# Method for the group separation of non-*ortho*-, mono-*ortho*- and multi-*ortho*-substituted polychlorinated biphenyls and polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans using activated carbon chromatography

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## Abstract

A cheap, efficient and reliable method for the separation of mono-*ortho*-/non-*ortho*-substituted polychlorinated biphenyls (PCBs), multi-*ortho*-PCBs and polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDDs/PCDFs) from one another and from other interferents was developed. A mixture of activated carbon AX-21 (50 mg) and Celite 545 as a carbon support (1:19) packed in a diposable tube was used. The compounds were gradually eluted with cyclohexane–dichloromethane–methanol (2:2:1) and toluene (a coplanar PCB fraction). PCDDs and PCDFs were regained by extraction of the inverted AX-21–Celite column with a small volume of toluene in a special miniaturized extraction apparatus. Recoveries from the column for PCBs and PCDDs/PCDFs varied from 63% to 100%. The method was used successfully for the fractionation of PCB technical formulations (Delor 103 and 105), municipal waste incinerator fly ash and biological samples (human adipose tissue, butter, egg and fish samples). The method is suitable for both mass spectrometric and electron-capture detection.

## 1. Introduction

The toxicity of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), mainly the most toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has been relatively closely investigated [1]. Recent studies [2] have also shown that polychlorinated biphenyl (PCB) congeners which are biochemically active [arylhydrocarbon hydroxylase (AHH) and ethoxyresorufin O-deethylase (EROD) induction] have planar or almost planar molecular structures, *i.e.*, have chlorine substitution of the aromatic rings in both *para* positions and in two or more of the four *meta* positions. In addition,

the analyses carried out following extensive human exposure to PCBs in Fukuoka (Japan) [3] and Taiwan [4] have showed that technical PCB mixtures such as Aroclors [5], Delors [6] and Kanechlors [7], in addition to several tens of PCB congeners, also contain substantially more toxic PCDFs as by-products. However, it has been found that some PCB congeners present in technical PCB mixtures [7] and environmental samples [8–10] also exhibit extremely high toxicity comparable to those of 2,3,7,8-substituted PCDDs and PCDFs [11].

There are at least three environmentally occurring coplanar non-*ortho*-substituted PCBs, namely 3,3',4,4'-tetra- (IUPAC No. 77 according

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to Ballschmiter and Zell [12]), 3,3',4,4',5-penta-
(No. 126), and 3,3',4,4',5,5'-hexachlorobiphenyl
(No. 169), with proposed TEFs (2,3,7,8-TCDD
toxic equivalency factors) of 0.01, 0.1, and 0.05,
respectively [13], and at least eight coplanar
mono-*ortho*-PCBs, namely 2,3',4,4',5-penta-
(No. 118), 2,3,3',4,4'-penta- (No. 105),
2',3,4,4',5-penta- (No. 123), 2,3,4,4',5-penta-
(No. 114), 2,3,3',4,4',5-hexa- (No. 156),
2,3,3',4,4',5'-hexa- (No. 157), 2,3',4,4',5,5'-hexa-
(No. 167) and 2,3,3',4,4',5,5'-heptachloro-
biphenyl (No. 189) with proposed TEFs of 0.001
[13], fulfilling the above-mentioned structural
conditions. As these TCDD-like PCB congeners
have been found in environmental and biological
samples mostly at substantially higher levels than
2,3,7,8-substituted PCDDs and PCDFs, their
contribution to the total TEQ (2,3,7,8-TCDD
toxic equivalent) dominates those of the PCDDs
and PCDFs [7,10,14–20].

For these reasons, it is important to deter-
mine, in addition to the "classical" PCBs (Nos.
28, 52, 101, 138, 153 and 180) and the 2,3,7,8-
substituted PCDDs and PCDFs, also the non-
*ortho*- and mono-*ortho*-PCBs.

As even high-resolution gas chromatographic
(HRGC)–mass spectrometric (MS) with selec-
ted-ion monitoring (SIM) peaks of the non-
*ortho*- and mono-*ortho*-PCBs may be overlapped
with those of multi-*ortho*-PCBs [6,21] and other
interfering co-extracted compounds, *e.g.*, DDE
and DDT, and in addition environmental levels
of multi-*ortho*-PCBs, mono-*ortho*-PCBs, non-
*ortho*-PCBs and PCDDs/PCDFs are very differ-
ent, it may be useful to divide precleaned or raw
sample extracts into at least two fractions con-
taining non-/mono-*ortho*-PCBs and PCDDs/
PCDFs. Carbon chromatography gives an oppor-
tunity for realising this demand.

Smith *et al.* [22] developed a very successful
method (which has been used in many variations
[8,16,23,24]) for PCDD/PCDF determination
based on cleaning up extracts of biological sam-
ples using potassium and caesium silicate, silica
gel and $H_2SO_4$–silica and active carbon AX-21
(50 mg) dispersed on glass fibres [25]. There are
many papers dealing with the testing of the
efficiency of various types of active carbon (*e.g.*,

Norit, Darco, AX-21, PX-21, Carbopack C,
Supelco SP-1, Altech SK-4, Wako, Carbosphere,
Baker, Separcol R-CARB cartridges) and the
suitability of various supports (*e.g.*, polyurethane
foam, sand, glass fibres, Chromosorb, Celite,
silica gel, polystyrene–divinylbenzene copoly-
mers) [17,24–36].

After the non-*ortho*- and mono-*ortho*-PCBs
had become compounds of interest because of
their toxicity, the ability of activated carbon to
retain these pollutants has started to be utilized
[9,10,15–17,19,20,23,31–41]. HPLC on porous
graphitic columns has also provided satisfactory
results in the field [18,42–44]. Various types of
semi-automated apparatus that include an active
carbon column with reverse elution have been
introduced [40,45–47]. On a Carbopack C col-
umn, it is even possible to separate non-*ortho*-
from mono-*ortho*-PCBs [35].

From our point of view, some of the above
methods have drawbacks, such as high elution
solvent consumption, expensive sorbents, low
recoveries, uncertain reproducibility, low ad-
sorption capacity, insufficient separation of com-
pounds of interest, a need for more complex
devices (HPLC, automation) and excessive back-
ground electron-capture detection (ECD) noise.

In this paper, we describe a cheap, reliable,
highly reproducible, robust and efficient method
based on the carbon chromatography that per-
mits the separation of two groups of toxic chloro-
aromatic pollutants, namely coplanar PCBs
(non-*ortho*- and mono-*ortho*-) and PCDDs/
PCDFs, from each other and from interfering
compounds, including di- to tetra-*ortho*-substi-
tuted PCBs.

## 2. Experimental

### 2.1. Reagents and standards

*Solvents.* n-Hexane, cyclohexane, dichlorome-
thane, methanol, toluene (all of pesticide quality
were obtained from Labscan, Dublin, Ireland)
and n-heptane (for residue analysis) from Merck
(Darmstadt, Germany).

*Adsorbents.* Super-A Activated Carbon AX-

21, lot No. 88049 (Anderson Development, Adrian, USA), Celite 545, 0.020–0.045 mm (Serva, Heidelberg, Germany), Florisil, 0.150–0.250 mm (Fluka, Buchs, Switzerland), silica gel 60, 0.063–0.200 mm (Merck) and ICN alumina B Super 1 (ICN Biomedicals, Eschwege, Germany) were used.

*Other materials.* Sodium sulphate, anhydrous granulated for residue analysis (Merck), sulphuric acid, GR, 95–97% (Merck), potassium hydroxide, GR (Merck), silver nitrate, GR (Medika, Bratislava, Slovak Republic), glasswool, silane treated (Chromatography Research Supplies, Addison, USA) and nitrogen, 99.99% (Linde-Technoplyn, Bratislava, Slovak Republic), purified by molecular sieve 5A and charcoal traps, were used.

