ECOREGION General advice
SUBJECT OSPAR request for review and update of the Technical Annexes to JAMP Guidelines for Monitoring of Contaminants in Biota and in Sediments

Advice summary

Three technical annexes to JAMP Guidelines for Monitoring of Contaminants in Biota and in Sediments (Technical annexes 2, 4, and 6) have been updated to reflect scientific and technical state of the art.

ICES advises that these revised annexes be used by OSPAR Contracting Parties in future.

Request

ICES is requested:

To review and update, as necessary, the following existing technical annexes to JAMP Guidelines:

a. contaminants in biota: Technical Annex 2 (Determination of metals)

b. contaminants in sediments: Technical Annexes 4 (Determination of mono-, di- and tributyltin in sediments: analytical methods) and 6 (determination of metals in sediments – analytical methods)

1) Examination of the current versions downloaded from the OSPAR website (section 'agreements') indicates that these are in need of review and update primarily in terms of updated literature and analytical detection limits (where given). The information is still relevant and there is nothing in there which prevents their being useful guidance.

In the light of the fact that TBT concentrations in the marine environment as shown around UK and the North Sea are now declining rapidly following completion of the implementation of the worldwide IMO ban on the use of TBT in antifouling paints on large vessels (Verhaegen et al., 2012; Law et al., 2012), when reviewed, consideration should also be given to refocusing the butyltins method on dibutyltin as the primary determinand rather than tributyltin, as this compound is still widely used in e.g. plastics and clothing.

2) The direct measurement of metals in environmental matrices such as biota and sediments is a recent development (cf. e.g. Maggi et al., 2009). This technique should be included in the technical guidelines

The updates should build on the latest developments as available to the relevant ICES working groups."

ICES advice

ICES advises that three technical annexes to the JAMP Guidelines for Monitoring of Contaminants in Biota and in Sediments (Technical annexes 2, 4, and 6) have been updated to reflect scientific and technical state of the art.

ICES advises that these revised annexes be used by OSPAR Contracting Parties in future.

For each technical annex, new techniques and developments and new key references were identified.

For both annexes relating to metals, a table has been added with commercially available certified reference materials, to support guidance on quality assurance and quality control. Descriptions relating to the sampling of fish and the sampling of eggs for metals have been clarified with some further added material.

For the technical annex relating to organotin compounds in sediments, new information on laboratory procedures such as extraction, digestion, derivatization and purification methods has been added.

Sources

ICES. 2014a. Report of the Marine Chemistry Working Group (MCWG). In preparation.

ICES. 2014b. Report of the Working Group on Sediments in Relation to Pollution (WGMS). In preparation.

Law, R. J., Bolam, T., James, D., Barry, J., Deaville, R., Reid, R. J., Penrose, R., and Jepson, P. D. 2012. Butyltin compounds in liver of harbour porpoises (*Phocoena phocoena*) from the UK prior to and following the ban on the use of tributyltin in antifouling paints (1992–2005 & 2009). Marine Pollution Bulletin, 64: 2576–2580.

Maggi, C., Berducci, M. T., Bianchi, J., Giani, M., and Campanella, L. 2009. Methylmercury determination in marine sediment and organisms by Direct Mercury Analyser. Analytica Chimica Acta, 641: 32–36.

Verhaegen, Y., Monteyne, E., Neudecker, T., Tulp, I., Smagghe, G., Cooreman, K., Roose, P., and Parmentier, K. 2012. Organotins in North Sea brown shrimp (*Crangon crangon* L.) after implementation of the TBT ban. Chemosphere, 86: 979–984.

Technical Annex 2: Metals in biota

This annex is intended as a supplement to the general guidelines which provides more information on overall sampling strategy. This annex is not a complete description or a substitute for detailed analytical instructions.

1 Species

1.1 Fish and shellfish

1.1.1 Criteria for the selection of species for temporal trend monitoring

Species for temporal trend monitoring should be selected in the light of information on stock composition and history. It is essential that long time-series using the same species with a fixed age-class interval, and preferably from a fixed location are obtained. Fish and shellfish species currently used for trend monitoring are listed in Tables 1 and 2 of the main guidelines.

1.1.2 Criteria for the selection of species for spatial distribution monitoring

The first choice species *Limanda limanda, Gadus morhua,* and *Mytilus edulis* or *Mytilus galloprovincialis* should be used if possible. The second choice species *Merlangius merlangus, Merluccius merluccius, Platichthys flesus*, and *Crassostrea gigas* should be used when none of the first choice species are available.

First choice species

Limanda limanda (dab)

Dab is a ground-dwelling species confined to the shelf seas. It has replaced the previously recommended plaice and flounder for the following reasons:

a. it is less mobile than plaice and flounder, thus it is more likely to represent the contamination of the area in which it is caught;
b. it has been used successfully in disease studies, thus complementary information from such studies would be available (in fish disease studies a length range for individual fish of 20–25 cm is used).

The southern distribution limit of dab is the north coast of Spain.

Gadus morhua (cod)

Cod normally live near the seabed but may also be pelagic. Cod occur in coastal areas and to 600 m depth. Cod may also be found in the open ocean and so may also be used for monitoring oceanic regions. The southern distribution limit of cod is at 45°N. A sampling size range of 30–45 cm is specified because cod of that size and age tend to feed on a fairly uniform diet.

Mytilus sp. (mussel)

Mytilus edulis occurs along almost all coasts of the Contracting Parties. It is therefore suitable for monitoring in nearshore waters (Bellas *et al.*, 2014; Green *et al.*, 2012; IFREMER, 2006). No distinction is made between *M. edulis* and *M. galloprovincialis* because the latter, which may occur along Spanish and Portuguese coasts, cannot easily be discerned from *M. edulis*. The two species accumulate metals in a comparable way. A sampling size range of 3–6 cm is specified to ensure availability in the entire maritime area. For monitoring in polluted areas, caged mussels may be transplanted from an unpolluted area and then left in the polluted area (Benedicto *et al.*, 2011; Søndergaard *et al.*, 2011) for e.g. one year before sampling and analyses. The results will reflect the last year's contamination in contrast to resident mussels that will reflect several years of contamination.

Second choice species

Platichthys flesus (flounder)

The distribution of flounder extends further south than that of dab and might therefore represent the flatfish of choice for certain Portuguese coastal areas and Spain's northwestern coastal areas. Flounder is not suitable for monitoring in open sea areas as it is primarily a coastal species. A sampling size range of 15–35 cm will include individuals of year-class 2, but otolith or other aging techniques will be needed to ensure that the correct year-class fish are analysed.

Merlangius merlangus (whiting)

Whiting can be caught in coastal waters and to 200 m depth. Its distribution is from Portugal to Iceland and Norway, thus covering all the maritime area subject to monitoring by Contracting Parties. It can be used as a substitute for cod. The sampling size range, 20–35 cm, may need adjustment in the light of future experience.

Merluccius merluccius (hake)

Hake live at 100-300 m along the shelf margins. The sampling size range is 20–35 cm; this range may need adjustment in the light of future experience.

Crassostrea gigas (Pacific oyster)

The Pacific oyster should be sampled in areas where *Mytilus* sp. is not available. Two-year old oysters are generally within the length range 9–14 cm. Sufficient individuals should be taken to ensure that the analysis is made on year-class 2 individuals.

1.2 Seabirds

Relevant references concerning the use of seabirds in contaminant monitoring programmes include Gilbertson (1987), Becker (1989, 1991), Becker *et al.* (1991, 1992), Walker (1992), Herzke *et al.* (2009), Miljeteig *et al.* (2009), and Dittmann *et al.* (2012).

Sterna hirundo (common tern)

The common tern is widely distributed over the European and North American Atlantic coasts as well as the Baltic Sea, but does not occur in Iceland. It feeds in marine, brackish, and fresh waters.

Haematopus ostralegus (oystercatcher)

The oystercatcher is widely distributed along the coasts of the Northwest Atlantic, including Iceland, and also occurs in the Baltic Sea. The species is not strictly marine as it also feeds inland. It feeds on benthos. In contrast to other seabirds, nest sites are accessible and the eggs within reach.

Uria aalge (guillemot)

The guillemot feeds in the open sea and nests on the coasts of northern Europe, in the Baltic Sea and on the North American coast.

2 Sampling

Two alternative sampling strategies are described: sampling to minimize natural variability and length-stratified sampling. In order to minimize natural variability, biological covariables should be controlled as far as possible (Viñas *et al.*, 2012).

2.1 Sampling to minimize natural variability

For fish, this can be achieved by sampling and analysing individually at least 12 young fish of the same sex, e.g. 2- to 3-year-old female fish. To assist the selection of the relevant length range in order to find individuals of the recommended age, it is advised to produce species-specific and region-related correlation graphs by use of existing data from the respective monitoring data base. An example is given in Appendix 1. Sampling in the field should bring sufficient fish ashore of relevant length classes to enable laboratory-based aging (through e.g. otolith reading) to ensure that the analysed sample is of the targeted age group.

