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Review

Critical review of the analysis of non- and mono-*ortho*-chlorobiphenyls

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Abstract

The various methods for the determination of non-*ortho* and mono-*ortho*-chlorobiphenyls are critically reviewed. Matrix, sample preparation, extraction, clean-up, fractionation and group separation methods, chromatographic separation (gas, liquid and supercritical fluid chromatography), as well as the various detection methods, multi-residue methods, quality control and method validation are discussed. For each topic, an overview is given of the current status of the field and recommendations for the most appropriate analytical approach are presented.

Contents

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1. Introduction

The diverse methods used to determine specific chlorinated biphenyls (CBs) in environmental samples have been well documented over the last few years [1–5]. For many congeners, an unequivocal separation from other compounds has been achieved and it has been possible to validate the method with appropriate reference materials [6–8]. However, the determination of many of the congeners occurring in environmental samples at ultra-trace levels (μg/kg or lower) still remains a problem. Attention should be focused on the valid measurement of these congeners since most have a specific toxicity. These are the planar CBs 77, 126 and 169 [9] as well as mono- or di-*ortho* substituted CBs 105, 118, 128, 156 and 157. The move toward the determination of the specific planar or toxic CBs has, however, tended to produce a number of publications which focus on these compounds alone [1,10,11]. This is an unfortunate and probably retrograde step since a balanced data set of a wider selection of CBs which have different biological activity [12] would be intrinsically more useful with very little additional effort on the part of the analyst.

Although there have been a small number of intercomparison exercises for the analysis of these compounds [13–17], there has been no co-oporate study to validate the measurement of toxic CBs in environmental matrices and there are no certified reference materials currently available to support this work.

This review, therefore, specifically focuses on the measurement of the toxic non-*ortho*- and mono-*ortho*-biphenyls in addition to the variety of other congeners measured for research and monitoring purposes.

It critically assesses the various methods presented in the literature in order to select those that will provide a more accurate and precise determination of these congeners.

Each chapter discusses these methods which are in current use or which have recently been developed for each stage of the analysis from extraction through to the final determination. A critical assessment is made in each section and recommendations are made on the most appropriate approach to take to give the current state of the art.

2. Matrix

2.1. Industrial formulations

Industrial formulations of polychlorinated biphenyls are the primary source of these environmental contaminants. The CB patterns found in environmental matrices have been compared to the patterns in technical mixtures like Aroclor, Kanechlor or Clophen. These mixtures are synthesised to a nominal degree of chlorination and generally show specific congener patterns for each formulation. However, the precise ratio of CBs will vary from batch to batch and between manufacturer for the same nominal degree of chlorination.

The practice of comparing CB patterns in environmental samples with those in technical mixtures can be misleading since mixtures emanating from different sources are mixed by diffusion, evaporation, adsorption onto solids at differing rates. Many congeners are metabolised while others bioconcentrate in lipophilic material. Therefore the final pattern in the environmental sample is often highly modified and may not resemble the original formulation or mixture of formulations.

Technical mixtures do not have a complex matrix to effect the analysis separation. Thus, the problems are confined to resolving co-eluting congeners [18].

Mullin et al. [19] synthesised the 209 congeners and studied their retention times on an SE-54 column, showing a number of co-eluting pairs of congeners. The studies of de Boer et al. [18] [51 congeners, using seven different gas chromatographic (GC) columns] and Larsen et al. [20] (140 congeners, five different GC columns) show the retention of key CBs on the more commonly used capillary gas chromatographic column phases. From these studies it is clear that another separation, in additional to a single-column GC either by multidimensional gas chro-

matography (MDGC) or pre-separation on other column systems, is necessary.

2.1.1. MDGC approach

Bowadt and Larsen [21] coupled two columns (CPSil-8, HT-5) in series to determine 86 CBs in technical mixtures (instead of 64 with single-column chromatography on CPSil-8).

Schulz et al. [22] use an SE-54 column as a first column which is coupled via a heart-cut onto an OV-210 column. Using this two-dimensional GC system, they separated all the congeners in Aroclor mixtures that were present above 0.05% w/w ($\equiv 50\ \mu g\ kg^{-1}$).

De Boer et al. [23] analysed Aroclor mixtures in order to determine specifically mono-*ortho* congeners using MDGC with an HP-Ultra 2 column and an HP-FFAP column. The different fractions of the first chromatogram were transferred by heart-cut to the second column in a separate oven with a separate temperature programme. The detection limit of this method was $0.5\ \mu g\ kg^{-1}$.

2.1.2. Liquid chromatography (LC) or high-performance liquid chromatography (HPLC)

The pre-separation on other column systems, e.g. HPLC, prior to GC is widely used in CB analysis. Jensen and Sundstrom [24] used charcoal pre-separation (Darco G-60 activated carbon) and obtained a level of determination of 50 ng/ml. De Boer et al. [25] used a porous graphitic carbon (PGC) column to separate the planar CBs in Aroclor 1254 prior to GC. The concentrations found in the Aroclor were CB 77 176.3 μg/g, CB 126 40.72 μg/g and CB 169 0.738 μg/g. Wells and Echarri [26,27] use a pyrenyl-silica column to successfully separate a mixture of 51 CBs and Halowax 1014.

In summary, MDGC is a very useful technique to separate co-eluting congeners. However it is not appropriate to use this technique to separate CBs which have concentrations that differ by a factor of more than 600 [28]. Thus the isolation of the non-*ortho*-CBs in Aroclors prior to measurement becomes critical. For advantages and drawbacks of these two approaches see also Sections 6, 7.2 and 7.5. In Tables 1 and 2 an overview is given of the results found so far in the most common technical products.

2.2. Environmental matrices

In contrast to the technical mixtures, the environmental matrices contain many compounds other than CBs so the sample preparation requires a selective extraction and additional clean-up prior to separation and detection. An overview of the range of matrices analysed for planar CBs is given in Tables 3, 4 and 5.

The concentration of CBs in environmental matrices is highly dependent upon the lipid content of the material. Samples which are low in lipids, e.g. sea water or sandy sediments, usually contain much lower concentrations of CBs compared with lipid-rich tissues such as marine mammal blubber [29]. In terms of sample size this means that the amount of material normally required for analysis is a function of the lipid content. De Voogt et al. [2] have reviewed the quantities of sample required both for analysis of the major CBs and for the determination of the non-*ortho*-CBs. The former ones being analysed routinely, the latter require a specific methodology because they occur at ~100–1000 times lower concentration.

Almost all sample extracts require some form of clean-up and group separation prior to the final determination. Methods which are classified as "quick", "rapid", or "direct" are usually only applicable to samples containing very high concentrations of CBs at a specific dump, waste or localised emission site. Where the lipid weight in the extract is particularly high, e.g. liver or fat extracts, then a specific step to remove the lipid should be included.

The initial sample intake mass can be modified in proportion to the detection limit of the final measurement. However, in most cases the amount of the non-*ortho*-CBs are often quite close to the limit of determination. The sample mass can be reduced in line with the final sample volume. For example 1000 g sediment can be extracted to give a 1-ml final volume from which 1 μl is injected. By reducing the final volume to 10 μl the intake mass can also be reduced to 10 g. This simple proportionation is often over-

Table 1
Planar CBs in technical mixtures

Mixture	Approach	CB-type	Concentration	Reference
Clophen A30	MDGC	77,	3900 μg/g	Schulz et al. [22]
		126, 169	ND	
Clophen A40	MDGC	77	6600 μg/g	Schulz et al. [22]
		126, 169	N	
Clophen A50	MDGC	126	800 μg/g	Schulz et al. [22]
		77, 169	ND	
Clophen A60	MDGC	126	4600 μg/g	Schulz et al. [22]
		77, 169	ND	
Aroclor 1242	MDGC	77	4500 μg/g	Schulz et al. [22]
		126, 169	ND	
Aroclor 1260	MDGC	169	500 μg/g	Schulz et al. [22]
		126, 77	ND	
or 1016 + 1254	MDGC	77,126,169	ND	Schulz et al. [22]
Aroclor 1254	PGC fractionation prior to GC analysis (elution with gradient solvent mixtures)	77	176.3 μg/g	de Boer et al. [25]
		126	40.72 μg/g	
		169	0.738 μg/g	
Aroclor 1242	PGC fractionation prior to GC analysis (elution with hexane only)	77	2500 μg/g	Al-Haddad [226]
		126	20 μg/g	
		169	ND	
Aroclor 1248		77	3400 μg/g	
		126	4.5 μg/g	
		169	ND	
Aroclor 1254		77	240 μg/g	
		126	80 μg/g	
		169	ND	
Aroclor 1260		77,126,169	ND	

ND = not detected.

looked in many laboratories, but is critical when the sample mass is limited, e.g. size of fish liver, or when an improvement in the detection limit is required. However, care must be taken with such small final volumes. Internal standards are mandatory to account for transfer losses and actual volumes. The purity of the solvent must be very high and the contamination from the laboratory surroundings and practices kept to an absolute minimum. Another way to lower the detection limit is to inject a larger volume of sample into the GC, e.g. by programmed temperature vaporising (PTV) [30]. These comments are generally applicable to the determination of CBs, but more so when the techniques are being used at their limit.

Fig. 1 shows a flow diagram of analyses dependent on the matrix.

Sections 4–8 of this review assess critically the separate steps in the scheme and section 11 gives recommendations on the various methods appropriate to different samples.

3. Sample pretreatment and recovery studies

3.1. Sample storage

Samples of sediment soil and tissue collected in the field are usually preserved by freezing immediately, either in the field, on board ship or at the laboratory. Rapid preservation is vital if

Table 2
Mono-*ortho* CBs in Aroclors (the values are given in %, w/w) determined with MDGC

CB	Aroclor				Laboratory[a]
	1242	1248	1254	1260	
74	2.3	4.1	1.3	0.09	a
	2.1	4.0	1.1	0.02	b
	2.17	–[b]	0.78	<0.05	c
114	0.04	0.13	0.19	0.03	a
	0.04	0.14	0.25	<0.01	b
	<0.05	–[b]	<0.05	<0.05	c
123	<0.02	0.09	0.14	<0.03	a
	0.04	0.09	0.22	<0.01	b
	<0.04	–[b]	0.81	<0.05	c
157	<0.01	<0.01	0.24	0.08	a
	<0.01	0.02	0.30	0.15	b
	<0.05	–[b]	<0.05	0.14	c
167	<0.03	0.03	0.45	0.18	a
	<0.01	0.03	0.39	0.13	b
	<0.05	–[b]	0.21	0.26	c
189	0.0001	0.0003	0.04	0.14	a
	<0.01	<0.01	0.04	0.10	b
	<0.05	–[b]	<0.05	0.11	c

[a] (a) DLO, Netherlands Institute for Fisheries Research [23]; (b) CEC Joint Research Center, Ispra [125]; (c) DMC, Institute for Marine Research, Kiel [22].
[b] Laboratory c did not measure Aroclor 1248.

the integrity of the sample is to be maintained. This is particularly important for samples where biological measurements such as enzyme activities are to be determined [12,31]. Borlakoglu et al. [31,32] froze parts of freshly killed pigeons directly after sectioning into small pieces to −20°C. Sediment cores should be sectioned and each sub-sample individually frozen in liquid nitrogen. Some core samplers allow the whole core to be frozen in situ prior to sectioning. This technique is preferable, if these facilities are available, since it allows the top interstitial fractions to be handled more easily.

Table 3
Matrices (inorganic) that have been analysed for non-*ortho*-PCBs

Inorganic matrices	Reference
Sediment	[182], [210], [76], [119], [227], [228]
Fly ash	[199], [223], [231], [232], [233], [234]
Soil	[111], [235], [236]
Water	[67], [237], [38]

Wherever possible the fish or animal should be dissected immediately and the individual tissue frozen in liquid nitrogen, rather than using the normal deep freeze which may take some hours to preserve the material [33]. The tissue should be stored in individual packs of approximately the size required for analysis to minimise subsampling thawed material. Refreezing thawed material for subsequent rethawing and analysis will cause degeneration of the tissue and compromise the analysis. Sediments and soils can be treated in different ways prior to extraction depending on the purpose of the programme [34]. Sediments are more conveniently stored as dried powders.

Most trace organic contaminants are associated with the organic fraction of the sediment, since they partition into the lipids and waxes on the sediment surface. The large proportion of the total organic carbon (TOC) is usually associ-

Table 4
Matrices (organic) analysed for planar PCBs

Matrice	Reference
Pigments	[238], [239]
Mineral oil	[105], [240], [241], [242]
Sewage sludge	[111], [229], [227], [55], [230]
Plants	[243]
Food	[9], [244], [57], [245], [230]
Faeces and urine	[96], [246]
Avian tissues	[31], [247], [32]
Eggs	[248], [249], [97]
Turtles	[250]
Fish and shellfish	[186], [114], [108], [251], [252], [253], [254], [47], [255], [215]
Fish oil	[256], [257]
Mammalian	[181], [75], [100], [258], [259], [260], [261], [262]
Mustelids	[206]
Horse fat	[101]
Bovine milk	[36], [263], [44]
Human blood and serum	[264], [265], [266], [267], [268], [42], [73], [269], [270]
Human milk	[53], [271], [36], [272], [135], [37], [273], [274], [275], [276], [277], [278]
Human adipose tissue	[53], [111], [266], [24], [279], [280]

ated with finer particles and an arbitrary value of 63 μm has been selected to isolate the organic fraction of the sediment [35]. When this fraction is required for a separate analysis it is advisable to wet sieve the sample, since dried sediments must be re-ground to break up the agglomerates. Re-grinding does not produce the original particle size distribution of the sediment or soil.

The sieved samples which are to be analysed for the less volatile components can be freeze dried or air dried at ca 35–40°C. The resultant sediment brick will require gentle grinding to obtain a free flowing powder.

Milk samples are most often directly freeze-dried before storage [36] or first frozen and only freeze-dried 48 h before analysis [37].

Water samples are best extracted within the same day. However, this is often impractical and small volumes, ca. 1–2 l, can be frozen. Plastic containers are not advised since CBs can adsorb onto the inner surfaces which subsequently need to be rinsed thoroughly with solvent. This can often be a source of contamination.

In most cases the concentration of CBs, particularly the non-*ortho*-CBs, are so low that it would be difficult to store the volume necessary to obtain a detectable mass of these congeners. Alternative methods include in situ extraction using resin columns [38] or a pressurised filtration and extraction system [39].

