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Chlorobiphenyls in marine sediments:
Guidelines for determination

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F. Smedes and J. de Boer

Abstract

The analysis of chlorinated biphenyls in sediments generally includes extraction with organic solvents, clean-up, removal of sulphur, column fractionation and gas chromatographic separation, mostly with electron capture detection. All of the steps in the procedure are susceptible to insufficient recovery and/or contamination. Different methods applied to each of these steps are discussed with their advantages and disadvantages. Where possible, quality control procedures are recommended in order to check the method's performance. Gas chromatographic conditions are discussed with regard to injection, separation, detection and system performance. In addition, the quality control aspects relating to calibrants, extraction, and clean-up are considered. These guidelines are intended to encourage and assist analytical chemists to critically (re)consider their methods and to improve their procedures and/or the associated quality control measures, where necessary.

Keywords: polychlorinated biphenyls, sediments, sample pretreatment, gas chromatography, quality control.

1   INTRODUCTION

Chlorinated biphenyls (CBs) were manufactured from 1930 until 1983 when their production was discontinued (de Voogt and Brinkman, 1989). They have been used extensively in electronic equipment, such as transformers and capacitors, and as plasticizers in paint and rubber sealants (Pearsson, 1982). Large quantities of CBs have reached the environment through leakage, disposal, evaporation, and by other means (Pearsson, 1982). Due to their persistence, their residence time in the environment is very long. In the aquatic environment, CBs tend to accumulate in sediments and biota because of their hydrophobic character and consequent low solubility in water. Sediments act as a sink for CBs and are, therefore, important in studies and monitoring of contaminants.

In the late 1970s, the Oslo and Paris Commissions (OSPAR) initiated a Joint Monitoring Programme (JMP), coordinated by the Joint Monitoring Group (JMG), as an extension of national monitoring programmes. CBs were seen as an important group of compounds to study. The JMG recognized that international comparability of data is of paramount importance and, consequently, guidelines were developed for the participants in this programme (Oslo and Paris Commissions, 1991). These guidelines mainly focused on sampling and only limited attention was given to analytical methods. However, in the case of CBs it was felt that more extensive guidelines were required in order to improve the analytical comparability of the data. The results of several international intercalibration exercises (Villeneuve and Mee, 1992; Kimbrough et al., 1994a; de Boer et al., 1994; de Boer and van der Meer, 1998a, 1998b) demonstrate that there is still a need to improve the quality of CB analyses in sediments. Although the adoption of a single method by all participating laboratories would, to a large extent, improve the comparability of data, there are various arguments against the implementation of a single method. Most analytical methods have both advantages and disadvantages, and the fact that comparable data can be produced by different methods gives additional credence to the results. These guidelines are not intended to give a detailed, rigid description of one method, but instead they offer a number of possibilities, with criticism of methods which have shown themselves to be less reliable than others.

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 the concentrations of other CBs, these determinands require an additional separation and concentration step (Haglund et al., 1990; de Boer et al., 1992c, 1992d; Hess et al., 1995).

2   GENERAL CONSIDERATION OF ANALYTICAL TECHNIQUES

Technical mixtures of CBs, such as Aroclor and Clophen, consist of many individual CB compounds and differ in their degree of chlorination and the distribution pattern of chlorine atoms (Ballschmiter and Zell, 1980). Theoretically, 209 CB congeners are possible; these congeners have been systematically numbered by Ballschmiter and Zell (1980). The CBs occurring in sediments usually originate from more than one technical product. For this reason and because of weathering effects, the distribution patterns of possible sources usually cannot be recognized in the sediments. CB contamination is therefore monitored by the determination of selected individual congeners (ICES, 1992). For the separation and detection of these congeners, high resolution capillary gas chromatography (GC) with electron capture detection (ECD) is generally applied. Prior to GC analysis, the CBs are extracted from the sediment using organic solvents. The extract requires an extensive clean-up, often consisting of several steps to isolate the CBs from other extracted compounds. For example, sulphur, which is often abundantly present in anoxic sediments, must be removed from the extract because it yields a

broad peak in the CB chromatogram. Clean-up techniques can give rise to losses of the analytes as well as contamination of the sample extract.

In these guidelines, a number of procedural steps are described with their limitations and advantages. Different combinations of steps can yield a number of procedures which can be applied to the analysis of CBs in sediment. Whichever procedure is adopted, each laboratory must demonstrate the validity of each step of its procedure. Additionally, the use of a second, different, method, in addition to the routine procedure, is recommended as a validation.

3   BLANKS AND CONTAMINATION

The very high sensitivity of the electron capture detector for CBs and the scope for concentration of sample extracts to very small volumes theoretically allows detection limits below 1 ng kg^{-1}. CBs are, however, widespread in the environment, which makes contamination during the analytical procedure inevitable. Consequently, the procedural detection limit is limited 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 an aliquot of 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 GC analysis.

All chemicals and adsorption materials should be checked for impurities and purified (e.g., by heating or extraction), if necessary. Glassfiber or paper Soxhlet thimbles should be extensively pre-extracted and the use of paper thimbles should preferably be avoided. Alternatively, all-glass Soxhlet thimbles with a G1 glass filter at the bottom can be used. The 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. If it is not possible to decrease the blank value sufficiently even after applying all precautions, potential contamination from the air (Balfanz *et al.*, 1993) should be checked.

Typical CB concentrations in air range from 0.1 ng m^{-3} for less volatile CBs to 2 ng m^{-3} for volatile CBs. However, laboratories built in the period 1960 to 1970 may have much higher CB concentrations in the air due to the use of CB-containing paints and rubber during that period (Benthe *et al.*, 1992). Paints and rubber (used between concrete elements of a building) may contain up to 25 % CBs. In addition, considerable CB contamination can result from exploded capacitors in fluorescent strip lighting (MacLeod, 1981). This contamination can be checked by measuring the CB concentrations in the air. As an alternative, a good estimate of the contamination can be made by placing a Petri dish with 2 g of C18-bonded silica in the laboratory for two weeks. After this period, the material is transferred to a glass column and eluted with 10 ml of 10 % diethylether in hexane. After concentration, the CB content can be measured by GC. Absolute amounts < 1 ng for each CB congener demonstrate that there will be no significant contamination from the air during a normal analytical procedure.

4   DRYING OF SEDIMENTS

Drying of sediments by evaporation of the water is primarily done for convenience. Dry sediments are more effectively homogenized, allowing accurate subsampling for parallel analyses for other determinands, such as moisture content and organic carbon. In addition, dried samples can be stored and transported more easily than wet samples. The absence of water in

the extract avoids laborious extraction with separation funnels, and makes the sample matrix more accessible to organic solvents which extract the CBs. As an alternative to drying via evaporation, several methods for binding the water can be applied. Alternative methods include the following:

A1   Chemical drying of samples can be performed by grinding with Na_2SO_4 or $MgSO_4$. When applying this method, one must be aware of the risk that inclusion of particles in the hydrated material can hinder the extraction. Large lumps containing too high a water content will not be sufficiently extracted. Therefore, intensive grinding and the addition of a sufficient quantity of drying salt, so as to result in a free-flowing powder, is of absolute importance for complete extraction. It is also essential that the operations of grinding and extraction are separated by a period of several hours to allow the water to desorb from the sediment organic matter and become fully bound to the drying salt.