For shellfish, a sample should be collected with the number of individuals large enough to be divided into at least three equal pools with each pool consisting of at least 20 animals and enough soft tissue for all analyses. The length of the individuals collected should be constant from year to year at each station, or should at least fall within a very narrow range, e.g. within 5 mm. To reflect recent levels of contamination, young individuals should be chosen.

2.2 Length-stratified sampling

Where successfully ongoing, length-stratified time-series should be continued.

2.2.1 Fish

Although several biological parameters are appropriate, length appears to be the parameter which can most easily be applied onshore and at sea and which has also been shown to be significant in many analyses. Much discussion has been devoted as to whether simple linear or log-linear (multiplicative) models give the better fit. General experience with other fish and other types of data indicate a preference for the log-normal model, at least for the present.

As the length dependence of the contaminant concentration is not well understood, sampling should keep the length/contaminant relationship under constant surveillance, i.e. the entire length range should be covered evenly. Care should be taken that the samples are not unduly clustered within a particular length interval. More length intervals could be used and the test of the hypothesized contaminant/length relationship becomes stronger if the lengths are evenly distributed. It is essential to keep the length stratification identical from one year to the next. The length range should be defined on the basis of practical considerations. For fish, the upper limit should be chosen in such a way that at least five fish 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. The length interval should be at least 2 cm in size. The length range should be split into five length intervals, which are of equal size after log transformation. For example, if the length range is 18-36 cm, then the interval boundaries could be (rounded to 0.1 cm) as follows:

18–20.7 20.8–23.8 23.9–27.3 27.4–31.3 31.4–36 cm.

2.2.2 Shellfish

For shellfish, the upper limit should be chosen in such a way that at least 20 mussels 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. The length interval should 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 after log transformation. For example, if the length range is 40-70 mm, then the interval boundaries could be (rounded to 1 mm) as follows:

a. five intervals: 40–45 46–50 51–56 57–63 64–70 mm.
b. three intervals: 40–48 49–58 59–70 mm.

2.3 Seabird eggs

2.3.1 Permission

Permission to collect the eggs must be received from the appropriate national authorities.

2.3.2 Sampling period and frequency

Eggs should be sampled annually at each site in May or June. Only clutches from the first laying cycle within a single year should be selected.

2.3.3 Number of eggs and sampling procedure

Eggs should only be taken from full clutches (i.e. common tern 3 eggs, oystercatcher 3–4 eggs). Eggs should not be taken from abandoned clutches. Only one egg should be taken from each clutch. Ten eggs should be selected in total (i.e. one egg from ten separate clutches) and it is important to choose the egg from each clutch randomly. As the eggs must be fresh (i.e. between 1 and 5 days incubation) information about the incubation stage of each egg is required. Two methods are recommended for determining incubation stage and sampling randomly:

a. Locate 12-15 clutches containing one egg only and mark these by placing a peg about 1 m from the nest. Mark the egg with a soft pencil. Check the clutches every other day until they are complete, marking each egg appropriately. Within two days of the last egg being laid, use a random number generator to ensure one egg is selected randomly from the completed clutch.

If such nest studies cannot occur and only one or two field visits are possible or desirable, then an alternative technique is:

b. Mark each of the eggs with a number and fill a suitable container with water and place the eggs in the water:

i. fresh eggs (i.e. of 1–2 days incubation) will lie on the bottom with the long axis parallel to the bottom;
ii. eggs of 3–6 days incubation will rest with the small end on the bottom of the beaker and the long axis forming an angle of 30–45°;
iii. eggs which float or stand vertically with the small end on the bottom are of more than 7 days incubation and this clutch should not be selected;
iv. for clutches where all eggs have been incubated less than 7 days, use a random number generator to ensure one egg is selected randomly from the completed clutch.

Each nest from which an egg has been taken should be marked, using a peg or some other type of marker, to ensure that a second egg is not taken. While still in the field the egg selected should be put into a numbered plastic egg box. The number of the box should be written on the shell of the egg in soft pencil. The clutch size from which the egg was taken, the nest number, and the sampling date should be recorded.

2.3.4 Materials

For each species, area, and year the following are required:

- nest pegs;
- a non-toxic, waterproof marker;
- a 1 litre plastic beaker;
- numbered egg boxes (e.g. for oystercatcher: 100 ml, polypropylene polyethylene, ø 55 × 73 mm, and for common tern: 50 ml, polystyrol/polyethylene, ø 41 × 49 mm).

3 Transportation

3.1 Fish and shellfish

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

3.2 Seabird eggs

Eggs should be kept cool during transport and frozen at −18°C as soon as possible after collection.

4 Pre-treatment and storage

4.1 Contamination

Sample contamination may occur during sampling, sample handling, pre-treatment and analysis (Oehlenschläger, 1994a), either from the environment, the containers or packing material used, the instruments used during sample preparation, or from the chemical reagents used during the analytical procedures. Controlled conditions are therefore required for all procedures, including the dissection of fish organs on-board ship. Relevant references concerning clean laboratories include Moody (1982), Mitchell (1982a), Boutron (1990), and Schmidt and Gerwinski (1994).

4.2 Fish

4.2.1 Dissection, storage, and drying

Ungutted fish should be wrapped separately in suitable material (e.g. polyethylene or polytetrafluorethylene) and frozen. The frozen samples should be stored in suitable containers to avoid damage. Subsamples (e.g. of liver) should be stored in a suitable acid-cleaned container and frozen or freeze-dried immediately. During freeze-drying, sample temperature should be maintained below 0°C to avoid the loss of volatile compounds. The individual samples should be clearly labelled and stored together in a suitable container. The frozen samples should be maintained at below −20°C until analysis. Freeze-dried samples should be stored in a dessicator. Subsamples for enzyme tests should be stored in vials suitable for storage in liquid nitrogen, labelled clearly and stored in liquid nitrogen until analysis.

The dissection must always be done by trained personnel on a clean bench, wearing clean gloves and using clean stainless steel knives which may be equipped with blades made of ceramics or titanium to reduce the risk of Cr and Ni

contamination. Colourless polyethylene tweezers are recommended for holding tissues during dissection. After each sample has been prepared, the tools should be cleaned regularly. The following procedure is recommended:

- wash in acetone or alcohol and high purity water;
- wash in HNO_3 p.a./high purity water diluted 1+1 (for tweezers diluted 1+6);
- rinse with high purity water.

4.2.2 Subsampling

To sample fish muscle, care should be taken to avoid including any epidermis or subcutaneous fatty tissue in the sample. Samples should be taken underneath the red muscle layer. In order to ensure uniformity the right side dorso-lateral muscle should be sampled. If possible, the entire right dorsal fillet should be homogenized or freeze-dried and subsamples taken for replicate dry weight and contaminant determinations. However, if 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 be used 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, in order to ensure comparability it is important to obtain the same portion of the muscle tissue for each sample (see Oehlenschläger, 1994b).

When dissecting the liver, care should be taken to avoid contamination from other organs (Viñas *et al.*, 2012). The whole liver should be homogenized or freeze-dried. However, if the amount of material to be homogenized would be too large, a specific portion of the liver should be chosen. In order to ensure comparability, this should always be the same part of the liver, preferably the middle part. Liver samples can be freeze-dried. Freeze-drying can be difficult for very fatty samples; in such cases the lipids can be extracted prior to freeze-drying. It must be ensured that no metals are extracted together with the lipids.

Where pooling of tissues is necessary, an equivalent quantity of tissue must be taken from each fish, *e.g.* a whole fillet from every fish. If the total quantity of tissue so yielded would be too large to be handled conveniently, the tissue may be subsampled, but a fixed proportion of each tissue must then be taken, e.g. 10% of each whole fillet or 10% of each whole liver or, for muscle tissue, 10% of the fish.

Personnel must be capable of identifying and removing the desired organs according to the requirements of the investigations.

4.3 Shellfish

4.3.1 Depuration

Mussels should be placed on a polyethylene tray elevated above the bottom of a glass aquarium. The aquarium should be filled with sub-surface (< approximately 1 metre) sea water collected from the same site as the samples and which has not been subject to known contamination from point sources. The aquarium should be aerated and the mussels left for 20–24 hours at water temperatures and salinity close to those from which the samples were removed.

4.3.2 Opening of the shells

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

4.3.3 Dissection and storage

The soft tissues should be removed and deep-frozen (−20°C) as soon as possible in containers appropriate to the intended analysis. The dissection must always be done by trained personnel on a clean bench wearing clean gloves and using clean stainless steel knives which may be equipped with blades made of ceramics or titanium to reduce the risk of Cr and Ni contamination. Colourless polyethylene tweezers are recommended for holding tissues during dissection. After each sample has been prepared, the tools should be cleaned regularly. The following procedure is recommended:

- wash in acetone or alcohol and high purity water;
- wash in HNO_3 p.a./high purity water 1+1 (for tweezers 1+6). This does not apply to parts of metal, e.g. stainless steel knives.
- rinse with high purity water.

