Multidimensional GC and Charcoal Separation Techniques in the Determination of non-*ortho* Cl chlorobiphenyls - A Comparison

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Abstract: *In spite of the potential suitability of multidimensional chromatography (MDGC) and activated carbon chromatographic techniques for the quantification of non-ortho Cl planar CBs in environmental samples, a number of analytical problems were encountered when both techniques were used in combination. Commercial charcoals behaved differently in their separation behavior of planar CBs while MDGC showed the presence of co eluting ECD active substances after carbon column separation. On the other hand, use of MDGC without preseparation of samples on carbon, often yielded concentrations of planar CBs below detection levels because the samples had to be diluted to avoid overloading of ortho, ortho' Cl CBs on the column. For these reasons, earlier reports may have over- and/or underestimated the concentrations of these planar CBs.*

Introduction: A congener-specific determination of toxic chlorobiphenyls such as 33'44'-T_4CB (#77), 33'44'5-P_5CB (#126) and 33'44'55'-H_6CB (# 169) in environmental samples is emphasized recently. However, measurement of these congeners constituting only a minor part of the CB mixture is difficult, the signals of non-*ortho* Cl CBs being generally masked by dominant signals of other CBs or co-extracted chemical entities. This applies equally to GC-ECD and GC-MS measurements. The interfering chemicals could be separated from the target components with multidimensional gas chromatography and determined with ECD (Duinker et al. 1988, Schulz et al. 1989). Alternatively, the affinity of of graphite for planar aromatic compounds was utilized in a conventional charcoal column chromatographic technique for the structural separation of CBs (Stalling et al. 1980, Tanabe et al. 1987). Difficulties in reproducing these published methods using locally available brands of charcoals are reported recently (van Arnhem 1989, Rinta-Säntti 1989 & Mes and Weber 1989). The experience gained at IFM, Kiel, NIOZ, Texel and Ehime University, Matsuyama was used in an exercise carried out in Kiel in 1989-90 to compare the usefulness of MDGC and various charcoals for the measurement of non-*ortho* Cl CBs in environmental samples.

Experimental: Materials: Three brands of charcoals were studied, *viz.* Wako Pure Chemical Industries, Japan, Baker Analytical Reagents, The Netherlands and

Alltech GmbH, Germany. Instrumental: MDGC ECD was carried out using a Siemens SICHROMAT-2 gas chromatograph equipped with two independent ovens and two ^{63}Ni electron capture detectors. The columns were 25m OV 54 (0.25 mm), 0.32 mm i.d, and 30m fused silica OV 210 (0.25 mm), 0.32 mm i.d. Most of the eluate of the first column, which was located in the first oven was allowed to pass through the "monitor detector". Activation of the live T piece forces a selected part of the eluate (in this case those fractions with CBs 77, 126, and 169) through the second column quantitatively. These are detected by the "main detector" (for details of this technique see Brinkman et al. 1988). Gas chromatography-mass fragmentographic data were obtained with SICHROMAT 2-8 and Finnigan Mat 800 MS. Analysis: Charcoals had been previously used and tested by two of the authors in their respective laboratories (Ehime University, Japan and NIOZ, The Netherlands). Samples of harbor seals (*Phoca vitulina*) were tested in Kiel using charcoal MDGC techniques. Samples were digested in alcoholic KOH and cleaned up on HPLC and Alumina. The NIOZ procedure involves two successive elution of the sample extract, first with 200 ml of 1:2 DCM in hexane and then with 150 ml of toluene, on Baker's charcoal. The sample extracts were also eluted on a similar basis with Wako charcoal. The method of Tanabe et al. (1989) was modified for this purpose. *Ortho* Cl PCBs were eluted using 30 ml of 20% DCM in hexane and 10 ml of 40% DCM in hexane. Planar CBs were eluted subsequently with 60 ml of 70:30 Benzene-Ethyl acetate.

In the normal procedure at Kiel, biological samples are ground with anhydrous Na_2SO_4 and extracted with hexane. The extracts are cleaned up on alumina and HPLC (Petrick et al. 1988). Blubber samples processed this way were chromatographed on Alltech charcoal. The method of Tarhanen et al. (1989) was modified for this purpose. That involves an initial 10 ml elution of 40:60 DCM:Hexane followed by 50 ml elution in toluene (reverse elution).

Recovery of non-*ortho* Cl CBs was tested using standards of high purity in all charcoals and found to be more than 85%. Additionally, 200 µg of Aroclor 1254 was chromatographed on these charcoals (only 10 µg was used on All* charcoal).

