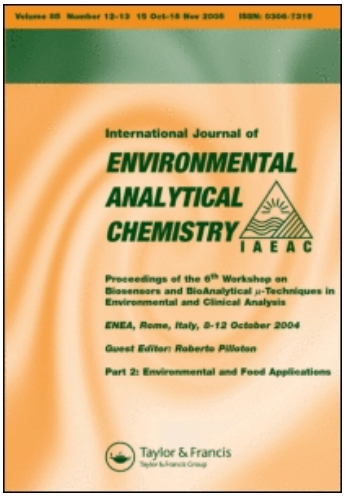

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Gas Chromatography-Negative Ion Chemical Ionization Mass Spectrometry for the Determination and Identification of Planar Polychlorinated Biphenyls in Biological Samples

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GAS CHROMATOGRAPHY-NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY FOR THE DETERMINATION AND IDENTIFICATION OF PLANAR POLYCHLORINATED BIPHENYLS IN BIOLOGICAL SAMPLES

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Planar and mono-ortho substituted polychlorinated biphenyls (PCBs) have been identified and quantified at very low concentrations in biological samples (herring and polecat samples) using an off-line combination of column liquid chromatography and gas chromatography-negative ion chemical ionization mass spectrometry (GC-NCIMS). The PCBs generate good mass spectra dominated by the molecular ion as the base peak. The dissociative electron capture mechanism is more pronounced for the higher chlorinated PCBs. The limits of detection of the planar and mono-ortho substituted PCBs under selected ion monitoring (SIM) conditions are 40-100 fg.

KEY WORDS: Planar PCBs, CG-NCIMS, biological samples.

INTRODUCTION

PCBs are lipophilic industrial pollutants which show bioaccumulating and biomagnifying properties. There have been reports indicating that the intrinsic PCB toxicity may be largely due to the presence of planar congeners (i.e., without ortho chlorines), probably because of their stereochemical similarity to 2,3,7,8-tetrachlorodibenzo-p-dioxins[1,2]. The structural requirements, in turn, depend on the number of ortho chlorine atoms and the presence of two para and at least two meta chlorine atoms on the biphenyl skeleton. The non-ortho planar PCBs which have the para/meta substitution, i.e., 3,4,3',4'-tetrachlorobiphenyl (isomer 77), 3,4,5,3',4'-pentachlorobiphenyl (isomer 126) and 3,4,5,3',4',5'-hexachlorobiphenyl (isomer 169) are biochemically the most active ones. Since they are present in trace quantities

only in commercial mixtures, an extremely sensitive and selective method is required for their detection and identification in biological and environmental samples.

Gas chromatography with electron capture detection (GC-ECD) and GC with mass spectrometric detection (GC-MS) in the electron impact (EI) ionization mode are the two methods which are currently used for the determination of the above and all other highly halogenated environmental contaminants. However, GC-ECD does not provide identification while the sensitivity of EI mass spectrometry is lower than that of GC-ECD [3-6]. Negative ion chemical ionization (NCI) mass spectrometry is a sensitive and selective method for monitoring organochlorine chemicals and can also be used for identification purposes. This technique has been applied to screening for environmental contamination by chlorinated aromatic pesticides and polychlorinated dibenzo-p-dioxins [7,8]. NCIMS has been applied to the determination of PCBs only in very few instances [9-11]. NCIMS, in some cases, was used only to verify the presence of co-eluting compounds and the actual analyses were carried out by GC-ECD. For example, when NCIMS was used to confirm the presence of cis- and trans-nonachlor in penguin livers, octachlorostyrene was also found to be present [10]. The NCI mass spectra obtained are often dominated by ions such as m/z 35 and 37, which makes identification more difficult.

