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A SIMPLE, NOVEL METHOD FOR THE QUANTITATIVE ANALYSIS OF COPLANAR (NON-ORTHO SUBSTITUTED) POLYCHLORINATED BIPHENYLS IN ENVIRONMENTAL SAMPLES

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ABSTRACT

A novel method is presented for the analysis of coplanar (i.e. non-ortho substituted) polychlorinated biphenyls (PCBs) in environmental samples. The new procedure combines methylene chloride extraction, gel permeation and Florisil chromatography. Recovery efficiencies and resolution of the Florisil column method are reported and compared to the carbon column method. It is shown that both the Florisil column and the Carbon column techniques give comparable results with recovery efficiencies for PCBs 77, 126 and 169 of nearly 100%. Excellent resolution and reproducibility for quantitative analysis of coplanar PCBs are obtained in complex PCB mixtures. The Florisil method is considerably simpler than the carbon column technique and better suited for routine analysis.

KEYWORDS

Coplanar PCB; Florisil/Carbon Chromatography.

INTRODUCTION

There is considerable evidence that most of the dioxin-like toxicity observed in aquatic ecosystems is related to non-ortho substituted PCBs [1,2]. Specifically, the coplanar congeners 3,3',4,4'-tetrachlorobiphenyl (IUPAC #77), 3,3',4,4',5-pentachlorobiphenyl (IUPAC #126) and 3,3',4,4',5,5'-hexachlorobiphenyl (IUPAC #169) are known to be strong inducers of both aryl hydrocarbon hydroxylase (AHH) and ethoxyresoruflin-o-deethylase (EROD). Safe[3] measured the TCDD toxicity equivalents of congeners 77, 126 and 169 to be 0.1, 0.4 and 0.1 respectively. In the aquatic environment, however, these congeners are observed at concentrations several orders of magnitude higher than that of TCDD. Kubiak[2], for example, concluded that over 90% of the dioxin-like toxicity associated with Forster's Terns in Green Bay could be accounted for by congeners 77, 126 and 169.

Despite the knowledge of the potential environmental hazard represented by these congeners in the environment, there is very little information on their distribution and dynamics in aquatic and terrestrial ecosystems. This is largely a result of the analytical difficulties encountered in quantifying these compounds in complex environmental samples[1,4]. To date, the most successful approaches have been the use of carbon column chromatography[5], or the use of HPLC chromatography using a 2-(1-pyrenyl) ethyldimethylsilylated silica column[6]. Both approaches, however, are laborious and not readily adaptable into routine laboratory procedures. The specialized nature of the carbon column and the HPLC approaches has resulted in a 'bottle neck' in developing priority information to quantify exposure dynamics of non-ortho substituted PCBs in the environment.

There is need for a technique that can be readily integrated into existing laboratory capabilities. A new approach is proposed herein, and is a simple extension of the Florisil column technique described by the Canada Wildlife Service[7] (CWS). The advantage of the new method is that it permits a complete assessment of all PCB congeners and many other organochlorines and pesticides without the addition of more complicated glassware or sample clean up techniques. The following study describes the technique in detail, and compares recoveries and results with the widely accepted carbon column approach.

MATERIALS AND METHODS

In order to compare the Florisil column with the carbon column technique, three independent studies were performed. The first was to determine and to compare recoveries of the two techniques by directly spiking the columns with standard solutions containing congeners 77, 126 and 169 at concentrations observed in environmental samples. Secondly, a complex mixture of Aroclors 1242:1254:1260 with 10 other organochlorinated compounds was enriched with congeners 77, 126 and 169 to compare the ability of the two techniques to resolve coelution problems. Finally, spiked samples (mink livers) were used to compare the two procedures using actual environmental samples.

Materials

Glass-distilled solvents were tested for interfering residues by GC-ECD analysis of concentrated aliquots. Anhydrous Na_2SO_4 (CALEDON) was heated in a muffle furnace at 650°C. Glass wool was soaked in dichloromethane (DCM) for 24 hrs., the excess DCM poured off, and the remaining DCM was evaporated by placing the glass wool in a fume hood. The glass wool was oven-heated overnight at 130°C.

The activated carbon AX-21 (64.92% moisture, ANDERSON DEVELOPMENT COMPANY) was extracted in a soxhlet with 50:50 acetone/DCM (v:v) to assure the carbon was free of interfering residues. All glassware was soap/water washed, heated at 350°C overnight, and then rinsed with acetone (3x), Petroleum ether (3x) and hexane (3x) before use.

