1.5.5.16 JAMP Guidelines for monitoring contaminants in biota and sediments

Request

ICES has received the following request from OSPAR:

Development of JAMP monitoring guidelines (OSPAR no. 2-2007)

To carry out the following development work with regard to the JAMP Guidelines for monitoring Contaminants in Sediments (OSPAR agreement 2002-16) and JAMP Guidelines for monitoring Contaminants in Biota (OSPAR agreement 1999-2) to ensure that monitoring guidance is in place to support a revised Co-ordinated Environmental Monitoring Programme.

a. develop draft technical annexes on monitoring of polybrominated diphenyl ethers and hexabromocyclododecane in sediments and biota following the structure of the existing technical annexes. SIME 2007 will be invited to clarify the congeners and compartments that are relevant for the development of monitoring guidance for brominated flame retardants.

b. review the existing technical annexes on PAHs to see whether they are adequate for monitoring of the alkylated PAHs and, as appropriate, prepare advice on any revisions that are necessary.

c. to develop a draft technical annex on monitoring of TBT and its breakdown products in biota”

Advice on point (a) has been provided previously.

Summary

Alkyl PAH in sediment and biota

The current OSPAR technical annex for PAH analyses required updating as clear guidance was required to ensure quantification of alkyl homologues of PAHs and alkyl substituted sulphur-heterocyclic PAHs in biota and sediment. The proposed updated technical guidelines are presented in annexes 1 and 2 and are recommended to OSPAR for adoption as part of their JAMP guidelines for monitoring contaminants.

The technical annex for the analyses of parent and alkylated PAHs in biota contains information for the selection of species, sampling techniques, sample transport, conservation, and sample treatment (including extractions, clean-up, and pre-concentration).

The analytical protocol follows the same technical principles as for the analysis of unsubstituted, parent PAHs. However, HPLC with fluorescence detection (HPLC-UVF) cannot be used for the detailed analysis of individual alkylated PAHs. Gas chromatography with mass spectrometry (GC-MS) is presently the preferred analytical technique for the analysis of both parent and alkylated PAHs.

Organotins in biota

The technical annex for organotins in biota, as appended at annex 3 is proposed for adoption by OSPAR as part of the Joint Monitoring and Assessment Programme (JAMP) Guidelines for Monitoring Contaminants in Biota.

Explanation

The full text of the response is found in the attached technical annexes.

Sources of information

ANNEX 1: Polyaromatic hydrocarbons in biota

Determination of parent and alkylated PAHs in biological materials

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) consist of a variable number of fused benzene rings. By definition, PAHs contain at least two fused rings. PAHs arise from incomplete combustion processes and from both natural and anthropogenic sources, although the latter generally predominate. PAHs are also found in oil and oil products, and these include a wide range of alkylated PAHs formed as a result of diagenetic processes, whereas PAHs from combustion sources comprise mainly parent (non-alkylated) compounds. PAHs are of concern in the marine environment for two main reasons: firstly, low molecular weight (MW) PAHs can cause tainting of fish and shellfish and render them unfit for sale; secondly, metabolites of some of the high MW PAHs are potent animal and human carcinogens — benzo[a]pyrene is the prime example. Carcinogenic activity is closely related to structure. Benzo[e]pyrene and the four benzo[ghi]perylene isomers all have a molecular weight of 252 Da; however, they are much less potent than benzo[a]pyrene. Less is known about toxicity of alkylated PAHs. However, one study has demonstrated that alkylated PAHs may have increased toxicity compared to the parent compound (Marvanova et al., 2008).

PAHs are readily taken up by marine animals both across gill surfaces (lower MW PAHs) and from their diet. They may bioaccumulate, particularly in shellfish. Filter-feeding organisms such as bivalve molluscs can accumulate high concentrations of PAHs, both from chronic discharges to the sea (e.g., of sewage) and following oil spills. Fish are exposed to PAHs both via uptake across gill surfaces and from their diet, but do not generally accumulate high concentrations of PAHs as they possess an effective mixed-function oxygenase (MFO) system which allows them to metabolise PAHs and to excrete them in bile. Other marine vertebrate and marine mammals also metabolise PAHs efficiently. An assessment of the exposure of fish to PAHs therefore requires the determination of PAH metabolite concentrations in bile, as turnover times can be extremely rapid.

There are marked differences in the behaviour of PAHs in the aquatic environment between the low MW compounds (such as naphthalene; 128 Da) and the high MW compounds (such as benzo[ghi]perylene; 276 Da) as a consequence of their differing physico-chemical properties. The low MW compounds are appreciably water soluble (e.g. naphthalene) and can be bioaccumulated from the dissolved phase by transfer across gill surfaces, whereas the high MW compounds are relatively insoluble and hydrophobic, and can attach to both organic and inorganic particulates within the water column. PAHs derived from combustion sources may actually be deposited to the sea already adsorbed to atmospheric particulates, such as soot particles. The sediment will act as a sink for PAHs in the marine environment.

2. Appropriate species for analysis of parent and alkylated PAHs

2.1 Benthic fish and shellfish

Guidance on the selection of appropriate species for contaminant monitoring is given in the OSPAR Joint Assessment and Monitoring Programme guidelines. All teleost fish have the capacity for rapid metabolism of PAHs, thereby limiting their usefulness for monitoring temporal or spatial trends of PAHs. Shellfish (particularly molluscs) generally have a lesser metabolic capacity towards PAHs, and so they are preferred because PAH concentrations are generally higher in their tissues. The blue mussel (Mytilus edulis) occurs in shallow waters along almost all coasts of the Northeast Atlantic. It is therefore suitable for monitoring in near shore waters. No distinction is made between Mytilus edulis and M. galloprovincialis because the latter species, which may occur along Spanish and Portuguese coasts, fills a similar ecological niche. A sampling size range of 30–70 mm shell length is specified to ensure availability throughout the whole maritime area. In some areas (e.g., the Barents Sea), other species may be considered. Recent monitoring studies have indicated a seasonal cycle in PAH concentrations (particularly for combustion-derived PAHs) in mussels, with maximum concentrations in the winter prior to spawning and minimum concentrations in the summer. It is particularly important, therefore, that samples selected for trend monitoring and spatial comparisons are collected at the same time of year, and preferably in the first months of the year prior to spawning.

For the purposes of temporal trend monitoring, it is essential that long time-series with either a single species or a limited number of species be obtained. Care should be taken that the sample is representative of the population and that it can be sampled annually. There are advantages in the use of molluscs for this purpose as they are sessile, and so reflect the degree of contamination in the local area to a greater degree than fish which are mobile and metabolise PAHs relatively efficiently. The analysis of fish tissues is often undertaken in conjunction with biomarker and disease studies, and associations have been shown between the incidence of some diseases (e.g., liver neoplasia) in flatfish and the concentrations of PAHs in the sediments over which they live and feed (Malins et al., 1988; Vethaak and Rheinallt, 1992). The exposure of fish to PAHs can be assessed by the analysis of PAH metabolites in bile, and by measuring the induction of mixed-function oxygenase enzymes which catalyse the formation of these metabolites.
3. **Transportation**

Live biota should be transported in closed containers at temperatures between 5°C and 10°C. For live animals it is important that the transport time is short and controlled (e.g., maximum of 24 hours). If biomarker determinations are to be made, then it will be necessary to store tissue samples at lower temperatures, for example, in liquid nitrogen at –196°C.

4. **Pre-treatment and storage**

4.1 **Contamination**

Sample contamination may occur during sampling, sample handling, pre-treatment, and analysis, due to the environment, the containers or packing materials used, the instruments used during sample preparation, and from the solvents and reagents used during the analytical procedures. Controlled conditions are therefore required for all procedures. In the case of PAHs, particular care must be taken to avoid contamination at sea. On ships there are multiple sources of PAHs, such as the oils used for fuel and lubrication, and the exhaust from the ship’s engines. It is important that the likely sources of contamination are identified and steps taken to preclude sample handling in areas where contamination can occur. A ship is a working vessel and there can always be procedures occurring as a result of the day-to-day operations (deck cleaning, automatic overboard bilge discharges, etc.) which could affect the sampling process. One way of minimizing the risk is to conduct dissection in a clean area, such as within a laminar-flow hood away from the deck areas of the vessel. It is also advisable to collect samples of the ship’s fuel, bilge water, and oils and greases used on winches, etc., which can be used as fingerprinting samples at a later date, if there are suspicions of contamination in particular instances.

Freeze-drying of tissue samples may be a source of contamination due to the back-streaming of oil vapours from the rotary vacuum pumps. Furthermore, drying the samples may result in losses of the lower molecular weight and more volatile PAHs through evaporation (Law and Biscaya, 1994).

4.2 **Shellfish**

4.2.1 **Depuration**

Depending upon the situation, it may be desirable to depurate shellfish so as to void the gut contents and any associated contaminants before freezing or sample preparation. This is usually applied close to point sources, where the gut contents may contain significant quantities of PAHs associated with food and sediment particles which are not truly assimilated into the tissues of the mussels. Depuration should be undertaken in controlled conditions and in clean seawater; depuration over a period of 24 hours is usually sufficient. The aquarium should be aerated and the temperature and salinity of the water should be similar to that from which the animals were removed.

4.2.2 **Dissection and storage**

Mussels should be shucked live and opened with minimal tissue damage by detaching the adductor muscles from the interior of at least one valve. The soft tissues should be removed and homogenised as soon as possible, and frozen in glass jars or aluminium cans at –20°C until analysis. Plastic materials must not be used for sampling and storage owing to possible adsorption of the PAHs onto the container material. As PAHs are sensitive to photo-degradation, exposure to direct sunlight or other strong light must be avoided during storage of the samples as well as during all steps of sample preparation, including extraction and storage of the extracts (Law and Biscaya, 1994). The use of amber glassware is strongly recommended.

When samples are processed, both at sea and onshore, the dissection must be undertaken by trained personnel on a clean bench wearing clean gloves and using PAH-free stainless steel knives and scalpels. Stainless steel tweezers are recommended for holding tissues during dissection. After each sample has been prepared, all tools and equipment (such as homogenisers) should be cleaned by wiping with tissue and rinsing with solvent.