*Standards.* n-Heptane solutions of di-*ortho*-PCBs [Nos. 28 (2,4,4'-trichlorobiphenyl, *i.e.*, mono-*ortho*-PCB congener), 52, 101, 138, 153 and 180 (400 ng ml$^{-1}$ each), 10 $\mu$g ml$^{-1}$ solutions were purchased from Labor Dr. Ehrenstorfer, Augsburg, Germany], mono-*ortho*-PCBs [Nos. 105, 114, 118, 123, 156, 157, 167 and 189 (200 ng ml$^{-1}$ each), 35 $\mu$g ml$^{-1}$ solutions were purchased from Amchro, Sulzbach/Taunus, Germany], non-*ortho*-PCBs [Nos. 77, 126 and 169 (400 ng ml$^{-1}$ each), 35 $\mu$g ml$^{-1}$ solutions were purchased from Amchro] $^{13}$C$_{12}$-labelled PCBs [Nos. 77, 126, 169, 105 and 118 (100 ng ml$^{-1}$ each), 40 $\mu$g ml$^{-1}$ solutions were purchased from Cambridge Isotope Labs., Woburn, MA, USA], PCDDs/PCDFs [1,2,4-TrCDD, 2,3,7,8-TCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, OCDD, and OCDF (TrCDD = trichlorodibenzo-*p*-dioxin; TCDF = tetrachlorodibenzofuran; HxCDD = hexachlorodibenzo-*p*-dioxin; OCDF = octachlorodibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin) (400 ng ml$^{-1}$ each), solids were either purchased from Cambridge Isotope Labs. or were kindly donated by Dr. K. Olie, University of Amsterdam], $^{13}$C$_{12}$-labelled 2,3,7,8-substituted PCDDs and PCDFs (50 ng ml$^{-1}$ TCDDs/TCDFs, 100 ng ml$^{-1}$ PeCDDs/pentachlorodibenzofurans (PeCDFs), 150 ng ml$^{-1}$ HxCDDs/hexachloro-

dibenzofurans (HxCDFs), 200 ng ml$^{-1}$ heptachlorodibenzo-*p*-dioxins (HpCDDs)/heptachlorodibenzofurans (HpCDFs) and 300 ng ml$^{-1}$ OCDD/OCDF), and some organochlorine pesticides [hexachlorobenzene (HCB), $\gamma$-benzene hexachloride ($\gamma$-HCH), *p,p'*-DDE and *p,p'*-DDT (1600, 400, 1600 and 400 ng ml$^{-1}$, respectively), solids were purchased from Supelco, Bellefonte, PA USA] were used as model compounds for the development of the separation method.

### 2.2. Samples

For the evaluation of the carbon separation method, the following samples were used.

*PCB technical formulations.* These were Delor 103, a technical PCB mixture corresponding to Aroclor 1242 (Chemko, Strážske, Slovak Republic), Delor 105, a technical PCB mixture corresponding to Aroclor 1254 (Chemko).

*Fly ash.* Fly ash from a municipal waste incinerator (MWI) in Bratislava (Slovak Republic) (10 g) was treated with 5% HCl, centrifuged, filtered through a Büchner funnel, dried at 50°C (maximum) and Soxhlet extracted with toluene for 24 h and either the residues were applied to an AX-21–Celite column or the toluene extract was cleaned up using a modified silica column and an ICN alumina B Super 1 column and the residues were then applied to the AX-21–Celite column [48,49].

*Biological samples.* The samples used were butter fat (pooled sample from eight Slovak producers), fish oil (pharmacy, Bratislava), oil from a cod liver tin (imported from Germany), shark fat (an n-hexane–acetone extract of shark meat purchased at a supermarket, Bratislava), catfish fat (an n-hexane–acetone extract of catfish meat purchased at a supermarket, Bratislava), egg fat (an egg obtained from a family farm, Sobotište, Slovak Republic; the yolk was mixed with anhydrous Na$_2$SO$_4$, a column was packed with the mixture and the fat was extracted with diethyl ether) and human fat (adipose tissue from an autopsy, Bratislava, extracted in the same way as the egg yolk). The

majority of lipids from the fat samples, if 1 g was taken for analysis, was removed with a Florisil column [60 g of Florisil activated at 600°C for 4 h and then overnight at 130°C, prewashed with 150 ml of n-hexane and analytes eluted with 300 ml of diethyl ether–n-hexane (3:50)]. A disposable column packed with 0.5 g of Florisil, 1 g of 44% $H_2SO_4$–silica, 0.5 g of Florisil and 1 g of anhydrous $Na_2SO_4$ was used for removing lipids from about 50-mg fat samples.

### 2.3. Carbon chromatography

*Preparation of AX-21–Celite mixture.* Activated carbon AX-21 (4 g) was thoroughly mixed with Celite 545 (76 g) to produce a 1:19 mixture. The mixture was purified using Soxhlet extraction with toluene for 8 h, dried in a vacuum oven at 100°C, then activated in a GC oven at 200°C under a nitrogen atmosphere for at least 8 h and stored in a desiccator.

*Preparation of AX-21–Celite column.* A disposable glass tube (30 cm × 10 mm O.D. × 8 mm I.D.) rinsed with acetone and n-hexane, including its outer surface [from this point the bottom part (11–12 cm) of the tube must not come into contact with hands or other contaminating surfaces], was perfectly sealed with a dense 1-cm silanized glass-wool plug which was shifted about 0.5–0.75 cm into the column. The tube was gradually packed with 0.1 g of Celite 545 and 1 g of the activated AX-21–Celite mixture, *i.e.*, the column contained 50 mg of AX-21 (the packing portions were always shaken with a vibration device). Carbon particles retained on the inner tube wall were rinsed off with a gentle stream of toluene (10 ml) from a pipette. After the carbon particles had settled, 0.1 g of Celite was poured into the toluene layer. As soon as the toluene had almost soaked into the column packing, the wall was rinsed with 3 ml of toluene in the same way as above. Finally, another 0.1-g portion of Celite was added and the column wall was again rinsed with 3 ml of toluene. The ready-to-use column is illustrated in Figure 1.

After the toluene rinse, the column was prerinsed with 5 ml of cyclohexane–dichloromethane–methanol (2:2:1) and finally with 5 ml
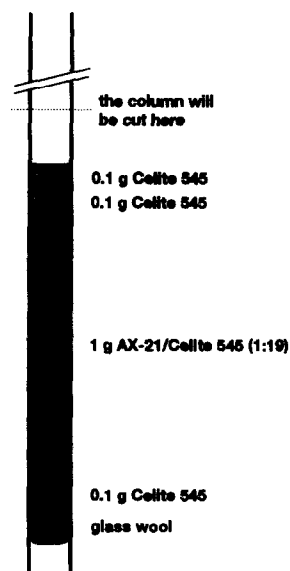


Fig. 1. Cross-section of the ready-to-use AX-21–Celite column. See text for description of adsorbent and column preparation.

of n-hexane. Then a sample extract (raw or precleaned) in 1 ml of n-hexane could be applied to the column. The sample container was then rinsed out with 3 × 0.5 ml of n-hexane.

*Elution.* Based on the elution profiles obtained, we decided on fractions as follows:

Fraction 1a: 4 ml of cyclohexane–dichloromethane–methanol (2:2:1), contains none of the model compounds (except approximately one third of p,p'-DDE and one quarter of p,p'-DDT);

Fraction 1b: 7 ml of cyclohexane–dichloromethane–methanol (2:2:1), contains multi-*ortho*-PCBs (no No. 28), γ-HCH, p,p'-DDT and p,p'-DDE;

Fraction 1c: 24 ml of cyclohexane–dichloromethane–methanol (2:2:1), contains none of the model compounds;

Fraction 2a: 2 ml of toluene, contains none of the model compounds;

Fraction 2b: 10 ml of toluene, contains the mono-*ortho*-PCBs, and the non-*ortho*-PCBs.

The column after air-drying overnight was carefully cut at about 1–1.5 cm above the packing and this empty space was filled with a dense plug of glass-wool. This shortened column (10–
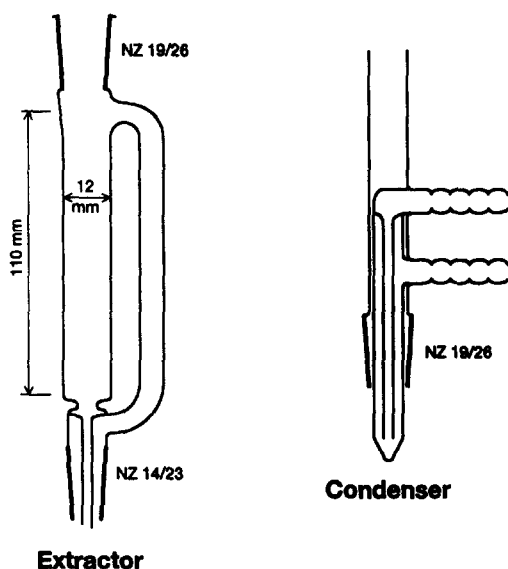
Fig. 2. Parts of the extraction apparatus (a conical-bottomed flask is not illustrated).

