# 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

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# 1. Background

This guideline provides advice on the analysis of polycyclic aromatic hydrocarbons (PAHs) in marine biota. It is suitable for PAH in non-vertebrate biota and for their metabolites (such as 1-hydroxypyre and 1-hydroxyphenanthren) in fish. The procedure described here covers analysis of alkylated PAHs, non-alkylated PAHs (parent) and their main metabolites.

All steps of the procedure are susceptible to contamination by traces of contaminants. Quality control measures are recommended in order to minimize a possible contamination of the sample originating from used chemicals, tools or surrounding. These guidelines are intended to encourage and guide scientific personnel to critically review their methods of sampling and to improve their procedures and quality assurance measures.

These guidelines are not intended as complete manual. If necessary, guidance should be sought from specialized and experienced laboratories.

Contracting parties should follow the HELCOM monitoring guideline but minor deviations from this are acceptable if the method achieves comparable results. Validation of the adopted analytical methods needs to be performed on the relevant matrix and concentration range e.g. by taking part in intercomparison studies or proficiency testing schemes (e.g. QUASIMEME or BEQUALM). These measures of quality assurance also cover a suitable sampling strategy and sample storage without contamination as described here.

## 1.1 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 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 system which allows them to metabolize PAHs and to excrete them via bile. An assessment of the exposure of fish to PAHs is therefore performed by the determination of PAH-metabolite concentrations in bile samples.

There are marked differences in the behavior 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 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.

## 1.2 Purpose and aims

The aim of the monitoring is to analyze the trace concentrations of PAHs or their metabolites in fish or shellfish. This includes the proper sampling, dissection and storage of the samples without contamination.

# 2. Monitoring methods

## 2.1 Monitoring features

Monitoring of organic pollutants (e.g. PAHs) in the marine environment should take into account the specific objectives of the monitoring program, including the quantitative objectives (e.g. spatial variation, time trends, effectiveness of measures). In this case the natural variability between the samples should be held low by an appropriate sampling. To minimize biological variability organisms from the same size class and sex (if possible) should be chosen to minimize biological variability.
All teleost fish have the capacity for rapid metabolism of PAHs. Therefore parent PAHs are found only in low concentrations in fish but PAH metabolites are found in reasonable concentration in fish bile. Shellfish (particularly molluscs) generally have a lesser metabolic capacity towards PAHs, and so PAH concentrations are generally higher in their tissues. 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 exposure of fish to PAHs can be assessed by the analysis of PAH-metabolites in bile. At offshore locations, the collection of appropriate shellfish samples may be problematic and the collection of fish samples may be simpler.

The sampling strategy for biological monitoring is described in detail in Combine, Part B, Annex B12, Appendix 1.

## 2.2 Time and area

The sampling areas should be located in the Baltic Sea and can be chosen regarding the aim of the monitoring program and the availability of organisms. Sampling frequency should be at least annually outside the spawning season.

## 2.4 Monitoring procedure

### 2.4.1 Monitoring strategy

When conducting an integrated chemical and biological effects sampling program where causes and effects should be regarded together, the sampling strategy used should comply with those used for biological effects monitoring. One important aspect is that the organisms should be randomly sampled as they appear in their size class and sex (if possible) regardless if they have visible effects or not.

### 2.4.2 Sampling method(s) and equipment

For sampling fish/shellfish for PAH/PAH-metabolites analysis contact to oil, fuel, exhaust gases or surfaces should be avoided. When samples are processed, both at sea or onshore, the dissection must be undertaken by trained personnel in a clean bench (if available) and wearing gloves. Alternative to a clean bench, clean

lab conditions can be chosen or the contamination during dissection can be excluded otherwise. These samples should not been stored with direct contact to plastic. Suitable materials for storage are glass, steel or aluminum pre-cleaned with ultra clean solvents. Samples could be dissected using e.g. stainless steel knives, hemostats, scalpels or tweezers and stored in glass vials or wrapped in aluminum foil before freezing. 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 aluminum, or should be wrapped in pre-cleaned aluminum foil and shock-frozen quickly in liquid nitrogen or in a blast freezer. The individual samples should be clearly and indelibly labelled and stored together in a suitable container at a temperature of -20 °C until analysis.

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 contamination which may result from back-streaming of oil from the rotary pumps used to generate the vacuum.

For sampling fish bile for PAH metabolite analysis the use of plastic and metal are minor problems. Bile samples should be taken with disposable needles (metal) and syringes (plastic) and stored frozen in any suitable vial. The tools should be replaced for every individual sampling. Pre-cleaning is not necessary. Contamination of blood should be strictly avoided because it may interfere with extraction.

