Manual for Marine Monitoring in the

COMBINE

Programme of HELCOM

Part B

General guidelines on quality assurance for monitoring in the Baltic Sea

Annex B-13

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

Appendix 2
Technical note on the determination of chlorinated biphenyls in sediment

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ANNEX B-13 TECHNICAL NOTE ON THE DETERMINATION OF HEAVY METALS AND PERSISTENT ORGANIC COMPOUNDS IN MARINE SEDIMENTS

ANNEX B-13 APPENDIX 2. TECHNICAL NOTE ON THE DETERMINATION OF CHLORINATED BIPHENYLS IN SEDIMENT

1. INTRODUCTION

These guidelines are based on the review papers by Smedes and de Boer (1994, 1998). The analysis of chlorinated biphenyls in sediments generally involves extraction with organic solvents, clean-up, removal of sulphur, column fractionation and gas chromatographic separation, mostly with electron capture or mass-spectrometric detection. All of the steps in the procedure are susceptible to insufficient recovery and/or contamination. Hence, quality control procedures are recommended in order to check the method performance. In addition, the quality control aspects relating to calibrants, extraction, clean-up, etc., are considered important. These guidelines are intended to encourage and assist analytical chemists to (re)consider their methods critically and to improve their procedures and/or the associated quality control measures, where necessary. It should be noted that these guidelines do not cover the determination of non-*ortho* substituted CBs. Due to the low concentrations of non-*ortho* CBs in sediments compared to those of other CBs, their determination requires an additional separation and concentration step.

These guidelines can also be applied for the determination of several other types of organochlorine compounds, e.g., chlorobenzenes, DDT and its metabolites, and hexachlorocyclohexanes. The recovery in the clean-up procedures must be checked carefully. In particular, treatment with H_2SO_4 results in loss of, e.g., dieldrin and endosulfanes. Also, the clean-up procedure with silver ions can result in low recoveries for certain pesticides.

It is neither possible nor desirable to provide fully detailed guidelines for the analysis of sediments. If necessary, guidance should be sought from highly specialized research laboratories. Whichever procedure is adopted, each laboratory must demonstrate the validity of each step of its procedure. The use of a second, different method, in addition to the routine procedure, is recommended as a validation. The analyses have to be carried out by experienced staff.

2. SAMPLING AND STORAGE

The major criteria for successful sediment sampling is to guarantee a fairly undisturbed sample stratification. (For further details about sampling, see the “Technical note on the determination of heavy metals in marine sediments”.) Plastic materials (except polyethylene or polytetra-fluorethene) must not be used for sampling due to adsorption of determinands to the container material.

The samples should be transported in closed containers; a temperature of 25 °C should not be exceeded. If the samples are not to be analysed within 48 hours after sampling, the sample has to be stored at 4 °C (short-term storage). Storage over several months is only possible for frozen (below -20 °C) and dried samples.

3. BLANKS AND CONTAMINATION

The procedural detection limit is determined by the blank value. In order to keep the blank value as low as possible, all glassware, solvents, chemicals, adsorption materials, etc., should be free of CBs or other interfering compounds.

Glassware should be washed thoroughly with detergents, heated to > 250 °C, and rinsed with an organic solvent prior to use.

All solvents should be checked for impurities by concentrating the volume normally used in the procedure to 10 % of the normal end volume. The presence of CBs and other compounds in the solvents can then be checked by gas chromatographic (GC) analysis.

All chemicals and adsorption materials should be checked for impurities and purified (e.g., by heating or extraction), if necessary. Glassfiber Soxhlet thimbles should be pre-extracted. The use of paper thimbles should be avoided. Alternatively, full glass Soxhlet thimbles with a G1 glass filter at the bottom can be used. Storage of these super-cleaned materials for a long period of time is not recommended, as laboratory air can contain CBs that will be adsorbed by these materials. The occurrence of blank values despite having taken all the above-mentioned precautions may be due to contamination from the air.

