CHROMSYMP. 2829

Chromatographic techniques in accurate analysis of chlorobiphenyls

N. Kannan*, G. Petrick, D. E. Schultz-Bull and J. C. Duinker

Institut für Meereskunde, Universität Kiel, Düsternbrooker weg 20, W-2300 Kiel-1 (Germany)

ABSTRACT

Accurate congener-specific determination of chlorobiphenyl congeners (all 209 congeners) is finally possible with the use of multidimensional gas chromatography–electron-capture detection techniques. The effectiveness of this technique for environmental analyses is enhanced by ultraclean laboratory practices, non-destructive extraction and clean-up steps and the use of low-volume, high-efficiency HPLC separation for various classes of organic contaminants. In the light of these new developments conventional procedures for chlorobiphenyl analysis are evaluated.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are anthropogenic compounds. Their presence in the environment has been studied in great detail for several reasons.

(1) Many representatives of this class of compounds (chlorobiphenyls; CBs) are persistent. Their ubiquitous presence in the environment has been demonstrated by the last four decades of research [1].

(2) Laboratory studies using *in vivo* and *in vitro* bioassays indicate that several CBs are inducers of drug-metabolizing enzymes, being able to affect various physiological processes such as reproduction, embryonic development, carcinogenesis and hormone- and vitamin-related control systems of these physiological processes [2].

(3) Several of these toxic symptoms noticed in laboratory experiments in animals have also been observed in victims of PCB poisoning and in occupationally exposed workers [3].

(4) Marine health programmes utilize biological indicators such as mussels to judge coastal contamination by CBs. It is proposed that "mussel watch" could be an early warning system for ecological catastrophes [4].

(5) CBs are even used as chemical tracers for observing certain biological phenomena such as population structure in marine mammals and migration pattern of birds and other animals [5].

(6) PCB mixtures are composed of many individual constituents (of all 209 CB congeners). These cover a large range of chemical and physicochemical properties in a systematic way [6]. Their distribution in the environment has been interpreted in theoretical models, involving molecular properties [7]. These models can be used to forecast the environmental behaviour of other less well studied compounds.

All the above-mentioned approaches, in one way or another, depend on reliable, accurate and unambiguous measurement of CBs in the material of choice.

PCBs differ in their physicochemical characteristics as well as in their toxic potencies. For example, non-*ortho* chlorine-substituted congeners such as 3,3',4,4'-tetrachlorobiphenyl (IUPAC No. 77), 3,3', 4,4',5-pentacholrobiphenyl (126) and 3,3',4,4',5,5'-hexachlorobiphenyl (HCB, 169) are potent inducers of enzymes, being far more toxic for certain biological end-points than other congeners [1]. On the

—————————
* Corresponding author.

other hand, lower chlorinated biphenyls (di- and trichlorobiphenyls, for example) and *ortho* chlorine-substituted congeners are more potent inhibitors of dopamine, an important neurotransmitter, than other congeners in pre-human primates [8]. The composition of PCBs in biological tissues and other environmental matrices differs greatly from the original source, *i.e.*, commercial PCBs, for various reasons such as metabolism, physical weathering etc. [9]. Taking into consideration such factors, the need for congener-specific analysis of CBs was stressed in the beginning of the last decade [10].

Although the use of high-resolution single-column gas chromatography (HRSCGC) is essential to reach this goal, no single column available can separate all 209 CB congeners. The use of mass spectrometry (MS) detection techniques to solve co-elution problems [11] may be not foolproof as fragmentation of co-eluting congeners with higher mass can generate interfering signals [12,13].

Mixtures of chlorobiphenyl can be analysed un-ambiguously in terms of the individual CBs by multidimensional gas chromatographic (MDGC) techniques [in combination with electron-capture detection (ECD)] [14–16]. Applying several thousand of "heart-cuts", the composition of a range of commercial PCB mixtures was determined [16,17]. Additional problems arise when "PCB" fractions of environmental samples are studied. Appropriate extraction and clean-up procedures are essential to minimize the presence of interfering compounds. We have developed an HPLC technique that is effective for biological tissues and other environmental matrices [15].

MDGC–ECD has been successfully used in the direct analysis of toxic non-*ortho* CBs, as they are usually present in very low concentrations; moreover they co-elute with other CBs that are present at considerably higher levels. Other methods have been suggested and applied for the analysis of this class of CBs, *e.g.*, various charcoal enrichment techniques [18]. The application of MDGC-ECD has shown some inherent weakness in the latter pre-GC separation techniques [19].

In view of these developments, it is highly desirable to sum up the procedures that are used for the accurate measurement of CBs. The current paper deals with important topics in CB analysis, such as clean laboratory practices (including solvent purity and blank problems), sampling, extraction, clean-up and pre-GC separation of environmental contaminants in column chromatography and quantitation of CBs.

MATERIALS AND METHODS

Since our laboratory procedures are directed towards meeting the severe requirements of MDGC–ECD, a brief description of this technique is given first.