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 fiber 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.

*Extraction and clean-up*

As PAHs are lipophilic, they are concentrated in the lipids of an organism, and a number of methods have been described for PAH extraction (see, e.g. Wang et al., 2017; Webster et al 2010). 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 considered as one of the 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. 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. 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 extraction should be followed by a suitable clean-up to remove those compounds which may interfere with the subsequent analysis. Different techniques may be used, both single 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. 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 (Lau et al., 2010) . The major advantages of using HPLC-based clean-up methods are their ease of automation and reproducibility. 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.

Bile samples are mixed with glucuronidas/sulfatase enzymes in order to convert conjugates present in the bile fluid into metabolites. The enzymatic reaction is then stopped by e.g. ethanol and the precipitated proteins are removed by centrifugation. After that, the sample is ready for HPLC-Fluorescence injection after filtration by a suitable syringe filter to avoid solid material in the HPLC-system. Quantification is usually performed by external calibration with certified standard solutions and can be supported by laboratory reference materials (Kammann, 2007).

*Selection of PAHs to be determined*

The choice of PAHs to be analyzed 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 programs 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. 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 analyzed in all cases, but an appropriate selection can be made from this list depending on the specific aims of the monitoring program to be undertaken.

If PAH in fish have to be analyzed PAH metabolites should be chosen instead of parent PAHs. The main metabolite 1-hydroxypyrene should be always the first choice.

**Table 1:** Compounds of interest for environmental monitoring for which the guidelines apply: parent PAHs, alkylated PAHs, PAH metabolites

| Parent PAH compound | MW | Alkylated PAH compound | MW | PAH metabolite | MW |
|---|---|---|---|---|---|
| **Naphthalene** | 128 | C1-Naphthalenes | 142 | 1-Hydoxypyrene | |
| **Acenaphthylene** | 152 | C2-Naphthalenes | 156 | 1-Hydroxyphenanthrene | |
| **Acenaphthene** | 154 | C3-Naphthalenes | 170 | 3-Hydroxybenzo(a)pyrene | |
| **Biphenyl** | 154 | C4-Naphthalenes | 184 | | |
| **Fluorene** | 166 | C1-Fluorenes | 180 | | |
| **Dibenzothiophene** | 184 | C2-Fluorenes | 194 | | |
| **Phenanthrene** | 178 | C3-Fluorenes | 208 | | |
| **Anthracene** | 178 | C1-Dibenzothiophenes | 198 | | |
| **Fluoranthene** | 202 | C2-Dibenzothiophenes | 212 | | |
| **Pyrene** | 202 | C3-Dibenzothiophenes | 226 | | |
| **Benz[a]anthracene** | 228 | C1-Phenanthrenes/ Anthracenes | 192 | | |
| **Chrysene** | 228 | C2-Phenanthrenes/Anthracenes | 206 | | |
| **Benzo[a]fluoranthene** | 252 | C3-Phenanthrenes/Anthracenes | 220 | | |
| **Benzo[b]fluoranthene** | 252 | C1-Fluoranthenes/Pyrenes | 216 | | |
| **Benzo[j]fluoranthene** | 252 | C2-Fluoranthenes/Pyrenes | 230 | | |
| **Benzo[k]fluoranthene** | 252 | 2,3-Benzanthracene | 228 | | |
| **Benzo[e]pyrene** | 252 | | | | |
| **Benzo[a]pyrene** | 252 | | | | |
| **Perylene** | 252 | | | | |
| **Indeno[1,2,3-cd]pyrene** | 276 | | | | |
| **Benzo[ghi]perylene** | 276 | | | | |
| **Dibenz[ah]anthracene** | 278 | | | | |

*Instrumental determination of PAHs*

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 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 analytes 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.

*HPLC*

Reversed-phase columns (e.g. 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 column should be maintained in a column oven heated to e.g. 30°C. HPLC-Fluorescence is widely used to quantify PAH-metabolites in bile fluids. The systems yielding the best sensitivity and selectivity utilize fluorescence detection which changes excitation/emission pairs over time. 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.

*GC-MS and GC-MS/MS*

For PAH analysis, the cleanliness of the liner is a 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.

*Calibration and quantification*

A range of fully deuterated parent PAHs is available for use as standards in PAH analysis. 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 only a single internal (e.g., phenanthrene-d10) or external calibration (e.g. 1-hydroxypyrene) have been used. If GC-MS is used, then a wider range of deuterated PAHs can be utilized as internal standards, both because of the wide boiling range of PAHs. 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). Calibration standards should be stored in the dark because some PAHs are photosensitive. Multilevel calibration with at least five calibration levels is preferred to adequately define the calibration curve. 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.

