

CONGENER-SPECIFIC CARBON ISOTOPE ANALYSIS OF TECHNICAL PCN AND PCB PREPARATIONS USING 2DGC-IRMS

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Introduction

Carbon isotope analysis is one of the most traditional approaches used in biogeochemical research for the last few decades. The isotopic composition of $^{13}\text{C}/^{12}\text{C}$ in bulk carbon has been measured in many kinds of environmental matrixes and supposed to be a useful indicator to understand biogeochemical circulation of carbon in ecosystems. With the recent advancements in instrumental analysis, compound-specific carbon isotope analysis (CSIA) is emerging as a hot issue in the research area of source determination of natural products¹. However, only limited investigations have been reported to date, regarding the CSIA of man-made chemicals such as polychlorinated biphenyls (PCBs). Although a traditional approaches such as fingerprinting, to determine sources of specific anthropogenic chemicals in environment has been in use for decades, those techniques are relatively more qualitative than quantitative; therefore, development of novel and reliable method is required to enable comprehensive understanding of sources and environmental destiny of man-made chemicals, especially those that are hazardous ones.

In this study, we developed a new approach of CSIA using two-dimensional gas chromatograph (2DGC) with combustion chamber (C)/ isotope ratio mass spectrometer (IRMS) and applied for “congener-specific” carbon isotopic analysis of PCBs and polychlorinated naphthalenes (PCNs). We clarified the need to solve the co-elution of individual chlorobiphenyl and chloronaphthalene congeners and to decrease the blank for “congener-specific” CSIA.

Materials and Methods

Kanechlor (Kanechlor 300, 400, 500 and 600), Aroclor (Arochlor 1221, 1242 and 1254) and individual PCB congeners 2,2',4-triCB (CB17), 2,3,5,6-tetraCB (CB65), 2,2',3,5,5',6-hexaCB (CB151) were purchased from GL Science in Japan and Manogen in the USA, respectively. Sovol, Trichlorodiphenyl and Chlorofen were kindly provided by Dr. Falandysz at the University of Gdansk. Halowax formulations, Halowax-Kit RC076 (HW 1000, 1001, 1013, 1014, 1051 and 1099) were from Analab. Inc. in the USA.

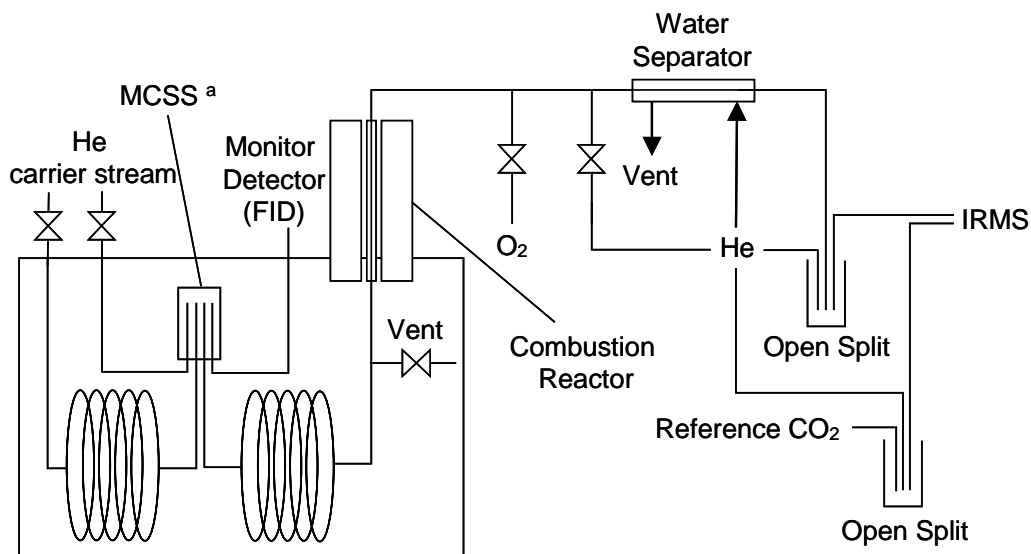


Fig. 1. Schematic view of the two dimensional GC combustion IRMS.

CSIA of PCBs was carried out using a 2DGC equipped with IRMS (Finnigan MAT252) in combination with GC Combustion III (Fig. 1.). 2DGC system was built from a Trace GC (Thermo electron) equipped with moving capillary stream switching (MCSS) technique². The MCSS technique cuts parts of effluents from the first column, which can transfer almost all of the target compounds into the second column.

