Analysis of 209 chlorinated biphenyl congeners using comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry in the 1-D mode followed by the 2-D mode

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Objectives

Define chlorinated biphenyl congeners.

Historical analytical challenge.

The chemical structures of chlorinated biphenyls

Introduction of GCxGC-TOFMS and chromatographic conditions

Two-Dimensional approach to PCB analysis

Summary and Conclusion.

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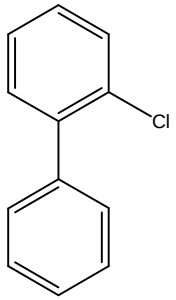
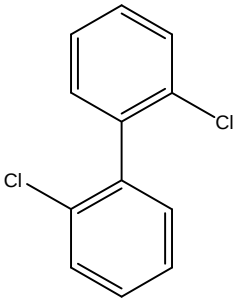
What are Chlorinated Biphenyls

- Ten homologs or distinct isomeric groups of compounds differing by the numbers of chlorines and hydrogens on a biphenyl ring
- They consist of mono, di, tri, tetra, penta, hexa, hepta, octa, nona, and deca substituted chlorines in the ten groups.
- Total of 209 related members or congeners
- They are considered ubiquitous environmental pollutants – found in marine plant and animal specimens, fish, bird eggs, and humans (M.D. Erickson 1986)

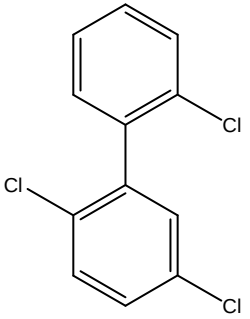
Chemical Structure of Each Class of Polychlorinated biphenyls