MDGC–ECD

MDGC allows a selected small fraction of the eluate of a high-resolution capillary column to enter a second capillary column with different characteristics for further separation. Thus, co-eluting compounds can be separated on the second column. The columns are in separate ovens and connected to separate detectors. The one connected to the first column is referred to as the "monitoring detector"; the other is the "main detector". Transfer of the selected fraction is regulated by a pneumatically controlled, valveless "live T-piece" unit. This is achieved without loss and dead volume problems. The system is optimized by regulating two gas flows in such a way that maximum and zero signals are obtained on the monitor and main detectors, respectively, when the flow is directed through the monitor detector and maximum and zero signals are obtained on the main and monitor detector, respectively, when the "heart-cut" is made.

A Siemens SiChromat-2 MDGC-ECD system is used. The first column is a 0.25-μm fused-silica SE-54 (25 m × 0.32 mm I.D.) column. The second column is a 0.25-μm fused-silica OV-210 (25 m × 0.32 mm I.D.) column. The gas pressure (hydrogen) was maintained at 0.8 bar on the first column and at 0.4 bar on the second column. Temperature programming conditions were: first column 140–250°C at 4°C min^{-1}, 12 min at 250°C; second column at 170°C until 20 min after injection, then increase to 240°C at 4°C min^{-1}.

MDGC–ECD is very sensitive and selective for CB components. Usually, the main detector is at least 50 times more sensitive than the monitor detector because the column load is small and the sample is pre-cleaned. The normal detection limit for CBs in this detector is 0.01 pg. MDGC–ECD

determination has certain advantages over MS detection. For example, owing to high-resolution separation combined with high-sensitivity ECD, even congeners with low relative contribution to a peak are determined with a better accuracy than is attainable by electron impact (EI) MS (e.g., CB-77 and -110). For similar reasons, congeners with the same number of chlorine atoms are separated and measured accurately in MDGC–ECD, which is not possible in single-column EI-MS.

The high sensitivity and selectivity of the MDGC–ECD technique as well as the extremely low levels of CBs in some of the samples (e.g., open ocean waters [20,21]) have forced us to apply severe measures to guarantee the integrity of samples with respect to the analytes. These are related to the quality of solvents, the effect of the atmosphere and pre-GC separations to eliminate interfering compounds.

It is a regular practice in our laboratory to screen every sample extract by GC–flame ionization detection (FID) and single-column GC–ECD, prior to MDGC–ECD analysis.

Reagents

The use of an MDGC–ECD system demands extremely pure solvents. Commercial products do not normally meet this requirement. Solvents are distilled under a nitrogen blanket without contact with laboratory air; in most cases two cycles are required. Distilled solvents are stored in glass ampoules (250 ml) sealed under a nitrogen atmosphere.

Laboratory chemicals such as chromatographic alumina and sodium sulphate are purchased commercially, cleaned overnight in organic solvents in a Soxhlet extractor and activated/dried. The clean materials are stored in clean and sealed glass ampoules; alumina in 2-g batches. Prior to their use with samples, their purity and activity is routinely checked in blank procedures.

Extraction and clean-up of environmental samples

A 2–3 g aliquot of biological tissue is homogenized with sodium sulphate and extracted in a special Soxhlet type of extractor [22] with 150 ml of 10% water in acetonitrile for 6 h. CBs and other contaminants are re-extracted from this solvent three times using 100 ml of double-distilled hexane. An aliquot is used for lipid determination.

Alumina column chromatography

The hexane extract is reduced in volume using a rotary evaporator in a nitrogen atmosphere. A 2-g aliquot of deactivated (10% with distilled water) alumina is packed on a glass column (8 mm I.D., 130 mm high, with solvent reservoir at the top) using n-hexane, and topped with sodium sulphate. The sample extract (ca. 200 μl) in hexane is transferred to the column and eluted with 12 ml of n-hexane under nitrogen pressure. This allows the separation of lipids from other organics.

HPLC clean-up

Sample extracts after alumina clean-up are reduced further in volume (ca. 100 μl) in a rotary evaporator in a nitrogen atmosphere. HPLC is carried out with a Constametric III pump with a Rheodyne injector on a stainless-steel column (200 × 4 mm I.D.) packed with Nucleosil 100-S, at a flow-rate of 0.5 ml/min. Details of the fractionation are given elsewhere [15]. The volume of the second HPLC fraction containing CBs and p,p'-DDE is reduced under nitrogen to ca. 50 μl and sealed in specially prepared glass microvials until analysis by MDGC–ECD.

RESULTS AND DISCUSSION

Most aspects of recent methodology for analysis of chlorobiphenyls are based on methods originally developed for packed-column GC–ECD analysis. They have been modified and refined in many respects to meet the requirements of capillary column GC–ECD. The use of MDGC–ECD has only further fortified this requirement. We have found some inherent problems associated with some of the original extraction, clean-up and chromatographic procedures. Great emphasis has also been placed in recent years on the measurement of certain toxic CB congeners at extremely low levels previously never attempted [12–14,23–25]. It is important to check whether conventional procedures are good enough for today's demands. Some experiments conducted in our laboratory to answer this question revealed that existing procedures require modifications. The results and comments are given in the following sections.