4.4 Seabird eggs

Before thawing, the weight of the egg (to the nearest 0.1 g including the shell), the length of the egg between poles and the breadth of the egg at the equator (to the nearest 0.1 mm using callipers) should be recorded. The egg should then be opened (this may occur during thawing) and the content carefully separated from the shell. After thawing, if the egg contains an embryo, the eye diameter or the "crown-tail" length of the embryo should be measured (to the nearest 0.1 mm using callipers). The content of the egg (i.e. the albumen and yolk) should be weighed (to the nearest 0.1 g) and homogenized in the same goblet for each egg (e.g. by an Ultra Turrax). The samples can then be analysed or deep-frozen for later analysis. The shell (including the shell-skin) should be washed with water and dried in laboratory air for at least a week before weighing (to the nearest 0.01 g). The shell thickness should be measured at three points along the egg equator.

5 Analysis

5.1 Preparation of equipment and reagents

Glassware and Teflon equipment should be washed extensively with diluted nitric acid (e.g. 1N HNO_3), distilled water and acidified metal-free deionized water, and should be rinsed immediately before using the following procedure involving acids or solvents. The blank from all plastic and glassware should be checked after the purification procedure. Acids, solvents, chemicals, and adsorption materials should be free of trace metals or organometallic compounds. If not they should be purified by appropriate methods. Acids should be checked by measuring blanks using the analytical procedure applied to the samples. If necessary, the acids should be purified by distillation, preferably under sub-boiling point conditions in a quartz distillation apparatus. If appropriate, chemicals and adsorption materials should be purified by exhaustive extraction with the solvents used for extraction of the metal compounds. Care should be taken to avoid contamination from laboratory air dust particles. Relevant references concerning reagents and materials include Moody *et al.* (1982, 1989), Tschöpel *et al.* (1980), Kosta (1982), Mitchell (1982b), Paulsen *et al.* (1989), and Luque de Castro and Luque García (2002).

5.2 Dry weight determination

Dry weight determinations should be carried out by air-drying homogenized subsamples of the material to be analysed to constant weight at 105°C. Freeze-drying could also be used for the dry weight determination.

5.3 Determination of metals

5.3.1 Homogenization and drying

When the analysis is undertaken, all fluids that may initially separate on thawing should be included with the materials homogenized. Wet or freeze-dried tissues should be homogenized. Homogenization of wet tissues should be performed immediately prior to any subdividing of the sample. Fresh tissue should be thoroughly homogenized to include any moisture and lipids that may have separated from the solid parts of the sample. Aliquots should be taken as soon as possible, either for direct analysis or for drying. When grinding samples after drying, classical techniques using a ball mill made of different materials should be used. References concerning sample pre-treatment include Klussmann *et al.* (1985), Luque de Castro and Luque García (2002), and Larsen *et al.* (2011a).

5.3.2 Digestion

The minimum requirements for the digestion procedure are the following:

- complete destruction of all organic material and mineralization of the sample;
- avoidance of loss of the elements to be determined;
- avoidance of contamination;
- a sampling size of minimum 200 mg dry material.

The following aspects should be considered as well:

- Digestion methods are preferred that use only small amounts of ultra pure reagents and chemicals;
- The method should be safe to handle (e.g. avoiding hydroperchloric acid);
- Some methods analyse directly and dissolution is not necessary e.g. AMA-254 for mercury analyses.
- A microwave digestion closed system is preferred for biota samples.
- The use of automated procedures is preferred.

Trace element analysis in biological tissues normally involves digestion of the sample with acids. Very pure acids are essential to ensure acceptable blanks. If "matrix-effects" prevail after sample digestion, three strategies may be followed:

- standard addition for calibration;
- chemical separation procedures;
- matrix modifiers.

Digestion methods have been described by, e.g. USEPA (1996), Barwick and Maher (2003), Rüdel *et al.* (2010), and Larsen *et al.* (2011b).

5.3.3 Instrumental determination

The appropriate instrumental equipment has to be chosen with regard to (i) the elements to be analysed, (ii) the concentration levels to be detected, and (iii) the matrix and the sampling processing prior to the measurement (e.g. digestion, pre-cleaning); for economic reasons account must also be taken of (iv) the typical throughput number of samples, and (v) investigation and operational costs.

For marine biota samples, all relevant monitoring programmes include mercury, cadmium, and lead as mandatory parameters. For analysing Cd and Pb from open-sea samples, e.g. flatfish liver of dab and plaice, Graphite Furnace Atomic Absorption Spectometry (GF–AAS) and Inductively-coupled Plasma (ICP) Mass Spectrometry (MS) are appropriate. For higher concentrated metals such as Cu and Zn, Flame-AAS, ICP–Atomic Emission Spectrometry (AES), or ICP–Optical Emission Spectrometry (OES) (weak for Pb) and Total Refection X-Ray Spectrometry (TXRF, weak for Cd) may also be used, but are not suitable to cover all obligating measurements in the required concentration range at very low concentrations without additional preconcentrating procedures.

For mercury cold vapour AAS systems are commonly used, as stand-alone device or addition to AAS systems. In recent years, direct measuring systems (e.g. AMA, PE SMS 100, and MLS DMA-80) for analysing mercury from liquid and solid samples without any preceeding digestion have become available, which have been proven to produce accurate and reliable results. A GF–AAS system equipped with a solid sample (autosampler) device for direct measuring and a high-resolution continuum source has also become available, which reduces the pretreatment of the samples and has only one source for all elements. Direct methods for analysing mercury using pyrolysis combined with a gold trap and fluorescence or atomic absorption detection are sensitive enough to measure biota samples directly (Carbonell *et al.*, 2009; Maggi *et al.*, 2009; Torres *et al.*, 2012). For the detection of hydride forming elements, such as arsenic, selenium, or antimony, nearly all manufactorers of AAS offer additional hydride add-on devices.

6 Analytical quality assurance

The programme planners must decide on the accuracy, precision, repeatability, limit of detection, and limit of determination required for each specific programme. Achievable limits of determination are as follows:

Cd	5 μg kg^{-1} wet weight
Hg	5 μg kg^{-1} wet weight
Pb	20 μg kg^{-1} wet weight
Cu	200 μg kg^{-1} wet weight

Relevant references concerning QA include HELCOM (1988), QUASIMEME (1992), Harms (1994), ICES (1995), and EC (2009).

6.1 Calibration solutions

For calibration purposes, single-element standard stock solutions at a concentration of 1000 mg l^{-1} are commercially available or can be prepared from the highest quality elements available (generally 99.999% purity) dissolved in high purity acid (usually 1 molar nitric acid). Single or mixed working element standard solutions for calibration purposes are prepared by taking aliquots of the standard stock solutions which are diluted using diluted acid as required. Both standard stock and working solutions are stored in polyethylene, borosilicate, or silica volumetric flasks. Borosilicate flasks must not be cleaned with alkaline solutions or heated above 70°C.

Working standard solutions at concentrations less than 100 μg l^{-1} should be prepared immediately before use, which is particularly important for mercury. The actual concentration of the element should be stated on the label together with the date of the preparation of all standard solutions. The calibration procedure must meet some basic criteria or

assumptions in order to give a best estimate of the true (but unknown) element content of the sample analysed. These are as follows:

- the masses or concentrations of standards for the establishment of the calibration function should be checked;
- the chemical and physical properties of the calibration standards must closely resemble those of the sample under investigation;
- the sample and calibration standard should be subjected to the same operational steps of the analytical procedure;
- the signals of repeatedly analysed calibration standards must be randomly distributed on either side of the calibration line.

Application of chemical separation procedures: Although relatively simple standards with a minimum of matrix matching are required, separation procedures that consist of several stages are prone to systematic errors due to both uncontrollable contamination and analyte losses, respectively.

6.2 Blanks

A procedural blank should be measured for each sample series and should be prepared simultaneously, using the same chemicals and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will lead to errors in quantification. Detailed information on how to reduce and control contamination is given by ICES (1995).

6.3 Accuracy and precision

A laboratory reference material (LRM), preferably a certified reference material (CRM), should be included in the analyses, with at least one LRM/CRM sample for each series of identically prepared samples.

The LRM must be homogeneous, well characterized for the determinands in question, and stability tests must have shown that it produces consistent results over time. The LRM should be of the same type of matrix (e.g. liver, muscle tissue, fat or lean fish) as the samples, and the determinand concentrations should occur in a comparable range to those of the samples. If the range of determinand concentrations in the sample is large (> a factor of 5) at least two LRMs should be included in each batch of analyses to cover the lower and upper concentrations. It is good practice to run duplicate analyses of a LRM to check within-batch analytical variability. The use of a freeze-dried LRM is a practicable alternative to a homogenized and frozen LRM. However, the efficiency of the preceding steps such as homogenization and drying cannot be checked. A quality control chart should be recorded for each metal. When introducing a new LRM, or when it is suspected from the control chart that there is a systematic error possibly due to an alteration of the LRM, another LRM (preferably a CRM) with a matrix as close as possible to the material analysed, should be used to check the reference material. Table A2.1 contains information on CRMs commercially available for use in marine monitoring.

