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Suitability of several carbon sorbents for the fractionation of various sub-groups of toxic polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans

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Abstract

Feasibility of several sorbents, Amoco PX-21, Carbosphere, Carbopack B and C and 2-(1-pyrenyl) ethyldimethylsilylated silica gel (PYE), for the fractionation of polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins and furans was investigated. Selection was based on their suitability for quantitative isolation of the target compounds with special attention for the most toxic mono- and non-*ortho*-CBs to prevent coelution with other congeners during the final determination by gas chromatography. Cost-effectiveness in terms of solvent and time consumption as well as feasibility for routine analysis and automation were considered additional merits of the methods compared. Final evaluation of the procedures providing the best results was done by comparison of the results obtained from the analysis of real-life samples. The results showed that, among the sorbents tested, Carbopack B and PYE were the most suitable for routine analysis. In particular, these sorbents allowed a more reliable determination of the toxic congeners and, consequently, of the toxic equivalents of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin content in environmental samples.

Keywords: Polychlorinated biphenyls; Polychlorinated dibenzo-*p*-dioxins; Polychlorinated dibenzofurans; Sorbents; Carbon sorbents; 2-(1-Pyrenyl) ethyldimethylsilylated silica

1. Introduction

Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are ubiquitous toxic environmental pollutants of great concern. Among the 209 possible PCBs, attention is usually focused on those that are approximate stereoisomers of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, i.e., non- and mono-*ortho* substituted PCBs, because these congeners show a similar toxicity as PCDD/Fs [1].

PCDD/F levels in environmental samples are much lower than those of PCBs [2] which, moreover, interfere in PCDD/F determination. Therefore,

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accurate PCDD/F analysis requires proper isolation of both classes of pollutants before final determination of PCDD/Fs by gas chromatography with high resolution mass spectrometry detection (GC–HRMS). Mono- and, particularly, non-*ortho* substituted PCBs are also minor contributors to the bulk of PCBs in real-life samples [3]. These relatively low concentrations, typically in the part per trillion (ppt) range in the case of non-*ortho* CBs, and the possibility of coelution with more abundant PCB congeners makes the accurate quantification of the most toxic PCB isomers with a single capillary column to be considered even nowadays as a not completely attained goal.

A variety of methods showing the feasibility of different types of sorbents for the isolation of PCDD/Fs from PCBs and for the simultaneous fractionation of the PCB classes have been published [4–7]. Due to the planar structure of these compounds, fractionation is (mainly) based on a charge transfer mechanism. Therefore, not surprisingly, carbon-based materials have typically provided the more satisfactory results when using open liquid chromatographic columns [8–11]. The advantages and shortcomings of these different approaches have been profusely discussed. However, to our knowledge, this discussion has been mainly based on the results reported by different laboratories rather than on the work by a single research group which has made the comparison of the different results sometimes difficult. Among the stationary phases currently available commercially for performing this kind of fractionation by high-performance liquid chromatography (HPLC), the best results have been reported when using porous graphitic carbon (PGC) [12,13] and, especially, 2-(1-pyrenyl) ethyldimethylsilylated silica gel (PYE) [3,14–17]. Despite their performance, in practice, none of these sorbents have substituted the open LC columns, which are still widely used for routine analyses in many laboratories. The new requirement in current legislations of counting for a contribution similar to that of the limit of detection (LOD) for those compounds found at non-detectable or quantifiable levels when calculating the total amount of toxic equivalents of 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (TEQs) in a sample [18] is an additional difficulty.

The goal of this study was to evaluate the feasibility of four carbon sorbents, Amoco PX-21, Carbosphere and Carbopack B and C, for the determination of selected toxic PCBs and PCDD/Fs. When necessary, experimental variables affecting this fractionation were re-optimised to improve the separation of classes. Special attention was given to the unambiguous determination of the most toxic PCBs, the non-*ortho* CBs (PCBs IUPAC Nos. 77, 126 and 169 [19]). Results were compared with those obtained by fractionation of the target compounds on a PYE column according to an earlier method [17]. The methods providing the best results were tested by analysing real-life samples and the TEQs were compared.