CB contamination through laboratory solvents

In the process of extraction, clean-up and GC–ECD analysis of environmental samples, solvents such as dichloromethane (DCM) are used in 200–500-ml volumes; these are concentrated to 2–100 μl before final determination in GC–ECD or GC–MS. Contamination originally present in the solvent is concentrated to possibly unacceptable levels. This can also occur when solvents are exposed to the atmosphere for longer periods.

Fig. 1 is a GC–ECD chromatogram of 100 ml of dichloromethane after HPLC clean-up. This solvent had been transported in a car in a capped bottle on a long drive during a pine needle-sampling exercise. The level of CB contamination that occurred in the solvent during the drive is unacceptable, especially when pine needles are used as indicators of atmospheric contamination [26]. Similar problems were noticed when DCM was transported on ships during research cruises. The significance of this factor in the analysis of water has been described before [20,21]. This problem can be solved by storing solvents under a nitrogen blanket in sealed ampoules, after distillation under nitrogen.

CB contamination from laboratory chemicals (solids)

Alumina or silica gel is often contaminated with compounds that interfere on the analysis of CBs. When activated in a drying cabinet, it becomes a very efficient sorbent for all vapours in the oven atmosphere. Thus, Soxhlet extraction and subsequent reactivation in a not perfectly clean oven may result in a product that is even more contaminated than before the intended purification. Drying, reactivation under vacuum and storage in sealed glass ampoules in small units (2–3 g of alumina) are recommended.

Alterations in CB concentration/composition during acid or alkali treatments

It is a practice in many laboratories to use strong acid or alkali to remove chemicals that interfere in CB analyses. We have checked whether such treatments maintain the integrity of CB residues in biological matrices. Four different tissues from a harbour seal (*Phoca vitulina*) with high (blubber), medium (liver) and low levels of CBs (brain and blood) were considered for this experiment.

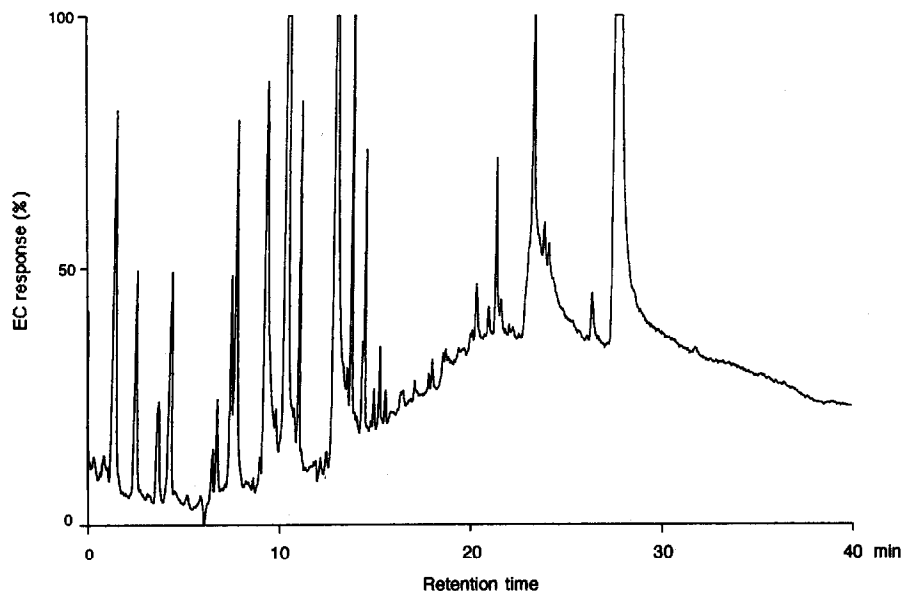These tissues were processed under completely

Fig. 1. A blank chromatogram of solvent dichloromethane. This solvent was carried in a closed glass bottle in a car for over 1000 km during pine needle sampling. A 100-ml portion of this solvent was tested after flash vacuum evaporation (under nitrogen) and HPLC clean-up. The HRGC–EC chromatogram shows air-borne contaminants which could interfere in the quantitation of CBs.
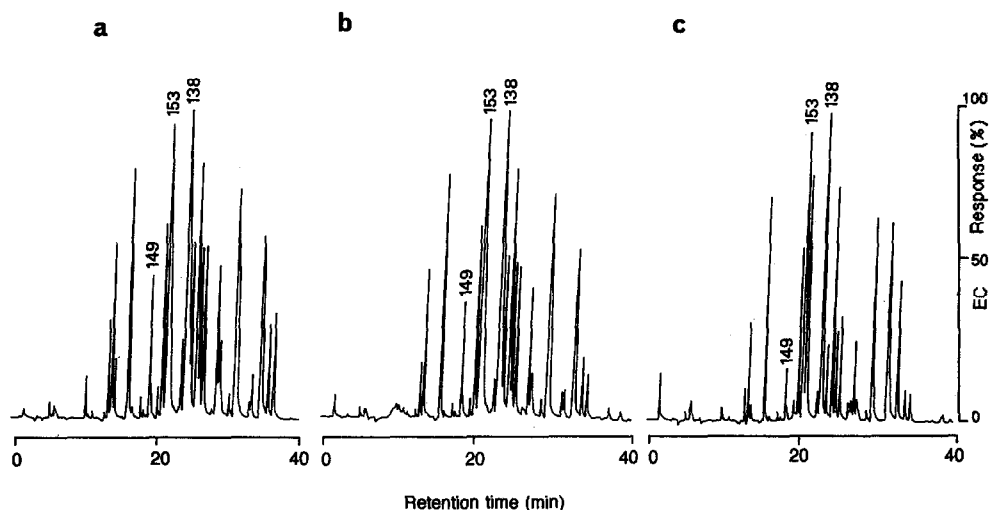
Fig. 2. HRGC–ECD: chromatogram of blubber (harbour seal) extract. (a) Processed according to our non-destructive methodology (see Materials and methods section for details). (b) Sample initially digested in 0.5 M ethanolic potassium hydroxide for 1 h, extracted and processed as in (a). (c) The extracted sample was treated with chromic acid, re-extracted and processed as in (a). Three CBs are identified by their IUPAC numbers as "markers".

