Manual for Marine Monitoring in the

COMBINE

Programme of HELCOM

Part B

General guidelines on quality assurance for monitoring in the Baltic Sea

Annex B-12

Technical note on the determination of heavy metals and persistent organic compounds in biota

Appendix 2
Technical note on the determination of polycyclic aromatic hydrocarbons in biota

ANNEX B-12 TECHNICAL NOTE ON THE DETERMINATION OF HEAVY METALS AND PERSISTENT ORGANIC COMPOUNDS IN BIOTA

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1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) consist of a variable number of fused aromatic rings. By definition, PAHs contain at least three fused rings, although in practice related compounds with two fused rings (such as naphthalene and its alkylated derivatives) are often determined and will be considered in these guidelines. 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. PAHs are of concern in the marine environment for two main reasons: firstly, low-molecular weight (MW) PAHs can be directly toxic to marine animals; 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, however, and benzo[e]pyrene and four benzofluoranthene isomers (all six compounds have a molecular weight of 252 Da) are much less potent. Some compounds (e.g., heterocyclic compounds containing sulphur, such as benzothiophenes and dibenzo-thiophenes) may also cause taint in commercially exploited fish and shellfish and render them unfit for sale.

PAHs are readily taken up by marine animals both across gill surfaces and from their diet, and 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 metabolize PAHs and to excrete them in bile. An assessment of the exposure of fish to PAHs therefore requires also the determination of PAH-metabolite concentrations in bile samples, as turnover times can be extremely rapid. Thus, the analysis of PAHs in fish muscle tissue should normally only be undertaken for food quality assurance purposes (Law and Biscaya, 1994).

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 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

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may actually be deposited to the sea already adsorbed to atmospheric particulates, such as soot particles. The majority of PAHs in the water column will eventually be either taken up by biota or transported to the sediments, and deep-water depositional areas may generally be regarded as sinks for PAHs, particularly when they are anoxic.

2. APPROPRIATE SPECIES FOR ANALYSIS OF PAHS

2.1 BENTHIC FISH AND SHELLFISH

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.

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 are obtained. Care should be taken that the sample is representative of the population and that sampling can be repeated 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. 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 ap 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 affect the formation of these metabolites. At offshore locations, the collection of appropriate shellfish samples may be problematic if populations are absent, sparse or scattered, and the collection of fish samples may be simpler. Generally, the analysis of PAHs in fish muscle tissue should only be considered for the purposes of food quality assurance.

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 before spawning.

2.2 FISH

Fish are not recommended for spatial or temporal trend monitoring of PAHs, but can be useful as part of biological effects studies or for food quality assurance purposes. The sampling strategy for biological effects monitoring is described in the OSPAR Joint Assessment and Monitoring Programme (JAMP).

3. TRANSPORTATION

Live mussels should be transported to the laboratory for sample preparation. They should be transported in closed containers at temperatures between 5 °C and 15 °C, preferably below 10 °C. For live animals it is important that the transport time is short and controlled (e.g., maximum of 24 hours).

Fish samples should be kept cool or frozen (at a temperature of -20 °C or lower) as soon as possible after collection. Frozen fish samples should be transported in closed containers at temperatures below -20 °C. 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. PRETREATMENT AND STORAGE

4.1 CONTAMINATION

Sample contamination may occur during sampling, sample handling, pretreatment 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, including the dissection of fish organs on-board a ship (see ANNEX B-13, Appendix 1). 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.

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 under controlled conditions and in filtered sea water; 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

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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 clean 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 homogenizers) should be cleaned.

4.3 FISH

4.3.1 DISSECTION AND STORAGE

The dissection of fish muscle and internal organs should be carried as soon as possible after collection. The details of fish muscle and liver dissection are given in ANNEX B-13, Appendix 1. If possible, the entire right side dorsal lateral fillet should be homogenized and sub samples taken for replicate PAH determinations. If, however, the amount of material to be homogenized would be too large, a specific portion of the dorsal musculature should be chosen. It is recommended that the portion of the muscle lying directly under the first dorsal fin is used in this case.