5. **Analysis**

5.1 **Preparation of materials**

Solvents and adsorptive materials must all be checked for the presence of PAHs and other interfering compounds. If found then the solvents, reagents, and adsorptive materials must be purified or cleaned using appropriate methods. Absorptive materials should be cleaned by solvent extraction and/or by heating in a muffle oven as appropriate. Glass fibre materials (e.g. Soxhlet thimbles and filter papers used in pressurised liquid extraction (PLE)) should be cleaned by solvent extraction or pre-baked at 450°C overnight. It should be borne in mind that clean materials can be re-
contaminated by exposure to laboratory air, particularly in urban locations, and so the method of storage after cleaning is of critical importance. Ideally, materials should be prepared immediately before use, but if they are to be stored, then the conditions should be considered critically. All containers which come into contact with the sample should be made of glass or aluminium, and should be pre-cleaned before use. Appropriate cleaning methods would include washing with detergents, rinsing with water of known quality, and finally solvent rinsing immediately before use.

5.2 Lipid determination

Although PAH data are not usually expressed on a lipid basis, the determination of the lipid content of tissues can be of use in characterising the samples. This will enable reporting concentrations on a wet weight or lipid weight basis. The lipid content should be determined on a separate subsample of the tissue homogenate, as some of the extraction techniques used routinely for PAHs determination (e.g., PLE with fat retainers, alkaline saponification) destroy or remove lipid materials. The total lipid content of fish or shellfish should be determined using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963) or an equivalent method such as Smedes (1999). Extractable lipid may be used, particularly if the sample size is small and lipid content is high. It has been shown that if the lipid content is high (>5%) then extractable lipid will be comparable to the total lipid.

5.3 Extraction

PAHs are lipophilic and so are concentrated in the lipids of an organism, and a number of methods have been described for PAH extraction (Ehrhardt et al., 1991). These methods generally utilise either Soxhlet extraction, or alkaline digestion followed by liquid-liquid extraction with an organic solvent. In the case of Soxhlet extraction, the wet tissue must be dried by mixing with a chemical drying agent (e.g., anhydrous sodium sulphate), in which case a time period of several hours is required between mixing and extraction in order to allow complete binding of the water in the sample. Samples are spiked with recovery standard and should be left overnight to equilibrate. Alkaline digestion is conducted on wet tissue samples, so this procedure is unnecessary.

Apolar solvents alone will not effectively extract all the PAHs from tissues when using Soxhlet extraction, and mixtures such as hexane/dichloromethane may be effective in place of solvents such as benzene and toluene, used historically for this purpose. Alkaline digestion has been extensively used in the determination of PAHs and hydrocarbons and is well documented. It is usually conducted in alcohol (methanol or ethanol), which should contain at least 10% water, and combines disruption of the cellular matrix, lipid extraction and saponification within a single procedure, thereby reducing sample handling and treatment. Solvents used for liquid-liquid extraction of the homogenate are usually apolar, such as pentane or hexane, and they will effectively extract all PAHs.

Alternatively extraction of wet or dry samples of biota may be carried out by pressurised liquid extraction (PLE). This is a more recent method, requiring less solvent and time for the extraction process. The wet biota sample is dried by mixing with sufficient anhydrous sodium sulphate to form a free flowing mixture and is packed into stainless steel extraction cells containing a glass fibre filter and sodium sulphate or glass powder to fill the cell. To ensure a better recovery samples may be extracted twice and extractions are performed at elevated temperatures and pressure.

5.4 Clean-up

Tissue extracts will always contain many compounds other than PAHs, and a suitable clean up is necessary to remove those compounds which may interfere with the subsequent analysis. Different techniques may be used, either singly or in combination, and the choice will be influenced by the selectivity and sensitivity of the final measurement technique and also by the extraction method employed. If Soxhlet extraction was used, then there is a much greater quantity of residual lipid to be removed before the analytical determination can be made than in the case of alkaline digestion. An additional clean-up stage may therefore be necessary. The most commonly used clean-up methods involve the use of deactivated alumina or silica adsorption chromatography. When applying fractionation, the elution pattern has to be checked frequently. This should be carried out in the presence of sample matrix, as that can partially deactivate the clean-up column, resulting in earlier elution of the PAHs than in a standard solution.

Gel permeation chromatography (GPC) and high performance liquid chromatography (HPLC) based methods are also employed (Nondek et al., 1993; Nyman et al., 1993; Perfetti et al., 1992). The major advantages of using HPLC-based clean-up methods are their ease of automation and reproducibility.

Isocratic HPLC fractionation of the extract can be used to give separate aliphatic and aromatic fractions (Webster et al., 2002). A metal-free silica column is used for the clean-up/fractionation as dibenzothiophene (DBT) can be retained on ordinary silica columns. The split time is determined by injection of a solution containing representative aliphatic and PAH standards. The silica column is regenerated by a cleaning cycle after a set number of samples. If PAHs are to be analysed by HPLC and there are significant amounts of alkylated PAHs present then the removal of the alkylated PAHs may be difficult.
5.5 Pre-concentration

In the methods suggested above, all result in an extract in which non-polar solvents are dominant. The sample volume should be 2 ml or greater to avoid errors when transferring solvents during the clean-up stages. Syncore parallel evaporators can be used with careful optimisation of the evaporation parameters. Evaporation of solvents using a rotary-film evaporator should be performed at low temperature (water bath temperature of 30°C or lower) and under controlled pressure conditions, in order to prevent losses of the more volatile PAHs such as naphthalenes. For the same reasons, evaporation to dryness must be avoided. When reducing the sample to final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the GC-MS include pentane, hexane, heptane, iso-hexane and iso-octane.

5.6 Selection of PAHs to be determined

The choice of PAHs to be analysed is not straightforward, both because of differences in the range of PAH compounds resulting from combustion processes and from oil and oil products, and also because the aims of specific monitoring programmes can require the analysis of different representative groups of compounds. PAHs arising from combustion processes are predominantly parent (unsubstituted) compounds, whereas oil and its products contain a much wider range of alkylated compounds in addition to the parent PAHs. This has implications for the analytical determination, as both HPLC-based and GC-based techniques are adequate for the determination of a limited range of parent PAHs in samples influenced by combustion processes, whereas in areas of significant oil contamination and following oil spills only GC-MS has sufficient selectivity to determine the full range of PAHs present. The availability of pure individual PAHs for the preparation of standards is problematic and limits both the choice of determinands and, to some degree, the quantification procedures which can be used. The availability of reference materials certified for PAHs is also rather limited. A list of target parent and alkylated PAHs suitable for environmental monitoring is given in Table A1.1. This differs both from the list previously developed within ICES specifically for intercomparison purposes, and the historic list of Borneff. In both cases, the lists were concentrated on a subset of parent (predominantly combustion-derived) PAHs due to analytical limitations. This approach completely neglects the determination of alkylated PAHs, which allows the interpretation of PAH accumulation from multiple sources including those due to oil inputs. It will not be necessary for all of these PAH compounds and groups to be analysed in all cases, but an appropriate selection can be made from this list depending on the specific aims of the monitoring programme to be undertaken.

Table A1.1 Compounds of interest for environmental monitoring for which the guidelines apply. For compounds in italics standards are not available for any isomers in this group.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>Compound</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>128</td>
<td>2, 3d-benzonaphthothiophene</td>
<td>234</td>
</tr>
<tr>
<td>C1-Naphthalenes</td>
<td>142</td>
<td>C1-234</td>
<td>248</td>
</tr>
<tr>
<td>C2-Naphthalenes</td>
<td>156</td>
<td>C2-Fluoranthenes/Pyrenes</td>
<td>230</td>
</tr>
<tr>
<td>C3-Naphthalenes</td>
<td>170</td>
<td>Benz[a]anthracene</td>
<td>228</td>
</tr>
<tr>
<td>C4-Naphthalenes</td>
<td>184</td>
<td>Chrysene</td>
<td>228</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>152</td>
<td>2,3-Benzanthracene</td>
<td>228</td>
</tr>
<tr>
<td>Acenaphthen</td>
<td>154</td>
<td>C1- Benz[a]anthracene/ Chrysene</td>
<td>242</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>154</td>
<td>C2- Benz[a]anthracene/ Chrysene</td>
<td>256</td>
</tr>
<tr>
<td>Fluorene</td>
<td>166</td>
<td>C3- Benz[a]anthracene/ Chrysene</td>
<td>270</td>
</tr>
<tr>
<td>C1-Fluorenes</td>
<td>180</td>
<td>Benzolo[a]fluoranthene</td>
<td>252</td>
</tr>
<tr>
<td>C2-Fluorenes</td>
<td>194</td>
<td>Benzolo[b]fluoranthene</td>
<td>252</td>
</tr>
<tr>
<td>C3-Fluorenes</td>
<td>208</td>
<td>Benzolo[f]fluoranthene</td>
<td>252</td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>184</td>
<td>Benzolo[k]fluoranthene</td>
<td>252</td>
</tr>
<tr>
<td>C1-Dibenzothiophenes</td>
<td>198</td>
<td>Benzolo[e]pyrene</td>
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<td>C2-Dibenzothiophenes</td>
<td>212</td>
<td>Benzolo[a]pyrene</td>
<td>252</td>
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<tr>
<td>C3-Dibenzothiophenes</td>
<td>226</td>
<td>Perylene</td>
<td>252</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>178</td>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>276</td>
</tr>
<tr>
<td>Anthracene</td>
<td>178</td>
<td>Benzolo[ghi]pyrene</td>
<td>276</td>
</tr>
<tr>
<td>C1-Phenanthrenes/Anthracenes</td>
<td>192</td>
<td>Dibenzolo[a,h]anthracene</td>
<td>278</td>
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<tr>
<td>C2-Phenanthrenes/Anthracenes</td>
<td>206</td>
<td>Benzolo[k]fluoranthene</td>
<td>252</td>
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<tr>
<td>C3-Phenanthrenes/Anthracenes</td>
<td>220</td>
<td>Cyclopenta[cd]pyrene</td>
<td>226</td>
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<tr>
<td>Fluoranthene</td>
<td>202</td>
<td>Naphtho[2,1-a]pyrene</td>
<td>302</td>
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<tr>
<td>Pyrene</td>
<td>202</td>
<td>Dibenzolo[a,e]pyrene</td>
<td>302</td>
</tr>
<tr>
<td>C1-Fluoranthenes/Pyrenes</td>
<td>216</td>
<td>Dibenzolo[a,f]pyrene</td>
<td>302</td>
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<tr>
<td>2, 1d-benzonaphthothiophene</td>
<td>234</td>
<td>Dibenzolo[a,l]pyrene</td>
<td>302</td>
</tr>
<tr>
<td>1,2d-benzonaphthothiophene</td>
<td>234</td>
<td>Dibenzolo[a,h]pyrene</td>
<td>302</td>
</tr>
</tbody>
</table>
5.7 Instrumental determination of PAHs