non-destructive procedures as practised in our laboratory. Another batch was digested in 0.5 M ethanolic potassium hydroxide at 80°C for 1 h. These samples were then extracted and processed as the first. A third batch was extracted like the first one; the concentrated extract was treated with chromic acid. After re-extraction of CBs in hexane the sample was processed like the first batch. The CB compositions in all these samples were determined by HRGC–ECD.

The chromatograms for blubber were very similar (Fig. 2). Three persistent congeners are labelled as markers. The ΣCB concentrations in samples are in the μg/g level. Apparently no dramatic changes are visible at these concentrations.

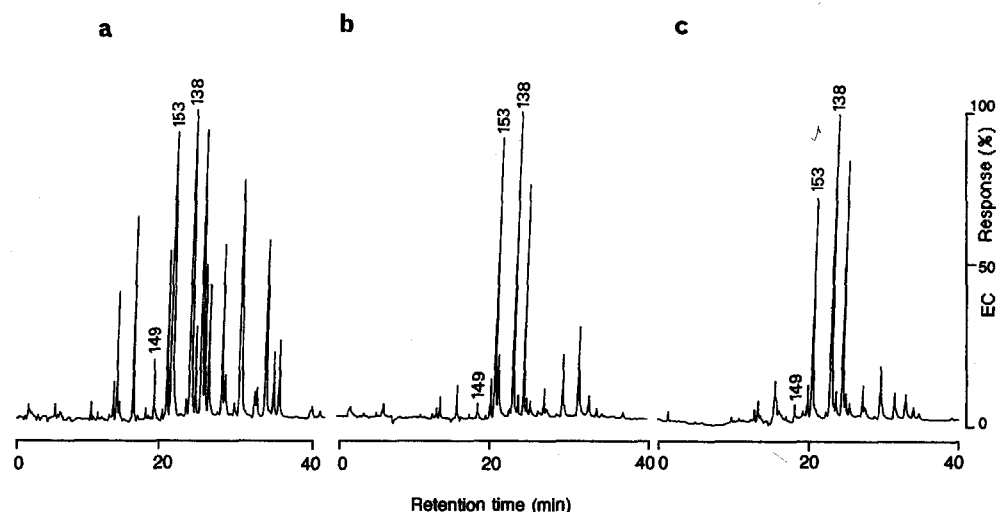Fig. 3 shows chromatograms of treated and

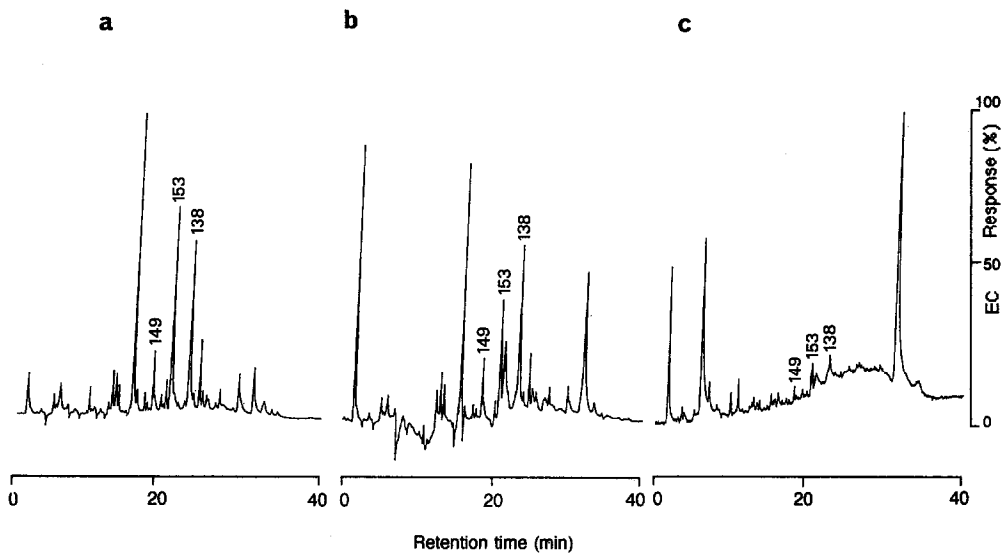Fig. 3. HRGC–ECD: chromatogram of the liver of a harbour seal. For explanation, see legend to Fig. 2.