2. Materials and methods

2.1. Chemicals

Cyclohexane, dichloromethane, *n*-hexane, isooctane, methanol and toluene (all pesticide quality) were obtained from SDS (Peypin, France). Two active charcoals, Amoco PX-21 (2–10 μm particle diameter) from Anderson Development (Adrian, USA) and Carbosphere (80–100 mesh) from Chrompack (Middelburg, The Netherlands), and two graphitic carbons, Carbopack B [as superclean ENVI-Carb solid-phase extraction (SPE) cartridges, 80–100 mesh] and Carbopack C (120–400 mesh) from Supelco (Bellefonte, PA, USA), were used in the study. Celite 545 (0.01–0.04 mm), Silica gel 60 (0.063–0.200 mm), potassium hydroxide and sulfuric acid (95–97% purity) were from Merck (Darmstadt, Germany), Florisil (60–100 mesh) from Floridin (Berkley Springs, WV, USA), granulated anhydrous sodium sulfate for residue analysis from J.T. Baker (Deventer, The Netherlands), and fiber glass and glass wool from Chromatography Research Supplies (Addison, USA).

A variety of PCBs including non-planar tetra- and tri-*ortho* CB congeners (PCBs IUPAC Nos. 45, 84, 88, 91, 95, 131, 132, 135, 136, 139, 144, 149, 171, 174, 175, 176, 178, 183, 196 and 197), semi-planar di-*ortho* CBs (PCBs 52, 101, 110, 129, 138, 153, 170, 180 and 194) and mono-*ortho* CBs (PCBs 105, 114, 118, 123, 156, 167, 157 and 189) and planar non-*ortho* CBs (PCBs 77, 126 and 169) were

included in the study. All of them were purchase from Ehrenstorfer (Augsburg, Germany) and the selection criteria were their relatively high abundance in environmental samples, toxicity and chromatographic reasons. A working stock solution containing 1000 pg/μl of each PCB was prepared in isooctane and used for further dilution and spiking. 1,2,3,4-Tetrachloronaphtalene (TCN, Ehrenstorfer) was selected as external standard for PCB determination by GC with electron-capture detection (ECD). $^{13}C_{12}$ isotopically labelled PCBs 77, 126 and 169 were used as internal standards (99% purity, Cambridge Isotope Laboratories, USA) for final determination of these compounds by GC–HRMS. EPA 1613 standard solutions (98% minimum purity, Wellington Laboratories, Guelph, Canada) were used for determination of the 2,3,7,8-PCDD/Fs by GC–HRMS [20].

Chicken and pork sausage samples used for PCDD/F determination were purchased from supermarkets in Madrid (Spain). Chicken and butter samples used for toxic PCB determination were provided by the National Institute of Public Health Folkehelsa (Oslo, Norway) as part of an interlaboratory study on the determination of PCBs 77, 126 and 169 in food conducted in 2000.

2.2. Procedures

2.2.1. Comparison of sorbents

Table 1 summarises the amounts of Amoco PX-21 [8,9,21], Carbosphere [10], Carbopack B [11] and Carbopack C [22,23] used to prepare the open LC columns. When required, the amount of other sorbents used to dilute the carbon has been specified. Solvents required for conditioning the column before using and, if possible, for reconditioning of the sorbent between consecutive samples are also included.

Analysis on the 5 μm Cosmosil 5-PYE column (250 mm×4.6 mm I.D., Nacalai Tesque, Promochem) was carried out at 25°C according to a previously reported method [17]. The HPLC system consisted of a Perkin-Elmer P10 pump (Perkin-Elmer, Norwalk, CT, USA), a Rheodyne 7125 injector valve equipped with a 20-μl loop and a Hewlett-Packard Series 1050 UV detector (Hewlett-Packard, Palo Alto, CA, USA) which was operated at 225 nm. *n*-Hexane was used as mobile phase at a flow ratio of 1 ml/min.