*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 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.

### 2.5 Data analysis

Data analysis for results is carried out as demanded by the respective analytical method.

To obtain a comprehensive picture of spatial PAH exposure in the environment concentrations of PAHs in shellfish and of PAH-metabolites in fish can be regarded together when appropriate threshold levels are applied.

## 3. Data reporting and storage

Data reporting, including QA information, should be in accordance with the requirements set by the relevant HELCOM bodies to ensure that all information for the assessment procedure to be applied are available, and using the ICES reporting. Information on the ICES data base (DOME) is available via the ICES-Website (see references).

All available data regarding ship position, net characteristics, speed, sampling date, time and GPS information should be stored carefully, because some of them are needed for reporting. Together with biological data and sample description all relevant data should be stored until reporting in their original formats. Both original data and reporting formatted data files should be stored in the reporting institutes for a suitable time after reporting.

## 4. Quality control

### 4.1 Quality control of methods

Quality assurance is a relevant part of all procedures from sampling to the final chemical analytical measurement (ICES, 2004). All procedures must be evaluated and controlled on a regular basis. For this purpose a quality assurance procedured scheme must be established and documented in each laboratory. This includes participation in interlaboratory proficiency testing schemes to ensure the long-term stability of the laboratory's performance, the use of reference materials and all required documentation. Variability and precision of the method, limit of determination, recoveries and similar crucial parameters of the methods should be part of the method description and been controled in regular intervals. QUASIMEME (www.wepal.nl) is a suitable proficiency testing scheme for testing analytical precision and accuracy between laboraties in environmental matrices. BEQUALM (www.bequalm.org) offers similar tests for PAH metabolites. Participation in QUASIMEME, BEQUALM or other suitable schemes (e.g. Kammann et al., 2013) should be regularly performed by the analytical lab.

To minimise the risk of contamination or the loss of determinands during sampling, storage, pre-treatment or analysis quality assurance measures should be applied to the sample from first contact to final measurement and for data reporting. All detailed QA data should be recorded in accordance with the QA procedures laid down in the relevant documents.

Training of personnel is part of quality assurance and of special importance regarding sampling. Only experienced personnel aware of possible contamination sources and trained in biological sampling should carry out sampling and storage. Detailed sampling schemes (Species, sex, numbers sizes etc) and sampling/storage protocols should be available as document on the ship and clearly communicated to the person in charge before the sampling has started.

### 4.2 Quality control of data and reporting

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 determinants 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 determinant 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. At regular intervals, the laboratory should participate in an intercomparison or proficiency exercise in order to provide an independent check on the performance.

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.

For quality control of data and reporting plausibility checks of the reported data should be done by the reporting institute. The correct upload to the data portals should be verified by a clean error log.

# 5. Contacts and references

## 5.1 Contact persons

Relevant experts can be contacted via HELCOM Expert Network on hazardous substances (EN-HZ) over the Co-Chairs:

Look up the actual chair persons under [HTTPS://HELCOM.FI/HELCOM-AT-WORK/GROUPS/STATE-AND-CONSERVATION/EN-HAZARDOUS-SUBSTANCES/](HTTPS://HELCOM.FI/HELCOM-AT-WORK/GROUPS/STATE-AND-CONSERVATION/EN-HAZARDOUS-SUBSTANCES/)

## 5.2 References

BEQUALM, BIOLOGICAL EFFECTS QUALITY ASSURANCE IN MONITORING PROGRAMMES (www.bequalm.org)

Budzinski, H. 2000. Combined protocol for analysis of PAHs and PCBs from sediments using focused microwave assisted (FMW) extraction at atmospheric pressure. Fresenius Journal of Analytical Chemistry, 367: 165–171.

During, R.A., Gaath, S. 2000. Microwave assisted methodology for the determination of organic pollutants in organic municipal wastes and soils: extraction of PCBs using heat transformer discs. Fres. J. Anal.Chem., 368: 684–688.

HELCOM Expert Network on hazardous substances (EN-HZ): HTTP://WWW.HELCOM.FI/HELCOM-AT-WORK/GROUPS/STATE-AND-CONSERVATION/EN-HAZARDOUS-substances/

ICES DOME: http://www.ices.dk/marine-data/data-portals/Pages/DOME.aspx

ICES. 2004. Chemical measurements in the Baltic Sea: Guidelines on quality assurance. Ed. by E. Lysiak-Pastuszak and M. Krysell. ICES Techniques in Marine Environmental Sciences, No. 35.149 pp.

