HELCOM

Guidelines on the determination of chlorinated hydrocarbons in sediment

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1. Introduction

These guidelines are based on the review from Smedes and de Boer (1994, 1998) and Eljarrat and Barceló (2009).

The analysis of chlorinated hydrocarbons 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 steps of the procedure are susceptible to insufficient recovery and contamination. Quality control measures are recommended in order to regularly monitor the performance of the method. These guidelines are intended to encourage and assist analytical chemists to critically review their methods and to improve their procedures and quality assurance measures, if necessary.

These guidelines can be applied for the determination of several types of chlorinated hydrocarbons, e.g., chlorinated biphenyls (CB), chlorobenzenes, DDT and its metabolites and hexachlorocyclohexanes. It should be noted that these guidelines do not cover the determination of non-*ortho* substituted CB. Due to the low concentrations of non-*ortho* CB in sediments comparing to those of other CB, their determination requires an additional separation and concentration step similar to the analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F).

These guidelines are not intended as complete laboratory manual. If necessary, guidance should be sought from specialized laboratories. Laboratories should demonstrate validity of each methodological step. Moreover, use of an alternative method, carried out concurrently to the routine procedure, is recommended for validation.

Contracting parties should follow the HELCOM monitoring guideline but minor deviations from this are acceptable if the method achieves comparable results. Validation of the adopted method needs to be performed on the relevant matrix and concentration range e.g. by taking part in intercomparison studies or proficiency testing schemes.

2. Sampling and storage

The major criterion for successful sediment sampling is to ensure undisturbed sample stratification. (For further details about sampling, see Annex B-13, Appendix 3 “Technical note on the determination of heavy metals in marine sediments” of the COMBINE manual.)

Plastic materials (except polytetra-fluorethene, PTFE) should not be used for sampling and storage due to the risk of adsorption of target compounds onto the container material. Samples should be transported in closed containers and preferentially at temperatures below 10 °C. The samples should be stored at 4 °C as soon as possible, but at least if they have not been analysed within 48 hours after collection (short-term storage). For long-term storage over several months the samples should be frozen below -20 °C or dried (Law and de Boer, 1995). When drying, avoid methods with substantial risk of losing volatile substances (see Chapter 4: Pretreatment).

3. Blanks and contamination

Basically, care should be taken to avoid contaminations during all steps of the analytical chain, including sampling, extraction and clean-up.

In order to reduce blank and sample contaminations to a minimum it is strongly recommended to pretreat all used glassware, solvents, chemicals, adsorption materials, etc., as follows:

- Glassware should be thoroughly washed with detergents and can be furthered cleaned, other than calibrated instruments, by heating at temperatures > 250 °C. The glassware should be rinsed with an organic solvent prior to use.

- All solvents should be analyzed for impurities by concentrating to 10 % of the regular final volume. This concentrate is then analysed similarly to a sample by HPLC or GC. The solvent blank should not contain target analytes or other interfering compounds in higher concentrations than specified by the laboratory.

- All chemicals and adsorption materials should be analyzed for impurities and purified (e.g., by heating or extraction), if necessary. Soxhlet thimbles should be pre-extracted. Glass fiber thimbles are preferred over paper thimbles. Alternatively, full glass Soxhlet thimbles, with a G1 glass filter at the bottom, can be used.

Storage of these supercleaned materials for a longer period is not recommended, as laboratory air might contain target compounds which can adsorb onto these materials. Therefore, contaminated blank samples might occur despite precautionary measures due to contamination from the air. Volatile compounds are usually the most common contaminants in blanksamples (Gremm and Frimmel, 1990). Therefore, if possible, critical steps should be done in a clean bench.

4. Pretreatment

The samples should be thoroughly homogenized before subsampling for analysis. The amount of samples usually depends on the expected concentrations. For the marine environment, the amount of sample should be equal to an amount representing 50–100 mg of organic carbon.

Chlorinated hydrocarbons can be extracted from wet or dried samples. However, storage, homogenization and extraction are easier to handle with dried samples.

Drying the samples at ambient or elevated temperatures as well as freeze-drying may alter the concentrations, e.g., by contamination or loss of compounds through evaporation (Law *et al.,* 1994). Therefore, potential losses and contaminations should be analyzed in advance, e.g. by exposing 1–2 g CIS-bonded silica to the drying conditions and subsequent extraction and analysis (clean-up can be omitted) (Smedes and de Boer, 1998). For evaluation of potential losses, analytes identical or similar to chlorinated hydrocarbons need to be added to the material. However, bear in mind that added analytes can behave differently from analytes that have interacted longer with the matrix material and therefore may be sorbed more strongly. To avoid contamination during freeze-drying, placing a lid with a hole of about 3 mm in diameter on the sample container is suggested.

