain biomarkers or chemical measurements, only females or only males are used (see relevant JAMP guidelines)
Size ranges	Dab: ≥ 15 cm (according to suggested new JAMP guidelines for externally visisble diseases).	For integrated monitoring encompassing chemistry, histopathology and biomarkers, the mid size group 20–24 cm is preferable,
	Flounder: ≥ 20 cm (according to suggested new JAMP guidelines for externally visisble diseases).	For integrated monitoring encompassing chemistry, histopathology and biomarkers, the mid size group 20–29 cm is preferable.
	Whiting: ≥ 15 cm (according to suggested new JAMP guidelines for externally visible diseases).	For integrated monitoring encompassing chemistry, histopathology and biomarkers, the mid size group 20–24 cm is preferable.
	Dragonet: ≥ 10 cm (according to suggested new JAMP guidelines for liver histopathology)	For integrated monitoring encompassing chemistry, histopathology and biomarkers, the size group 10–15 cm is preferable.
Sample size	Depending on the parameter measured, according to JAMP Guidelines.	Sample sizes have to fulfil statistical requirements for spatial and/or temporal trend monitoring. Preferably, all measurements should be done in individual fish and pooling should be avoided (with the possible exception of contaminant measurements).
Sampling time and frequency	Sampling for all parameters should be carried out at the same time, outside the spawning season, and at least once a year in the same time window	Justification is provided in the OSPAR JAMP Guidelines

SUBJECT	PARAMETER	COMMENT
Sampling location	Sampling for all parameters should be carried out at the same site	The location, size and number of sampling sites depend on the purpose of the monitoring. For offshore sampling targeted at fish, it is recommended to use ICES statistical rectangles as sampling sites. A number of repeated samplings (= hauls) (replicates) should be carried out in each of these rectangles. For coastal and estuarine waters, sites should be selected based on existing WFD chemical/biological monitoring sites. A minimum requirement would be to use three sampling sites, reflecting a range of impacted to unimpacted sites.
Chemical determinands	Metals: Hg, Cd, Pb, Cu, Zn CBs: ICES 7 CBs + CB77, CB81, CB126, CB169 + CB105, CB114, CB123, CB156, CB157, CB167, CB189. Brominated flame retardants: congeners of the penta-mix, octa-mix and deca-mix PBDE formulations; hexabromocyclododecane, tetrabromobisphenol-A. Lindane. TBT	In addition, in situ PAH measurements (eg., using UV-fluorescence spectrometry) may be employed under specific circumstances (e.g. after oil spill or PAH-related point source discharges). Besides the contaminants already covered by the OSPAR CEMP, there are a number of other compounds from the OSPAR List of Chemicals for priority action that should be monitored because of their toxicity and environmental relevance. The list provided is, therefore, not complete.
Biological effects measurements	Biological effect techniques as specified in the OSPAR JAMP Guidelines for contaminant-specific and general biological effects monitoring. Additional techniques: e.g. Comet assay, AChE, DR-CALUX, VTG	Some of the CEMP/JAMP biological effects techniques (and the associated Guidelines/Technical Annexes) need to be revised, e.g. in the light of their contaminant-specificity. Further biological effects techniques with a known responsiveness to relevant contaminants should be considered for incorporation (see list in WGBEC 2004).
Supporting parameters	Length, weight, age, somatic indices, stage of gonadal maturation, grossly visible anomalies, lesions, parasites, lipid, population structure, hydrography (temperature, salinity, oxygen content)	In the list, parameters are provided that are known to affect both the biological effects responses and the concentration of contaminants. The data can be of use for normalisation.

Table 7.3.2 Overview of selected methods for integrated bivalve monitoring (2005 WKIMON Report, revised).

SUBJECT	PARAMETER	COMMENT
Species	Primary species: *Mytilus edulis* Alternate species: *Mytilus galloprovincialis, Crassostrea gigas, Ostrea edulis*	Across the OSPAR area, the 1st choice shellfish is not available in all areas. In such cases, other species should be selected, such as oysters. For *Mytilus* sp., speciation studies are recommended in order to confirm species identity.
Sex	females and/or males	For certain biomarkers or chemical measurements, only females or only males are used (see relevant JAMP guidelines)
Size range	Mussel: 3–6 cm Pacific oyster: 9–14 cm	Based on JAMP Guidelines for chemical monitoring
Sample size	Depending on the parameter measured, according to JAMP Guidelines.	Sample sizes have to fulfil statistical requirements for spatial and/or temporal trend monitoring. For some parameters, sample size has still to be defined. Preferably, all measurements should be done in individual mussels and pooling should be avoided
Sampling time and frequency	Sampling for all parameters should be carried out at the same time, outside the spawning season, and at least once a year in the same time window	Justification is provided in the OSPAR JAMP Guidelines

SUBJECT	PARAMETER	COMMENT
Sampling location	Sampling for all parameters should be carried out at the same site.	The location, size and number of sampling sites depend on the purpose of the monitoring. For coastal and estuarine waters, sites should be selected based on existing WFD sites. A minimum requirement would be to use three sampling sites, reflecting a range of impacted to unimpacted sites. For offshore studies, caging of mussels should be considered.
Chemical determinands	Metals: Hg, Cd, Pb, Cu PAHs: EPA 16 + NPD CBs: ICES 7 + CB 77,81,126,169 + CB 105,114,123,156,157, 167,189 Brominated flame retardants: congeners of the penta-mix, octa-mix and deca-mix PBDE formulations; hexabromocyclododecane, tetrabromobisphenol-A. Lindane Organotin compounds	In addition, total hydrocarbon measurements (eg., using UV-fluorescence spectrometry) may be employed under specific circumstances (e.g. after oil spill or PAH-related point source discharges). Besides the contaminants already covered by the OSPAR CEMP, there are a number of other compounds from the OSPAR List of Chemicals for priority action that should be monitored because of their toxicity and environmental relevance. The list provided is not complete.
Biological effects measurements	Biological effects techniques as specified in the OSPAR JAMP Guidelines for contaminant- specific biological effect monitoring. Additional techniques: AChE, metallothionein, scope for growth, lysosomal stability, histopathology	Some of the CEMP/JAMP biological effects techniques (and the associated Guidelines/ Technical Annexes) need to be revised. Further biological effects techniques with a known responsiveness to relevant contaminants should be considered for incorporation (see WGBEC list of recommended techniques). For some of the contaminants of concern, there is still lack of appropriate biological effects techniques.
Supporting parameters	Shell length, shell and soft body weight, stage of gonadal maturation, grossly visible anomalies, lesions, parasites, lipid, population structure, sampling depth, hydrography (temperature, salinity, oxygen content, turbidity), nutrients/eutrophication	In the list, parameters are provided that are known to affect both the biological effects responses and the concentration of contaminants. The data can be of use for normalisation.

Table 7.3.3 Overview of methods and species for integrated gastropod/organotin monitoring (2005 WKIMON Report, revised).

SUBJECT	PARAMETER	COMMENT
Species	Intertidal species: *Nucella lapillus Nassarius reticulata* *Littorina littorea* Offshore species: *Buccinum undatum* *Neptunea antiqua*	
Sex	Females and/or males	
Size range	Size ranges are to be selected in accordance with the JAMP Guidelines	
Sample size	Depending on the parameter measured, according to JAMP Guidelines.	All measurements should be done in individual gastropods and pooling should be avoided.
Sampling time and frequency	Sampling for all parameters should be carried out at the same time, outside the spawning season, and at least once a year in the same time window	Justification is provided in the OSPAR JAMP Guidelines.
Sampling Location	Sampling for all parameters should be carried out at the same site.	The location, size and number of sampling sites depend on the purpose of the monitoring. For coastal and estuarine waters, sites should be selected based on existing WFD sites. A minimum requirement would be to use three sampling sites, reflecting a range of impacted to unimpacted sites. For point sources and shipping lanes, see relevant JAMP guidelines.
Chemical Determinands	Organotin compounds	Guidelines for chemical measurements in biota are in preparation through ICES.
Biological effects measurements	Imposex or intersex (species-dependent endpoint)	
Supporting parameters	Shell length, lipid, hydrography (temperature, salinity, oxygen content, turbidity), organotin compounds in sediment.	

Table 7.3.4 Environmental parameters for inclusion in monitoring programmes (water) (2005 WKIMON Report).

SUBJECT	PARAMETER	COMMENT
Chemistry	Salinity, nutrients, oxygen	
Chemical determinands	Metals: Hg, Cd, Pb, Cu, Zn PAHs: EPA 16 + Naphthalene, phenanthrene, dibenzothiophene and their alkylated derivatives CBs: ICES 7 CBs Brominated flame retardants: congeners of the penta-mix, octa-mix and deca-mix PBDE formulations; hexabromocyclododecane, tetrabromobisphenol-A. Lindane Organotin compounds	Consideration should be given to bioavailability. To answer the JAMP question relating to concentrations approaching background or zero, there may be a requirement to measure a broader range of chemicals.
Physical	Temperature, content of suspended matter	
Biology	Phyto- and zooplankton	Information might be useful in the case of specific events, such as blooms affecting fish health

Table 7.3.5 Environmental parameters for inclusion in monitoring programmes (sediment) (2005 WKIMON Report).