Table A2.1 Certified reference materials for metals in marine organisms.

Code	Organization	Matrix
ERM-CE278k	IRMM[1]	Mussel tissue
ERM-BB422	IRMM	Fish muscle
BCR-463	IRMM	Tuna fish
DOLT-4	NRC[2]	Dogfish liver
DORM-4	NRC	Fish
LUTS-1	NRC	Non defatted lobster hepatopancreas
TORT-3	NRC	Lobster hepatopancreas
SRM 2976	NIST[3]	Mussel tissue
SRM 1946	NIST	Lake fish tissue

[1]IRMM: Institute for Reference Materials and Measurements (Europe).
[2]NRC: National Research Council (Canada).
[3]NIST: The National Institute of Standards and Technology (USA).

Additionally a duplicate of at least one sample should be run with every batch of samples. Each laboratory should participate in interlaboratory comparison studies and proficiency testing schemes on a regular basis, preferably at an international level.

6.4 Data collection and transfer

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

7 Data recording and reporting parameters

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

The following parameters should be recorded although they may serve different purposes, e.g. internal sampling protocols, and QA or requirements of the data base of the assessing body:

7.1 Sampling and biological parameters

Fish

- location of catch (name, latitude and longitude);
- date of collection and time (start and end time of trawling operations, GMT);
- mean trawling depth;
- type of gear;
- irregularities and unusual conditions;
- name and institution of sampling personnel;
- for each individual:
  - the species, its length, total weight, sex, age, reproductive status (GSI);
  - sample type (*e.g.* muscle, liver);
  - total tissue weight of the dissected organ;
- the number of individuals and data specified for pooled samples.

Shellfish

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

Seabird eggs

- location of sampling site (name, latitude and longitude);
- species;
- estimated number of pairs of the species breeding in the sampling area;
- date of collection;
- estimated or known laying date;
- size of clutch from which the egg was taken;
- number of eggs in the sample from the site;
- irregularities and unusual conditions;
- name and institution of sampling personnel;
- for each egg:
  - weight (to the nearest 0.1 g);
  - length and breadth (between poles and the equator to the nearest 0.1 mm);
  - content weight exclusive of shell (to the nearest 0.1 g);
  - shell thickness (to the nearest 5 μm) taking the mean of triplicate measurements with a micrometer;
  - shell weight (to the nearest 0.01 g);
  - embryo length (to the nearest 0.1 mm) or eye diameter of the embryo (to the nearest 0.1 mm).

7.2 Analytical and quality assurance parameters

- LRM and CRM results for the metals listed in Section 7.3 below;
- mean soft tissue dry weight and method of determining water content if this differs from air drying to constant weight at 105°C;
- descriptions of the digestion and instrumental determination methods used;
- the determination limit for each element. The limits should not exceed the values in Section 6;
- measurement uncertainty;
- the relevant QA information according to the requirements specified in the programme;
- the mean tissue lipid weight and method of extraction could also provide valuable information.

7.3 Parameters

- Elements of interest for monitoring programmes for which these guidelines apply:
  - cadmium (total);
  - mercury (total);
  - lead (total);
  - zinc;
  - copper**.**

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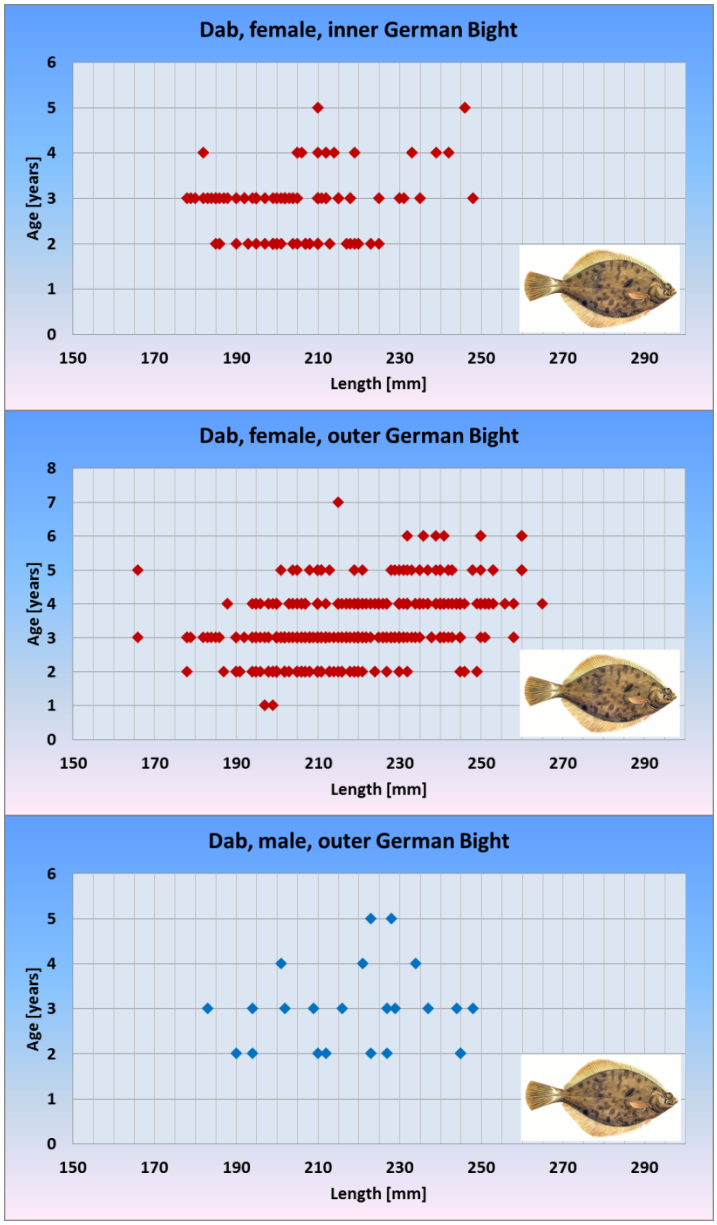
Appendix 1

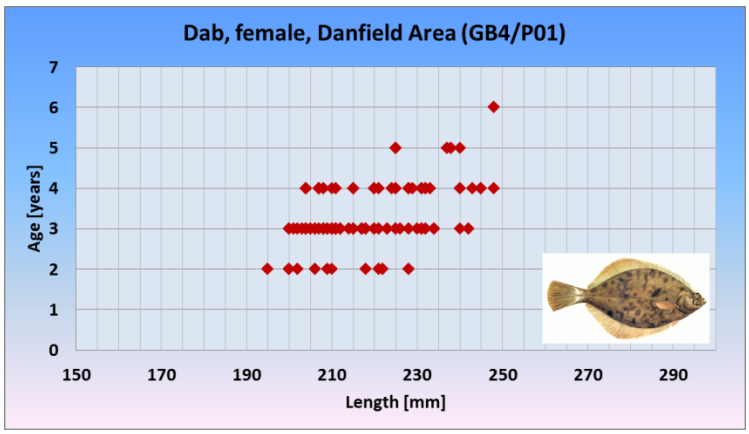
Examples of age–length relations for fish by species and regions.

These relationships can be used to select individuals of the recommended age by using the length measurement when sampling on-board ship.

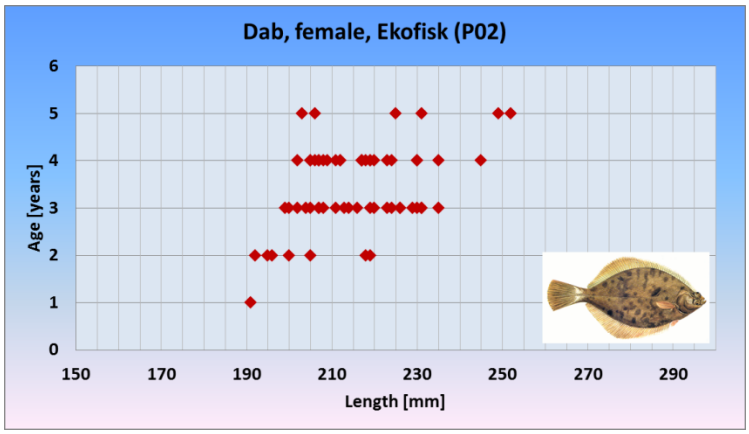
The laboratories of the contracting parties performing metal analysis in biota should use their databases to produce comparable information for their specific species and regions.