11 cm long) was placed in an inverted position in a miniature Soxhlet-like extractor heated by a sand-bath (see Figs. 2 and 3). The empty space at the top of the column (0.5–0.75 cm) should always be filled with dripping toluene to ensure a column flow. Tri- to octachlorinated dibenzo-p-dioxins and dibenzofurans were extracted for 3 h with 10 ml of toluene (fraction 3).

## 2.4. GC–ECD and GC–MS

*GC–ECD.* For most elution and recovery studies an HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a $^{63}$Ni electron-capture detector operated at 320°C with nitrogen at 60 ml min$^{-1}$ as make-up gas was used. A 60 m × 0.25 mm I.D. DB-5 fused-silica capillary column (J & W Scientific, Folsom, CA, USA) at 210 kPa helium column head pressure connected with a splitless injector at 280°C (purge time 1 min) was held at 110°C for 1.5 min, then programmed to 200°C at 30°C min$^{-1}$ and from 200°C to 315°C at 2.5°C min$^{-1}$, and held at the final temperature until elution of peaks had ceased. The peaks were integrated by an Apex 2.0 chromatographic computer integrator.
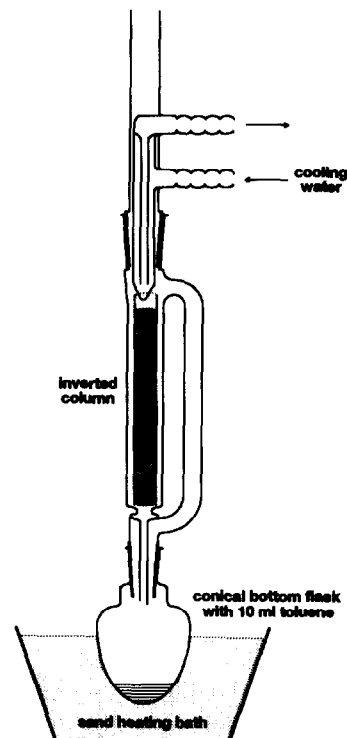


Fig. 3. Schematic diagram of the extraction of PCDDs and PCDFs retained on the AX-21–Celite column.

*GC–MS I.* An HP 5840A gas chromatograph (Hewlett-Packard) with a 60 m × 0.25 mm I.D. DB-5MS fused-silica capillary column (J & W Scientific) combined through a direct interface with an HP 5985A mass spectrometer in the selected-ion monitoring mode (GC–MS-SIM) was used for the detection of non-, mono- and multi-ortho-PCBs and organochlorine pesticides. Two or three m/z values corresponding to the most abundant ions from molecular clusters of PCBs and HCB ([M]$^+$, [M + 2]$^+$ and/or [M + 4]$^+$) were monitored. For p,p'-DDE, p,p'-DDT and γ-HCH, ions at m/z 246.00 and 248.00, m/z 235.00 and 237.00 and m/z 180.95 and 218.95, respectively, were used. The GC–MS conditions were as follows: column temperature, initially 110°C for 1 min, then program at 30°C min$^{-1}$ to 200°C and at 2.5°C min$^{-1}$ to 305°C; splitless injection port temperature, 280°C; purge time, 1 min; injection volume, 2 μl; carrier gas, helium;
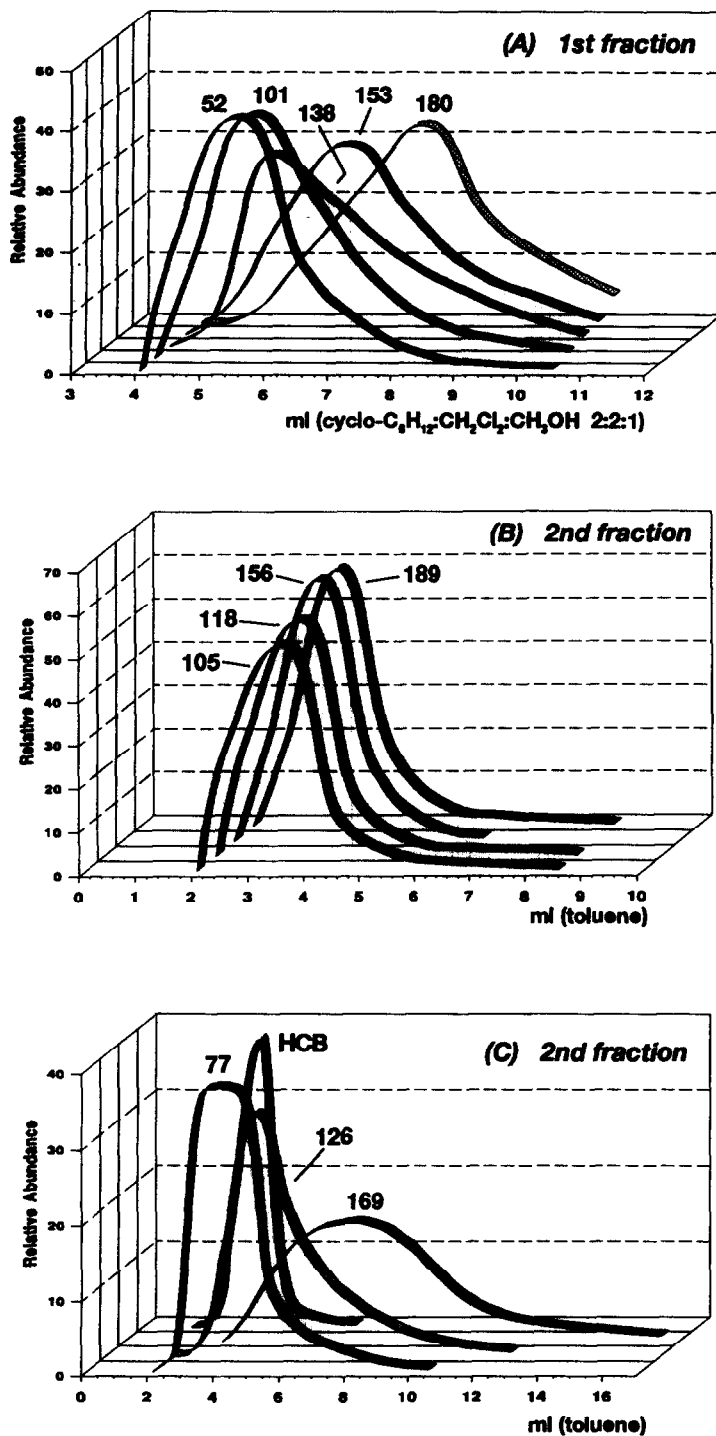
Fig. 4. Elution profiles of (A) di-*ortho*-substituted PCBs, (B) mono-*ortho*-PCBs and (C) non-*ortho*-PCBs + HCB on the AX-21–Celite column. Elution conditions as described in the text; 1-ml subfractions were collected and analysed by HRGC–ECD.

column head pressure, 190 kPa; direct interface temperature, 275°C and ionization energy, 70 eV.

*GC–MS II.* An HP 5890 Series II gas chromatograph (Hewlett-Packard) with a 30 m × 0.25 mm I.D. SP-2331 fused-silica capillary column (Supelco) combined through a direct interface with an HP 5970B mass-selective detector (Hewlett-Packard) in the SIM mode was used for PCDD and PCDF detection. Two or three *m/z* values corresponding to the most abundant ions from molecular clusters of PCDDs and PCDFs ($[M]^+$, $[M+2]^+$ and/or $[M+4]^+$) were monitored. The GC–MS conditions were as follows: column temperature, initially 120°C for 1 min, then programmed at 25°C $min^{-1}$ to 200°C, at 1°C $min^{-1}$ to 230°C and at 10°C $min^{-1}$ to 260°C; splitless injection port temperature, 280°C; purge time, 1.5 min; carrier gas, helium; column head pressure, 70 kPa; direct interface temperature, 260°C; and ionization energy, 70 eV.