SUBJECT	PARAMETER	COMMENT
Chemistry	TOC, water content, Al, Li	Al and Li (or other elements as appropriate to the sediment type) are used for normalisation of contaminant concentrations.
Chemical determinands	Metals: Hg, Cd, Pb, Cu, Zn PAHs: EPA 16 + Naphthalene, phenanthrene, dibenzothiophene and their alkylated derivatives CBs: ICES 7 CBs+ CB77, CB81, CB126, CB169 + CB105, CB114, CB123, CB156, CB157, CB167, CB189. Brominated flame retardants: congeners of the penta-mix, octa-mix and deca-mix PBDE formulations; hexabromocyclododecane, tetrabromobisphenol-A. Lindane Organotin compounds	Consideration should be given to bioavailability. To answer the JAMP question relating to concentrations approaching background or zero, there may be a requirement to measure a broader range of chemicals.
Physical	Sediment type, particle size, colour index, information on anthropogenic disturbances, sedimentation rates, current flow rates	Anthropogenic disturbance such as trawling or sand and gravel extraction may affect the sediment structure.
Biology	Benthic fauna	

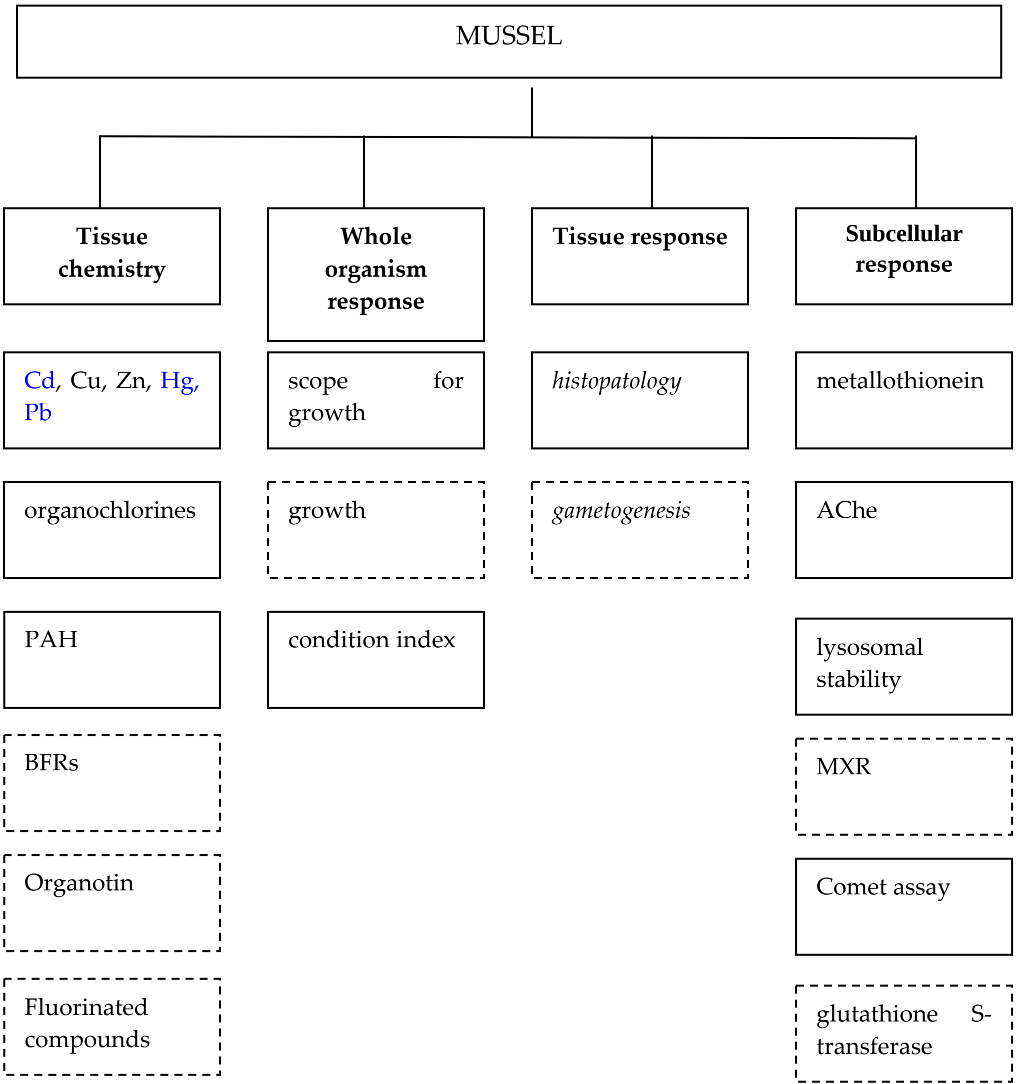


Figure 7.3.1 (p. 13 in 5.1 OSPAR DRAFT integrated guidelines.doc). ***Revised figure.***

7.3.2 Sampling strategies for integrated fish and bivalve monitoring

The integration of contaminant and biological effects monitoring requires a strategy for sampling and analysis that includes the

- sampling and analyses of same tissues and individuals;
- sampling of individuals for effects and chemical analyses from the same population as that used for disease and/or population structure determination at a common time;
- sampling of water, the water column and sediments at the same time and location as collecting biota; and
- more or less simultaneous sampling for and determination of primary and support parameters (e.g. hydrographic parameters) at any given location.

Sampling strategies for the integrated fish and shellfish schemes are proposed below (Figures 7.3 and 7.4). These are driven primarily by the assessment of external diseases and macroscopic liver nodules (fish) and histopathology (bivalves), since these require the largest number of individuals. A sub-sample of individuals within the primary sample is further sampled for liver histopathology (fish) and biomarkers (fish and bivalves) to meet Criteria 1 and 2.

In the specified target species, further sub-sampling of the same individuals for chemical analysis is often restricted by insufficient remaining tissue, e.g. liver in fish. In order to meet Criteria 2, sub-samples for chemical analysis are taken from the same combined hauls/population as those for disease/biomarkers.

In order to integrate sediment, water chemistry and associated bioassay components, with the fish and bivalve schemes, sediment and water samples should be collected at the same time as fish/bivalve samples and from a site or sites that are representative of the defined station/sampling area. (See Section 7.2.2).

Additional integrated sampling opportunities may arise from trawl/grab contents, for example, gastropods for imposex or benthos and these should be exploited where possible/practicable.

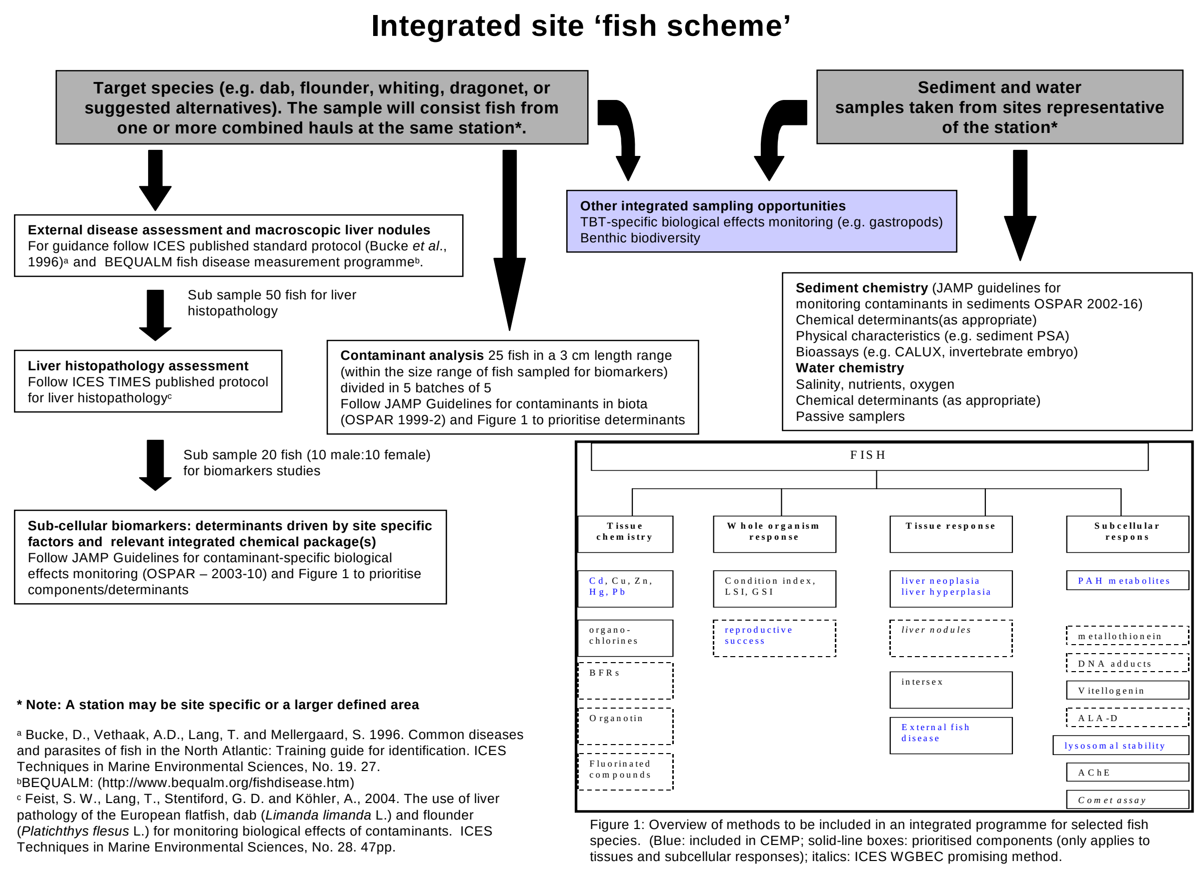


Figure 7.3.2 Sampling strategy for integrated fish monitoring.

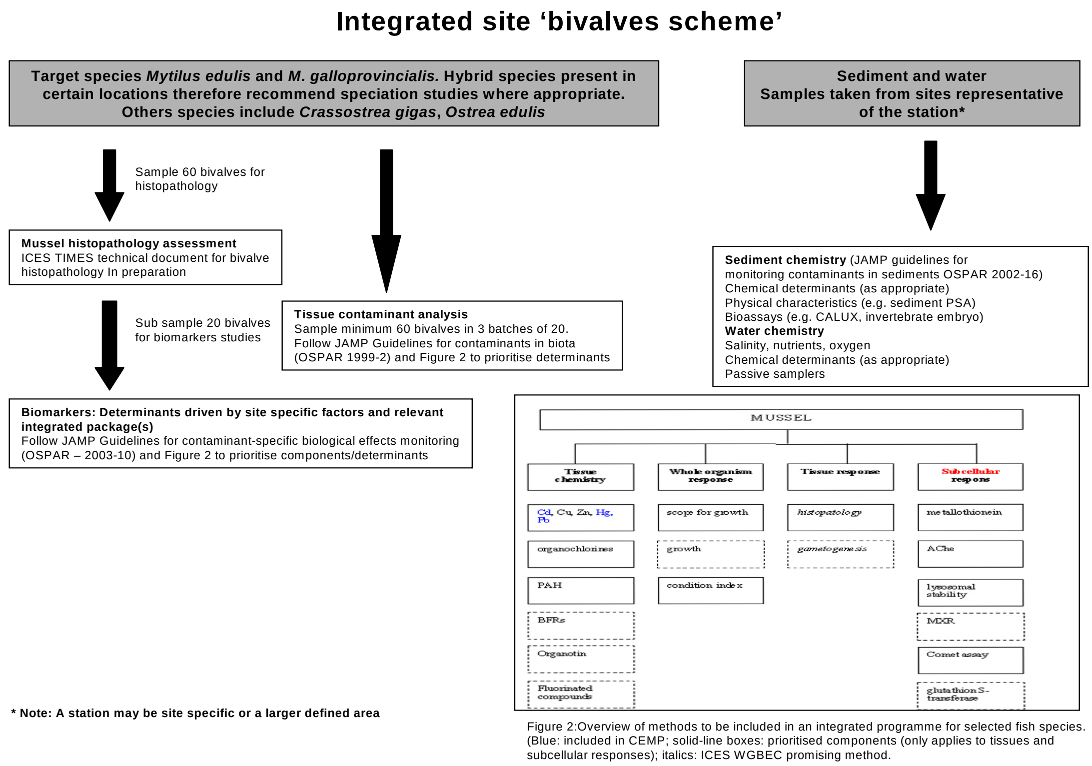


Figure 7.3.3 Sampling strategy for integrated bivalve monitoring.