Chemical drying of samples can be performed by grinding with Na_2SO_4, or $MgSO_4$ until the sample reaches a sandy consistency. It is essential that several hours elapse between grinding and extraction to allow for complete dehydration of the sample. Residual water will decrease extraction efficiency.

5. Extraction and clean-up

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

Other extraction and clean-up methods than those described below may be used, provided that the methods have been tested and found equivalent to established methods regarding e.g. recovery.

5.1. Extraction of wet sediments

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, in particular 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. Extraction will be continued with a mixture of polar and apolar solvents such as acetone/ hexane or methanol/ dichloromethane. It has to be kept in mind that hexane and dichloromethane is a lot more toxic than similar solvents such as pentane, heptane, cyclohexane, isohexane. For complete extraction at least three subsequent extractions are required and a contact time of up to 24 hours with the solvent should be sufficient to complete the desorption of the chlorinated hydrocarbons from the sediment.

Soxhlet extraction or extraction by pressurized liquid extraction such as ASE of wet sediments should be conducted in two steps. First, a polar solvent, such as acetone, is used to extract the water from the sediment. In a second step the collecting flask is replaced and the extraction will be continued using a less polar solvent or solvent mixture such as acetone/hexane or toluene. Thereafter, the extracts will be combined.

To separate the water and keep the chlorinated hydrocarbons in a solvent that is compatible with the continued analysis different methods can be used. For example, water will be added to the combined extracts and the chlorinated hydrocarbon compounds will be extracted to a non-polar solvent. Another possibility is to add Na_2SO_4 to bind water.

Extraction should be conducted with a sufficient number of extraction cycles. Extraction efficiency should be analyzed for different types of sediments through a second extraction step. The extracts will be analysed separately and compared. A recovery of more than 90 % during the first extraction step is considered adequate.

5.2. Extraction of dry sediments

For dried sediments pressurized liquid extraction (e.g. ASE) is frequently applied to extract chlorinated hydrocarbons. The use of a mixture of a polar and a non-polar solvent, e.g., 25 % (v/v) acetone/hexane is recommended for sufficient extraction efficiency. A higher content of polar solvent increases extraction efficiency, but it has to be removed prior to gas chromatographic analysis.

Alternatively to ASE, extraction can be conducted with a regular Soxhlet, a hot Soxhlet with at least 50 to 60 extraction cycles (approximately 8 hours for the hot Soxhlet) or by microwave extraction. Supercritical fluid extractions have also been demonstrated, but have not found wide application due to low reproducibility compared to the other technique (Law et al, 2011).

Extraction should be conducted with a sufficient number of extraction cycles. Extraction efficiency should be analyzed for different types of sediments through a second extraction step. The extracts will be analysed separately and compared. A recovery of more than 90 % during the first extraction step is considered adequate.

Prior to any concentration steps, a keeper (high-boiling solvent, e.g. a high-boiling alkane or toluene) should be added. Make sure that the keeper does not interfere with the analytes of interest in the instrumental analysis.

5.3. Removal of sulphur and sulphur-containing compounds

The crude extracts usually require clean-up to remove co-extracted compounds (Wise *et al.,* 1995). Due to chlorophyll-like compounds extracted from the sediment, the raw extract is usually colored and also contains sulphur and sulphur-containing compounds, oil, PAH compounds and other natural and anthropogenic compounds. Selection of the appropriate clean-up method depends on the subsequent instrumental method to be used for analysis. Copper powder, wires, or gauze are the most common ways to remove the sulphur directly from an organic solvent. Copper can be applied during or after sediment extraction. Ultrasonic treatment might improve the removal of sulphur. If sulphur appears to be present in the final extract, the amount of copper used was insufficient and the clean-up procedure must be repeated.

Be aware that a prolonged contact between the sample and the copper may degrade some chlorinated pesticides.

Another possibility to remove sulphur is to add an aqueous saturated Na_2SO_3 solution 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 with a polar solvent which is miscible with water, the Na_2SO_3 solution can be added directly after the extraction. If the extraction mixture also contains a non-polar 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 non-polar 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 Na_2SO_3 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.

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 chlorinated hydrocarbons 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.

Sometimes the use of multiple methods may be necessary for different samples. Several methods leave aromatic sulphur compounds in the extract which will elute from the GC column at the same retention time as the lower chlorinated biphenyls. The major part of these compounds can be removed by eluting a non-polar extract over a column containing silica loaded with concentrated sulphuric acid.