## 3. Results and discussion

Activated carbon AX-21 was chosen because of its sufficient adsorption capacity, activity stability, low price and its commonly reported use [8,16,20,22,23,25,26,28,31,36,37,41,46,50]. Celite 545 as a physical support for the activated carbon, when packed in a gravity column, fulfils the conditions stipulated for inertness, purity, perfect immobilization of the carbon and acceptable column flow. To achieve a sufficient adsorption capacity, up to 50 mg of AX-21 was mixed with 950 mg of Celite 545, *i.e.*, at a ratio of 1:19 (5%, w/w). However, this amount of activated carbon necessitates a high elution volume of toluene so that the adsorbed PCDDs and PCDFs (especially OCDD and OCDF) can be eluted even from the inverted AX-21–Celite column. Moreover, the fractions 1a, 1b, 1c, 2a and 2b bring lead to lower chlorinated dioxins and dibenzofurans being transported through
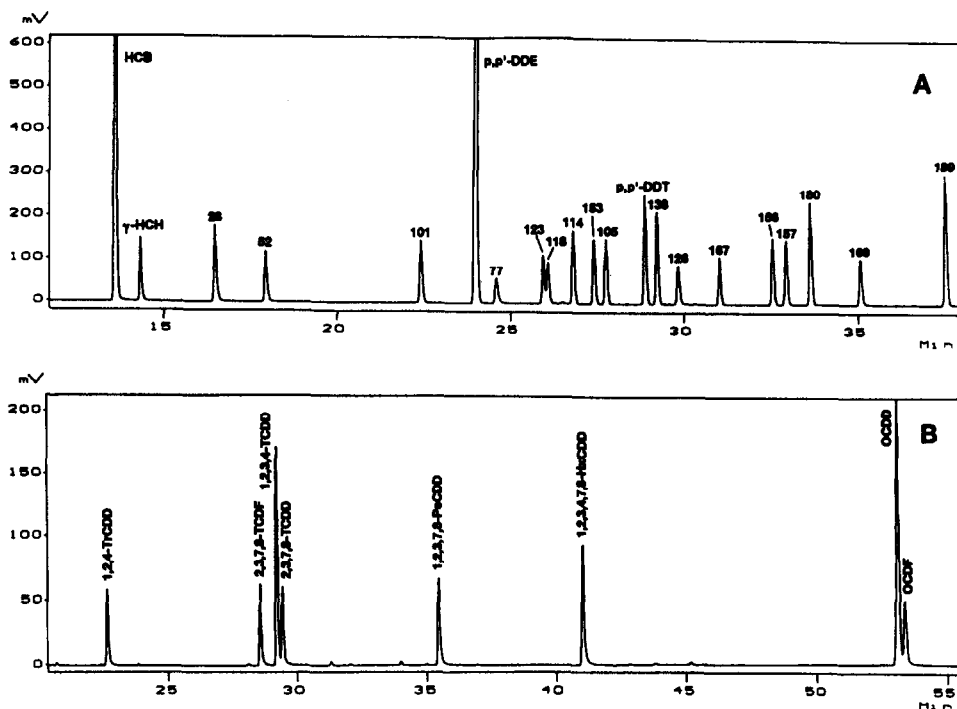


Fig. 5. HRGC–ECD (DB-5, 60 m) of (A) six "classical" PCB congeners (Nos. 28, 52, 101, 138, 153 and 180), three non-*ortho*-substituted PCBs (Nos. 77, 126 and 169), eight mono-*ortho*-PCBs (Nos. 123, 118, 114, 105, 167, 156, 157 and 189) and some organochlorine pesticides (HCB, γ-HCH, *p,p'*-DDE, and *p,p'*-DDT) and (B) some PCDDs and PCDFs used for carbon chromatographic method development. HRGC–ECD conditions as described in the text.

almost the whole of the column packing (2,3,7,8-TCDD traces begin to appear in the 16th ml of toluene). This means that TCDDs will be on the top of the inverted column and for their elution a larger volume of toluene should be applied. To decrease the elution volume as much as possible we made an original miniaturized "Soxhlet-like" extractor, the dimensions of which are adjusted to the inverted AX-21–Celite column (see Figs. 2 and 3).

A mixture of cyclohexane, dichloromethane and methanol (2:2:1, v/v/v) as an eluting solvent was selected because of its capability to elute non-planar molecules of non-polar, intermediate polar and polar compounds from carbon adsorbents. The elution curves of HCB and of the most important di-, mono- and non-*ortho*-substituted PCBs on the AX-21–Celite column are shown in Fig. 4. The curves were constructed from the abundance values of some of the

Table 1
Average recoveries of some congeners of PCDDs, PCDFs, di-*ortho*-, mono-*ortho*-, and non-*ortho*-PCBs, HCB, γ-HCH, p,p'-DDE and p,p'-DDT from the AX-21–Celite column

| Compound | Amount added (ng) | Recovery (%)[a] | | | Standard deviation (%) |
|---|---|---|---|---|---|
| | | Fraction 1b | Fraction 2b | Fraction 3 | |
| PCB-28 | 20 | –[b] | 22 | – | 4.8 |
| PCB-52 | 20 | 88 | – | – | 15.2 |
| PCB-101 | 20 | 98 | – | – | 16.8 |
| PCB-138 | 20 | 99 | – | – | 20.2 |
| PCB-153 | 20 | 97 | – | – | 18.5 |
| PCB-180 | 20 | 98 | – | – | 15.2 |
| PCB-77 | 10 | – | 82 | – | 5.3 |
| PCB-126 | 10 | – | 91 | – | 4.0 |
| PCB-169 | 10 | – | 77 | 21 | $4.4_{fr\ 2b}/4.0_{fr\ 3}$ |
| PCB-123 | 10 | – | 84 | – | 4.8 |
| PCB-118 | 10 | – | 82 | – | 5.3 |
| PCB-114 | 10 | – | 80 | – | 5.8 |
| PCB-105 | 10 | – | 84 | – | 4.0 |
| PCB-167 | 10 | – | 88 | – | 4.4 |
| PCB-156 | 10 | – | 90 | – | 7.3 |
| PCB-157 | 10 | – | 86 | – | 6.2 |
| PCB-189 | 10 | – | 100 | – | 5.8 |
| HCB | 80 | – | 40 | – | 16.0 |
| γ-HCH | 20 | 77 | – | – | 10.2 |
| p,p'-DDE | 80 | 62 | – | – | 12.2 |
| p,p'-DDT | 20 | 76 | – | – | 18.5 |
| 1,2,4-TrCDD | 5 | – | – | 63 | 9.0 |
| 2,3,7,8-TCDF | 5 | – | – | 71 | 7.3 |
| 1,2,3,4-TCDD | 10 | – | – | 74 | 10.2 |
| 2,3,7,8-TCDD | 5 | – | – | 75 | 11.6 |
| 1,2,3,7,8-PeCDD | 5 | – | – | 85 | 12.9 |
| 1,2,3,4,7,8-HxCDD | 5 | – | – | 85 | 11.5 |
| OCDD | 5 | – | – | 64 | 12.9 |
| OCDF | 2.5 | – | – | 64 | 9.0 |

[a] 4 ml of cyclohexane–dichloromethane–methanol 2:2:1 (fraction 1a), 7 ml of the same mixture (1b), 24 ml of the same mixture (1c), 2ml of toluene (2a), 10 ml of toluene (2b), "Soxhlet-like" extraction (fraction 3) and other chromatographic conditions are described in the text. Average of six determinations.
[b] Dashes indicate that the compounds were not detected or were present in negligible concentrations.

compounds, as are separated in Fig. 5, determined by HRGC–ECD in captured 1-ml carbon column subfractions.