A2   Drying with water-adsorbing material (Al_2O_3, SiO_2, etc.) (Japenga *et al.*, 1987) obviates the problem in method A1, but the water is not bound irreversibly and can easily be released when polar solvents are used for extraction. Therefore, a benzene/hexane mixture is applied for the extraction (Japenga *et al.*, 1987). However, the use of benzene is strongly discouraged.

A3   Application of air drying in an oven at elevated temperature (40 °C, for example) may cause losses by evaporation, depending on the volatility of the CBs present and the nature of the sediment, e.g., the organic carbon content. Applying this method to a sandy sample with a low organic carbon content will easily result in losses. Air drying at ambient temperature is successfully employed in some laboratories, but the low water uptake of air at ambient temperature leads to an extended exposure to laboratory air which can significantly increase the CB contents of the sample (Alcock *et al.*, 1994). This will particularly have an effect when CB concentrations are low, as is usually the case for marine sediments.

A4   Freeze-drying is becoming a more popular technique. Losses through evaporation are diminished by keeping the temperature in the evaporation chamber below 0 °C.

Drying of the samples by evaporation of the water can be risky, as both loss of compounds through evaporation and uptake from the air may occur. In addition, cross-contamination cannot be excluded. Experiments in one of the authors' laboratories showed that no loss of CBs (CB18 and higher) occurred during freeze-drying (evaporation temperature –5 °C) over a ten-day period. However, an unexpected increase in the hexachlorobenzene concentration occurred, demonstrating that contamination can also result. For freeze-drying of tissue homogenates, Norstrom and Won (1985) reported that no loss through evaporation occurred for compounds with boiling points equal to or higher than that of hexachlorobenzene.

The advantages of dried sediments with regard to storage, homogenization, and extraction usually compensate for the effort needed to control the drying process. It is recommended that every drying procedure applied should be verified by parallel wet analysis of samples with both low and high levels of CBs. The routine control procedure should verify that the evaporation of analytes and occurrence of contamination are minimized. Losses should be checked by submitting a sediment with a low organic carbon content to the drying procedure and subsequently analysing both the dry as well as the wet sediment. Exposing C18-bonded silica to the drying process in parallel with the samples provides a sensitive test for (cross)contamination. The analysis is similar to that described for the determination of contamination from the air.

Method A3 is not recommended for the reasons outlined above, while method A2 can only be applied if a suitable alternative for benzene can be found. Methods A1, the safest drying method with regard to losses, and A4 are those most commonly applied.

5   EXTRACTION

The contaminants should be isolated from the sediment and transferred to an organic solvent prior to further analysis. Three factors determine the yield of an extraction procedure:

a)   the solubility of CBs in the extraction solvent;

b)   accessibility of the extraction solvent to the sediment surface and sediment organic matter;

c)   the exposure or extraction time.

The solubility determines the equilibrium that can be reached at a certain stage of the extraction, while accessibility and extraction time are more related to the kinetics of the extraction. An apolar organic solvent, such as hexane, may have a high solubility for CBs but does not have access to the inner part of sediment organic matter because this material contains many polar groups such as amines, phenols, and carboxylic acids which prevent the hexane from entering the material. In the case of wet sediment, CBs must desorb from the sediment to the water phase before they can be transferred to the hexane. This desorption step is extremely slow (10–30 days), especially in aged sediments (Karickhoff, 1984). As a result, the extraction efficiency using hexane is nearly zero. To penetrate into the organic matter, the addition of a polar solvent, such as methanol or acetone, is needed. This solvent replaces the water and accelerates the desorption as the solubility of CBs in the aqueous phase becomes much higher (Li and Andren, 1994). In addition, an elevated temperature will also promote the desorption process.

For dried sediments, the process described above is also valid but less pronounced. The absence of water allows the use of more apolar solvents, but a mixture of both a polar and an apolar solvent is recommended, while extraction with medium polarity solvents will also suffice. The yield for (Soxhlet) extraction with hexane appears to be significantly lower than with more polar solvents (Kimbrough *et al.*, 1994b).

Another difficulty can be the presence of graphite carbon-like materials such as coals in the sediment. Also for this type of material, acetone has a better extraction potential than hexane, but toluene is preferred for such materials. Most marine sediments, however, do not contain significant quantities of these materials.

Extraction methods include the following listed below:

