Manual for Marine Monitoring in the

COMBINE

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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 3
Technical note on the determination of chlorinated biphenyls and organochlorine pesticides in biota

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

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1. Background

This guideline provides advice on biological sampling and sample handling for the analysis of persistent organic pollutants in marine biota. It is suitable for organochlorine pesticides (OCPs) and chlorinated biphenyls (CBs) such as PCB, DDT and metabolites, HCH isomers, HCB, and dieldrin and dioxin-like PCBs if their concentrations are comparable to those of other PCB.

The procedure described here covers analysis of CBs and OCPs in marine fish and shellfish.

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. These measures of quality assurance also cover a suitable sampling strategy and sample storage without contamination as described here.

1.1 Introduction

The analysis of chlorinated biphenyls (CBs) and organochlorine pesticides (OCPs) in fish samples generally involves extraction from the respective matrix with organic solvents together with lipids, followed by clean-up and gas chromatographic separation with electron capture (GC-ECD) or mass spectrometric (GC-MS) detection.

The analytical procedure is liable to systematic errors due to insufficiently optimized gas chromatographic conditions, determinant losses (evaporation, unsatisfactory extraction yield), and/or contamination from laboratory ware, reagents and the laboratory environment. It is therefore essential that the sources of systematic errors are identified and eliminated as far as possible.

In the following paragraphs, the JAMP guidelines (OSPAR, 2012) have been taken into consideration.

1.2 Purpose and aims

The aim of the monitoring is to analyse the trace concentrations of CBs and OCPs in tissues of 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 persistent organic pollutants such as chlorinated biphenyls (CBs) and organochlorine pesticides (OCPs) in marine biota should take into account the specific objectives of the monitoring programme, including the quantitative objectives (e.g. spatial variation, time trends, effectiveness of measures). In this case biological variability between the samples should be reduced by an appropriate sampling. To minimize

biological variability only healthy organisms from the same size class and sex (if possible) in good condition should be chosen.
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.

2.2 Time and area

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

2.4 Monitoring procedure

2.4.1 Monitoring strategy

The researcher should consider advantages and disadvantages of sample dissection on board of the vessel compared to dissection in the laboratory regarding sample contamination. If dissection on board is not desired the organisms can be frozen on board and dissected later in the laboratory under conditions of maximum protection against contamination.

2.4.2 Sampling method(s) and equipment

For sampling fish/shellfish for organic chemical analysis contact to plastics, rubber, oil, fuel, exhaust gases or surfaces should be avoided. These samples should not been stored with direct contact to plastic. Suitable materials for storage are glass, steel or aluminium pre-cleaned with ultra clean solvents. Al nylon bags (Rilsan bags) are suitable for storing samples of mussels, fish and sediment. Samples could be dissected using e.g. stainless steel knives, hemostats, scalpels or tweezers and stored in glass vials or wrapped in aluminium foil before freezing.

2.4.3 Sample handling and analysis

Pre-treatment of laboratory ware and reagents; contamination control

Glassware, reagents, solvents, and other laboratory equipment that comes into contact with the sample material to be analysed should be free of impurities that interfere with the quantitative determination of CBs and OCPs following Webster et al. 2013.

For cleaning purposes, the following procedures should be followed:

1. Glassware should be thoroughly washed with detergents, dried with acetone and rinsed with a non-polar solvent such as *n*-pentane, hexane or toluol.

2. Glass fibre Soxhlet thimbles should be pre-extracted with an organic solvent. The use of paper Soxhlet thimbles should be avoided. Alternatively, glass fibre thimbles or full glass Soxhlet thimbles, with a G1 glass filter at the bottom, are recommended.

3. Solvents should be checked for impurities using GC after concentrating the volume normally used in the procedure to 10 % of the final volume. If necessary, solvents can be purified by controlled re-distillation and rectification over KOH in an all-glass distillation column.

4. Reagents and column adsorption materials should be checked for contamination before use by extraction with an organic solvent (e.g., *n*-pentane) and analysis by GC, using ECD or MS detector.

5. Laboratory air can also be contaminated with CBs, OCPs or compounds interfering with the CB/OCP analysis. A good estimation of the contamination of the air can be found by placing a petri dish with 2 grams of C18-bonded silica for two weeks in the laboratory. After this period, the material is transferred to a glass column and eluted with 10 ml of 10% diethylether in hexane. After concentrating the eluate, the CB concentrations can be measured. Absolute amounts of <1 ng show that the contamination of the air is at an acceptably low level in that laboratory (Smedes and de Boer, 1994).