All experiments were carried out four times at the two levels of concentration investigated, 500 pg/μl and 100 pg/μl.

2.3. Real-life samples

Sample preparation was carried out according to published methods [24,25]. Briefly, the chicken and the pork sausage samples were homogenised with a silica–anhydrous sodium sulfate (1:1, w/w) mixture, loaded in a glass column, spiked with $^{13}C_{12}$ PCB 77, 126, 169 and $^{13}C_{12}$ 2,3,7,8-PCDD/Fs and extracted with acetone–hexane (1:1, v/v). Butter was dissolved in hexane and spiked with the compounds quoted above. Subsequent fat removal was carried out on a multilayer clean-up column containing silica and silica modified with sulfuric acid (44%, w/w) using *n*-hexane as solvent. This extract contained the target compounds and was used for further evaluation of the sorbents selected for fractionation of the analytes. The fat content was determined gravimetically after separate extraction of a sub-sample with chloroform–methanol (1:1, v/v) [26].

With Amoco PX-21, the extraction, clean-up of the extracts and final fractionation on the carbon column was carried out on-line by coupling of the columns used in the different steps according to a procedure previously described [27].

All experiments were carried out in duplicate.

2.4. PCB and PCDD/F analysis

The determination of the *ortho*-substituted CBs in the collected extracts was performed by GC–ECD (HP-5890 Series II). Samples were injected in the splitless mode (splitless time, 1 min) in a capillary BPX-5 column (60 m×0.25 mm I.D., 0.25 μm film thickness) from SGE (Australia). The column temperature was programmed from 60°C (2 min) to 180°C at 50°C/min, then to 230°C (40 min) at 4°C/min, and finally to 270°C (10 min) at 4°C/min. The injector and detector temperatures were 280°C

Table 1
Experimental conditions used for the PCB and PCDD/F fractionation in the sorbents tested. Frames indicate classes of compounds collected as separate fractions

Sorbent	Amount (mg)	Sorbent dilution (w/w)	Conditioning[a] (solvent, ml)	Reconditioning[b] (solvent, ml)	Tetra-+ tri-*ortho* CBs	Di-*ortho* CBs	Mono-*ortho* CBs	Non-*ortho* CBs	PCDD/Fs
Amoco PX-21	45	1:14, glass fiber	Dcm (250)+Tol (1000)	[Tol (150)+Met (150) +Tol (150)][c] +Cyc–Dcm (80:20, 170)	Cyc–Dcm (80:20, 350)			Dcm–Tol (80:20, 120)	[Tol (170)][c]
Carbosphere	2000	–	Tol (reflux 3 weeks)	Tol (reflux 1 week)	Dcm (30, reflux 1 h)		Tol (25)	Tol (30, reflux 2 h)	[Tol (30, reflux 24 h)][c]
Carbopack B	250	–	[Tol (20)][c]+Hex (20)	Disposable	Hex (15)		Hex–Tol (91:1, 20)	Hex–Tol (75:25, 20)	[Tol (60)][c]
Carbopack C	3600	1:4.5, celite	Tol (2)+Dcm–Met –Tol (75:20:5, 1) +Cyc–Dcm (1:1, 1) +Hex (2)	Disposable	Hex (2)+Cyc–Dcm (1:1, 1)			Dcm–Met –Tol (75:20:5, 1)	Tol (15)
PYE	–	–	–	Not required	Hex (5.1)	Hex (0.5)	Hex (2.2)	Hex (6)	

See Section 2.1 for identification of the target compounds included in each class. Cyc: Cyclohexane; Dcm: dichloromethane; Hex: *n*-hexane; Met: methanol; Tol: toluene.
[a] Solvent(-s) used for conditioning of the sorbent before using.
[b] Solvent(-s) used for reconditioning of the sorbent between two consecutive samples.
[c] Elution in the back flow direction.

and 300°C, respectively. Nitrogen was used as carrier gas at a column head pressure of 22.4 p.s.i. and as make-up gas (60 ml/min) (1 p.s.i.=6894.76 Pa).