This agenda item focused on the chemical-biological effect approach being developed by OSPAR and the discussion was based around the draft document produced by OSPAR via WKIMON III.

A document cited by OSPAR for reference on sampling strategy and design from ICES WGSAEM did not exist.

Conclusion

The guidelines presented above are considered by the WKIMON group as a first attempt to draft a technical annex to support the integrated chemical-biological effect approach. Further development on the draft is essential.

Recommendation

Complete draft technical annex initiated at WKIMON IV. Work to be undertaken by WKIMON or its follow-on group to be completed by December 2008.

Justification

Needed to complete the OSPAR integrated chemical-biological effect guidelines.

8 Develop a draft technical annex on recommended packages of chemical and biological methods for monitoring on a determinant basis to ensure that chemical and biological methods are well matched and that chemical analyses underpin biological effects monitoring TOR d

Review of CEMP requirements

This agenda item was addressed by reviewing the chemical determinants listed in the OSPAR CEMP and pre-CEMP (ASMO, 2007a) and considering the most appropriate chemical analyses and biological effects techniques that could be applied in an integrated fashion to monitor for these compounds in the marine environment.

Some general points concerning integrated monitoring were noted during this process:

a) In some cases the list of contaminants that should be reported under the CEMP (and pre-CEMP) may be insufficient for an integrated approach. In order to aid interpretation of biological effects measurements, an integrated assessment may require data on related contaminants which would elicit a response on the biological effects components of the methods packages. Determinants additional to those required under the CEMP have therefore been added to the packages below.

b) It was felt that a fully 'integrated' approach to monitoring should include passive sampling of contaminants as part of the package of methods. This will provide information on availability of contaminants in sediments and allows for temporally integrated sampling of contaminants in water. (Guidelines for the application of passive samplers are available from ICES WGMS).

c) The biological effects techniques applied to these packages of methods are listed either in the ICES WGBEC recommended techniques list (WGBEC, 2007) or form part of the fish and shellfish methods packages proposed in the draft JAMP guidelines for integrated monitoring and assessment of contaminants and their effects (ASMO 2007b). The biological effects methods included here are separated into those appropriate for monitoring selected fish species, shellfish (mussels) and bioassays (sediment, water and *in vitro* tests).

d) It should be noted that the biological effects methods listed here are those which may form part of an overall integrated monitoring package and are likely to be affected by the OSPAR priority contaminants in question. Many of the effects measurements listed are 'general' biological effects which are indicative of stress or health status of marine organisms or general toxicity in the sediments and water column. These may be affected by a wide range of contaminants and are not specific to the contaminants in question. Therefore, for each group of substances the most specific and relevant biological effects techniques have also been highlighted.

e) These packages of methods should be considered supplemental to the existing JAMP guidelines for contaminant specific (OSPAR-2003–10) and general (1997–7) biological effects monitoring and the JAMP Guidelines on contaminants in biota (OSPAR 1999–2) and sediment (OSPAR 2002–16). The JAMP guidelines provide more detailed background on the biological effects and chemical analysis methods referred to here and the necessary cofactors that should be recorded for these techniques. The packages of methods presented here combine contaminant-specific effects with the general biological effects methods that are likely to respond to the contaminants. They also deal with groups of contaminants not addressed by the contaminant specific guidelines and propose further integration of techniques such as passive sampling and invertebrate methods for metals.

The priority chemical determinants from the OSPAR CEMP and pre-CEMP are as follows (taken from ASMO, 2007a). The Appendices referred to are CEMP appendices.

The following components of the CEMP are to be measured on a mandatory basis:

- the heavy metals cadmium, mercury and lead in biota and sediment (Appendix 2);
- the PCB congeners CB 28, CB 52, CB 101, CB 118, CB 138, CB 153, and CB 180 in biota and sediment (Appendix 3);
- the PAHs anthracene, benz[a]anthracene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, fluoranthene, ideno[1, 2, 3-cd]pyrene, pyrene and phenanthrene in biota and sediment (Appendix 4);
- TBT in sediment (biota voluntary / pre-CEMP) (Appendix 5).

The following components are currently part of the pre-CEMP and are to be measured on a voluntary basis:

- the brominated flame retardants HBCD and PBDEs 28, 47, 66, 85, 99, 100, 153, 154 and 183 in biota and sediment, and BDE 209 in sediment (Appendix 8);
- the planar PCB congeners CB 77, 126 and 169 in biota. Monitoring of those congeners in sediment should be undertaken only if levels of marker PCBs are e.g. 100 times higher than the Background Assessment Concentration (Appendix 9);
- the alkylated PAHs C1-, C2-, and C3-naphthalenes, C1-, C2- and C3-phenanthrenes, and C1-, C2- and C3-dibenzothiophenes and the parent compound dibenzothiophene in biota and sediment (Appendix 10);
- PFOS in sediment, biota and water (Appendix 12);
- Polychlorinated dibenzodioxins and furans in biota and sediment (Appendix 13);

8.1 Methods package for metals

Although cadmium, mercury and lead are the only mandatory metal determinants under the CEMP, other metal species are needed to interpret the biological effects data as part of an integrated package. Additional metal species needed include copper and zinc. Metals analysis should be performed on sediments and biota collected from the same times and locations where possible. Cofactors for sediment analysis are also required including aluminium and lithium. DGTs present the opportunity to undertake passive sampling for metal species to allow temporally integrated sampling of water and measure availability of metals in sediments.

Metal-'specific' biological effects measurements include metallothionein, ALA-D and oxidative stress, although both metallothionein and oxidative stress responses are known to be affected by other contaminants. ALA-D is lead-specific and can be measured in fish blood, although it has limited use/expertise across the ICES/OSPAR community and it is recommended that it is applied only in areas where lead contamination is perceived to be a problem or where chemical monitoring indicates that concentrations are e.g. significantly above background.

ALA-D is relevant for fish only. Metallothionein can be applied to fish liver and mussel digestive glands although best results are obtained from mussels. There are a number of oxidative stress measurements that can be made in both fish and mussels which could add value to an integrated package of metals methods, but due to the lack of standardised methods, QA and assessment criteria it is suggested that this method is not an essential part of the metals package.

A number of 'general' biological effects measurements in fish and shellfish will be affected by environmental metal contamination and these are shown in Figure 8.1 below. *In vivo* bioassays are also relevant measurements for the effects of metals.

Metallothionein in mussels and ALA-D in fish are considered the most specific/relevant biological effects methods for metals.

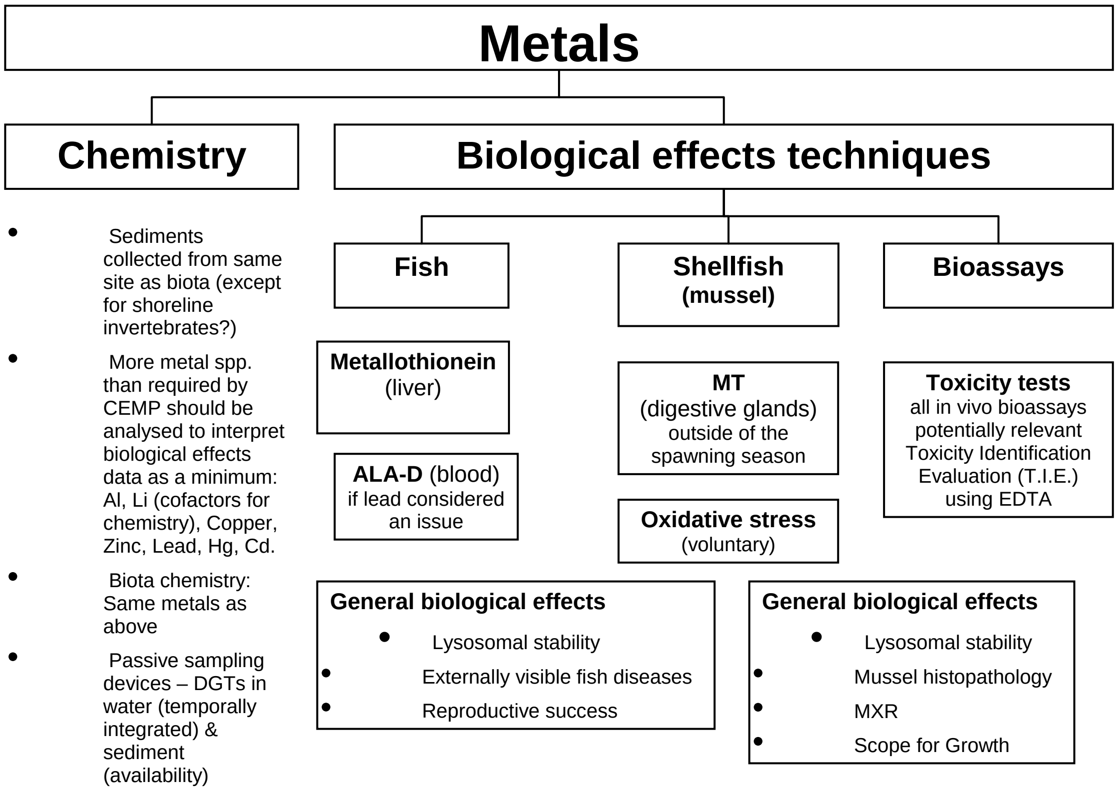


Figure 8.1 Package of chemical and biological effects methods relevant to monitoring for metals. The most specific / relevant biological effects methods are highlighted.

8.2 Methods package for PCBs, polychlorinated dibenzodioxins and furans

Due to the similarity of their toxicological effects, a single methods package was proposed for both PCBs and polychlorinated dibenzodioxins and furans. In addition to the OSPAR CEMP required determinants, additional CBs may cause biological effects and their analysis should be included in an integrated monitoring approach. These include co-planar CBs CB105 and CB 156. A variety of passive sampling devices (e.g. silicone rubber) offer the potential for temporally integrated sampling of these compounds from water and investigation of their availability in sediments and these should be employed where possible.