Sample pretreatment and Clean-Up

To ensure complete extraction of the lipophilic CBs and OCPs from biological sample matrices, it is essential to dry the material and disrupt the cell walls of the biological matrix to be analysed. This can be achieved by using Ultra Turrax mixing or grinding of the sample with a dehydrating reagent, such as Na_2SO_4, followed by multiple solid/liquid extraction with a mixture of polar and non-polar solvents (e.g., acetone/hexane or methanol/dichloromethane) using accelerated solvent extraction (ASE) or Soxhlet extraction. It is essential to allow complete binding of the water present in the sample with the dehydrating reagent prior to starting the extraction step. The extraction efficiency must be checked for different types and amounts of biological matrices to be investigated (see 'recovery section').

The crude lipid extract obtained from sample pretreatment requires a clean-up in order to remove co-extracted lipophilic compounds that interfere with the gas chromatographic determination of CBs and OCPs. Effective removal of high molecular weight compounds can be achieved by gel permeation chromatography (GPC). For further clean-up and/or fractionation an additional SPE clean-up may be required. Normal-phase solid/liquid chromatography, using deactivated Al_2O_3 or deactivated silica as adsorbents and hexane or iso-octane as solvents, is an appropriate technique for the separation of the determinants from lipids or other interfering compounds.

Determination by gas chromatography

Because of the large number of organochlorine compounds to be determined, high resolution gas chromatography (GC) with fused silica capillary columns is necessary. Detection with MS or MS/MS is widely used. However, ECD can be suitable too.

Hydrogen is the preferred carrier gas and is indispensable for columns with very small inner diameters. As a compromise to safety aspects, helium is also acceptable.

In order to achieve sufficient separation, capillary columns should have a length of 30 or 60 m, an internal diameter of < 0.25 mm (for diameters below 0.18 mm the elevated pressure of the carrier gas needs special instrumentation) and a film thickness of the stationary phase of < 0.25 µm. For routine work, the SE 54 (Ultra 2, DB 5, RTx 5, CP-Sil 8) phase (94 % dimethyl-, 5 % phenyl-, 1 % vinyl-polysiloxane) or medium polar columns (CP-Sil 19, OV-17, OV 1701, DB 17) have been shown to give satisfactory chromatograms. A second column with a stationary phase different from that used in the first column, may be used for confirmation of the peak identification, if ECD is used or MS does not separate homologues.

Splitless and on-column injection techniques may both be used. Split injection is not recommended because strong discrimination effects may occur. Other techniques such as temperature-programmed or pressure-programmed injection may have additional advantages, but should be thoroughly optimized before use. The reproducibility of injection is controlled by the use of an internal standard not present in the sample.

Quantitative analysis is performed by comparing the detector signal produced by the sample with that of defined standards. The use of an electron capture detector (ECD) sensitive to chlorinated compounds or - more generally applicable - a mass selective detector (MS or MS/MS) is essential.

Due to incomplete separation, several co-eluting compounds can be present under a single detector signal. Therefore, the shape and size of the signal have to be critically examined. With a MS used as detector, either the molecular mass or characteristic mass fragments should be recorded for that purpose. If only an ECD is available, the relative retention time and the signal size should be confirmed on columns with different polarity of their stationary phases.

Calibration

Stock solutions of individual organohalogen compounds should be prepared using iso-octane as the solvent and weighed solid individual standard compounds of high purity (> 99 %) or by dilution of certified standard solutions. Stock solutions can be stored in measuring flasks in a refrigerator or in a dessiccator with a saturated atmosphere of iso-octane, but losses can easily occur, particularly when storing in refrigerators. Loss of solvents in stock solutions can be controlled by recording the weight and filling up the missing amount before a new aliquot is taken.

The GC should be calibrated before each batch of measurements. Mixtures of standard solutions are commercially available, e.g. a set of six standard solutions for CB determination or five standard solutions for OCP determination. Standards used for multilevel calibration should be regularly distributed or repeated over the sample series, so that matrix and non-matrix containing injections alternate.

For the purpose of determining recovery rates as well as quantification, one or more appropriate internal standards should be added to each sample at the beginning of the analytical procedure. Ideal internal standards are CBs which are not present in the sample and which do not interfere with other CBs. All 2,4,6-substituted CB congeners are, in principle, suitable. With GC/MS, C13 or 13C-labelled CBs should preferably be used as internal standards. More as one internal standard could be used to cover different physical properties of the analytes and mirror losses during extraction or clean-up process linked to e.g. volatility or lipophilicity. The maximum of internal standard coverage is to match every analyte with its own C13- or 13C-labelled standard. This strategy is sometimes chosen when low concentrated analytes and high matrix load occur at the same time (coplanar PCB).

2.5 Data analysis

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

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 (https://www.ices.dk/data/data-portals/Pages/DOME.aspx).

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 procedural 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 controlled in regular intervals. QUASIMEME (www.wepal.nl) is a suitable proficiency testing scheme for testing analytical precision and accuracy between laboratories in environmental matrices. Participation in QUASIMEME or other suitable schemes should be regularly performed by the analytical lab.

To minimise the risk of contamination or the loss of determinants 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

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/

5.2 References

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