The outlet of the first column was diverted to the inlet of the second column, both installed in a dome shaped glass tip, located inside a GC oven. These cut functions

were programmed according to the retention time of target congeners on monitor detector (FID) connected to a dome. Individual isomers of PCBs and PCNs were separated by a less-polar capillary column, 30 m x 0.25 mm i.d. x 0.25 DB-5 (J&W Scientific, USA), and PCB and PCN congeners cut by MCSS were separated again on another column (Rtx-200, 15 m x 0.32 mm i.d. x 0.25 μ m, Restek, USA).

After passing through the second column, separated PCB and PCN congeners were combusted to CO₂ in an oxidation reactor through a T-peace connection at the exit of the chromatographic column. The combustion reactor consisted of an alumina tube packed with metal oxide (copper, nickel and platinum wires). Moisture resulting from the combustion was removed from the sample stream via a tubular water separator (Nafion membrane). Reoxidation was performed once a day.

CO₂ produced by combustion of chromatographic effluents continuously enters the ion source of the MAT 252 IRMS through an open split. Ion currents were measured for *m/z* 44, 45 and 46 continuously. Ions were generated by electron impact (70 eV), and accelerating voltage was 10 kV. Isotopic compositions were reported in parts per thousand, per mill (‰), deviation from those of isotopic standard reference material using the conventional δ notation:

$$\delta^{13}\text{C} (\text{‰}) = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$. The CO₂ reference gas was calibrated by isotope ratio reference gas from Oztech Co. in the USA (SHOKO-944C), $\delta^{13}\text{C} = -40.84$ vs the Pee Dee Belemnite.

The stability of the system and background were checked daily. CB17, 65 and 151 were analyzed at the beginning and at the end of sample measurements for quality control. The standard deviations for repeated measurements of these 3 standards were 0.4‰ throughout the analysis. Samples containing different concentrations, ranging from 7 to 62 ng carbon were analyzed in triplicate to test the combustion of carbon to CO₂ under the system conditions specified. The peak area was linear and $\delta^{13}\text{C}$ values were stable with a corresponding standard deviation that ranged from 0.06 to 0.26 for these PCB congeners.

Each technical PCB and PCN preparation was analyzed at multiple times. Calculated isotope ratios were reported as below the limit of obtaining stable data if peak area was not above the threshold amount (300 mV peak high).

Results and discussion

$\delta^{13}\text{C}$ value for technical PCBs: The 2DGC separation enabled a significant improvement in the sensitivity of individual PCB isomers at least by an order of magnitude better than traditional GC methods because of low background and less coeluted peaks. FID and IRMS chromatogram of Kanechlor 500 are shown in Fig. 2. FID chromatogram shows separation of PCB congeners in a technical PCB mixture on the first column. IRMS chromatogram for m/z 44 (CO_2) shows separation of selected PCB congeners that were cut by MCSS to solve the co-elution of several isomers. In a CSIA by single GC/C/IRMS, all organic compounds in samples are transferred to the oxidation reactor and combusted to CO_2 . It means that combustion chamber has to handle a bulk of carbon for oxidation, which results in high background and low sensitivity. On the other hand, 2DGC/C/IRMS by MCSS, only target compounds that are selected using the monitor detector (FID, in this case) are transferred to oxidation chamber via a second column. The background for MS is shifted from column bleed due to ramping of oven temperature. $\delta^{13}\text{C}$ value for the test sample is calculated by comparison between reference isotope ratios and that of the sample. Estimated sensitivity of carbon using 2DGC/C/IRMS is less than 5 ng, which corresponds to 100 – 500 ng of individual PCB congeners.