B1   Extraction methods utilizing mixing techniques are often used to extract wet sediments although there is no reason not to apply them to dried sediments, also. Extraction is stimulated by shaking, Ultra Turrax mixing, ball mill tumbler or ultrasonic treatment. Water-miscible solvents, such as methanol, acetone (Kerkhof *et al.*, 1982), and acetonitrile (Ozretich and Schroeder, 1986), are used, especially in the first step. The extraction potency of the first step is low as there will be a considerable amount of water in the liquid phase. This step can also be regarded as a water removal step, after which the extraction is continued with a mixture of solvents (acetone plus hexane or methanol plus dichloromethane (Brown *et al.*, 1980)). For an accurate extraction, at least three subsequent extractions are needed and the contact time (24 hours) with the solvent
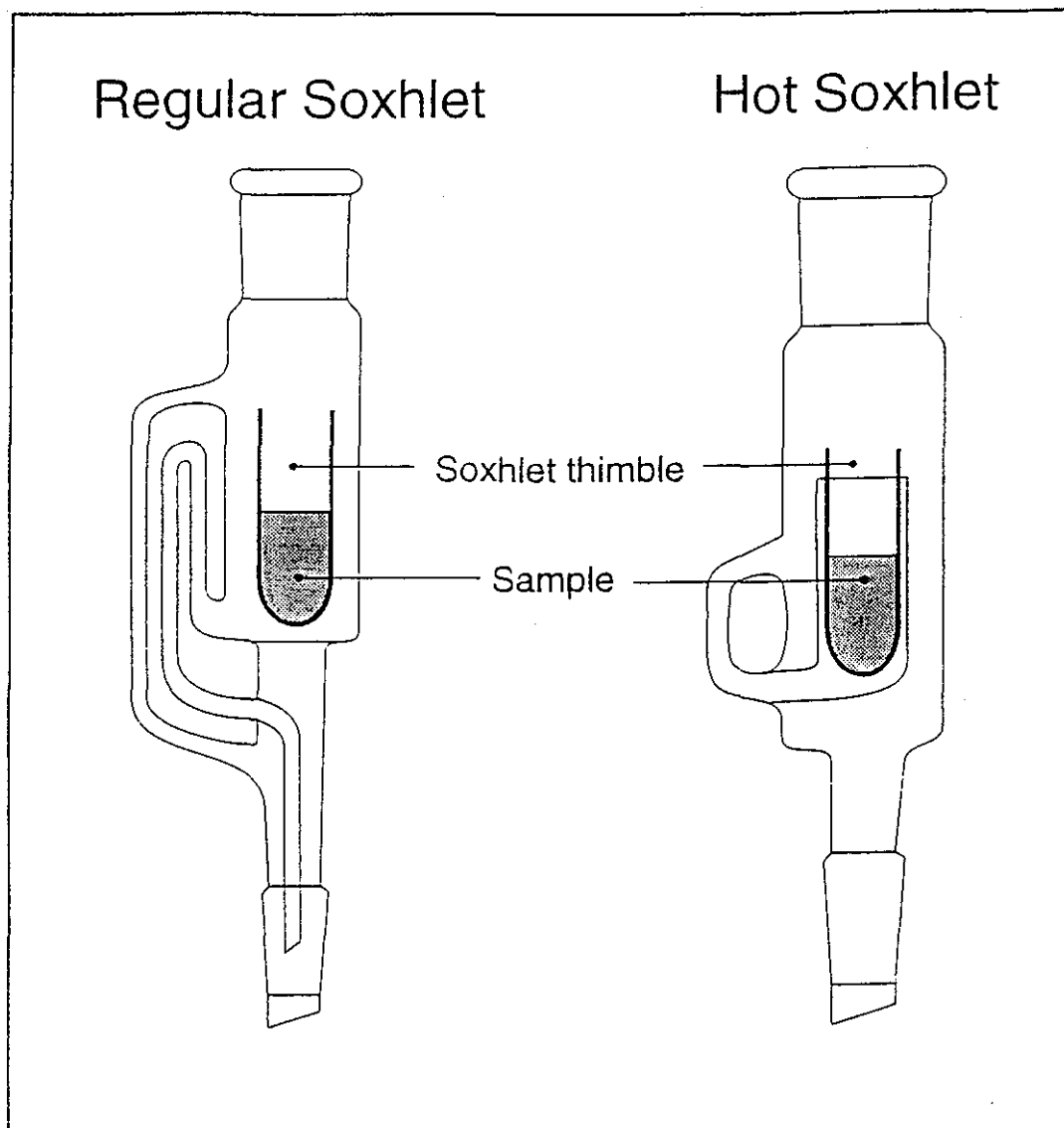
should be sufficient (Brown *et al.*, 1980; Kerkhof *et al.*, 1982) to complete the desorption of CBs from the sediment. Subsequently, the CBs are extracted into an apolar solvent such as hexane by liquid-liquid extraction. Water is added both to enhance the efficiency of extraction into hexane and to remove the polar solvent (e.g., methanol, acetone) from the hexane. This extraction method is laborious, but on the other hand no drying of the sample is necessary.

B2 For dried sediments, Soxhlet extraction is the most frequently applied technique to extract CBs. The absence of water allows the use of a wide variety of solvents. The most commonly used solvents are: dichloromethane, pure or mixed with acetone or hexane, and acetone-hexane. With a mixture of a polar and an apolar solvent, it is important to find a balance between the solvents. A mixture containing a greater proportion of the polar solvent has an increased extraction potency, but the polar solvent has to be removed thereafter. For this removal, laborious extraction with water (see also method B1) should preferably be avoided. Therefore, a good choice of solvent is 25 % acetone in hexane. This forms an azeotrope of 56 % acetone for the actual extraction, and in the concentration step after the extraction almost all of the acetone can be removed through azeotropic distillation, thereby avoiding laborious shaking with separation funnels.

Two Soxhlet types are available: (1) the regular Soxhlet with a siphon and the thimble outside the vapour stream, and (2) the so-called hot Soxhlet, in which the thimble is placed in a holder within the vapour stream. In the latter case, the extraction takes place at the boiling temperature of the solvent mixture, thus promoting the desorption process. Often the liquid in the thimble is boiling, in this way improving the exchange between the liquid and solid phases. Both types are illustrated in Figure 1. A considerable amount of time is needed for a complete extraction: at least 16 hours for a regular Soxhlet and 8 hours for a hot Soxhlet. In addition, the number of revolutions must be sufficient, but a rate of revolution twice as high does not automatically shorten the extraction time proportionately, as desorption is also time and temperature dependent.

B3 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 (4 hours). Then the flask is replaced and the extraction is continued with a mixture of acetone plus hexane (1+3) according to method B2. Subsequently, the extracts are combined and, after addition of water, the CBs are extracted to hexane.

B4 Supercritical fluid extraction (SFE) is a relatively new method which is currently being applied within a number of research laboratories, mainly for dry sediments. Results obtained to date appear very promising. The use of solvent modifiers (Hawthorne *et al.*, 1993) or the combination of SFE with solid phase sorption (Bøwadt *et al.*, 1993) may be necessary. The optimum conditions are still under investigation, so it is not possible to prescribe strict guidelines regarding this method at the present time.

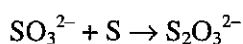**Figure 1.** Examples of a regular Soxhlet and a hot Soxhlet.

In principle, all of the methods described are suitable for the extraction of CBs from sediments. However, Soxhlet extraction is recommended over mixing methods (Kimbrough *et al.*, 1994b), especially for dry samples. The extraction times indicated above are generally more than sufficient, but shorter times cannot be recommended as the necessary extraction time can vary for different matrices and the equipment used. Demonstrating a high recovery of spikes does not guarantee that all CBs have been extracted from the sediment. This makes validation of the selected method essential.

6   SULPHUR REMOVAL AND PRIMARY CLEAN-UP

The crude extract requires a clean-up because many other compounds are co-extracted in addition to the compounds of interest. This extract will be coloured due to the presence of chlorophyll-like compounds extracted from the sediment, and it will also contain sulphur, oil, PAHs and many other natural and anthropogenic compounds.

There are several methods for the removal of sulphur. These include a reaction of S with SO_3^{2-} to $S_2O_3^{2-}$, contact of S with a heavy metal (Hg, Cu, Cd, Ag, etc.) to form a metal sulphide, gel permeation chromatography (GPC), and saponification. They are described below:

C1   The method of Jensen *et al.* (1977) is well known, but it is rather laborious. An aqueous saturated Na_2SO_3 solution is added to the 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. On shaking, the TBA ion forms an ion pair with the sulphite ion which is introduced into the organic phase where the reaction takes place:

$$SO_3^{2-} + S \rightarrow S_2O_3^{2-}$$

Subsequently, water is added to remove the isopropanol. The aqueous phase is quantitatively extracted with hexane.