There are no truly specific biological effects measurements available for PCBs, polychlorinated dibenzodioxins and furans. The most relevant are considered to be induction of CYP1A/EROD activity in fish liver and application of the dioxin receptor based in vitro test, DR-CALUX.

Several other general biological effects measurements in fish and shellfish may respond to exposure to these compounds and are given below in Figure 8.2. DR-Calux is considered the most useful *in vitro* bioassay technique although chronic *in vivo* bioassays may also be relevant.

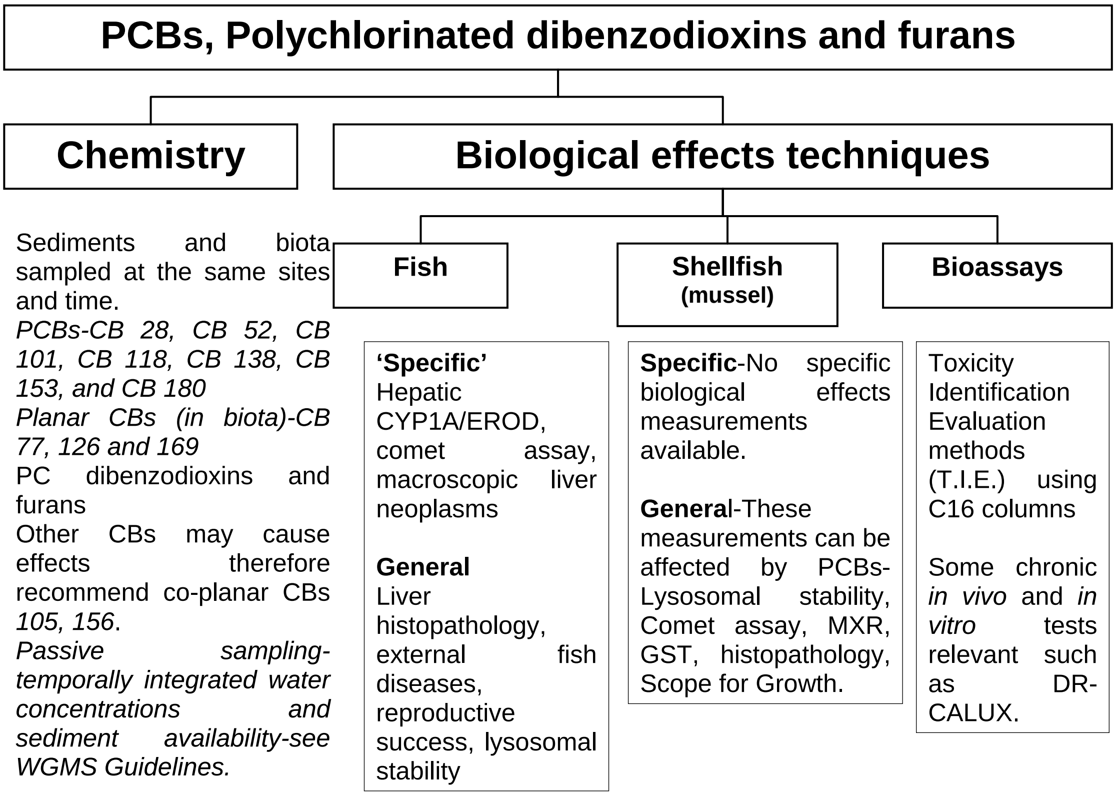


Figure 8.2 Package of chemical and biological effects methods relevant to monitoring for PCBs polychlorinated dibenzodioxins and furans. The most specific/relevant biological effects methods are highlighted.

8.3 Methods package for PAH and alkylated PAH

Due to similar toxicological effects, a single package of methods is proposed for PAH and alkylated PAH. The package of methods is similar to Figure 8.2 above although chemical determinants should be analysed in sediment and shellfish for biota only. Due to rapid metabolism in finfish, PAH should be analysed as metabolites in bile rather than parent compounds in liver or flesh. As above, passive sampling should also be applied where possible.

Additional specific biological effects are applicable for PAH/alkylated PAH. These include PAH metabolites in fish bile and DNA adducts in fish liver. The most relevant/specific biological effects techniques are highlighted as induction of hepatic CYP1A/EROD, DNA adducts and the DR-CALUX *in vitro* bioassay.

General biological effects measurements will also respond to exposure to these compounds and are given in Figure 8.3 below.

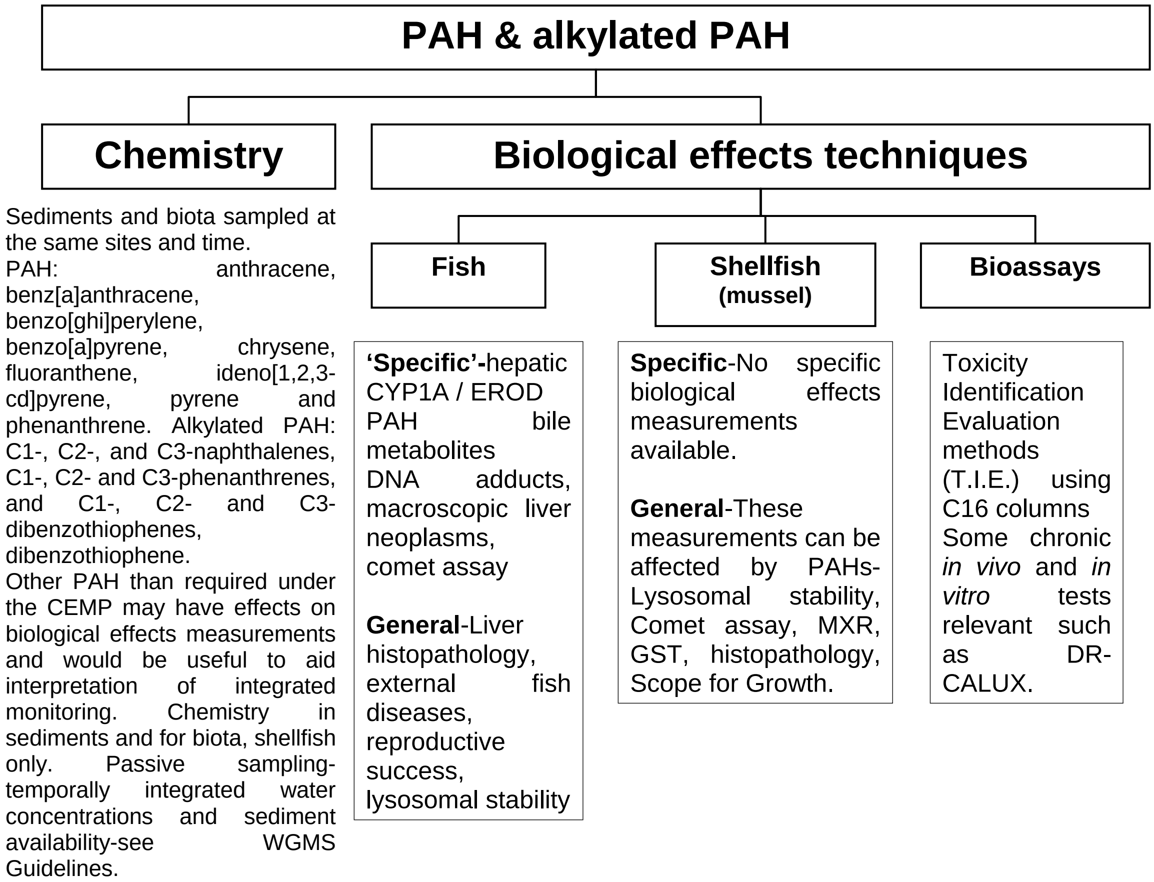


Figure 8.3 Package of chemical and biological effects methods relevant to monitoring for PAH and alkylated PAH. The most specific / relevant biological effects methods are highlighted.

8.4 Organotins

It was felt that the package of methods appropriate for organotin monitoring was already very well described by the JAMP guidelines on organotin-specific monitoring and included a suite of parameters relevant to imposex/intersex in gastropods, TBT, DBT, MBT, TPhT, DPhT, MPhT in sediments (for offshore monitoring) and in biota where appropriate (voluntary). It was noted that passive sampling for organotins may become an option for integrated monitoring of organotins in the future. It was also noted that bivalve embryo bioassays are sensitive to dissolved TBT at ng/L level.

8.5 BFRs

It was noted that there are currently very few biological effects methods available and tested in a monitoring context for measuring the effects of these compounds. The determinants required for CEMP are HBCD and PBDEs 28, 47, 66, 85, 99, 100, 153, 154 and 183 in biota and sediment, and BDE 209 in sediment. Passive sampling is also relevant as described above in Section 8.2.

There are no specific biological effects techniques available. Thyroid hormone receptor assays in fish blood are relevant but have not been well field tested, nor is this an ICES recommended technique. Recent studies on the toxicological properties of these compounds in fish suggest that there are limited overt effects that can be detected by existing techniques.

8.6 PFOS

PFOS analysis in sediment, biota and water is included in the list of pre-CEMP determinants, however no specific biological effects techniques are recommended here. It was noted that the compound may have endocrine disrupting effects and that some ED-relevant endpoints may be appropriate along with general biological effect measurements such as reproductive success. A battery of short-term low volume bioassays (*in vitro* and *in vivo*) using extracts can be used to perform a first screening/assessment of unintended impacts and novel contaminants (see background document on water bioassays). These extracts can be derived from water, sediment, biota and/or passive samplers. Information obtained from bio-analysis can also be used as input for the design of future monitoring programmes and the development of appropriate higher-level biological effects techniques biomarkers. However, a package of methods relevant to PFOS would require further consideration.

References

ASMO 2007a. Proposal for revising the OSPAR Coordinated Environmental Monitoring Programme (CEMP). ASMO 07/13/1.

ASMO 2007b Draft JAMP guidelines for integrated monitoring and assessment of contaminants and their effects. ASMO 07/6/8-E.

WGBEC 2007. Report of the ICES Working Group on Biological Effects of Contaminants, 2007.