Chromatographic data were acquired in a System Gold acquisition data system (Beckman, CA, USA). Identification of the PCB congeners in the different fractions was based on the comparison of the peak retention time of each compound relative to TCN with those of the standard solution analysed under the same experimental conditions. The calibration curves used for quantification had coefficients of correlation better than 0.92. The absolute LODs were in the range 0.2–10 pg/μl.

The determination of the non-*ortho* CBs and PCDD/Fs in the collected fractions was performed on a Fisons 8000 Series GC system (Fisons Instruments, Milan, Italy) equipped with a HRMS (AutoSpec-Ultima, Micromass, Manchester, UK) in the single ion monitoring (SIM) mode at a minimum resolution of 10 000. Ionisation was carried out by electron impact (EI) at 37 eV. The temperature of the source and the transfer line were 250°C and 280°C, respectively. Samples were injected in the splitless mode (splitless time, 1 min) in a capillary DB-5 column (60 m×0.25 mm I.D., 0.25 μm, J&W Scientific, Folsom, CA, USA). The column temperature was programme from 140°C (1 min) to 200°C (1 min) at 20°C/min and then to 300°C (20 min) at 3°C/min to complete the program. The injector temperature was 280°C. Helium was used as carrier gas at a head pressure of 7.3 p.s.i. For PCDD/Fs [20] and PCB 77 the $[M]^+$ and $[M+2]^+$ ions were monitored while the $[M+2]^+$ and $[M+4]^+$ ions were selected for PCBs 126 and 169.

3. Results and discussion

3.1. Comparison of sorbents

Table 2 summarises the results obtained when the sorbents tested were examined for the fractionation of the PCBs and PCDD/Fs using the conditions of Table 1. Ranges reported are the average values of the recoveries obtained at the two spiking levels investigated, 500 pg/μl and 100 pg/μl, for the compounds selected in the fraction in which they were expected to elute (see Section 2.1 and Table 2 for identification of the PCB congeners included in each sub-group). Therefore, in those cases in which a compound split into two consecutive fractions, the recovery value reported here for that particular compound will apparently be lower that those previous published using a similar approach but in which the *total* recovery has been calculated. See Table 1 to identify those classes that were really collected as separate fractions in the different procedures assayed.

Under the experimental conditions used in this study, all sorbents tested were able to separate PCDD/Fs from PCBs. However, except in the case of Carbopack C, elution of the PCDD/Fs in the back flow direction was recommended (or even mandatory). Although it was not checked in the present study, according to the literature, the elution of PCDD/Fs can be achieved on a PYE column by direct elution with a large amount of *n*-hexane [16]. However, to reduce the amount of solvent required for the extraction of this class of compounds both backflush of PCDD/Fs [28] or direct elution with

Table 2
Mean recoveries (as %) of the PCBs and PCDD/Fs studied in the expected fraction

Sorbent	Tetra-+tri-*ortho* CBs	Di-*ortho* CBs	Mono-*ortho* CBs	Non-*ortho* CBs	PCDD/Fs
Amoco PX-21	89–112	92–112	90–115	90–92	71–105[a]
Carbosphere	98–105	95–103	65–115	95–106	94–102[a]
Carbopack B	70–113	98–109	75–100	92–96	71–90[a]
Carbopack C	98–114	97–118	95–115	11–45	81–105
PYE	82–98	88–104[b]	84–108[c]	90–97	–

See Table 1 for experimental conditions and Section 2.1 for identification of the target compounds included in each class.
[a] Elution in the back flow direction.
[b] The range includes the recoveries of the tri-*ortho* CBs 84, 132, 171, 174 and 196 which fully eluted within this fraction.
[c] The range includes the recoveries of the di-*ortho* CBs 138, 170 and 194 which fully eluted within this fraction.

more selective solvents such as toluene [3] have been proposed. In the latter case, 35 ml of toluene were required for the quantitative elution of PCDD/Fs from a PYE column of similar dimensions to that used in the present study. In general, carbon sorbents require larger amounts of aromatic solvents, typically toluene, and/or longer extraction times for the elution of PCDD/Fs (Table 1). This was particularly the case for the active carbon sorbents investigated: Amoco PX-21 required 170 ml of toluene for the quantitative backflushing of the toxic PCDD/Fs; Carbosphere required 24 h refluxing with 30 ml of toluene. However, in general, good recoveries were obtained for PCDD/Fs with all methodologies evaluated (globally in the 71–105% range).