Standards

Standards of 3,3',4,4'-tetrachlorobiphenyl (#77), 3,3',4,4',5-pentachlorobiphenyl (#126) and 3,3',4,4',5,5'-hexachlorobiphenyl (#169) were obtained from Acustandard in iso-octane (35 µg/mL). Standards for chromatographic analysis were developed in a hexane solution containing the three coplanar congeners at concentrations of 35 ng/mL. To check for potential coelution problems, a calibrated standard solution containing 10 organochlorinated compounds with a mixture of Aroclors 1242:1254:1260 (1:1:1) was obtained from the CWS Laboratory, Ottawa. In order to spike mink liver tissue, a methanol spiking solution (3.5 ng/mL) containing congeners 77, 126 and 169 was developed.

Spiking of the Carbon and Florisil Columns

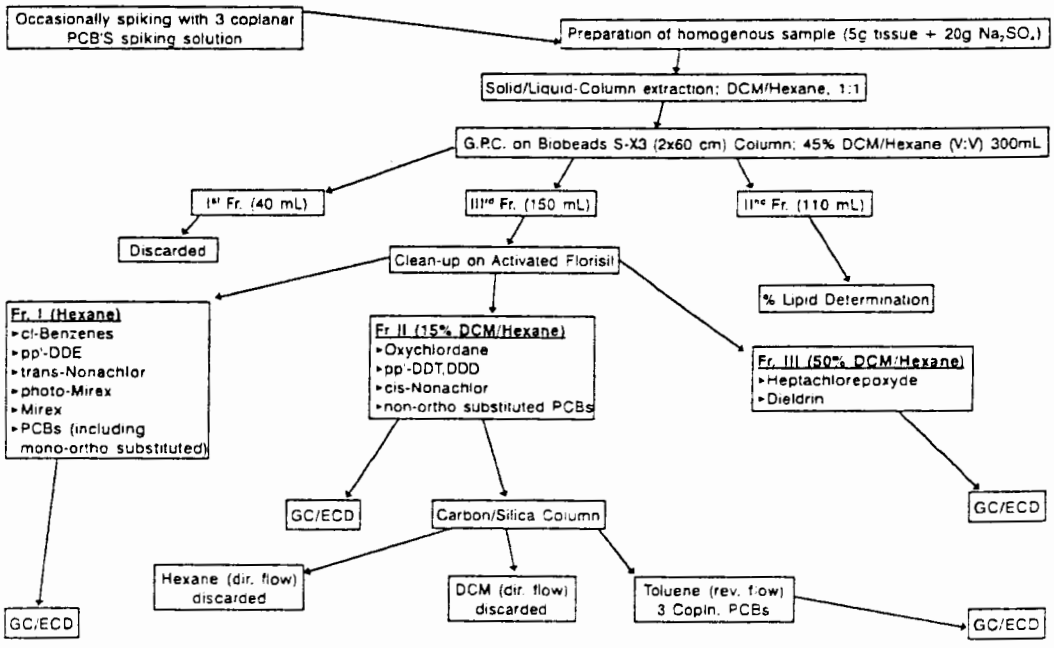
Florisil and carbon columns were spiked with standard solutions of congeners 77, 126 and 169 to yield final concentrations of 0.35, 0.7, 1.4, 2.8, 3.5 and 7.0 ng/mL. Columns were further evaluated by enriching an Aroclor mixture 1242:1254:1260 (1:1:1) with the above concentrations of the coplanar congeners.

Mink liver samples had total PCB level under 100 μg/kg wet weight, and the non-ortho substituted congeners were below detection. The mink samples (n = 18) were fortified with a spiking solution (3.5 ng/mL) to yield a reference material with a concentration of 0.7 μg/kg for each coplanar PCB. The liver was then analyzed in order to quantify the accuracy and precision of the two procedures.

Analytical Procedures

Figure 1 summarizes the procedure to quantify the concentrations of congeners 77, 126 and 169 in mink samples using both the Florisil column and carbon column techniques. Essentially, a sample is extracted, using DCM/Hexane (1:1). The sample is then run on a Gel Permeation

Figure 1. **Flow Chart of Analytical Procedures**



Chromatography column, and the third fraction, containing the contaminants of interest, is transferred to a Florisil column.