9 Review progress with the ICON demonstration project TOR e

9.1 Current position

The objective of the practical ICON Workshop is to assess the health of North Sea ecosystems through an international sea-going workshop and subsequent integrated assessment of the impact of contaminants on the North Sea. ICON aimed at: (1) to try out the core components of the WKIMON integrated chemical biological effect methods and guidelines (demonstration component) (2) investigate a range of additional biological effects techniques (research component). The ICON initiative was started in 2006, led by Ketil Hylland (Norway) and with potential funding from the Norwegian oil industry and Research Council. A last steering group meeting was held in Copenhagen ICESHQ in May 2007 to discuss the program. Commitments were made by various MS at that time to have ship time and scientific resource available to do the work. In December 2007, it became clear that no further Norwegian funding would be available to support the activities in ICON.

The group discussed how best to take ICON forward, as OSPAR had expressed their support for such an opportunity to field test integrated monitoring schemes. It was decided that a realistic option will be to run a nation-based, but coordinated, programme. The UK, Germany, France, NL and Spain informed the WG that they would be willing to participate in such a reduced ICON project, including the following aspects:

9.2 Future programme

The revised program should focus on the demonstration component rather than the research component. It should contain a smaller number of stations and species than were included in the original proposal for ICON. In addition, the revised ICON should also look at existing good data sets, and select a data set for analysis as a case study that might be suitable for inclusion as box text in QSR2010 (action UK, John Thain and Brett Lyons).

The following components/sites/species should be included:

Coastal sites (Seine, Wadden Sea, Firth of Forth, Devon coast, Spain (MEDPOL), possibly Iceland):

- Mussel scheme and passive samplers: all sites
- Fish scheme (flounder only): Seine, Wadden Sea, Scotland

Offshore sites 5 or 6 sites (NW Helgoland, Doggerbank and Firth of Forth, Iceland, western Baltic Sea, Seine; and possibly a Norwegian JAMP site).

- Fish scheme (dab only): all sites

It was noted that the selected sites should be preferably already be established as JAMP sites, and for which background chemical information and other biological effects data are already available. The importance to set up assessment criteria for the selected species and methods in due time was also noted.

Round the table, the following offers of contributions of ship time and other resources were made:

John Thain (CEFAS, UK): Has a cruise on Endeavour in June.

Dick Vethaak (NE): has time for a limited amount of additional work in September in association with existing monitoring programmes. He plans to sample (flounders and mussels) one site in the Wadden See for the full WKIMON programme, including passive sampling. No research ship is required in this inshore area.

Thierry Beurgeot (FR): Has ship time in September for sampling work in a pilot study in Seine estuary. Hopes to obtain more funding, but IFREMER can give two weeks of ship time to collect flounder. If funding is available, mussels in cages and passive samplers will be deployed in the Seine estuary.

Thomas Lang (GE): Has a three week research cruise in September, and could visit all stations in original draft ICON programme. Will observe fish diseases (external) and some histopathology in flatfish.

Matt Gubbins (FRS, UK): has approximately one week of time on FRV Scotia in October. The Scottish Government is keen on the programme, particularly at sites of direct relevance to Scotland, such as the Firth of Forth. Expected that the programme will concentrate on fish sampling and biomarkers, possibly with fish disease, and PS.

Concha Martinez (ES): plans to work in April in one area, three caged mussel sites along a gradient, and also sample fish. No experience of PS. Happy for people to make additional analyses on their samples.

Summary of locations

Coastal sites	Murcia gradient	Mussels
	Seine estuary	Flounder + mussels
	Wadden See	Flounder + mussels
	Forth gradient	Flounder + mussels
	Whitsand bay or North coast of Devon	Flounder + mussels
Offshore	Helgoland	Dab
	Dogger	Dab
	Forth	Dab
	Baltic	Dab
	Iceland	Dab

A "lean and mean" steering committee should be set up to redesign and co-ordinate the revised program, flesh out the details and take it forward to WGBEC2008 in March. The tasks in the coming months are:

1) Review the leadership of the project and create a new Steering Group
2) Decide on how the sampling exercise should be coordinated
3) Ensure that all determinands are covered and what organizations can contribute
4) Draw up a timetable for the project
5) Coordinate the distribution of samples (and intercalibration where required)
6) List requests for additional samples for research purposes.

ACTION

John Thain would contact Ketil Hylland to convey the discussions of the WKIMON IV meeting and with Thomas Laing and Dick Vethaak would liaise to organise a steering group meeting in early March to be held at the Johann Heinrich von Thünen-Institute in Germany.

10 Any other business.

10.1 Progressing the work initiated under WKIMON

The participants agreed that significant progress had been made at the WKIMON III and IV meetings and that momentum to complete the work on the "integrated chemical-biological effects approach" needs to be maintained. However, it was noted that the status of WKIMON (convened as a workshop) was no longer tenable within the OSPAR or ICES system. It was agreed that the work being undertaken by the WKIMON group would take up to three years to complete. With this in mind, it was proposed that a new group be formed within the ICES/OSPAR setup, perhaps a joint Study Group, to complete the outstanding work. Tasks for the group were identified and recommendation put forward (see recommendation below). It was noted that in order to complete many of the outstanding tasks in time for the ICON project and QSR 2010 the "new group" would need to meet the autumn of 2008.

11 Final report to SIME and onward transmission to ASMO and ICES (ACME and MHC)

The meeting concluded at 16:30 hr on 7th February 2008. The report was compiled after the meeting and subsequently amended by a written procedure.

12 Recommendations

12.1 Recommendation to create a follow-on Study Group

It was proposed that WKIMON be disbanded. It was further proposed to create a new Study Group (SG) on Integrated Monitoring of Chemicals and Biological Effects (SGIMCEB). It would have a life of up to three years.

The SG would be administered by ICES, but should be a joint ICES-OSPAR group. Other conventions could also be included formally, or else could be invited to send delegates. In this respect:

- ICES countries can nominate members through Delegates
- OSPAR can nominate members
- SG chair can invite people to the first meeting.
- WKIMON should make a recommendation for the new SG
- WKIMON should give some information about the proposed area of work, tasks, etc.
- WKIMON can suggest a chairman.

Justification

That WKIMON be disbanded, having held series of successful meetings and contributed significantly to the development of integrated approaches to environmental monitoring of contaminants and their effects in coastal and open-sea areas. That specific items, identified in the list of Recommendations / Actions below, should be transferred to the terms of reference for a new ICES/OSPAR Study Group on Integrated Monitoring of Chemicals and Biological Effects, as outlined below.

12.2 Recommendation that a joint ICES/OSPAR Study Group on Integrated Monitoring of Chemicals and Biological Effects (SGIMCEB) be created; it is recommended that the chairman should be Dr Ian M Davies (UK), and should hold its first meeting in autumn 2008

Recommend that a joint ICES/OSPAR Study Group on Integrated Monitoring of Chemicals and Biological Effects (SGIMCEB) be created under the chairmanship of Dr Ian M Davies (UK), and should hold its first meeting in autumn 2008.

Justification

There is increasing emphasis on integrated forms of data collection, analysis and assessment as part of the drive towards ecosystem approaches to environmental management. ICES laboratories have submitted significant amounts of data to the ICES DOME on both the concentrations of contaminants in the marine environmental and of their biological effects. ICES WGs such as WGMS, MCWG and WGSAEM have contributed strongly over the years to developing and validating assessment criteria and tools for chemical measurements, and the novel task is now to develop assessment tools for biological effects that can be integrated with the assessment methods currently used by OSPAR MON in assessing contaminant data. This proposed Group has its foundations in the outputs from WKIMON which has identified the need for further research for the development of better assessment tools for OSPAR and potentially for HELCOM use, and will link into regional scale assessments against the objectives of the Marine Strategy Directive. The focus of the group is therefore the development of methods that are relevant to ICES advisory role for both open sea and inshore (transitional) waters, and which may need to take account of regional variability. The SG would sit between the research emphasis of WGBEC and the application of their work in the ICES advisory context, which requires a wider range of expertise (chemists, statisticians, etc.) than is available in WGBEC.

Tasks

The tasks of the proposed Study Group are:

1) To develop and validate assessment criteria for biological effects measurements (e.g. molecular or cellular biomarkers) which are indicative of potential significant effects at higher levels or organisation (e.g. in organs or whole organisms).

2) To develop data reduction and presentation tools that effectively communicate integrated assessments of chemical and biological effects measurements.

3) To plan and assess the outcomes of field and desk-based exercises to test and validate integrated approaches to monitoring and data analysis.

4) To develop templates for documentation that ensure that documentation of the scientific background, analytical methods, assessment criteria, and data reduction and presentation tools are coherent, cohesive, and compatible.

The following action points below should also be taken into account as tasks directed to the first meeting of SGIMCEB. If the SG is not approved, then alternative ways to address these questions will be required:

ACTIONS

Actions from Agenda item 3 and 4 combined concerning background documents and assessment criteria:

To develop a seasonal model of background activities of EROD in flatfish for use in the assessment of CEMP monitoring data, and review and amend the OSPAR Background Document for EROD. To organise a Workshop on background activity of EROD in flatfish to undertake this work, in Ostend for three days at the end of September/early October. This is an outstanding and important need identified at WKIMON III. Invitations to be sent to specialists in this field, in liaison with ICES WGBEC.

Action: Local convenors, Patrick Roose and Kris Cooreman (Be).

To collate data on the variability of vitellogenin concentrations in fish blood from areas unimpacted by oestrogenic contaminants by 31 March to allow calculation of Background Assessment Concentrations for vtg.

Action: Ian Davies (UK).

To review the outcome from SIME2008 discussions on assessment criteria for metabolites of PAHs in bile and determine whether further development is required before OSPAR MON 2008.

Action: Chairs, in time for meeting of SGIMCEB.

Review and amend the draft Background Document on metabolites of PAHs in bile in time for meeting of SGIMCEB.

Action: Norway.

To update the Background Document on DNA adducts and revise the proposed assessment criteria in time for meeting of SGIMCEB.

Action: Brett Lyons (UK).

Propose amended text for the OSPAR Guideline on fish disease monitoring by 1 March.

Action: Thomas Lang and Werner Wosniok (DE).

To develop a new short section for the OSPAR Guideline on Biological effects monitoring on imposex in gastropods by 1 March.

Action: Matt Gubbins (UK).

To develop new text for the OSPAR Guideline on Biological effects monitoring in intersex in fish in time for meeting of SGIMCEB.

Action: Steve Feist (UK).

That the Background Documents and Guideline texts on metallothionein and ALA-D by reviewed and amended as necessary by SGIMCEB.

Action: SGIMCEB.

Chairs to clarify with OSPAR Secretariat the process of version control, amendment, approval and adoption for Background Documents, Guidelines and Technical Annexes.