The greatest sensitivity and selectivity in routine analysis for parent PAH is achieved by combining HPLC with fluorescence detection (HPLC-UVF) or capillary gas chromatography with mass spectrometry (GC-MS). However, for the analysis of parent and alkylated PAHs GC-MS is the method of choice. In terms of flexibility, GC-MS is the most capable technique, as in principle it does not limit the selection of determinands in any way, while HPLC is suited only to the analysis of parent PAHs. In the past, analyses have also been conducted using HPLC with UV-absorption detection and GC with flame-ionisation detection, but neither can be recommended for alkylated PAHs because of their relatively poor selectivity. Both in terms of the initial capital cost of the instrumentation, and the cost per sample analysed, HPLC-UVF is cheaper than GC-MS. With the advent of high-sensitivity benchtop GC-MS systems, however, this cost advantage is now not as marked as in the past, and the additional information regarding sources available makes GC-MS the method of choice.

Limits of determination within the range of 0.05 to 0.5 µg kg\(^{-1}\) wet weight for individual PAH compounds should be achievable by GC-MS. However this limit can be lowered in routine analysis.

5.7.1 GC-MS

The three injection modes commonly used are splitless, on-column and PTV (programmed temperature vaporiser). Automatic sample injection should be used wherever possible to improve the reproducibility of injection and the precision of the overall method. If splitless injection is used, the liner should be of sufficient capacity to contain the injected solvent volume after evaporation. For PAH analysis, the cleanliness of the liner is also very important if adsorption effects and discrimination are to be avoided, and the analytical column should not contain active sites to which PAHs can be adsorbed. Helium is the preferred carrier gas, and only capillary columns should be used. Because of the wide boiling range of the PAHs to be determined and the surface-active properties of the higher PAHs, the preferred column length is 25–50 m, with an internal diameter of 0.15 mm to 0.3 mm. Film thicknesses of 0.2 µm to 1 µm are generally used; this choice has little impact on critical resolution, but thicker films are often used when one-ring aromatic compounds are to be determined alongside PAHs, or where a high sample loading is needed. No stationary phase has been found on which all PAH isomers can be resolved; the most commonly used stationary phase for PAH analysis is 5% phenyl methylsilicone (DB-5 or equivalent). This will not, however, resolve critical isomers such as benzo\([b]\), \([j]\) and \([k]\)fluoranthenes, or chrysene from triphenylene. Chrysene and triphenylene can be separated on other columns, if necessary such as a 60 m non polar column such a DB5MS. For PAHs there is no sensitivity gain from the use of chemical ionisation (either positive or negative ion), so analyses are usually conducted in electron-impact mode at 70eV. Quadrupole instruments are used in single ion monitoring to achieve greater sensitivity. The masses to be detected are programmed to change during the analysis as different PAHs elute from the capillary column. In SIM the molecular ion is used for quantification. Qualifier ions can be used to confirm identification but they are limited for PAHs. Triple quadrupole mass spectrometry can also be used and will give greater sensitivity. Some instruments such as ion-trap and time of flight mass spectrometers exhibit the same sensitivity in both modes, so full scan spectra can be used for quantification.

An example of mass spectrometer operating conditions in SIM mode is given in Table A1.2. The ions are grouped and screened within GC time windows of the compounds. In general the number of ions should not be greater than 20. The dwell time is important parameter and should be close for each ion. For GC capillary column analysis a dwell time should not be shorter than 20 ms, while a sum of a dwell in each retention time windows should not be greater than 500 ms. An example of conditions that can be used along with dwell times are shown in Table A1.2.
Table A.1.2  Example of operational conditions for the GC-MS analysis of parent and alkylated PAHs.

<table>
<thead>
<tr>
<th>Group N°</th>
<th>Retention time (min)</th>
<th>Dwell time (ms)</th>
<th>Ions in group (AMU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.00</td>
<td>100</td>
<td>128 136 142</td>
</tr>
<tr>
<td>2</td>
<td>21.00</td>
<td>100</td>
<td>152 156 160</td>
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<tr>
<td>3</td>
<td>23.70</td>
<td>100</td>
<td>154 164 168 170</td>
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<td>4</td>
<td>26.80</td>
<td>80</td>
<td>166 176 180 182 184</td>
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<td>5</td>
<td>31.60</td>
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<td>12</td>
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<td>100</td>
<td>252 256 264 266</td>
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<tr>
<td>13</td>
<td>59.00</td>
<td>100</td>
<td>266 276 278 288</td>
</tr>
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</table>

Alkylated homologues of PAHs (C1–C4), mainly associated with petrogenic sources, contain a number of different isomers that can give very complex but distinct distribution profiles when analysed by GC-MS. Integration of each isomer separately is difficult for most alkylated PAHs. 1- and 2-Methyl naphthalene give well resolved peaks that can be quantified separately. C1-Phenanthrene/anthracene gives five distinct peaks corresponding to 3-methyl phenanthrene, 2-methyl phenanthrene, 2-methyl anthracene, 4- and 9-methyl phenanthrene and 1-methyl phenanthrene. These may be integrated as a group or as separate isomers. For all other alkylated PAHs the area for all isomers may be summed and quantified against a single representative isomer. This method will lead, however, to an overestimation of the concentration as may include non alkylated PAHs. Examples of integrations of both parent and alkylated PAHs are shown in Appendix 1.

6.  Calibration and quantification

6.1 Standards

The availability of pure PAH compounds are limited. Although most of the parent compounds can be purchased as pure compounds, the range of possible alkyl-substituted PAHs is vast and only a limited selection of them can be obtained. PAH standards are available for at least one isomer of most alkyl group listed in Table A1.1. A range of deuterated PAHs (normally 5 to 7) should be used as internal standards to cover the range of PAHs being analysed in samples. A range of fully-deuterated parent PAHs is available for use as standards in PAH analysis. Suitable standards could range from d8-naphthalene to d14-dibenz[a,h]anthracene. Crystalline PAHs of known purity should be used for the preparation of calibration standards. If the quality of the standard materials is not guaranteed by the producer or supplier (as for certified reference materials), then it should be checked by GC-MS analysis. Solid standards should be weighed to a precision of 10–5 grams. Calibration standards should be stored in the dark because some PAHs are photosensitive, and ideally solutions to be stored should be sealed in amber glass ampoules or sealed GC vials. Otherwise, they can be stored in a refrigerator in stoppered measuring cylinders or flasks that are gas tight to avoid evaporation of the solvent during storage.

6.2 Calibration

Multilevel calibration with at least five calibration levels is preferred to adequately define the calibration curve. In general, GC-MS calibration is linear over a considerable concentration range but may exhibit a change of slope at very low concentrations. Quantification should be conducted in the linear region of the calibration curve. A separate calibration curve may be used where sample concentrations are very low. An internal standard method should be employed, using a range of deuterated PAHs as internal standards.

6.3 Recovery

The recovery of analytes should be checked and reported. Given the wide boiling range of the PAHs to be determined, the recovery may vary with compound group, from the volatile PAHs of low molecular weight to the larger compounds. Deuterated standards can be added in two groups: those to be used for quantification are added at the start of the analytical procedure, whilst those from which the absolute recovery will be assessed are added prior to GC-MS injection. This allows the recovery to be calculated.
7. Analytical Quality Control

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination which they consider acceptable. Achievable limits of determination for each individual component are as follows:

- for GC-MS measurements: 0.05 μg kg⁻¹ ww;

Further information on analytical quality control procedures for PAHs can be found elsewhere (Law and de Boer, 1995). A procedural blank should be measured with each sample batch, and should be prepared simultaneously using the same chemical reagents and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will result in errors in quantification. The procedural blank is also very important in the calculation of limits of detection and limits of quantification for the analytical method. In addition, a laboratory reference material (LRM) should be analysed within each sample batch. The LRM must be homogeneous and well-characterised for the determinands of interest within the analytical laboratory. Ideally, stability tests should have been undertaken to show that the LRM yields consistent results over time. The LRM should be of the same matrix type (e.g. mussels) as the samples, and the determinand concentrations should be in the same range as those in the samples. Realistically, and given the wide range of PAH concentrations encountered, particularly in oil spill investigations, this is bound to involve some compromise. The data produced for the LRM in successive sample batches should be used to prepare control charts. It is also useful to analyse the LRM in duplicate from time to time to check within-batch analytical variability. The analysis of an LRM is primarily intended as a check that the analytical method is under control and yields acceptable precision, but a certified reference material (CRM) of a similar matrix should be analysed periodically in order to check the method bias. The availability of biota CRMs certified for PAHs is very limited, and in all cases the number of PAHs for which certified values are provided is small. At present, only NIST 1974a (a frozen wet mussel tissue) and NIST 2974 (a freeze-dried mussel tissue) are available. At regular intervals, the laboratory should participate in an intercomparison or proficiency exercise in which samples are circulated without knowledge of the determinand concentrations, in order to provide an independent check on performance.