Table 5
Non- and mono-*ortho*-CBs in various matrices

Exhaust/flue gas	[232], [233], [65], [281], [282], [147], [231], [60], [283]
Ambient air	[58], [284], [285], [286]
Arctic air	[287], [288]
Laboratory air	[57]

3.2. Spiking and recovery studies

3.2.1. Principles of recovery measurements

Recovery measurements are one of the more difficult and ill-defined aspects of trace organic analysis. These measurements are often completed, with the minimum number of replicate determinations over a limited concentration range, to optimistically justify the use of a method. Experiments designed to obtain the efficiency of the analytical method often implicitly assume that this also includes the efficiency of extraction from the matrix. The term recovery is generally misinterpreted and many papers which

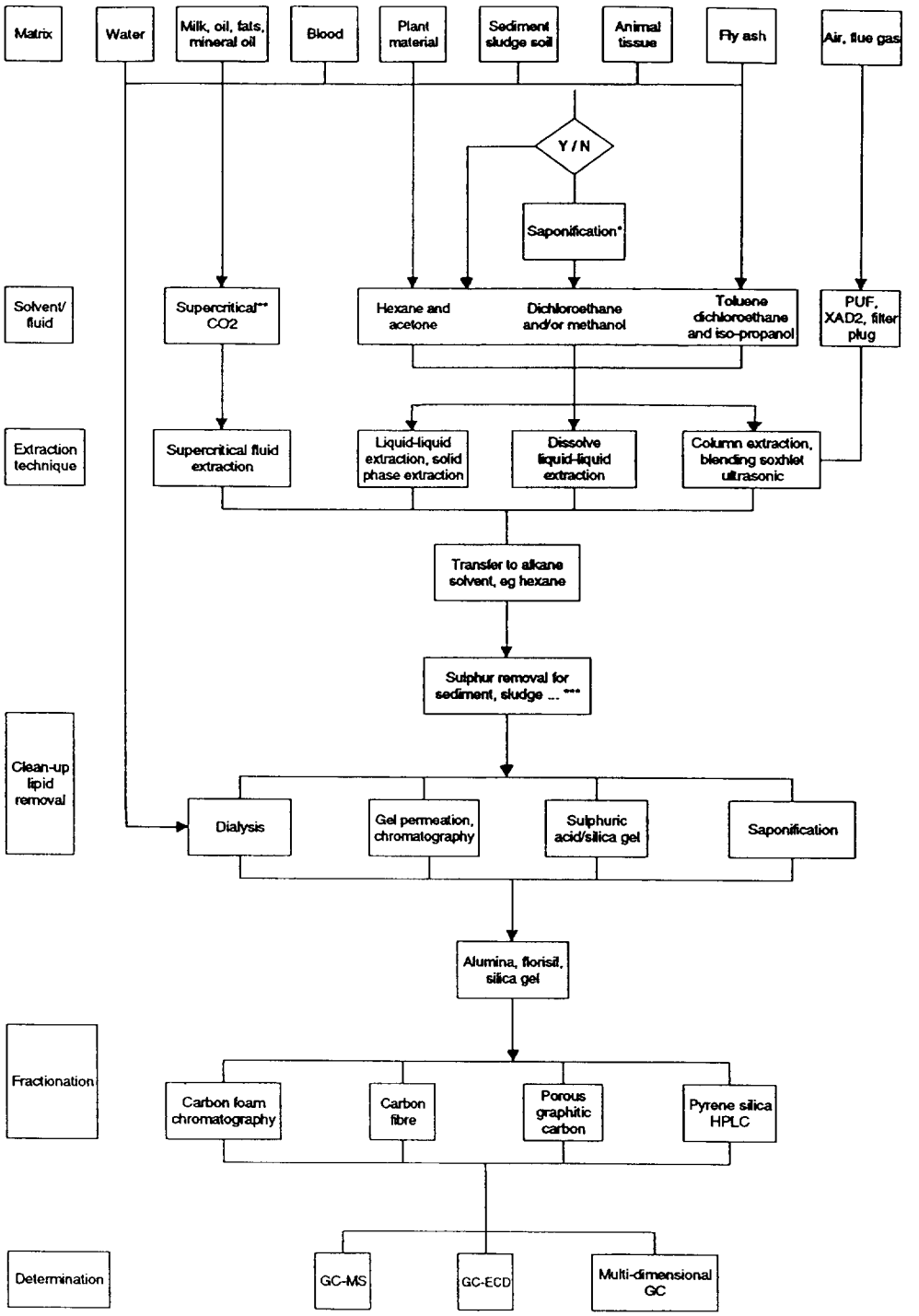

Fig. 1. Schematic flow diagram for the determination of CBs including non-*ortho* congeners in environmental matrices. (Modified according to Wells [4]). PUF = Polyurethane foam. (*) Can be made before or/and after extraction; (***) except for water; (***) can be made before or after column clean-up.

report recovery data from sediment and biota can serve to prolong this problem.

The basic requirement is to estimate how much of the determinant has been removed from the natural matrix by a given extraction technique. However, the widespread practice of simply adding a known amount of the determinant to the matrix, usually in an organic solvent, prior to extraction and subsequent analysis, does not answer this question. This type of spiked sample analysis will determine the accuracy and precision of the subsequent analytical steps, but does not necessarily measure the efficiency of extraction.

To determine the efficiency of extraction it is imperative that the contaminant is bound to the matrix in a similar configuration to that which exists in the environment. The extraction efficiency can then be measured for that determinant in a specific matrix configuration.

At present, water is the only matrix where this can be achieved in a relatively straightforward way. The determinants are added below the surface of the sample in a small ca. 1–2 ml volume of water miscible solvent. The water must be completely mixed and allowed to stand at least overnight prior to extraction to allow the contaminants to come into equilibrium with the other organic materials, particularly humic materials. The spiked water sample must be analysed in its entirety, including the inner surfaces of the container, either separately or as a single determination.

Wet sediments can be dosed with known amounts of the determinant by adding the contaminants in a small volume ca. 2 ml of water miscible solvent such as acetone, to the sample and the interstitial water. The sediment and pore water are mixed thoroughly in a closed container for not less than 24 h and then allowed to settle for a further 24 h period prior to a final mix. The sediment can be subsequently freeze-dried or drained of any excess water and extracted as a wet sediment. The filtered pore water should also be analysed. If the organics are mixed completely with the sediment and are given sufficient time to adsorb and diffuse into the sediment surface, then most lipophilic, hydrophobic determinants with an adsorption coefficient of $>10^2$ will be almost completely associated with the organic fraction in the sediment [40]. The sample should be analysed in its entirety to reduce any errors associated with the heterogeneity of the sample.

Organic contaminants can only be fully bound into biological tissue through feeding or exposure studies. The animal distributes the trace organics throughout the body, partitioning and possibly metabolising the material in the process. The dose can also contain a small fraction of a radiolabelled tracer, e.g. ^{14}C, which could be measured directly in a specific tissue after sacrificing the animal. The concentration in the tissue from the scintillation measurement of the radiolabelled material can be compared to the amount determined by organic extraction, clean-up and analysis by GC or GC–mass spectrometry (GC–MS) to determine the full recovery of the method. Such experiments are extremely costly and time consuming and can rarely be justified on the basis of determining extraction efficiency alone.

Alternative methods are less expensive, but should not be regarded as a measure of extraction efficiency per se. Where it is not possible to obtain an absolute comparison with a spiked value it is necessary to select a method which gives the highest recovery of the determinant from the natural matrix using an exhaustive or comparative technique. Unspiked tissue samples are extracted sequentially by the same method to determine the time required for the maximum removal of the contaminants. Normally, the time period for each set of conditions, e.g. solvent or temperature/pressure supercritical fluid extraction (SFE) is extended until the subsequent extraction contains none of the analyte(s). Direct comparison of different methods or the same technique with different conditions can be made. Unfortunately, prior to the emergence of SFE, only a few detailed comparisons [41] have been made.

3.2.1.1. Recovery measurements in practice

In the initial stages of any method development, recovery measurements are made to opti-

mise the conditions of extraction and sample treatment. It is difficult to be categorical about the percentage level of recovery which is regarded as acceptable for a method. Clearly, some workers accept values of <60%. Where methods give a recovery ca. <75% it is essential to determine whether this low value is dependent on the specific type of matrix structure, e.g. percentage organic carbon in the sediment or lipid in tissue. Methods with such a low recovery are also likely to have a greater variance associated with the precision of the measurement. In all cases there should be an estimate of the recovery for the batch of samples being analysed. Data on recovery are often limited to a few initial measurements (ca. 1–5) at a single or, at the most, two different levels of concentration, which is an inadequate basis to make any further corrections to the final data. Since the only purpose for making recovery measurements is to be able to apply a correction factor to the data subsequently produced, it is vital to have a good estimate of the errors associated with that recovery. Where the recovery is variable and sufficiently low to warrant the data being corrected, then this recovery measurement must be made at the time of the analysis. It is insufficient to use recovery information obtained during the initial validation of the method to correct measurements made some while afterwards.

Regular, routine sample recovery measurements[1] can be made using the method of standard addition. The matrix is spiked with the determinants in a small volume of solvent at a level which is ca. 50%, 100%, 150% and 200% above the estimated level in the sample. A number of independent replicates should be made at each level. Provided that sufficient material is available the sample can be analysed prior to spiking. In case of limited size, e.g. small fish livers, a number of samples may be pooled and homogenised for such recovery experiments.

[1] Sample recovery measurements are a measure of the efficiency of the analytical method and do not necessarily include a measure of the extraction efficiency, as explained above.

Standard addition to wet sediment should be made in a water miscible solvent, e.g. acetone or methanol. Any convenient solvent can be used to spike dry sediment. Standard addition to tissue samples can be made by first spiking a small amount of silica and allowing the solvent to evaporate. The silica is then ground with the tissue prior to extraction and left at least 2 h to equilibrate.

Following the analysis of the spiked samples the data are plotted to determine the average recovery and the confidence interval of the method (Fig. 1). Once this recovery is established then a single or duplicate recovery sample can be analysed at regular intervals to check the validity of the regression. In this way a series of data are obtained over a period of time to give a long-term estimate for the efficiency of the method.

Isotope dilution mass spectrometry (IDMS) is a more elegant method to overcome the whole problem of sample recovery [33,42,43]. The ^{13}C-labelled isotope of the determinant is added to the sample at the commencement of the analysis and the ratio of the labelled and unlabelled compounds are measured by MS. This technique eliminates the need for recovery measurements and automatically accounts for any losses in the determination. The two main limitations of this method are the cost and availability of the labelled compounds and the need to use MS or atomic emission detectors (AED) as a detector.

3.3. Keeper technique

The more volatile congeners such as CB 77 can be trapped by adding a "keeper" to the solutions during clean-up procedures to prevent loss through evaporation. Van Rhijn et al. [44] added dodecane to the extracts before clean-up.

4. Extraction methods

4.1. Liquid–liquid extraction

Gómez-Bellinchón et al. [45] made an intercomparison study between liquid–liquid extrac-

tion (LLE) and solid-phase extraction (using polyurethane foam and Amberlite XAD-2) to analyse sea water. The LLE was more effective, especially for higher volumes, as the solid adsorbants seemed to release some compounds after the extraction of a certain volume of extracted water (300 l for Amberlite and 400 l for the polyurethane foam). On the other hand, the liquid–liquid extractor has a larger size and must be controlled continuously in terms of stirring. Thus, in the case of field methods, polyurethane foam can be the adsorbent of choice.

Nam et al. [46] made a comparative study between SFE and LLE of 11 organochlorine pesticide residues and chlorobiphenyls, as Aroclor 1260, in blood and milk. The biological fluids were spiked at levels between 1 and 20 $\mu g\,kg^{-1}$, adsorbed onto Florisil and extracted with CO_2. An aliquot of the same sample was solvent extracted with hexane (whole blood) or cyclohexane–dichloromethane (1:1) (milk). The recovery of organo-chlorines (OCs) from the hexane extraction of the blood ranged between 77% and 107% and for the SFE from 72% to 91%. The recovery from the spiked milk, for the LLE, ranged between 72% and 110% and for the SFE it was between 77% and 91%. Although the recoveries were lower for the SFE the purpose of this study was to attempt to obtain the maximum recovery without the need for further clean-up.

Johansen et al. [47] extracted crab and Norheim et al. [48] polar bear tissues according to a method of Brevik [49]. In the case of the polar bear tissue, the method comprised addition of 10 ml H_2O to ca. 1 g macerated fat sample, 3 ml 0.3% NaCl to 5 g macerated liver tissue, extraction with acetone (15 ml) and *n*-hexane (10 ml) by ultrasonic disintegration and repetition of the extraction using 5 and 10 ml acetone and *n*-hexane, respectively. In the case of the crab tissue, the extraction of 10–20 g sample needed 40 ml cyclohexane, 30 ml acetone and 15 ml water for ultrasonic digestion.

Wells [50] has reviewed LLE methods for CBs in sea water. Off-line and batch extraction require large solvent volumes and tend not to exhaustively extract the congeners from the colloidal or particulate phase. Hermans et al. [51] have overcome these problems by using counter-current flow extractions. Kelly et al. [39] have developed a pressurised extraction and filtration system (PEFS) for extracting up to 30 l of sea water in situ to determine low levels of CBs (15 $pg\,l^{-1}$).

4.2. Solid-phase extraction

When tissue or sediment contain low levels of contaminants the sample mass has to be proportionally increased. Some workers [52] have homogenised large sample masses (500 g) prior to blending. However, bulk tissue is difficult to handle without large scale equipment. A simple alternative is to use a column extraction system. Smaller quantities of tissue can be ground with sodium sulphate and added to the glass column (1000×100 mm I.D.). Beck et al. [53] studied human adipose tissue, Huckins et al. [54] and Schmidt and Hesselberg [55] studied fish. Schmidt and Hesselberg present a variant of this method. They not only added Na_2SO_4, but also sea sand to improve the grinding of the material. Solvents, such as dichloromethane, were added and the tissue soaked for 30 min. The dichloromethane was slowly drained from the column and the extraction repeated. All extracted lipids and associated non-polar contaminants are removed with ca. 500 ml of solvent. Other solvents such as hexane–acetone and petroleum ether–ethyl acetate mixtures are commonly used.

Both water and air are matrices to which solid-phase adsorption allows the extraction of high volumes. Wells [50] has briefly reviewed the techniques for the extraction of sea water. Polyurethane foam is frequently used for the extraction of CBs [56–64]. Some groups have successfully used XAD-2 [61,64], although the resins are easily contaminated, primarily by monomer resin from freshly exposed surfaces. In an unusual study by Weistrand et al. [66], the laboratory air was so heavily contaminated that glass dishes were sufficient to collect the CBs in the air. Electrical waste, stored close to the laboratory, was found to be the cause of the laboratory contamination. Vreuls et al. [67] used

an on-line SPE–thermal desorption system to introduce large amounts of water into a GC. Although the system has been described as an extraction system it should be considered as a sample introduction system with enrichment capacity.

4.3. Blending and ultrasonic extraction

The simplest extraction technique for solid matrices is to blend or ultrasonicate the sample with an appropriate organic solvent at room temperature. Apart from the polarity of the solvent, the efficiency of the extraction is dependent upon the homogeneity of the matrix, and the mixing-ultrasonication-blending-soaking time. The mixture of sample and organic solvent is separated by filtration and washing with solvent.

Blending has been used for soils, sediments and milk [68,69] plants, animal tissue, fish and shellfish [70,71], but in general is preferred for biological tissues. Jensen et al. [71] and Jansson et al. [70] used an Ultra-Turrax blender and solvent extraction in a separating funnel for a wide range of polychlorinated and polybrominated contaminants in fish and animal tissue.

Storr-Hansen and Cederberg [72] have examined seal tissue and found levels down to 45 pg/g Σ (non-*ortho*-CBs). They blended the tissue with an Ultra-Turrax blender and extracted it with 3×40 ml of DCM–MeOH (2:1) per 8 g sample. Ryan et al. [73] studied blood samples from patients of the Yusho and Yu-Cheng rice oil poisonings. A polytron homogeniser was used with a mixture of ethanol–hexane–aqueous saturated $(NH_4)_2SO_4$ in the ratios 1:2:1 for a 1-min extraction. The compounds of interest were polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) and planar PCBs. PCDDs/Fs were found at levels of 1 to 5 μg/kg and the CBs 126 and 169 were at lower levels.

4.4. Acid and alkaline treatment for extraction purposes

The extraction of sediment or tissue can be significantly improved by means of reactive extraction mixtures. These involve mostly acids [74] or alkalines [75] or silica gels which are impregnated with acids or alkalines.

Kannan et al. [76,77] used ethanolic KOH for sediments. Smith et al. [74] used a reactive column where wet sediment or tissue is ground with sodium sulphate and put on a column with 20 g 40% sulphuric acid–silica gel, then with 10 g potassium silicate and lastly 5–10 g sample. With recoveries of 106, 96 and 77% for the congeners 77, 126 and 169, respectively, they detected levels of 90 pg/g (77), 11 pg/g (126) and <10 pg/g (169).

Remberger et al. [78] attempted to extract both the "free" and the "bound" fractions with acetonitrile–hexane–methyl *tert.*-butyl ether solvent mixture. However, a higher recovery (25–100%) was obtained by using methanolic potassium hydroxide. Wells et al. [8] reported the same improvement with saponification for the recovery of CBs from sewage sludge during an intercomparison exercise.

4.5. Vapour-phase extraction (Bleidner)

Schuphan et al. [79] have used the "Bleidner" vapour-phase extraction technique for the determination of organochlorine pesticides (OCPs) and CBs in lake sediment and compared the results with traditional Soxhlet extraction. The advantage of the Bleidner distillation is that it avoids the time-consuming steps of drying, conventional extraction and clean-up. The thawed sediment was mixed with distilled water and an antifoaming agent, and the aqueous phase distilled into a flask containing iso-octane, which was subsequently used to extract the distillate. Direct measurement of the OCPs and the CBs were made by capillary GC–ECD. The Soxhlet method required the sediment to be dried. The pore water was separated from the solid and extracted with *n*-hexane–toluene (9:1). The moist sediment was exhaustively dried with phosphorous pentoxide prior to Soxhlet extraction with *n*-hexane–toluene (9:1) for 20 h.

The recoveries of the CBs by the Bleidner technique declined with increasing chlorination (CB 28 98% to CB 180 43%) and were likely to be a function of the decrease in volatility of the

congeners. The method, therefore, although rapid for some volatile, non-bound hydrophobic organics is not suitable for a wide application as an extraction technique.