Action: Ian Davies and John Thain (UK).

Agenda item 5

A follow-on group to WKIMON should take this approach forward to develop and validate the process with real data sets and data generated through the OSPAR ICON demonstration project. To develop EAC-equivalent assessment criteria for biomarkers i.e. responses leading to concern for wellbeing/health at organ or whole organism level.

Action: Follow on group of WKIMON.

To validate the scheme proposed by WKIMON for the assessment of biological effects data, when the necessary assessment criteria are available.

Agenda item 6

To review the partial draft of a Technical document on sampling for integrated chemical and biological effects monitoring, and to ensure that the document is compatibility with the current OSPAR Integrated Guidelines.

Action follow on WKIMON group.

Agenda item 7

To review and comment on draft text on packages of chemical and biological effects measurements to address particular OSPAR priority contaminants by 14 February.

Action: All WKIMON members.

Agenda item 8

Reflecting the support of WKIMON for the progress of the ICON demonstration project, to form a new Steering Group for the project. John Thain would contact Ketil Hylland to convey the discussions of the WKIMON IV meeting and with Thomas Lang and Dick Vethaak would liase to organise a steering group meeting in early March to be held at the Johann Heinrich von Thünen-Institute in Germany.

Action: Thomas Lang to host.

Agenda item 9

Ian Davies would investigate the possibility of continuing the WKIMON work within the ICES system, and with the support of OSPAR. See recommendation above.

To progress tasks identified in the Recommendations from WKIMONIV as to be completed in time for the meeting of SGIMCEB.

Agenda item 10

To pass the completed text to SIME for comment.

Action: Chairs.

Annex 1: List of participants

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Annex 2: Agenda

WKIMON IV Agenda

Date of meeting: 09:30 on Tuesday** 5th–**16:30 on Thursday 7th February 2008

at: ICES, H. C. Andersens Boulevard 44–46, DK-1553 Copenhagen V, Denmark.

1) Adoption of agenda.

2) Review and note the Terms of Reference (WKIMON IV; 2007/2/ACOM49).

3) Assessment criteria: Review the progress with the Fish Disease Index and progress with the development of assessment criteria for biological effects methods. Where possible develop further and complete and/or develop a timetable for completion as appropriate.

4) To review the current status of background documents for biological effects techniques and, as appropriate make arrangements for any further work needed to ensure they reflect further developments on assessment criteria.

5) Undertake further development of a generic assessment framework and method for the integrated biological effect-chemical approach to monitoring and build what is available into a draft technical annex.

6) In support of the integrated chemical-biological effect approach, develop an initial draft of a technical annex on sampling design and supporting parameters.

7) Develop a draft technical annex on recommended packages of chemical and biological effects for monitoring on a determinant basis to ensure that chemical and biological methods are well matched and that chemical analysis underpins biological effects monitoring.

8) Review progress with the ICON demonstration project. Develop a programme and timetable to ensure that the proposed integrated chemical-biological effect approach is validated and its application to both specific CEMP issues and to the general health assessment of a region.

9) Any other business.

10) Finalise a report for SIME by 18th Feb and onward transmission to ASMO and ICES (ACME and MHC).

Annex 3: Terms of Reference

2007/2/ACOM49 The Fourth **ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-sea Areas** [WKIMON IV] (Co-chairs: Ian Davies designated by OSPAR and John Thain designated by ICES) will meet in ICES Headquarters, Copenhagen 5–7 February 2008 to:

a) complete the development of assessment criteria for specific biological effects methods.

b) undertake further development of a generic assessment framework and method for the integrated biological effect-chemical approach to monitoring and build what is available into a draft technical annex.

c) develop an initial draft technical annex on the sampling design and overall parameters of the integrated monitoring approach.

d) Develop an initial draft technical annex on recommended packages of chemical and biological effects for monitoring on determinant basis to ensure that chemical and biological methods are well matched and that chemical analysis underpin biological effects monitoring.

e) review the organisation and scope of the international ICON Workshop of integrated biological effects and chemical monitoring and its application to both specific CEMP issues and to the general health assessment of a region.

WKIMON IV will report for the attention of ACOM and MHC for onward transmission to OSPAR (SIME) by 15 February 2008.

NB: At present OSPAR has not yet decided to support this Workshop in 2008. The ICES Secretariat will communicate these revised draft ToRs to the OSPAR Secretariat with a request for support of the Workshop. If a Co-chair person cannot be designated by OSPAR, ICES will try to find a solution.

Supporting Information

Priority:	The current activities of this group are directed towards developing an integrated approach to monitoring and assessment of both chemical and effects for application within OSPAR monitoring programmes. Consequently these activities are considered to have a very high priority.
Scientific justification and relation to action plan:	Action Plan No: 1. Term of Reference a) Finalisation of ongoing work from previous WKIMON meetings Term of Reference b) Required to underpin the application of the integrated approach to monitoring. It is recognised that this will need a critical review and appraisal of current methods on integrated assessments (e.g. Fullmonti, REGNS, models from Canada etc.) with a view to developing a recommendation for methods to specifically characterise the OSPAR maritime area and present a readily understandable assessment. Guidance on how to tackle different time scales should be included. Term of Reference c) Overall and general considerations about the integrated monitoring approach which will provide guidance to WGBEC and WGSAEM in relation to OSPAR request No. 8. Term of Reference d) Conclusion of work by recommendation of packages of chemical and biological effects for monitoring on determinant basis Term of Reference e) Plans were initiated at WKIMON III to take advantage of a Norwegian initiative in the area of North Sea Health. Various planning meetings will have occurred during 2007 (ie since WKIMON III) and a wider review of the plans and progress is required to ensure that they adequately take account of the interests of OSPAR in developing integrated monitoring for Regional assessments.
Resource requirements:	The research programmes which provide the main input to this group are already underway, and resources are already committed. The additional resource required to undertake additional activities in the framework of this group is negligible.
Participants:	The Group is normally attended by some 17–22 members.
Secretariat facilities:	None.
Financial:	No financial implications.
Linkages to advisory committees:	The reports of the WKIMON go to advice drafting on the answer to request from OSPAR on an integrated approach to monitoring and assessment of both chemical and effects.
Linkages to other committees or groups:	The reports of the WKIMON go to MHC as well as to the MCWG and WGMS.
Linkages to other organizations:	SIME and ASMO under OSPAR

Annex 4: Technical Minutes of RGChem-May 13–14, 2008

1 Members of the Review Group

Chair

Paul Keizer, Vice-Chair ICES ACOM

Members

Jarle Klungsøyr (Norway)

José Fumega (Spain)

Les Burridge (Canada)

Claus Hagebro (ICES)

The members did their work by correspondence from May 1–12, 2008 and then the Chair and two members (Fumega and Klungsøyr) met at ICES Headquarters on May 13th and 14th as the Advice Drafting Group (ADG). The morning of May 13th any outstanding issues from the technical review were resolved. These minutes were left open until technical issues relating to the EAC Advice (2.1.1) were resolved and then they were reviewed and accepted by correspondence.

2 Request

The Review Group was responsible for the technical review of the draft advice text from ICES Expert Groups in response to a number of requests from OSPAR. Identification of the responses was made difficult due to the rewording in some of the EG resolutions, the carryover of some requests from previous years, and unsolicited updates provided by some EGs. The advice will have to be carefully documented to make certain that OSPAR is clear to which requests ICES is responding. The 2007 requests from OSPAR were specifically:

2.1 Current requests from OSPAR

2.1.1 Development of proposals for Environmental Assessment Criteria

To ensure EACs are available for assessments of concentrations of hazardous substances in the marine environment, which will contribute to the QSR 2010. This request will be based upon the following:

(i) review proposals from The Netherlands for updated EACs for PCBs according to the 2004 methodology.

(ii) peer review of proposals for updated EACs for PAHs developed by The Netherlands;

(iii) review the basis for the updated EACs for metals in sediments proposed in 2004, with particular emphasis on the selection of partition coefficients and, as appropriate, the development of alternative proposals for these EACs;

(iv) development of proposals for updated EACs for components included in the pre-CEMP, giving emphasis to the development of EACs for HBCD;

ICES should seek to ensure that work is well co-ordinated with work on environmental quality standards of the Priority Substances Working group under the

Water Framework Directive Common Implementation Strategy and the associated Expert Group on EQS.

Subsequent to the meeting of OSPAR SIME ICES was directed to focus on responding to items (i) and (ii).

2.1.2 Tools for coordinated monitoring of dioxins, planar CBs and PFOS

To prepare the following tools to support the coordinated monitoring of dioxins, planar CBs and PFOS under the OSPAR CEMP:

a) technical annexes to the JAMP Guidelines for monitoring Contaminants in Sediments (OSPAR agreement 2002-16) and JAMP Guidelines for monitoring Contaminants in Biota (OSPAR agreement 1992–2) according to the structure of the existing technical annexes covering the following:

(i) monitoring of dioxins in biota and sediments, taking into account advice from SIME 2007 that monitoring of dioxins in sediments should only be carried out in specific areas (such as sedimentation areas or estuaries) because of time lag (10–12 years) in deposition of quantities required for sampling;

(ii) monitoring of PFOS in sediments, biota and water;

b) to review the existing technical annexes on monitoring of chlorinated biphenyls in biota and sediment and propose revisions so that they are adequate for monitoring of planar CBs in these compartments, taking into account advice from SIME that monitoring in sediments should be undertaken only if levels of marker PCBs are e.g. 100 times higher than the BACs and that for biota monitoring of concentrations in seabird eggs could provide an alternative matrix.

2.1.3 To develop background concentrations for dioxins

This request is actually part of the request in 2.1.2 but was handled separately.

2.1.4 Further development of guidance on integrated monitoring and assessment of chemicals and biological effects

To complete the development of JAMP guidance for integrated monitoring of chemicals and their biological effects through preparing technical annexes on:

(i) survey design. The purpose is to provide guidance on the selection of representative stations, taking into account requirements under the Water Framework Directive and the proposed Marine Strategy Directive, and for the selection of stations for integrated monitoring. This work should build on work by WGSAEM 2007 relating to the spatial design of monitoring programmes and should take into account the approach taken by the UK in re-designing their station network;

(ii) groups of biological effects methods to be deployed to address specific questions. This should provide guidance on recommended packages of chemical and biological effects for monitoring on determinand basis to ensure that chemical and biological methods were well matched and that chemical analysis underpinned biological effects monitoring.

Only item (ii) was dealt with during this meeting since item (i) was directed to WGSAEM who will not meet until the fall of 2008.