When dissecting the liver, care should be taken to avoid contamination from the other organs. If bile samples are to be taken for PAH-metabolite determinations, then they should be collected first. If the whole liver is not to be homogenized, a specific portion should be chosen in order to ensure comparability. Freeze-drying of tissue samples cannot be recommended for PAH determination, due to the contamination which may result from back-streaming of oil from the rotary pumps used to generate the vacuum.

If plastic bags or boxes are used, then they should be used as outer containers only, and should not come into contact with tissues. Organ samples (e.g., livers) should be stored in pre-cleaned containers made of glass, stainless steel or aluminium, or should be wrapped in pre-cleaned aluminium foil and shock-frozen quickly in liquid nitrogen or in a blast freezer. In the latter case, care should be taken that the capacity of the freezer is not exceeded (Law and de Boer, 1995). Cold air should be able to circulate between the samples in order that the minimum freezing time can be attained (maximum 12 hours). The individual samples should be clearly and indelibly labelled and stored together in a suitable container at a temperature of -20 °C until analysis. If the samples are to be transported during this period (e.g., from the ship to the laboratory), then arrangements must be made which ensure that the samples do not thaw out during transport. Sub samples for biomarker determinations should be collected immediately after death in order to minimize post-mortem changes in enzymatic and somatic activities, and stored in suitable vials in liquid nitrogen until analysis.

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 clean 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 homogenizers) should be cleaned.

When pooling of tissues is necessary, an equivalent quantity of tissue should be taken from each fish, e.g., 10 % from each whole fillet.

5. ANALYSIS

5.1 PREPARATION OF MATERIALS

Solvents, reagents, and adsorptive materials must be free of PAHs and other interfering compounds. If not, then they must be purified using appropriate methods. Reagents and absorptive materials should be purified by solvent extraction and/or by heating in a muffle oven, as appropriate. Glass fibre materials (e.g., Soxhlet thimbles) are preferred over filter papers and should be cleaned by solvent extraction. It should be borne in mind that clean materials can be re-contaminated by exposure to laboratory air, particularly in urban locations, and so 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, and should be pre-cleaned before use. Appropriate cleaning methods would include washing with detergents, rinsing with water, and finally solvent-rinsing immediately before use. Heating of glassware in an oven (e.g., at 400°C for 24 hours) can also be useful in removing PAH contamination.

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 characterizing the samples. The lipid content should be determined on a separate subsample of the tissue homogenate, as some of the extraction techniques used routinely for PAH determination (e.g., alkaline saponification) destroy lipid materials. The total fat weight should be determined using the method of Smedes (1999) or an equivalent method.

5.3 DRY WEIGHT DETERMINATION

Generally PAH data are expressed on a wet weight basis, but sometimes it can be desirable to consider them on a dry weight basis. Again, the dry weight determination should be conducted on a separate sub sample of the tissue homogenate, which should be air-dried to constant weight at 105 °C.

5.4 EXTRACTION AND CLEAN-UP

PAHs are lipophilic and so are concentrated in the lipids of an organism, and a number of methods have been described for PAH extraction (see, e.g., Ehrhardt *et al.,* 1991). The preferred methods generally utilize either Soxhlet extraction, or alkaline digestion followed by liquid-liquid extraction with an organic solvent. Microwave-assisted solvent extraction can be mentioned as one of the modern techniques being applied to PAH analysis (Budzinski *et al.,* 2000; During and Gaath, 2000; Vázquez Blanco *et al*., 2000; Ramil Criado *et al.,* 2002). In the case of Soxhlet extraction, the wet tissue must be dried by mixing with a chemical 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. Alkaline digestion is conducted on wet tissue samples, so this procedure is unnecessary. In neither case can the freeze-drying of the tissue prior to extraction be recommended, owing to the danger of contamination from oil back-streaming from the rotary pump (which provides the vacuum) into the sample. Non-polar 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. For these reasons, it should be the method of choice. Solvents used for liquid-liquid extraction of the homogenate are usually non-polar, such as pentane or hexane, and they will effectively extract all PAHs.