After the elution volumes for the individual groups of the compounds had been determined according to the elution curves (see Figure 4), recovery tests ($n = 6$) were performed. A mixture of standards (see Fig. 5) was used for the tests. Average recoveries and standard deviations for fractions 1b, 2b and 3 are presented in Table 1. When the recoveries are <80%, the losses are probably caused by irreversible adsorption (PCDDs and PCDFs), during solvent evaporation (No. 28, HCB, $\gamma$-HCH) or by eluting in other fractions ($p,p'$-DDE and $p,p'$-DDT in fraction 1a, No. 28 in fraction 1c and No. 169 in fraction 3). The recovery of No. 169 can be improved by increasing the elution volume of fraction 2b from 10 ml to 16–18 ml of toluene, provided that PCDD/PCDF losses are

of little importance, e.g., the PCDDs and PCDFs are not going to be determined.

In our laboratory we also currently use a gravity column packed with 3 g of ICN alumina B Super 1, which is able to separate PCBs and PCDDs/PCDFs between the following two fractions: (A) PCBs Nos. 28, 52, 101, 138, 153, 180, 123, 118, 114, 167, 156, 157, 189 and partly (about 30–50%) 105 (2% $CH_2Cl_2$ in $n$-hexane, 25 ml); and (B) di- to octaCDD/CDF, No. 77, 126, 169 and the remainder of No. 105 (50% $CH_2Cl_2$ in $n$-hexane, 30 ml).

The efficiency of the method was tested using various real samples such as technical PCB mixtures (Delor 103 and Delor 105), MWI fly ash, fish, butter, egg and human adipose tissue. We observed that the presence of higher lipid amounts (>0.2 g) applied on the 1-g AX-21–Celite column resulted in decreased retention of the analytes, e.g., some of mono-ortho-PCBs
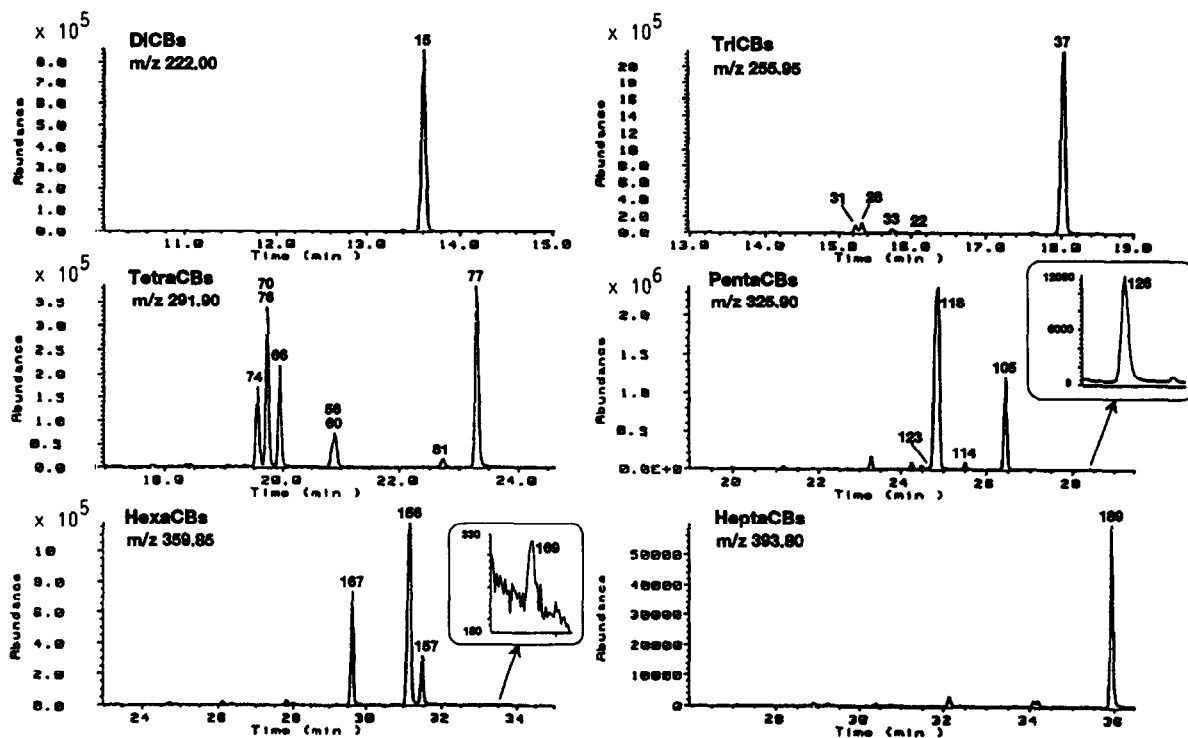


Fig. 6. HRGC–MS-SIM (DB-5MS, 60 m) of mono-ortho- and non-ortho-substituted di- to heptachlorobiphenyl congeners present in the fraction 2b from the AX-21–Celite column, i.e., in 10 ml of toluene after 4 ml of cyclohexane–dichloromethane–methanol (2:2:1) (fraction 1a), 7 ml of the same eluting solvent (fraction 1b), 24 ml of the same eluting solvent (fraction 1c) and 2 ml of toluene (fraction 2a). Sample fractionated: 1 mg of Delor 105 (for details, see the text).

partly eluted in fraction 1c. For this reason it is necessary to remove the lipids as much as possible either by chemical treatment (decomposition with sulphuric acid or alkali metal hydroxides) or by chromatography (Florisil, gel permeation). Florisil chromatography is less suitable because a more polar eluting solvent (e.g., 6% diethyl ether in n-hexane) must be used in order that the coplanar PCBs can elute, with the consequence that part of the lipids (triglycerols of unsaturated fatty acids present mainly in

vegetable and fish oil) elute together with the PCBs.

Mass fragmentograms of non-/mono-ortho-PCBs separated by the carbon column from a 1-mg sample of Delor 105 are presented in Fig. 6. Tetra- to hexaCDFs were determined in fraction 3. Levels of the compounds mentioned above are given in Table 2. The TEQs calculated from non-ortho- and mono-ortho-PCBs found in Delor 103 and Delor 105 are substantially higher than those from PCDDs/PCDFs (62 versus 2.7

Table 2
Levels of HCB, mono-ortho- and non-ortho-substituted PCBs, PCDDs and PCDFs in PCB technical mixtures of Slovak provenance (Delor 103 and Delor 105)[a] and a fly ash sample from a municipal waste incinerator in Bratislava

| Compound | Concentration[b] | | |
|---|---|---|---|
| | Delor 103 ($\mu$g g$^{-1}$) | Delor 105 ($\mu$g g$^{-1}$) | MWI flyash (ng g$^{-1}$) |
| HCB | 2.6 | 4.2 | 59 000 |
| PCB-77 | 5 270 | 1 020 | 0.74 |
| -126 | 32 | 56 | 1.1 |
| -169 | N.D. | N.D. | 0.26 |
| -81 | 355 | 75 | 0.15 |
| PCB-118 | 3 300 | 14 700 | 0.82 |
| -114 | 160 | 270 | 0.11 |
| -105 | 1 600 | 4 800 | 0.63 |
| -167 | 85 | 4 300 | 0.24 |
| -156 | 190 | 8 700 | 0.51 |
| -157 | 15 | 1 700 | 0.27 |
| -189 | 29 | 350 | 0.32 |
| 2378-TCDF | 2.0 | 2.2 | |
| Other TCDFs | 57 | 10 | |
| 12378- + 12348-PeCDF | 1.1 | 0.69 | |
| 23478-PeCDF | 4.8 | 0.54 | |
| Other PeCDFs | 14 | 3.4 | |
| 123478- + 123479-HxCDF | N.D. | 0.27 | |
| Other HxCDFs | N.D. | N.D. | |
| PCDDs, total | N.D. | N.D. | 247 |
| PCDFs, total | 79 | 17 | 128 |
| PCDDs + PCDFs | 79 | 17 | 375 |
| I-TEQ[c] | 2.7 | 0.55 | 5.2 |
| TEQ[d] | 62 | 51 | 0.13 |