The recovery during clean-up should be analyzed 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 or copper can result in low recoveries for certain pesticides.

5.4. Further clean-up

Clean-up using normal phase chromatography is the most appropriate technique for the separation of the chlorinated hydrocarbons from other compounds. Using non-polar solvent, e.g., hexane or *iso*-octane, chlorinated hydrocarbons usually elute very rapidly.

All polar solvents used during extraction or sulphur-removal 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 the primary clean-up step through which usually a sufficiently clean extract for a gas chromatography- electron capture detector (GC-ECD) analysis of the sample is achieved, given that sulphur has been removed.

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

For high activity silica (overnight at 180 °C), the retention of chlorinated hydrocarbons is negligible while PAH compounds are more strongly retained. The chlorinated hydrocarbons are eluted with non-polar solvents. Upon using more polar solvents (e.g., hexane/acetone) some interfering organochlorine pesticides might become eluted**.**

When GPC is used for removing sulphur (see 5.3 REMOVAL OF SULPHUR AND SULPHUR-CONTAINING COMPOUNDS) the removal of high molecular weight material can also be incorporated into the procedure. GPC does not separate chlorinated hydrocarbons from other compounds in the same molecular range (such as organochlorine pesticides), so additional clean-up is usually required.

For the separation of chlorinated hydrocarbons from lipids or oil components reverse-phase high-performance liquid chromatography (RP-HPLC) can be used. Due to the use of aqueous solvents in RP- HPLC solvents need to be changed from polar to non-polar and *vice versa*. Another option is the use of strong acid (e.g. H_2SO_4) to degrade the lipids; however, it may also degrade some pesticides.

6. Gas chromatography

In particular, for the large number of CB congeners (a total of 209) high-resolution capillary gas chromatography (GC) is the method of choices. However, 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 from other ECD-detectable compounds. For example, the seven common indicator-PCBs should be analysed. If there is a desire to separate and analyse all congeners, it is recommended to use multidimensional gas chromatography (MGC) that makes use of two successive columns of different selectivity or polarity. However, the optimization is difficult (co-elution of some PCBs) and it is not routinely applied at commercial laboratories. Alternatively two ECD detectors and two parallel columns with different selectivity or polarity can be used, reducing the detection limit by a factor of 2 but improving the selectivity of co-elution PCBs by choosing the column with least overlap for suspected co-elutions.

Another option is to use GC-MS instrumentation for more selective determination.

For all GC methods, parameters have to be optimized.

6.1 Column dimensions

Column dimensions for the determination of chlorinated hydrocarbons are:

- length: minimum 50 m, and
- inner diameter (i. d.): maximum 0.25 mm.
- film thickness: 0.2 µm to 0.4 µm

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 above 500 kPa, which are often not compatible with regular GC instruments. Also, the risk of leakage increases.

6.2 Stationary phases

A wide range of stationary phases can be used for the separation of chlorinated hydrocarbons (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 non-polar, chemically bonded phases. Stationary phases that separate chlorinated hydrocarbons 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.

6.3 Carrier gas

Preferentially, 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}.

6.4 Injection techniques

The two systems commonly used are splitless and on-column injection. Split injection should not be used due to strong discrimination effects. 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 may 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. 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.

An auto-sampler should be used.

6.5 Temperature programming

The temperature programme must be optimized for sufficient separation of the chlorinated hydrocarbons. A separation time of 60 to 120 minutes can be neccessary. 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 see Smedes and de Boer (1998).

6.6 Detection

The use of a mass spectrometer (MS) or tandem mass spectrometer (MS/MS) is highly recommended. MS gives the possibility to use ^{13}C labelled internal standards. Different ionization methods have been reported: Electron impact ionization (EI), Negative chemical ionization (NCI) or electron capture negative ionization (ECNI). Another used detector for the analysis of chlorinated hydrocarbons is the electron capture detector (ECD), but injection of chlorinated or oxygen-containing solvents should be avoided. NCI and ECNI is extremely sensitive for penta- to decachlorinated CBs (approximately ten-fold better than ECD), but can be less sensitive for less chlorinated PCBs (Law et al, 2011).

6.7 Identification

Usually, the compounds in the sample are identified based on their retention times as compared to those of the standard compounds analyzed under the same conditions. Moreover, upon using GC-MS compound characteristic mass fragments serve as additional identifiers.

7. Quantification

Automatically processed chromatograms should be reviewed if, e.g., the baseline is set correctly.

For calibration purposes a multilevel calibration with at least five concentration levels is recommended. The calibration curve should be linear and cover the working range. Obtained calibrations should be regularly validated in terms of precision and accuracy.