In this work NCI mass spectra of PCB congeners with a different number of chlorine atoms have been compared and the analytical potential of the technique for real-life applications has been explored. To this end, column liquid chromatography (LC) using a stationary phase known to discriminate between planar, mono-ortho substituted and almost all other PCBs has been off-line combined with GC-NCIMS (using methane as a reagent gas) for the separation and the determination of the planar (isomers 77, 126 and 169) and two mono-ortho substituted (isomers 105 and 118) PCBs in biological samples.

EXPERIMENTAL

Chemicals

The solvents n-hexane, dichloromethane, n-pentane, iso-octane and diethylether (distilled before use) were of pesticide grade from Merck (Darmstadt, Germany). Anhydrous sodium sulphate, alumina B Super I and silica gel 60 (0.063-0.200 mm) were purchased from J.T. Baker (Deventer, The Netherlands), ICN Biomedicals (Eschwege, Germany) and Merck, respectively. The PCB standards were obtained from Promochem (Wesel, Germany). Helium carrier gas and the reagent gas methane (99.99% purity) were supplied by Hoekloos (Schiedam, The Netherlands).

The alumina and silica gel used as sorbents for clean-up were activated for 24 h at 200°C and 140°C, respectively. They were deactivated with potassium hydroxide and sulphuric acid (both used after previous washing with n-hexane) as indicated below.

Sample preparation and extraction

Two types of sample were analysed. Herring oil was obtained as a gift from the Swedish Environmental Protection Agency (Solna, Sweden) and polecats from different locations in the Netherlands were selected on the basis of differences in habitat. All polecats used in this study were killed by traffic.

Approximately 0.1-2 g of a biological sample were weighed and homogenised and then dried with sodium sulphate [11]. The PCBs were extracted with 150 ml of dichloromethane:n-pentane (1:1, v/v) in a Soxhlet extractor for 6 h. The extract was evaporated to 1 ml using a gentle stream of nitrogen.

For clean-up a 25 cm x 11 mm I.D. glass column (only for polecats) was packed from the bottom with 3 x 1 g of silica (deactivated with 10% KOH for eliminating strongly coloured interferences, 44% H_2SO_4 and 22% H_2SO_4 for oxidizing lipids) and 2 g of 5% water-deactivated alumina. A layer of 1 cm of anhydrous sodium sulphate was placed on top of each portion of silica and alumina. The column was prewashed with 10 ml of hexane:diethylether (97:3, v/v). The 1-ml PCB-containing extract was added to the column, the first 4 ml of eluate (hexane:diethylether, 97:3) were discarded and the next 15 ml which contain the relevant PCBs were collected. Nitrogen was employed to reduce the volume to 1 ml and the concentrated extract was added to a silica column (3 g; 0.5% water-deactivated) which was eluted with hexane:diethylether (97:3). The first 15 ml were collected and evaporated to 100 ul using nitrogen. The remainder was quantitatively transferred to a 300 ul microvial and evaporated to 50 ul.

The separation of planar from non-planar PCBs was performed on a 15 cm x 4.6 mm I.D. Cosmosil PYE 5 column which contains 2-(1-pyrenyl)ethyldimethylsilylated silica (Nacalai Tesque, Kyoto, Japan; delivered by Promochem) using hexane at a flow rate of 0.5 ml/min as eluent. The fraction containing the non-planar and mono-ortho substituted PCBs eluted between 3 and 8.5 min while the fraction which contains the planar PCBs, eluted between 8.5 and 14.2 min. Both fractions were evaporated to 1 ml under a stream of nitrogen. Finally, 2 ml of iso-octane and 100 ul of iso-octane containing 2,4,5,2',4',5'-hexachlorobiphenyl (PCB congener 153) as an internal standard were added and further evaporation was carried out to reduce the volume of the non-planar and planar fractions to 1 ml and 25 ul, respectively.