The repeatability of the methods assayed, which was calculated as the average of the values found at the two spiking levels tested, was satisfactory with relative standard deviations (RSDs) below 9% for all classes of target compounds.

As regards PCBs, all sorbents tested except Carbopack C, allowed the quantitative isolation of the non-*ortho* CBs from the rest of the *ortho*-substituted CBs included in the study.

Carbopack C has typically been used as an additional clean-up step prior to GC–HRMS of the PCDD/Fs which effectively reduces the risk of interference during the analysis of these compounds in real-life samples (see below) [22,23]. In fact, this treatment did not affect the final recoveries of PCDD/Fs (81–105%). However, contrary to previously published results [22,23], in the present study Carbopack C did not enable the separation between the toxic mono- and non-*ortho* CBs studied. The unacceptably low recoveries obtained for the most toxic PCBs, the non-*ortho*-substituted congeners, in their fraction (range, 11–45%) ruled this sorbent out for any further consideration in the study.

Satisfactory recoveries were obtained for the non-*ortho* CBs with the other sorbents evaluated (range, 90–106%). However, it is important to point out that several experiments were carried out in the present study in order to improve the separation of the non-*ortho* CBs and the other *ortho*-substituted CBs originally achieved by Krokos et al. using Amoco PX-21 [8]. The final experimental conditions allowed a distinctly better separation between these two classes of PCBs than previously reported [9,21] with recoveries in the range 89–115% and 90–92% for *ortho*-substituted CBs and non-*ortho* CBs, respectively. Despite various attempts and the relatively large amount of carbon used, no further fractionation among the different sub-groups of *ortho*-substituted CBs was achieved with this sorbent. That is, the coelution problems affecting the quantification of the mono-*ortho* CBs studied were obviously not solved with Amoco PX-21.

Therefore, it was concluded that, of the investigated sorbents, only Carbosphere and Carbopack B provided an adequate separation among the two more environmentally relevant classes of PCBs, mono- and non-*ortho* CBs, and the rest of the *ortho*-subtituted CBs. This kind of fractionation can also be achieved on a PYE column [17]. Acceptable recoveries which are in the range of those previously published [10], were 65–115% for the mono-*ortho* CBs with Carbosphere, and 75–108% with Carbopack B and PYE. The lower value in these ranges corresponded to PCB 189 which partially eluted in the non-*ortho* CB fraction. In the case of PYE, moreover, PCBs 138, 170 and 194 eluted in the mono-*ortho* CB fraction. However, it is important to note that on none of the sorbents did the elution of these particular congeners in a sub-group differ from the expected one to affect adversely the proper quantification of the toxic mono- and non-*ortho* CBs. More importantly, all three sorbents, Carbosphere, Carbopack B and PYE, provided total isolation of PCBs 77 and 126 from PCBs 110, 129 and 178, which allows the accurate quantification of the non-*ortho* CBs.

The main practical limitation of Carbosphere was the relatively high background level of the extracts collected from this column even when the sorbent was refluxed with toluene for 3 weeks before use (Table 1). The background level detected in the Carbosphere extracts made the use of selective detectors such an MS mandatory for accurate determination of all the target compounds. Although reusing the column was in principle possible, the long time required for cleaning the sorbent (1 week) detracted from its use in routine analysis. In the case of the other activated sorbent used in this study, i.e., Amoco PX-21, background levels were never as high as for Carbosphere and the performance of the column was found to improve with reusing and