4.6. Small-scale extraction

The mass of sample taken for analysis is primarily dependent on four factors: (i) the amount of material available; (ii) the concentration of the determinant; (iii) the heterogeneity of the sample; and (iv) the method of analysis. Most conventional solvent extraction techniques currently consume more sample than is required, use more extraction solvent than is necessary and ultimately only analyse 1/1000 of the material prepared, e.g. 1 μl from 1 ml.

These small-scale extraction techniques [80] can be used in conjunction with "on-line" LC–GC or LC–MS to utilise the whole extract in the final determinations. This approach can significantly reduce the size of sample required and the volume of solvent used. Steinwandter [80] has applied this technique to the analysis of pesticide residues in fruit and vegetables. A small mass, e.g. 5 g of fruit, water content >70%, is macerated and extracted with acetone or acetonitrile. A third non-polar solvent, petroleum ether or dichloromethane, is added to the binary system which is then subsampled in situ for further analysis. However, small-scale extraction is generally unsuitable for most environmental matrices where the concentration of CBs demands a relatively high sample intake mass.

4.7. Soxhlet extraction

Soxhlet extraction has been used for the isolation of non-polar and semi-polar trace organics from a wide variety of sediments, soils, animal and plant tissues (see Table 6). The size of the systems can vary, but the more common configurations use between 100 and 200 ml solvent to extract between 20 and 200 g of sediment and 1 and 100 g of biological tissue[2]. Larger systems can be used, but require proportionally more solvent. It is essential to match the solvent polarity to the solute solubility and to thoroughly wet the matrix with the solvent when extraction commences. Animal and fish tissue are first

[2] The mass of biological tissue is usually less since it is ground with sodium sulphate prior to extraction, reducing the overall tissue mass which can be placed in the Soxhlet.

Table 6
Overview Soxhlet extraction

Matrix	Analytes	Solvent	Duration	Reference
Sediment	PCDD/Fs	Benzene	16 h	[82]
Sediment	PAHs, PCDD/Fs, PCBs (including planars)	Toluene	24 h	[83]
Sediment	OCDD, TCDF, CB77	DCM	Overnight	[119]
Soil/sludge	PAHs	DCM	3 h	[289]
Sediment	23 PCBs (incl CB77)	Hexane–acetone	16 h	[182]
Soil	CBs 77, 126, 169	Hexane–acetone	8–12 h	[111]
Biological tissues	CBs 77, 126, 169	Hexane–DCM	8–12 h	[111]
Fish	PCDD/Fs	DCM	%[a]	[252]
Fish	CBs 28, 52, 101, 118, 138, 153, 180	Ethyl acetate	18 h	[290]
Fish	PCBs congener-specific	*n*-Pentane, DCM, *n*-pentane–DEE[b], hexane–acetone	6 h	[41]
Blubber		Hexane	4 h	[291]

[a] EPA method 8290.
[b] DEE = diethyl ether.

macerated and then ground with sodium sulphate and silica. This grinding reduces the water content and helps to open up the tissue structure.

Non-polar solvents like *n*-hexane have been used to extract non-polar contaminants like OCPs and CBs. While these solvents are relatively efficient for removing organochlorines from fatty tissues which have a predominance of triglycerides, they are not completely extracted from low fat tissue. De Boer [41] made a comparative study of the extraction efficiency of different solvents for CBs in fatty and lean fish tissues. The comparison was made between pike perch, perch, bream, roach and eel and the solvents were *n*-pentane, *n*-pentane–diethyl ether (1:1), dichloromethane and acetone–*n*-hexane (1:9). He also compared these extractions with the saponification of the tissue with 40% potassium hydroxide in ethanol (1:1) at 90°C for 4 h prior to extraction. The main conclusions of this work were that samples should be left for a minimum of 2 h to dry completely after grinding with sodium sulphate. A longer time was unnecessary. Extraction with non-polar solvents like *n*-alkanes took considerably longer (>6 h) and were not as effective as polar solvents like dichloromethane in removing the CBs and the lipid. This was less evident for fatty (triglyceride) tissue, reflecting the relative distribution of CBs bound onto the phospholipids and the partition into the neutral lipids. A minimum of 6-h extraction with a polar solvent was recommended. Although higher recoveries were obtained by initially saponifying, van der Valk and Dao [81] found that prolongation of the hydrolysis reaction at temperatures above 70°C and >1 h resulted in a loss of CB 180. Wells and Echarri [26] also found that the highly chlorinated CBs were also dechlorinated and hydrolysed.

Sediments and soils need to be thoroughly wetted to obtain an efficient extraction. Surface tension of the solvent across the pores of a dry sediment are sufficient to prevent complete diffusion of the liquid into the micro-cavities of the sediment. Non-polar solvents do not readily wet the surface of dry sediments and are too immiscible with water to be able to penetrate the wet material. This problem can largely be overcome by (i) wetting the sediment with an electrolyte, e.g. 1% ammonium chloride overnight; and (ii) using a binary mixture such as acetone–hexane or dichloromethane which has sufficient polarity and a water solubility to wet the surface. Another possibility is to have the surface of the sediment wet enough to use the Soxhlet-Dean-Stark extractor. The principle of the method, described by Lamparski et al. [82], uses an azeotropic distillation combined to the Soxhlet. Because the surface is wet with water there is little surface tension and aromatic solvents like benzene or toluene can be used to improve the extraction efficiency for planar non-polar molecules. The azeotropic mixture is distilled and is collected in the Dean-Stark-trap which allows the water content in the sediment to be determined at the same time. Lamparski et al. [82] used benzene to determine dioxins and furans, Zebühr et al. [83] modified the procedure, using toluene, for the determination of CBs. Toluene is less carcinogenic than benzene and preferable for laboratory use. Some workers have saponified the sediment to remove the waxes and lipids prior to extraction. In some cases this technique can result in an improved recovery [8]. One disadvantage of the Soxhlet extraction is that sulphur is also extracted from sediments/soils by this method and must be removed at a later clean-up step [84].

4.8. Supercritical fluid extraction (SFE)

The attraction of SFE as an extraction technique is directly related to the unique properties of the supercritical fluid. These fluids have a low viscosity, high diffusion coefficients, low toxicity and low flammability, all of which are clearly superior to the organic solvents normally used. Carbon dioxide is the most common supercritical fluid to be used, since it is inexpensive and has a low critical temperature (31.3°C) and pressure (72.2 atm). Other less commonly used fluids include nitrous oxide, ammonia, fluoroform, methane, pentane, ethanol, sulphur hexafluoride and dichlorofluromethane. Most of these fluids

are clearly less attractive as solvents in terms of toxicity or as environmental friendly chemicals. Commercial SFE systems are available, but some workers have also made inexpensive modular systems [85].

An advantage of SFE is that extracts are much cleaner, so additional clean up of the extracts is not necessary in most cases. In the study of Nam et al. [84] sediments were extracted with SFE and Soxhlet to make a comparison between the extraction methods regarding elemental sulphur, which could interfere with CB/pesticide analysis using GC–ECD. They found that only 2% of the sulphur was present in SFE extracts. On the other hand, the Soxhlet extracts contain so much sulphur that it caused a severe problem to analyse pesticides.

Bavel et al. [86] investigated the optimisation of different SFE parameters for PCDD/PCDFS and CBs from fly ash. They investigated the effects of temperature (40, 70, 100°C), pressure (100, 300, 500 atm), time (10, 30, 50 min) and entrainer addition (toluene, propanol, methanol). They investigated the extraction efficiency of ^{13}C-labelled PCDD/PCDFs, non-*ortho* CB 77, 126, 169 and non-planar CBs 101, 153, 202 for the different conditions. The CBs were recovered, but at low efficiencies. Two factors significantly influenced the recovery of the non-*ortho* congeners. These were temperature and time of the static extraction. Low temperature and low static time increased the recovery efficiency. For the non-planar CBs 101, 153 and 202, the temperature was again important, but pressure was more influential than static time. Better recoveries were obtained at low temperature and high pressure. The polarity of the entrainer was not important for both groups of CBs. Other studies have shown that the polarity of the entrainer is important for the extraction of PCDD/PCDFs from different matrices (Onsuka and Terry [87]). In conclusion, CBs could be selectively extracted from PCDD/PCDFs and it seems that there are differences between the optimum SFE parameter settings between planar and non-planar CBs.

A recent study of Hüsers et al. [88] showed that high recoveries were found for the extraction of PCDD/PCDFs from spiked polyurethane foam with fly ash and soil using SFE. They made a comparison between Soxhlet extraction and SFE. The extraction conditions were 395 atm, 90°C, 3 h, CO_2 modified with 5% toluene as supercritical fluid. Their conclusion was that SFE had slightly lower recoveries than Soxhlet, but the major problem was that the reproducibility was unsatisfactory. This is in contrast to the results of Onuska and Terry [87] who find higher recoveries of 2,3,7,8-TCDD spiked sediment using SFE than Soxhlet extraction (SFE conditions 310 atm, 40°C, 30 min). Soxhlet extraction also showed the greatest variability in recovery. An explanation for the higher recovery is probably found in the differences in supercritical fluids and temperature used between the studies of Hüsers et al. [88] and Onuska and Terry [87]. The study of Onuska and Terry [87] showed that a combination of superfluid carbon dioxide or nitrous oxide and a mixture of 2% methanol results in the highest recoveries (100%) of tetrachlorodibenzo-*p*-dioxin from the sediment. This is in contrast to the experiments where only carbon dioxide was used which gave recoveries of 48% in 30 min. Increased extraction time did not show significant improvement.

Some improvement in the level of selectivity in SFE of biological tissue has been made by the addition of solid adsorbents to the tissue in order to bind the lipids while the contaminants are extracted. Johansen et al. [89] ground fish tissue with anhydrous sodium sulphate and basic alumina prior to extracting with CO_2 at a fluid density of 0.57 g cm^{-3}. Recoveries ranged between 70 and 86%. The extracts were cryofocused prior to reinjection by thermal desorption with little or no interference from any lipid. This method of extraction gave promising evidence of improved selectivity, but interferences from other co-extracted materials still required further separation in most samples prior to the final determination.

Due to the low level of planar CBs in some biological matrices, their analysis requires high amounts of sample (5–10 g) lipid equivalent. At present such high amounts of lipids cannot be treated successfully by the extraction with SFE.

The same problem is found for sediments. The most promising SFE studies have used extraction cells which do not contain more than a few grams of material. This limitation on sample intake mass means that portions of the sample have to be extracted sequentially to analyse the mass of material necessary to detect the trace CBs.

4.9. Critical evaluation of extraction techniques

In principle, the extraction of environmental samples takes place in two stages: (i) separation of the phases, e.g. particulates from water or water from the solid matrix; (ii) separation of the CBs from the matrix.

It is important to remember that the extraction of organic contaminants such as CBs from the matrix is both kinetically and thermodynamically controlled [90]. Simply increasing the polarity or match of the solvent to determinant, e.g. dichloromethane or toluene, may not significantly reduce the required contact time. The CBs do not reside on the outside surfaces of the matrix to be "washed" off. Cells in tissues and cavities in sediment particles must be penetrated by the solvent which must subsequently be replaced to effect a complete extraction.

Water can be treated as a single matrix or after separation of particulates [51,91]. The choice depends on (i) the analytical question; and (ii) the level of suspended solids. Where water contains more than 2–5 $mg\,l^{-1}$ it would be advantageous to filter (e.g. 0.45 μm) the suspended material and treat the two phases separately. Extraction of CBs from aqueous samples is not straightforward and a simple separation by shaking is not always adequate. Extraction is highly dependent on the amount of dissolved organic carbon (DOC). Hermans et al. [51] required a continuous LLE for several hours to exhaustively extract all the congeners. Addition of 10% methanol or isopropanol is often necessary to improve the extraction of these highly hydrophobic analytes in the presence of significant quantities of DOC. Spiking samples to test the extraction efficiency is possible, but it is necessary to allow sample and spike to equilibrate at least by mixing overnight prior to extraction [3,50,90].

Trace levels of CBs have been extracted from seawater using XAD resins [38]. This is a useful technique, but put a heavy demand on the very stringent cleaning procedure to ensure a low blank from the resin matrix.

The separation of water from the solid matrices is confined primarily to three techniques: (i) freeze drying; (ii) air drying; and (ii) grinding with a drying agent, such as anhydrous sodium sulphate.

The advantage of freeze drying is that the samples, being less bulky, are easier to handle and a smaller, more concentrated mass is produced for subsequent extraction. For sediments and soils it is necessary to start from a matrix with a known water content. Care is required to prevent cross contamination when drying samples particularly when they may have very different levels of the determinant. Meticulous cleaning to remove any particles from an earlier batch is especially necessary when drying a series of samples from different locations. The second disadvantage of freeze drying is that some of the lower chlorinated CBs can be lost by this technique. This is particularly important for other organochlorine pesticides, e.g. hexachlorocyclohexanes (HCHs), which may be determined in the same sample extract. Freeze drying biological tissue with a high lipid content, e.g. sea mammal blubber, may not serve any advantage since there is little water to remove from these materials. Even fatty fish tissue, e.g. herring or mackerel, may also be problematic as the level of oil may prevent the tissue residue from forming into a solid powder which is easy to handle. The main advantage of this method is that low fat tissue, which can also mean low contaminant levels, can be usefully bulked.

Normally air drying at ca. 35–40°C is usually most suited to larger amounts of soils and sediments. The disadvantage, as with freeze drying is that the resultant material is usually a solid cake which must be subsequently ground to a powder which will not have the same physical characteristics as the original material. Therefore

any particle size fractionation (e.g. <63 μm) must be done with the original wet sample.

The main alternative is the use of mild drying agents such as anhydrous sodium sulphate. This technique is used primarily for drying biological tissue. Silica can be added to the mix as the sample is ground to facilitate the rupture of the cell membranes. The disadvantage is that it is labour intensive, difficult to automate, and produces rather a bulky sample to extract once the sample and drying agent are mixed.

Most extraction techniques have been developed on the basis of a specific requirement. For example, column extraction, where the solvent percolates through the sample in a chromatographic style column was developed to cope with large sample masses. Provided the correct conditions of extraction rate and solvent polarity are observed most techniques have been shown to be adequate for the purpose. All extraction techniques must be validated either by spiking [50] or by exhaustive extraction [3].

Some techniques are specifically not recommended such as the vapour-phase (Bleidner) method [79]. This method has a number of inherent recovery difficulties without any real advantage. It is reported as a combined extraction and clean-up technique, however a large number of other organics also co-distil with the CBs making further clean-up and/or group separation necessary. The most well used and optimised methods are Soxhlet extraction and blending/ultrasonic mixing, with column extraction being used where the bulk of the sample cannot be handled by the other methods.

Many of the above classical methods are to be modified to reduce the use of toxic chlorinated solvents and alternative solvents or methods must be found. For the classical methods a useful option is methyl-*tert.*-butyl ether. It is as efficient as dichloromethane when used to extract CBs from low fat tissue in a Soxhlet, provided the extraction time is increased to ca. 6–8 h. (See above for comment on kinetic and thermodynamic aspects of extraction) [92].

SFE is still a developing technique which has a number of distinct advantages. The solvent used, usually CO_2 with or without modifier, is non-toxic and the extracts can contain much lower quantities of more polar co-extractants. A practical drawback of the method is its relatively high cost. The main disadvantage of the SFE CO_2 is that it is often not available in sufficient purity. In some instances additional clean-up may not be necessary, however at the ultra-trace level at which the toxic CBs occur it is almost always the case that the sample will require further treatment. However, the development of on-line clean-up with SFE followed by adsorption columns would appear to be very promising. At the moment another key limitation of SFE is the reproducibility of the technique which is less than that of Soxhlet extraction. In many instances Soxhlet has a higher recovery than SFE and when the solvent is sufficiently modified, e.g. with methanol, then the level of co-extracted lipids can be the same as that obtained from Soxhlet extraction. The key critical parameters of temperature, pressure and time of extraction, which are important in the quantitative recovery of CBs, have not been optimised for each of the key environmental matrices, the conditions being dependent upon the matrix composition, e.g. organic carbon level in sediments. The other main limitation to this method at present is that the limited sample size (2–10 g) is often too small for the measurement of the toxic CBs. Table 7 gives an overview of the characteristics of various extraction methods.

5. Clean-up methods

The chromatographic materials used to isolate the planar CBs, such as HPLC and GC columns, are highly sensitive to trace amounts of lipophilic material which affect the active surfaces of the stationary phase and degrade the resolving power of the column. An effective clean-up procedure is therefore, essential. Since the toxic CBs are present in most samples at the ultra-trace level the sample mass required is larger than that which is used for measuring the routine monitoring CBs. It follows, therefore, that the amount of lipophilic material in the extract will also be much greater.