Dab, Southern North Sea

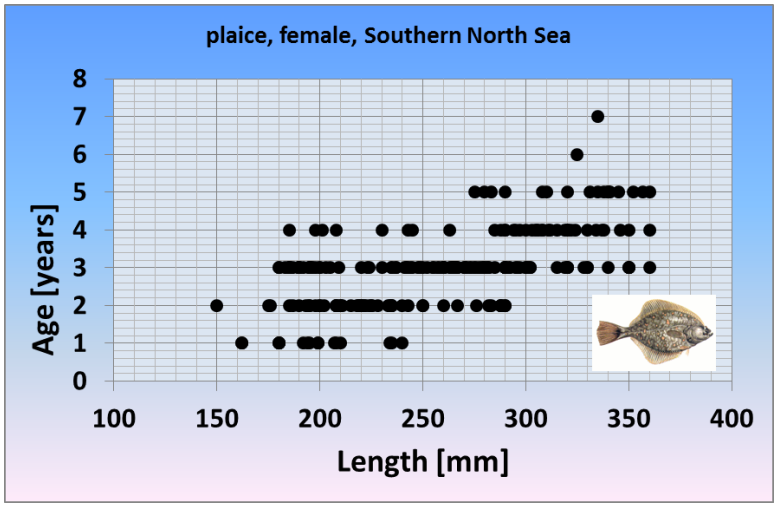


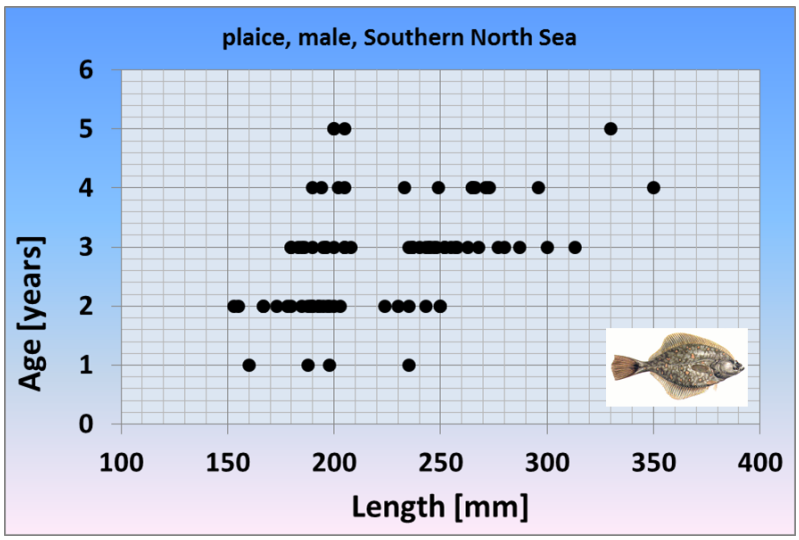


Dab, Central North Sea



Plaice, Southern North Sea, outer German Bight




plaice, male, Southern North Sea
Age [years]
0
1
2
3
4
5
6
100
150
200
250
300
350
400
Length [mm]

Technical Annex 4: Determination of mono-, di-, and tributyltin in sediments – analytical methods

This annex is intended as a supplement to the general guidelines. It is not a complete description or a substitute for detailed analytical instructions. The annex provides guidelines for the measurement of organtins in marine sediment in monitoring programmes. Organotins can originate from several sources. In addition to the previous use of antifouling agents on ship hulls, organotins can also be emitted to the environment from their use as fungicides or stabilizers for plastic materials (Fent, 2006; Fent and Muller, 1991; Hoch, 2001).

Target compounds include tributyltin (TBT), dibutyltin (DBT), and monobutyltin (MBT), and also triphenyltin (TPhT), diphenyltin (DPhT), and monophenyltin (MPhT). The method can be optimized to analyse other target organotins such as octyltins.

In order to assess the analytical results of organotin compounds in sediments, covariables must be measured as potential normalizers (e.g. grain-size distribution, organic carbon content, carbonate content). For samples "diluted" with sand, the sample intake size can be increased. For very sandy samples, isolation of the fine fraction by sieving might be required.

Organotin analyses are complex and sensitive operations. It is essential that they are carried out by suitably accredited or similarly qualified staff.

1 Sampling and storage

Storage of sediment samples is preferably done in cleaned glass, but containers of other materials such as polycarbonate or aluminium may also be suitable. In these cases, it is important to ensure that adsorption of and contamination by organotin compounds does not occur. Since photochemical degradation during storage has been reported for the aqueous phase (Quevauviller and Donard, 1991), the samples should be protected from light. Samples should be frozen after collection. For longer-term storage, the samples should be placed in a freezer with or without freeze-drying. Under these conditions, samples can be stored for over a year (Gomez-Ariza *et al*., 1994).

2 Transportation

Samples should be kept cool or frozen after collection. Samples should be transported in closed containers at temperatures between 5 °C and 15°C, preferably < 10°C. Frozen samples should not be defrosted.

3 Blanks and contamination

The complete analytical procedure should be checked for blank values, i.e. all solvents, chemicals, and adsorptive materials should be checked for potential sources of contamination or interference. If a contamination has been localized, measures must be taken to avoid it (e.g., cleaning, different suppliers, etc.).

Although butyltin compounds are not likely to occur in the laboratory environment, in solvents, or in most chemicals, commercial derivatization reagents sometimes contain significant concentrations of various (butyl)tin species. This can be solved by purchasing from other suppliers or by preparing the reagent in the laboratory.

Glassware should be treated thoroughly with concentrated HCl or HNO_3 and rinsed with deionized water and acetone prior to use. Alternatively, the glassware can be heated in an oven at 450°C or above after going through the standard glassware cleaning procedure.

4 Pre-treatment

Before taking a subsample for analysis, samples should be sufficiently homogenized. Especially samples from harbours can contain paint particulate matter irregularly distributed in the sample, thereby affecting the representativeness of the subsample. This can only be avoided when intensive mixing techniques (e.g. ballmill) are applied. Homogeneity can be checked by analysing several subsamples (e.g. five). Sediment samples from the marine environment are more homogeneous than those from harbour areas, as contamination in marine sediments usually derives from the water phase as mediated by tidal water movements. Less polluted samples are often more homogeneous than highly polluted samples. Because the size of the sample intake for analysis is inversely related to the pollution level, the intake will be small when the risk of heterogeneity is high. For this reason, multiple analyses might be appropriate for the higher concentration levels. The sample intake is usually around 1–5 g (dry weight), but some methods do not allow the use of more than 1 g (see also Section 5.5).

Most extraction methods can deal with wet as well as dry samples. Analysis of wet samples saves laborious drying procedures, but dry samples are more easily homogenized and stored. In general, organotins can be analysed from the same sample collected to monitor other substances such as polychlorinated biphenyls (PCBs). Since mono-, di-, and tributyltin are ionic compounds and strongly sorbed to the sediment, it is unlikely that losses through evaporation during air-drying or freeze-drying will occur. Air-drying has been reported possible up to 50°C, but because other related compounds (i.e. phenyltins) decompose, freeze-drying is preferred (Gomez-Ariza *et al*., 1994). Whichever drying procedure is used, the suitability with regard to cross-contamination and losses should always be tested (Quevauviller and Donard, 1991). If sieving is required, avoid contact with plastics. The use of stainless steel equipment is strongly recommended.

5 Analysis

5.1 Preparation of materials

Solvents, chemicals, and adsorption materials must be free of organotin compounds or other interfering compounds (see also Section 3). If they are not they should be purified using appropriate methods or replaced with clean materials. Solvents should be checked by concentrating the volume normally used in the procedure to 10% of the final volume and then analysing for the presence of organotin compounds and other interfering compounds by gas chromatography (GC). If necessary, the solvents can be purified by redistillation. Chemicals and adsorption materials should be purified by extraction and/or heating. Glass fibre materials (e.g. thimbles for Soxhlet extraction) should be pre-extracted. Alternatively, full glass thimbles with a G1 glass filter at the bottom can be used. Generally, paper filters should be avoided in filtration and substituted by appropriate glass filters. As all super-cleaned materials are prone to contamination (e.g. by the adsorption of organotin compounds and other compounds from laboratory air), materials ready for use should not be stored for long periods. All containers, glassware, etc. which come into contact with the sample must be made of appropriate material and must have been thoroughly pre-cleaned.

5.2 Dry weight determination

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

5.3 Calibration and preparation of calibration solutions

5.3.1 Calibration

Multilevel calibration with at least five calibration points is preferred to adequately define the calibration curve. Standard preparation can be done in two ways, depending on the methods of extraction/derivatization used:

i) by using alkyltin salts, then proceeding to the derivatization step as for samples (for hydridization or ethylation followed by purge-and-trap analysis, there is no other appropriate way than using alkyltin salts);
ii) by using commercially readily available derivatized standards.

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

A new calibration solution should always be cross-checked to the old standard solution.