GC-MS system

A Hewlett Packard (HP; Palo Alto, CA, U.S.A.) 5989A MS Engine, equipped with a dual EI/CI source, in conjunction with a HP 5890 (Series II) gas chromatograph and a HP-UX 98578 X data system was used for GC. Separations were performed on a 12 m x 0.2 mm i.d. HP-1 (crosslinked methyl silicone gum with 0.33 um film thickness) fused-silica capillary column. An amount of 2 µl of each sample was injected onto the GC using the split/splitless injector in the splitless mode with a purge delay of 0.75 min. The column was introduced directly into the ion source and its temperature was programmed from 90°C (for 1 min) at 25°C/min to 270°C. The injector temperature was set at 250°C, the transfer line at 280°C and the ion source at 150°C or 200°C. Helium was used as carrier gas and methane

as reagent gas for chemical ionization. The MS Engine was tuned on m/z 264, 414 and 633 (negative ions) corresponding to perfluorotributylamine, at the same pressure as used during analysis.

RESULTS AND DISCUSSION

Mass spectra of PCBs

There are few recent papers regarding the NCI mass spectrometry of PCBs (cf. above). If GC-NCIMS has therefore to be used for the identification and trace-level determination of PCBs in biological samples, the NCI spectra of these pollutants should be studied in some detail.

NCI mass spectra of seven selected tetra- to deca-substituted PCBs are shown in Figure 1; these were obtained by introducing low-pg amounts of the PCB congeners into the gas chromatograph (cf. Figure 2 below). The mass spectra demonstrate that non-dissociative electron capture plays the major role. According to Pellizzari et al. [12], mass spectra of PCBs with one to five chlorine atoms show the predominant ions at m/z 35 and 37, while molecular ions have a low abundance. In their study, with congeners containing six chlorines, the molecular ion became more apparent and for higher degrees of chlorination, the molecular ion became the base peak. However, our results show that already for PCBs with four chlorine atoms, $M^{-\cdot}$ is the most abundant mass cluster. Actually, only NCI mass spectra of PCBs with three chlorine atoms or less were dominated by m/z 35 and 37. For the rest, it should be noted that the mass spectra of the isomers with 4, 5 and 6 chlorine atoms in Figure 1 are those of the planar congeners 77, 126 and 169, respectively. In agreement with our results, Stemmler et al. [13] showed that a HP 5985 B quadrupole mass spectrometer generates a lower abundance of low-mass ions (such as Cl) than other instruments.

NCI is known to generate relatively simple mass spectra but they can be affected by changes in the physical and geometrical parameters of the ion source [13-16]. The different results obtained by Pellizzari et al. [12] and in our study may indeed well be due to differences in temperature and/or the reagent gas pressure of the ion source. Some authors even indicate that the spectra of polyhalogenated compounds are sensitive to minor variations in sample concentration and water/oxygen contamination [17,18]. Our studies show that for PCBs with a higher number of chlorine atoms the dissociative capture of the thermal electrons tends to increase. The effect appears to be more pronounced for the planar PCBs (cf. spectrum of congener 169). This can be attributed to an increase of the product ions, since a higher substitution with electronegative chlorine atoms enhances the stabilization of a negative charge. This is in agreement with the data of Erhardt-Zabik et al. [19] who observed a decrease in molecular ion abundance as the PCB congener was substituted with nine or ten chlorine atoms. Finally, enhancing non-dissociative electron capture requires slow electrons which can be obtained by moderation. Good moderating capacities are expected from H-containing compounds [18] and methane was therefore preferred as electron moderating gas in the present study.