Table 7
Evaluation of extraction techniques

	Ease of automation	Maximum mass of sample	Time of extraction	Cost-initial outlay	Cost-running expenses	Ease of optimisation
Soxhlet	Possible	Moderate	Moderate	Moderate	Low	Easy
Blending ultrasonic	Difficult	Large	Rapid	Moderate	Low	Difficult
Chemical modification	Difficult	Large	Slow	Very low	Very low	Moderate
Vapour phase separation	Difficult	Moderate	Slow	Moderate	Low	Complex
Column extraction	Possible	Large	Slow	Low	Low	Difficult
SFE	Easy	Small	Very rapid	Very high	High	Complex

Extracts containing ca. 250 mg of lipid from biological tissue or 50 mg of lipid from sediment is usually sufficient to determine the more abundant CBs in all but the cleanest samples. However, the larger sample size for the planar CB analysis can result in extracts containing up to 10–20 g of lipid, all of which must be efficiently removed prior to further sample treatment [2,4,93]. Traces of lipid, e.g. 1 mg, may only be a fraction of the total extract (ca. 1:10 000) but this will become very significant when the sample is concentrated to 1 ml. This amount of lipid remaining in the extract will degrade a carbon or pyrenyl column which are used for the separation of CBs. Most CBs are relatively robust chemicals so that either saponification or concentrated sulphuric acid treatment can be used to degrade the lipids. However, Kannan et al. [11] found that saponification and treatment with strong acids attack some congeners, so non-destructive methods of lipid removal may, in some cases, be more applicable.

5.1. Non-destructive lipid removal

5.1.1. Gel permeation chromatography (GPC)

Most workers [54,94–97] use BioBeads SX3 (200–400 mesh) in a range of column sizes and solvents. Separation has been made primarily between lipid material >500 Da which is the first to elute from the column followed by the smaller molecules which include most of the organic contaminants that accumulate in biological tissue. This separation is particularly appropriate for the determination of trace organic contaminants in fish since most of these compounds are bio-accumulated via the gill filaments, which have an upper molecular size cut-off of ca. 500 Da. Haglund et al. [98] use the SX3 BioBeads only to remove the lipid traces remaining after a sulphuric acid treatment.

GPC, or as it is sometimes known, size-exclusion chromatography (SEC), has several key advantages over other methods currently available. The method is non-destructive and, it can be fully automated [4]. It is also more applicable to the isolation of "unknown" contaminants where there is little information on the polarity or chemical functionality of the molecule. Adsorption chromatography is not able to isolate groups of compounds with very different polarities or structure in a single small fraction. GPC can also handle a larger mass of lipid in each sample. Columns of ca. 500 × 25 mm I.D.

can cope with up to 500 mg of fat whereas the adsorption columns are limited to ca. 50 mg g^{-1} of lipid. It is possible to increase the size of the adsorption column to remove ca. 250 mg of lipid, but larger volumes of solvent are required to elute the more polar organics.

One main disadvantage of the GPC system is that it is difficult to remove all of the lipid [99]. Ford et al. [100] use a 60 g SX-3 BioBead column on an automated 1002B GPC system. But the remaining traces of lipids had to be removed with an additional silica column. Since the triglycerides elute prior to the smaller contaminants the "tail" of the lipid peak intrudes into the second fraction. The amount of lipid in the "tail" becomes significant because there is a relatively large mass of triglyceride that has to be removed relative to the concentration of the contaminants. Grob and Kälin [99] found that much of the tailing was caused by lipid trapped in the injection port and the connecting tubing. Although this contamination was reduced by appropriate switching, the lipid was not completely eliminated. Even a 0.01% carry-over from 1 g of lipid will leave an unacceptably high level of co-extractant in the extract. Until this inherent problem can be solved the low-molecular-mass fraction usually requires further clean-up to remove the trace lipids, e.g. SiO_2 prior to analysis.

Tuinstra et al. [101] have used GPC for the removal of animal fat in the determination of planar CBs and Haglund et al. [98], Haglund [102] and Jansson et al. [70] had a very similar system to separate the CBs in extracts of reindeer tissue, fish liver and seal blubber.

5.1.2. Adsorption columns/solid-phase clean-up

The use of adsorption chromatography for clean-up of lipid samples is well established. Alumina, silica and Florisil [72,103,104] have been used in different mesh sizes, levels of activity and column sizes to separate the CBs from co-extracted materials and in group separation schemes to isolate the CBs from OCPs and other trace organics. The absorbents have been used separately and in combination to reduce the sample handling and analysis time. Each column system must be fully validated, not only for the particular physical configuration selected, but also for each new batch of adsorbent. Although 12–24 samples can be cleaned-up simultaneously, the technique is highly labour intensive and should be eventually superseded by an automatic "on-line" system. Alumina impregnated with 20% potassium hydroxide has been used effectively for the clean-up of mineral oil and waste oil containing CBs [105].

Huckins et al. [54] used silica and sulphuric acid silica columns for CB enrichment and removal of the remaining lipids after GPC-lipid removal.

Extracts from plant material and some types of organically rich sediments can give particular problems from negative peaks in the GC from aryl and alkyl hydrocarbons and from oxy and nitro heteroaromatics. In addition to the LC methods of clean-up, HPLC has been used to selectively remove these interferences and to isolate organochlorine compounds other than CBs. Greinvall et al. [106] have used a 5 μm 300 × 3.9 mm I.D. μBondapak aminopropyl silica column with hexane as an eluant to successfully separate PAH [107] and CBs from chloroparaffins, polychlorocamphenes (PCC) and OCPs in addition to other extraneous material. Alumina and silica columns were applied by de Boer et al. [108] after saponification for a further clean-up of biological sample extracts prior to a CB group separation on a PGC column. An extensive list of references on the use of alumina and silica is given by Lang [5].

5.1.3. Dialysis

Polyethylene (PE) film of pore size ca. 50 μm can be used to dialyse the organic extract to isolate the CBs from the fat. Around 10 g of fat in 15–20 ml cyclopentane placed in a cut section of pre-washed, PE "lay flat" tubing overnight in a beaker of cyclopentane will dialyse around 95% or more of the CBs into the surrounding solvent. The PE has a molecular mass cut-off of around 500 Da and acts as a static size-exclusion

membrane. This method is simple and effective in principle [109], but requires large solvent volumes since the solvent has to be changed several times to ensure complete CB recovery. Lipids also co-extract during the procedure up to 1–2% of the total lipid.

5.1.4. Partitioning

Jensen et al. [110] developed a partitioning system for the removal of lipids. The method is a cyclic procedure and the non-lipids are transferred into acetonitrile. Hexachlorobenzene has been used as reference because of its low partitioning coefficient between oil and acetonitrile. Five batches of 10 times the oil volume were required to transfer 96.6%. This method is only able to reduce the fat content. If the lipids are present at levels higher than trace levels, more than 10% of the lipids are transferred to the acetonitrile phase.

5.2. Destructive lipid removal

5.2.1. Oxidative dehydration (sulphuric acid treatment)

The main alternative, destructive clean-up method to saponification is oxidative dehydration with concentrated sulphuric acid. Concentrated sulphuric acid as such may be brought in contact with the lipid extract in an organic solvent (e.g. pentane or hexane) [111] or it can be adsorbed onto silica gel [74,112]. The degradation primarily removes lipids and wax ester, but many other co-extractants are also removed at the same time [113–115]. The main advantage of this technique is that it is fast, efficient and can remove large quantities of lipid (20 g or more). A column of 50 g of silica–sulphuric acid can remove 10 g of lipid from an extract. Wells and Echarri [26] found that when removing such large quantities of fat, the microfine carbon formed tended to retain a small percentage of the CBs (ca. 2–3%) on the column. There was no difference in retention between the planar and non-planar CBs. The volume required to elute the CBs can be reduced by using dichloroethane in place of *n*-hexane[3]. Kannan et al. [11] found recently that chromic acid treatment degrades planar CBs. At the trace level this can be very significant.

5.2.2. Saponification

Lipids can be saponified by heating the extract in a small volume of solvent with 20% ethanolic potassium hydroxide at ca. 70°C for 30 min. Van der Valk and Dao [81] found that CB 180 was partially degraded during the saponification of sewage sludge when temperatures were above 70°C for more than 30 min. However, when a standard CB mixture in hexane was treated in the same way there was no measurable degradation. Metal particles present in the sewage sludge may possibly act as a catalyst for the dechlorination.

Wells and Echarri [26] initially used CB 209 as a recovery standard, but were only able to obtain ca. 50% from the saponification at 60°C in 20% ethanolic potassium hydroxide. They repeated the saponification experiment with a fish oil spiked with a mixture of 51 CBs (National Research Council of Canada CLB standard) and found that CBs 170, 194, 195, 201, 205, 206, 207 and 209 were all hydrolysed with CB 170 degrading by ca. 10% over 90 min to CB 209 which was almost completely eliminated in 30 min. All other CBs, including CB 180 in the CLB mixture were not affected by the reaction at temperatures below 70°C. This hydrolytic degradation only affects the more chlorinated CB, but could have implications for recovery experiments where CB 189 is used as a surrogate standard.

Kannan et al. [11] compared a solution treated with 0.5 *M* ethanolic potassium at 80°C for 1 h to a solution treated with a non-destructive method (see section 7.1.5). Depending on the nature of the matrix several congeners degraded notably (i.e. 149, 153, 138). Saponification of lipids in

[3] Safety: Extreme care should be exercised when handling the silica–sulphuric acid powder. The silica powder may become airborne with handling large quantities and is, in effect, microfine concentrated acid. The material should always be handled in a fully operational fume cupboard and a filter mask should be worn.

fish and marine mammal samples prior to the determination of non-*ortho*-CBs was successfully applied by de Boer et al. [108,116]. Ethanolic KOH was used during 8 h at 80°C. Recoveries of different spikes of CBs 77, 126 and 169 were 84–107%.

5.3. Sulphur removal

Elemental sulphur is present in most soils and sediments, and is sufficiently soluble in most common organic solvents to make its removal necessary prior to analysis by GC–ECD or GC–MS [117]. The most effective methods available are (i) reaction with mercury or a mercury amalgam [118] to form mercury sulphide; (ii) reaction with copper to form copper sulphide [119]; or (iii) reaction with sodium sulphite in tetrabutylammoniumhydroxide (Jensen's reagent) [120]. Removal of the sulphur with the mercury or copper requires the metal surface to be clean and reactive. For environmental reasons the use of mercury is discouraged. For small amounts of sulphur it is possible to include the metal in a clean-up column. However, if the metal surface becomes covered with the metal sulphide the reaction will cease and the metal will need to be cleaned with dilute nitric acid. Schuphan et al. [79] developed a method to remove large amounts of sulphur. The micro-column used contains 1 g Cu-amalgam and is eluted with 5 ml *n*-hexane. The method of Tan et al. [121] seems to be easier to handle but is also limited by the surface of activated copper powder. Electrolytically purified copper powder is activated by soaking in 6 *M* HCl for 3 min and after rinsing with water and methanol, small amounts of the powder are added until the colour of the powder does not change. For larger amounts of sulphur it is more effective to shake the extract with Jensen's reagent [120]. In that method an aqueous saturated Na_2SO_3 solution is added to the hexane extract. Tetrabutylammonium (TBA) salts and isopropanol are added to the mixture for a better transfer of the $(H)SO_3^-$ ions to the organic phase. The TBA ion forms an ion pair with the sulphite ion which is introduced in the organic phase where the reaction takes place. Water is subsequently added to remove the isopropanol. The aqueous phase is extracted with hexane. Japenga et al. [122] developed a method in which the Na_2SO_3 was loaded on an alumina column.

Silver nitrate, loaded onto silica is also a very efficient sulphur removing agent [117,123,124] although also less appropriate from an environmental point of view. Finally, sulphur may be removed by saponification (see section 5.2.1 and by GPC [125]).

5.4. Combined techniques

Often the use of a single technique for fat separation alone is not sufficient and a combination of methods is required, for example GPC and alumina columns or saponification, alumina and silica columns [108,116]. The clean-up is normally followed by a fractionation of CBs and chlorinated pesticides, often on silica gel, and group separation of CBs (see section 6).

A method for the clean-up and separation of several groups of analyses in milk was developed by Noren [126]. The maximum sample size was 10 g for medium fat milk. Böhm et al. [36] developed a three-column system (30 g silica/6 g phenyl ethyl RP material/7 g silica) using solvents like DMF–water, petroleum ether and acetonitrile. They succeeded in removing 2–3 g lipids. Concentrated HCl was needed in this method.

5.5. Critical evaluation of clean-up methods

Clean-up of biota and sediment samples prior to CB analysis is a laborious task and mainly requires a combination of different techniques which cannot easily be automated. Of the non-destructive techniques for lipid removal GPC is the only technique for which automated systems are available. A relatively high amount of fat (up to 1 g) can also be removed by using this technique. It is, however, very difficult to remove all lipids at one time. A tail of a few tenths of percents of lipids will generally be collected together with the CBs and may effect the subsequent GC analysis. A repeated GPC step will

solve this problem, but of course is even more laborious. Adsorption chromatography on alumina columns may be used in combination with or instead of GPC. Alumina very efficiently removes fat from extracts (ca 150 mg g^{-1} alumina). A drawback may be that other compounds may pass through the alumina column often causing negative peaks in the final chromatograms. Florisil and silica may be used as alternatives for alumina. These materials are often used in combination with alumina for fractionation of CBs and organochlorine pesticides. They are very sensitive to water content and the elution patterns of the determinants should be checked with each batch of adsorbent.

The use of dialysis for fat removal is an elegant method which has only recently been applied and may offer some alternative for the future. Destructive methods such as treatment with sulphuric acid and saponification may also be used, but more labile compounds such as some organochlorine pesticides are often degraded with these methods. Degradation of CBs has been reported for these methods, but saponification has been successfully applied for the determination of planar CBs in biota, showing good recoveries.

Table 8 shows some characteristics of the techniques for lipid-removal. Various methods are available for the removal of sulphur from sediment extracts. Treatment with mercury or silver nitrate/silica columns may be very efficient, but not recommendable for environmental reasons. Copper powder, added to an extract or as a column, copper wire or gauze may be an alternative, but when high sulphur concentrations are present this may be a laborious method because the copper surface requires regular cleaning. The method of Jensen (shaking with TBA) may, therefore, be preferred, although this method too is laborious. By using saponification or GPC, sulphur removal may be efficiently combined with the removal of lipids.

6. Fractionation/group separation

Normally, it is necessary to make a series of group separations prior to the final resolution of the CBs and organochlorines by high resolution gas chromatography. The cleaned-up extract, at this stage, will contain other organohalogens such as OCPs, polychlorinated naphthalenes (PCNs), PCDDs, PCDFs, polychlorinated camphenes (PCCs) (Toxaphene) as well as the CBs [70]. The OCPs and the more abundant CBs are normally the predominant groups of OCs, and these can easily be separated on silica gel [103,104] and determined separately. Most of the PCCs can also be separated from the CBs and the OCPs on the silica gel by increasing the polarity of the solvent from a *n*-alkane to between 5 and 15% methyl *tert.*-butyl ether in hexane [103]. This separation is normally adequate when the predominant monitoring CBs are to be determined.

However, with the exception of CB 118, and to a minor extent CB 105, all mono-*ortho*-CBs and the non-*ortho*-CBs are present at substantially lower concentrations compared with the remaining CBs. It is, therefore, necessary to separate the non-*ortho* and mono-*ortho*-CBs into different groups since: (i) the range of concentrations of the CBs is normally too large for all congeners to be measured without additional dilution or concentration; and (ii) some of the key CBs are not resolved on a single GC column, regardless of the column phase.

The methods available for the isolation of the CBs into separate fractions, prior to GC analysis, utilise the spatial planarity of these compounds.