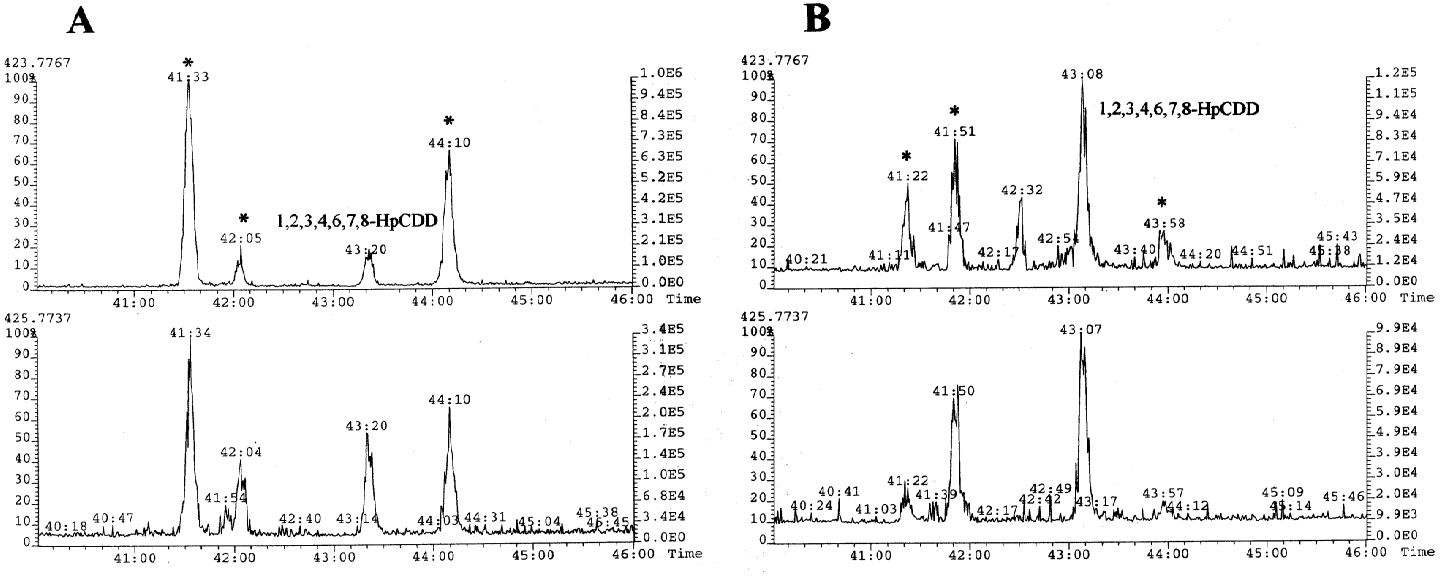

Fig. 1. Fragmentograms of the 1,2,3,4,6,7,8-HpCDD found in a pork sausage sample after (A) isolation from PCBs on a Florisil column and (B) when the Florisil extract of PCDD/Fs was submitted to additional clean-up on Carbopack C. Impurity: *.

Table 3
Comparison of the sorbents studied on the basis of their practical characteristics

Sorbent	Variability among batches	Sensitivity to lipids	Background level	Solvent consumption	Time consumption	Possibility of reutilisation	Possibility of automation	Cost/effectiveness
Amoco PX-21	+++	+	+++	++++	+++	++++	+++	++
Carbosphere	+++	−	++++	++	++++	+	−	+
Carbopack B	+	−	+	++	++	−	++	+++
Carbopack C	++++	+	++	−	++	−	−	−
PYE	−	++++	−	−	+	++++	++++	++++

−: very low; +: low; ++: moderate; +++: high; ++++: very high.

ageing [9]. However, the background level made still difficult to use ECD.

According to these considerations, the prepacked SPE columns of Carbopack B and, especially, the PYE HPLC column were preferred over the rest of the laboratory-packed carbon columns. In fact, among the sorbent tested, PYE was the only one able to provide a (relatively) satisfactory fractionation of one additional class of PCBs, the di-*ortho* CBs, from most of the tri- and tetra-*ortho* CBs included in the study [17]. Only a few tri-*ortho* CBs eluted in this particular fraction (Table 2), but the fractionation achieved ensured the accurate determination of the relatively abundant di-*ortho* CBs investigated.