4. PRETREATMENT

CBs can be extracted from wet or dried samples. Storage, homogenization, and extraction are much easier when the samples are dry. However, drying the samples may alter the concentrations, e.g., by loss of compounds through evaporation or by contamination.

Before taking a subsample for analysis, the sample should be sufficiently homogenized.

Chemical drying of samples can be performed by grinding with Na_2SO_4 or $MgSO_4$ until the samples reach a sandy consistency. It is essential that the operations of grinding and extraction are separated by at least several hours to allow proper binding of the water and avoid insufficient extraction.

Freeze-drying is becoming a more popular technique. Losses through evaporation are diminished by keeping the temperature in the evaporation chamber below 0 °C. Possible losses or contamination must be checked. Contamination during freeze-drying is reduced by putting a lid, with a hole of about 3 mm in diameter, on the sample container.

5. EXTRACTION AND CLEAN-UP

5.1. EXTRACTION

The target compounds must be extracted from the sediment with an organic solvent prior to further analysis.

Wet sediments are extracted by mixing with organic solvents. Extraction is enhanced by shaking, ultra-turrax mixing, ball mill tumbler, or ultrasonic treatment. Water-miscible solvents, such as methanol, acetone, and acetonitrile, are used, especially in the first step. The extraction efficiency of the first step is low as there will be a considerable amount of water in the liquid phase. The extraction is continued with a mixture of polar and apolar solvents (acetone plus hexane, or methanol plus dichloromethane). For complete extraction, at least three subsequent extractions are needed and the contact time (24 hours) with the solvent should be sufficient to complete the desorption of the CBs from the sediment.

Wet sediments can also be extracted utilizing a Soxhlet, but this is best done in two steps. First, a polar solvent, such as acetone, is used to extract the water from the sediment. Then the flask is replaced and the extraction is continued with a mixture of, e.g., acetone/hexane.

In both cases, water should be added to the extracts and the CBs should be extracted by an apolar solvent such as hexane.

For dried sediments, Soxhlet extraction is the technique most frequently applied to extract CBs. The use of a mixture of a polar and an apolar solvent (e.g., acetone/hexane) is recommended for sufficient extraction efficiency. A good choice is 25 % acetone in hexane. A higher content of the polar solvent increases the extraction efficiency, but the polar solvent has to be removed prior to gas chromatographic analysis. The extraction can be carried out with a regular Soxhlet or a hot Soxhlet. At least 50 to 60 extraction cycles should be performed (approximately 8 hours for the hot Soxhlet). The extraction efficiency must be checked for different types of sediments by a second extraction step. These extracts should be analysed separately.

Supercritical fluid extraction (SFE) is a relatively new method. The optimal conditions are still under investigation. A new static extraction system applying high temperature and high pressure also seems to be a promising technique.

In principle, all the methods described are suitable for the extraction of CBs from sediments. For dry samples, however, Soxhlet extraction is recommended over mixing methods.

Prior to any concentration step, a keeper (high-boiling alkane) must be added.

5.2. REMOVAL OF SULPHUR AND SULPHUR-CONTAINING COMPOUNDS

The crude extract requires a clean-up as many compounds other than CBs are co-extracted. This extract will be coloured due to chlorophyll-like compounds extracted from the sediment, and it will also contain sulphur and sulphur-containing compounds, oil, PAHs, and many other natural and anthropogenic compounds.

An aqueous saturated Na_2SO_3 solution is added to a hexane extract. In order to allow transfer of the HSO_3-ions to the organic phase, tetrabutylammonium (TBA) salts and isopropanol are added to the mixture. Subsequently, water is added to remove the isopropanol. The aqueous phase is then quantitatively extracted with hexane (Jensen *et al.,* 1977). If the extraction is performed by a polar solvent miscible with water, the Na_2SO_3 solution can be added directly after the extraction. If the extraction mixture also contains an apolar solvent, then, depending on the ratio of the solvents, the addition of TBA and isopropanol may not be necessary. Any excess $Na_2SO_3$and reaction products can be removed by the addition of water and partitioning between the apolar solvent and water.