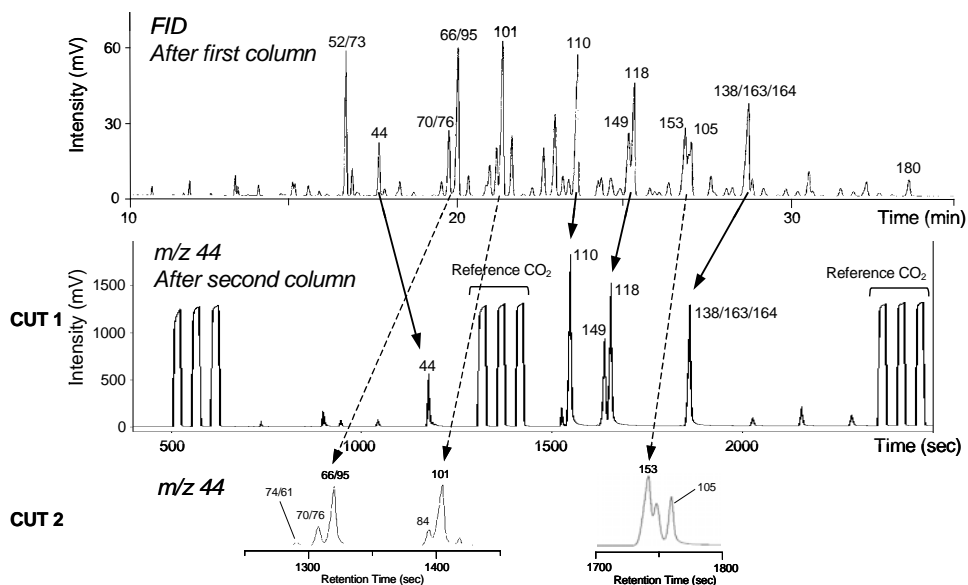


Fig. 2. FID chromatogram and MAT252 IRMS mass chromatogram of m/z 44 for Kanechlor 500. Peaks are expressed by IUPAC number.

First and second columns were DB-5 and Rtx-200, respectively.

Totally, thirty-one PCB congeners were measured in ten technical PCB preparations. Fig. 3 shows the comparison of $\delta^{13}\text{C}$ values of Aroclor 1254 measured in this study and from literature^{3,4}. There were variations in $\delta^{13}\text{C}$ values for same congeners in these three reports. Only $\delta^{13}\text{C}$ values for CB153 and CB138 were within $\pm 0.5\%$, while the other ones had higher variability. Drenzek *et al.* (2002) reported bulk $\delta^{13}\text{C}$ values for technical PCBs from three different suppliers⁵. They found Aroclor mixtures had a narrow range of $\delta^{13}\text{C}$ values with a mean value of $-26.17 \pm 0.37\%$ (n = 10). There was no apparent bias between suppliers. The precision of GC/C/IRMS is generally better than 0.5%. The reason for the large difference in $\delta^{13}\text{C}$ values among the three reports may not only be due to the source of the material (suppliers), but also due to analytical error. Thus, it is important to consider international calibration, based on stable standard materials, for CISA.

Table 1. $\delta^{13}\text{C}$ values for 10 technical PCBs.

congener	structure	Kanechlor	Kanechlor	Kanechlor	Kanechlor	Aroclor	Aroclor	Aroclor	Sovol	Trichloro-	Chlorofen
		300 a 41	400 a 48	500 a 53	600 a 60	1221 b 21	1242 b 41	1254 b 53	41	diphenyl 53	70
biphenyl						-25.21					
1	2-					-27.17					
3	4-					-24.66					
10/4	2,6-/2,2'-	-23.91					-25.87				-22.66
8/5	2,4'-/2,3'-	-24.04					-26.22				-23.07
18	2,2,5'-	-25.30	-24.17				-26.85				-23.94
16/32	2,2,3-/2,4,6-	-25.38	-23.19				-26.72				-24.66
31/28	2,4,5-/2,4,4'-	-25.39	-24.14				-26.70				-24.77
33/20/53	2,3,4'-	-25.64	-24.02				-26.34				-24.62
52/73	2,2,5,5-/2,3,5,6-	-26.40	-25.76	-25.70			-27.72	-26.31	-23.43		-25.13
43/49	2,2,3,5-/2,2,4,5'-	-25.94	-25.52	-24.21			-27.01	-25.00			-24.61
47/75/48	2,2,4,4'-/2,4,4,6-/2,2,4,5'-		-25.31				-27.20				
44	2,2,3,5'-	-26.03	-25.50	-24.92			-27.32	-25.26			-24.61
59/42	2,3,3,6-/2,2,3,4'-		-25.66				-27.61				-25.86
74/61	2,4,4,5-/2,3,4,5'-		-25.14				-26.68				
70/76	2,3,4,5-/2,3,4,5'-		-24.79	-22.43			-26.72	-23.99	-21.97		
66/95	2,3,4,4'-/2,2,3,5,6-		-25.32	-25.82	-24.99		-26.95	-26.45	-23.56	-25.23	
89/101/90	2,2,3,4,6-/2,2,4,5,5'/2,2,3,4,5'-		-28.47	-27.56	-24.81			-27.38	-27.07		
110	2,3,3,4,6-		-27.48	-26.26				-27.30	-25.29		
149/139	2,2,3,4,5,6-/2,2,3,4,4,6-			-27.50	-25.90			-27.80			
118/106	2,3,4,4,5-/2,3,3,4,5'-		-26.21	-25.93				-26.44	-25.12		
153/132/168	2,2,4,4,5,5-/2,2,3,3,4,6-/2,3,4,4,5,6-	-26.40	-27.00	-25.48				-27.15	-25.10		-22.99
105	2,3,3,4,4'-		-25.78	-26.13				-27.10	-24.98		
164/163/138	2,3,3,4,5,6-/2,3,3,4,5,6-/2,2,3,4,4,5,6-			-27.10				-27.46	-26.35		
182/167	2,2,3,4,4,5,6-/2,2,3,4,4,5,6-						-25.76				-25.43
174	2,2,3,3,4,5,6'-						-27.71				
180	2,2,3,3,4,4,5,5'-						-27.25				-24.89
170/190	2,2,3,3,4,4,5,5,6-						-26.48				
199	2,2,3,3,4,5,5,6-						-28.87				-26.57
203/196	2,2,3,4,4,5,5,6-/2,2,3,3,4,4,5,6-						-28.17				-25.83
194	2,2,3,3,4,4,5,5,5'-						-26.53				-25.90
206	2,2,3,3,4,4,5,5,6-										-27.78
average		-25.34	-25.46	-25.88	-26.81	-25.68	-26.85	-26.47	-24.76	-24.47	-25.61
maximum value		-23.91	-23.19	-22.43	-24.81	-24.66	-25.87	-23.99	-21.97	-22.66	-22.99
minimum value		-26.40	-28.47	-27.56	-28.87	-27.17	-27.72	-27.80	-27.07	-25.86	-27.78