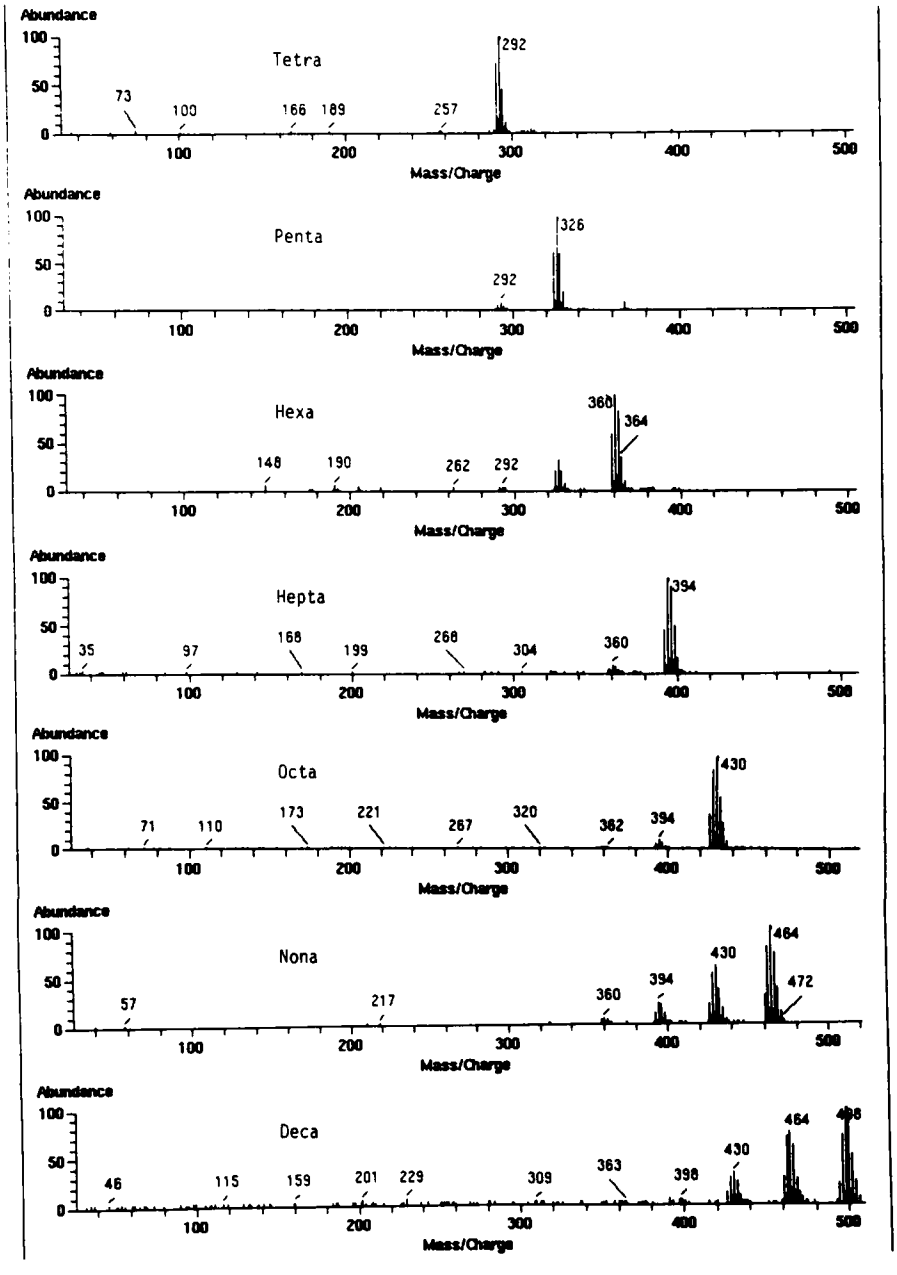

Figure 1 NCI mass spectra of seven PCB congeners (tetra-deca, scan range, 30-505 amu) using methane as reagent gas. GC-NCIMS conditions are given in Experimental section.