[a] The AX-21–Celite column was used for the group separation; PCB-77, 126, 169, 105 and 118, PCDDs and PCDFs were quantified by spiking $^{13}$C$_{12}$-surrogates; concentrations of remaining PCB congeners and HCB were calculated using MS-SIM responses of their standards and the recoveries as are given in Table 1.
[b] N.D. = Not detected (<0.05 $\mu$g g$^{-1}$ for PCDFs and 0.3 $\mu$g g$^{-1}$ for PCBs).
[c] Toxic equivalents were calculated from 2,3,7,8-substituted PCDDs and PCDFs (values are not given) using international toxic equivalency factors according to NATO/CCMS [51].
[d] Toxic equivalents calculated from mono-ortho- and non-ortho-PCBs using toxic equivalency factors according to ref. 13.
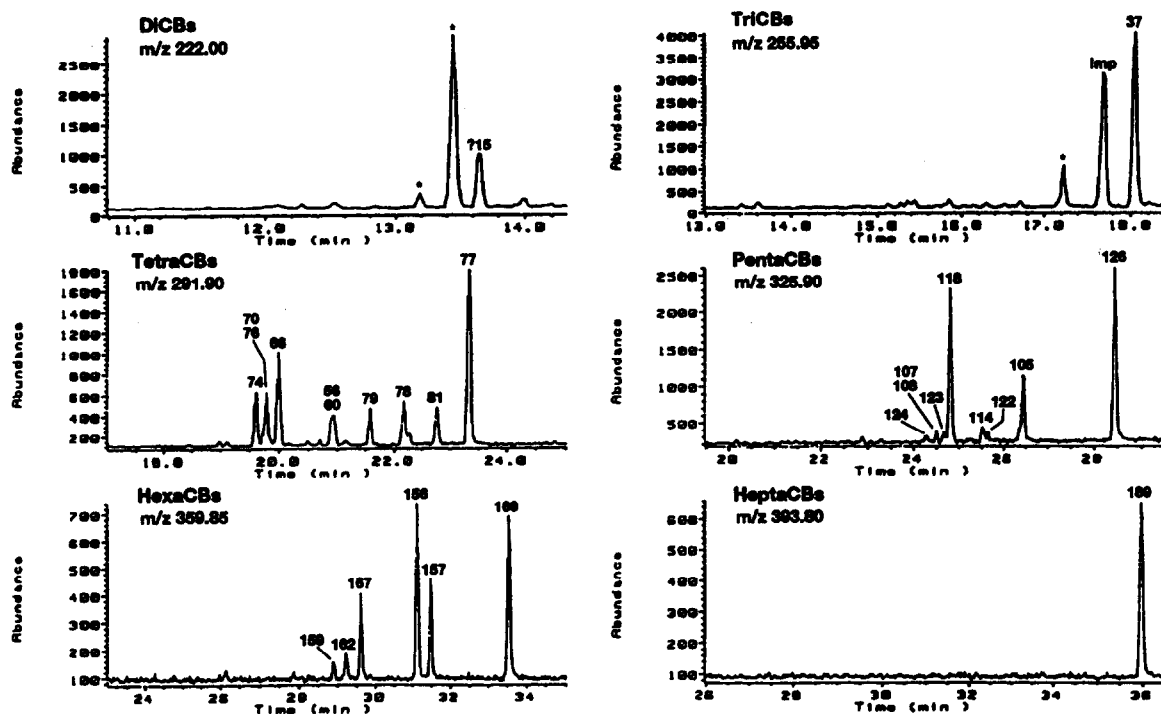
Fig. 7. HRGC–MS-SIM (DB-5MS, 60 m) of mono-*ortho*- and non-*ortho*-substituted di- to heptachlorobiphenyl congeners present in fraction 2b from the AX-21–Celite column. Sample fractionated: 10 g of fly ash from a municipal waste incinerator (for details, see the text). Asterisks indicate unidentified PCB congeners.
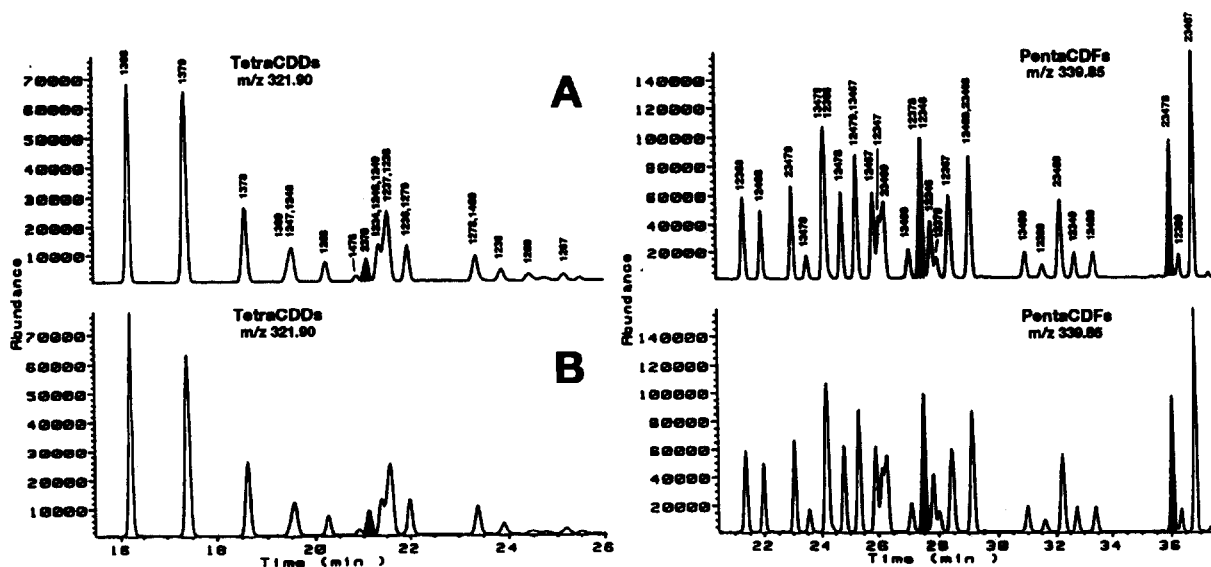


Fig. 8. Comparison of HRGC–MS-SIM (SP 2331, 30 m) of tetraCDDs and pentaCDFs found in the fractions 2b of a toluene extract of MWI fly ash (10 g) after (A) "classical" clean-up using an $H_2SO_4$–KOH–AgNO$_3$–silica column and a basic alumina column (upper traces) and (B) no clean-up (lower traces).