Japenga *et al.* (1987) developed a column method for the removal of sulphur and sulphur-containing compounds. The column material is made by mixing an aqueous solution of Na2SO3 with Al_2O_3. Some NaOH is also added to improve the reaction with sulphur. Subsequently, the material is dried under nitrogen until a level of deactivation equivalent to 10 % water is reached. Storage must be under nitrogen because sulphite in this form may easily be oxidized to sulphate. Eluting the extract (hexane) through a

column filled with this material results in removal of the sulphur in combination with further clean-up of the sediment extract. The sulphur removal properties are somewhat difficult to control.

Mercury, or copper powder, wire, or gauze remove the sulphur directly from an organic solvent. Although mercury is appropriate for removing sulphur, it should be avoided for environmental reasons. Copper can be applied during or after Soxhlet extraction. Ultrasonic treatment might improve the removal of sulphur. If sulphur appears to be present in the final extract, the amount of copper or mercury used was insufficient and the clean-up procedure must be repeated.

Silver ions strongly bind sulphur and sulphur compounds. Loaded on silica, $AgNO_3$ is a very efficient sulphur-removing agent. It can be prepared by mixing dissolved $AgNO_3$ with silica and subsequently drying under nitrogen. Compounds containing aromatic rings are strongly retained, but for CBs this retention is reduced, probably due to shielding of the rings by the chlorine atoms. Retained compounds can easily be eluted by using cyclohexene, or another solvent with double bonds, as a modifier.

Elemental sulphur is strongly retained on a polystyrene divinylbenzene copolymer column as generally applied for gel permeation chromatography (GPC). In addition, this method combines the removal of sulphur with a clean-up.

All of these methods have advantages and disadvantages. Sometimes the use of multiple methods may prove necessary for different samples. Several methods leave some aromatic sulphur compounds in the extract which will elute from the GC column at the same retention time as the lower CBs. The major part of these compounds can be removed by eluting an apolar extract over a column containing silica loaded with concentrated sulphuric acid.

5.3. FURTHER CLEAN-UP

As CBs are apolar, clean-up using normal phase chromatography is the most appropriate technique for their separation from other compounds. Using an apolar solvent, e.g., hexane or iso-octane, as an eluent, CBs normally elute very rapidly. All polar solvents used in the extraction or sulphur-removal step should be removed before further clean-up. The last concentration step is usually performed by evaporation with a gentle stream of nitrogen. Evaporation to dryness should always be avoided.

Deactivated Al_2O_3 (5–10 % water) is often used as a primary clean-up. Al_2O_3 normally gives a sufficiently clean extract for a gas chromatography electron capture detector (GC-ECD) screening of the sample, provided that sulphur has been removed.

Deactivated SiO_2 (1–5 % water) does not retain CBs (including planar CBs) and only slightly retains polycyclic hydrocarbons when eluted with hexane or iso-octane.

For high activity silica (overnight at 180 °C), the retention of CBs is negligible while PAHs are more strongly retained. The CBs and a few organochlorine compounds are eluted with apolar solvents. When using more polar solvents (e.g., hexane/acetone), some interfering organochlorine pesticides are eluted.

When GPC is used for removing the sulphur, the removal of high molecular weight material can also be incorporated into the procedure. GPC does not separate CBs from other compounds in the same molecular range (such as organochlorine pesticides), so additional clean-up is usually required.

For the separation of CBs from lipids or oil components, reversed-phase high-performance liquid chromatography (HPLC) can be used. Due to the use of aqueous solvents in reversed-phase HPLC, the samples have to be transferred several times between polar and apolar solvents.