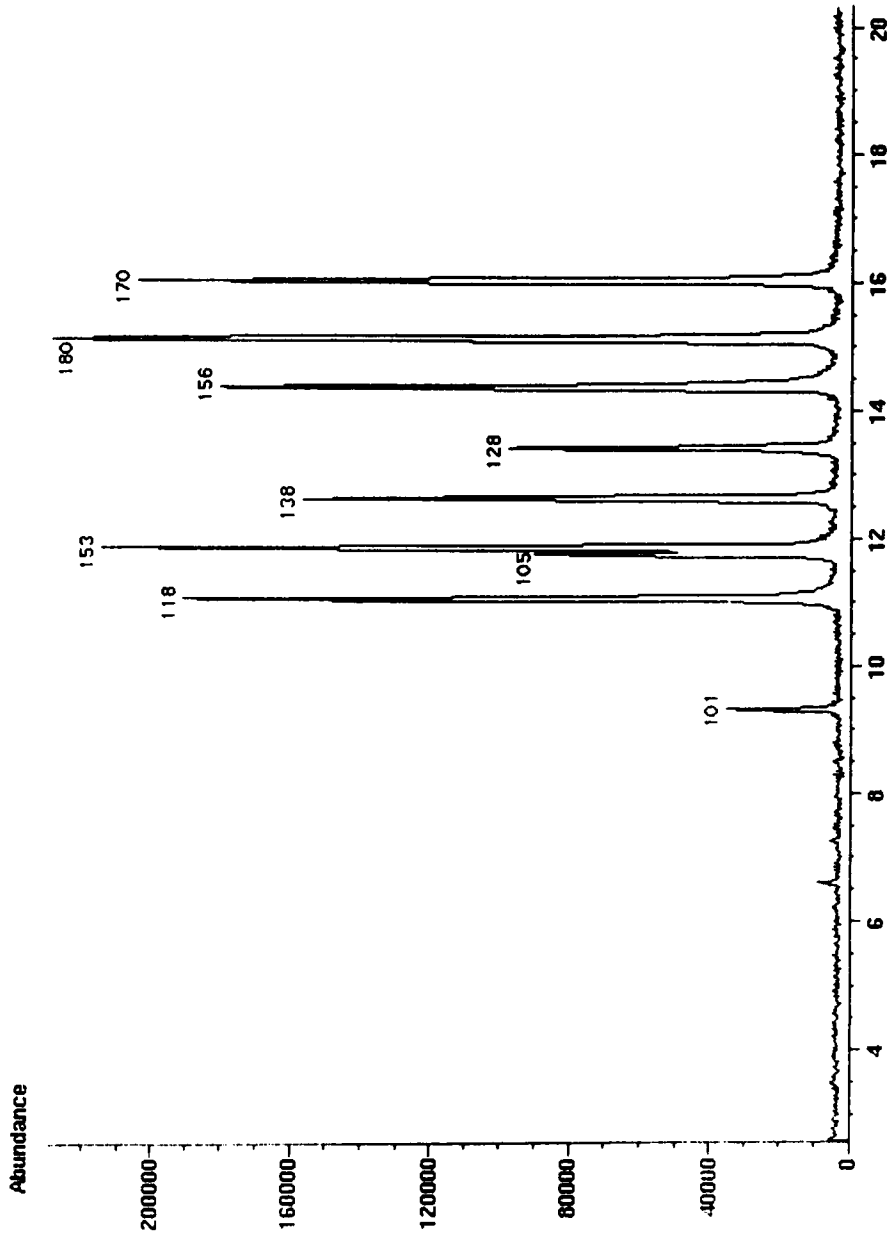

Figure 2 GC-NCIMS chromatogram of a standard mixture of PCBs (IUPAC numbers indicated) in iso-octane (0.7 pg each) obtained under full-scan (30-505 amu) conditions. GC-NCIMS conditions are given in Experimental section.

Figure 2 shows a GC-NCIMS chromatogram obtained under full-scan (30-505 amu) conditions after injecting a standard mixture of PCBs in iso-octane (0.7 pg each). Despite the short run time of 16 min most peaks are nicely resolved. Obviously, even under full-scan conditions the limits of detection are expected to be in the range of 100 fg for most of the congeners.