6.1. Activated carbon and adsorption columns

Activated carbon has been used extensively to separate the non-*ortho* and the mono-*ortho* CBs from the remaining congeners [2]. The early attempts at using carbon columns were directed towards the separation of the PCDDs and PCDFs from other organochlorines and are covered in the review by Erickson [127]. In 1974 Jensen and Sundstrom [24] used an activated charcoal column (200 × 15 mm I.D. Darco G-60) to separate the four groups of PCBs in Clophen technical mixtures. The tetra-*ortho*-CBs, group 1, and the tri-*ortho*-CBs, group 2, were eluted with tetrahydrofuran. The mono- and di-*ortho*-

Table 8
Comparison of lipid-removal techniques

Technique	Requirement							
	Lipid-removal	Destruction of CBs	Destruction of other compounds	Recovery	Amount of time spent	Amount of work invested	Batch variability	Automation
GPC	Two steps necessary	None observed	None observed	Good	High	High	None observed	Easy
Florisil alumina silica	Few lipids removed or large amount of adsorbent used	None observed	None observed	Good	Fair	Fair	All adsorbents were batch dependent	Difficult
Dialysis	Fair	None observed	None observed	Good	High	Fair	None observed	Difficult
Acetonitrile back-extraction	Fair	None observed	None observed	Good	High	High	None observed	Difficult
Sulphuric acid (and or sulphuric acid/silica)	Good	None observed	Organochlorine pesticides	Good	Low	Fair	Negligible	Difficult
Saponification	Good	Mainly congeners with fully chlorinated aryl-ring	Organochlorine pesticides	Fair	Low	Fair	Negligible	Difficult

CBs, group 3, and the non-*ortho*-CBs, group 4 were eluted with benzene. Following this some workers used polyurethane foam to disperse the activated carbon. Huckins et al. [54] and Stalling et al. [128] used AMOCO PX-21 15% (w/w) on the polyurethane foam. Stalling et al. eluted the CBs into five fractions from tetra to non-*ortho*-CBs with a step gradient of toluene–cyclohexane (2:98 to 100:0).

Tanabe et al. [93] reverted to using a simple activated carbon column. After removing the co-extracted lipophilic material by saponification they adsorbed the OCs onto the carbon column from the hexane extract. The OCPs and non-planar compounds were eluted with dichloromethane–hexane (1:5), the non-*ortho*-CBs were eluted with benzene–ethyl acetate (1:1) and the PCDDs and PCDFs with toluene. The recoveries for CB 77, CB 126 and CB 169 were >90% at the 10 $\mu g\,kg^{-1}$ level and >60% at the 0.1 $\mu g\,kg^{-1}$ level.

6.1.1. Polyurethane foam/glass fibre/activated carbon

Polyurethane foam as a support [129] for activated charcoal has had limited success due to the breakdown of the foam with polar solvents. Glass fibre was used as an alternative substrate for the activated carbon [74,130,131], with the planar molecules being trapped on the carbon from the extract in dichloromethane–benzene (1:1) and backflushed from the column with toluene. Ford et al. [100] have a similar semi-automated system with mixtures of hexane and DCM and reversed-elution with toluene.

Wilson-Yang et al. [132] used a carbon/glass fibre column (180 × 4 mm I.D.) to separate the non-*ortho*-CBs from the *ortho*-CBs. The sample was eluted under nitrogen (ca. 30 psi) with dichloromethane in hexane (1:3, 10 ml) to remove the CBs 28, 52, 60, 138, 166, 170 and 158, followed by dichloromethane to elute CBs 123, 118, 114, 157, 105, 167, 156 and 189. The flow and pressure were reversed and the planar CBs 77, 81, 126 and 169 were eluted with toluene (20 ml). The samples were spiked at levels between 70 and 200 $\mu g\,kg^{-1}$ and average recoveries were 60%, 72% and 36% for CBs 77, 126 and 169, respectively. Schwartz et al. [97] developed an HPLC system with gradient elution with quite good recoveries from 80–90% for the planars 77, 126 and 169. Krokos et al. [133] have a fully automated method with recoveries of 122, 87 and 93% for the CBs 77, 126 and 169 respectively at the pg/kg level.

6.1.1.1. Silica gel/activated carbon

Sericano et al. [134] used a 1:20 mix of activated AX-21 carbon and low-pressure silica gel LPS-2. They tested the efficiency of the column with an Aroclor 1254 and a dolphin blubber extract spiked with CB 77, 81, 126 and 169 at 50 $\mu g\,kg^{-1}$. The first eluate of dichloromethane–cyclohexane (50 ml) contained the *ortho*-CBs and the second eluate of toluene (40 ml) contained the planar CBs 77, 81, 126 and 169. The recoveries were reported to be between 82 and 96%. Kuehl et al. [43] mix Amoco PX-21 with silica gel in a weight ratio of 7:2. This method, had a detection limit of 0.2 $\mu g/kg$.

6.1.1.2. Carbopack C/mixed with Celite

Storr-Hansen and Cederberg [72] used a Carbopak C/Celite column (200 × 10 mm I.D.). After cleaning the column in situ the *ortho*-CBs were eluted with hexane (50 ml) and the non-*ortho*-CBs 37, 77, 81, 126 and 169 were removed with toluene (15 ml). The planar CBs were determined by IDMS using the ^{13}C-labelled CBs with recoveries of (79 ± 14)% for CB 77, (80 ± 15)% for CB 126, (83 ± 17)% for CB 169 (n = 63). The spiking level was 4 ng equivalent to 400 $ng\,kg^{-1}$ in the sample. The advantage of using Carbopak C over the more conventional activated carbon means that the CBs can be eluted with a smaller volume of less polar solvent to remove the *ortho*-CBs and the non-*ortho*-CBs can be eluted without having to backflush them from the column.

Atuma and Andersson [40] used active coal (SP-1) on chromosorb to separate the 2-4-*ortho*-CBs and 4,4′-DDE with hexane dichloromethane, and the mono-*ortho* and non-*ortho*-CBs by eluting with toluene. The more *ortho* and non-*ortho*-CBs were subsequently separated using Carbopack C and eluting with hexane and

toluene respectively. The [^{13}C]CBs were used as surrogate standards for CB 77, 126 and 169, CB 189 for the mono-*ortho* and CB 53 for the 2-4 *ortho*-CBs. Dewailly et al. [135] also work with a Carbopack C/Celite column to fractionate non-*ortho*-CBs and PCDD/Fs. They found that their method using high-resolution mass spectrometry could detect 25, 210 and 221 pg/g for the CBs 77, 126 and 169 respectively in human breast milk. On drawback of using this column is, however, that PCNs coelute in the same fraction as the CBs [136].

6.1.1.3. Charcoal

The attraction of using activated charcoal is that it has a high affinity for organics even at the ultra-trace level, it is inexpensive, readily available and easy to use. However, there are also some significant drawbacks to its use in the present application.

It is essential to separate the mono-*ortho*-CBs as well as the non-*ortho*-CBs from the other congeners, since both groups contribute to the overall toxicity of the PCBs. The mono-*ortho*-CBs are present at considerably higher concentrations and therefore may contribute as much, if not more than the planar CBs to the overall toxicity, and possible biological effect. The recovery of planar CBs from active carbon at the level which occur in environmental samples has not always been fully quantitative. However low recoveries can be overcome by using IDMS, but the impurities in the carbon have proved to be a greater problem. Jansson et al. [112] made a multi-residue study with a charcoal fractionation step. By spiking their samples they found 51–120% recoveries. Kannan et al. [137] made an extensive and thorough comparison of six activated charcoals (Wako, Baker Analytical, Serva-SK-4, Anderson, AX-21, Alltech, SK-4 type, and Merk) type using the MDGC–ECD techniques developed by this group [138] to determine the efficiency and separation of these CBs. They use a test mixture containing 0-4 *ortho*-CBs to optimise the separation prior to testing on a batch of Aroclor 1254 which had been previously characterised [38]. With the advantage of the MDGC it was possible to clearly identify the problems of separation with these materials. They concluded that the activated charcoals tested were not able to completely separate the non-*ortho*-CBs from the dominant *ortho*-CBs. In particular the co-elution of CB 110 with CB 77, and of CBs 129 and 178 with CB 126 was sufficient to prevent the quantification of the planar congeners. They concluded that data for CB 77 and CB 126 obtained by these forms of activated charcoal, when not using MDGC, are likely to be an overestimate. They also found that it was difficult to obtain a clean blank with these activated charcoals even after extensive cleaning. These impurities are a major drawback of this method which has been explicitly and comprehensively reported for the first time. Also high amounts of solvents are necessary to collect the non-*ortho*-PCBs from the charcoals in comparison to the HPLC columns.

Lundgren et al. [139] examined Amoco PX21 in an HPLC column. They collected 17 fractions and found a rather complicated elution scheme for the PCBs according to their substitution pattern. But the planar PCBs were all completely and solely eluted in the 17th fraction which was collected after 40 until 60 min of elution. They used a gradient in solvent mixture of DCM in hexane and toluene. Athanasiadou et al. [140] used six successive columns of which the first two were a mixture of charcoal with Celite and the 3rd and 4th columns of pure charcoal. The purpose of separation is the preparation of material for toxicity tests. The separation is thus not pushed to the ultra-trace level and can thus not be used for analytical purposes.

6.1.1.4. Florisil

Lazar et al. [141] compared Florisil as fractionation column to activated carbon columns. For both techniques similarly good recoveries are found (nearly 100%). But the Florisil column seems to be considerably simpler. Storr-Hansen and Cederberg [72] investigated the use of adsorption chromatography to separate the non-*ortho*-CBs from the remainder of the CBs. The initial work on Florisil reported by Kamops et al. [142] and more recently by Fernandez et al. [143] and Harrad et al. [111] was repeated with the

inclusion of alumina and silica in these studies. Normally these absorbents are adjusted to a specific activity with water in order to remove co-extracted materials from soil, sediment, or tissue matrices. In this case Storr-Hansen and Cederberg [72], heated the adsorbents at 450°C to fully activate the material which was used without further deactivation. A mixture of CBs, including the non-*ortho* congeners, was reapplied to a column (200 × 10 mm I.D.) containing activated basic alumina (6 g) and eluted with hexane. Two observations were made. Firstly, the elution volume for the bulk of the CBs increased from around 10–20 ml to 150 ml, and secondly, the planar congeners, CBs 37, 81, 77, 126 and 169 were only partially eluted after some 300 ml. The similar studies with active Florisil gave approximately the same pattern of retention of the planar CBs, but with a smaller elution volume. The elution pattern with activated silica did not drastically alter from the normal deactivated (ca. 3–5%) adsorbent with the CBs eluting with 50 ml of hexane. Storr-Hansen and Cederberg [72] concluded that the lack of selectivity by the SiO_2 was due to the different –OH bonding. An alternative explanation is also possible. Highly activated silica is very hygroscopic and even when the column is prepared rapidly at room temperature, the adsorbent will become deactivated to at least 1% due to the surrounding water vapour in the laboratory atmosphere. When the alumina and Florisil were deactivated to >3% with water the separation between the planar and non-planar CBs disappeared and all CBs were eluted with 20–30 ml hexane.

Harrad et al. [111] also used Florisil, activated to 130°C for 16 h, to separate the planar CBs from the *ortho*-CBs. The sample was added to a 1 g column in a pasteur pipette and first eluted with hexane (10 ml) to remove the bulk of the CBs and then eluted with dichloromethane to isolate CB 77, CB 126, and CB 169. Recoveries ranged from 78% to 107% with accuracies of between 26–33% for spiked samples which had been previously cleaned-up with sulphuric acid. This method was applied to human milk, adipose tissue, avian tissue, soil and sewage sludge using ^{13}C-analogs with the final determination by IDMS.

These adsorption methods are suitable for the separation of the planar CB from the other *ortho*-CBs only and the matrix must be thoroughly cleaned-up prior to this separation being made. Normal lipid removal cannot be made simultaneously on the same column.

6.2. Porous graphitic carbon and pyrenyl-silica columns

The porous graphitic carbon (PGC) HPLC packing developed by Knox et al. [144] was used to separate CBs, PCDDs and PCDFs by Creaser and Al-Haddad [145]. The PGC has a surface area of 150 $m^2 g^{-1}$ and mean particle size of 7 μm, with a pore volume of 2 $cm^3 g^{-1}$ which is ideal for use in an HPLC system. The CBs were chromatographed on the column (50 × 4.7 mm I.D., Shandon Southern, UK) with *n*-hexane. The *ortho*-CBs were eluted in the first 10 ml using a flow-rate of 1 ml min^{-1}. The non-*ortho*-CBs were eluted with a further 90 ml of hexane. The column was backflushed with a further 200 ml of hexane to remove the PCDDs and PCDFs. The backflush volume can be reduced by using toluene in place of hexane. The advantage of such a system is that the *ortho* and non-*ortho*-CBs can be separated using a single solvent with an HPLC system that can be fully automated.

Tuinstra et al. [101] used the same PGC "Hypercarb" column, but with a solvent mix of dichloromethane–cyclohexane (1:1) at 2 ml min^{-1} to separate the toxic CBs in animal fat. The increase in polarity reduced the elution volume of the CBs. The first fraction contained the *ortho*-CBs. The solvent was then switched to toluene to remove the non-*ortho*-CBs, again to reduce the elution volume. The column was completely washed free of toluene with the starting solvents prior to the next sample. Other laboratories have recently used the Hypercarb PGC-columns for the analysis of planar PCBs [108,116,146,147]. Engberg and Storr-Hansen [146] developed a method using hexane and a hexane–toluene mixture as solvents which resulted in elution profile where 5% of the mono-*ortho*-CBs were found in the same fraction as non-*ortho*-CBs.

Hong et al. [148] used the PGC column to separate the non-*ortho*-CBs in human milk. They used the same single hexane solvent as Creaser and Al-Haddad [145], but reversed the flow after the elution of the *ortho*-CBs to speed up the recovery of the second fraction.

Zebühr et al. [83] used two coupled HPLC columns to improve the isolation of the CBs according to their *ortho* substitution pattern. They coupled an aminopropyl RP column (250 × 10 mm I.D.) with a Hypercarb column (100 × 4.7 mm I.D.). All extracts of fish tissue were cleaned up prior to this HPLC separation to remove the lipids. The samples were eluted with *n*-hexane through the amino propyl column to isolate the aliphatics and mono-cyclic compounds, e.g. hexachlorobenzene. The second fraction containing the dicyclics PCDD/Fs, PCNs and CBs, was switched to the second Hypercarb column. The polyaromatics were removed from the first column by backflushing. The dicyclic fraction were firstly eluted from the PGC with hexane (4-2 *ortho*-CBs) and then with hexane–dichloromethane (1:1) to remove the mono-*ortho*-CBs. The PGC column was washed with dichloromethane–methanol (1:1) and then backflushed with toluene at 40°C to isolate the PCDD/Fs and the non-*ortho*-CBs together.

The activated carbon and the porous graphitic carbon is complemented by the silica bond phase, 2-(1-pyrenyl) ethyldimethylsilyated (PYE) silica [98,149] which can separate the *ortho*- and non-*ortho*-CBs on the basis of the degree of planarity and chlorination. This column material separates structurally similar molecules with different π-electron densities resulting from the spatial configuration of the aryl rings, and has sufficient resolution to isolate the non-, mono- and other *ortho*-CBs. Initially, this type of column was, like the activated carbon and the PGC, used to separate the toxic CBs 77, 126 and 169. However it is also possible to separate other key CBs which can co-elute on the 5% phenyl methyl GC column (SE-54, CPSil 8 type). This column, therefore, has the potential to remove a number of ambiguities that sometimes occur in the final determination of the toxic CBs where MDCG is not available. Haglund et al. [149] have used this column to determine the mono- and non-*ortho*-CBs in reindeer, herring and seal tissue and Wells and Echarri [26] have measured a similar suite of CBs in seal, dolphin, porpoise and whale with a similar separation technique, and more recently for fish tissue [27].

Using the retention pattern of 168 CBs available on this PYE column [26,27,99] it has been possible to isolate three different fractions which will isolate predominantly the tri- and di-*ortho*-CBs (fraction I) from the mono-*ortho* (fraction II) and the non-*ortho*-CBs (fraction III). The elution order of the CBs is not solely dependent upon the degree of *ortho* substitution. Temperature effects the elution pattern and retention time of the CBs, therefore the column should be thermostatically controlled. Furthermore, a number of the more highly chlorinated CBs elute in the second and third fractions. For example, the effect on retention of the *ortho*-substitution with the more highly chlorinated CBs is seen by comparing CBs 205, 206 and 209, where one aryl ring is fully substituted. The second ring has a 3′,4′,5′ pattern for CB 205, which elutes in fraction III. By adding one further *ortho* atom to the ring the elution of CB 206 now becomes split between fractions I and II, and the fully substituted CB 209 with the last *ortho*-chloro position substituted elutes completely in fraction I [26].

These fractions separate pairs of CBs which have similar retention times on an SE-54 CPSil 8 type column and overcome some of the problems encountered by Kannan et al. [137] using the activated carbon columns without MDGC. CB 129 and CB 178 which normally co-elute with CB 126 are separated into fractions I and II, and CB 110 (fraction II) is fully separated from CB 77 (fraction III). The two CBs 138 and 163 are also separated into fractions I and II, respectively [26], although a full 100% split is difficult to maintain since any small shift in retention times of 3 to 5 s can result in ca. 5% of the more abundant CB 138 in fraction II.