5.4. CONTROL OF EXTRACTION AND CLEAN-UP

The check of extraction and clean-up can be performed by analysing a reference material. To check the clean-up and concentration steps, it is recommended to pass a standard solution through the entire procedure. This standard solution is used for the determination of the recovery for the sample series. Additionally, an internal recovery standard should be added to each sample before extraction, to check for recovery during the analytical procedures. If major losses have occurred, then the results obtained should not be reported. CB29 is suggested as a recovery standard because, owing to its high volatility, losses due to evaporation are easily detected. CB29 elutes relatively late from alumina and silica columns. Small peaks that may be present in the gas chromatogram at the retention time of CB29 do not hinder the use of this CB because the recovery standard only indicates major errors in extraction or clean-up.

In case GC/MS is applied, labelled CBs can be used as recovery standards. This allows correction for recovery, provided that each chlorination stage is represented.

6. GAS CHROMATOGRAPHY

Because of the large number of CB congeners (a total of 209), high-resolution capillary gas chromatography (GC) is the method of choice for the determination of CBs. The analysis of CBs in sediments should focus on the determination of selected individual congeners. As it is currently impossible to separate all CBs in technical mixtures and to separate them from other ECD-detectable compounds, it is recommended that two columns of different selectivity (polarity) are used for analysis. For more reliable separation of CBs, multidimensional gas chromatography (MDGC) is the preferred method. This technique is especially valuable for specific separations, but still needs basic investigations before routine application is possible.

For all GC methods, parameters have to be optimized.

COLUMN DIMENSIONS

Column dimensions for the determination of CBs are:

- length: minimum 50 m, and
- inner diameter: maximum 0.25 mm.

Greater resolution can be obtained by reducing the inner diameter to 0.20 mm or less. Below a diameter of 0.15 mm the carrier gas pressure rises to values greater than 500 kPa, which are not compatible with normal GC equipment. Also, the risk of leakage increases.

The film thickness should be between 0.2 µm and 0.4 µm.

STATIONARY PHASES

A wide range of stationary phases can be used for the separation of CBs (e.g., 94 % dimethyl-, 5 % phenyl-, 1 % vinyl polysiloxane, or 7 % phenyl-, 7 % cyanopropyl-, 86 % methyl-siloxane). The use of more polar phases is sometimes limited as their maximum temperatures are not as high as for apolar, chemically bonded phases. Stationary phases that separate CBs on the basis of molecular size, such as the liquid crystal phase, should not be used for monitoring purposes since they do not provide sufficient reproducibility.

CARRIER GAS

Preferably, hydrogen should be used as the GC carrier gas. When using columns with very small inner diameters, the use of hydrogen is essential. The linear gas velocity should be optimized. Appropriate settings for 0.25 mm i.d. columns range from 20–40 cm s^{-1} and for 0.15 mm i.d. columns from 30–50 cm s^{-1}.

INJECTION TECHNIQUES

The two systems commonly used are splitless and on-column injection. Split injection should not be used because strong discrimination effects may occur. Other techniques such as temperature-programmed or pressure-programmed injection may have additional advantages, but should be thoroughly optimized before use. The volume of the liner should be large enough to contain the gas volume of the evaporated injected solvent. When the liner is too small, memory effects can occur due to contamination of the gas tubing attached to the injector. Very large liner volumes can cause a poor transfer of early eluting components, so that peaks due to those analytes will be reduced or even disappear. An auto-sampler should be used. In addition, the use of a light packing of (silylated) glass wool in the liner improves the response and reproducibility of the injection, but some organochlorine pesticides such as DDT may be degraded when this technique is applied.

TEMPERATURE PROGRAMMING

The temperature programme must be optimized for a sufficient separation of the CB congeners. An analysis time of 60–120 minutes is inevitable. In addition to a reproducible temperature programme, a fixed equilibration time is important for a correct analysis and constant retention times.

For further details and recommendations, Smedes and de Boer (1998) should be consulted.