8. Data reporting

The calculation of results and the reporting of data can represent major sources of error, as has been shown in intercomparison studies for PAHs. Control procedures should be established in order to ensure that data are correct and to obviate transcription errors. Data stored on databases should be checked and validated, and checks are also necessary when data are transferred between databases. Data should be reported in accordance with the latest ICES reporting formats.

9. References


Revision of the Nordtest Methodology for oil spill identification, http://www.nordicinnovation.net/nordtestfiler/tec499.pdf


ANNEX 1 – APPENDIX 1

Examples of integration of parent and alkylated PAHs analysed by GC-MS. The standards used for the calibration of the alkylated PAHs are asterixed.
Ion 178.00 (177.70 to 178.70): EPG1132.D data.ms

Ion 192.00 (191.70 to 192.70): EPG1132.D data.ms

Ion 206.00 (205.70 to 206.70): EPG1132.D data.ms

Ion 220.00 (219.70 to 220.70): EPG1132.D data.ms
4-methyldibenzothiophene

C1-DBT

C2-DBT

C3-DBT
benzofluoranthenes

benzo[e]pyrene

benzo[a]pyrene

pyrene

indeno[123-cd]pyrene

benzo[ghi]perylene
ANNEX 2

Technical annex: Polyaromatic hydrocarbons in sediments

Determination of parent and alkylated PAHs in sediments

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) consist of a variable number of fused aromatic rings. By definition, PAHs contain at least two fused rings. PAHs arise from incomplete combustion processes and from both natural and anthropogenic sources, although the latter generally predominate. PAHs are also found in oil and oil products, and these include a wide range of alkylated PAHs formed as a result of diagenetic processes, whereas PAHs from combustion sources comprise mainly parent (non-alkylated) PAHs. Metabolites of some of the high MW PAHs are potent animal and human carcinogens – benzo[α]pyrene is the prime example. Carcinogenic activity is closely related to structure. Benzo[e]pyrene and the four benzo[a]anthracene isomers all have a molecular weight of 252 Da, however they are much less potent than benzo[α]pyrene. Less is known about toxicity of alkylated PAHs. However, one study has demonstrated that alkylated PAHs may have increased toxicity compared to the parent compound (Marvanova et al., 2008).

This Technical Annex provides advice on the analysis of parent and alkylated polycyclic aromatic hydrocarbons (PAH) in total sediment, sieved fractions, and suspended particulate matter. The analysis of in sediments generally includes extraction with organic solvents, clean-up, high performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection or gas chromatographic (GC) separation with flame ionisation (FID) or mass spectrometric (MS) detection (e.g., Fetzer and Vo-Dinh, 1989; Wise et al., 1995). All steps in the procedure are susceptible to insufficient recovery and/or contamination. Quality control procedures are recommended in order to check the performance of the method. These guidelines are intended to encourage and assist analytical chemists to critically reconsider their methods and to improve their procedures and/or the associated quality control measures, where necessary.

These guidelines are not intended as a complete laboratory manual. If necessary, guidance should be sought from highly specialised research laboratories. Whichever procedure is adopted, each laboratory must demonstrate the validity of each step of its procedure. In addition, the use of a second (and different method), carried out concurrently to the routine procedure, is recommended for validation. The analyses must be carried out by experienced staff.

2. Pre-treatment and Storage

2.1 Contamination

Sample contamination may occur during sampling, sample handling, pre-treatment and analysis, due to the environment, the containers or packing materials used, the instruments used during sample preparation, and from the solvents and reagents used during the analytical procedures. Controlled conditions are therefore required for all procedures. In the case of PAHs, particular care must be taken to avoid contamination at sea. On ships there are multiple sources of PAHs, such as the oils used for fuel and lubrication, and the exhaust from the ship’s engines. It is important that the likely sources of contamination are identified and steps taken to preclude sample handling in areas where contamination can occur. A ship is a working vessel and there can always be procedures occurring as a result of the day-to-day operations (deck cleaning, automatic overboard bilge discharges, etc.) that could affect the sampling process. It is advisable to collect samples of the ship’s fuel, bilge water, and oils and greases used on winches, etc., which can be used as fingerprinting samples at a later date, if there are suspicions of contamination in particular instances.

Freeze-drying of sediment samples may be a source of contamination due to the back-streaming of oil vapours from the rotary vacuum pumps. Furthermore drying the samples may result in losses of the lower molecular weight, more volatile PAHs through evaporation (Law et al., 1994).

Plastic materials must not be used for sampling and storage owing to possible adsorption of the PAHs onto the container material. Samples should be transported in closed containers; a temperature of 25°C should not be exceeded. If the samples are not analysed within 48 hours after sampling, they must be stored at 4°C (short-term storage). Storage over several months is only possible for frozen, (i.e., below −20°C) and/or dried samples (Law and de Boer, 1995).

As PAHs are sensitive to photo-degradation, exposure to direct sunlight or other strong light must be avoided during storage of the samples as well as during all steps of sample preparation, including extraction and storage of the extracts (Law and Biscaya, 1994). The use of amber glassware is strongly recommended.
2.3 Blanks

The procedural detection limit is determined by the blank value. In order to keep the blank value as low as possible, PAHs or other interfering compounds should be removed from all glassware, solvents, chemicals, adsorption materials, etc., that are used in the analysis. The following procedures should be used:

- glassware should be thoroughly washed with detergents and rinsed with an organic solvent prior to use. Further cleaning of the glassware, other than calibrated instruments, can be carried out by heating at temperatures >250°C;
- all solvents should be checked for impurities by concentrating the amount normally used to 10% of the normal end volume. This concentrate can then be analysed by GC and should not contain significant amounts of PAHs or other interfering compounds;
- all chemicals and adsorption materials should be checked for impurities and purified (e.g., by heating or extraction), if necessary. Soxhlet thimbles should be pre-extracted. Glassfibre thimbles are preferred over paper thimbles. Alternatively, full glass Soxhlet thimbles, with a G1 glass filter at the bottom, can be used. The storage of these supercleaned materials for a long period is not recommended, as laboratory air can contain PAHs that will be absorbed by these materials. Blank values occurring despite all the above-mentioned precautions may be due to contamination from the air. The most volatile compounds will usually show the highest blanks (Gremm and Frimmel, 1990).
- Glassfibre filters used for the PLE (pressurised liquid extraction) method should be heated at 450°C overnight.

3. Pre-treatment

Before taking a subsample for analysis, the samples should be sufficiently homogenised. The intake mass is dependent on the expected concentrations. For the marine environment, as a rule of thumb, the mass of sample taken for analysis can be equal to an amount representing 50–100 mg organic carbon. PAHs can be extracted from wet or dried samples. However, storage, homogenisation and extraction are much easier when the samples are dry. Care must be taken if freeze-drying samples for the reasons described in 2.1. Possible losses and contamination have to be checked. Contamination can be checked by exposing 1–2 g C18-bonded silica to drying conditions and analysing it as a sample (clean-up can be omitted) (Smedes and de Boer, 1997). Contamination during freeze-drying can be reduced by placing a lid, with a hole about 3 mm in diameter, on the sample container, while evaporation of the water is not hindered.

4. Extraction and clean-up

Exposure to light must be kept to a minimum during extraction and further handling of the extracts (Law and Biscaya, 1994). Since photo-degradation occurs more rapidly in the absence of a sample matrix, first of all the standard solution used for checking the recovery of the procedure will be affected, allowing a proper detection of the influence of light. The most photo-sensitive PAH is benzo[a]pyrene, followed by anthracene.

4.1 Wet sediments

Wet sediments should be extracted using a stepwise procedure by mixing with organic solvents. Extraction is enhanced by shaking, Ultra Turrax mixing, ball mill tumbling or ultrasonic treatment. Water-miscible solvents, such as acetone, methanol, or acetonitrile, are used in the first step. The extraction efficiency of the first step will be low as there is a considerable amount of water in the liquid phase. For sufficient extraction, at least three subsequent extractions are needed. The contact time with the solvent should be sufficient to complete the desorption of the PAHs out of the sediment pores. Heating by microwave or refluxing will accelerate this process.

When utilising a Soxhlet, the extraction of wet sediments should be conducted in two steps. First, a polar solvent, such as acetone, is used to extract the water from the sediment, then the flask is replaced and the extraction continued with a less polar solvent or solvent mixture (e.g., acetone/hexane). Thereafter, the extracts must be combined. For both batch and Soxhlet extraction, water must be added to the combined extracts and the PAHs must be extracted to a non-polar solvent.

Extraction of wet sediments by pressurised liquid extraction (PLE) is a more recent method, requiring less solvent and time for the extraction process. Wet sediment is dried by mixing with sufficient anhydrous sodium sulphate to form a free flowing mixture and is packed into stainless steel tubes for extraction. Extractions are performed at elevated temperatures and pressures. Various extracting solvents (DCM, acetone, methanol, acetonitrile, hexane, DCM: acetone [1:1], hexane:acetone [1:1] were investigated by Saim et al. (1998) and as long as the solvent polarity was >1.89 (i.e. all
except hexane) no significant differences were noted. Extraction temperatures can be manipulated to suit the analytical requirements.

### 4.2 Dry sediments

Although all the methods mentioned above can also be used for dried sediments, Soxhlet extraction is the most frequently applied technique to extract PAHs from dried sediments. Medium-polar solvents such as dichloromethane or toluene, or mixtures of polar and non-polar solvents can be used. When using dichloromethane, losses of PAHs have occasionally been observed (Baker, 1993). Although toluene is not favoured because of its high boiling point, it should be chosen as solvent when it is expected that sediment samples contain soot particles. For routine marine samples, the use of a mixture of a polar and a non-polar solvent (e.g., acetone/hexane (1/3, v/v)) is recommended.

The extraction can be carried out with a regular or a hot Soxhlet (Smedes and de Boer, 1997). A sufficient number of extraction cycles must be performed (approximately 8 hours for the hot Soxhlet and 12 to 24 hours for normal Soxhlet). The extraction efficiency has to be checked for different types of sediments by a second extraction step. These extracts should be analysed separately.