Fig. 4. HRGC–ECD: chromatogram of the blood of a harbour seal. For explanation, see legend to Fig. 2.

untreated liver samples at ng/g ΣCBs per g of lipid. Several congener peaks that appear in Fig. 3a are missing from the samples treated with acid and alkali. There is no drastic difference in the composition of marker CBs between these samples.

Concentration (ΣCBs) levels in blood and brain were 300–500 pg/g. The composition of CBs in these low-concentration samples is affected drastically by harsh treatment with akali and acid (Figs. 4 and 5).

Chromic acid seems to affect the CB composition in blood severely (Fig. 4c). On the other hand, potassium hydroxide treatment affected the CB composition in seal brain (Fig. 5b).

In many cases, tissues with high CB concentration levels such as blubber and adipose tissue have been selected for analysis to characterize body levels. Measurement of CBs in blood is increasingly popular in human health programmes [24,27], and brain
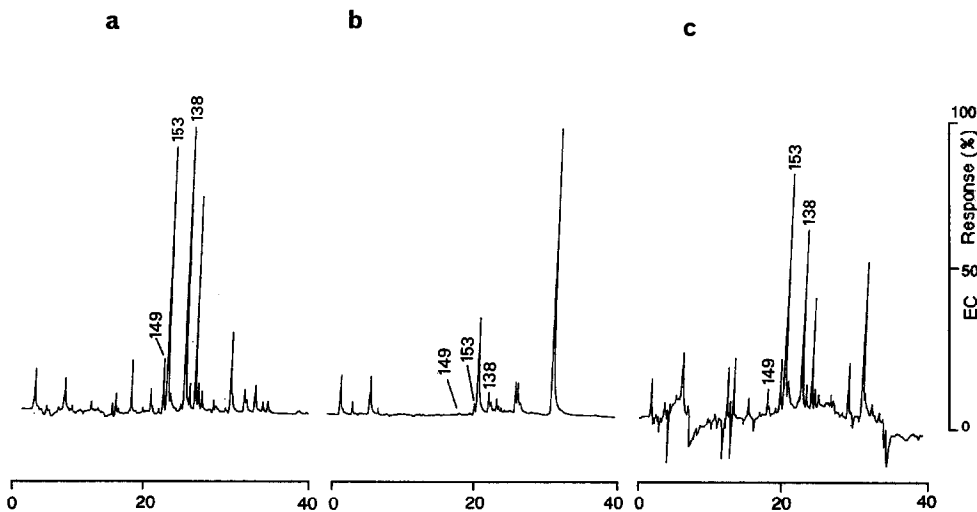

Fig. 5. HRGC–ECD: chromatogram of the brain of a harbour seal. For explanation, see legend to Fig. 2.

tissue is studied frequently as a measure of the effect of CBs on animal behaviour [8]. The high sensitivity of ECD for the detection of CBs requires only small amounts of sample (typically 2–5 g). The total amounts of CBs in such samples will be in the range obviously affected by alkali and acid treatments.

This effect may remain unnoticed in experimental protocols using spikes at levels that exceed natural concentrations (radiolabelled standards or otherwise). Moreover, standards that are added prior to or after the extraction of the sample may not represent the original integrated status in which contaminant and biological/environmental matrix exist. There are reasons to believe that the effect of saponification or acidification on CB composition could be matrix specific. For example, Van der Valk and Dao [28] noticed that degradation of CBs and HCB occurred during alkaline saponification at 90°C of an extract from sewage sludge. It was reasoned that very small particles originating from the sludge could have catalysed the degradation. Similar degradation to CBs was noticed in sediment samples in our laboratory.

There is an additional reason to believe that environmental sample matrix can trigger composi-

tional change in CBs when treated with acids or alkali [29]. When a standard solution containing pure CB congeners (IUPAC Nos. 110, 77, 129, 126, 202 and 156, in the order of elution in a SE-54 column) at the 10-pg level was treated with chromic acid, the chemical loss was minimal. However, we noticed during heart-cuts that chromic acid introduced impurities (oxidation products?) that co-eluted with CB-126 and CB-156 (Fig. 6). This phenomenon cannot be detected in single-column (SC) GC–ECD analysis, resulting in a large over-estimation of CB-126 and CB-156. Similar effects are anticipated in the use of strong oxidizing agents such as fuming sulphuric acid and concentrated sulphuric acid.