Florisil Column Chromatography

Florisil (60 - 100 μm mesh from Supelco) was activated overnight at 130°C, and poured into a glass column (1 cm x 35 cm) filled with hexane. To the top of the column, 2 cm of anhydrous Na_2SO_4 was added.

After transferring the sample to the column, the column is eluted with 50 mL of hexane and Fraction 1 collected. The column is further eluted with 50 mL 15% DCM/Hexane (v:v) to yield Fraction 2. Fraction 3 was collected by eluting the column with 65 mL 50% DCM/Hexane (v:v). Each fraction was evaporated to a final volume of 2 mL.

Carbon/Silica Column Separation of Non-Ortho Chlorinated PCBs

A 5% carbon/silica gel mixture containing AX-21 activated carbon (ANDERSON DEVELOPMENT COMPANY) and Silica Gel (100 - 200 μm Mesh from SUPELCO), activated overnight at 130°C. This mixture was cooled to room temperature in a desiccator prior to preparing the glass column. The glass column consisted of 0.6 cm x 10 cm glass tube with similar ground joints (7/25) at both ends, to match a 50 mL glass reservoir. A 2 cm bed of 5% carbon/silica mixture was filled (dry) between 2 x 1 cm glass wool beds. The column was rinsed concurrently with 15 mL of toluene, 15 mL of DCM, and 15 mL of hexane to activate the column. A 1.5 - 2 mL concentrated sample extract was added to the top of the column using a Pasteur pipette. The column was eluted with 30 mL volume of hexane, and the elute was collected as Fraction 1. The column was then eluted with 30 mL of DCM and the elute (Fraction 2) was collected. The column was then inverted and eluted with 30 mL of toluene, (Fraction 3) which contained the non-ortho chlorinated substituted PCBs. Fraction 3 was evaporated (after adding 4 mL of Iso-octane), then transferred to a graduated container and diluted with hexane to an appropriate volume.

GC/ECD Capillary Conditions

The three fractions separated in the Florisil column clean up step, and the Fraction 3 in Carbon/Silica column separation were analyzed separately on a HP-5890 GC/ECD equipped with a HP-3396 integrator and an HP-7673 autosampler.

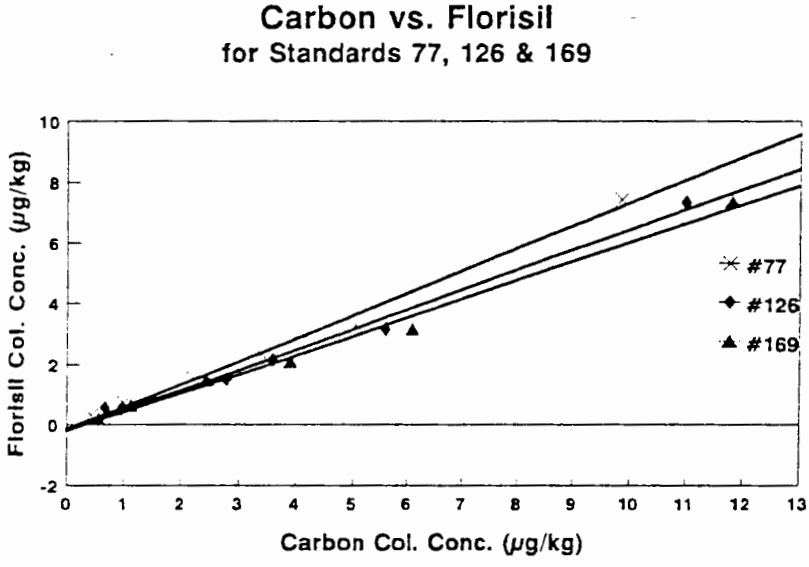
Column:	30 m x 0.25 mm i.d. with 0.25 mm DB-5 film thickness (J & W)	
Inj. Temperature:	250°C.	
Carrier Gas:	He (30 cm/sec, measured at 100°C)	
Make-up Gas:	Ar/CH_4 (95%/5%) at 40 mL/min.	
Oven Temperature Program:	Initial Temp.:	100°C
	Initial Time:	0.5 min.
	Rate:	3°/min.
	Final Temp.:	270°C
	Final Time:	8 min.