Calibration solutions should be stored in a refrigerator in gas-tight containers to avoid evaporation of solvent during storage. It is important to determine the expiry date of standard dilutions in order to avoid a concentration shift due to deterioration of analytes or evaporation of solvents.

5.3.3 Isotope dilution–mass spectrometry

Isotope dilution–mass spectrometry technique (IDMS), can be used as an alternative quantification method (Monperrus *et al.*, 2003; Centineo *et al.*, 2004).

5.4 Extraction

Organotin compounds are strongly bound to particulate matter. The binding forces to the sediment have a dualistic character. Whereas tributyltin is mainly bound by hydrophobic forces, mineral binding dominates for monobutyltin because of its high electrical charge (e.g. the binding characteristic of trace metals). To achieve complete extraction, the butyltin compounds have to be released from the sediment, i.e. the binding must be diminished and the solubility in the extraction solvent must be maximized.

Different approaches can be applied to extract organotins from sediments:

- Acidic digestion followed by *in situ* derivatization with simultaneous extraction to an organic phase.
- Leaching under acidic conditions with simultaneous extraction of the compounds to an organic phase, as applied with different acids, solvents, and complexing agents.

To maintain a logical order, "*in situ* derivatization" will be discussed as a derivatization technique (see below) and not as an extraction technique. Furthermore, the use of recovery internal standards (RIS) to check the procedural steps is discussed separately below.

5.4.1 Acidic digestion followed by *in situ* derivatization

Digestion techniques that add hydrochloric acid or acetic acid can be used to extract organotins, while stirring or shaking the sample. Another possibility is the use of ultrasonic treatment.

5.4.2 Leaching and subsequent extraction to an organic phase

When extracting organotin compounds with an organic phase immiscible with water (e.g. DCM, diethylether, hydrocarbons, etc.), much higher acid concentrations (6 M HCl) can be applied without obstructing the derivatization. High acid concentrations will leach most of the monobutyltin from the sediment, but the high electrical charge of the monobutyltin^{3+} ion will not allow complete extraction to an organic phase. Under these strongly acidic conditions, the addition of complexing agents, e.g. tropolone (2-hydroxy-2,4,6-cycloheptatrienone) or diethyldithiocarbamate (Zhang *et al*., 1991; Quevauviller, 1996) is not expected to have much effect. Just like the sediment, the agent will be protonated and consequently lose (much of) its complexation ability. When applied, the effectiveness of complexing agents should be critically evaluated. Furthermore, large amounts of agents in the extract may affect the chromatography.

Quantitative extraction of all butyltin compounds to pentane is possible only under strongly acidic conditions when HBr (6 M) is used (Gomez-Ariza *et al*., 1995). The presence of bromide ions is essential to promote the extraction to the organic phase (pentane) through the formation of neutral ion-pairs. For tributyltin, it was shown that the distribution coefficient between octanol and water increased from 10^2 to 10^6 after the addition of 1 M bromide (Weidenhaupt, 1995).

Gomez-Ariza *et al*. (1995) used a "sediment:6 M HBr: pentane" ratio of 1:5:10 (g/v/v) for extraction. The leaching time was set to one hour, followed by an extraction of one hour. For completeness a second extraction with pentane is recommended. The pentane extract obtained can safely be concentrated, as the ionic butyltin compounds will not evaporate easily. This low risk of evaporation also allows transfer to other solvents if required for derivatization or analysis. The residue can be subjected to chromatographic methods such as high performance liquid chromatography (HPLC) that directly analyses the butyltin compounds in their ionic form. For GC methods, the butyltin compounds are derivatized to their hydride or tetra-alkyl form.

5.5 Derivatization

Derivatization can either be performed after extraction or simultaneously with extraction.

Sodium Tetraethylborate (NaBEt4): Derivatization with this reagent has been developed to minimize the analysis time. The NaBEt4 procedure allows a simultaneous extraction–derivatization in a buffered medium (optimum pH 4–5). NaBEt4 derivatization produces more thermally stable derivatives. However, NaBEt4 is extremely air sensitive; since it is considered as pyrophoric, care must be taken to keep its chemical integrity. Although solutions in water have been shown to be stable for about one month at 4°C, it is recommended to prepare them freshly for use.

Solutions of the reagent in an organic solvent (e.g.tetrahydrofuran, methanol, or ethanol) seem to be more stable (Smedes *et al.*, 2000). After the addition of sodium tetraethylborate (e.g. 1 to 4 ml of 2–5% solution in water or organic solvents), the mixture is shaken vigorously (Wilken *et al.*, 1994).

Although ethylation in the aqueous phase is very fast, the derivatization is limited by the desorption kinetics. Multiple additions have been applied, but a continuous addition of the reagent using a peristaltic pump supported by effective mixing conditions is more appropriate. In this way, the reagent is always present and every butyltin molecule desorbed from the sediment is immediately derivatized and extracted, which also makes the desorption process continuous. However, this very intensive derivatization may lead to the formation of boroxin, a six-angle ringed ethylborane. This compound is very reactive to the (bonded) phases used in gas chromatographic columns, affecting the column efficiency and mass spectrometric (MS) response. The boroxin is not removed by the normal phase column clean-up procedure usually applied, but can be degraded by the addition of an alkaline aqueous solution with a pH above 12. Ethylated organotin compounds will not be affected.

Since organic matter also reacts with the sodium tetraethylborate, the amount of sample that can be used is limited. As a rule of thumb, the sample intake should represent about 20–50 mg organic carbon which is, in practice, 1 g fine material (dry weight).

If simultaneous extraction and *in situ* derivatization is used, 5 to 10 ml of organic solvent (hexane or pentane) must be added before derivatization. The extraction of the derivative itself is quantitative but to isolate the whole organic phase, a second extraction is necessary. Usually centrifugation is required to separate the phases.

Grignard reagent, sodium diethyldithiocarbamate (NaDDTC) and sodium borohydrate (NaBH4) are alternative derivatization agents which can be used on organic phase extracts from sediment leachates. These reagents are not widely used anymore. Methods are described in Waldock *et al.* (1989) and Morabito *et al.* (2000).

5.6 Clean-up

Whether a clean-up step must be applied depends on the sample type, separation (GC or LC), and detection method used. Furthermore, the nature of the extract determines whether a clean-up step is possible. In the literature, no clean-up procedures are reported for aqueous/methanol leachates. Clean-up is not necessary when the butyltin compounds are determined by purge-and-trap analysis, which acts as a superb clean-up. However, extraction methods using an organic solvent will co-extract many kinds of other compounds from the sample, such as sulphur and sulphur-containing compounds, oil, and many other natural and anthropogenic compounds.

In addition to co-extracted substances, the extract will contain by-products of the derivatization. Using sodium tetraethylborate for derivatization, compounds such as boroxin, diethylsulphide, and diethyltrisulphide can be formed in rather large quantities (Section 5.5). If the basic wash has not yet been conducted, it should be added here as a clean-up step. The ethylsulphides usually do not disturb the instrumental analysis. Also, co-extracted substances usually do not visually disturb the chromatogram because most detection methods are very selective. Nevertheless, a large amount of matrix in the sample can affect the chromatography when the loading capacity of the column is exceeded, and can influence the detector response (e.g. MS). A decrease in the amount of matrix is always favourable for instrumental analysis and therefore a clean-up is recommended.

Generally, a simple SiO_2, Al_2O_3, or Florisil column clean-up is sufficient for sample clean-up. Alkylated tin compounds are as non-polar as PCBs and elute rapidly with hexane. Nevertheless, highly activated materials are not recommended, as the organotin compounds may degrade during elution. Using 2 g of SiO_2 deactivated with 1–5% water, or Al_2O_3 with 5–10% water in a glass column, organotin compounds usually elute in 5–10 ml hexane or pentane. Elution patterns should always be checked for each batch of column material.

5.7 Pre-concentration

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

5.8 Instrumental determination

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

High performance liquid chromatography is an alternative approach. It mainly uses fluorescence, ultraviolet, and more recently inductively coupled plasma optical emission spectrometry (ICP–OES), inductively coupled plasma mass

spectrometry (ICP–MS), and mass spectrometry detectors such as atmospheric pressure chemical ionization mass spectrometry (APCI–MS–MS) and electrospray ionization mass spectrometry (ESI–MS).

ICP–MS and (P)FPD detectors, equipped with a 610 nm band-pass filter selective for tin compounds have been applied widely because of their inherent selectivity and sensitivity. (P)FPD has been shown to have greater selectivity and lower detection limits (by a factor of 25 to 50 times) for organotin compounds than those obtained with conventional FPD (Bravo *et al.*, 2004).

5.8.1 Gas chromatography

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

5.8.2 High performance liquid chromatography

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

6 Quality assurance

References of relevance to QA procedures include HELCOM (1988), HELCOM COMBINE manual (HELCOM, 2014), QUASIMEME (1992), Oehlenschläger (1994), ICES (1996), Morabito *et al.* (1999), and EU (2009).