Problems in the measurement of non-ortho chlorine-substituted CB congeners

The importance of measuring aryl hydrocarbon hydroxylase (AHH)-inducing CB congeners (especially CB-77, -126 and -169) in environmental samples was realized from the mid-1980s. A charcoal enrichment method was proposed for these congeners during the 1986 Dioxin conference [18], and preliminary results on their levels in human and
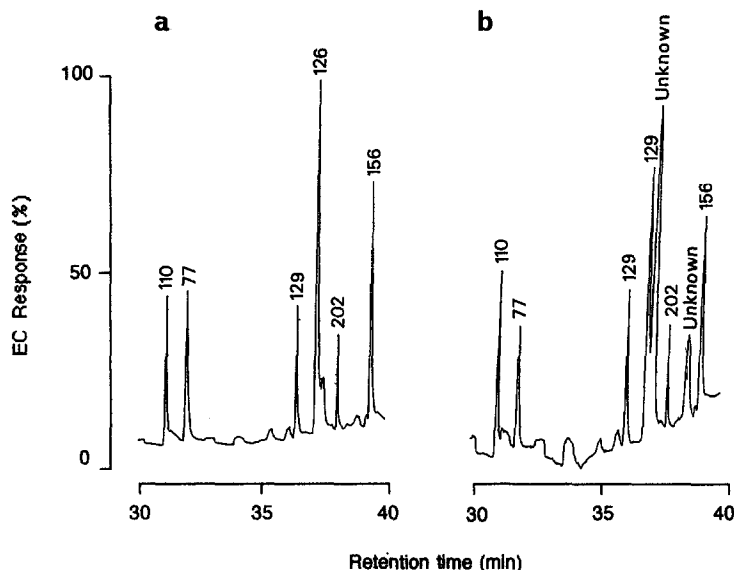

Fig. 6. MDGC–ECD chromatogram of three "heart-cuts" performed for the separation of CBs 77/110; 126/129; 202/156 and co-eluting impurities. (A) Approximately 100 pg standard mixture of toxic CBs. (b) The same after chromic acid treatment. Acid treatment introduced co-eluting impurities/byproducts, which could cause overestimations of CB-126 and -156 in single-column GC-ECD.

animal tissues were reported [30]. A more direct approach to measure these congeners with the use of MDGC–ECD was developed shortly afterwards [14]. Subsequent publications from these two groups revealed the occurrence of non-*ortho* CBs in commercial PCBs [16,31], in sediments [32], in water [20,21,33], in human tissues [34,35] and in wildlife [23,36]. A literature survey shows that at least 50 publications have been devoted to the analytical chemistry of these toxic CB congeners in the last 6 years [37]. Also, dioxin researchers are interested increasingly in these compounds, which resemble 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (TCDD) in their mode of biological action.

The analysis of these congeners is hampered by their extremely low concentrations in the presence of co-eluting congeners at much higher concentrations [16]. Only a few methods for the direct measurement of non-*ortho* CBs are known. These are MDGC–ECD [14], GC–GC–MS [38,39] and GC–NCI-MS (negative chemical ionization) [13].

The possibilities of MDGC–ECD have been described earlier in this paper. Mass spectrometric methods can also solve some fundamental problems. However, co-eluting congeners occurring at high concentrations relative to the target non-*ortho* congener can interfere through fragmentation of the parental ion even when the chlorine numbers differ from the target compound. Schmidt and Hesselberg [13] have shown recently that even NCI has this problem. Co-elution of CB-110 with CB-77 posed the greatest challenge for their methodology. In methane NCI, CB-110 produced a small amount of tetrachlorobiphenyl fragment ion (m/z 292) interfering in the quantitation of CB-77 (m/z 292 was the quantitation ion of choice for CB-77). Similar interference occurred in the determination of CB-126 by CB-129. They report that the error will be 15% for CB-126 and 70–100% for CB-77, if no correction is applied.

All other methods involving non-*ortho* CBs utilize an intermediate enrichment step before final determination by GC–ECD or GC–MS. The materials of choice for this purpose are activated charcoals [19,40], porous graphitic carbon [41], florisil [42], alumina [43] and 2(1-pyrenyl) ethyldimethylsilylated silica (PYE) [44]. These materials are used essentially to isolate the non-*ortho* CBs from the rest of the congeners before measurement.

Several brands of activated charcoals that are in current use in various methodologies were tested for this efficiency in comparison with direct measurements in MDGC–ECD [19]. CB-77, -126 and -169 were measured in Aroclors and in seal blubber using charcoal techniques and MDGC–ECD. The charcoals were generally very efficient, but they did not result in a *complete* separation of the class of CBs that was involved in the study. As a result, a small portion of *ortho* CBs was left out in the non-*ortho* fraction. This was in the range 0.032–2.2% for CB-110 and 0.0018–0.0087% for CB-178. The concentrations of these co-eluting compounds are large enough to interfere when they co-elute with CB-77 and CB-126, respectively. The latter congeners occur at <0.01% of the total CBs. In conclusion, Kannan et al. [19] pointed out that these congeners could not be accurately determined using SC-GC techniques with either ECD or MS detection. This was confirmed in a recent study using HRGC–HRMS (electron impact ionization) [12]. It was observed that although the carbon/silica gel column separates the co-planar CBs (non-*orthos*) from the non-planar CBs very effectively, the concentration of CB-110 in the original sample (fish and marine mammal) was often so much larger than that of CB-77 that even a small percentage of CB-110 remaining in the co-planar fraction resulted in an interference at m/z 292, the quantitation ion for CB-77. They also noticed co-elution of CB-158 (2,3,3',4,4',6-H_6CB), CB-185 (2,2',3,4,5,5',6-H_7CB) and CB-129 with CB-126 when using a DB-5 capillary column. To avoid overestimations in HRGC–HRMS, they proposed a peak area correction factor, which had to be done manually. These recent observations point out clearly that most published reports on non-*ortho* CBs without such strict quality control measures carry an element of uncertainty in them.