Prior to running a series of samples and standards, the GC or HPLC systems should be equilibrated by injecting at least one sample extract. In addition, standards used for multilevel calibrations should be

regularly distributed over the sample series so that matrix-and non-matrix-containing injections alternate. A sample series should include:

- a procedural blank;
- a laboratory reference material;
- at least five standards;
- one standard sample treated similarly to the samples for determination of the recovery.

The limit of quantification usually depends on the purpose of the investigation. The limit of quantification that can be achieved depends on the blank sample, the sample matrix, concentrations of interfering compounds, and the amount of sample. However, a limit of quantification of 0.1 ng g^{-1} (dry weight, fraction < 2 mm) or better should be attained for single compound analysis. The method for calculating the limit of determination should follow the advice in Part B-4.2.3 (COMBINE manual).

8. Quality Assurance

A number of measures should be taken to ensure sufficient quality of the analysis. Six main areas can be identified:

1. extraction efficiency and clean-up;
2. calibrant and calibration;
3. system performance;
4. long-term stability;
5. internal standards; and
6. frequent participation in interlaboratory proficiency testing schemes (e.g. QUASIMEME two times a year, www.quasimeme.org)

8.1. Extraction efficiency and clean-up

Extraction efficiency and clean-up can be controlled by analysing reference materials (Annex B-7). To determine the recovery rates of the clean-up and concentration steps, it is recommended to pass a standard solution (see 8.5. INTERNAL STANDARDS) through the entire procedure. The addition of corresponding internal standards to the samples is preferred.

If major losses have occurred, the results should not be reported.

8.2 Calibrant and calibration

Basically, calibration solutions should be stored in ampoules at a cool, dark place. Weight loss during storage should be recorded for all standards.

Calibration solutions from certified crystalline compounds should be used. However, the laboratory should have the appropriate equipment and expertise to handle these hazardous crystalline substances. Alternatively, certified compound solutions can be used. Preparation of two independent stock solutions allows cross-checks of the standard solutions if necessary.

8.3 System performance

The performance of the GC system can be monitored through regularly analyzing the resolution of two closely eluting compounds. A decrease in resolution indicates deteriorating GC conditions.

The signal-to-noise ratio of a low concentrated standard can give information on the condition of the detector. For example, a dirty MS-source can be recognized by the presence of a higher background signal, together with a reduced signal-to-noise ratio if not used in the SIM mode.

8.4 Long-term stability

One laboratory reference sample should be included in each series of samples. A quality control chart should be recorded for selected chlorinated hydrocarbons. 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.

8.5 Internal standards

Internal standards should be added to all standards and samples either in a fixed volume or by weight and should not interfere with the target analytes.

If possible, it is preferable to have internal standards corresponding as much as possible to each analyte, e.g. using isotopically labeled compounds combined with mass spectrometry as detection technique (e.g. pp-DDT-D8, isotopically labelled CBd).

After clean-up and before GC analysis, an additional internal standard can be added to evaluate the recovery of the internal standards added before clean-up.

8.6 Interlaboratory proficiency testing schemes

Each laboratory analysing sediments should participate in interlaboratory studies on the determination of chlorinated hydrocarbons in sediments on a regular basis (e.g. QUASIMEME offers the possibility to take part twice a year, www.quasimeme.org).

7. References

Eljarrat, E., Barceló, D. 2009. Chlorinated and brominated organic pollutants in contaminated river sediments. In: Contaminated sediments. The Handbook of Environmental Chemistry Vol. 5T. Springer

Japenga, J., Wagenaar, W.J., Smedes, F., and Salomons, W. 1987. A new rapid clean-up procedure for the simultaneous determination of different groups of organic micropollutants in sediments; application in two European estuarine sediment studies. Environ. Techn. Lett. 8: 9–20.

Jensen, S., Renbey, L., and Reutergårdh, L. 1977. Residue analysis of sediment and sewage sludge for organochlorines in the presence of elemental sulfur. Analytical Chemistry, 49: 316–318.

Law, R.J., Webster, L., Theobald, N. Rumney, H.S. and de Boer, J. (2011) Organic Micropollutants. *In* Chemical Marine Monitoring: Policy Framework and Analytical Trends, Quevauviller, P. Roose, P. And Verreet, G. John Wiley /Sons, Ltd.

Smedes, F., and de Boer, J. 1994. Guidelines for the determination of chlorobiphenyls in sediments. Quimica Analytica, 13: 100–108.

Smedes, F., and de Boer, J. 1998. Chlorobiphenyls in marine sediments: Guidelines for determination. ICES Techniques in Marine Environmental Sciences, No.21. 24 pp.