PLE can also be used for the extraction of freeze-dried sediments. Instead of anhydrous sodium sulphate to dry the sediment the sample is mixed with a clean sand or diatomaceous earth to increase the surface area of the sediment. The same solvent mixtures detailed above for wet sediment extraction can be used for the dry sediments. Supercritical fluid extraction (SFE) has also been used for the extraction of organic compounds. The optimum conditions may vary for specific sediments (e.g., Dean et al., 1995; Reimer and Suarez, 1995).

### 4.3 Clean-up

The crude extract requires a clean-up to remove the many other compounds which are co-extracted (e.g., Wise et al., 1995). Due to chlorophyll-like compounds extracted from the sediment, the raw extract will be coloured and also contain sulphur and sulphur-containing compounds, oil, and many other natural and anthropogenic compounds. Selection of the appropriate clean-up method depends on the subsequent instrumental method to be used for analysis. Prior to the clean-up, the sample must be concentrated and any polar solvents used in the extraction step should be removed. The recommended acetone/hexane mixture will end in hexane when evaporated because of the formation of an azeotrope. Evaporation can be done either using a rotary evaporator or parallel evaporating systems such as Syncore. Especially for the rotary evaporator, care should be taken to stop the evaporation in time at about 5 ml. For further reducing the volume, a gentle stream of nitrogen should be applied. The extract should never be evaporated to dryness. The drawback of the rotary evaporator is that more volatile components may be lost during the nitrogen drying stage whilst the heavier components stick to the glassware. The Buchi Syncore Analyst also uses glass tubes but the system is sealed, avoiding contamination from the lab air during evaporation. It does not use a nitrogen stream, thus reducing the loss of volatiles and if the flushback module is fitted the sides of the tubes are rinsed automatically thus reducing the loss of the heavier components.

For removing more polar interferences from the extract, deactivated aluminium oxide (10 % water), eluted with hexane, as well as silica or modified silica columns, e.g., aminopropylsilane, eluted with toluene or a semipolar solvent mixture such as hexane/acetonitrile (95/5, v/v) or hexane/dichloromethane (98/2, v/v), can be used. Gel permeation chromatography (GPC) can be used to remove high molecular weight material and sulphur from the extracts.

For GC-MS analysis, sulphur should be removed from the extracts, in order to protect the detector. This can be achieved by the addition of copper powder, wire or gauze during or after Soxhlet extraction. Copper can also be added to the PLE cell, however, this is not always sufficient and further treatment with copper may be required following extraction. Ultrasonic treatment might improve the removal of sulphur. As an alternative to copper, other methods can be used (Smedes and de Boer, 1997).

Aliphatic hydrocarbons originating from mineral oil interfere with the flame ionisation detection. They can be removed from the extract by fractionation over columns filled with activated aluminium oxide or silica. The first fraction eluting with hexane is rejected. The PAHs elute in a second fraction with a more polar solvent, e.g., diethylether or acetone/hexane. When applying fractionation, the elution pattern has to be checked frequently. This should be carried out in the presence of sample matrix, as that can partially deactivate the clean-up column, resulting in earlier elution of the PAHs than in a standard solution.

Gel permeation chromatography (GPC) and high performance liquid chromatography (HPLC) based methods are also employed (Nondek et al., 1993; Nyman et al., 1993; Perfetti et al., 1992). The major advantages of using HPLC-based clean-up methods are their ease of automation and reproducibility.
Isocratic HPLC fractionation of the extract can be used to give separate aliphatic and aromatic fractions (Webster et al., 2002). A metal free silica column is used for the clean up/fractionation as dibenzothiophene (DBT) can be retained on ordinary silica columns. The split time is determined by injection of a solution containing representative aliphatic and PAH standards. The silica column is regenerated by a cleaning cycle after a set number of samples. If PAHs are to be analysed by HPLC and there are significant amounts of alkylated PAHs present then the removal of the alkylated PAHs may be difficult.

4.4 Pre-concentration

In the methods suggested above, all result in an extract in which non-polar solvents are dominant. The sample volume should be 2 ml or greater to avoid errors when transferring solvents during the clean-up stages. Syncore parallel evaporators can be used with careful optimisation of the evaporation parameters. Evaporation of solvents using a rotary-film evaporator should be performed at low temperature (water bath temperature of 30°C or lower) and under controlled pressure conditions, in order to prevent losses of the more volatile PAHs such as naphthalenes. For the same reasons, evaporation to dryness must be avoided. When reducing the sample to final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the GC-MS include pentane, hexane, heptane, iso-hexane, and iso-octane.

5. Selection of PAHs to be determined

The choice of PAHs to be analysed is not straightforward, both because of differences in the range of PAH compounds resulting from combustion processes and from oil and oil products, and also because the aims of specific monitoring programmes can require the analysis of different representative groups of compounds. PAHs arising from combustion processes are predominantly parent (unsubstituted) compounds, whereas oil and its products contain a much wider range of alkylated compounds in addition to the parent PAHs. This has implications for the analytical determination, as both HPLC-based and GC-based techniques are adequate for the determination of a limited range of parent PAHs in samples influenced by combustion processes, whereas in areas of significant oil contamination and following oil spills only GC-MS has sufficient selectivity to determine the full range of PAHs present. The availability of pure individual PAHs for the preparation of standards is problematic and limits both the choice of determinands and, to some degree, the quantification procedures that can be used. The availability of reference materials certified for PAHs is also rather limited. A list of target parent and alkylated PAHs suitable for environmental monitoring is given in Table A2.1, and this differs both from the list previously developed within ICES specifically for intercomparison purposes, and the historic list of Borneff. In both cases, the lists were concentrated on a subset of parent (predominantly combustion-derived) PAHs due to analytical limitations. This approach completely neglects the determination of alkylated PAHs, which allows the interpretation of PAH accumulation from multiple sources including those due to oil inputs. It will not be necessary for all of these PAH compounds and groups to be analysed in all cases, but an appropriate selection can be made from this list depending on the specific aims of the monitoring programme to be undertaken.
Table A2.1 Compounds of interest for environmental monitoring for which the guidelines apply. For compounds in italics standards are not available for any isomers in this group.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>Compound</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>128</td>
<td>Benzo[b]naptho[2,3-d]thiophene</td>
<td>234</td>
</tr>
<tr>
<td>C1-Naphthalenes</td>
<td>142</td>
<td>C1-benzonaphthothiophenes</td>
<td>248</td>
</tr>
<tr>
<td>C2-Naphthalenes</td>
<td>156</td>
<td>C2-Fluoranthenes/Pyrenes</td>
<td>230</td>
</tr>
<tr>
<td>C3-Naphthalenes</td>
<td>170</td>
<td>Benz[a]anthracene</td>
<td>228</td>
</tr>
<tr>
<td>C4-Naphthalenes</td>
<td>184</td>
<td>Chrysene</td>
<td>228</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>152</td>
<td>2,3-Benzanthracene</td>
<td>228</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>154</td>
<td>C1- Benz[a]anthracene/ Chrysene</td>
<td>242</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>154</td>
<td>C2- Benz[a]anthracene/ Chrysene</td>
<td>256</td>
</tr>
<tr>
<td>Fluorene</td>
<td>166</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1-Fluorenes</td>
<td>180</td>
<td>Benzo[a]fluoranthene</td>
<td>252</td>
</tr>
<tr>
<td>C2-Fluorenes</td>
<td>194</td>
<td>Benzo[b]fluoranthene</td>
<td>252</td>
</tr>
<tr>
<td>C3-Fluorenes</td>
<td>208</td>
<td>Benzo[j]fluoranthene</td>
<td>252</td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>184</td>
<td>Benzo[k]fluoranthene</td>
<td>252</td>
</tr>
<tr>
<td>C1-Dibenzothiophenes</td>
<td>198</td>
<td>Benzo[e]pyrene</td>
<td>252</td>
</tr>
<tr>
<td>C2-Dibenzothiophenes</td>
<td>212</td>
<td>Benzo[a]pyrene</td>
<td>252</td>
</tr>
<tr>
<td>C3-Dibenzothiophenes</td>
<td>226</td>
<td>Perylene</td>
<td>252</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>178</td>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>276</td>
</tr>
<tr>
<td>Anthracene</td>
<td>178</td>
<td>Benzo[ghi]perylene</td>
<td>276</td>
</tr>
<tr>
<td>C1-Phenanthrenes/Athracenes</td>
<td>192</td>
<td>Dibenzo[a,h]anthracene</td>
<td>278</td>
</tr>
<tr>
<td>C2-Phenanthrenes/Athracenes</td>
<td>206</td>
<td>Benzo[k]fluoranthene</td>
<td>252</td>
</tr>
<tr>
<td>C3-Phenanthrenes/Athracenes</td>
<td>220</td>
<td>Cyclopenta[cd]pyrene</td>
<td>226</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>202</td>
<td>Naphtho[2,1-ae]pyrene</td>
<td>302</td>
</tr>
<tr>
<td>Pyrene</td>
<td>202</td>
<td>Dibenzo[a,e]pyrene</td>
<td>302</td>
</tr>
<tr>
<td>C1-Fluoranthenes/Pyrenes</td>
<td>216</td>
<td>Dibenzo[a,i]pyrene</td>
<td>302</td>
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<tr>
<td>Benzo[b]naptho[2,3-d]thiophene</td>
<td>234</td>
<td>Dibenzo[a,k]pyrene</td>
<td>302</td>
</tr>
<tr>
<td>Benzo[b]naptho[1,2-d]thiophene</td>
<td>234</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. Instrumental determination of PAHs

The greatest sensitivity and selectivity in routine analysis for parent PAH is achieved by combining HPLC with fluorescence detection (HPLC-UVF) or capillary gas chromatography with mass spectrometry (GC-MS). However, for the analysis of parent and alkylated PAHs GC-MS is the method of choice. In terms of flexibility, GC-MS is the most capable technique, as in principle it does not limit the selection of determinands in any way, while HPLC is suited only to the analysis of parent PAHs. In the past, analyses have also been conducted using HPLC with UV-absorption detection and GC with flame-ionisation detection, but neither can be recommended for alkylated PAHs because of their relatively poor selectivity. Both in terms of the initial capital cost of the instrument and the cost per sample analysed, HPLC-UVF is cheaper than GC-MS. With the advent of high-sensitivity benchtop GC-MS systems, however, this cost advantage is now not as marked as in the past, and the additional information regarding sources available makes GC-MS the method of choice.

