Baltic Marine Environment Protection Commission

Working Group on the State of the Environment and Nature Conservation

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Background

HELCOM guidelines for contaminants are currently being revised. In accordance with STATE & CONSERVATION 2-2015 (para 2MA.3), Lead Country Germany submits draft Monitoring guideline on biological material sampling and sample handling for the analysis of persistent organic pollutants (PAHs, PCBs and OCPs) and metallic trace elements in the HELCOM area as a contribution to the ongoing revision of HELCOM monitoring guidelines.

The Monitoring guideline on biological material sampling and sample handling for the analysis of persistent organic pollutants (PAHs, PCBs and OCPs) and metallic trace elements is currently described in Annex B-12, Appendix 1 in the COMBINE manual. The draft for new guidelines includes updates on procedure. The QA/QC section is expanded. The guidelines were also revised by HELCOM EN-HZ and comments received from this working group, especially from Estonia and Denmark are included in the new guidelines.
All changes are presented in track changes.

Action requested

The Meeting is invited to:

- consider, amend as needed, and endorse the draft Monitoring guideline on biological material sampling and sample handling for the analysis of persistent organic pollutants (PAHs, PCBs and OCPs) and metallic trace elements.

Monitoring guideline on biological material sampling and sample handling for the analysis of persistent organic pollutants (PAHs, PCBs and OCPs) and metallic trace elements

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1. Background

This guideline provides advice on biological sampling and sample handling for the analysis of persistent organic pollutants and metallic trace elements in marine biota. They are suitable for hazardous substances: trace metals and organic compounds including chlorinated compounds (such as PCB, DDT and metabolites, HCH isomers, HCB and dieldrin), parent and alkylated PAHs, PAH metabolites, brominated flame retardants such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), perfluorinated compounds (PFCs), organotin compounds (TBT and its breakdown products), dioxins, furans and dioxin-like PCBs.

The procedure described here covers sampling of 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

Shellfish or fish have to be dissected while they are in good condition. If biological tissue deteriorates, uncontrollable losses of determinants, cross-contamination from other deteriorating tissues and organs or problems of determining wet weight may occur. To avoid this, shellfish samples and individual fish specimens must be dissected at sea if adequate conditions prevail on board, or be frozen immediately after collection and transported frozen to the laboratory using appropriate inert packing materials to avoid the contamination from package, where they are dissected later.

If the option chosen is dissection on board the ship, the following criteria must be met:

1. The work must be carried out by personnel capable of identifying the species and removing the desired organs according to the requirements of the investigations; and
2. The risk of contamination from work space or equipment must have been minimized by selection and/or pretreatment of the equipment and work space on board.
3. Only experienced personnel aware of possible contamination sources should carry out sampling, dissection and storage. Information and training of the personnel is necessary as part of quality assurance.

1.2 Purpose and aims

The aim of the monitoring is to analyse the trace concentrations of chemical contaminants 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 (PAHs, PCBs and OCPs) and metallic trace elements in 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 introduction of additional variability between the samples should be reduced by appropriate sampling. If possible only healthy organisms from the same sex (fish) and size class in good condition should be chosen to minimize biological variability.
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 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 in line with the aim of the monitoring program and the availability of organisms. Sampling frequency should be annual, and for fish outside the spawning season.

2.4 Monitoring procedure

2.4.1 Monitoring strategy

In general, sampling and storage should be performed without contamination.

The researcher should consider advantages and disadvantages of sample dissection on board the vessel compared to dissection in the laboratory with respect to sample contamination. If dissection on board is not desired the organisms can be frozen on board and dissected later in the laboratory with optimal protection against contamination. A clean bench can help to minimize metal contamination via dust and small particles.

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2.4.2 Sampling method(s) and equipment

Every biota sample should be characterized and described in a suitable sample protocol. For fish at least species, length, weight and, if possible, sex should be reported. For shellfish species, length, total weight and soft body weight are necessary. When sampling biota for organic chemical analysis contact to plastics, rubber, oil, fuel, exhaust gases or contaminated 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. 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.

When sampling fish bile for PAH metabolite analysis plastic and metal are no general problem. Bile samples should be taken with disposable needles (metal) and syringes (plastic) and stored in any suitable vial. The tools should be replaced for every individual sampling. Pre-cleaning is not necessary.

Sampling biota for trace metal analysis tools made of metal should be avoided and e.g. coulorless plastic tweezers or ceramic knifes are suitable for dissection. These samples could be stored in plastic or glass tubes or nylon bags (Rilsan bags). Contact to dust, exhaust gases or particles of ship paint should be avoided. After each sample has been prepared, including the samples of different organs from the same individual, the tools should be changed or cleaned.