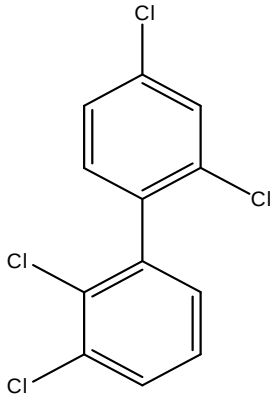
2-Chloro-biphenyl



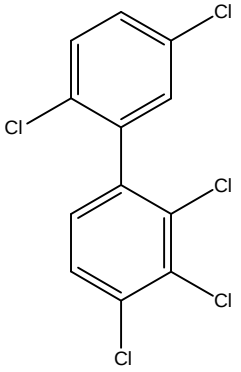
2,2'-Dichloro-biphenyl



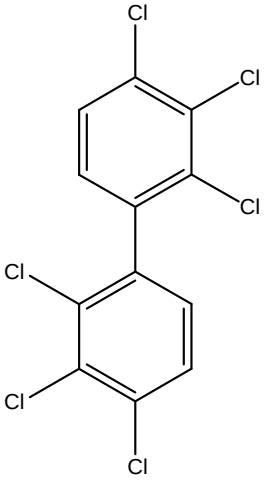
2,5,2'-Trichloro-biphenyl



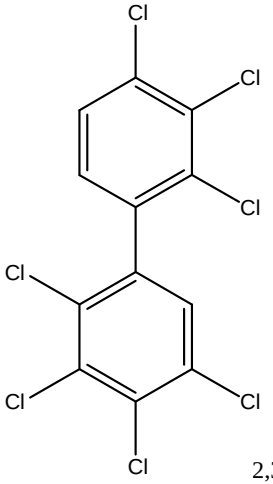
2,3,2',4'-Tetrachloro-biphenyl



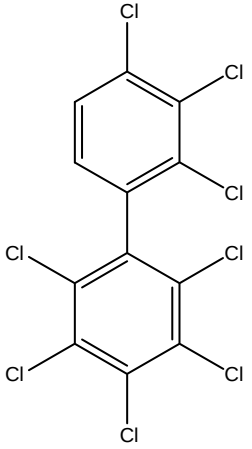
2,3,4,2',5'-Pentachloro-biphenyl



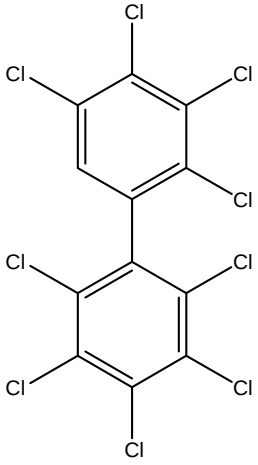
2,3,4,2',3',4'-Hexachloro-biphenyl



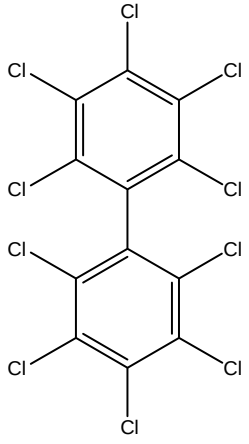
2,3,4,5,2',3',4'-Heptachloro-biphenyl



2,3,4,5,6,2',3',4'-Octachloro-biphenyl



2,3,4,5,6,2',3',4',5'-Nonachloro-biphenyl



2,3,4,5,6,2',3',4',5',6'-Decachloro-biphenyl

Historical Analytical Challenges

- Chromatographers have used all conceivable chromatographic techniques in their quest to distinguish the 209 congeners, all to no avail.
- Different columns with varying phases have been employed
- Heart-cutting technique was also attempted with modest results

Comprehensive Two-Dimensional GC with Time-of-Flight Mass Spectrometer

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Two ovens are contained inside the GC, hence the name GCxGC, each with opposite polarity columns (Rtx-PCB, 40m 0.18 mmID x 0.18 µm and DB-17, 1m 0.1 mmID x 0.1 µm film)

Multiple windows are opened to enable multitasking of the software, and to view instrument response as control parameters are adjusted.

GCxGC-TOFMS Chromatographic Conditions: Carrier gas (Helium) 1.2 mL/min

Acquisition rate: 100 spectra/sec

Volume injected: 1µL of sample

Acquisition: 45 – 550 m/z

Oven 1: 70 °C (0.5min) to 150 °C (0 min) at 10 °C /min, to 250 °C at 1 °C /min, to 275 °C (15min) at 4 °C /min

Oven 2: 85 °C (0.5min) to 165 °C (0 min) at 10 °C /min, to 265 °C at 1 °C /min, to 290 °C (15min) at 4 °C /min

Transfer line temperature: 300 °C

Modulation time: 4 sec

Detector gain: 1650V

Modulator temperature offset: 30 °C

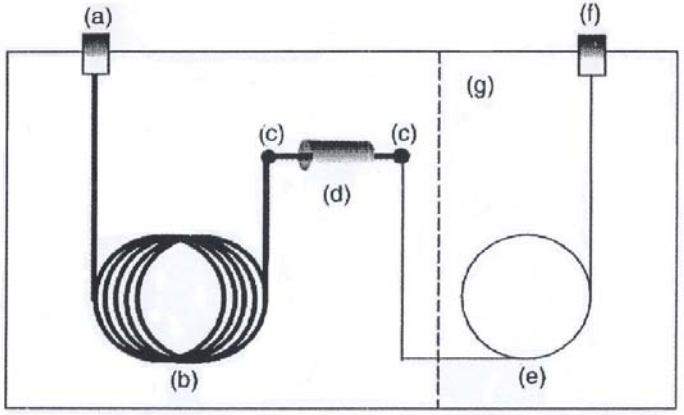

Illustration of a GC × GC system: (a) injector, (b) first column, (c) connectors of columns, (d) modulator, (e) second column, (f) detector, (g) second oven (in some cases) (from Marriott & Shellie 2002).

Chlorinated Biphenyl Congeners Arranged by Group to Provide Approximately Equal Signal Intensities