A 3 μL sample was injected using a splitless injection mode.

RESULTS

Florisil and carbon columns were spiked with a range of concentrations (0.35 to 7.0 ng/mL) of congeners 77, 126 and 169. A linear relationship was found between the two techniques (*Figure 2*). As the slopes were not significantly different ($P<0.001$ ANCOVA), there is no bias in either technique in quantifying these three congeners. The r^2 for direct spiking of congeners

Figure 2. **Direct Spiking of Congeners 77, 126 and 169.**



77, 126 and 169 on Florisil and carbon columns were 0.992, 0.991 and 0.991 respectively.

Results illustrate
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Figure 3. Direct

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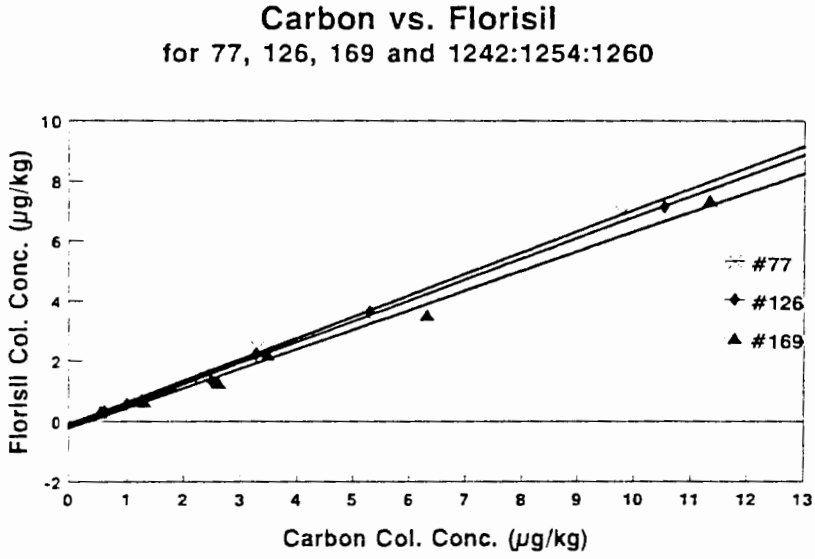
Results illustrated in *Figure 3*, show a linear relationship between the carbon and Florisil techniques. Here the Florisil and carbon columns were spiked with a range of concentrations of congeners 77, 126, and 169, as previously mentioned, but fortified with an Aroclor mixture 1242:1254:1260 (1:1:1). The r^2 for congeners 77, 126 and 169 using the two techniques were

Figure 3. **Direct Spiking of Congeners 77, 126 and 169 and Aroclor Mixture.**



0.998, 0.997 and 0.992 respectively. There was no significant difference in the slopes or the intercepts ($P < 0.001$ ANCOVA).

One of the major concerns of quantifying non ortho substituted PCBs was the coelution with other PCB congeners (eg . 77 coelutes with 110). Florisil columns spiked with an enriched Aroclor mixture described above did not have coelution problems, and separation pattern and recoveries obtained with Florisil are summarized in *Table 1*.

Table 1. Separation Pattern and Percentage Recoveries of OCC and PCBs Including Coplanar Congeners Using an Activated Florisil Column

SPIKING SOLUTION	COMPOUND	% RECOVERY		TOTAL % RECOVERY
		FRACTION I	FRACTION II	
AROCLOR 1242/1245/1260 1:1:1 + 77,126,169 1:1:1	1,2,3,5 TCB	100	-	100
	1,2,3,4 TCB	93	-	93
	QCB	93	-	93
	HCB	89	2	91
	OCS	94	-	94
	TRANS-NONACHLOR	77	19	96
	pp'-DDE	93	-	93
	pp'-DDT	38	63	101
	PHOTOMIREX	96	-	96
	MIREX	94	-	94
	PCB 31/28	90	-	90
	PCB 52	99	-	99
	PCB 44	91	-	91
	PCB 66/95	93	-	93
	PCB 60	97	-	97
	PCB 101	94	-	94
	PCB 99	92	-	92
	PCB 97	94	-	94
	PCB 87	93	-	93
	PCB 110	91	-	91
	PCB 118	95	-	95
	PCB 146	99	-	99