2.2 Carryover and updated advice

2.2.1 Proposals for background concentrations in biota with priority to metals in fish and shellfish

This is a carryover from a request from OSPAR for 2007. MCWG had agreed to a potential approach to providing this advice at its 2007 meeting, noting that more work would have to be done before MCWG 2008.

2.2.2 Update of proposed background concentrations for alkylated PAHs in sediments

WGMS 2007 studied the available information from Scotland, Norway and France, and used those for an initial set of proposed Background Concentrations. WGMS 2007 recognized that these Background Concentrations had been obtained from very limited datasets and so they could not have high confidence in the values. WGMS 2007 recommended that they be forwarded to OSPAR with a view to them being used on a trial basis in data assessments.

WGMS also recommended that work be undertaken to extend the data set underlying these estimations, to include data from other areas, such as the Baltic Sea and to add any additional data to the database. New data had been identified by Norway, UK and Sweden. The WG agreed to address the problem intersessionally and make further recommendations in 2008.

2.2.3 Technical annexes for PAH in biota and sediment and for TBT in sediment

This is in response to a request from OSPAR which was directed to MCWG and WGMS 2007.

3 Sources of Advice

The reports of the following Expert Groups were sources of information for the ICES advice:

- MCWG (items 2.1.2, 2.1.3, 2.2.1, 2.2.3)
- WGMS (items 2.1.1, 2.1.2, 2.2.2, 2.2.3)
- WGBEC (items 2.1.1, 2.1.4), and
- WKIMON (items 2.1.1, 2.1.4)

4 Technical Review

4.1 Current Requests from OSPAR

4.1.1 Development of proposals for Environmental Assessment Criteria

Regarding

(i) review proposals for updated EACs for PCBs according to the 2004 methodology.

There appears to be consensus that the proposed approach has a great deal of uncertainty and is not suitable for recommending EACs that could be used for the assessment.

- The proposal attempts to apply criteria to a small number of individual compounds that may or may not be representative of a class of compounds consisting of 209 congeners, not to mention metabolites. Although this has been done before in earlier OSPAR documents, for example, there appears to be recognition in both the proposal and the WGBEC comments that much more work is necessary before experts are content with the process or outcome. Guidelines from Canada and the US (sediment quality) are given for PCBs but not for individual congeners and the guidelines are very similar to those for dioxins suggesting that they are based on the toxicity of planar PCBs.

- In general, the WGBEC is rightly concerned that setting EACs for individual congeners may not be as conservative or cautionary as necessary since:
    - Recent data indicate that exposure to low concentrations of non-planar PCBs may result in sublethal responses not previously identified for these types of compounds.
    - Choice of data used to calculate an EAC has significant influence on the resulting number.
    - PCBs do not exist in isolation. Therefore having EACs for individual compounds may ignore the presence and effects of others.
- Most of the data for environmental concentrations of PCBs is for the non-planar congeners that are generally considered less toxic than the dioxin-like planar PCBs. Thus field observations of PCB toxicity is correlated with measured concentrations of the non-planar PCBs when the toxicity may in fact be due to the presence of unmeasured planar PCB congeners. Using the EACs calculated for individual congeners to assess the status of PCB contamination would thus be misleading.
- Given the uncertainty that appears to exist, not only regarding individual EACs but also the method(s) of determining these EACs, the WGBEC's overall recommendation to re-evaluate the EACs should be accepted.

Detailed comments

1) Given the importance of the potential application of these proposed values, the request for listing of relevant references in the proposal from The Netherlands is justified. WGBEC reviewers have cited several recent papers that could influence the derivation of an EAC. An annex or appendix showing references would serve several valuable purposes:

1.1) allows independent review of the literature, the quality of the data and therefore invites comments on the validity of the individual EAC.

1.2) Allows identification of knowledge gaps and areas where research is required to fulfill regulatory responsibilities.

1.3) Provides a valuable resource for authors striving to develop similar criteria.

2) The EAC for CB153 seems very high. The point raised by the WGBEC and the response by the author is a good summary of the difficulty that apparently exists in agreeing on how best to set these criteria for PCBs. The problem exists to some degree due to the lack of environmental data for the more-toxic planar PCBs.

3) The discussion surrounding secondary poisoning is similar to that for direct effects. The WGBEC provides a number of references, in this case, that support their argument for a more precautionary approach to assessing PCBs. The recommendation by WGBEC seems reasonable. The

response of the Netherlands indicates an interest in discussing other approaches suggesting recognition that they "don't have it right."

4) WGBEC has provided a number of publications indicating that non-planar PCBs (and their metabolites) can have sub-lethal effects that are not the same as those observed with dioxin-like PCBs. The recommendation of the WGBEC should be accepted and techniques developed to determine EACs for the non-planar PCBs recognizing these other sub-lethal effects.

5) Obtaining toxicity data for PCBs from top predators is very difficult. Work with salmon may be possible but working with some tunas and sharks is far more difficult. Data are readily available on PCB levels in top predators but effects data are not. The goal, while worthwhile, may not be attainable.

6) The EACs for PCBs in organisms needs to be expressed in relation to the lipid content. This is a very important point particularly in discussions of human health or intake of PCBs by humans where PCB-laden organs may not be consumed. The Netherlands have addressed this in a separate report to OSPAR SIME but the report should be added as an appendix and a paragraph added to the EAC document to draw the reader's attention to that appendix and to its significance.

7) WGBEC concluded that the proposed EACs for PCBs in sediments do not pose a problem for the JAMP since the EACs are in all cases greater than the proposed BCs. While this is correct WGMS did not consider the issue that WGBEC has raised regarding the appropriateness of using the EACs for individual PCB congeners given the nature of the data available for the assessment.

Regarding

(ii) peer review of proposals for updated EACs for PAHs developed by the Netherlands;

WGBEC 2008 reviewed and generally agreed with proposals from the Netherlands for EACs for PAHs. There appear to be no areas of concern regarding the actual EAC or the status of that EAC (firm, conditional, etc.).

Detailed comments

1) The request for listing of relevant references is justified. Although the author says they have accessed only a limited data base for this exercise, they also indicate that others (OSPAR) have reviewed the literature. It is clear that EACs will change with availability of data, a reference list therefore increases the strength of the EAC. An annex or appendix showing references would serve several valuable purposes:

1.1) allows independent review of the literature, the quality of the data and therefore invites comments on the validity of the individual EAC.

1.2) Allows identification of knowledge gaps and areas where research is required to fulfill regulatory responsibilities.

1.3) Provides a valuable resource for authors striving to develop similar criteria.

2) The question of phototoxicity is obviously a complicated one. The WGBEC correctly suggests that data based on field or environmentally relevant concentrations are not common. They also contend that lab-based data

should be considered with environmental relevance (in terms of concentrations) acting as a weighting factor and that the precautionary principle be invoked. It is unclear from the response of the EAC author whether or not they intend address this concern. WGBEC's approach seems reasonable.

3) WGBEC's request to consider secondary poisoning from food sources is reasonable. However, a brief perusal of the literature supports the author's contention that few data are available particularly for uptake by aquatic organisms. It is suggested that the Netherlands be encouraged to proceed with preparing a paragraph to describe the issue and to identify research gaps that should be addressed.

Regarding

(iii) review of the basis for the updated EACs for metals in sediments proposed in 2004, with particular emphasis on the selection of partition coefficients and, as appropriate, the development of alternative proposals for these EACs;

(iv) development of proposals for updated EACs for components included in the pre-CEMP, giving emphasis to the development of EACs for HBCD;

ICES should seek to ensure that work is well co-ordinated with work on environmental quality standards of the Priority Substances Working group under the Water Framework Directive Common Implementation Strategy and the associated Expert Group on EQS.

These latter two points were not addressed by WGBEC as a result of the final direction that WGBEC received from the OSPAR SIME 2008 meeting.

4.1.2 Tools for coordinated monitoring of dioxins, planar CBs and PFOS

(i) monitoring of dioxins in biota and sediment.

The MCWG was only able to respond to part of this request and this fact had been communicated to OSPAR prior to the MCWG meeting so that OSPAR could identify priorities if it wished. During the MCWG meeting in 2008 several useful suggestions were made regarding future work needed to complete the response. Since dioxins and planar CBs are often analysed together MCWG suggests that these two compound groups be included in the same technical annex. The technical annex can be based on that for chlorinated biphenyls, but the methodology will need to be revised. In collaboration with MCWG and WGMS a subgroup is going to prepare draft technical annexes for dioxins/co-planar PCBs in biota and sediment for review and revision at MCWG 2009. ICES should discuss this with OSPAR to make certain that they understand what is being proposed and that they agree with this approach.

In the OSPAR request these is no list of individual compounds that the Technical Annex should cover. MCWG suggest that compounds with chorine substitution in the 2, 3, 7, and 8-positions are most toxic and obviously should be included in analyses. A table with a proposal of compounds that the guideline should cover is given.

Information on the analyses of dioxins and planar PCBs is available from a number of scientific reports and publications. Examples are available from the EU projects DIFFERENCE and DIAC, which have specifically explored low-cost analytical methods analysis of dioxins and planar CBs as alternatives to HRMS. Information on these projects is presented in previous MCWG reports (MCWG 2004 and 2005).

The Annex 7 of the WGMS report contains a first draft of a Technical Annex for PCDDs and PCDFs in marine sediments.

WGMS proposed some amendments to the technical annex for CBs. The work will continue intersessionally, with a view to completing the task at WGMS 2009. WGMS proposed that the existing Technical Annex 2 (of the JAMP sediment monitoring guidelines) on CBs should be amended to include planar CBs. This proposal should be cleared with OSPAR.

(ii) Monitoring of PFOS in sediment, biota and water.

MCWG discussed the necessity for broadening the list of compounds to include other perfluorinated compounds (PFCs) as well as PFOS. They recommended that perfluorooctanoic acid (PFOA) should also be included as a parameter in biota, sediment and water monitoring because of the relatively large quantity that is produced and its widespread occurrence in the marine environment. Longer chain PFCs (C >7) should be included in biota and sediment monitoring because of their high bioaccumulation potential. It was also recommended that short chain (C4 to C6) sulfonates should be considered for inclusion in monitoring programmes because they are replacing PFOS and they also show high persistence; these should be preferably monitored in water.

MCWG indicated the need for more research on these compounds to clarify relevant processes and emission pathways in the environment.