C2   If the extraction was performed by a polar solvent miscible with water (methods B1 and B2), then a Na_2SO_3 solution can be added directly after the extraction. If the extraction mixture also contains an apolar solvent, then whether or not the SO_3^{2-} has access to the organic phase depends on the ratio of the solvents. If this access is adequate, the addition of TBA and isopropanol is unnecessary. Any excess of Na_2SO_3 and reaction products can be removed by the addition of water and partitioning between the apolar solvent and water.

C3   Japenga *et al.* (1987) developed a column method for the removal of sulphur. 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. The material is then dried under nitrogen until a level of deactivation equivalent to 10 % water is reached. Storage must be under nitrogen as 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 the primary clean-up of the sediment extract.

C4   Mercury, copper powder, and copper gauze remove the sulphur directly from an organic solvent by sulphide formation. Exposing the extract to hexane-washed copper powder (Jacobs *et al.*, 1992) will remove the sulphur from solution. This may also be done by inserting a copper gauze into the hexane extracts. Both methods can be applied during or after Soxhlet extraction. The application of a copper gauze is elegant, as it can potentially be reused after treatment with nitric acid and subsequent washing with water and methanol. However, the amount of copper may appear to be insufficient when sulphur is present in high concentrations. If sulphur appears to be present in the final extract injected in the GC, addition of copper powder to the vial and a two-minute ultrasonic treatment is generally sufficient to complete the removal of sulphur.

C5   Silver ions strongly bind sulphur and sulphur compounds (Dunn *et al.*, 1987). Loaded on SiO_2, $AgNO_3$ is a very efficient sulphur-removing agent. It can be prepared by mixing dissolved $AgNO_3$ with SiO_2 and subsequently drying under nitrogen. Compounds containing aromatic rings are strongly retained (Eganhouse, 1986), 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 bounds, as a moderator.

C6   Elemental sulphur is strongly retained on a polystyrene-divinylbenzene copolymer column, as generally applied for gel permeation chromatography (GPC) (Johnson *et al.*, 1978; Schlabach *et al.*, 1993). It elutes later than the void volume when using either dichloromethane or tetrahydrofuran. CBs elute at the expected molecular range and can be isolated in that way. In addition, this method combines the removal of sulphur with a clean-up.

C7   Another possible step which removes sulphur, but also acts as a general clean-up, is saponification (Tuinstra, 1986). The concentrated extract is mixed with ethanolic potassium hydroxide and heated. The conditions for saponification are critical, as too high temperatures and too long saponification times may cause decomposition of higher chlorinated compounds such as hexa- to deca-CBs, due to the presence of trace metals in the sediment that can act as a catalyst (van der Valk and Dao, 1988). The maximum temperature during saponification should therefore never exceed 70 °C, while the saponification should not last longer than one hour.

All 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 times as the lower CBs. The major part of these compounds can be removed by eluting a hexane extract over a column with $2 \, g \, SiO_2$ loaded with 44 % concentrated sulphuric acid. However, this material poses a greater hazard in handling than sulphuric acid itself and should be handled with utmost care. For environmental reasons, the use of mercury (Brannon and Karn, 1990) should be avoided, while silver columns should only be used when regular methods have failed.

7   COLUMN CLEAN-UP AND FRACTIONATION

As CBs are non-polar, fractionation using normal phase chromatography is the most appropriate technique for the separation of CBs from other compounds. All polar solvents used in the extraction or sulphur removal step should be removed before further clean-up. This can be done by azeotropic distillation or evaporation in the presence of a keeper (high boiling alkane). When low boiling (40–80 °C) solvents are used, 2 ml iso-octane (2,2′,4-trimethylpentane) can be added prior to Kuderna Danish or rotary evaporation. The last concentration step is usually performed by evaporation with a gentle stream of nitrogen. Evaporation to dryness should always be avoided.

Using hexane or iso-octane as an eluent, CBs normally elute very rapidly in most clean-up systems based on normal phase chromatography.

The following methods are available:

D1   Deactivated Al_2O_3 (5–10 % water content) is used very often as a primary clean-up. In method C3 it is combined with a reagent for the removal of sulphur. CBs pass through this column almost unretained when eluted with hexane, iso-octane or pentane. Provided that the sulphur has been removed, Al_2O_3 normally gives a sufficiently clean extract for a GC-ECD screening of the sample. When fully activated (dried at 180 °C), Al_2O_3 retains all CBs and, as alkanes are not retained, the elution separates this type of oil compounds from CBs. However, the high activity is very sensitive to moisture and decreases rapidly after contact with the sample. Consequently, the sample capacity of highly active Al_2O_3 is relatively low.

D2   Florisil is one of the oldest materials used in the clean-up for CB and organochlorine pesticide analysis. It is a mixture of several inorganic oxides, with SiO_2 and MgO as the main substances. As it is a mixture, the composition varies from batch to batch. In its most active form, it retains planar CBs while the other CBs elute almost unretained. Highly activated Florisil can lose its activity easily. Because of the batch-to-batch variation, the use of this material does not contribute to the robustness of the procedure. When using Florisil, the elution pattern should be established for each batch.

D3   Deactivated silica (SiO_2) (1–5 % water) does not retain CBs (including planars) and only slightly retains polycyclic aromatic hydrocarbons (PAHs) when eluted with hexane or iso-octane. For high activity SiO_2 (overnight at 180 °C), the retention of CBs is also negligible while PAHs are more strongly retained. Therefore, it is an ideal material for the isolation of all the CBs. However, when organochlorine pesticides are also to be determined in the same extract, deactivation of the silica with a few percent of water is necessary. For standardization it is recommended that, prior to adding the water, the silica is first heated overnight at 200 °C. Control of the activity of the silica is essential, as the fractionation between, e.g., CBs and organochlorine pesticides may be affected by variations in activity.

D4   When GPC is applied for removing the sulphur (method C6), the removal of high molecular weight material can also be incorporated into the procedure. Separation is obtained on polystyrene-divinylbenzene copolymer columns (e.g., Biobeads). Very apolar solvents such as dichloromethane (Fernández *et al.*, 1992) or mixtures such as dichloromethane/hexane (1:1), ethylacetate/toluene (3:1) (Johnson *et al.*, 1976) are applied as eluents to prevent adsorption of the analytes to the column material. Also, elevated temperatures can be applied to influence adsorption while permeation is unaffected (Bicking and Wilson, 1991). GPC does not separate CBs from other compounds, such as organochlorine pesticides, in the same molecular range and so an additional fractionation is usually required.

D5   In reversed-phase chromatography, the CBs elute during a solvent gradient from 70 % to 90 % methanol (Brodsky and Ballschmiter, 1989) together with numerous other compounds of the same polarity. Most of the common extraction methods yield an extract containing an apolar solvent which cannot be injected directly for reversed-phase chromatography, so compounds have to be transferred between solvents several times, for example, before injection and after elution. However, when only polar solvents are used, for example in the extraction of wet sediments, the application of reversed-phase columns could be considered as a clean-up. In the elution of an acetonitrile extract over SPE C18 with acetonitrile, high molecular weight hydrocarbons (i.e., lipids and grease)

are strongly retained, while CBs elute in the first few column volumes (Ozretich and Schroeder, 1986; Walters, 1990).