From a practical point of view, it is worth mentioning that the methods based on the use of the disposable SPE cartridges of Carbopack B and the PYE HPLC column involved smaller volumes of solvent and shorter analysis times (e.g., 14 ml of *n*-hexane and 12 min for PCB fractionation and only 35 ml of toluene for PCDD/Fs direct elution [3] on PYE) than the laboratory-made of Amoco PX-21 and Carbosphere columns (Table 3). Carbopack B and PYE did not introduce background interferences and both sorbents showed a good reproducibility between batches, which was not always the case for the other activated carbons investigated. For obvious reasons, PYE can be considered as more suitable for (semi-)automation or coupling of the sample preparation method with the subsequent fractionation of the target compounds than the Carbopack B SPE cartridges. On the other hand, the extreme sensitivity of PYE to lipids [15] is a shortcoming of this HPLC column.

3.2. Application to real-life samples

As an illustration of the need for an accurate fractionation of toxic PCBs and PCDD/Fs studied for their reliable quantification in real-life samples, a variety of foodstuffs were submitted to different fractionation procedures of the target compounds.

Table 4 shows the PCDD/F concentrations of a medium fat meat (chicken, 3.8% fat on a fresh mass basis) and a fatty meat (pork sausage, 25% fat on a fresh mass basis) (i) after isolation from PCBs on a Florisil column [2], (ii) when the Florisil extract containing the PCDD/Fs was submitted to further purification with Carbopack C, and (iii) when the original extract was directly fractionated on Carbopack B. In all cases, results are the average of two separate analyses. In both types of samples, further clean-up of the Florisil extracts containing the PCDD/Fs on Carbopack C resulted in a reduction of the background level which allowed the quantification of some congeners that were not detected in the Florisil extracts. The reduction of the LODs also contributed to a more accurate determination of the TEQs. The total TEQs calculated for the Florisil extracts of chicken and pork sausage (0.89 and 1.85 pg/g on a fresh mass basis) were reduced to 0.19 and 0.27 pg/g, respectively, after additional clean-up on Carbopack C. In addition, the latter TEQ values agreed with those obtained when using Carbopack B (0.11 and 0.23 pg/g for chicken and pork sausage, respectively), which proved the feasibility of Carbopack B for complex environmental samples.

As a typical example, Fig. 1 shows the fragmentograms of the 2,3,7,8-HpCDD in the pork sausage

Table 4
PCDD/Fs and TEQs concentrations (pg/g on a fresh mass basis) in chicken muscle and pork sausage determined using various clean-up and fractionation methods

	Sample					
	Chicken			Pork sausage		
	Florisil	Florisil+Carbopack C	Carbopack B	Florisil	Florisil+Carbopack C	Carbopack B
2,3,7,8-TCDF	0.08	0.06	0.06	0.17	0.16	0.15
1,2,3,7,8-PCDF	0.07	0.07	0.06	0.18	0.12	0.12
2,3,4,7,8-PCDF	0.4[a]	0.08	0.08	0.5[b]	0.18	0.17
1,2,3,4,7,8-HxCDF	0.2[a]	0.12	0.13	0.2[a]	0.21	0.19
1,2,3,6,7,8-HxCDF	0.2[a]	0.06	0.05	0.2[a]	0.11	0.11
2,3,4,6,7,8-HxCDF	0.2[a]	0.05	0.05	0.3[a]	0.11	0.11
1,2,3,7,8,9-HxCDF	0.5[b]	0.07[b]	0.02[b]	0.3[b]	0.1[b]	0.06[b]
1,2,3,4,6,7,8-HpCDF	0.14	0.19	0.17	0.30	0.32	0.33
1,2,3,4,7,8,9-HpCDF	0.2[a]	0.06[b]	0.02[b]	0.2[a]	0.1[a]	0.08[b]
OCDF	0.15	0.29	0.26	0.4	0.3	0.28
2,3,7,8-TCDD	0.03[b]	0.01[b]	0.005[b]	0.02[b]	0.01[b]	0.007[b]
1,2,3,7,8-PCDD	0.5[b]	0.08[b]	0.01[b]	0.8[b]	0.06[b]	0.04[b]
1,2,3,4,7,8-HxCDD	0.1[b]	0.04[b]	0.01[b]	2[a]	0.07[b]	0.04[b]
1,2,3,6,7,8-HxCDD	0.1[b]	0.08	0.07	2[b]	0.11	0.10
1,2,3,7,8,9-HxCDD	0.1[b]	0.04	0.05	2[a]	0.07[b]	0.06[b]
1,2,3,4,6,7,8-HpCDD	0.26	0.3	0.3	6[a]	0.72	0.74
OCDD	0.99	0.97	0.92	6	5.5	5.32
TEQ	0.89	0.19	0.11	1.85	0.27	0.23