a GC Science, b Manogen

$\delta^{13}\text{C}$ values of individual PCB congeners ranged from -21.97 (CB70/76, Sovol) to -28.87% (CB199, KC 600) (Table 1). There was a trend of decrease in isotope compositions with increasing chlorine number in each Kanechlor (Fig.4). This

phenomenon is also affected by chlorine composition pattern. For example, $\delta^{13}\text{C}$ values for CB95, which co-eluted with CB66, were the highest and CB101 were the lowest among pentachlorinated biphenyls in KC 400 and 500. Similar to the study by Jarman *et. al.* (1998), CB70 (2,3',4',5-) was isotopically heavier than CB52 (2,2',5,5'-) in every technical PCBs ³. The averages of $\delta^{13}\text{C}$ values for homologues are shown in Fig.4. Increased chlorine number caused increased ^{13}C depletion in all PCBs.

Table 2. $\delta^{13}\text{C}$ values for Halowax series

Congener	Seriese Cl weight (%) Structure	HW 1000	HW 1001	HW 1013	HW 1014	HW 1051	HW 1099
		23	27	49	55	60	49
2/1	2,4-1-	-23.59					
5/6/7/12	1,4-/1,5-/1,6-/2,7-	-26.30	-22.37				
9	1,8-	-25.50					
21/24/14	1,3,7-/1,4,6-/1,2,4-		-24.72	-23.00			-24.05
23	1,4,5-		-23.50				-22.21
33/34/37	1,2,4,6-/1,2,4,7-/1,2,5,7-		-25.30	-24.34	-23.97		-24.40
38/40	1,2,5,8-/1,2,6,8-		-25.64	-24.42	-22.91		-24.59
46	1,4,5,8-		-25.09	-23.86	-23.01		-23.98
52/60	1,2,3,5,7-/1,2,4,6,7-					-23.03	
61	1,2,4,6,8-			-24.37	-22.90		
59	1,2,4,5,8-			-25.19	-23.47		
71/72	1,2,4,5,6,8-/1,2,4,5,7,8-				-25.64		
65	1,2,3,4,5,8-				-26.01		
73/74	1,2,3,4,5,6,7-/1,2,3,4,5,6,8-					-21.68	
75	1,2,3,4,5,6,7,8-					-24.92	
	Average	-25.13	-24.44	-24.20	-23.87	-23.30	-23.84
	maximum value	-23.59	-22.37	-23.00	-22.90	-21.68	-22.21
	minimum value	-26.30	-25.64	-25.19	-26.01	-24.92	-24.59

$\delta^{13}\text{C}$ value for technical PCNs:

$\delta^{13}\text{C}$ values of individual congeners in Halowaxes ranged from -21.68 (CN73/74, HW 1051) to -26.30 ‰ (CN5/6/7/12, HW 1000) (Table 2). Similar to PCBs, isotope compositions decreased with increasing chlorine number in each Halowax (Fig.4). HW 1000 and HW 1051 were at the two extremes of their $\delta^{13}\text{C}$ values because of the reason that their profile of congener contents were different and that they had only few congeners.