Limits of determination within the range of 0.05 µg kg⁻¹ dry weight for individual PAH compounds should be achievable by GC-MS.

6.1 GC-MS

The three injection modes commonly used are splitless, on-column and PTV (programmed temperature vaporiser). Automatic sample injection should be used wherever possible to improve the reproducibility of injection and the precision of the overall method. If splitless injection is used, the liner should be of sufficient capacity to contain the injected solvent volume after evaporation. For PAH analysis, the cleanliness of the liner is also very important if adsorption effects and discrimination are to be avoided, and the analytical column should not contain active sites to which PAHs can be adsorbed. Helium is the preferred carrier gas, and only capillary columns should be used. Because of the wide boiling range of the PAHs to be determined and the surface-active properties of the higher PAHs, the preferred column length is 25–50 m, with an internal diameter of 0.15 mm to 0.3 mm. Film thicknesses of 0.2 µm to 1 µm are generally used; this choice has little impact on critical resolution, but thicker films are often used when one-ring aromatic compounds are to be determined alongside PAHs, or where a high sample loading is needed. No stationary phase has been found on which all PAH isomers can be resolved; the most commonly used stationary phase for PAH analysis is 5% phenyl methylsilicone (DB-5 or equivalent). This will not, however, resolve critical isomers such as
benzo[\textit{b}, \textit{j}] and \textit{k} fluoranthenes, or chrysene from triphenylene. Chrysene and triphenylene can be separated on other columns, if necessary such as a 60 m non-polar column such a DB5MS. For PAHs there is no sensitivity gain from the use of chemical ionisation (either positive or negative ion), so analyses are usually conducted in electron-impact mode at 70eV. Quadrupole instruments are used in single ion monitoring to achieve greater sensitivity. The masses to be detected are programmed to change during the analysis as different PAHs elute from the capillary column. In SIM the molecular ion is used for quantification. Qualifier ions can be used to confirm identification but they are limited for PAHs. Triple quadrupole mass spectrometry can also be used and will give greater sensitivity. Some instruments such as ion-trap and time of flight mass spectrometers exhibit the same sensitivity in both modes, so full scan spectra can be used for quantification.

An example of mass spectrometer operating conditions in SIM mode is given in Table A2.2. The ions are grouped and screened within GC time windows of the compounds. In general the number of ions should not be greater than 20. The dwell time is an important parameter and should be close for each ion. For GC capillary column analysis a dwell time should not be shorter than 20 ms, while a sum of a dwell in each retention time windows should not be greater than 500 ms. An example of conditions that can be used along with dwell times are shown in Table A2.2.

Table A.2.2 Example of operational conditions for the GC-MS analysis of parent and alkylated PAHs.

<table>
<thead>
<tr>
<th>Group</th>
<th>Retention time (min)</th>
<th>Dwell time (ms)</th>
<th>Ions in group (AMU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.00</td>
<td>100</td>
<td>128 136 142</td>
</tr>
<tr>
<td>2</td>
<td>21.00</td>
<td>152</td>
<td>156 160</td>
</tr>
<tr>
<td>3</td>
<td>23.70</td>
<td>154</td>
<td>164 168 170</td>
</tr>
<tr>
<td>4</td>
<td>26.80</td>
<td>166</td>
<td>176 180 182 184</td>
</tr>
<tr>
<td>5</td>
<td>31.60</td>
<td>178</td>
<td>184 188 194 196 198</td>
</tr>
<tr>
<td>6</td>
<td>35.30</td>
<td>192</td>
<td>198</td>
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<tr>
<td>7</td>
<td>36.60</td>
<td>206</td>
<td>212</td>
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<tr>
<td>8</td>
<td>39.40</td>
<td>202</td>
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<td>44.65</td>
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<tr>
<td>10</td>
<td>45.30</td>
<td>226</td>
<td>228 230 234 240</td>
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<tr>
<td>11</td>
<td>48.58</td>
<td>242</td>
<td>248</td>
</tr>
<tr>
<td>12</td>
<td>52.00</td>
<td>252</td>
<td>256 264 266</td>
</tr>
<tr>
<td>13</td>
<td>59.00</td>
<td>266</td>
<td>276 278 288</td>
</tr>
</tbody>
</table>

Alkylated homologues of PAHs (C1–C4), mainly associated with petrogenic sources, contain a number of different isomers that can give very complex but distinct distribution profiles when analysed by GC-MS. Integration of each isomer separately is difficult for most alkylated PAHs. 1- and 2-Methyl naphthalene give well resolved peaks that can be quantified separately. C1-Phenanthrene/anthracene gives five distinct peaks corresponding to 3-methyl phenanthrene, 2-methyl phenanthrene, 2-methyl anthracene, 4- and 9-methyl phenanthrene and 1-methyl phenanthrene. These may be integrated as a group or as separate isomers. For all other alkylated PAHs the area for all isomers may be summed and quantified against a single representative isomer. This method will, however, lead to an overestimation of the concentration as may include non alkylated PAHs. Examples of integrations of both parent and alkylated PAHs are shown in Appendix 1.

7. Calibration and quantification

7.1 Standards

The availability of pure PAH compounds are limited. Although most of the parent compounds can be purchased as pure compounds, the range of possible alkyl-substituted PAHs is vast and only a limited selection of them can be obtained. PAH standards are available for at least one isomer of most alkyl group listed in Table A2.1. A range of deuterated PAHs (normally 5 to 7) should be used as internal standards to cover the range of PAHs being analysed in samples. A range of fully-deuterated parent PAHs is available for use as standards in PAH analysis. Suitable standards could range from d8-naphthalene to d14-dibenz[a,h]anthracene. Crystalline PAHs of known purity should be used for the preparation of calibration standards. If the quality of the standard materials is not guaranteed by the producer or supplier (as for certified reference materials), then it should be checked by GC-MS analysis. Solid standards should be weighed to a precision of 10-5 grams. Calibration standards should be stored in the dark because some PAHs are photosensitive, and ideally solutions to be stored should be sealed in amber glass ampoules or sealed GC vials. Otherwise, they can be stored in a refrigerator in stoppered measuring cylinders or flasks that are gas tight to avoid evaporation of the solvent during storage.
Calibration

Multilevel calibration with at least five calibration levels is preferred to adequately define the calibration curve. In general, GC-MS calibration is linear over a considerable concentration range but may exhibit a change of slope at very low concentrations. Quantification should be conducted in the linear region of the calibration curve. A separate calibration curve may be used where sample concentrations are very low. An internal standard method should be employed, using a range of deuterated PAHs as internal standards.

7.3 Recovery

The recovery of analytes should be checked and reported. Given the wide boiling range of the PAHs to be determined, the recovery may vary with compound group, from the volatile PAHs of low molecular weight to the larger compounds. Deuterated standards can be added in two groups: those to be used for quantification are added at the start of the analytical procedure, whilst those from which the absolute recovery will be assessed are added prior to GC-MS injection. This allows the recovery to be calculated.

8. Analytical quality control

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination, which they consider acceptable. Achievable limits of determination for each individual component using GC-MS are 0.05 µg kg\(^{-1}\) dry weight.

Further information on analytical quality control procedures for PAHs can be found elsewhere (Law and de Boer, 1995). A procedural blank should be measured with each sample batch, and should be prepared simultaneously using the same chemical reagents and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will result in errors in quantification. The procedural blank is also very important in the calculation of limits of detection and limits of quantification for the analytical method. In addition, a laboratory reference material (LRM) should be analysed within each sample batch. The LRM must be homogeneous and well characterised for the determinands of interest within the analytical laboratory. Ideally, stability tests should have been undertaken to show that the LRM yields consistent results over time. The LRM should be of the same matrix type (e.g., liver, muscle, mussel tissue) as the samples, and the determinand concentrations should be in the same range as those in the samples. Realistically, and given the wide range of PAH concentrations encountered, particularly in oil spill investigations, this is bound to involve some compromise. The data produced for the LRM in successive sample batches should be used to prepare control charts. It is also useful to analyse the LRM in duplicate from time to time to check within-batch analytical variability. The analysis of an LRM is primarily intended as a check that the analytical method is under control and yields acceptable precision, but a certified reference material (CRM) of a similar matrix should be analysed periodically in order to check the method bias. A marine sediment (NIST SRM 1941b)\(^1\) is available, with certified values for 24 PAHs and a further 44 as reference (non-certified) values. At regular intervals, the laboratory should participate in an intercomparison or proficiency exercise in which samples are circulated without knowledge of the determinand concentrations, in order to provide an independent check on performance.

9. Data reporting

The calculation of results and the reporting of data can represent major sources of error, as has been shown in intercomparison studies for PAHs. Control procedures should be established in order to ensure that data are correct and to obviate transcription errors. Data stored on databases should be checked and validated, and checks are also necessary when data are transferred between databases. Data should be reported in accordance with the latest ICES reporting formats.