Analysis of biological samples

The extraction of PCBs from biological samples and the removal of interfering co-extracted compounds can be accomplished by a variety of well established methods [20]. Liquid-liquid extraction is mostly preferred to ensure sufficient solvent penetration into the sample and was also used in our study (cf. Experimental section). The separation of planar and non-planar PCBs can be achieved by employing different crystalline forms of active carbon as the packing material in LC [21,22]. In carbon chromatography the separation occurs on the basis of molecular geometry and the number of chlorine atoms [23] which is more efficient than separation on the basis of analyte polarity on Florisil or silica. In this work, a nice and fast isolation of planar PCBs was achieved using a 2-(1-pyrenyl) ethyldimethylsilylated silica column [24,25]. Less than 10 ml of a single solvent (hexane) sufficed to separate the planar and non-planar fractions. This favourably contrasts with carbon chromatography, where much higher volumes of various mobile phases and longer times are needed for a complete separation [22]. The selectivity of the PYE column is probably due to π-π interactions between the electron clouds of the planar PCBs and the pyrene moieties of the stationary phase.

After extraction of the biological samples and LC preseparation, the PCBs were analysed by GC-NCIMS under selected ion monitoring (SIM) conditions using methane as reagent gas. The extracted ion chromatogram obtained after injection of the planar-PCB-containing fraction of the herring oil extract is shown in Figure 3. Relevant analytical data for the three planar as well as two mono-ortho substituted PCBs are listed in Table 1. These results were obtained using an ion source temperature of 200°C. When the ion source temperature was

Table 1 Analytical data of planar and mono-ortho substituted PCBs in herring oil under SIM conditions

PCB congener	t_R* *(min)*	*LOD*** *(fg)*	*Base peak*	*Conc. (ng/g)*
planar				
3,4,3',4'-tetra (77)	7.3	70	292	0.35
3,4,5,3',4'-penta (126)	7.9	35	326	0.27
3,4,5,3',4',5'-hexa (169)	8.6	55	360	0.08
mono-ortho substituted				
2,3,4,3',4'-penta (105)	7.7	95	326	10.5
2,4,5,3',4'-penta (118)	7.5	55	326	38.4

* 2 µl of sample injected onto the GC column using splitless mode.
** Signal-to-noise ratio, 3/1; injection of standard solutions.

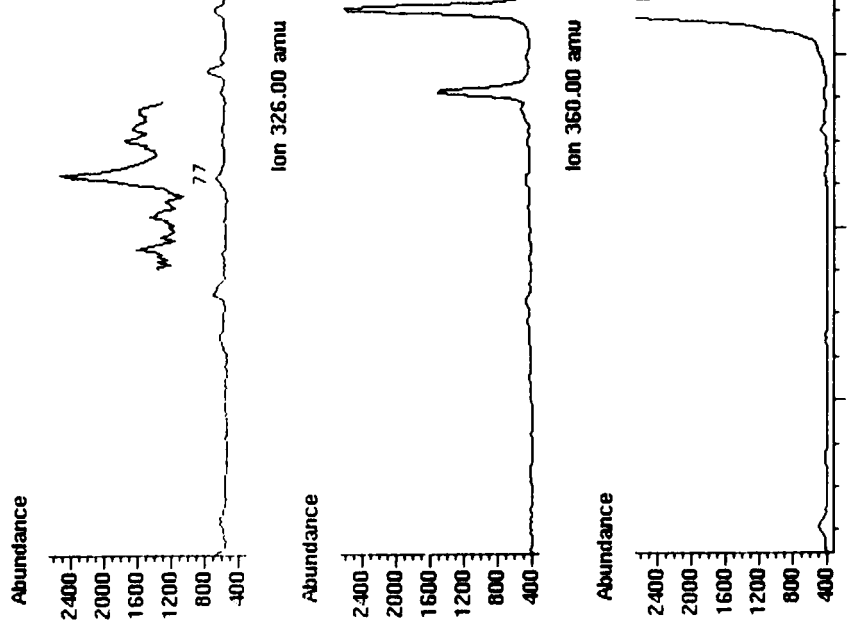

Figure 3 Extracted ion chromatograms of a herring oil extract under SIM (m/z 292, 326, 360) conditions. GC-NCIMS conditions are given in Experimental section; however, source temperature at 200°C.