ppm and 51 *versus* 0.55 ppm, respectively). In accordance with results published by Kannan *et al.* [32], we observed that about 99.5% of No. 110 co-eluting with No. 77 on a DB-5 column is removed in the first fractions on our AX-21–Celite column. However, the remainder of No. 110 in environmental samples can still interfere when ECD is used.
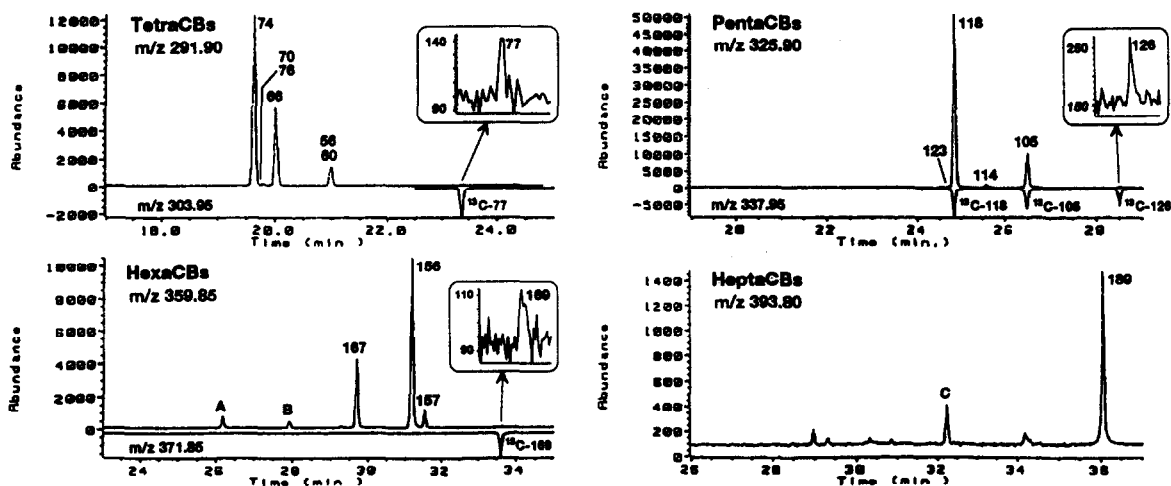
Fig. 9. HRGC–MS-SIM (DB-5MS, 60 m) of mono-*ortho*- and non-*ortho*-substituted tetra- to heptachlorobiphenyl congeners present in the AX-21–Celite fraction 2b. Peaks oriented negatively (abundance is adjusted) belong to $^{13}C_{12}$-surrogates. Sample fractionated: 1 g of fat from human adipose tissue cleaned up on a Florisil column (for details, see the text).
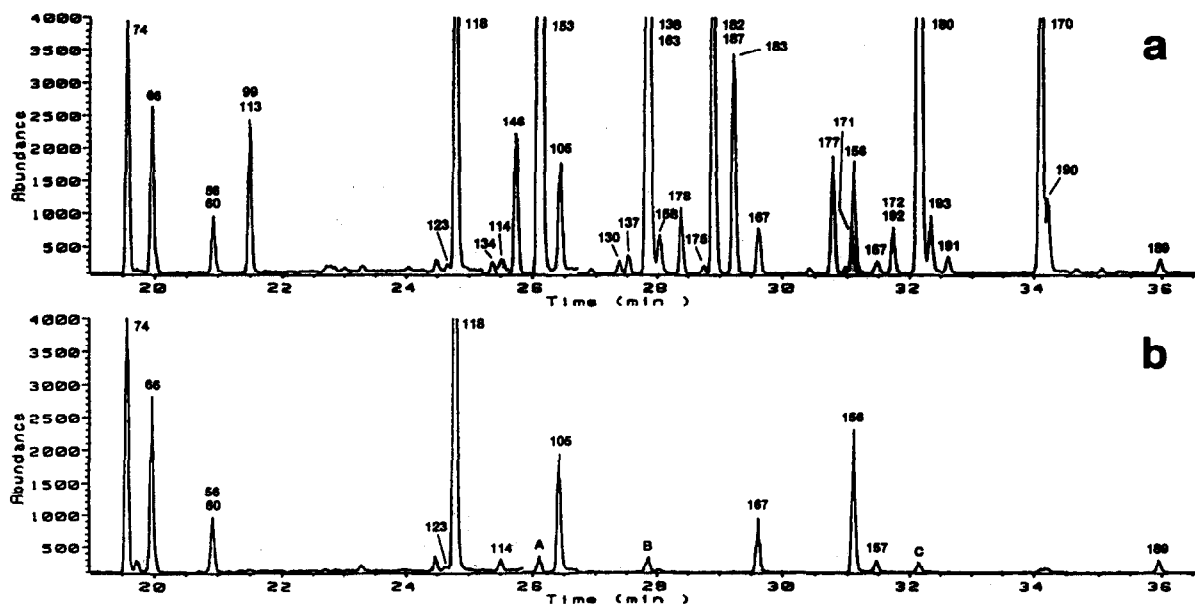
Fig. 10. Summed HRGC–MS-SIM (DB-5MS, 60 m) of tetra- to heptaCBs found in (a) a human fat sample cleaned-up on an $H_2SO_4$–silica column and (b) the AX-21–Celite fraction 2b of the sample in (a) (for details, see the text). The peaks labelled A, B and C represent negligible remainders of PCB congeners Nos. 153, 138 and 180, respectively.

Mass fragmentograms of di- to heptaCBs present in the AX-21–Celite column fraction 2b of an MWI fly ash sample extract are shown in Fig. 7. Although the raw extract was applied directly to the carbon column, the mass fragmentographic patterns of non-/mono-*ortho*-PCBs and PCDDs/PCDFs were congruent with those for the same fly ash sample extracted parallelly, cleaned up using the "classical" procedure to remove precursors of PCDDs and PCDFs such as polychlorinated phenoxyphenols and diphenyl ethers (an $H_2SO_4$–KOH–AgNO$_3$–silica column and a basic $Al_2O_3$ column) and also fractionated on the carbon column (Fig. 8). However, no statistic evaluation was made. It is noteworthy that in the fly ash sample the concentrations of Nos. 77, 126 and 169 congeners were higher than those of the individual mono-*ortho*-PCBs. Mono-*ortho*-PCB, non-*ortho*-PCB and PCDD/PCDF

concentrations are given in Table 2. In this instance the TEQ value calculated from the PCDDs and PCDFs is higher than the total TEQ from non-*ortho*- + mono-*ortho*-PCBs (5.2 *versus* 0.13 ppb).

Mass fragmentograms of tetra- to heptaCBs detected in fraction 2b (1 g of human fat analysed; fat removed using the Florisil column) are shown in Fig. 9.

The capability of the AX-21–Celite column to remove multi-*ortho*-PCBs and other interferents present in a human adipose tissue sample (50 mg) is illustrated by comparison of the summed mass fragmentograms of tetra- to octaCBs present in a human fat sample after the $H_2SO_4$–silica clean-up + AX-21–Celite fractionation and after AX-21–Celite fractionation only, as shown in Fig. 10. Fig. 11 shows the results of HRGC–ECD of the above-mentioned samples.
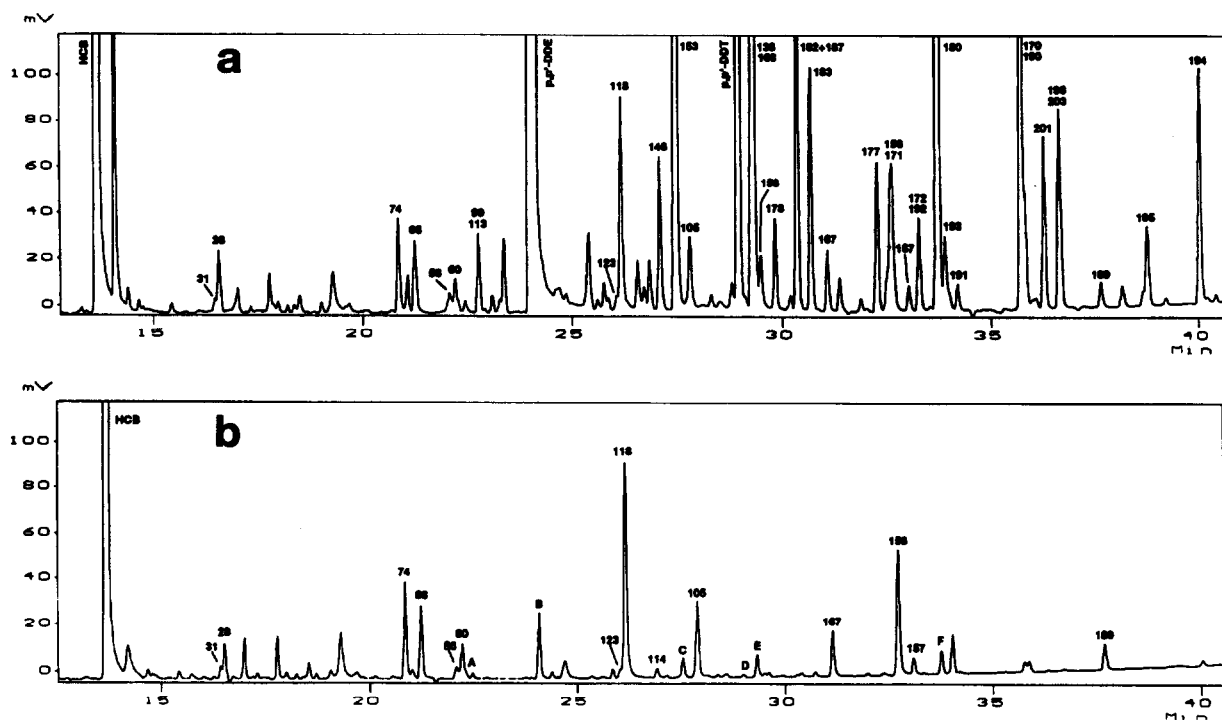


Fig. 11. HRGC–ECD chromatograms (DB-5, 60 m) of (a) a human fat sample cleaned up on an $H_2SO_4$–silica column and (b) the AX-21–Celite fraction 2b of the sample in (a) (for details, see the text). The peaks labelled A, B, C, D, E and F represent negligible remainders of No. 101, *p,p'*-DDE, No. 153, *p,p'*-DDT, No. 138 and No. 180, respectively.

The method presented was also successfully verified using egg, butter and fish (shark and catfish meat, fish oil and cod liver).