10. References


\(^1\) More info on [https://srmors.nist.gov/view_detail.cfm?srn=1941B](https://srmors.nist.gov/view_detail.cfm?srn=1941B)


ANNEX 2 – APPENDIX 1

Example integrations of parent and alkylated PAHs analysed by GC-MS. The standards used for the calibration of the alkylated PAHs are asterixed.
Ion 184.00 (183.70 to 184.70): EPG1132.D\_data.ms

Ion 198.00 (197.70 to 198.70): EPG1132.D\_data.ms

Ion 212.00 (211.70 to 212.70): EPG1132.D\_data.ms

Ion 226.00 (225.70 to 226.70): EPG1132.D\_data.ms

Abundance

Time

DBT

4-methyldibenzothiophene

C1-DBT$	extsuperscript{*}$

C2-DBT$	extsuperscript{*}$

C3-DBT$	extsuperscript{*}$

4-methyldibenzothiophene
Cyclopenta[c,d]pyrene

benz[a]anthracene

benzo[c]phenanthrene

benz[b]anthracene

chrysene

2-methylchrysene

dimethylbenz[a]anthracene
 Ion 252.00 (251.70 to 252.70): QU576.D\data.ms

benzofluoranthenes
benzo[e]pyrene
benzo[e]pyrene

perylene

 Ion 276.00 (275.70 to 276.70): QU576.D\data.ms

indeno[123-cd]pyrene
benzo[ghi]perylene

ANNEX 3

Technical Annex: Organotin compounds in biota

This annex is intended as a supplement to the general guidelines. It is not a complete description or a substitute for detailed analytical instructions. The annex provides guidelines for the measurement of organotins, in marine biota in monitoring programmes. Target compounds include tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) and also triphenyltin (TPhT), diphenyltin (DPhT), and monophenyltin (MPhT).

1. Species

Target species for the monitoring of organotin compounds are shellfish, in particular bivalves like *Mytilus edulis* or *Mytilus galloprovincialis*. *Mytilus edulis* occurs in shallow waters along almost all coasts of the Contracting Parties. It is therefore suitable for monitoring in nearshore waters. No distinction is made between *M. edulis* and *M. galloprovincialis* because the latter, which may occur along the coast from Spain and Portugal to the southern coasts of UK, cannot easily be discerned from *M. edulis*. A sampling size range of 3-6 cm is specified to ensure availability throughout the whole maritime area. The Pacific oyster (*Crassostrea gigas*) should be sampled in areas where *Mytilus sp.* is not available. The sampling size should be within the length range 9-14 cm to ensure individuals of the 2 year age class.

Gastropods can also be used for TBT indicators, for instance in relation to biological effect monitoring. However, gastropods do not feed as continuously as bivalves and have a higher capacity of TBT metabolism, possibly resulting in a higher variability of TBT body burdens in gastropods compared with bivalves. In addition, correlation between imposex and TBT body burdens in the environment can be low, because of a time-lag between current TBT levels and imposex induced irreversibly in the early life stages and also because of non-continuous feeding strategies. In some sensitive gastropod species, imposex can also be induced by TBT at lower levels than analytical detection limits generally achieved.

2. Sampling

Two alternative sampling strategies are described: sampling to minimise natural variability and length-stratified sampling. References of relevance to sampling and statistics include Gilbert (1987); Bignert et al. (1993 and 1994); Nicholson and Fryer (1996); and Nicholson et al. (1997). Advice on sampling strategies for temporal trend and spatial monitoring in shellfish are provided in OSPAR’s general JAMP Guidelines for Monitoring Contaminants in Biota and in Technical Annex 1: Organic Contaminants.

3. Transportation

Samples should be kept cool and frozen at <-20°C as soon as possible after collection. Length and weight should be determined before freezing. Live mussels should be transported in closed containers at temperatures between 5-15°C, preferably <10°C. Frozen samples should be transported in closed containers at temperatures < -20°C. More rigorous conditions will be necessary for samples for biological effects monitoring, e.g. storage in liquid nitrogen.

4. Pre-treatment and storage

4.1 Contamination

Sample contamination may occur during sampling, sample handling, pre-treatment, and analysis (Oehlenschläger, 1994), due to the environment, the containers or packing material used, the instruments used during sample preparation or from the chemical reagents used during the analytical procedures. Controlled conditions are therefore required for all procedures, including the dissection of organisms on board ship.

4.2 Depuration

Mussels should be placed on a polyethylene tray elevated above the bottom of a glass aquarium. The aquarium should be filled with sub-surface sea water collected from the same site as the samples and which has not been subject to contamination from point sources if possible. The aquarium should be aerated and the mussels left for 20-24 hours at water temperatures and salinity close to those from which the samples were removed.

4.3 Opening of the shells

Mussels should be shucked live and opened with minimum tissue damage by detaching the adductor muscles from the interior of one valve. The mussels should be inverted and allowed to drain on a clean towel or funnel for at least 5 minutes in order to minimise influence on dry weight determinations.
4.4 Dissection and storage

The soft tissues should be removed and deep frozen (-20°C) as soon as possible in containers appropriate to the intended analysis. TBT is stable in cockles and oysters stored at -20°C in the dark over a 7 month period. Longer storage can cause significant loss of TBT due to degradation (Gomez-Ariza et al., 1999). The dissection of the soft tissue must be done under clean conditions on a clean bench by scientific personnel, wearing clean gloves and using clean stainless steel knives. After each sample has been prepared, the tools should be cleaned regularly. Washing in acetone or alcohol and high purity water is recommended. When the analysis is eventually undertaken, all fluids that may initially separate on thawing should be included with the materials homogenised. Homogenisation should be performed immediately prior to any sub-dividing of the sample.

5. Analysis

5.1 Preparation of materials

Solvents, chemicals and adsorption materials must be free of organotin compounds or other interfering compounds. If not they should be purified using appropriate methods. Solvents should be checked by concentrating the volume normally used in the procedure to 10% of the final volume and then analysing for the presence of organotin compounds and other interfering compounds using a GC. If necessary, the solvents can be purified by redistillation. Chemicals and adsorption materials should be purified by extraction and/or heating. Glass fibre materials (e.g. thimbles for Soxhlet extraction) should be pre-extracted. Alternatively, full glass thimbles with a G1 glass filter at the bottom can be used. Generally, paper filters should be avoided in filtration and substituted for by appropriate glass filters. As all super cleaned materials are prone to contamination (e.g. by the adsorption of organotin compounds and other compounds from laboratory air), materials ready for use should not be stored for long periods. All containers, skills, glassware etc. which come into contact with the sample must be made of appropriate material and must have been thoroughly pre-cleaned. Glassware should be extensively washed with detergents, heated at >250°C and rinsed immediately before use with organic solvents or mixtures such as hexane/acetone. Alternatively, all glassware can be washed in 10% HCl (or even in concentrated HCl) and then rinsed with distilled water.

5.2 Lipid determination

Organotin data are not usually expressed on a lipid basis. Lipid content is not a good normalisator because of poor correlations to organotin content. However, the determination of the lipid content of tissues can be of use in characterising the samples. If required, the lipid content should be determined on a separate subsample of the tissue homogenate, as some of the extraction techniques used routinely for organotin determination may destroy lipid materials. The total fat weight should be determined using the method of Bligh and Dyer (1959) or Smedes (1999).

5.3 Dry weight determination

Dry weight determinations should be carried out by air-drying homogenised sub-samples of the material to be analysed to constant weight at 105°C.

5.4 Determination of organotins by gas chromatography

5.4.1 Calibration and preparation of calibrand solutions

5.4.1.1 External calibration

When using an external calibration, multilevel calibration with at least five calibration points is preferred to adequately define the calibration curve. Standards preparation can be done in two ways depending on the methods of extraction/derivatisation used:

i) by using alkyltins salts then proceed to the derivatisation step as for samples (for hydridisation or ethylation followed by purge-and-trap analysis, there is no other appropriate way than using alkyltin salts);
ii) by using commercially readily available derivatised standards (e.g. Quasimeme http://www.quasimeme.org/).

Standard solutions can be prepared in (m)ethanol or another solvent depending on the instrumental method used. Addition of an internal standard (tripropyltin chloride TPrTCI or 13C labelled or deuterated TBT if using GC analysis with mass selective detection) to all standard and samples solutions is recommended. When using tripropyltin chloride, which is an underivatised standard, the recovery efficiency of the whole procedure can be determined.

A new calibration solution should always be cross-checked to the old standard solution.
Calibrand solutions should be stored in a refrigerator in gas tight containers to avoid evaporation of solvent during storage. It is important to determine the expiry date of standard dilutions in order to avoid a concentration shift due to deterioration of analytes or evaporation of solvents.

5.4.1.2 Isotope Dilution-Mass Spectrometry

When using Isotope Dilution-Mass Spectrometry technique (IDMS), external calibration is not required.

5.4.2 Homogenisation and drying

Homogenisation should be carried out on fresh tissue. Care should be taken that the sample integrity is maintained during the actual homogenisation and during drying. When the analysis is undertaken, all fluids that may initially separate on thawing should be included with the materials homogenised. Homogenisation should be performed immediately prior to succeeding procedures. When grinding samples after drying, classical techniques using a ball mill can be used. Cryogenic homogenisation of dried or fresh materials at liquid nitrogen temperatures using a PTFE device (cf. Iyengar and Kasperek, 1977) or similar techniques can be applied (cf. Iyengar, 1976; Klussmann et al., 1985).

5.4.3 Extraction

Release of organotin compounds from the biological matrix is a critical step, due to the strong matrix binding of the analytes and possible species degradation. Recovery standards should be added prior to extraction, however correction procedures should be used with care as equilibration between the spiked and the target compounds is not always guaranteed. Different extraction techniques are commonly used, such as microwave assisted extraction, mechanical shaking and digestion. Microwave assisted extraction (MAE) as well as mechanical shaking provide quantitative recoveries with negligible degradation of the TBT compounds (Centineo et al., 2004). However, it must be taken into account that considerable loss of DBT, due to degradation was reported for MAE. Digestion techniques can be used to extract butyltins, though species degradation is not always under control using this technique. Mechanical shaking seems to be a suitable technique. Alternatively, pressurised liquid extraction (accelerated solvent extraction) can be used to extract organotin compounds. Extraction usually takes place in an aqueous methanolic acidic environment, with subsequent extraction to an organic phase, such as pentane or hexane. Acidic conditions enhance the extraction efficiency, acetic acid is usually preferred to other acids to ensure stability of butyltins compounds. Complexing agents such as tropolone are often employed. Extraction can be performed on wet as well as on freeze-dried samples. Wet tissue must be dried by mixing with anhydrous sodium sulphate or other anhydrous materials.