SPIKING SOLUTION	COMPOUND	% RECOVERY		TOTAL % RECOVERY
		FRACTION I	FRACTION II	
	PCB 153	98	-	98
	PCB 105	76	17	93
	PCB 141	92	-	92
	PCB 138	94	-	94
	PCB 182/187	94	-	94
	PCB 183	90	-	90
	PCB 174	93	-	93
	PCB 171	93	-	93
	PCB 172	80	-	80
	PCB 180	96	-	96
	PCB 170/190	94	-	94
	PCB 201	92	-	92
	PCB 203	93	-	93
	PCB 195	98	-	98
	PCB 194	111	-	111
	PCB 189	105	-	105
3 PCBs STANDARD MIXTURE	PCB 77	-	109	109
	PCB 126	-	109	109
	PCB 169	-	115	115

Using spiked (0.7 μg/kg) mink liver samples (n = 18), the two techniques were compared to measure recovery efficiencies (***Table 2***).

Table 2. **Florisil and Carbon Column Recovery Data**

FLORISIL		% RECOVERY			STANDARD DEVIATION (%)	NUMBER OF SAMPLES
CONGENER	SRM (μg/kg)	MIN	MAX	MEAN		
77	0.7	64	134	92	14.5	18
126	0.7	77	165	104	16.9	18
169	0.7	73	165	120	17.1	18
CARBON		**% RECOVERY**			**STANDARD DEVIATION (%)**	**NUMBER OF SAMPLES**
CONGENER	SRM (μg/kg)	MIN	MAX	MEAN		
77	0.7	60	154	111	22.8	18
126	0.7	77	138	108	17.6	18
169	0.7	67	170	117	23.2	18

Analysis of variance indicated there was no significant difference between the two techniques. Carbon column chromatography yielded mean percent recoveries of 111±23%, 108±18%, and 117±23% for congeners 77, 126 and 169 respectively. Florisil chromatography yielded 92±15%, 104±17%, and 120±17%. Generally, the carbon and Florisil column techniques were closely comparable in both accuracy and precision.

DISCUSSION

Non-ortho substituted congeners have been observed at elevated concentrations in the Great Lakes, and when considered in terms of TCDD toxic equivalents, are potentially responsible for most of the toxicity observed in aquatic ecosystems[2]. The limited success of quantifying the exposure dynamics of these chemicals limits the evaluation of the risk of toxicological effects. For example, in the Great Lakes, total PCB concentrations have been observed to be decreasing

in fish and herring gull populations. If the non-ortho substituted congeners represent most of the toxicological activity of the chlorinated biphenyls, then it is important to know if levels of these compounds have decreased accordingly. Unfortunately, there is insufficient data on these critical chemicals because only a few laboratories presently have the ability to quantify them.

This study shows that both the carbon and Florisil column techniques are excellent techniques for the quantitative analysis of coplanar PCBs. The Florisil column technique, however is a simple modification for most routine laboratories to adapt, thus, providing potential to increase the knowledge base as to the distribution and levels of coplanar PCBs. Also, it was noted that carbon column chromatography can produce noisy baselines with negative peaks interfering with the analysis (eg congener 126). It was observed that the elution characteristics of the carbon column were somewhat inconsistent over time introducing errors in chromatography. It is probable that the activated Florisil column method is more suitable than the carbon column method for automation.

The one concern encountered with the Florisil technique is variability in batches of Florisil. This has been previously reported in the literature[8]. A variety of Florisil was investigated and confirmed these earlier observations. Problems were most noticeable with congener 110, which would elute in both Fraction 1 and 2, and had retention times similar to that of congener 77. This can be avoided by previously testing the characteristics of the Florisil, and by careful programming of the gas chromatography.

Another potential problem is coelution with toxaphene. Although this was not a problem with the mink liver samples used in this study, other organisms might have elevated levels of toxaphene. It is suggested, that if toxaphene is suspected in the sample, then the sample should be cleaned up on a silica column, before being transferred to Florisil.

In summary, the Florisil column approach can greatly benefit the study of the dynamics of non-ortho substituted PCBs in aquatic and terrestrial ecosystems. The technique is inexpensive and provides a comprehensive analysis of PCBs in environmental samples. Being much simpler than the carbon column approach, most laboratories will be able to better quantify the toxicological risks of PCBs using the Florisil column approach.

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M. Tomczak assisted in the preparation of the figures and the final text.

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ABSTRACT

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