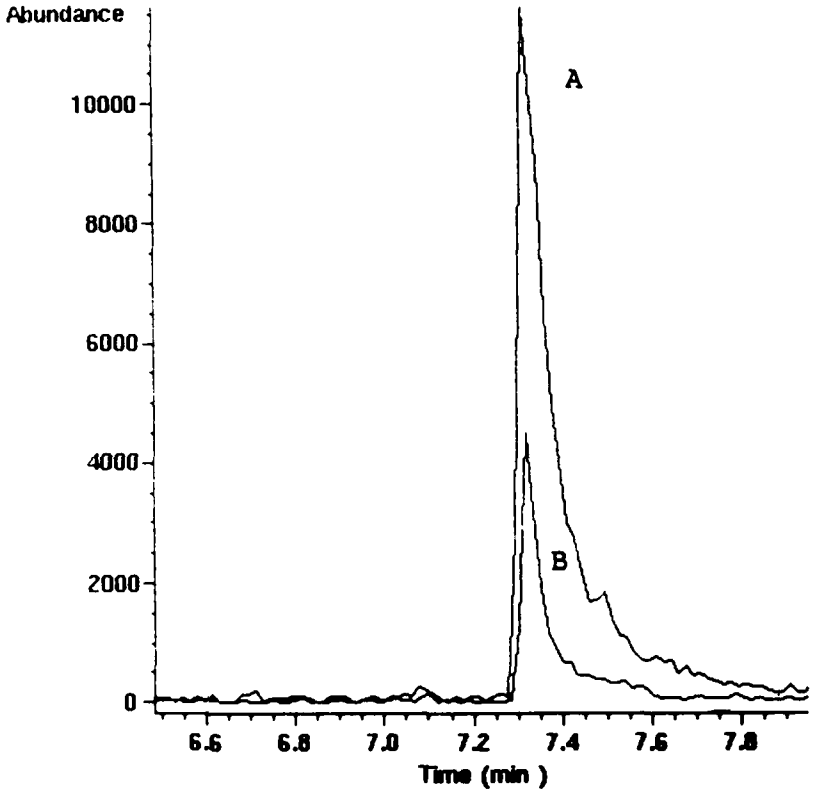

Figure 4 Response of planar PCB congener 77 in GC-NCIMS at source temperature of (A) 150°C and (B) 200°C.

reduced to 150°C the peak intensities increased, particularly for PCB congener 77 (cf. [26]). This is demonstrated in Figure 4 by the 3-fold increase in signal intensity shown by the latter congener. Unfortunately, too small an amount of sample had been provided to allow another

Table 2 Concentrations of planar PCBs (ng/g of lipid) in muscle and anal gland secrete of polecats in The Netherlands

Location	*Sex**	*Organ/ tissue***	*Concentration of PCB No.* 77	126	169
Kinderdijk	M	I	1.9	9.6	2.0
Voorthuizen	F	II	0.6	0.5	0.2
Voorthuizen	F	I	2.3	1.0	nd***
Kadijk-Terwolde	M	II	0.6	0.2	nd
Kadijk-Terwolde	M	I	3.5	1.4	0.6
Barneveld	M	II	1.1	2.1	0.5
Barneveld	M	I	0.2	2.6	0.7

* M: male, F: female.
** I : anal gland secrete; II: muscle.
*** nd: not detected.