5.4.4 Derivatisation

5.4.4.1 Alkylation

Grignard reagent: A variety of Grignard reagents is used for alkylation reactions in derivatisation. The smaller the alkylation group, the more volatile the products of derivatisation, and the greater the losses during the transfer and work up. This method is time-consuming and requires very dry conditions and non-protic solvents. The use of Grignard reagents is hazardous as they react violently with protic solvents such as water, acid, alcohol, ketones and appropriate safety precautions must be taken. A liquid-liquid extraction step is necessary to isolate the derivatised organotins. However, unlike hydride derivatives of butyltins which may degrade in hours or days, the tetraalkyl derivatives formed with Grignard reagents are very stable (Morabito et al., 2000). Derivatisation with Grignard reagents include extra steps in the analytical procedure as clean up of excess Grignard reagent with acid is required.

Sodium Tetraethylborate (NaBEt₄): Derivatisation with this complexing agent has been developed to minimise the analysis time. The NaBEt₄ procedure allows a simultaneous extraction-derivatisation in a buffered medium (optimum pH 4-5). NaBEt₄ derivatisation produces more thermally stable derivatives. However, NaBEt₄ is extremely air sensitive, since it is considered as pyrophoric, care must be taken to keep its chemical integrity. Although solutions in water have been shown to be stable for about 1 month at 4 °C, it is recommended to prepare them freshly for use. Solutions of the reagent in an organic solvent (e.g. tetrahydrofuran, methanol or ethanol) seem to be more stable (Smedes et al., 2000). The determination of organotin compound in complex matrices, such as biological matrices with high lipid content, has led to several problems, including low recovery and low derivatisation efficiency. A clean-up step might be subsequently required.

Sodium Diethyldithiocarbamate (NaDDTC): NaDDTC is preferable to Grignard reagents as it does not require anhydrous conditions but it does not simultaneously derivatise and extract like NaBEt₄. Yet this step can be combined with Grignard reagent to provide better derivatisation for a wider spectrum of organotins.
5.4.4.2 Hydride generation
The butyltin species are converted to an hydride form by sodium tetrahydroborate (NaBH₄). Hydride generation produces a large volume of hydrogen as a by-product, which facilitates the purging of butyltin hydrides from a large volume of sample.

5.4.5 Clean-up
The most commonly used clean-up methods involve the use of alumina or silica adsorption chromatography. For the latter, phenyltin compounds like triphenyltin may not co-elute with butyltins. Gel permeation chromatography and similar high performance liquid chromatography (HPLC) based methods are also employed. The major advantages of using HPLC-based clean-up methods are their ease of automation and reproducibility.

5.4.6 Pre-concentration
Evaporation of solvents using a rotary evaporator should be performed under controlled temperature and pressure conditions, and the sample volume should be kept above 2 ml. Evaporation to total dryness should be avoided. To reduce the sample volume even more, e.g. to a final volume of 100 µl, solvents like pentane or hexane can be removed by concentration with a gentle stream of nitrogen. Only nitrogen of a controlled high quality should be used. Iso-octane is recommended as a keeper for the final solution to be injected into the GC.

5.5 Instrumental determination
Most of the analytical techniques developed for the speciation of organotin compounds are based on GC. GC remains the preferred separation technique owing to its high resolution and the availability of sensitive detectors such as (pulsed) flame photometry ((P)FPD), mass spectrometry (MS) or inductively coupled plasma- mass spectrometry (ICP-MS)

As an alternative approach, high performance liquid chromatography has become a popular technique. It mainly uses fluorescence, ultraviolet, and more recently inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), and mass spectrometry detectors such as atmospheric pressure chemical ionisation mass spectrometry (APCI-MS-MS) and electrospray ionisation mass spectrometry (ESI-MS). ICP-MS and (P)FPD detectors have been applied widely because of their inherent selectivity and sensitivity. (P)FPD has been shown to have greater selectivity and lower detection limits (by a factor of 25 to 50 times) for organotin compounds than those obtained with conventional FPD (Bravo et al., 2004).

5.5.1 Gas chromatography
The two injection modes commonly used are splitless and on-column injection. Automatic sample injection should be used wherever possible to improve the reproducibility of injection and the precision of the overall method. If splitless injection is used, the liner should be of sufficient capacity to contain the injected solvent volume after evaporation. Helium must be used for GC-MS, GC-FPD and GC-ICP-MS. The preferred column length is 25–30 m, with an internal diameter of 0.15 mm to 0.3 mm. Film thicknesses of 0.3 µm to 1 µm are generally used. The most commonly used stationary phase for organotin analysis is 5% phenyl methyl siloxane. Mass spectrometric analyses are usually conducted in electron-impact mode at 70eV.

5.5.2 High Performance Liquid Chromatography
All stainless steel parts of the HPLC system that come into contact with the sample should be replaced by polyether ketone (PEEK) components. Reverse phase columns (e.g. octadecylsilane C18) are commonly used (Wahlen and Catterick, 2003) and the mobile phase can consist, for example, of a mixture of acetonitrile, water and acetic acid with 0.05% triethylamine, pH 3.1–3.4 (65:25:10 variable depending on columns used).

5.5.3 Detection
Flame photometry (FPD), equipped with a 610 nm band-pass filter, selective for tin compounds), mass spectrometry (MS) or inductively coupled plasma-mass spectrometry (ICP-MS) are mainly used as detectors for gas chromatography and high performance liquid chromatography.

6. Quality assurance
References of relevance to QA procedures include HELCOM (1988); HELCOM COMBINE manual, QUASIMEME (1992); Oehlenschläger (1994); ICES (1996); and Morabito et al. (1999).

6.1 System performance
The performance of the instrumentation should be monitored by regularly checking the resolution of two closely eluting organotin compounds. A decrease in resolution points to deteriorating instrumental conditions. A dirty MS-source can
be recognised by the presence of an elevated background signal together with a reduced signal-to-noise ratio. Chromatograms should be inspected visually by a trained operator.

6.2 Recovery

The recovery should be checked and reported. One method is to add an internal (recovery) standard to each sample immediately before extraction (e.g. tripropyltin) and a second (quantification) standard immediately prior to injection (e.g. tetrapropyltin). The recovery of MBT may be lower than for other organotin compounds, probably because of a lower derivatisation efficiency.

When using Isotope Dilution-Mass Spectrometry technique, the loss of target analytes is compensated. However, the recovery should still be calculated and should be between 50% and 150%.

6.3 Calibrand solutions and calibration

See Section 5.4.1.

6.4 Blanks

A procedural blank should be measured for each sample series and should be prepared simultaneously using the same chemicals and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will lead to errors in quantification. Even if an internal standard has been added to the blank at the beginning of the procedure, a quantification of peaks in the blank and subtraction from the values obtained for the determinands must not be performed, as the added internal standard cannot be adsorbed by a matrix.

6.5 Accuracy and precision

A Laboratory Reference Material (LRM) should be included, at least one sample for each series of identically prepared samples. The LRM must be homogeneous, well characterised for the determinands in question and stability tests must have shown that it produces consistent results over time. The LRM should be of the same type of matrix (e.g. liver, muscle tissue, fat or lean fish) as the samples, and the determinand concentrations should occur in a comparable range to those of the samples. If the range of determinand concentrations in the samples is large (> factor of 5) two reference materials should be included in each batch of analyses to cover the lower and upper concentrations. The data produced for the LRM in successive sample batches should be used to prepare control charts. It is also useful to analyse the LRM in duplicate from time to time to check within-batch analytical variability. The analysis of an LRM is primarily intended as a check that the analytical method is under control and yields acceptable precision, but a certified reference material (CRM such as ERM-CE 477 (mussel, certified for TBT, DBT, MBT) or NIES No. 11 (fish tissue certified for TBT and non certified reference value for TPhT)) of a similar matrix should be analysed periodically in order to check the method bias. Additionally a duplicate of at least one sample should be run with every batch of samples. Each laboratory should participate in interlaboratory comparison studies and proficiency testing schemes on a regular basis, preferably at an international level.

6.6 Data collection and transfer

Data collection, handling and transfer must take place using quality controlled procedures.

7. Data recording and reporting parameters

The calculation of results and the reporting of data can represent major sources of error, as has been shown in intercomparison studies for organotin compounds. Control procedures should be established in order to ensure that data are correct and to avoid transcription errors. Data stored in databases should be checked and validated, and checks are also necessary when data are transferred between databases.

Data reporting should be in accordance with the requirements of the monitoring programme and with the latest ICES reporting formats. Results should be reported according to the precision required for the programme. In practice, the number of significant figures is defined by the performance of the procedure.

The following parameters should be recorded:
7.1 Sampling and biological parameters

Shellfish
- location of sampling site (name, latitude, and longitude);
- date and time of sampling (GMT);
- sampling depth with respect to low tide (for sub-tidal sites only);
- irregularities and unusual conditions;
- name and institution of sampling personnel;
- number of pooled samples;
- number of individuals in pool;
- mean, minimum and maximum length and standard deviation;
- mean dry shell weight;
- mean soft tissue weight (wet weight);
- condition index.

7.2 Analytical and quality assurance parameters

- LRM and CRM results for a set of organotin compounds, reported on a wet weight basis;
- descriptions of the extraction, cleaning and instrumental determination methods;
- mean tissue lipid weight and method of extraction;
- the mean soft dry weight and method of determining water content if this differs from air drying to constant weight at 105°C (if sufficient material is available);
- the detection limit for each organotin compound. Specific performance criteria, including detection limits and precision, are usually set by the programme. A typical detection limit for single contaminants is 1 µg/kg wet weight, although this might be difficult to achieve for phenyltins compounds.
- QA information according to the requirements specified in the programme.

7.3 Lipids

- total lipids (e.g. Bligh and Dyer, 1959; or Smedes, 1999) (expressed as % or g/kg wet weight).

7.4 Parameters

- organic contaminants of interest to monitoring programmes for which these guidelines apply: organotin compounds suite required for analysis
  - Butyltin compounds: Tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT)
  - Phenyltin compounds: Triphenyltin (TPhT), diphenyltin (DPhT) and monophenyltin (MPhT)
8. References