6.1 System performance

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

6.2 Recovery

The recovery should be checked and reported. It does not guarantee that extraction is complete for the more aged compounds already present in the sample, but nevertheless complete recovery is a minimum requirement for the assumption that extraction is complete. One method is to add an internal (recovery) standard to each sample immediately before extraction (e.g. tripropyltin) and a second (quantification) standard immediately prior to injection (e.g. tetrapropyltin).

Correction for recovery is advised against as it is most likely not representative of the actual recovery of aged compounds and is only a measure of how well the procedure has been performed. However, when it is local practice to correct for recoveries, three recovery standards (a mono-, di-, and trialkyltin) are required because of the different properties of the three butyltin compounds. The uncorrected values should be reported in brackets to show the elevation due to the recovery correction. Results of analyses that show recoveries lower than 50% should be rejected or the samples should be re-analysed.

When using the isotope dilution–mass spectrometry technique, the loss of target analytes is compensated. However, the recovery should still be calculated and should be between 50% and 150%.

6.3 Blanks

A procedural blank should be measured for each sample series and should be prepared simultaneously, using the same chemicals and solvents as for the samples. Its purpose is to determine if sample contamination has occurred from compounds that interfere with the peaks in the GC-analysis. Such contamination will lead to errors in quantification. If interference is found then materials and reagents should be checked for contamination. It is not possible to correct (after

the event) for this interference because the internal standard in the blank has not been absorbed by a matrix (e.g. sediment).

6.5 Accuracy and precision

A laboratory reference material (LRM) or certified reference material (CRM) should be included, at least one sample for each series of identically prepared samples. The LRM/CRM must be homogeneous, well characterized for the determinands in question, and stability tests must have shown that it produces consistent results over time. The LRM/CRM should be preferably of the same type of matrix as the samples, and the determinand concentrations should occur in a comparable range to those of the samples. If the range of determinand concentrations in the samples is large (> a factor of 5), two reference materials should be included in each batch of analyses to cover the lower and upper concentrations.

The data produced for the LRM/CRM in successive sample batches should be used to prepare control charts. It is also useful to analyse the LRM/CRM 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 (such as CRM 646 or PACS-2) of a similar matrix should be analysed periodically in order to check the method bias, ideally twice a year as a minimum. Additionally, a duplicate of at least one sample should be run with every batch of samples. Each laboratory should participate in interlaboratory comparison studies and proficiency testing schemes on a regular basis, preferably at an international level.

6.6 Data collection and transfer

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

7 Data recording and reporting parameters

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

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

7.2 Analytical and quality assurance parameters

- LRM and CRM results for a set of organotin compounds, reported on a dry weight basis.
- Descriptions of the extraction, cleaning, and instrumental determination methods.
- The detection limit for each organotin compound. Specific performance criteria, including detection limits and precision, are usually set by the programme. A typical detection limit for single contaminants is 1 µg kg^{-1} (dry weight) of Sn, although this might be difficult to achieve for phenyltins compounds.
- QA information according to the requirements specified in the programme.

7.3 Parameters

- Organic contaminants of interest to monitoring programmes for which these guidelines apply – butyltin compounds: tributyltin (TBT) and dibutyltin (DBT).
- This technical annex also provides guidance on the determination of monobutyltin (MBT), phenyltin, and octyltin compounds.

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Technical Annex 6: Determination of metals in sediments – analytical methods

1 Introduction

This technical annex provides guidance on the determination of metals (including metalloids and some non-metals like Se) in whole sediment and in sieved fractions. Determinations of metals can be achieved by acid digestion of the sediment followed by analysis of the digest solution by spectroscopic or spectrometric methods, or by non-destructive techniques such as X-ray fluorescence analysis (XRF), instrumental neutron activation analysis (INAA), etc. The guidelines are intended to assist those analytical chemists that are starting up metal analyses in sediments, as well as those already performing such analyses. They do not provide full details on specific laboratory procedures. Further guidance may be sought from specialized laboratories and publications (e.g. Loring and Rantala., 1991; Popek, 2003) or general guidance for selection of analytical methods (e.g. Larsen *et al.*, 2011). Analyses should be carried out by accredited or similarly qualified staff and the procedure should be validated, for example in accordance with the EU's QA/QC Directive (EU, 2009).

Metals may occur in both fine and sand fractions of sediments. However, most natural and anthropogenic substances (metals and organic contaminants) show a much higher affinity to fine particulate matter than the coarse fraction. Iron and manganese oxy-hydroxide coatings, and constituents such as organic matter and clay minerals, contribute to the affinity for contaminants for this fine material.

Total methods, such as procedures involving total dissolution of sediment samples with hydrofluoric acid (HF) prior to analysis, or non-destructive methods without digestion such as neutron activation analysis (INAA) and X-ray fluorescence analysis, determine total metal contents in the whole sediment sample. In contrast, methods using a partial digestion with only strong acids, e.g. nitric acid or *aqua regia*, mainly measure metals in the fine fraction, and only extract small amounts of metals from the coarse fraction. For fine material, similar results have been obtained using both total and strong partial methods (Smedes *et al.*, 2000; QUASH/QUASIMEME intercalibrations).

2 Sampling, pre-treatment, and storage

Sampling sediments for metals analysis should preferably be done using cleaned plastic equipment, but this may not always be possible (e.g. at sea). Where metal sampling gear such as grabs must be used, care must be taken to avoid contamination of the sample, for instance by sub-sampling only sediment that has had no contact with the walls of the sampling device (maintain at least 1 cm distance from sides). Sample thickness should be chosen according to the monitoring purposes.

For recurrent spatial and temporal monitoring, the upper 2 cm of the sediment are sampled. For other purposes, such as retrospective assessment of temporal changes, core samples can be taken. If knowledge exists about the sedimentation rate, the sampling strategy can be based on this knowledge.

Sediments can be stored in closed plastic or glass containers. Samples must be sieved to 2 mm after sampling to remove large debris as well as large detritus and benthic organisms. Otherwise biotic material will deteriorate during further sample handling like storage, freezing, or ultrasonic treatment, and become part of the sediment sample. Metal concentration will be expressed as a fraction of the sieved sample. Samples may then be further wet sieved to a smaller size fraction. Further details on sieving procedures are available in the Technical Annex 5: Normalization of contaminant concentrations in sediments.

For total analysis, metals are usually not very sensitive with regard to storage conditions. If the sample is to be used for measurement of other parameters, then those may determine how to store the samples. For total analysis of metals the sample can be stored at 4°C for a few weeks and for extended periods when frozen at –20°C, although direct wet sieving is preferred. For prolonged storage, freeze-drying of samples can be considered. In this case contamination and losses of contaminants during freeze-drying have to be checked, in particular for volatile parameters (e.g. volatile organics) to be analysed in the same samples. In contrast to the generic guidelines, it should be noted that air-drying is not appropriate due to high contamination risks. Besides, samples may be difficult to disaggregate and mineral structures may be affected. The stability of metals during sediment storage was studied by, e.g. Guevara-Riba *et al.* (2006) and Linsinger *et al.* (2011).

Once sieved and dried, samples should be homogenized and ground to a fine powder in a non-contaminating mill (e.g. made of agate or silicon nitride), and stored in plastic or glass containers until analysis.

3 Blanks and contamination

Any contact between the samples and metals should be avoided. If metallic implements are required during sampling (e.g. grab jaws), they should be of stainless steel and contact between the subsample and metal should be minimized.

Plastic and glassware should be cleaned using a laboratory washing machine incorporating an acid wash, or by an equivalent cleaning procedure. Some plastic ware may not need to be cleaned before first use for metals work, but this feature must be thoroughly examined (e.g. using acid leaching tests) before proceeding with any real samples.

Blanks should be taken through the whole procedure. In practice, this will generally represent the time from acid addition to a sample container through to the final measurement. There should be at least one analytical blank in a batch of 10–20 samples, representing 5–10% of the sample number.

For core-samples, care should be taken not to contaminate lower samples with upper samples in the process of cutting up the sediment core.

4 Digestion

4.1 Hydrofluoric acid digestion

HF digestions should be performed in polytetrafluorethylene (PTFE or PFA) vessels or equal quality, since the vessel must be metal-free and resist attack by the acid itself. Dried samples (normally 0.2–1 g) should be accurately weighed into the vessel. Under fume extraction, the acid(s) are added. Some workers add HF first and leave the mixture to stand overnight, others add HF, nitric acid, or *aqua regia* (see below); others use a perchloric acid mixture, etc. In general, the mixtures are left to stand for a certain period of time (1 hour – overnight) to avoid problems with violent reactions, which may be prompted by the presence of organic matter in the sediment. Note that perchloric acid and organic matter can promote an explosive reaction, so this acid must be handled with great caution if applied to sediments. Specially designed fume hoods should be used for HF and perchloric acid treatments.