The retention times using the pyrenyl column are very repeatable within a batch calibration, but are susceptible to change if the sample contains any lipid residues. Both Haglund et al. [98] and Wells and Echarri [26] found it essential to remove all lipophilic co-extracted materials prior to separating the CBs on the PYE column.

The column efficiency can be maintained by regular flushing of the column with ethyl acetate between sample batches [26,27].

The PYE HPLC column is ideally suited for separating organics on the basis of their planar structure. Wells and Echarri [26,27] investigated the elution profile of other groups of planar compounds that have AHH/EROD (aryl hydrocarbon hydroxylase/7-ethoxy resorufin O-deethylase) activity and can also interfere with the determination of the toxic CBs. These were the polynuclear aromatic hydrocarbons (PAHs), the PCNs and the PCDDs and PCDFs.

Like the activated carbon and the PGC, the PCDDs and the PCDFs can be separated from the CBs on the PYE column. However, it is also possible to separate the PCDDs and the PCDFs into their own fraction according to the degree of chlorination. In view of the high k' values for the hepta- and octa-chloro isomers it is preferable to remove these by backflushing or by increasing the polarity of the solvent.

The potential interference from PCN can also be reduced with the PYE column. The penta- and hexa-CNs elute in fraction III along with the non-*ortho*-CBs. But the predominant CNs which occur in the PCN formulations such as the Halowax 1041 (56% Cl) are separated by GC from the planar CBs [27].

6.3. Two-dimensional HPLC

Bandh et al. [150] use the PYE column in combination with a nitro-column for a two dimensional HPLC technique to separate PAHs, PCNs, PCDDs/Fs and co-planar CBs from other organic traces.

6.4. Critical evaluation of the fractionation and group separation methods

Gravity columns of alumina and silica (deactivated to various degrees), Florisil or graphitic carbon have been shown to fractionate chlorinated aromatic compounds with much less reproducibly than HPLC columns. In addition, each batch of alumina or silica has to be calibrated on each occasion to establish the elution pattern of the determinants. Also the column material itself can only be used once. One of the main disadvantages of active charcoal is the impurities in the charcoal which made it difficult to obtain low blank values. Another drawback of this method, in comparison to the HPLC columns is the relatively high amount of solvents needed to collect the non-*ortho*-CBs from the charcoal, which unnecessarily increased blank values. The PYE column uses small amounts of hexane (15 ml) to separate di- to tetra-*ortho* from the mono-*ortho* and from the non-*ortho*-CBs. Finally, these active charcoal systems cannot be automated to the same degree as the HPLC columns. Six or twelve samples can be fractionated in parallel, but no autosampling or sequential automation is readily available. These disadvantages are overcome by using the HPLC columns. A possible advantage of the adsorption columns is a decreased risk for cross contamination between samples since they are disposable.

Porous graphitic carbon columns (HPLC application) can be operated in reversed-flow mode to recover the more easily adsorbed fractions like dioxins. This is not the case for the pyrenyl-silica column. Extracts injected onto the HPLC columns must be lipid-free, especially for the PYE column, otherwise retention time shifts of the CBs occur. This in contrast to active charcoal, which are more tolerant of high amounts of lipids. The PYE column has to be thermally regulated. In general, cooling gives better separation characteristics and longer column-life. Multidimensional systems, often developed for the separation of specific congener pairs, can actually be considered to fulfil fractionation tasks. The use of a pyrenyl-silica column in combination with a nitro-column is here certainly an efficient tool. Table 9 summarises the most common fractionation techniques.

7. Chromatographic separation

Various groups have studied the separation of CBs which are commonly determined in environmental samples [2,18,21,38,72,151–155].

Despite the use of more complex chromato-

Table 9
Comparison of fractionation techniques

Technique	Requirement				
	Separation	Batch variability	Lipid sensitivity	Interference from column material	Automation
Gravity columns of alumina silica or Florisil	Difficult	High	Low	Low	Difficult
Charcoal	Fair	High	Low	High	Difficult
Porous graphitic carbon-HPLC	Good	Low	High	Low	Easy
Pyreneyl silica-HPLC	Good	Low	Very high	Very low	Easy

graphic techniques such as MDGC or serial-coupled GC, no GC methods have been reported that were able to separate directly the non-*ortho*-CBs from the other PCBs. Kannan et al. [137] concluded that for the determination of non-*ortho*-CBs a pre-separation from other CBs is necessary because of the extremely low concentration of the non-*ortho*-CBs, both in technical PCB mixtures and in environmental samples. They reported a 600-fold difference as a maximum between CBs 77 and 110, beyond which a separation is only possible with MDGC. The concentration difference between the three non-*ortho*-CBs and the major CBs in most environmental samples varies between ca. 100 and 3000 [108].

For mono- and di-*ortho*-CBs a direct GC separation is possible under various conditions. This section will mainly focus on the separation of mono- and di-*ortho*-CBs. Since the GC of non-*ortho*-CBs after pre-separation is relatively easy.

7.1. Single-column GC

Single-column high-resolution GC is normally the final stage of a CB analysis. The selection of the capillary column's physical parameters, stationary phase and GC conditions is crucial in obtaining a single uncompromised signal resulting from the elution of one congener, free from interference from other CBs and co-extracted material. The overall optimisation of the GC is essential if the maximum column efficiency is to be obtained [7,8]. In addition to the optimal sample introduction and column installation, the capillary column separation of the CBs is dependent on (a) carrier gas; (b) column parameters; and (c) choice of stationary phase(s).

7.1.1. Carrier gas

Hydrogen is preferred as a carrier gas. It offers a good resolution, even at a higher gas velocity, since the height equivalent to a theoretical plate (HETP) is relatively unaffected by the flow-rate above the optimum [18,152]. The resolution of both helium and nitrogen declines as the gas velocity is increased. Helium may be used as an alternative to hydrogen, but the extremely high pressures with very narrow columns of <0.15 mm I.D. creates practical difficulties [152]. Helium may be used for columns with internal diameters >0.20 mm although the resolution obtained will be less than when hydrogen is used. Nitrogen is, in principle, not suitable for use in capillary GC because it drastically reduces the column efficiency, although it has been used for some MDGC applications [23].

7.1.2. Column parameters

The column dimensions are not very critical for the determination of non-*ortho*-CBs, provided that a pre-separation has been made. The three CBs, 77, 126 and 169 and the internal standard may easily be separated, particularly where ^{13}C-labelled standards are used. This can easily be obtained on a relatively short, medium bore column. If, however, there is any doubt concerning the presence of other CBs or other planar compounds in the same fraction, then the problem can be resolved by using columns with a length of 50 m and 0.25 mm I.D.. These minimum column dimensions have been determined as a result from several inter-laboratory studies on mono- and di-*ortho*-CBs [13–15,156]. A reduction of the internal diameter down to 0.15 mm will further improve the separation of the CBs [152]. A film thickness of 0.30 μm is recommended in order to enable a separation of the early eluting CBs such as CB 28 and CB 31, particularly on medium-polar columns. A thinner film thickness may be used on apolar columns to shorten the analysis time. A film thickness of only 0.1 μm is sufficient for the analysis of the non-*ortho*-CBs 77, 126 and 169.

7.1.3. Stationary phases

The only complete set of retention time data so far available for all 209 congeners was reported by Mullin et al. [19] for the SE-54 stationary phase 5% diphenyl–1% vinyldimethylsiloxane. For this reason, most CB analyses have been carried out on columns with this phase. However, additional GC retention data has become available for a number of CBs on different columns. De Boer et al. [18] measured the retention times of 51 CBs on seven narrow bore columns, CPSil 8, CPSil 12, CPSil 19, CPSil 88 and C_{18} from Chrompack, SB Smectic from Lee Scientific, and the FFAP from Hewlett-Packard. Bowadt and Larsen [21] coupled a 50 m × 0.25 mm I.D. (0.26 μm film thickness) CPSil 8 with a 25 m × 0.22 mm I.D. HT-5 column. The HT-5 is a newer column coated with 1,2-dicarba-*closo*-dodecarborane polydimethylsiloxane which is a high temperature (>300°C) material based on the "Dexil 410" packed-column liquid phase. Eighty-four CBs were resolved by ECD and 108 CBs by MS using this coupled system. The separations and peak assignments were given, but no retention data. Larsen et al. [157] made a comparison of the separation of the toxic CBs from the potentially interfering CBs on eight different phases and a further study [20] of the separation of 140 CBs in technical mixtures on five different phases with data on all potentially overlapping peaks. From the work of de Boer et al. [18] and Larsen et al. [157], it was concluded that a CPSil 19 column offers better possibilities for the separation of the most important CBs than a SE-54 type column.

7.2. Combined techniques

7.2.1. Serial-coupled columns

CB analysis using serial coupled GC–ECD and GC–MS was applied by Larsen and Bowadt [158] and Bowadt and Larsen [21]. They reported an additional separation of 26 CBs compared to single-column GC, but, on the other hand, 10 CBs now co-eluted where previously they were separated with single-column GC. A drawback of this system is the long retention times that are required. This makes the method less attractive for routine applications. For the separation of specific CBs it may, however, be useful. This technique is not of interest for the determination of non-*ortho*-CBs only.

7.2.2. Parallel-coupled columns

In parallel-coupled GC or dual-column GC two columns are attached via a retention gap to one injector and, on the other side, each column to one detector. In this way the CB analysis can be carried out on two different stationary phases at the same time. For a reliable quantification it is essential that both columns have exactly the same dimensions. Otherwise discrimination may occur, which would lead to different results on the two columns. Storr-Hansen reported the determination of mono- and di-*ortho*-CBs by dual-column GC–ECD [72,159]. Similar determinations were also reported by Luckas et al. [160]. Natzeck et al. [161] used two dual-column

GC systems for the separation of the non-*ortho*-CBs 77, 126 and 169 from other CBs after a pre-separation on Carbopack. Bøwadt and Johansson [162] reported the use of a 60 m × 0.25 mm I.D., 0.25 μm DB 17 column in parallel with a 25 m × 0.25 mm I.D., 0.25 μm CPSil 8 column in series with a 25 m × 0.22 mm I.D., 0.10 μm HT5 (1,7-decarba-*closo*-dodecarboranedimethylpolysiloxane) column. The column was selected because of a good separation of CBs and organochlorine pesticides [163]. For the determination of the mono- and di-*ortho* congeners a pre-separation of CBs and other possible compounds which might interfere in the CB chromatogram such as organochlorine pesticides (including chlordane and toxaphene) and flame retardants (brominated biphenyls and diphenylether) is, however, recommended.

7.2.3. Multidimensional gas chromatography

MDGC is one of the most effective techniques to separate CBs and avoid interference from other congeners or other compounds [11,138,164–167]. The present configuration of the MDGC [118] normally uses a non-polar phase on the first column such as SE-54 or CPSil 8 to make the initial, well characterised separation. The sample is chromatographed on this first column to a point just prior to the elution of the unresolved peaks. The column flow is then switched into the second column of a different, usually more polar phase, e.g. CPSil 19, CPSil 88, for the duration of the elution of these unresolved peaks only. This technique is widely-called a heart-cut and can be made with a precision of seconds. Multiple heart-cuts can be made during one run [23]. The inherent resolving power of the system is in the coupling of two columns of different polarity and the isolation of a relatively few compounds on the second column. This technique is ideal as a check for co-elution when carrying out congener-specific determinations in complex mixtures in general. So far every attempt to determine all CBs by single column GC has been unsuccessful. Where resolution is improved for some CBs on alternative stationary phases, it is lost for other congeners. Co-eluting compounds can be detected by MDGC–ECD at a level of a few percents [168]. MDGC–ECD is, however, not suitable [137] for the direct analysis of the non-*ortho*-CBs 77, 126 and 169 which normally occur at 1000-fold lower concentrations than the major CBs. Heart-cuts of non-*ortho*-CBs and other CBs co-eluting on the first column are easily overloaded because of the large difference in concentration and full separation on the second column cannot be obtained. The concentration difference between mono-*ortho*-CBs and the remaining congener is a factor of 100 or less so MDGC–ECD may be used successfully to clarify the identity of CBs in technical PCB mixtures [23,28,166] and in environmental samples [153].

A disadvantage may be the relatively long analysis times required. This may be overcome by the use of shorter second columns. The use of two independent GC ovens is preferred, because it offers more flexibility in temperature programmes [138,153].

Schulz et al. [22] re-examined commercial Aroclor and Clophen industrial formulations of PCB using MDGC to completely characterise the CB content above the 0.05% level. Each PCB mixture was initially chromatographed on an SE-54 and the chromatogram separated into distinct regions containing one or two peaks which were individually heart-cut into the second OV 210 or C-87 column. Some of the unresolved peaks on the SE-54 would be better resolved on a narrow bore, rather than an 0.32 mm I.D. column, but the MDGC information is extremely valuable since it specifically confirms the absence of 77 CBs from these formulations above the 0.05% level. The combination of these data with the retention data obtained by Larsen et al. [20] for the 140 CBs on five different stationary phases give a complete set of retention data for all CBs that occur in the formulations on all five phases, with the exception of CBs 69, 75, 96 and 182.

The choice of the two columns in MDGC determines the separation power of the system [18]. The greater the difference in selectivity of the two stationary phases used, the better resolution is obtained. Liquid crystalline column phases, such as SB-Smectic columns, are an

alternative to the choices available. Normally the separation of CBs on most stationary phases is determined by polarity, but with the liquid crystalline columns, which were initially developed for use in SFC, the separation of the CBs is dependent on molecular shape. SB-Smectic columns have successfully been applied for the determination of CBs by MDGC [18,169,153]. A drawback of this type of stationary phase is the high bleed, i.e. loss of column material at higher temperatures. MDGC is still not a routine technique, but it is a powerful research technique for isomer-specific CB analysis at trace level [158].

7.3. Liquid chromatography (LC–GC coupling)

On-line liquid chromatography–gas chromatography (LC–GC) has been applied to the determination of CBs [170,171]. No application has been described for the determination of non- and mono-*ortho*-CBs. A group separation of CBs is reported by Welch and Hoffmann [172], who used a micro capillary LC column packed with an electron-acceptor stationary phase, 5 μm 2,4-dinitrophenylmercapto-propylsilica. Detection was carried out by GC–MS.

7.4. Supercritical chromatography and SFE–GC coupling

Similarly to LC–GC no application is known at the moment of SFC or SFE for the determination of planar CBs. Several basic studies on the performance of SFE have been carried out by Hawthorne and co-workers [173–175]. Most studies on SFE of CBs have focused on sediments [176–179]. Lipids are normally co-extracted using SFE to remove PCBs from biota. Although lipids can be separated afterwards, this co-extraction makes the SFE approach less attractive because little time is gained in this way. In fact, the procedure may even become more time-consuming because the samples must be freeze-dried prior to SFE. Bøwadt et al. [180] developed an SFE method to extract CBs from biota leaving the bulk of lipids in the original matrix. An additional clean-up is, however, recommended for more fatty tissues. The results were comparable with those obtained after Soxhlet extraction from the same samples. Detection limits were 0.5–2 ng/g and standard deviations were less than $\pm$ 5% at CB concentrations of 0.8–134 ng/g.

7.5. Critical evaluation of separation methods

It is essential that a pre-separation, e.g. by HPLC, is carried out prior to the gas chromatographic separation of non-*ortho*-CBs. A pre-separation is useful also for the determination of mono- and di-*ortho*-CBs, but a direct GC determination for these CBs is possible under correct optimised GC conditions.

The major mono-*ortho*-CBs 105, 118, 156 and the di-*ortho*-CBs can be determined by single-column GC. Information from several intercomparison exercises [8,14,15] indicates that capillary columns should have minimum lengths of 50 m and maximum internal diameters of 0.22 mm, but preferably 0.20 mm. Further reduction down to 0.15 mm is recommended, although one should be aware of possible leakages and that pressure regulators with a range up to 400 kPa are required. The optimum film thickness ranges from 0.2–0.4 μm.

There is a complete set of retention times for all CBs only for the SE-54 stationary phase. There is, however, information on retention times of most CBs for a number of other columns. These data are essential since SE-54 type columns do not offer the optimum separation for the single-column GC determination of CBs. Slightly more polar columns such as CPSil 19 are to be preferred.

Serial-coupled GC may offer more resolution, but the analysis time is generally longer, which makes this option less attractive for routine purposes. Parallel-coupled GC may be more accessible for routine applications with the advantage that two chromatograms of different columns are obtained in the analysis time needed for one column. The system may be vulnerable with narrow bore columns ($<$0.20 mm I.D.), because connections may start to leak at higher gas pressures.