This is the first report of $\delta^{13}\text{C}$ values of individual congeners of PCNs. As it is important to carry out inter-laboratory calibration of CSIA and necessity of reliable standard materials, domestic standardization of CSIA in Japan has been initiated in collaboration with National Metrology Institute of Japan.

SPECIAL INSTRUMENTAL TECHNIQUES AND PATTERN RECOGNITION

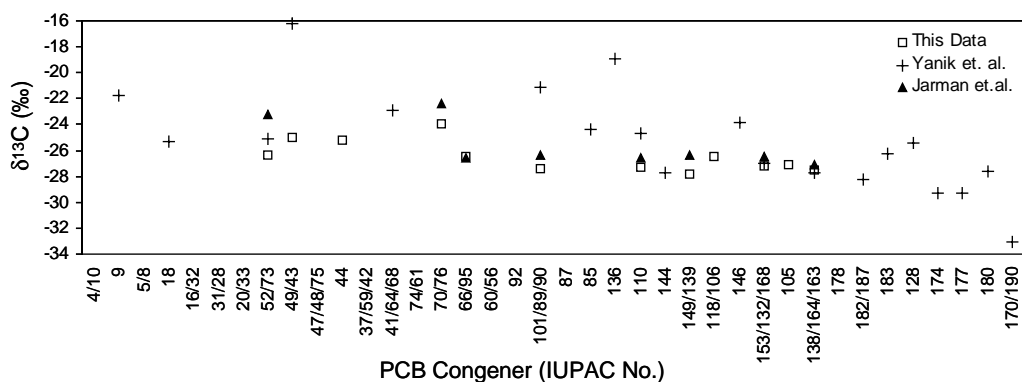


Fig. 3. Comparison of $\delta^{13}\text{C}$ values for Aroclor 1254 between this study and those by Yanik⁴ and Jarman³.

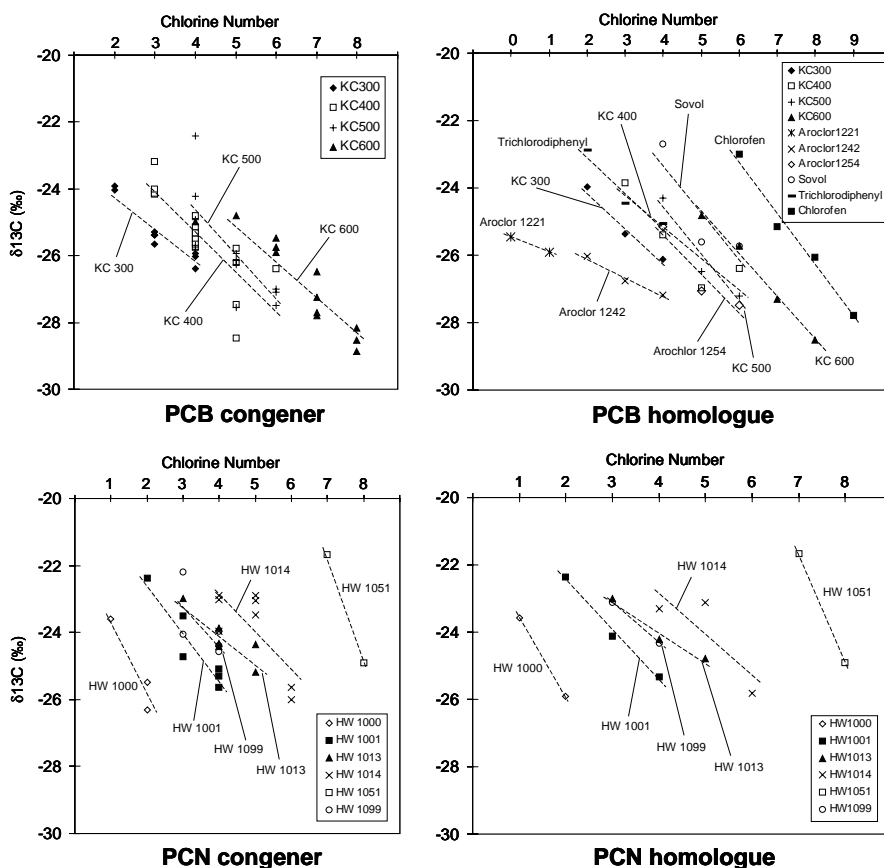


Fig. 4. $\delta^{13}\text{C}$ values for PCB and PCN congeners and each homologues in technical formulations. # $\delta^{13}\text{C}$ values for each homologue were represented by average of isomer values.

Acknowledgments

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