## 4. Acknowledgements

## 5. References

[1] Environmental Health Criteria 88: Polychlorinated Dibenzo-p-dioxins and Dibenzofurans, World Health Organization, Geneva, 1989.
[2] P. de Voogt, D.E. Wells, Reutergårdh and U.A.Th. Brinkman, Int. J. Environ. Anal. Chem., 40 (1990) 1.
[3] J. Nagayama, M. Kuratsune and Y. Masuda, Bull. Environ. Contam. Toxicol., 15 (1976) 9.
[4] P.H. Chen, J.M. Gaw, C.K. Wong and C.J. Chen, Bull. Environ. Contam. Toxicol., 25 (1980) 325.
[5] G.W. Bowes, M.J. Mulvihill, B.R.T. Simoneit, A.L. Burlingame and R.W. Risebrough, in F. Cattabeni, A. Cavallaro and G. Galli (Editors), Dioxin—Toxicological and Chemical Aspects, SP Medical and Scientific Books, New York, 1978, Ch. 8, p. 79.
[6] A. Kočan, J. Petrík and I. Holoubet, in T. Damstra, L.S. Birnbaum, M.J. Charles, J.C. Corton, R. Deriksen, W.F. Greenlee and G.W. Lucier (Editors), DIOXIN'91, Proceedings of the 11th International Symposium on Chlorinated Dioxins and Related Compounds, Research Triangle Park, NC, September 23–27, 1991, University of North Carolina, Chapel Hill, 1991, p. 332.
[7] S. Tanabe, N. Kannan, T. Wakimoto, R. Tatsukawa, T. Okamoto and Y. Masuda, Toxicol. Environ. Chem., 24 (1989) 215.
[8] T.R. Schwartz, D.E. Tillitt, K.P. Feltz and P.H. Peterman, Chemosphere, 26 (1993) 1443.
[9] C.-S. Hong, B. Bush and J. Xiao, Ecotoxicol. Environ. Saf., 23 (1992) 118.
[10] É. Dewailly, J.P. Weber, S. Gingras and C. Laliberté, Bull. Environ. Contam. Toxicol., 47 (1991) 491.

[11] S. Safe, Environ. Carcin. Ecotoxicol. Rev., C9 (1991) 261.
[12] K. Ballschmiter and M. Zell, Fresenius'Z. Anal. Chem., 302 (1980) 20.
[13] S. Safe, Chemosphere, 25 (1992) 61.
[14] L.M. Smith, T.R. Schwartz and K. Feltz, Chemosphere, 21 (1990) 1063.
[15] J. Tarhanen, J. Koistinen, J. Paasivirta, P.J. Vuorinen, J. Koivusaari, I. Nuuja, N. Kannan and R. Tatsukawa, Chemosphere, 18 (1989) 1067.
[16] D.G. Patterson, Jr., C.R. Lapeza, Jr., E.R. Barnhart, D.F. Groce and V.W. Burse, Chemosphere, 19 (1989) 127.
[17] Ch.-S. Hong and B. Bush, Chemosphere, 21 (1990) 173.
[18] L.G.M.Th. Tuinstra, J.A. van Rhijn, A.H. Roos, W.A. Traag, R.J. van Mazijk and P.J.W. Kolkman, J. High Resolut. Chromatogr., 13 (1990) 797.
[19] D.T. Williams and G.L. LeBel, Chemosphere, 22 (1991) 1019.
[20] W.M. Jarman, S.A. Burns, R.R. Chang, R.D. Stephens, R.J. Norstrom, M. Simon and J. Linthicum, Environ. Toxicol. Chem., 12 (1993) 105.
[21] J. Krupčík, A. Kočan, J. Petrík, P.A. Leclercq and K. Ballschmiter, Chromatographia, 35 (1993) 410.
[22] L.M. Smith, D.L. Stalling and J.L. Johnson, Anal. Chem., 56 (1984) 1830.
[23] H. Beck, A. Dross and W. Mathar, Chemosphere, 19 (1989) 1805.
[24] M. Nygren, M. Hansson, M. Sjöström, C. Rappe, P. Kahn, M. Gochfeld, H. Velez, T. Gent-Guenther and W.P. Wilson, Chemosphere, 17 (1988) 1663.
[25] L.M. Smith, Anal. Chem., 53 (1981) 2152.
[26] J.N. Huckins, D.L. Stalling and W.A. Smith, J. Assoc. Off. Anal. Chem., 61 (1978) 32.
[27] L.J. Babiak and A.S.Y. Chau, J. Assoc. Off. Anal. Chem., 62 (1979) 1174.
[28] P.H. Cramer, R.E. Ayling, K.R. Thornburg, J.S. Stanley, J.C. Remmers, J.J. Breen and J. Schwemberger, Chemosphere, 20 (1990) 821.
[29] K. Norén, A. Lundén, J. Sjövall and A. Bergman, Chemosphere, 20 (1990) 935.
[30] A.K.D. Liem, A.P.J.M. de Jong, J.A. Marsman, A.C. den Boer, G.S. Groenemeijer, R.S. den Hartog, G.A.L. de Korte, R. Hoogerbrugge, P.R. Koostra and H.A. van't Klooster, Chemosphere, 20 (1990) 843.
[31] D.W. Kuehl, B.C. Butterworth, J. Líbal and P. Marquis, Chemosphere, 22 (1991) 849.
[32] N. Kannan, G. Petrick, D. Schulz, J. Duinker, J. Boon, E. van Arnhem and S. Jansen, Chemosphere, 23 (1991) 1055.
[33] L. Zupaičič-Kralj, J. Jan and J. Marsel, Chemosphere, 23 (1991) 841.
[34] E. Storr-Hansen and T. Cederberg, Chemosphere, 24 (1992) 1181.
[35] S.S. Atuma and Ö. Andersson, Chemosphere, 27 (1993) 1.

[36] P. Schmid, M.E. Suter-Hofmann and Ch. Schlatter, *Chemosphere*, 18 (1989) 1741.

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

[38] S. Tanabe, N. Kannan, T. Wakimoto and R. Tatsukawa, *Int. J. Environ. Anal. Chem.*, 29 (1987) 199.

[39] M. Athanasiadou, S. Jensen and E. Klasson Wehler, *Chemosphere*, 23 (1991) 957.

[40] W.E. Turner, S.G. Isaacs and D.G. Patterson, Jr., *Chemosphere*, 25 (1992) 805.

[41] R. Lazar, R.C. Edwards, C.D. Metcalfe, T. Metcalfe, F.A.P.C. Gobas and G.D. Haffer, *Chemosphere*, 25 (1992) 493.

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

[43] J. de Boer, C.J.N. Stronck, F. van der Valk, P.G. Wester and M.J.M. Daudt, *Chemosphere*, 25 (1992) 1227.

[44] C.-S. Hong, B. Bush, J. Xiao and E.F. Fitzgerald, *Chemosphere*, 24 (1992) 465.

[45] C.R. Lapeza, Jr., D.G. Patterson, Jr., and J.A. Liddle, *Anal. Chem.*, 58 (1986) 713.

[46] C.A. Ford, D.C.G. Muir, R.J. Norstrom, M. Simon and M.J. Mulvihill, *Chemosphere*, 26 (1993) 1981.

[47] R.R. Chang, W.M. Jarman, C.C. King, C.C. Esperanza and R.D. Stephens, *Chemosphere*, 20 (1990) 881.

[48] A. Kočan, J. Petrík, J. Chovancová, L. Neubauerová, and M. Bezačinský, *Chemosphere*, 23 (1991) 1473.

[49] A. Kočan, A. Zemek, and M. Popl, *Collect. Czech. Chem. Commun.*, 56 (1991) 1221.

[50] *Method 1613: Tetra- through Octa- Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS (Revision A)*, US Environmental Protection Agency, Alexandria, VA, 1990.

[51] D.G. Barnes (Editor), *Pilot Study on International Information Exchange on Dioxins and Related Compounds: International Toxicity Equivalency Factors (I-TEF) Method of Risk Assessment for Complex Mixtures of Dioxins and Related Compounds*, Report No. 176, North Atlantic Treaty Organization/Committee on the Challenges of Modern Society, US Environmental Protection Agency, Washington, DC, 1988.