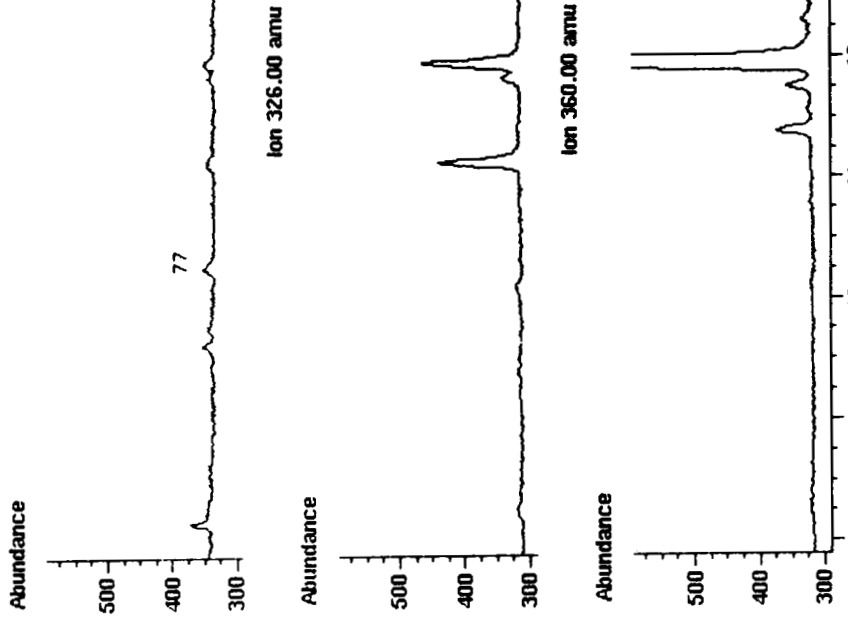

Figure 5 Extracted ion chromatograms of the extract of a polecat anal gland secrete sample from BARNEVELD under SIM conditions. GC-NCIMS conditions are given in Experimental section; source temperature, 150°C.

analysis at the optimum temperature. For the rest, the data in Table 1 indicate that detection limits of 40-100 fg (under SIM conditions) can be obtained with real-life samples.

Table 2 summarizes data on the concentrations of the three planar PCBs in muscle and anal gland secrete from polecats found at various locations in The Netherlands. These data were collected as part of an extended biomonitoring/toxicological programme that is presently in progress [11]. Analysis of these samples was carried out using an extended temperature programme, viz. from 90°C (for 1 min) at 25°C/min to 180°C and then at 4°C/min to 270°C, improved resolution being required because of the complicated matrix of most of these samples. As is clear from Figure 5, this was especially true for PCB congener 169 which elutes close to a large interfering peak. The combined results again demonstrate that low- and sub-ng/g levels of the planar PCBs can be detected and quantified in biological samples using a rather restricted amount (0.1-2 g) of sample.

The planar PCB levels found by us are of the same order of magnitude as those found in recent studies. As regards the literature data, the three planar PCB congeners have been detected in the blubber of a finless porpoise using GC-ECD (0.71-16.2 ng/g level on wet basis [22]) and in horse fat (0.3-10.9 ng/g [27]). In the latter instance, gel permeation chromatography and porous graphitic carbon LC were combined with GC-EIMS [27]. The latter authors observed rather large discrepancies when quantification was carried out using GC-ECD instead of GC-MS. This was probably due to interferences showing up in GC-ECD. This again indicates that detection and identification by means of, preferably NCI (cf. ref. [19]), mass spectrometry should be recommended for the ultra-trace level analysis of PCB-containing biological matrices.

CONCLUSIONS

The off-line combination of LC and GC-NCIMS provides a very sensitive and selective technique for the identification and quantitative determination of planar and mono-ortho substituted PCBs in complex biological samples. The good selectivity of the system is partly due to the separation efficiency of the pyrenyl-type LC column used to separate planar from non-planar PCBs prior to their introduction onto the GC column. It also arises from the good performance of the Hewlett Packard MS Engine mass spectrometer which provides the molecular ion as the most abundant cluster in NCIMS for all PCBs with four or more chlorine atoms. Detection limits in the SIM mode typically are 40-100^fg. In the present study, this has allowed the detection of the planar PCB congeners 77, 126 and 169 as well as two mono-ortho substituted PCBs in herring oil and organs from various polecats at the level of 0.1-10 ng/g of lipid.

Acknowledgments

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