The most commonly used methods are Al_2O_3 with 5–10 % water as a rigid primary fractionation followed by elution over SiO_2 with hexane. For this last step, SiO_2 is preferred over Florisil because of the batch-to-batch variations of the latter. GPC is also regularly applied as primary fractionation. A drawback of GPC is that CBs have to be transferred to hexane for further fractionation. The solvent transfers necessary in the application of reversed-phase chromatography do not make this method attractive for routine analyses.

8   COMBINATION OF METHODS

From the methods described above, several analytical schemes can be composed for the separation of CBs from sediment. In Figure 2, a few selected methods for application to dried sediments are presented in a flow chart. This scheme is not intended to be complete, but only presents the most practical methods for routine application. For dry sediments, only Soxhlet extraction is considered because of its simple and exhaustive character. In the clean-up steps, more variations are possible also within one method scheme; for example, the scheme shown on the far left in Figure 2 applies method C1 for sulphur removal after the extract has been concentrated. However, when the extract contains a polar solvent, the first concentration step of the extract can be omitted and the reagents suggested by Jensen *et al.* (1977) can be added to it, as described in method C2.

The Al_2O_3 (5–10 % H_2O) and SiO_2 fractionation can be combined in one column, thereby avoiding an intermediate concentration step.

Although the sulphur-removal properties of method C3 are somewhat difficult to control, the rapidity of the C3–D3 methods combination is very attractive. The use of copper for sulphur removal is a good alternative, but not superior (Andersson and Holwit, 1994).

Both GPC and saponification combine the removal of sulphur with a primary clean-up that makes the use of Al_2O_3 (5–10 % H_2O) optional, as indicated by a dotted line in Figure 2.

Wet sediment is extracted by mixing or Soxhlet, as shown in Figure 3. Sufficient extraction time is very important and the extraction is always performed in multiple steps. The first extraction is necessary to remove the water, after which the extraction is continued with organic solvents. The combined extracts contain sufficient polar solvent for a direct addition of Na_2SO_3 to remove the sulphur (method C2) before water is added and the CBs are extracted to hexane. After concentration, the clean-up and fractionation steps are the same as in the procedure for dried sediments.

Figure 2. Scheme of methods for the isolation and clean-up of CBs from dried sediments prior to gas chromatographic analysis. Codes in the figure refer to corresponding codes in the text. Dotted borders indicate optional steps.

Figure 3. Scheme of methods for the isolation and clean-up of CBs from wet sediments prior to gas chromatographic analysis. Codes in the figure refer to corresponding codes in the text. Dotted borders indicate optional steps.
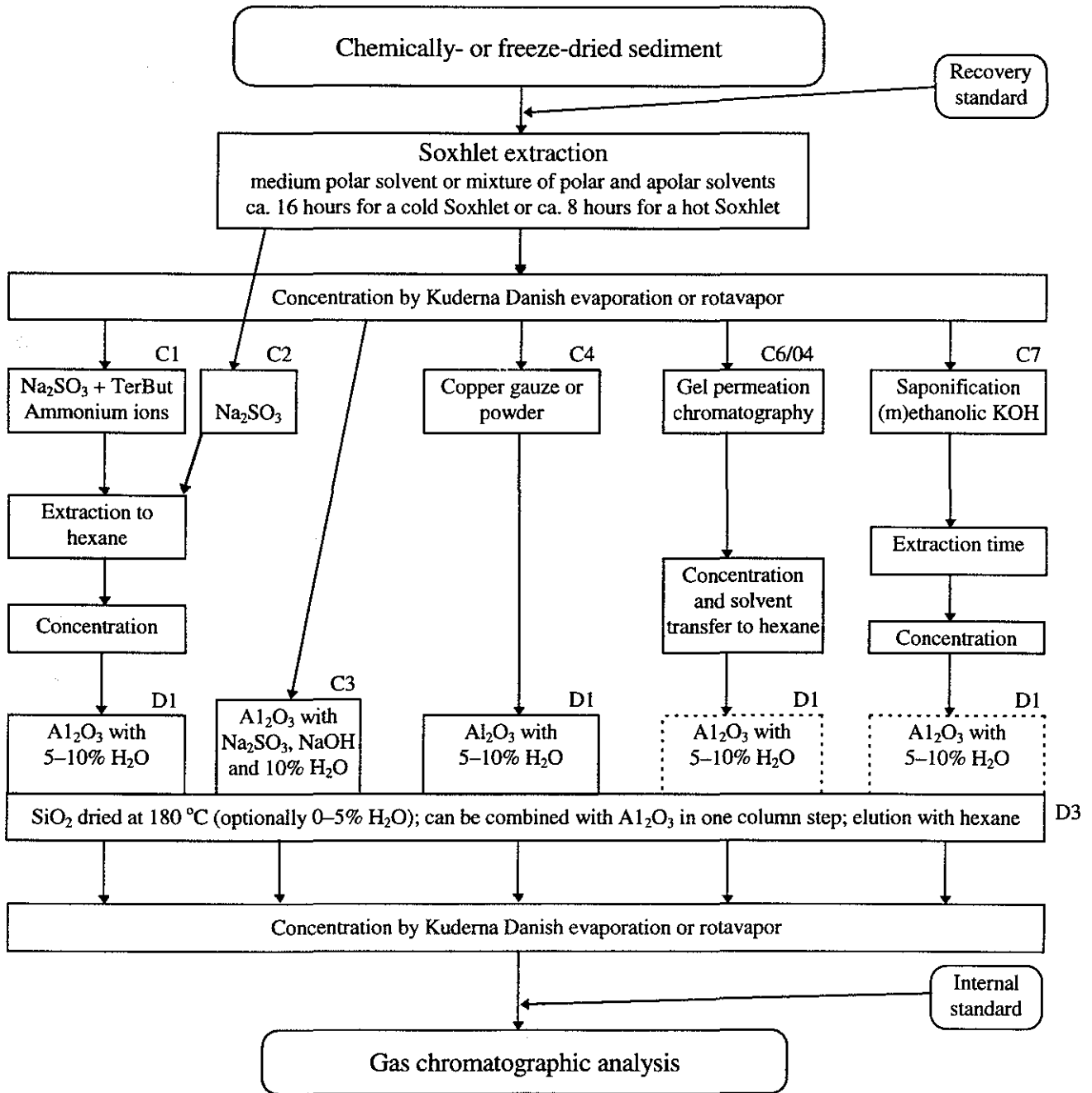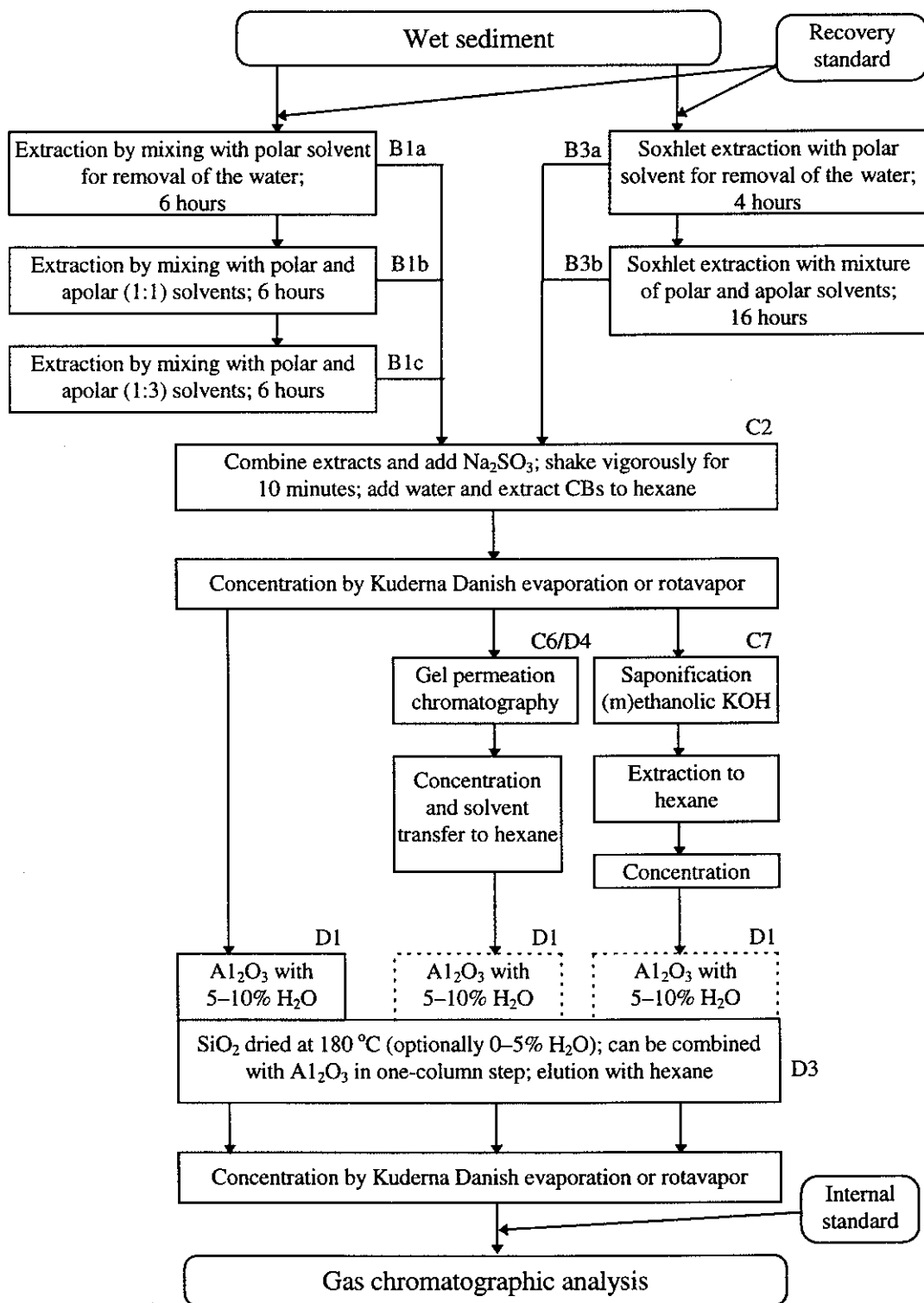
Wet sediment

