

28 Technical annex: recommended packages of chemical and biological methods for monitoring on a determinant basis

Matt Gubbins, Patrick Roose, Ian M. Davies, and Dick Vethaak

28.1 Review of CEMP requirements

This technical annex was prepared by reviewing the chemical determinants listed in the OSPAR Coordinated Environmental Monitoring Programme (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:

- 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.
- 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 allow for temporally integrated sampling of contaminants in water. (Guidelines for the application of passive samplers are available from ICES Working Group on Marine Sediments in Relation to Pollution (WGMS).)
- The biological effects techniques applied to these packages of methods are listed either in the ICES Working Group on Biological Effects of Contaminants (WGBEC) recommended techniques list (ICES, 2007c) or form part of the fish and shellfish methods packages proposed in the draft Joint Assessment and Monitoring Programme (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 to monitoring selected fish species, shellfish (mussels), and bioassays (sediment, water, and *in vitro* tests).
- 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.
- 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-fold 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);
- perfluorooctane sulfonate (PFOS) in sediment, biota, and water (appendix 12);
- polychlorinated dibenzodioxins and furans in biota and sediment (appendix 13).

28.2 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. Diffusive gradients in thin films (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. It is recommended that it be applied only in areas where lead contamination is perceived to be a problem or where chemical monitoring indicates that concentrations are significantly above background.

ALA-D is relevant only for fish. 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 owing to the lack of standardized methods, quality assurance, 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 28.1. *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.

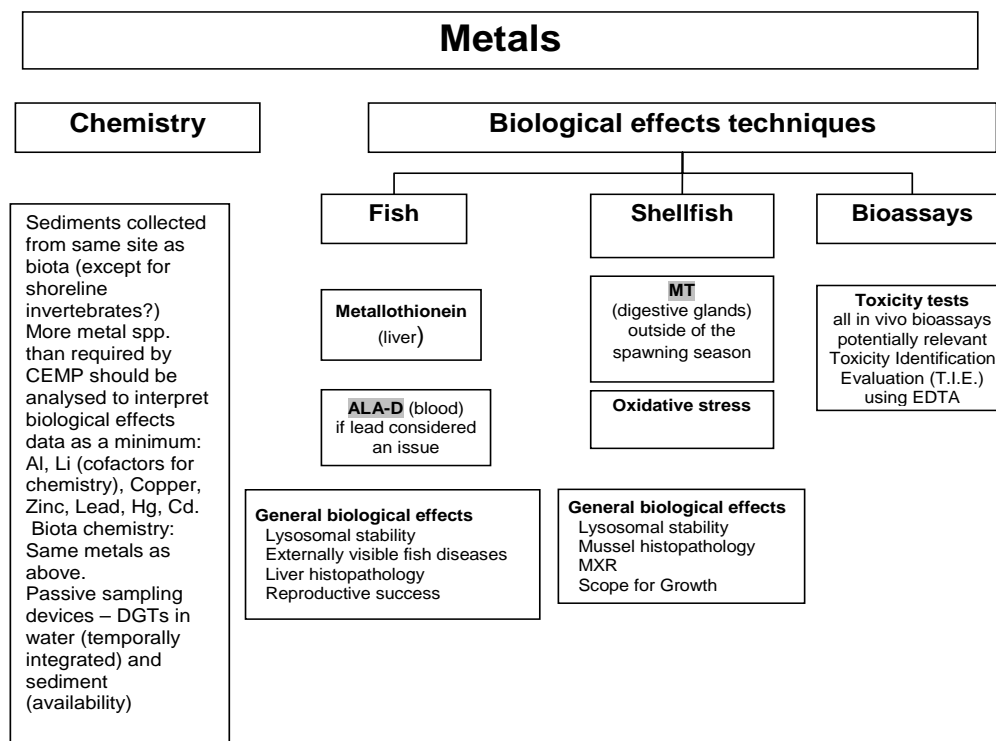


Figure 28.1. Package of chemical and biological effects methods relevant to monitoring for metals. The most specific/relevant biological effects methods are highlighted (bold, shade).

28.3 Methods package for PCBs, polychlorinated dibenzodioxins, and furans

Because of the similarity of their toxicological effects, a single methods package was proposed for polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins, and furans. In addition to the OSPAR CEMP required determinants, additional chlorinated biphenyls (CBs) may cause biological effects, and their analysis should be included in an integrated monitoring approach. These include coplanar CBs CB105, and CB156. 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 shown in Figure 28.2. DR-CALUX is considered the most useful *in vitro* bioassay technique, although chronic *in vivo* bioassays may also be relevant.