MCWG considers that it may not be necessary to prepare a Technical Annex on PFOS analysis in water as there is an ISO proposal in development. The ISO proposal for analysis of PFCs in water is at an advanced stage and is due for revision April 2008. In connection with the preparation of the ISO guideline, an inter-comparison exercise has been performed with sea water included as a matrix. Although the proposed procedure is suitable for application to sea water the documented application range does not fit the requirements of OSPAR (10 pg/L to10 ng/L). These comments have been given to the ISO group by Germany. MCWG 2009 will review the revised ISO proposal to consider whether it meets the requirements of OSPAR for a technical annex for PFOS in seawater.

A technical annex was prepared on the analyses of perfluorinated compounds (PFCs) in biota by the ICES Marine Chemistry Working Group. The main contributors were Lutz Ahrens, Norbert Theobald, Rosana Bossi, Katrin Vorkamp, Philippe Bersuder, Ralf Ebinghaus and Evin McGovern. The objective of the technical annex is to provide advice on the analysis of PFCs in biota. The document is of good quality and should be published, after some minor technical editing, as an overview guideline on the analyses of PFCs in biota.

During the RGChem meeting there was a discussion about the effectiveness of this process, i.e. the RG/ADG review and ACOM approval, for these technical annexes. It was agreed that it would be more reasonable to handle them as a publication and a potential mechanism would be through publication in the ICES TIMES series. The draft text from the Expert Group(s) could be provided to client as a preliminary draft which would be finalized when the TIMES publication was released. It is likely that in some instances several technical annexes would be published in one TIMES edition. This suggestion needs to be brought to the ACOM and also needs to be discussed with the Expert Groups and then agreed upon by OSPAR and clients in general. The Expert Groups may have to spend more time producing the annexes but this would be offset by the fact that the TIMES reports are authored and are citable.

There was insufficient time to produce a technical annex for sediments at the meeting. Much of the analytical information included in technical annex for PFCs in biota is directly relevant to sediment analysis and a MCWG subgroup in collaboration with WGMS will work intersessionally to provide a draft for finalisation at MCWG 2009.

4.1.3 To develop background concentrations for dioxins

WGMS has provided some preliminary information and MCWG briefly discussed the topic but both have deferred this work until 2009. This will be a difficult task but WGMS and MSWG need to coordinate their efforts to insure consistency in their approach. They should consider whether or not the approach taken for proposing the BCs for alkylated PAHs would be applicable here.

4.1.4 Further development of guidance on integrated monitoring and assessment of chemicals and biological effects

Biological effects

No new technical annexes for this topic have been prepared in 2008 by WKIMON IV and WGBEC.

A single methods package was proposed for both PCBs and polychlorinated dibenzodioxins and furans due to the similarity of their toxicological effects (WKIMON IV 2008; WGBEC 2008). In addition to the OSPAR CEMP required determinands, additional PCBs may cause biological effects and their analysis should therefore be included in an integrated monitoring approach. These include the co-planar CBs like CB105 and CB 156.

There are no truly specific biological effects measurements available for PCBs, polychlorinated dibenzodioxins, and furans. The most relevant are considered to be induction of CYP1A/EROD activity in fish liver and application of the dioxin receptor based in vitro test, DR-CALUX.

Several other general biological effects measurements in fish and shellfish may respond to exposure to these compounds. DR-CALUX is considered the most useful *in vitro* bioassay technique although chronic *in vivo* bioassays may also be relevant. These methods are listed in the WKIMON IV 2008 and WGBEC 2008 reports.

4.2 Carryover requests and updated advice

4.2.1 Proposals for background concentrations in biota with priority to metals in fish and shellfish

MCWG rightly notes (reiterates) that the concept of a single BC for these naturally occurring metals throughout the OSPAR area is flawed and furthermore there is no sound methodology to determine natural background (pre-industrial) concentrations for these metals in biota. MCWG chose to identify, with a number of important limitations, "low" concentrations of the metals of interest in mussels but found that there was insufficient good quality data to define similar concentrations in fish. MCWG did not comment on the biological activity of these metals and the potential variation due to the stage in the life history of the mussels. However this was one of the reasons for not making recommendations for BCs for fish.

4.2.2 Update of proposed background concentrations for alkylated PAHs in sediments and biota

WGMS 2007 proposed BCs for alkylated PAHs in sediments based on limited data from samples identified as being from "pristine" areas. WGMS 2008 has provided revised estimates based on the "down-core" concentrations determined in 3 cores from Scottish waters, 2 cores from the Bay of Biscay, and 1 core from Norway. These concentrations are almost without exception lower than those proposed by WGMS 2007. WGMS rightly notes that this is a preliminary proposal based on a very limited data set and that more data are needed in order to develop some reasonable level of confidence in the proposed BCs.

In pursuing the details regarding a "deep" core sample from the Baltic there was some confusion over the source of this information. It was determined that this was a personal communication from Per Johnson of Sweden. This should be noted in the WGMS report.

With the caveats noted for the proposed "low concentrations" for metals in mussels, MCWG 2008 proposed values for some alkylated PAHs in shellfish.

The Review Group noted that in a number of instances values were being given in this and other reports with a questionable number of significant figures. Expert Groups should consider this point in future recommendations since the number of significant figures sends an implicit message about the precision and accuracy of the values.

4.2.3 Technical annexes for PAH in biota and sediment and for TBT in sediment

These technical annexes were prepared in response to an OSPAR request that MCWG and WGMS received in 2007. The annexes appear to be complete and of good quality. Consideration should be given to publication in the TIMES series which will require some additional work on the documents with respect to the identification and referencing of background and supporting material (see note under 4.1.2).

5 List of actions

5.1 For the Expert Groups

1. WGBEC has provided a number of publications indicating that non-planar PCBs (and their metabolites) can have sub-lethal effects that are not the same as those observed with dioxin-like PCBs. The recommendation of the WGBEC should be accepted and techniques developed to determine EACs for the non-planar PCBs recognizing these other sub-lethal effects.

ACTION: In general there needs to be a discussion with WGBEC/ISGIMC and OSPAR regarding the future of EACs. If this concept is to be pursued for the preliminary assessment under the MSFD then considerable work needs to be done.

2. WGMS concluded that the proposed EACs for PCBs in sediments do not pose a problem for the JAMP since the EACs are in all cases greater than the proposed BCs. While this is correct WGMS did not consider the issue that WGBEC has raised regarding the appropriateness of using the EACs for individual PCB congeners given the nature of the data available for the assessment.

ACTION: It would be useful to have the reaction of WGMS to the concerns raised by WGBEC.

3. There was insufficient time to produce a technical annex for PFCs in sediments at the MCWG meeting. Much of the analytical information included in technical annex for PFCs in biota is directly relevant to sediment analysis and a MCWG subgroup in collaboration with WGMS will work intersessionally to provide a draft for finalisation at MCWG 2009. Furthermore the progress of ISO on the PFOS in water guidelines will be reviewed and a recommendation will be made regarding its appropriateness as a guideline for marine water samples.

ACTION: Note the request for intersessional work.

4. Regarding the development of background concentrations of dioxin in water, sediment, and biota. WGMS has provided some preliminary information and MCWG briefly discussed the topic but both have deferred this work until 2009. This will be a difficult task but WGMS and MCWG need to coordinate their efforts to insure consistency in their approach. They should consider whether or not the approach taken for proposing the BCs for alkylated PAHs would be applicable here.

   A single methods package was proposed for both PCBs and polychlorinated dibenzodioxins and furans due to the similarity of their toxicological effects (WKIMON IV 2008; WGBEC 2008). In addition to the OSPAR CEMP required determinands, additional PCBs may cause biological effects and their analysis should therefore be included in an integrated monitoring approach. These include the co-planar CBs like CB105 and CB 156.

ACTION: Coordination is required between MCWG and WGMS (chairs) on this as well as ICES approaching OSPAR to seek their agreement to the proposed approach.

5. Regarding proposals for background concentrations in biota with priority to metals in fish and shellfish:

   MCWG did not comment on the biological activity of these metals and the potential variation due to the stage in the life history of the mussels although was one of the reasons for not making recommendations for fish.

ACTION: Ask WGMS to comment on this.

6. The Review Group noted that in a number of instances values were being given in these reports with a questionable number of significant figures. Expert Groups should consider this point in future recommendations since the number of significant figures sends an implicit message about the precision and accuracy of the values.

ACTION: For the attention of the Expert Groups.

5.2 For OSPAR

1. Regarding the technical annexes for monitoring of dioxins, planar PCBs, and PFOS in biota and sediment, in the OSPAR request there is no list of compounds that the Technical Annex should cover. MCWG suggest that compounds with chorine substitution in the 2, 3, 7, and 8-positions are most toxic and obviously should be included in analyses. A table with a proposal of compounds that the guideline should cover is given.

ACTION: This should be commnunicated to OSPAR for agreement on the list of compounds to be included.

2. Since dioxins and planar CBs are often analysed together MCWG suggests that these two compound groups be included in the same technical annex. The technical annex can be based on that for chlorinated biphenyls, but the methodology will need to be revised. In collaboration MCWG and WGMS a subgroup is going to prepare draft technical annexes for dioxins/co-planar PCBs in biota and sediment for review and revision at MCWG 2009. ICES should discuss this with OSPAR to make certain that they understand what is being proposed and that they agree with this approach.

ACTION: Request agreement from OSPAR on the approach.

5.3 For ICES

1. Identification of the responses was made difficult due to the rewording in some of the EG resolutions, the carryover of some requests from previous years, and unsolicited updates provided by some EGs. The advice will have to be carefully documented to make certain that OSPAR is clear to which requests ICES is responding.

ACTION: A database of non-recurring advice is needed to keep track of the requests and the ICES response.

2. During the RGChem meeting there was a discussion about the effectiveness of this process, i.e. the RG/ADG review and ACOM approval, for these technical annexes. It was agreed that it would be more reasonable to handle them as a publication and a potential mechanism would be through publication in the ICES TIMES series. The draft text from the Expert Group(s) could be provided to client as a preliminary draft which would be finalized when the TIMES publication was released. It is likely that in some instances several technical annexes would be published in one TIMES edition. This suggestion needs to be brought to the ACOM and also needs to be discussed with the Expert Groups and then agreed upon by OSPAR and clients in general. The Expert Groups may have to spend more time producing the annexes but this would be offset by the fact that the TIMES reports are authored and are citable.

ACTION: This needs to go to the ACOM for approval in principle and then a detailed procedure prepared and implemented.

6 Approval of the minutes

The minutes were approved by members of the Review Group with all issues resolved on May 23, 2008.