The following procedures are recommended for cleaning tools used for preparing samples:

1) for the analysis of metallic trace elements

- a) Wash in acetone or alcohol and high purity water.
- b) Wash in HNO_3 (p.a.) diluted (1+1) with high purity water. Tweezers and haemostates in diluted (1+6) acid.
- c) Rinse with high purity water.

2) for the analysis of CBs and OCPs

- a) Wash in acetone or alcohol and rinse in high purity water
- b) Clean with hexane or other high purity extraction solvent used in the later sample preparation

The surface used during dissection should be cleaned in the same manner. The tools must be stored dust-free when not in use. The dissection room should be kept clean and the air should be free from particles.

Fish muscle and liver samples dissection

Fish species recommended for monitoring in the Baltic Sea are dab, flounder, cod, herring, perch and eelpout (Table 1). The fish species should be chosen dependent on the sampling method and availability in the area (coastal/off-shore). Preferably 15-20 but a minimum of 12 individuals should be sampled from a suitable size class. All fish should be analysed individually for contaminants. As for dab, perch and flounder, sex can be determined easily and only females should be chosen.

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Table 1: Fish *species* sampling characteristics

Fish Species	Habitat	Number	Size [cm]	Age [year]	Sex	Tissue
Cod (Gadus morhua)	Off shore		25-40	1-3		
Dab (Limanda limanda)	Off shore		18-30	1-3	Single sex, females	Muscle for Hg, bile for PAH metabolites, liver for all other determinants
Eelpout (Zoarces viviparous)	Coastal			2-3	Single sex, preferably females	
Flounder (Platichthys flesus)	Coastal/off shore	Preferably 15-20 at least 12	15-35	1-3	Single sex, females	
Perch(Perca fluviatilis)	Coastal		15-20	2-5	Single sex, females	Muscle for organic contaminants and Hg, liver for other trace metals
Herring (Clupea harengus)	Off shore		15-30	1-2		Muscle for organic contaminants and Hg, bile for PAH metabolites, liver for other trace metals.

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For fish sampling, commercial catches can be used if fish transport to the laboratory does not take longer than 24 hours. The fish must be transported on ice. The dissection then takes place at the laboratory.

For analysis of fish muscle, the epidermis and subcutaneous tissue should be carefully removed from the fish. Samples should be taken under the red muscle layer. In order to ensure uniformity of samples, the right side dorso-lateral muscle should be taken as the sample. If possible, the entire right dorsal lateral filet should be used as a uniform sample, from which subsamples can be taken after homogenizing for replicate dry weight and contaminant determinations. If, however, the amount of material obtained by this procedure is too large to handle in practice, a specific portion of the dorsal musculature should be chosen for the sample. It is recommended that the portion of the muscle lying directly under the first dorsal fin should be utilised in this case. As both fat and water content vary significantly in the muscle tissue from the anterior to the caudal muscle of the fish (Oehlenschläger, 1994), it is important to obtain the same portion of the muscle tissue for each sample.

To sample liver tissue, the liver must be identified in the presence of other organs such as the digestive system or gonads (Harms and Kanisch, 2000). The appearance of the gonads will vary according to the sex of the fish and the season. After opening the body cavity bile fluid samples should be taken first using disposable needles and syringes. Bile fluid samples should not contain traces of blood. After that, the connective tissue around the liver should be cut away and as much as possible of the liver is removed in a single piece. Bile fluid should not contaminate the liver tissue.

When fish samples which have been frozen at sea are brought to the laboratory for analysis, they should be dissected as soon as the tissue has thawed sufficiently. The dissection of fish is easiest when the material, at least the surface layers of the muscle tissue, is half frozen. For dissection of other organs, the thawing must proceed further, but it is an advantage if, for example, the liver is still frozen. It should be noted that any loss of liquid or fat due to improper cutting or handling of the tissue makes the determinations of dry weight and fat content, and consequently the reported concentrations of determinants, less accurate. After muscle preparations and bile sampling, the liver should be completely and carefully removed while still partly frozen to avoid water and fat loss. Immediately after removing it from the fish, the liver should be returned to the freezer so that it will be completely frozen prior to further handling. This is particularly important for cod liver.