Recovery standard

Extraction by mixing with polar solvent for removal of the water; 6 hours — B1a

B3a — Soxhlet extraction with polar solvent for removal of the water; 4 hours

Extraction by mixing with polar and apolar (1:1) solvents; 6 hours — B1b

B3b — Soxhlet extraction with mixture of polar and apolar solvents; 16 hours

Extraction by mixing with polar and apolar (1:3) solvents; 6 hours — B1c

C2 — Combine extracts and add Na_2SO_3; shake vigorously for 10 minutes; add water and extract CBs to hexane

Concentration by Kuderna Danish evaporation or rotavapor

C6/D4 — Gel permeation chromatography

C7 — Saponification (m)ethanolic KOH

Concentration and solvent transfer to hexane

Extraction to hexane

Concentration

D1 — Al_2O_3 with 5–10% H_2O

D1 — Al_2O_3 with 5–10% H_2O

D1 — Al_2O_3 with 5–10% H_2O

SiO_2 dried at 180 °C (optionally 0–5% H_2O); can be combined with Al_2O_3 in one-column step; elution with hexane — D3

Concentration by Kuderna Danish evaporation or rotavapor

Internal standard

Gas chromatographic analysis

9 GAS CHROMATOGRAPHY

9.1 General Considerations

Because of the large number of CB congeners (a total of 209) (Ballschmiter and Zell, 1980), high resolution capillary GC is the method of choice for CB determination. Due to differences between the CB patterns in sediments and in technical PCB mixtures such as Aroclor, Clophen, etc., total PCB determination by packed column GC leads to inaccurate results (Musial and Uthe, 1983; Alford-Stevens *et al.*, 1985). However, also when capillary GC is applied, the use of technical mixtures for calibration can result in a severe bias for the total CB content (Eganhouse and Gossett, 1991). Therefore, analysis of CBs in sediments should focus on the determination of selected individual congeners.

As it is currently impossible to separate all 209 CBs, even by capillary GC, it is recommended that two columns are used for analysis. They should be selected in such a way that they provide additional information due to their differing selectivity: CBs that coelute on one column should be separated on the other. The extract should be injected once for each column, or injected onto parallel coupled columns which are connected to one injector (Storr-Hansen, 1991). In the latter case, the length and diameter of both columns should be identical, or the extract will not be divided equally between the two columns.

For very small column diameters, 0.15 mm or less, difficulties may be encountered because of the very high pressures that are required, as leakage at the connectors may result.

Instead of parallel coupling of the columns, they may also be connected serially (Larsen *et al.*, 1992a). Drawbacks of this technique may be that the analysis time is relatively long and it allows no confirmation as only one chromatogram is obtained. Good selection of the columns may provide a very good resolution for specific pairs of CBs, but separation of all congeners is still not possible.