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, both 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 alumina or silica adsorption chromatography, but gel permeation chromatography and similar 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.

5.5 PRE-CONCENTRATION

The sample volume should be 2 cm^3 or greater to avoid errors when transferring solvents during the clean-up stages. 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 should 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 gas chromatograph (GC) or GC-MS include pentane, hexane, heptane and *iso*-octane, whereas for HPLC analyses acetonitrile and methanol are commonly used.

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

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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 1. In both cases, the list was 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 1: Compounds of interest for environmental monitoring for which the guidelines apply

Compound	MW	Compound	MW
Naphthalene	128	C2-Phenanthrenes/Anthracenes	206
C1-Naphthalenes	142	C3-Phenanthrenes/Anthracenes	220
C2-Naphthalenes	156	Fluoranthene	202
C3-Naphthalenes	170	Pyrene	202
C4-Naphthalenes	184	C1-Fluoranthenes/Pyrenes	216
Acenaphthylene	152	C2-Fluoranthenes/Pyrenes	230
Acenaphthene	154	Benz[a]anthracene	228
Biphenyl	154	Chrysene	228
Fluorene	166	2,3-Benzanthracene	228
C1-Fluorenes	180	Benzo[a]fluoranthene	252
C2-Fluorenes	194	Benzo[b]fluoranthene	252
C3-Fluorenes	208	Benzo[j]fluoranthene	252
Dibenzothiophene	184	Benzo[k]fluoranthene	252
C1-Dibenzothiophenes	198	Benzo[e]pyrene	252
C2-Dibenzothiophenes	212	Benzo[a]pyrene	252
C3-Dibenzothiophenes	226	Perylene	252
Phenanthrene	178	Indeno[1,2,3-cd]pyrene	276
Anthracene	178	Benzo[ghi]perylene	276

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Compound	MW	Compound	MW
C1-Phenanthrenes/ Anthracenes	192	Dibenz[ah]anthracene	278

5.7 INSTRUMENTAL DETERMINATION OF PAHS

Unlike the situation for chlorobiphenyls (CBs), where GC techniques (particularly GC-ECD) are used exclusively, two major approaches based on GC and HPLC are followed to an equal extent in the analysis of PAHs. The greatest sensitivity and selectivity in routine analyses are achieved by combining HPLC with fluorescence detection (HPLC-UVF) and capillary gas chromatography with mass spectrometry (GC-MS). 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-ionization detection, but neither can be recommended because of their relatively poor selectivity.

Intercomparison exercises have demonstrated a serious lack of comparability between specific hydrocarbon concentrations measured in different laboratories and using both analytical approaches described above (Farrington *et al.,* 1986). An interlaboratory performance study has been carried out within the QUASIMEME laboratory testing scheme in order to assess the level of comparability among laboratories conducting PAH analyses and to identify improvements in methodology (Law and Klungsøyr, 1996; Law *et al.,* 1998, QUASIMEME).

Limits of determination within the range of 0.2 to 10 µg kg-1 wet weight for individual PAH compounds should be achievable by both GC-MS and HPLC-UVF techniques.