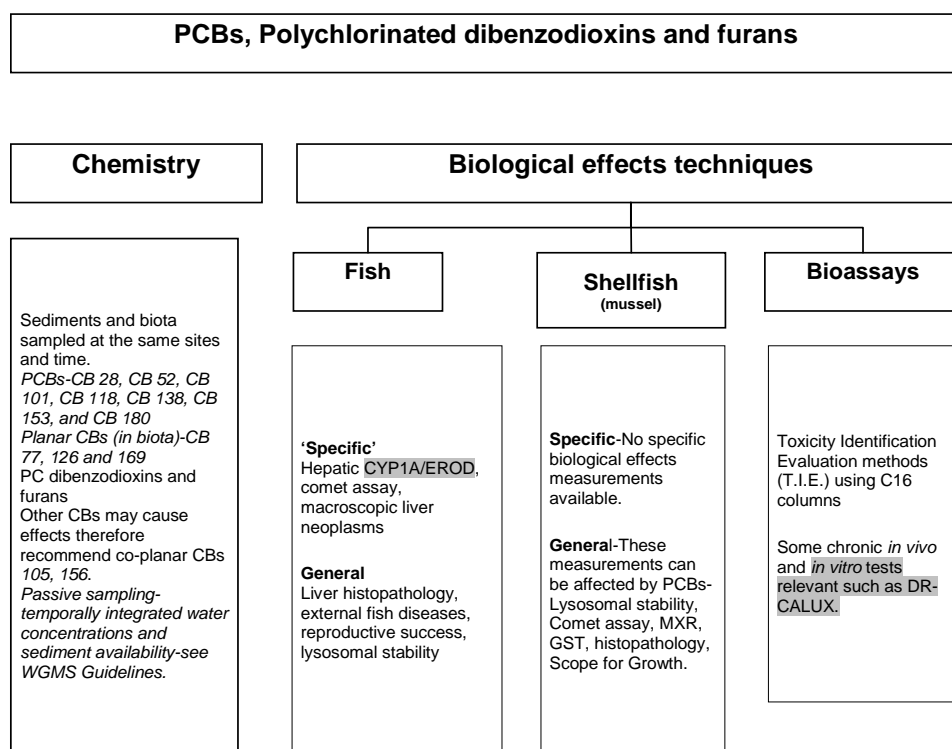


Figure 28.2. Package of chemical and biological effects methods relevant to monitoring for PCBs, polychlorinated dibenzodioxins, and furans. The most specific/relevant biological effects methods are highlighted (bold, shade).

28.4 Methods package for PAHs and alkylated PAHs

Because of their similar toxicological effects, a single package of methods is proposed for polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs (Figure 28.3). The package of methods is similar to Figure 28.2 above, although chemical determinants should be analysed in sediment and shellfish only for biota. Because of their rapid metabolism in finfish, PAHs should be analysed as metabolites in bile rather than as parent compounds in liver or flesh. As above, passive sampling should also be applied where possible.

Additional specific biological effects are applicable for PAHs/alkylated PAHs. 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 28.3.

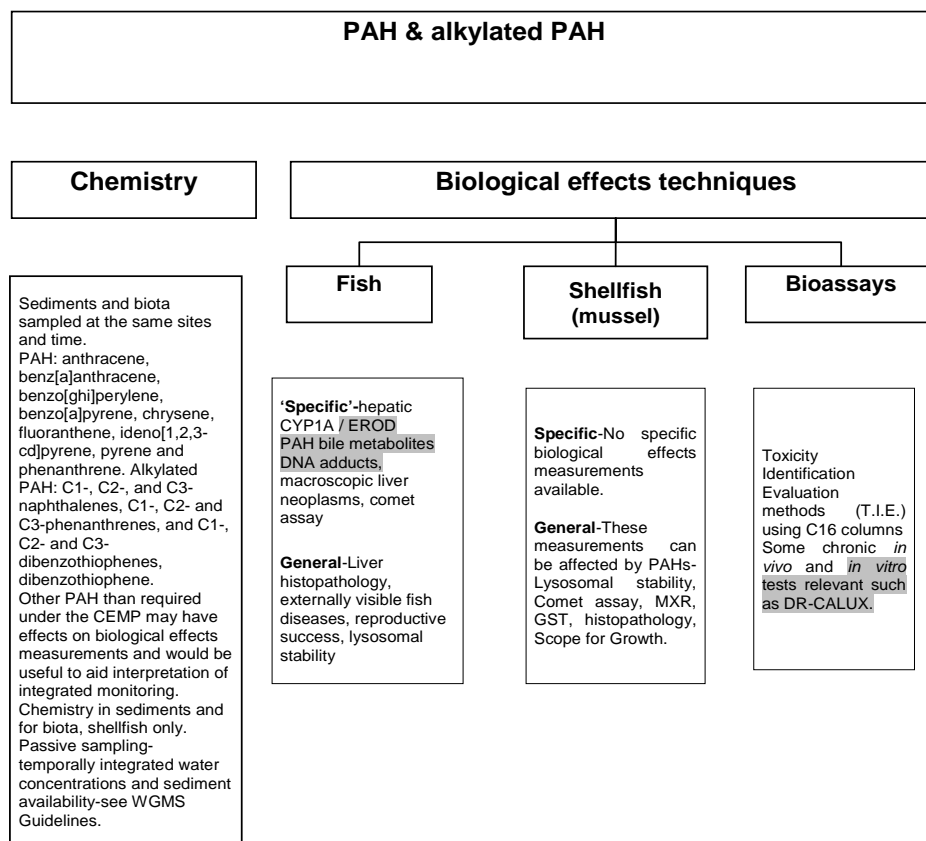


Figure 28.3. Package of chemical and biological effects methods relevant to monitoring for PAHs and alkylated PAHs. The most specific/relevant biological effects methods are highlighted (bold, shade).

28.5 Organotins

It was felt that the package of methods appropriate to 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 future. It was also noted that bivalve embryo bioassays are sensitive to dissolved TBT at the ng l⁻¹ level.

28.6 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 brominated flame retardants (BFRs). 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.

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.

28.7 PFOS

Perfluorooctane sulfonate (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 endocrine disruptor-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 bioanalysis 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.