Several high-resolution GC columns offer satisfactory separation of these particular congeners and are used successfully for these measurements [45–47]. However, none of these columns, except SE-54 (5% phenyl methyl silicone), has been fully characterized for retention time indices for all 209 CB congeners [48]. In the absence of such data for other columns, congener-specific analysis for chlorobiphenyls would be only partial.

The need for chemical purity of solvents and

reagents used in CB analysis was discussed above. Charcoals are also reagents. They have some problems that are unique to this class. The activated charcoal can be produced from various sources such as combustion of petroleum compounds, palm oil, coconut shells and other vegetable sources. The impurity that can arise from these materials is also varied and unpredictable. Extensive cleaning of these materials is a prerequisite for their use. Five brands of charcoals that were used in an earlier study [19] were scanned by scanning electron microscopy (SEM), which revealed that the surface characteristics were very different for different charcoals (which resulted in different elution profiles for different charcoals [19]). The SEM studies also indicated that silicon, sulphur and metals such as potassium, calcium, iron and aluminium are present in these charcoals at per cent levels. It is yet to be shown whether these metals present in the charcoals can cause some catalytic degradation of CBs. This possibility may arise when acidified silica gel columns are combined with charcoal columns in automated clean-up procedures.

Quantitation of CBs: need for uniformity

It is surprising that commercial PCBs such as Aroclors, Clophens and Kanechlors are still used as standards for qualitative and quantitative analysis of CBs in environmental samples. Except in accidental spills and in continuous discharge from PCB manufacturing plants, the PCBs that reach the environment are modified by various physicochemical and biochemical mechanisms over a period of time. The irrelevance of quantifying environmental CB residues using commercial PCBs has been pointed out by various research groups [9,10]. An isomer-specific principal component analysis [49] and a linear inverse regression method [7] have demonstrated quantitatively that CB residues in environmental samples (fish, turtles [49] and water, shrimps, fish, marine mammals [7]) are by no means described adequately by Aroclors such as 1242, 1248, 1252 and 1260. An alarming situation was pointed out recently [50]. Samples with CB concentrations as high as 10 000–50 000 μg/kg were reported "undetected" in the Superfund's contract laboratory programme (CLP) because it required reports in terms of Aroclors.

It is also not uncommon that CBs in environmental samples are quantitated on the basis of isomer groups [51]. Again, only limited information can be extracted from such isomer data.

The most modern and accurate approach is to quantitate CBs in environmental matrices on the basis of individual congeners. Standards that represent selected persistent and toxic congeners can also be used, depending on the situation [36,52]. It is important to base the quantitation on a reliable high-resolution chromatographic technique, such as using SE-54 capillary columns. All the modern research on CBs clearly indicates that it is time that PCBs are quantitated and studied exclusively as individual chemical entities rather than as an uncharacterized mixture of compounds.

ACKNOWLEDGEMENT

REFERENCES

1 S. Safe, CRC Crit. Rev., 13 (1984) 319.
2 A. Brouwer, in C. H. Walkers (Editor), The Role of Enzymes in Regulating the Toxicity of Xenobiotics, University of Reading, Reading, 1991, p. 731.
3 R. D. Kimbrough, Annu. Rev. Pharmacol. Toxicol., 27 (1987) 87.
4 E. D. Goldberg, Sci. Total Environ., 100 (1991) 17.
5 S. Tanabe, B. G. Loganathan, A. Subramanian and R. Tatsukawa, Mar. Pollut. Bull., 18 (1987) 561.
6 G. R. Shaw and D. W. Connel, Environ. Sci. Technol., 18 (1984) 18.
7 J. C. Duinker, D. E. Schulz and G. Petrick, Chemosphere, 23 (1991) 1009.
8 R. F. Seegal, B. Bush and W. Shain, Chemosphere, 23 (1991) 1941.
9 S. Safe, L. Safe and M. Mullin, J. Agric. Food Chem., 33 (1985) 24.
10 J. C. Duinker, M. T. J. Hillebrand, K. H. Palmork and S. Wilhelmsen, Bull. Environ. Contam. Toxicol., 25 (1980) 956.
11 A. L. Alford-Stevens, Environ. Sci. Technol., 20 (1986) 1194.
12 D. W. Kuehl, B. C. Butterworth, J. Libal and P. Marquis, Chemosphere, 22 (1991) 849.
13 L. J. Schmidt and R. J. Hesselberg, Arch. Environ. Contam. Toxicol., 23 (1992) 37.
14 J. C. Duinker, D. E. Schulz and G. Petrick, Anal. Chem., 60 (1988) 478.
15 G. Petrick, D. E. Schulz and J. C. Duinker, J. Chromatogr., 435 (1988) 241.
16 D. E. Schulz, G. Petrick and J. C. Duinker, Environ. Sci. Technol., 23 (1989) 852.