For unambiguous determination of CBs, multidimensional GC (MDGC) is the preferred method (Guenther *et al.*, 1989; de Boer and Dao, 1991a). In MDGC a heart-cut of the chromatogram of the first column is transferred to a second column, which is installed in a separate oven. If the proper selection of columns is made, all CBs can in principle be separated. This technique is especially valuable for specific separations (de Boer and Dao, 1991b), but still needs basic investigation before routine application is possible. MDGC systems with two columns in one GC oven are, in general, less selective. Also, the use of wide-bore columns (Schulz *et al.*, 1989) in MDGC should be avoided, because too much of the separation efficiency is lost.

9.2 Column Dimensions

Several interlaboratory studies have demonstrated that the minimum column dimensions for the determination of CBs should be the following:

> length: minimum 50 m; internal diameter: maximum 0.25 mm.

More resolution can be obtained by reducing the internal diameter to 0.20 mm or less (de Boer and Dao, 1989). A practical barrier is found around 0.15 mm because the carrier gas pressure then rises to values greater than 500 kPa, which are not compatible with normal GC equipment.

The film thickness should be between 0.2 and 0.4 mm (Ettre, 1985; Seferovic *et al.*, 1986). A thinner film results in a faster chromatogram, but induces a loss in resolution especially in the early part of the chromatogram. Thicker films result in excessive retention times, whilst the separation is not improved.

9.3   Stationary Phases

The retention times of all CB congeners have been determined for only one stationary phase, SE-54 (95 % methyl-, 5 % phenyl-siloxane) (Mullin *et al.*, 1984). Nevertheless, for a wide range of other stationary phases information on the retention times of most CBs is available (Larsen *et al.*, 1992a, 1992b, 1992c; de Boer *et al.*, 1992a). The influence of coelution of CB congeners in several marine reference materials was investigated by Schantz *et al.* (1993). An overview of possible coelutions of CBs on a few frequently used stationary phases is given in Table 1. The use of more polar phases is sometimes limited, as their maximum temperature is not as high as for apolar, chemically bonded, phases.

Stationary phases that separate CBs on the basis of molecule size rather than on polarity, such as the liquid crystal phase SB-Smectic (Swerev and Ballschmiter, 1987; Guenther *et al.*, 1989; Larsen *et al.*, 1992c), offer a CB chromatogram which is completely different from those obtained using the more common phases. Distinct disadvantages of these liquid phases are, however, the high level of bleed, low maximum temperatures, and reaction with the hydrogen carrier gas.

Table 1. Possible coelution of CBs on the most common stationary phases.

Congener	SE-54/CP-Sil 8	OV-1701/CP-Sil 19	SP-2330/CP-Sil 88
CB28	CB31	CB31	
CB31	CB28	CB28	
CB52	CB73	CB49	
CB101	CB84 CB90		CB55
CB105	CB132	CB141	CB129 CB174
CB118			CB81 CB123 CB140
CB138	CB160 CB163	CB158 CB160 CB163 CB176	
CB153	CB105 CB132		CB110
CB156	CB171 CB202		
CB180		CB157 CB197	CB197

9.4 Carrier Gas

Hydrogen should preferably be used as the GC carrier gas. For safety reasons, precautions should be taken to prevent explosions due to leakage of hydrogen. Alternatively, helium may be used, but the use of hydrogen is preferred because the H/u-curve is not as steep and, therefore, a better resolution is obtained at a higher gas velocity. When using columns with very small internal diameters (e.g., 0.15 mm), as recommended above, the use of hydrogen is essential.

The linear gas velocity, measured by determining the retention time of the solvent peak or another unretained component, 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}.

9.5 Injection Techniques

As for all other parameters, optimization is essential prior to the use of an injection technique. The two systems commonly used are splitless and on-column injection. Other techniques, such as temperature-programmed or pressure-programmed injection, may have additional advantages, but should also be thoroughly optimized before use. Split injection cannot be used because strong discrimination effects occur.

In splitless injection, the split flow is stopped during injection and continued after the sample is transferred to the column. The volume of the liner should be large enough to contain the gas volume of the evaporated injected solvent quantity. At a temperature of 270 °C and a pressure of 40 psi, 1 ml iso-octane represents approximately 100 ml of vapour volume. However, a liner must have a larger volume (0.2–0.5 ml) to absorb the expansion which occurs after injection (Grob, 1994). When the liner is too small, memory effects can occur due to contamination of the gas tubing attached to the injector (Grob, 1994). This can be checked by injecting iso-octane after analysis of a CB standard. Smaller injection volumes are preferred, but this of course increases the detection limit. 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 altogether.

The use of an autosampler is a prerequisite for obtaining an acceptable reproducibility. In addition, the use of a light packing of (silylated) glass wool in the liner improves the response and reproducibility of the injection (Grob and Neukom, 1984), but some organochlorine pesticides such as DDT may be degraded when this technique is applied. The positioning of the column in the injector is important and the instructions of the GC manufacturer should be followed. The splitless time should be optimized to avoid discrimination. This can be done by injecting a solution containing an early-eluting and a late-eluting CB, using different splitless times. Starting with a splitless period of 0.5 minutes, the peak height of the late-eluting compound will presumably increase relative to that of the first compound. The optimum is found at the time when the peak height stabilizes. The split ratio is normally set at 1:25 and is not really critical. The septum purge, normally 2 ml min^{-1}, should be stopped during injection until the splitter is opened. The temperature of the injector should preferably be about 10 °C above the maximum temperature of the oven programme, but should not exceed the maximum temperature permitted for the column and for the septum used. A regular replacement of the septum is necessary in order to prevent small particles of the septum from entering the liner.

For on-column injection, the optimum temperature programme of the oven and the optimum initial temperature should first be selected. Due to the variety of on-column injectors, a detailed optimization procedure cannot be given. Often more parameters are important; it is

recommended to apply a simplex procedure. More information on optimization of on-column parameters may be obtained from Snell *et al.* (1987).

9.6   Temperature Programming

Next to the choice of GC columns with appropriate dimensions, it is of utmost importance to select a suitable temperature programme for the GC oven. Many analysts tend to save some time on chromatographic analysis. Given the time used for each sample until then (sampling, sieving, drying, extraction and clean-up), this may well be inefficient. For a sufficient separation of the CB congeners, an analysis time of 60–120 minutes is inevitable.

An example of how an optimal balanced temperature programme can be developed is given here. First, an injection is made utilizing a linear temperature programme at a rate of $1\ ^{\circ}C\ min^{-1}$ starting directly after injection. Then the programme rate is adjusted so that 40–45 minutes elapse between the elution of CB28 and CB180 (for 50 m x 0.2 mm columns). From the chromatogram thus obtained, the temperature at the time 15 minutes before the elution of CB28 is taken and the programme rate from injection to this temperature is set at $30\ ^{\circ}C\ min^{-1}$. If the resolution is still insufficient for a group of compounds, an isothermic period or a temporary lower programme rate can be inserted starting at a temperature 10–20 °C lower than their elution temperature. After elution of the last compound of interest, a high programme rate can be applied to advance to the maximum temperature followed by a hold for 15–20 minutes to promote a rapid cleaning of the column. For accurate analysis and constant retention times, it is important that, in addition to a reproducible temperature programme, the equilibration time until the next injection is also constant.