5.8 HPLC

Reversed-phase columns (e.g., octadecylsilane (RP-18)) 15–30 cm in length are used almost exclusively, in conjunction with gradient elution using mixtures of acetonitrile/water or methanol/water. A typical gradient may start as a 50 % mixture, changing to 100 % acetonitrile or methanol in 40 minutes. This flow is maintained for 20 minutes, followed by a return to the original conditions in 5 minutes and 5–10 minutes' equilibration before the next injection. The use of an automatic injector is strongly recommended. Also, the column should be maintained in a column oven heated to 10–30°C. The systems yielding the best sensitivity and selectivity utilize fluorescence detection. As different PAH compounds yield their maximum fluorescence at different wavelengths, for optimum detection of PAHs the wavelengths of the detector should be programmed so that the excitation/emission wavelengths detected are changed at pre-set times during the analytical determination. For closely eluting peaks, it may be necessary to use two detectors in series utilizing different wavelength pairs, or to affect a compromise in the selected wavelengths if a single detector is used. As the fluorescence signals of some PAHs (e.g., pyrene) are quenched by oxygen, the eluents must be degassed thoroughly. This is usually achieved by continuously bubbling a gentle stream of helium through the eluent reservoirs, but a vacuum degasser can also be used. Polytetrafluorethylene

(PTFE) tubing must not then be used downstream of the reservoirs as this material is permeable to oxygen; stainless steel or polyether-etherketone (PEEK) tubing is preferred.

5.9 GC-MS

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. 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 25–30 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; 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. These separations can be made on other columns, if necessary. For PAHs there is no sensitivity gain from the use of chemical ionization (either positive or negative ion), so analyses are usually conducted in electron-impact mode at 70 eV. The choice of full-scan or multiple-ion detection is usually made in terms of sensitivity. Some instruments such as ion-trap mass spectrometers exhibit the same sensitivity in both modes, so full-scan spectra are collected, whereas for quadrupole instruments greater sensitivity is obtained if the number of ions scanned is limited. In that case, the masses to be detected are programmed to change during the analysis as different PAHs elute from the capillary column.

6. CALIBRATION AND QUANTIFICATION

6.1 STANDARDS

A range of fully deuterated parent PAHs is available for use as standards in PAH analysis. The availability of pure PAH compounds is limited (Annex B-7). 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. In HPLC, where the resolving power of the columns is limited and the selectivity less than that which can be obtained using MS detection, only a single internal standard is normally used (e.g., phenanthrene-d10), although fluoranthene-d10 and 6-methyl chrysene, among others, have also been used. If GC-MS is used, then a wider range of deuterated PAHs can be utilized, both because of the wide boiling range of PAHs present and because that allows the use of both recovery and quantification standards. Suitable standards could range from naphthalene-d8 to perylene-d10. It is always recommended to use at least two and preferably three internal standards of hydrocarbons of small, medium, and high molecular weight (e.g., naphthalene-d8, phenanthrene-d10, perylene-d12. Crystalline PAHs of known purity

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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. 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 exhibits non-linear behaviour when the mass of a compound injected is low due to adsorption. Quantification should be conducted in the linear region of the calibration curve, or the non-linear region must be well characterized during the calibration procedure. For HPLC-UVF, the linear range of the detection system should be large, and quantification should be made within the linear range. External standardization is often used with HPLC due to the relatively limited resolution obtainable with this technique as generally employed.

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. For GC-MS analysis, 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 ensures that the calculated PAH concentrations are corrected for the recovery obtained in each case. In the case of HPLC, where only a single deuterated PAH standard is used, it is more common to assess recovery periodically by carrying a standard solution through the whole analytical procedure, then assessing recovery by reference to an external standard. This technique does not, however, correct for matrix effects, and so may be used in conjunction with the spiking of real samples.

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: 0.2 µg kg-1 ww;

- for HPLC measurements: 0.5–10 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. Test materials from the former runs of QUASIMEME Laboratory Proficiency Testing can be used as Laboratory Reference Material. The LRM must be homogeneous and well characterized 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. The availability of biota CRMs certified for PAHs is very limited (Annex B-7; QUASIMEME), and in all cases the number of PAHs for which certified values are provided is small. At regular intervals, the laboratory should participate in an intercomparison or proficiency exercise in order to provide an independent check on the 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 in 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.

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Annex B-12 Technical note on the determination of heavy metals and persistent organic
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