HF is corrosive and toxic. It is therefore necessary to either remove the acid or render it less harmful to the measurement instruments. The acid may either be boiled off, which requires specialized facilities to extract the toxic fumes, or neutralized with boric acid (H_3BO_3), which is toxic itself.

Samples may be digested in a programmable heating block, with HF removal by evaporation. Alternatively, microwave digestions provide a rapid way to digest sediments. Some systems may allow the evaporation of HF, but in general microwaves use closed systems which allow pressure and temperature effects to rapidly dissolve the sediment. The most common methods use polytetrafluorethylene (PTFE or PFA) lined and sealed digestion vessels (Nakashima *et al.* 1988; Loring and Rantala, 1990). Since these closed systems retain the HF, boric acid is added after the HF digestion to complex remaining HF and make the resulting solution less hazardous, as well as preventing aluminium fluoride precipitation. The solution should be made up to volume with ultra-pure water and left to stand for at least 24 hours prior to analysis to precipitate excess boric acid. It is also possible to use adjusted amounts of boric acid and heat the digest to accelerate the process (Maham *et al.*, 1987). Typical methods are described, for example, in Cook *et al.* (1997), Jones and Laslett (1994), Wu *et al.* (1996), and Quelle *et al.* (2011).

If HF is to be removed by evaporation, care should be taken to ensure that mercury is not lost from sample solutions (Delft and Vos, 1988). It can be difficult to avoid mercury contamination with total digestion, but usually mercury is not bound strongly, so mercury can alternatively be analysed using strong acid digestion or by direct analysis (Taylor *et al.*, 2012).

4.2 Strong acid digestion

Partial digestions follow broadly similar procedures to HF digestions, as described above, for example using HNO_3 or *aqua regia* and deionized water to ca. 0.5 g sample. Microwave digestion is the preferred technique, but alternative methods applying high pressure and temperature can be used. The method used needs to be checked. Adequate performance is achieved when the digestion dissolves all the Al and Li from the clay fraction. It can easily be tested whether a method meets this requirement through parallel analyses of very fine grained samples by the partial method in use and by a total method, e.g. HF. If results for Al and Li do not differ significantly, the partial method used is sufficiently strong. To optimize the tests and to further normalize results, sieving to 20 or 63 µm grain size can be used, also reducing problems with detection limits in sandy sediments. A more general discussion on normalization can be found in the Technical Annex 5: Normalization of Contaminant Concentrations in Sediments.

If the partial method results in lower contents than the total method, the conditions for the partial digestion such as time, temperature, acid concentration, etc. need to be adjusted. Usually boiling with *aqua regia* is insufficient for a complete dissolution of Al. Historically, *aqua regia* has been used for strong acid digestions, but hydrochloric acid produces interferences for multi-element analysis by ICP and Cd in graphite furnace, so concentrated nitric acid alone may be used as a substitute (Christensen *et al.*, 1982; Krumgalz and Fainshtein, 1989; Koopmann and Prange, 1991). However, collision or reaction cell technology in ICP–MS can be used to reduce the interfering effect of chloride and other multi-element interferences, down to levels of < 1% mass overlap for double charged or multi-element species, thus minimizing correction formulas for standard mass-corrections.

5 Analysis and detection

Analysis of metals in solution resulting from digestion may be performed by a variety of means, but usually involves spectrometric or spectroscopic detection. Flame or graphite furnace atomic absorption spectroscopy used to be the primary method for analysis of metals (Welz, 1985). Alternatively, non-destructive methods, i.e. XRF (e.g. Jenkins, 1999; Potts, 1992; Williams, 1987; Bertin, 1984; Parsons *et al.*, 2013) and INAA (Alfassi, 1998), which do not require a preceding digestion step, can be used. Multi-element techniques like inductively coupled plasma attached to either an emission spectrometer (ICP–AES) or mass spectrometer (ICP–MS) allow much more rapid analysis of a wide range of metals (Kimbrough and Lauenstein, 2006; Duzgoren-Aydin *et al.*, 2011; Castillo *et al.*, 2012).

Interferences in the analysis may arise through the presence of other components in the sample. Use of at least 3-point standard additions may highlight where these occur and can be used to correct for suppression or enhancement effects. Interferences occurring with multi-element analytical techniques can be complex and require accredited or similarly qualified personnel to identify and minimize such effects (Cook *et al.*, 1997).

Mercury can be detected by fluorescence spectrometry or cold vapour atomic absorption spectrometry. Direct methods for analysing mercury using pyrolysis combined with a gold trap and fluorescence or atomic absorption detection are sensitive enough to measure sediments directly (Maggi *et al.*, 2009; Kelly *et al.*, 2012). ICP–MS is also sufficiently sensitive to measure Hg, but care should be taken about controlling carry-over memory effects.

It should be ensured that the limits of detection of the analytical technique selected meets the requirements of the respective monitoring programme. Typical detection limits using different methods are given in Table A6.1.

Table A6.1 Typical limits of detection for the determination of metals with different techniques (in mg kg^{-1} d.w.) based on typical sample intakes (0.5–1 g).

	Al	Li	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
AAS / flame	5	0.2		0.5	5	2		5	5	10
AAS / graphite furnace, hydride technique, cold vapour	< 1	< 1	0.2	0.02	< 1	< 1	0.05	< 1	< 1	-
ICP–AES with hydride generation	10	10	10 1	0.5	1	1	-	2	5	1
ICP–MS	40	0.1	1	0.01	0.2	0.1	0.05	0.2	0.2	2
X-ray fluorescence analysis (XRF)	1000	-	-	-	10	10	-	10	10	20
Neutron activation analysis (INAA)	-	-	0.3	1	0.8	-	0.1	-	-	2
Fluorescence, AAS spectrometry (direct or cold vapour/hydride generation)	-	-	0.2	-	-	-	0.005	-	-	-
Direct Mercury Analyser (AA)							0.005			

6 Metal speciation

Several methods are in use to examine metal speciation in sediments, mainly by use of sequential extraction (e.g. Gleyzes *et al.*, 2002; Scouller *et al.*, 2006; Sutherland, 2010; Duzgoren-Aydin *et al.*, 2011), but currently also by passive samplers (for metals primarily DGTs) in porewater (Peijnenburg *et al.*, 2014).

7 Limits of detection

The limit of detection for each metal is normally determined by analysing a blank solution (containing acid to the dilution that is present in the sample) at least ten times. The limit of detection is calculated from three times the standard deviation of the blank taken through the whole procedure. For typical limits of detection, see Table A6.1.

8 Calibration and standards

Calibrations are usually performed using multi-element stock solutions and at least a 4-point calibration covering the range of concentrations expected in the samples. Multi-element solutions are commercially available, and may be used provided that they are of a similar matrix to the analyte. A crosscheck solution from a separate batch, or from a different supplier or an internal reference standard, should be used to check the calibration. Differences should not exceed 10%.

For non-destructive methods, appropriate certified reference sediments are required for calibration purposes.

9 Quality assurance

Every determinand should have its own Quality Control and Quality Assessment (QC – QA) scheme that includes regular blanks and calibration checks, the use of internal reference materials and certified reference materials, and quality control charts. A system suitability check should be included in each batch to confirm that the measuring instrument is operating correctly. In each batch of samples at least one standard addition (from the start of the digestion) should be included to demonstrate that matrix effects do not occur, and also a duplicate sample.

At least one laboratory reference material should be included in each batch of samples in order to check the long-term performance. A quality control chart should be constructed for selected metals, including warning and alarm limits. If the warning limits are exceeded, the method should be checked for possible errors. When alarm limits are exceeded, the results should not be reported.

Certified reference materials (CRMs) for sediments are commercially available for both total methods and partial digestion methods. The data provided by such materials allow a check, independent of laboratory standards, of the analytical performance. Table A6.2 contains information on certified reference materials available for use in marine monitoring.

Table A6.2 Certified reference materials for metals in marine sediments.

Code	Organization	Matrix
BCR 277R	IRMM[1]	Estuarine sediment
BCR 320R	IRMM	Channel sediment
BCR CC580	IRMM	Estuarine sediment (only Hg and CH_3Hg)
BCR 667	IRMM	Estuarine sediment
HISS-1	NRC[2]	Marine sediment
MESS-3	NRC	Marine sediment
PACS-2	NRC	Marine sediment (Harbour)
SRM 1646a	NIST[3]	Estuarine sediment
SRM 1944	NIST	Marine sediment
SRM 2702	NIST	Marine sediment

[1]IRMM: Institute for Reference Materials and Measurements (Europe).
[2]NRC: National Research Council (Canada).
[3]NIST: The National Institute of Standards and Technology (USA).

Participation in an international proficiency-testing scheme, e.g. QUASIMEME is highly recommended to improve comparability between laboratories. Relevant quality assurance data should be reported, e.g. to ICES, together with concentration data.

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