Shellfish sampling

The blue mussel *(Mytilus edulis)* occurs in shallow waters along many coasts of the Baltic Sea. It is therefore suitable for monitoring in near shore waters. No distinction is made between *M. edulis*, *M. gallopovincialis,* and *M. trossulus* because the latter species fill a similar ecological niche. Also other members of Mytilus spp can be selected if they are regarded as ecological comparable to *Mytilus edulis* and the above mentioned species are not present.

Two alternative sampling strategies can be used to minimize the influence of natural size variability: size selected sampling or length-stratified sampling.

1) A sampling size range of 2–7 cm shell length is specified to take into account the availability throughout the whole Baltic maritime area. In areas with lower salinities smaller length range (e.g. 1-3 cm) is allowable. The age should be in the range of 3-5 years.

2) For length-stratified sampling, the upper limit of shell length should be chosen in such a way that at least 20 individuals in the largest length interval can easily be found. The length stratification should be determined in such a way that it can be maintained over many years for the purposes of temporal trend monitoring. The length interval shall be at least 5 mm in size. The length range should be split into at least three length intervals (small, medium, and large) which are of equal size

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after log transformation. Mussels can be collected by a bottom grab or picked by hand (diver/on foot), and sorted on shore or onboard. The number of specimens needed for analysis depends on their length, e.g. 80-100 individuals are necessary to get sufficient material within the length range of 4-5 cm.

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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 at -20 °C until analysis. Alternatively the samples could be frozen on board and further processed in the laboratory if no adequate conditions to prevent contamination are available.

Contamination of mussel samples by sediment is a problem. To minimize the potential of contamination mussels should be placed on a polyethylene tray elevated above the bottom of a glass aquarium. The aquarium should be filled with filtered 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 to 24 hours at water temperatures and salinity close to those from which the samples were collected.

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2.4.3 Sample handling and analysis

For shellfish one pooled sample from the homogenised material of size sorted individuals should be analysed. When shucked on board the samples should be stored in suitable containers. Material from single fish specimens should be packaged and stored individually.

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- Samples for analysis of metallic trace elements can be stored in polyethylene, polypropylene, polystyrene or glass containers.
- Samples for analysis of CBs and OCPs should be packaged in precleaned aluminium foil or in precleaned glass containers.
- Bile samples for PAH metabolite analysis should be stored in disposable plastic vials which didn't need to be precleaned

Liver tissue can deteriorate rapidly at room temperature. Consequently, samples should be frozen as soon as possible after packaging. They can be frozen rapidly by immersion in liquid nitrogen or blast freezing, but both these techniques need care. Whatever system is used, freezing a large bulk of closely packed material should be avoided. Once frozen, samples can be stored in a deep freezer at temperatures of -20ºC or below. Each sample should be carefully and permanently labelled. The label should contain at least the sample's identification number, the type of tissue, and the date and location of sampling.

2.5 Data analysis

For sampling no special data analysis is required. All samples were treated and processed individually. Data analysis of the 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 template/method. 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, GPS and other relevant 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

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original formats. Both original data and reporting formatted data files should be stored by the reporting institutions for a suitable time after reporting.

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4. Quality control

4.1 Quality control of methods

Quality assurance (QA) is a relevant part of all procedures from sampling to the final chemical analytical measurement. All procedures must be evaluated and controlled on a regular basis. For this purpose a quality assurance procedure 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 maintenance of 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.

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 documents 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 data should be done by the reporting institution. The correct upload to the data portals should be verified by a clean error log.

Furthermore, it is recommended to store raw data in a way which is closely linked to reporting format regarding e.g. field names, units, formats (e.g. date, time, GPS) or other accompanying information. This strategy helps to avoid unnecessary conversion mistakes.

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 :

- Berit Brockmeyer, E-mail: berit.brockmeyer@bsh.de
- Jaakko Mannio, E-mail: jaakko.mannio@ymparisto.fi

5.2 References

Harms, U.; Kanisch, G. (2000): Intra-Individual Variability of levels of lead detected in the liver of Baltic cod (Gadus morhua). Mar. Poll. Bull., 40 / 8, 710-712

Oehlenschläger, J. 1994. Quality assurance during sampling onboard. *In* ICES/HELCOM Workshop on Quality Assurance of Chemical Analytical Procedures for the Baltic Monitoring Programme. Ed. by G. Topping and U. Harms. Baltic Sea Environment Proceedings No. 58: 82-84.

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

5.3 Additional literature

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 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

JAMP Guidelines for Monitoring Contaminants in Biota. Update 2010. Revision 2012. OSPAR Commission, London, UK.) https://www.ospar.org/documents?d=32414

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