DETECTION

The most frequently used detector for CB analysis is the electron capture detector (ECD). Injection of chlorinated or oxygen-containing solvents should be avoided. The use of a mass selective detector (MSD) or

even a mass spectrometer (MS) as a detector for CB analysis is becoming more common and generally applicable. Negative chemical ionization (NCI) is extremely sensitive for penta- to decachlorinated CBs (approximately ten-fold better than ECD). Electron impact ionization (EI) may be used as an alternative ionization method, but for most CBs the sensitivity of this method is ten-fold lower than for ECD.

SYSTEM PERFORMANCE

The performance of the GC system can be monitored by regularly checking the resolution of two closely eluting CBs. A decrease in resolution indicates deteriorating GC conditions. The signal-to-noise ratio yields information on the condition of the detector. A dirty ECD-detector or MS-source can be recognized by the presence of a higher background signal, together with a reduced signal-to-noise ratio.

LONG-TERM STABILITY

One laboratory reference sample should be included in each series of samples. A quality control chart should be recorded for selected CBs. If the warning limits are exceeded, the method should be checked for possible errors. When alarm limits are exceeded, the results obtained should not be reported.

A certified reference material should be analysed at least twice a year, and each time the procedure is changed. Each laboratory analysing sediments should also participate in interlaboratory studies on the determination of CBs in sediments on a regular basis.

7. IDENTIFICATION

The presence of a single chlorobiphenyl compound is proved if the retention time of the substance corresponds with that of the same compound in the standard solution analysed under the same conditions on both columns. Using a GC/MS system additionally, the molecular mass or characteristic mass fragments (chlorine cluster), is a suitable way to prove the identification of individual CBs.

8. QUANTIFICATION

CB determinations should always be carried out using calibration solutions prepared from crystalline CBs. Preferably, certified CBs should be used. Two independent stock solutions of different concentrations should always be prepared simultaneously to allow a cross-check to be made. Calibration solutions should preferably be stored in ampoules in a cool, dark place. For all containers with standards, the weight loss during storage should be recorded.

After clean-up and before GC analysis, at least one internal standard is added for volume correction.

The ideal internal standard is a CB which is not found in the samples and does not co-elute with other CBs, e.g., CB 29, CB 112, CB 155, CB 198, or all 2,4,6-substituted CB congeners. Alternatively, 1,2,3,4-tetrachloronaphthalene can be used.

Internal standards should be added in a fixed volume or weighed to all standards and samples.

Since the ECD has a non-linear response curve, a multilevel calibration with at least five concentration levels is strongly recommended. A point-to-point calibration is preferred. If that option is not available, a linear working range can be identified, which allows the use of linear regression within this range. Alternatively, a non-linear fit can be used. If regression is applied, the standards should always be recalculated as samples and checked against their nominal values. Deviation from the nominal values should not exceed 5 %.

When the chromatogram is processed by using automated integrators, the baseline is not always set unambiguously, and always needs visual inspection. The use of peak heights is recommended for quantification.

The GC system should be equilibrated by injecting at least one standard or sample omitting any further evaluation prior to a series of samples and standards. In addition, standards used for multilevel calibration should be distributed regularly over the sample series, so that matrix and non-matrix containing injections alternate. A sample series should consist of:

1) a procedural blank;

2) a laboratory reference material;

3) at least five standards;

4) one standard solution that has been treated in the same manner as the samples (recovery determination).

When using a GC/ECD system with two columns of different polarities, the more reliable result should be reported.

The limit of determination should depend on the purpose of the investigation. A limit of 0.1 ng g^{-1} (dry weight, fraction < 2 mm) or better should be reached. The method for calculating the limit of determination should follow the advice in Part B-4.2.3 (COMBINE manual). The limit of determination that can be achieved depends on the blank, the sample matrix, the concentrations of interfering compounds, and the quantity of sediment used for analysis.

9. REFERENCES

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