Kammann, U. 2007. PAH metabolites in bile fluids of dab (Limanda limanda) and flounder (Platichthys flesus) – spatial distribution and seasonal changes. Environ Sci Pollut Res, 14(2): 102-108.

Kammann, U., Askem, C., Dabrowska, H., Grung, M., Kirby, M.F., Koivisto, P., Lucas, C., McKenzie, M., Meier, S., Robinson, C., Tairova, Z.M., Tuvikene, A., Vuorinen, P.J., Strand, J. 2013. Interlaboratory proficiency testing for measurement of the PAH metabolite 1-hydroxypyrene in fish bile for marine environmental monitoring, Journal of AOAC International, 96(3): 635-641.

Lau EV, Gan S, Ng HK. 2010 Extraction techniques for polycyclic aromatic hydrocarbons in soils. Int J Anal Chem. 2010:398381. .

Law, R.J., de Boer, J. 1995. Quality assurance of analysis of organic compounds in marine matrices: Application to analysis of chlorobiphenyls and polycyclic aromatic hydrocarbons. *In* Quality Assurance in Environmental Monitoring—Sampling and Sample Pretreatment, pp. 129–156. Ed. by P. Quevauviller. VCH Publishers, Weinheim, Germany.

QUASIMEME (www.wepal.nl)

Ramil Criado M. 2002. Optimization of microwave-assisted extraction method for the analysis of PCBs in ash samples. Journal of Chromatography A, 1: 1–9.

Vázquez Blanco, E., López Mahía, P., Muniategui Lorenzo, S., Prada Rodríguez, D., and Fernández Fernández, E. 2000. Optimization of microwave-assisted extraction of hydrocarbons in marine sediments: comparison with the Soxhlet extraction method. Fresenius Journal of Analytical Chemistry, 366: 283–288.

Wang W, Meng B, Lu X, Liu Y, Tao S. 2007. Extraction of polycyclic aromatic hydrocarbons and organochlorine pesticides from soils: a comparison between Soxhlet extraction, microwave-assisted extraction and accelerated solvent extraction techniques. Anal Chim, 602(2):211-22.

Webster,L., Tronczynski,J., Korytar,P., Booij,K., Law,R. 2010. Determination of parent andalkylated polycyclic aromatic hydrocarbons (PAHs) in biota and sediment. ICES Techniques in Marine Environmental Sciences. No.45.26pp.

## 5.3 Additional literatur

Galgani F, Martínez-Gómez C, Giovanardi F, Romanelli G, Caixach J, Cento A, Scarpato A, Benbrahim S, Messaoudi S, Deudero S, Boulahdid M, Benedicto J, Andral B. 2011. Assessment of polycyclic aromatic

hydrocarbon concentrations in mussels (Mytilus galloprovincialis) from the Western basin of the Mediterranean Sea. Environ Monit Assess. 172(1-4):301-317

Guidance Document No 25 on chemical monitoring of sediment and biota under the Water Framework Directive. European Commission Technical Report 2010-41. https://circabc.europa.eu/sd/a/7f47ccd9-ce47-4f4a-b4f0-cc61db518b1c/Guidance%20No%2025%20-%20Chemical%20Monitoring%20of%20Sediment%20and%20Biota.pdf

Guidance Document No 32 on Biota Monitoring (the Implementation of EQSbiota) under the Water Framework Directive. European Commission Technical Report - 2014 - 083. https://circabc.europa.eu/sd/a/62343f10-5759-4e7c-ae2b-12677aa57605/Guidance%20No%2032%20-%20Biota%20Monitoring.pdf

Guidance Document No. 33 on analytical methods for boita monitoring under the Water Framework Directive. European Commission Technical Report - 2014 – 084. https://circabc.europa.eu/sd/a/9cf535ba-14f2-4f0f-b75e-e334ad506caf/Guidance%20No%2033%20-%20Analytical%20Methods%20for%20Biota%20Monitoring.pdf

Liguori L, Heggstad K, Hove HT, Julshamn K. 2006 An automated extraction approach for isolation of 24 polyaromatic hydrocarbons (PAHs) from various marine matrixes. Anal Chim Acta. 573-574:181-8.

Zelinkova Z, Wenzl T. Polycycl Aromat Compd. 2015 The Occurrence of 16 EPA PAHs in Food - A Review. 35(2-4):248-284. doi: 10.1080/10406638.2014.918550. Epub 2015 Nov 16. PMID: 26681897