[a] LOD, compound not detected.
[b] LOD, compound detected but below LOQ.

sample with and without additional clean-up on Carbopack C of the PCDD/F fraction obtained with Florisil. The figure shows that this additional treatment contributed to reduce the background level and to eliminate impurities (see first and last eluting peaks in this chromatographic window).

Finally, Table 5 shows the TEQs calculated for toxic PCBs in a chicken muscle and a butter sample

Table 5
TEQs of PCBs (pg/g on a fresh mass basis) in chicken muscle and butter determined without and with Carbopack B fractionation

PCBs	Chicken muscle		Butter	
	Without fractionation[b]	With fractionation[f]	Without fractionation	With fractionation
Mono-*ortho*[a]	0.28	0.28	0.38	0.38
77	0.09[c]	0.0008 (0.0003)	0.13	0.004 (0.003)
126	6.33[d]	0.090 (0.094)	0.78	0.68 (0.97)
169	0.03[e]	0.002 (0.002)	0.02[e]	0.02 (0.02)
Total	6.73	0.38	1.31	1.08

Consensus values for the non-*ortho* CBs in these samples are mentioned in parentheses.
[a] Including PCBs 105, 114, 118, 123, 156, 157, 167 and 189.
[b] As determined with GC–ECD on a DB.5 column.
[c] Coelution with PCB 110.
[d] Coelution with PCBs 129 and 178.
[e] LOD.
[f] As determined with GC–ECD for mono-*ortho* CBs and GC–HRMS for PCBs 77, 126 and 169.

included in an interlaboratory exercise dealing with the determination of the non-*ortho* CBs. Mutual agreement between the TEQs calculated for these three PCBs and their corresponding consensus values supported the validity of the proposed Carbopack B method.

4. Conclusions

Despite many years of work in the field, accurate quantification of the individual toxic congeners of PCBs and PCDD/Fs in routine analysis of environmental samples is still not a completely achieved goal. Coelution problems and the high background levels can lead to an overestimation of the TEQs in environmental samples. Current legislation requirements of adding a contribution equal to that of the LOD in the case of non-detected toxic congeners is an additional difficulty.

Feasibility of four carbon sorbents, Amoco PX-21, Carbosphere, Carbopack B and Carbopack C, and one HPLC stationary phase, PYE, for the fractionation of various sub-groups of toxic PCBs and PCDD/Fs has been compared. PYE and the (still not so widely used) Carbopack B SPE cartridges provide the most satisfactory isolation of the PCDD/F, non-*ortho* CB and mono-*ortho* CB sub-groups form the other di-, tri- and tetra-*ortho* CBs investigated. The low background level found in the extracts of these sorbents and the good reproducibility between batches are additional merits. Therefore, both sorbents are valuable alternatives for solving the most pressing problems affecting the accurate determination of the toxic PCBs and PCDD/Fs and, consequently, for reliable determination of the TEQs in real-life samples.

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