9.7   Detection

The most frequently used detector for CB analysis is the electron capture detector (ECD). Most modern ECDs consist of a radioactive ^{63}Ni foil with an activity of 370 MBq. The advantage of this detector is its sensitivity to halogenated compounds. Detection limits of 0.1 pg may be obtained for higher chlorinated CBs. ECD-response factors for CBs are given by Mullin *et al.* (1984). Injection of chlorinated or oxygen-containing solvents should be avoided. The temperature of the ECD should be optimized. A range is normally given by the manufacturer; optimum temperatures generally vary between 280 °C and 350 °C. A higher temperature, especially in combination with hydrogen as a carrier gas, is preferred as this will keep the Ni-foil clean. When the chromatogram contains large negative peaks following positive (analyte) peaks, it is necessary to clean the detector. For safety reasons, the cleaning of the radioactive source should be carried out by the manufacturer.

The use of mass spectrometry (MS) as the detection technique for CB analysis is becoming more common. This is mainly due to the availability of more sensitive and easier to handle mass spectrometers. Negative chemical ionization (NCI), also called electron capture negative ion (ECNI) mass spectrometry, is extremely sensitive for penta- to deca-CBs (approximately ten-fold better than ECD). Unfortunately, CBs with fewer than four chlorine atoms cannot be measured with this technique, because the sensitivity to these compounds is very low. 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 that for ECD.

9.8   Quantification

One drawback of the ECD is its poor linearity. Therefore, the injection of different dilutions of CB standard solutions during a series of samples (multilevel calibration) is strongly recommended. Alternatively, the linear range of the ECD can be determined (Wells *et al.*, 1988; de Boer *et al.*, 1992b), and samples should then be diluted or concentrated to fit within this range. As the different CB congeners are present in the sample in diverse concentrations, more than one injection per sample may therefore be necessary. Because of the generally low CB concentrations in sediments, the extracts are often already maximally concentrated, which does not allow for additional manipulation. Therefore, multilevel calibration is preferred for sediment samples.

When the chromatogram is processed by using automated integrators, the baseline is not always set unambiguously, and always needs visual inspection. A small error in the baseline has a much greater impact on measured peak area than on measured peak height. Furthermore, peak areas suffer more from possible errors in case of unresolved peaks than do peak heights. Therefore, the use of peak heights is recommended for quantification (de Boer *et al.*, 1992b).

When using GC/ECD, the system should be equilibrated by injecting at least one sample prior to a series of samples and standards. Normally the injector, column, and detector need some time to stabilize and in the first injection a lower response is normally found. This first sample is not quantified, as its purpose is to deactivate adsorption sites within the GC system. Therefore, the injection of a standard should always be preceded by injection of a sample. In addition, standards used for the multilevel calibration should be distributed regularly over the sample series so matrix- and non-matrix-containing injections alternate.

A sample series should consist of:

1)   a procedural blank;

2)   an internal reference material;

3)   four or five standards to enable a proper multilevel calibration;

4)   one standard that has been treated similarly to the samples (recovery determination);

5)   samples, one of which is in duplicate (preferably from a former or later series).

10   QUALITY ASSURANCE

A number of measurements should be taken to ensure a sufficient quality of the analysis. Four main areas can be identified: calibrant (and calibration), system performance, control of extraction and clean-up, and control of long-term stability.

10.1   Calibrants and Calibration

CB determinations should always be carried out using calibration solutions prepared from crystalline CBs. Certified CBs should preferably be used. A number of certified crystalline CBs can be obtained from, e.g., the EC Measurements, Standards, and Testing Programme (Brussels). Utmost attention should be paid to the preparation of calibration solutions, and two independent solutions should always be prepared simultaneously to allow a cross-check to be made. Detailed information on the precautions to be taken when weighing and diluting

calibration solutions of CBs can be obtained from Wells *et al.* (1992) and Topping *et al.* (1992). 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.

One or more internal standards, added in a fixed volume or weight to all standards and samples, should always be used to control the final concentration step and the GC analysis. The ideal internal standard is a CB which is not found in the samples and does not coelute with other CBs, for example, CBs 29, 112, 155, 198 (numbering according to Ballschmiter and Zell (1980)). Alternatively, 1,2,3,4-tetrachloronaphthalene (TCN) or homologues of dichloroalkylbenzylether (Wells *et al.*, 1985) can be used. For an internal check on the recovery throughout the whole procedure, one CB congener not contained in technical formulations should be added to each sample (see also below).

10.2   System Performance Control Points

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 can be recognized by the presence of a higher background signal, together with a reduced signal-to-noise ratio (see also Section 9.7, above).

The presence of adsorptive sites in the system can be recognized when a higher response factor is obtained for compounds in samples than is seen in standards, or when convex calibration lines are obtained. The signal obtained from octachloronaphthalene seems to represent a useful indicator to identify a deteriorating GC column.

10.3   Control of Extraction and Clean-up

For the control of extraction and clean-up, it is recommended that a standard solution be passed through the entire procedure, from the initial extraction to the final determination. This standard is used to determine the recovery for the sample series. An internal standard should also be added to each sample to check for losses. This recovery standard should not be used to correct any data, but only as a control on the whole procedure (additions are indicated in Figures 2 and 3). If major losses have occurred, the results obtained should not be reported, and the determination should be repeated after corrective actions have been taken.

CB29 is suggested as the recovery standard because, owing to its high volatility, losses due to evaporation are easily detected. CB29 elutes relatively late from alumina and silica columns. Therefore, losses due to clean-up may also be detected by use of this CB. Small peaks that may be present in the chromatogram at the retention time of CB29 do not hinder the use of this CB because the recovery standard only controls major errors in extraction or clean-up.

10.4   Long-term Stability

One internal reference sample should be included in each series of samples. This sample should be taken from a large, homogeneous batch of sediment that can serve as an internal reference material over a long period. A quality control chart should be recorded (de Boer and van der Meer, 1998b). If the warning limits are exceeded, the method used should be checked for possible errors. When the alarm limits are exceeded, the results obtained should not be reported. A certified reference material should be analysed 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.

11 CONCLUDING REMARKS

As stated above, it is neither possible nor desirable to recommend a definitive method for the analysis of CBs in sediments. It is the task of the analyst to demonstrate that the procedure applied is well calibrated and under control, i.e., that no significant losses or contamination occur during drying, extraction, clean-up, or any other sample handling procedure. Appropriate quality control measures should be taken to control the procedures. As intercomparison exercises are usually performed with oven-dried or freeze-dried sediments, for reasons of stability and homogeneity, errors made in the laboratory during the drying step can easily be overlooked. Therefore, special attention should be paid to the drying of sediments.

12 ACKNOWLEDGEMENT

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