For a direct determination of the mono-*ortho*-CBs which normally occur at lower concentrations, such as CBs 114, 123, 157, 167 and 189, MDGC is recommended [23]. This technique is also extremely helpful for the determination of the composition of any other peak in a chromatogram. Because of the relatively long analysis times and because only a few CBs can be determined at any one time, MDGC is not really suitable as a routine technique for CB determinations. It is, however, essential to solve any question of co-elution. The selection of a set of columns to be used in combination in MDGC is very important in this respect.

LC–GC and SFE/SFC are both relatively new techniques which until now have not been applied for the determination of non- and mono-*ortho*-CBs. SFE offers good possibilities for the determination of CBs in sediments. When applying SFE for the determination of CBs in biota, the interference of lipids is still not completely solved, which makes extra manipulations necessary. Particular difficulties arise when multiple fractions must be isolated from each sample by the LC, for example different groups of compounds, e.g. CBs and OCPs. The sample also needs to be separated into fractions when similar compounds are present at considerably different concentrations or where chromatographic overlap is to be avoided [3].

An overview of the current recommended ways of separation for some groups of CBs is given in Table 10.

8. Final determination

The power of analytical instrumentation currently available makes it possible to detect toxic organic contaminants at concentrations below $1 \cdot 10^{-12}$ (= pg/g) in environmental samples. Such low detection limits are essential if these contaminants are to be measured with the accuracy and precision required for correlation with biological effects data. In turn, these detection requirements place additional demands on the sample extraction, clean-up and group separation schemes particularly as more chemicals are added on the list of determinants.

As studies of Beck and co-workers [53,181] proved, there is often a detection limit for the ECD around 0.1 μg/kg. However, normal levels of planar PCBs may be in the low ng/kg range.

8.1. Electron-capture detection

8.1.1. Calibration

The earlier measurements of PCBs were dependent on industrial formulations to calibrate the detector and to quantify the total PCB

Table 10
Separation techniques for different groups of congeners

Congener	Group		
	Major mono-*ortho*-CBs 105,118,156	Minor mono-*ortho*-CBs 114, 123, 157, 167, 189	Non-*ortho*-CBs 77, 126, 169
Single-column GC without pre-separation	Possible with care	Difficult	Currently not possible
MDGC	Easy	Easy	Difficult
Single column GC with pre-separation on:			
Adsorption charcoal	Easy	Difficult	Difficult
PGC-HPLC PYE-HPLC	Easy	Easy	Easy

concentration in the sample [128]. This calibration was usually achieved by peak matching and summing selected peaks to produce a total value. Considerable effort has gone into the production of individual, pure chlorobiphenyls, initially by individual research laboratories and now by commercial companies and reference material suppliers. Selected CBs are available as certified or well characterised materials for monitoring and for toxicological studies. Of the 209 CBs, 124 are currently commercially available and these cover more than 85% of the CBs found in the environment. The main CBs which are not widely available have the substitution patterns 2,3,5 (2), 2,3,6 (6), 2,6 (8), 3,5 (12), 2,3,4,6 (9), and 2,3,5,6 (9). The values in parenthesis are the number of each substitution pattern currently not available.

The calibration of the GC using PCB formulations, a legacy from the earlier work, should now be regarded as inappropriate laboratory practise [182,183]. It is difficult to attain the necessary level of accuracy and precision required and to probe the present problems relating the environmental processes and toxicological studies [184] with these formulations.

Some earlier Aroclor reference materials have been re-analysed to determine their individual CB content. These materials have been used as secondary standards to support the continuing transition from formulation-total PCB based data to the single congener analysis [112]. Maack and Sonzogni [185] have used Aroclor formulations for calibration and have then calculated the individual congeners using published data on the congener content of the specific formulation. While these data may be sound for that particular batch of the formulation, the between-batch variability of these formulations is too large to reliably translate the individual CB data for use in a calibration procedure.

Williams et al. [186] has proposed the use of total CB concentrations to predict the 2,3,7,8 tetrachlorodibenzo-*p*-dioxin equivalent concentrations (TEQs) of the non-*ortho* and mono-*ortho* in fish. They concluded that this was possible within a factor of 2–5. While this approximation may be a suitable, inexpensive, screening technique there are some major disadvantages of such a technique. When the toxic equivalent concentrations are close to the permitted limits then a precision of a factor of 2–5 will be insufficient and more detailed and accurate studies will be required. Also the ratio of toxic planar CBs and more persistent and abundant CBs will change between different organs within a fish, between species and populations of the same species as a result of exposure to different sources of PCB, metabolism, and seasonal variations. Such ratios are not sufficiently constant for the detailed studies correlating planar compounds and biological effect.

The preparation, maintenance and use of the calibration solutions still remains as one of the main sources of error in the measurement of CBs.

The response curves of the electron-capture detector (ECD) to halocarbons have been fully reviewed [187]. The limitations of the linear range of the ECD are well known and documented, and most workers have attempted to calibrate the detector using the most linear portion of the response curve. However, with the recent improvement in the precision and accuracy of trace CB measurement it is necessary for the calibration curve to be within ± 5% of the true detector response. Under these circumstances it is more appropriate to regard the detector as having a non-linear response and making the calibration accordingly. Single-point calibration does not have sufficient accuracy and bracketing calibration points, covering the upper and lower end of the working range, are only valid if the measurement is made in the more linear portion of the ECD response curve. A substantial proportion of environmental sample extracts contain CBs at the ultra-trace level which only register in the non-linear region of the detector. The measurement of the non-*ortho* and mono-*ortho*-CBs at the ultra-trace level along with the more abundant congeners will span a significant proportion of the ECDs working range. Under such circumstances it is necessary to use a multi-point calibration to reduce the errors which can occur, particularly at lower concentrations where the deviation from lineari-

ty is the greatest [6,188]. With the stability of modern GC instrumentation and detectors it is, a priori, sufficient to establish a multi-point calibration twice a week in preference to using a single- or two-point calibration on a daily basis. The calibration can be verified with each batch of samples (ca. 10) by including one calibration solution at approximately the same concentration as anticipated in the samples. If the check calibrant is >5% from the expected value the detector should be recalibrated. This calibration schedule allows the response–mass profile to be maintained over a wider range without an extensive reduction of sample through-put.

8.1.2. Thermal stability

To maintain the correct level of stability of the calibration the ECD must be optimised at the correct operating temperature and be thermally stable. It must also remain uncontaminated from less volatile material which can gradually elute from the column. There are different detector temperatures mentioned in literature. Lee and Chau [189] use a ^{63}Ni detector at 300°C whereas Johansen et al. [47] prefer higher temperature at about 330°C. Normally, the higher detector temperature (320–340°C) will be sufficient, but when contamination increases the baseline signal of the detector it can normally be cleaned in situ by replacing the make-up gas with hydrogen at a temperature of ca. 400°C for 30 min.

Lower flow-rates for the make-up gas can give a substantial increase in sensitivity, however, the response becomes increasingly non-linear at these low flows [36]. This may not be so obvious from a simple plot of response against mass, but is clear when a response/mass vs. mass plot is made.

8.2. Mass spectrometry

8.2.1. High-resolution mass spectrometry (HRMS)/low-resolution mass spectrometry (LRMS) and mass-selective detection (MSD)

Tuinstra et al. [101] use high-resolution MS (resolution 8000) and the electron-impact (EI) mode to determine the planar, non-*ortho*-CB 77, CB 126 and CB 169 in animal fat. They made novel use of CB 153 as a syringe standard since the endogenous CB 153 is removed during the porous graphitic carbon clean-up. Another possibility is the use of CB 101 as syringe standard [108], [116]. The PFK lock mass at m/z 316.9824 was used to maintain the accurate mass marking and prevent mass drift, but it was also invaluable in determining any potential interferences. The advantages of high-resolution MS in selectivity and sensitivity are well known. However, one disadvantage of this technique is that co-eluting material, although not positively detected by the selected mass, will competitively reduce the ionisation of the determinant within the ion source and so decrease the signal of the CB. When the lock mass is used this interference can be detected as the PFK signal, which should remain constant, also declines.

A similar interference has been reported during a quality check on a calibration solution of the non-*ortho*-CBs [4]. The low resolution quadrupole negative-ion chemical ionisation (NCI)–MS total-ion trace gave a higher variance for CB 169, which was traced to the presence of a phthalate impurity when the solution was re-analysed in the EI mode.

The actual resolution used by an operator of HRMS can vary, for example, from 6000 [47] to 10 000 [44]. In general, HRMS has lower detection limits (low ng kg^{-1} range for planar PCBs) then LRMS [47,136]. Asplund et al. [190] obtained levels in the low μg kg^{-1} range with a MSD system. Himberg and Sippola [191] have used a quadrupole instrument as detector of a MDGC–MS system. They found a detection limit of 0.05 ng kg^{-1} for the CBs 126 and 169.

8.2.2. Electron-impact (EI) or negative- and positive-ion chemical ionisation (NCI, PCI)

The CB intercomparison programme of the Community Bureau of Reference (BCR) between 1982–1985 [8] concluded that, whilst MS gave additional specificity to the identification of the CBs, the measurements were not sufficiently precise for certification purposes. However, recent improvements in MS hardware and sensitivity and selectivity, particularly in the negative-ion chemical ionisation (NCI)–MS [192-194]

mode, have made this detector considerably more amenable to the identification and measurement of CBs at the trace level and with similar precision to the ECD. An overview for the choice of EI-, PCI- and NCI-mode is given by Barceló [192].

The similarity of most CB mass spectra sometimes decreases the specificity of the technique in identifying each congener, particularly when GC peaks are unresolved. Roos et al. [193] were able to identify a second CB with EI and NCI–MS under the peak of CB 138 which could not be separated on the more commonly used phases, e.g. CPSil 5, CPSil 8, or CPSil 19. They tentatively assigned the identity of the peak as CB 163, which has a similar retention time to CB 138 on these columns [19]. This was confirmed by Larsen and Riego [154] after they synthesised the CB 163 and were able to separate these two congeners on a very polar bis-cyanoproplyphenyl phase (SP-2330) and by de Boer and Dao [108]. However, CB 138 also co-elutes with CB 158 on this polar column [26]. These studies have confirmed that most data reported on CB 138 is, in fact, a composite value for CB 138 plus CB 163. Since both congeners are relatively persistent, and relatively insensitive to environmental modification most workers will continue to determine the sum of these congeners until a routine separation is available. According to Haraguchi et al. [195] single-ion monitoring (SIM) can improve the sensitivity by one order of magnitude over the TIM-mode.

NCI–MS has the advantage that many of the CB response factors (R_Fs) are comparable with the ECD. Prior to NCI–MS being available all ECD samples were concentrated, often to very small volumes, for analysis by MS–EI or PCI–MS. This degree of sample concentration had the added disadvantage that impurities became apparent and that other compounds with a similar MS response would mask the CB, making confirmation difficult.

A comparison of the R_F values for 33 CBs obtained by ECD and NCI–MS was made by Wells et al. [196], who used hydrogen as the moderator gas to generate the negative ions with a two-fold increase in sensitivity for the CBs compared with methane. Other advantages of using hydrogen are: (a) a single gas system; (b) a cleaner ion source for considerably longer periods; and (c) a similar carrier gas. Haraguchi et al. [195] found that the NCI-mode was 500–1000 times more sensitive for the analysis of CB-$MeSO_2$ metabolites than the EI-mode. Detection limits for penta- and heptachlorobiphenyl according to Barcelo [192] are also higher for EI (3 pg) than for NCI (0.03–0.5 pg according congener), both in SIM-mode. The NCI–MS R_Fs for the congeners with less than 3–4 chloro atoms are substantially lower than those of the more chlorinated CBs, but the response is not dependent on the number of chlorine atoms alone. Quite small changes in structure can also significantly change the R_Fs. CB 149, 2,3,6,2′,4′,5′-hexachlorobiphenyl had an ECD R_F of 256 which was drastically reduced to 11 by using NCI–MS. By comparison CBs 151, 2,3,5,6,2′5′-hexachlorobiphenyl has the same number of chloro atoms as CB 149, but with the chloro in the 4′ position moving to the 5′ position on the first ring. Here CB 151 has an ECD R_F of 145 and a NCI–MS R_F of 355. In the EI-mode, Martelli et al. [197] found differences in relative response of a factor two within groups of homologue CBs.

Bowadt et al. [198] compared NCI with PCI-modes. They found that the sensitivity of PCBs in NCI increases dramatically within an increase in number of chlorine atoms while the opposite is the case for PCI. So were the detection limits for PCB 28 200 μg/l (PCI) and 700 μg/l (NCI) and for PCB 80 253 μg/l (PCI) and 2.2 μg/l (NCI) in a full scanning run.

8.2.3. Isotope-dilution MS

A great number of studies have made use of isotope-dilution techniques in the determination of planar PCBs [42–44,72,73,199,200]. Patterson et al. [42] used isotope-dilution MS (IDMS) to determine non-*ortho* chloro and *ortho*-substituted CBs in human serum. They synthesised over 50 ^{13}C-labelled CBs normally found in human tissue and determined each CBs using either EI–or NCI–MS. The analytical coefficient of variation was estimated to be between 12–

18% at the parts-per-quadrillion level. Although the recoveries of CB 126 and CB 169 were low, the IDMS technique compensates for this in each sample giving an acceptable estimate of these congeners in the serum. The recoveries for CB 77 were, however, high due to interferences from the carbon column clean-up. Kuehl et al. [43] used IDMS to separate and quantify the co-eluting couple of CBs 77 and 110. Storr-Hansen and Cederberg [72] improved their detection limit from 0.1 mg/g (ECD analysis) down to 0.005 mg/g using the IDMS technique. Finally, van Rhijn et al. [44] used isotope-dilution techniques in order to determine the planar PCBs and dioxins simultaneously. Their method allows the quantification of the interference between CB 169, and 1,2,3,7,8-PCDD as well as between CB 126 and 2,3,7,8-TCDD. The recoveries are low (30–50%), but known.

8.2.4. Ion trap mass spectrometry detection (ITD)

An alternative MS technique is ion-trap mass spectrometry. The operation of the ion trap differs from other MS techniques, especially in the ionisation process, where the ions are trapped electronically, i.e. spatially focused. This process is controlled to obtain sufficient ions for detection. After the ionisation/trapping process, ions are made unstable according to their mass, and the destabilised ions are transferred to the electron multiplier detector outside the trap itself. An ion trap is, in effect, a three-dimensional quadrupole. The ion trap was developed by Paul and Steinwedel [203] and has been described in detail by Dawson [201] and Todd [202]. Nowadays it is usually coupled with gas chromatography. The GC–ITD has been used for CB analysis in biota (Bergman et al. [204]), in mineral oil (Salomon et al. [205]) and for the detection of non-*ortho*-CBs in mustelids (Leonards et al. [206]).

With this technique, it is possible to obtain a full scan spectrum at low concentrations, for CBs in the low-ppb range. The detection limit can be increased by a factor of 2 for most compounds using narrow mass ranges (NMR) instead of using a full scan of the ion trap. This may be compared to a conventional GC–MSD where the detection limits are lowered using SIM instead of a full scan. However, the disadvantage of using SIM mode is that it decreases the overall confirmatory power of the technique. The combination of a low detection limit and the possibility of unequivocal identification of CBs in environmental samples makes ion-trap detection a very useful technique in environmental analysis.

Today, MS–MS is a new option available for the ion trap, which gives a new dimension to the problems involved in CB analysis. MS–MS can resolve interfering isomer pairs of CBs which could not be separated with single MS using a SE-54 type capillary column. Also detection limits can again be lowered using MS–MS instead of single MS.

8.3. Other detectors

8.3.1. Flame ionisation detection

The flame ionisation detector seemed to be an attractive alternative to the ECD for GC analysis of organic compounds [207]. However, the detection limit is far too high (0.5 ppm) to determine organochlorines at a trace level, or planar CBs which tend to occur at ultra-trace levels [208]. Guenther et al. [169] used it as their monitoring detector (after the first column) in a MDGC system which seems to be a more realistic and satisfying application.

8.3.2. Atomic emission detection

A great number of compounds can be identified, after GC separation, by atomic emission detection [118]. This multi-element detector can include N, P and especially halogens like Cl and Br for the simultaneous determination. Müller and Camman [209] have detected CB 77 in synthetic mixtures and have examined Clophen A60 mixtures. The source uses a microwave-induced plasma and a special interference filter to improve the sensitivity.