17 N. Kannan, D. E. Schulz-Bull, G. Petrick and J. C. Duinker, *Int. J. Environ. Anal. Chem.*, 47 (1992) 201.

18 N. Kannan, S. Tanabe, T. Wakimoto and R. Tatsukawa, *Chemosphere*, 16 (1987) 1631.

19 N. Kannan, G. Petrick, D. Schulz, J. Duinker, J. Boon, E. van Arnhem and S. Jansen, *Chemosphere*, 23 (1991) 1055.

20 D. E. Schulz, G. Petrick and J. C. Duinker, *Mar. Pollut. Bull.*, 19 (1988) 526.

21 D. E. Schulz-Bull, G. Petrick and J. C. Duinker, *Mar. Chem.*, 36 (1991) 365.

22 M. Ehrhardt, *ICES Tech. Mar. Environ. Sci.*, 4 (1987) 14.

23 N. Kannan, S. Tanabe, M. Ono and R. Tatsukawa, *Arch. Environ. Contam. Toxicol.*, 18 (1989) 850.

24 D. G. Patterson, Jr., C. R. Lapeza, E. R. Barnhart, D. F. Groce and V. W. Burse, *Chemosphere*, 19 (1989) 127.

25 P. M. de Voogt, D. E. Wells, L. Reutergårdh and U. D. T. Brinkman, *Int. J. Environ. Anal. Chem.*, 40 (1990) 1.

26 G. Ericksson, S. Jensen, H. Kylin and W. Strachan, *Nature*, 341 (1989) 42.

27 M. Luotamo, J. Järvisalo and A. Aitio, *Environ. Health Perspect.*, 60 (1985) 327.

28 F. van der Valk and Q. T. Dao, *Chemosphere*, 17 (1988) 1735.

29 J. C. Duinker, D. Schulz-Bull, G. Petrick and N. Kannan, presented at the *12th International Symposium on Chlorinated Dioxins and Related Compounds, Tampere, Finland, August 24–28, 1992.*

30 S. Tanabe, N. Kannan, A. Subramanian, S. Watanabe, M. Ono and R. Tatsukawa, *Chemosphere*, 16 (1987) 1965.

31 N. Kannan, S. Tanabe, T. Wakimoto and R. Tatsukawa, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 451.

32 N. Kannan, S. Tanabe, T. Okamoto, R. Tatsukawa and D. J. H. Phillips, *Environ. Pollut.*, 62 (1989) 223.

33 G. Petrick, D. E. Schulz-Bull and J. C. Duinker, *Z. Wasser-Abwasser-Forsch.*, 25 (1992) 115.

34 N. Kannan, S. Tanabe and R. Tatsukawa, *Arch. Environ. Health*, 43 (1988) 11.

35 S. Tanabe, N. Kannan, T. Wakimoto, R. Tatsukawa, T. Okamoto and Y. Masuda, *Toxicol. Environ. Chem.*, 24 (1989) 215.

36 J. C. Duinker, D. Schulz and G. Petrick, *Mar. Pollut. Bull.*, 19 (1988) 19.

37 C. S. Creaser, F. Krokos and J. R. Startin, *Chemosphere*, 25 (1992) 1981.

38 E. Sippola and K. Himberg, *Fresenius' Z. Anal. Chem.*, 339 (1991) 510.

39 K. Himberg and E. Sippola, presented at the *12th International Symposium on Chlorinated Dioxins and Related Compounds, Tampere, Finland, August 24–28, 1992.*

40 J. N. Huckins, D. L. Stalling and J. D. Petty, *J. Assoc. Off. Anal. Chem.*, 63 (1980) 750.

41 C. S. Creaser and A. Al-Haddad, *Anal. Chem.*, 61 (1989) 1300.

42 L. R. Kamops, W. J. Trotter, S. J. Young, A. C. Smith, J. A. G. Roach and S. W. Page, *Bull. Environ. Contam. Toxicol.*, 23 (1979) 51.

43 E. Storr-Hansen, M. Cleemann, T. Cederberg and B. Jansson, *Chemosphere*, 24 (1992) 323.

44 P. Haglund, L. Asplund, U. Järnberg and B. Jansson, *Chemosphere*, 20 (1990) 887.

45 K. Ballschmitter, W. S. Schäfer and H. Buchert, *Fresenius' Z. Anal. Chem.*, 326 (1987) 253.

46 L. M. Smith, T. R. Schwartz, K. Feltz and T. J. Kubiak, *Chemosphere*, 21 (1990) 1063.

47 J. de Boer, Q. T. Dao and R. van Dortmond, *J. High Resolut. Chromatogr.*, 15 (1992) 249.

48 M. D. Mullin, C. M. Pochni, S. McCrindle, M. Romkes, S. H. Safe and L. M. Safe, *Environ. Sci. Technol.*, 18 (1984) 468.

49 T. R. Schwartz, D. L. Stalling and C. L. Rice, *Environ. Sci. Technol.*, 21 (1987) 72.

50 M. D. Erickson, in O. Hutzinger and H. Fiedler (Editors), *Organohalogen Compounds*, Vol. 2, Eco-Informa Press, Bayreuth, 1990, p. 29.

51 A. L. Alford-Stevens, W. L. Budde and T. A. Bellar, *Anal. Chem.*, 57 (1985) 2452.

52 V. A. McFarland and J. U. Clarke, *Environ. Health Perspect.*, 81 (1989) 225.