Results and Discussion: Problems were encountered in reproducing the procedure of Tanabe et al. (1987) using Baker, Sigma and Fisher charcoals (van Arnhem 1989, Rinta-Sántti 1989 & Mes and Weber 1989). Excessive background noise, poor removal of *ortho*-Cl PCBs and low recovery of non-*ortho* Cl were some of the problems. The first two carbons also needed excessive solvents (>350 ml) and high pressure. The direct measurement of non-*ortho* Cl PCBs in a complex mixture using MDGC in this laboratory, was used to test these carbons. It was noted that co-planar CBs were retained differently in charcoals (probably, on the availability of active graphite structures) and we found that none of the single procedures works effectively for different charcoals. For example Alltech charcoal effectively removed 99% of CBs in 10 ml of solvent mixture (Table 2). Extensive recovery & blank checks using pure CB congeners were

essential prerequisite in charcoal chromatography. Results of MDGC analysis of five seal samples using Baker and Wako charcoals are given in Table 1. While CB 77 measurements were quite agreeable between those charcoals, the values for CBs 126 & 169 varied by more than a factor of 2 in some samples. It was also noted that there was a significant contribution of CB 110 to the "CB 77" peak. Though charcoal selectively retains planar CBs, other CBs and compounds of biological/environmental origin do appear in the cleaned-up extract in amounts to interfere the trace level determination of non-*ortho* Cl CBs. Thus, reliable measurements of CBs 77,126 & 169 on a single SE-54 HR-GC-ECD, even after charcoal clean-up is doubtful. Apart from these negative conclusions on charcoal we also found an important possible use of this material. As can be observed from Table 2, CBs 77 and 126 were detected at low, but measurable concentrations in Aroclor 1254 by MDGC-ECD after carbon

Table 1. Concentration (ng/g) of non-*ortho* Cl CBs in seal blubber. Values after charcoal chromatography-MDGC measurements.

Sample No.	33'44'-T_4CB		33'44'5-P_5CB		33'44'55'-H_6CB	
	Wako	Baker	Wako	Baker	Wako	Baker
1	0.28	0.38	0.53	1.8	0.14	0.44
2	0.15	0.10	0.25	0.46	0.05	0.05
3	0.04	0.07	0.45	0.35	0.04	0.24
4	0.20	0.20	0.51	0.48	0.16	0.37
5	0.05	0.04	1.08	0.78	0.33	0.18

Table 2. Percent of selected PCB congeners in the non-*ortho* Cl fraction after charcoal - MDGC analysis of Aroclor 1254 (wt/wt)

PCB No.	Structure	Reported values	% wt/wt after charcoal chromatogr. Wako	Baker	Alltech
118	23'44'5	6.39[a]	0.034	0.039	0
110	233'4'6	5.85[a]	0.11	0.10	0.0019
138	22'344'5'	3.20[a]	0.01	0.0074	0
128	22'33'44'	2.07[a]	0.0044	0.0022	0
180	22'344'55'	0.38[a]	0.0053	0.0023	0
187	22'34'55'6	0.32[a]	0.0026	0	0.02
183	22'344'5'6	0.17[a]	0.002	0.0011	0
174	22'33'456'	0.34[a]	0.0019	0.0023	0
77	33'44'	0.06[b]	0.057	0.033	0.022
126	33'44'5	0.0046[b]	0.018	0.006	0.0072

Values of a & b are from a - Schulz et al. (1989) & b Kannan et al. (1987)

separation. These congeners could not be detected by MDGC without charcoal separation because of the presence of too high amounts of 110 relative to 77 in the peaks transferred to the main column in the MDGC. So, the use of carbon offers a new dimension to the MDGC analysis.(see also Fig.1). In the described procedures use of strong oxidants such as chromic acid was carefully avoided as a clean-up because quantitative loss of 77,126 & 169 was noticed in this laboratory. In summary, it can be concluded that the additive use of both carbon chromatography and MDGC is necessary for precise quantification of this class of highly toxic CB congeners.

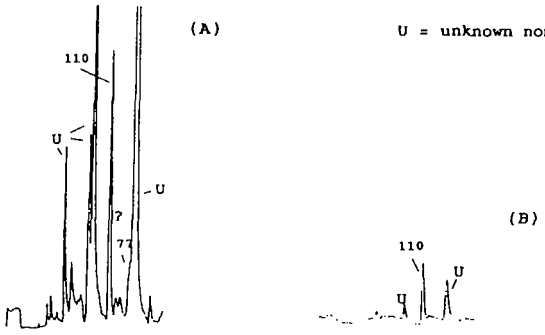

Figure 1. "77 cluster" of peaks in MDGC after a 'heart cut'. Several unknown peaks appeared in direct MDGC measurement of blubber (*Phoca vitulina*) sample (A) and were eliminated/reduced after charcoal clean-up (B). In spite of these rigorous separations some compounds escape to cause over estimations of planar CBs, if not careful.

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