8.3.3. GC–Fourier transform infrared detection

Several laboratories evaluated the possibility of using infrared spectrometry as GC detection. Fuoco et al. [210] found that FT-IR is a suitable

detection technique after pre-separation on a PGC column. They have a detection limit below 0.1 μg/kg.

8.3.4. Spectroscopic detection techniques with liquid chromatography

Liquid chromatography is predominantly used as a tool in the clean-up procedure for the analysis of PCBs. The separation capabilities and lower sensitivity of LC systems make their application in the determination of CBs less attractive. More common applications involve the quantification of "total PCB" as biphenyl after a dechlorination step (e.g. Kuchen et al. [211]). Two LC systems with spectroscopic detection techniques have been described, however, which appear to be selective and sensitive for non-*ortho*-CBs. The selectivity arises from differences in the electronic properties of *ortho* and non-*ortho*-CBs. The singlet ground state and lowest triplet state of the biphenyl moiety have preferably a planar conformation. *Ortho*-substituted CBs have a non-planar configuration with a higher freedom of rotation about the central C–C bond, which results in a relatively short lifetime and higher reactivity of the lowest excited triplet state as compared to the non-*ortho* planar CBs. The difference in lifetimes of the lowest excited triplet state is utilised.

The first approach involves sensitised room temperature phosphorescence in liquid solutions (RTPL). The eluent contains biacetyl. The CBs are excited with λ_{ex} ~260 nm and reach the lowest triplet state through intersystem crossing. Upon collision with biacetyl molecules energy transfer takes place resulting in RTPL of this compound. Limits of detection in the order of 0.5 ng absolute have been reported for a number of non-*ortho*-CBs (Donkerbroek et al. [212]). Recently, a detection technique employing dioxetane chemiluminescence following photochemical in-line generation of singlet oxygen has been described (Niederländer et al. [213]). CBs are brought to lowest triplet state. In this state, they react with oxygen (triplet ground state) to produce singlet oxygen. The latter reacts with 1,2-diethoxyethene to produce the 3,4-diethoxy-1,2-dioxethane. This compound decomposes thermally under generation of chemiluminescence. An LC system consisting of a PYE column for separation and the chemiluminescence detection described is reported to have detection limits of 0.7, 1.6 and 3.3 ppb for CBs 77, 126 and 169, respectively. These limits are comparable to those reported for EI–LRMS. The method was capable of detecting CBs 77, 126 and 169 in herring oil. Unfortunately, no quantitative data were reported, so that the accuracy cannot be assessed. It is concluded that LC coupled with spectroscopic detection techniques have perspectives for non-*ortho*-CBs, but that more research is required to validate these methods.

8.4. Critical evaluation for final determination methods

The "classical" GC–ECD system is capable of producing accurate and precise data provided that optimised clean-up and separations are carried out. Mass-spectrometric detection is applied increasingly, which combines high sensitivity and selectivity with unequivocal confirmation. It should be noted, however, that in general 50-m columns are used for analysis with GC–ECD and 25 m for GC–MS. The shorter lengths are often selected for GC–MS due to the increase in background resulting from column bleed, however, with stable GC phases longer columns can still be used without decreasing the LOD. This implies that the higher selectivity of mass spectrometry is partially compromised by the lower separation. This problem may be overcome by the use of MS–MS.

NCI–MS proves to be a highly sensitive technique for CBs with, depending on the structure, more than 4 Cl atoms. It combines a sensitivity comparable to or better than ECD with the possibility of confirmation.

GC separation and detection with ECD or MS techniques are the method of choice for routine analysis, in particular for non-*ortho* compounds. All other detection principles dealt with are not recommended for routine applications, owing to poor selectivity or sensitivity and/or insufficient validation.

9. Multi-residue methods

The environmental analytical chemist is placed somewhat in a dilemma. The cost of sampling, and, in many cases, the irreplaceable nature of the samples makes it imperative to obtain as much chemical information as possible from the material collected. Inevitably, samples will be obtained for specific chemical measurements, but in addition to the primary objectives, other qualitative information can prove invaluable. However, the wide angle analytical approach, which continues to add compounds to a single scheme, will ultimately lead to conflicting methodological requirements and an unacceptable level of compromise. As a result, two types of sample preparation schemes have emerged which reflect the extremes of this approach. Firstly, the highly specific method, for the determination of a single or very small group of similar compounds, and secondly, multi-residue schemes which cover the preparation and separation of the maximum number of compounds possible for broadly based chemical information.

At present the serial "on-line" approach is difficult to fully incorporate into the multi-residue scheme [70] in which a large number of compounds are separated into groups and determined in parallel. The advantages of the multi-residue method are: (1) an extensive analysis of expensive and sometimes irreplaceable samples, especially those taken from remote sites, e.g. open ocean or from specific experiments; (2) correlation of data of different determinants within a single analysis to reduce variability; (3) the reduction of analytical effort at the sample preparation stages.

The determination of polychlorinated dioxins and furans is probably the nearest to the analysis of planar PCBs. As the molecules are considered to be structurally very much the same (they are iso-steric), the extraction and clean-up procedures are the same and only the final separation or fractionation techniques differs for each group of compound. The study of van Rhijn et al. [44] is an example for the simultaneous determination of planar PCBs and PCDD/Fs.

Paasivirta et al. [214] specifically examined planar polycyclic aromatic compounds (PCB, PCDD/F, PCBz, PCN, anthracenes, fluorenes and fluorenones) separated according to their LC elution pattern. Sericano et al. determined planar PCBs in oyster [215] and dolphin blubber [134] along with PAHs. Wise et al. [107] also determined PAHs and PCBs using one analytical procedure. The recovery of the PAH determination with GC–MS (some extraction and partly some clean-up as PCBs) is compared to another method (only applied for PAHs) and very similar results were found.

Bergqvist et al. [216] examine chlorinated phenols, pesticides, PCBs and PCDD/Fs in one procedure, with an aliquot of the homogenised sample taken for the analysis of volatile compounds. Tarhanen et al. [217] try to retain the volatile compounds in the sample by grinding the sample with Na_2SO_4 for homogenisation and drying for 2 d at room temperature. On the contrary, Zebühr et al. [83] have an extraction method where the sample has not to be dried and thus the volatile chlorinated phenols can be analysed with the bulk of less volatile compounds.

Krahn et al. [218] have developed a multi-residue scheme for the determination of OCs and PAHs in sediment and biota. In this scheme, the preparation is semi-automated with GPC to separate the biogenic material from both the PAHs and the OCs and the sulphur in the sediment samples. The faecal sterols were separated and cleaned up with an amino-cyano HPLC column prior to derivitisation with bis-(trimethylsilyl)trifluoro-acetamide. The previous manual scheme used alumina and silica clean-up columns, which took considerably more time.

Bandh et al. [150], finally developed a semi-automated method which involves 2D-HPLC with a nitro-column and a pyrenyl-column. After Soxhlet extraction with Dean-Stark-trap, the toluene extract is reduced in volume and passed through a 10% deactivated silica-gel column. The HPLC system separates several fractions. The first fraction contains aliphatics and monocyclic aromatics. The second fraction contains PCBs, PCNs and PCDD/Fs and is further separated by the pyrenyl column.

10. Quality control and method validation

Quality control for the analysis of CBs has improved over the last 10–15 years [13–16]. After the initial development of an analytical method, it is necessary to validate each stage of the process. Many factors influence the analysis and the subsequent quality of the data from the source of calibrants and calibration to the maintenance of instruments and management of the quality of each step. Schemes for the assurance of the quality of analysis have been developed. A good example for an extensive validation of laboratories and methods and the quality of those has been given by Rymen et al. [219] for dioxin and by the analysis of CBs [13,17].

10.1. Application of quality control methods

Most of the principles of quality control and quality management are well established (Taylor [220]). However, in most cases it is a question of application of these techniques and a broad based education of those undertaking the analysis.

Wells et al. [221] showed the need for continuous assessment schemes, learning and communication programs as well as additional steps which lead to the preparation of certified reference materials. Wells and Kelly [222] reported progress in the quality assurance of environmental trace organic analysis and outlined the significance of laboratory control for marine monitoring programs. On behalf of the International Council for the Exploration of the Sea, de Boer et al. [14,15,156] undertook a series of stepwise learning exercises for the improvement of the measurement of CBs in marine media. This approach has been continued (Wells and de Boer [13]) in the EC-QUASIMEME project. These include the mono-*ortho* congeners CBs 118, 105, 156. Further intercomparison exercises for the measurement of non-*ortho* and mono-*ortho*-CBs in fish oil have also been undertaken (de Voogt et al. [17]). The between-laboratory variance for these exercises was 40–60% for the non-*ortho*-CBs. Within the recent QUASIMEME project the between-laboratory agreement for the mono-*ortho*-CBs (CBs 118, 105, 156) has improved significantly.

10.1.1. Sources of error in the analysis of PCBs

The sources of error for the determination of the mono-*ortho*-CBs and non-*ortho*-CBs are quite similar with the exception that the difficulties in analysis are magnified at the ultra-trace level.

The primary requirement is for pure, certified calibration materials. Where these are not currently available, a source of well characterised materials of known purity (Promochem GmbH, Wesel, Germany) should be used. Some of the major errors occur during preparation, storage and checking of standard calibration solutions [6]. The following points have an essential influence on the quality of the preparation of a calibrant in solution:

–The use of pure, certified or well characterised solids.

–Calibration of the balance prior to the preparation of standard solutions, and control of the balance during use.

–Minimise external influences on the balance (sunlight, heat, draft).

–Minimisation of electric charges [6].

–Only use solvents of known, tested purity and check ($\times 100$ concentration by ECD or MS).

–Check all new solutions in intercomparison or prepare a second, independent solution.

–Control the calibration solution mass by mass and not mass by volume.

–Use iso-octane as solvent.

–Use moisture resistant labels or permanent markers, not paper labels.

–Store calibration solutions at 0–5°C in sealed ampoules when possible.

After preparation the solutions should be ampouled under inert gas (e.g. argon) and stored in a cool (0–5°C) and dark place. Working solutions can be stored in conical flasks and with care; the weight loss should be limited to a maximum of 2% over a six to nine month period. Weight losses due to evaporation of solvent in the refrigerator or at the bench must

be monitored and an adjustment made by addition of solvent. However, it is preferred that working solutions are also ampouled at the required strength to minimise such losses. Wells [4] has reported the main sources of errors in calibration and determination which is shown in the Table 11.

To overcome much of the variability in the analysis many workers use ^{13}C-labelled homologes as internal standards for the determi-

Table 11
Summary of the main sources of error in the determination of CBs in environmental matrices

Problem	Symptom	Remedy
Calibration	Accuracy is concentration dependent	Use multi-point calibration to mirror detector response. Check optimum make up gas flow for maximum "linearity" not maximum sensitivity.
Inaccurate calibration solution	High/low bias in intercalibrations	Prepare independent calibration solutions and double check with a second laboratory.
Calibration solution evaporation	Calibration response increases with time	Control calibrations solutions by weight. Use new, ampouled solutions.
Impure calibrants	Multiple peaks in single calibration solution	Use certified calibrants.
	Bias from low calibrant response	Check all calibration solutions by FID as well as ECD.
Internal standards	All determinands have lower or higher values than expected	Incorrect addition of internal standard. Only use compound that do not occur in samples.
Poor GC resolution	Shoulders, unresolved doublets or single peaks. Results biased high	Use 50 m narrow bore (0.22 mm I.D.) columns. Check peak purity with a second/third column, MS and/or MDGC. Optimise carrier gas flow-rate. Only use He or H_2.
High mass discrimination	Tail-off of sensitivity with retention time	Clean splitless injector. Optimise split temperature/time. Use on-column injection.
Co-extracted material	Noisy baseline Negative peaks	Improve clean-up. Sulphuric acid and/or aminopropyl HPLC clean-up.
	Interference from other OCs	Use RP-HPLC and PGC or pyrenyl HPLC to remove PCNs, PCDD/Fs and CBs.
Poor recovery	Low or varied results	Check and validate method. Use surrogate recovery standards CB 53, CB 189, ^{13}C-labelled CBs.

Reference: D.E. Wells [4].

nation of the planar, non-*ortho*-CBs, in particular CBs 77, 126 and 169. The advantages of IDMS are well known and have been used to great effect in the closely related analytical field of dioxin analysis [44,200,224,225]. This technique reduces the error associated with false positive values which can occur at the ultra-trace level, but it may not be essential where there has been adequate clean-up and separation of the non-*ortho*-CBs, e.g. HPLC-PYE or hypercarb column. When the extract is sufficiently clean the ECD may be more advantageous in terms of ease of use and stability.

GC-optimisation has been studied in intercomparisons [14,15]. More recently, the GC–ECD system has been examined by Megginson et al. [188] and criteria for optimisation of the gas chromatograph and the detector have been reported on a very practical level.

An expression of mono- or non-*ortho* substituted CBs in terms of PCDD toxic equivalent factors is still very vague and can be a source of error in further calculation or interpretation. de Boer and Brinkman [28] showed that a 400% error can be introduced into data for these CBs by applying the toxic equivalence values proposed by different authors. Reports should contain the original CB raw data to permit further study.

According to Wells et al. [8] validation of a new method should include an intercomparison of extraction efficiencies and clean-up steps and an intercalibration of the whole procedure. It is not usually sufficient to develop a method in one laboratory without a substantial independent verification of the technique. This is especially true of the determination of CBs at the ultra trace level.

As an alternative to the intercomparison exercises it would be possible to use a suitable reference material, but currently only non-planar CBs are certified in environmental matrices.

11. Conclusions and recommendations

The following conclusions are made as a result of a number of careful studies on this field by the authors and a number of other workers.

The initial difficulty in determining non-*ortho*-CBs is the amount of sample required to obtain a sufficient quantity of the analyte. Such requirements on sample size may place restrictions on the extraction method, e.g. SFE. Conversely, the sample itself may be limited, e.g. viscera from small fish.

Generally it is better to reduce the final sample volume and make the final determination on as much of the extract as possible, e.g. 1 μl from 10 μl, or on a higher injection volume, e.g. by PTV injection.

While this makes the best use of the sample it is essential to remove all traces of lipophilic material. Whichever method of clean-up is used it is essential to check the efficacy of the lipid removal, for example by GC–FID.

Pure crystalline solid CBs should be used to prepare the calibration solutions. Any solution purchased for calibration purposes should be checked against known and verified standards. This applies both to the ^{13}C-labelled and non-labelled congeners. The calibration solution should be cross-checked by independent analysis through an interlaboratory exchange scheme or through a Laboratory Testing Scheme. The between laboratory agreement for calibration solution should be $< \pm 10\%$.

A multilevel calibration of both the ECD and MS should be used. The detector should be regarded as essentially non-linear and the calibration should not be extrapolated beyond the calibration range. This will help to reduce serious errors which are known to occur at this stage. The ^{13}C-labelled CBs should be used wherever possible as standards for the determination of the non-*ortho*-CBs which occur at much lower concentrations. This will not only account for instrument fluctuations, but for the recovery of the whole method. The CBs should be separated not only from co-extracted materials but from each other prior to detection. This is most usefully achieved either by HPLC prior to GC using a PYE or PGC column or by MDGC using two columns with very different separation characteristics.

Data obtained using the ECD should be verified by MS, at least for some samples of each batch. All measurable traces of lipids should be

removed prior to the final GC detection and preferably prior to group separation on the PYE or PGC–HPLC. This can be done by checking the sample using GC–FID. Excess lipids will only serve to degrade the capillary or HPLC column, contaminate the detector and compromise the chromatographic resolution. Both instruments and results suffer as a result.

The analysis of CBs is sufficiently complex to consider the determination as a separate scheme rather than part of a wider multi-residue programme. The determination of each congener should not be compromised by lack of separation into the required fractions or by a method that is unsuitable for other determinants, e.g. not using concentrated sulphuric acid if dieldrin is included in part of the scheme. Two separate fractions for the different analyses can easily be made at the post extraction stage or by undertaking separate extraction. The maximum number of CBs should be measured in any one scheme. Focusing on a small group of congeners such as the non-*ortho*-CBs in isolation is not recommended. This gives a significantly more useful data base both for monitoring and research purposes relating to biological effects. This approach will also assist in the overall improvement of measurement of CBs since attention is paid to all aspects of the analysis for a wide range of congeners. Confirm the validation of the method by including a quality control programme in the laboratory and by participating in a national or international laboratory testing scheme. If this is not available then a less formal interlaboratory sample exchange scheme can provide participants with some positive feedback on the quality of the measurement made.

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