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Group	Mono	Di	Tri	Tetra	Penta	Hexa	Hept	Octa	Nona	Deca
A	1[a](0.20)[b]	4(0.40)	16(0.75)	40(1.25)	82(1.50)	128(3.00)	170(3.40)	194(3.25)	206(3.25)	209(3.00)
B	2(0.20)	5(0.40)	17(0.85)	41(1.50)	83(2.00)	129(3.00)	171(4.00)	195(4.05)	207(4.00)	
C	3(0.20)	6(0.40)	18(0.85)	42(1.50)	84(2.00)	130(3.00)	172(4.00)	196(4.05)	208(4.00)	
D		7(0.40)	19(0.85)	43(1.50)	85(2.00)	131(3.00)	173(4.00)	197(4.25)		
E		8(0.40)	20(0.85)	44(1.50)	86(2.00)	132(3.00)	174(4.00)	198(4.25)		
F		9(0.40)	21(0.85)	45(1.50)	87(2.00)	133(3.00)	175(4.00)	199(4.25)		
G		10(0.40)	22(0.85)	46(1.50)	88(2.00)	134(3.00)	176(4.00)	200(4.25)		
H		11(0.40)	23(0.85)	47(1.50)	89(2.00)	135(3.00)	177(4.00)	201(4.25)		
I		12(0.40)	24(0.85)	48(1.50)	90(2.00)	136(3.00)	178(4.00)	202(4.25)		
J		13(0.40)	25(0.85)	49(1.50)	91(2.00)	137(3.00)	179(4.00)	203(4.25)		
K		14(0.40)	26(0.85)	50(1.50)	92(2.00)	138(3.00)	180(4.00)	204(4.25)		
L		15(0.40)	27(0.85)	51(1.50)	93(2.00)	139(3.00)	181(4.00)	205(4.25)		
M			28(0.85)	52(1.50)	94(2.00)	140(3.00)	182(4.00)			
N			29(0.85)	53(1.50)	95(2.00)	141(3.00)	183(4.00)			
O			30(0.85)	54(1.50)	96(2.00)	142(3.00)	184(4.00)			
P			31(0.85)	55(1.50)	97(2.00)	143(3.00)	185(4.00)			
Q			32(0.85)	56(1.50)	98(2.00)	144(3.00)	186(4.00)			
R			33(0.85)	57(1.50)	99(2.00)	145(3.00)	187(4.00)			
S			34(0.85)	58(1.50)	100(2.00)	146(3.00)	188(4.00)			
T			35(0.85)	59(1.50)	101(2.00)	147(3.00)	189(4.00)			
U			36(0.85)	60(1.50)	102(2.00)	148(3.00)	190(4.00)			
V			37(0.85)	61(1.50)	103(2.00)	149(3.00)	191(4.00)			
W			38(0.85)	62(1.50)	104(2.00)	150(3.00)	192(4.00)			
X			39(0.85)	63(1.50)	105(2.00)	151(3.00)	193(4.00)			
Y				64(1.50)	106(2.00)	152(3.00)				
Z				65(1.50)	107(2.00)	153(3.00)				
2A				66(1.50)	108(2.00)	154(3.00)				
2B				67(1.50)	109(2.00)	155(3.00)				
2C				68(1.50)	110(2.00)	156(3.00)				
2D				69(1.50)	111(2.00)	157(3.00)				
2E				70(1.50)	112(2.00)	158(3.00)				
2F				71(1.50)	113(2.00)	159(3.00)				
2G				72(1.50)	114(2.00)	160(3.00)				
2H				73(1.50)	115(2.00)	161(3.00)				
2I				74(1.50)	116(2.00)	162(3.00)				
2J				75(1.50)	117(2.00)	163(3.00)				
2K				76(1.50)	118(2.00)	164(3.00)				
2L				77(1.50)	119(2.00)	165(3.00)				
2M				78(1.50)	120(2.00)	166(3.00)				
2N				79(1.50)	121(2.00)	167(3.00)				
2O				80(1.50)	122(2.00)	168(3.00)				
2P				81(1.50)	123(2.00)	169(3.00)				
2Q					124(2.00)					
2R					125(2.00)					
2S					126(2.00)					
2T					127(2.00)					

[a] Chlorinated biphenyl IUPAC Number
[b] Concentration in µg/mL

Polychlorinated Biphenyl Congener Classes with Identification Ions

PCB Congener Isomers Classes	Numbers of PCB isomers per congener class	Molecular Formula	Identification ions $M^{+\cdot}$	$(M - Cl)^{+}$	$(M - 2Cl)^{+\cdot}$
Monochlorobiphenyl	3	$C_{12}H_9Cl$	188	153‡	-
Dichlorobiphenyl	12	$C_{12}H_8Cl_2$	222	187‡	152‡
Trichlorobiphenyl	24	$C_{12}H_7Cl_3$	256	221‡	186‡
Tetrachlorobiphenyl	42	$C_{12}H_6Cl_4$	290 (292)*	255	220
Pentachlorobiphenyl	46	$C_{12}H_5Cl_5$	324 (326)	289 (291)*	254
Hexachlorobiphenyl	42	$C_{12}H_4Cl_6$	358 (360)	323 (325)	288 (290)*
Heptachlorobiphenyl	24	$C_{12}H_3Cl_7$	392 (394)	357 (359)	322 (324)
Octachlorobiphenyl	12	$C_{12}H_2Cl_8$	426 (430)	391 (393)	356 (358)
Nonachlorobiphenyl	3	$C_{12}HCl_9$	460 (464)	425 (429)	390 (392)
Decachlorobiphenyl	1	$C_{12}Cl_{10}$	494 (498)	459 (463)	424 (428)

* Largest component of the isotope cluster is in parentheses, if not the first member.

‡ HCl losses are also observed for lower chlorinated congeners, resulting in one mass unit lower masses than in the Table.

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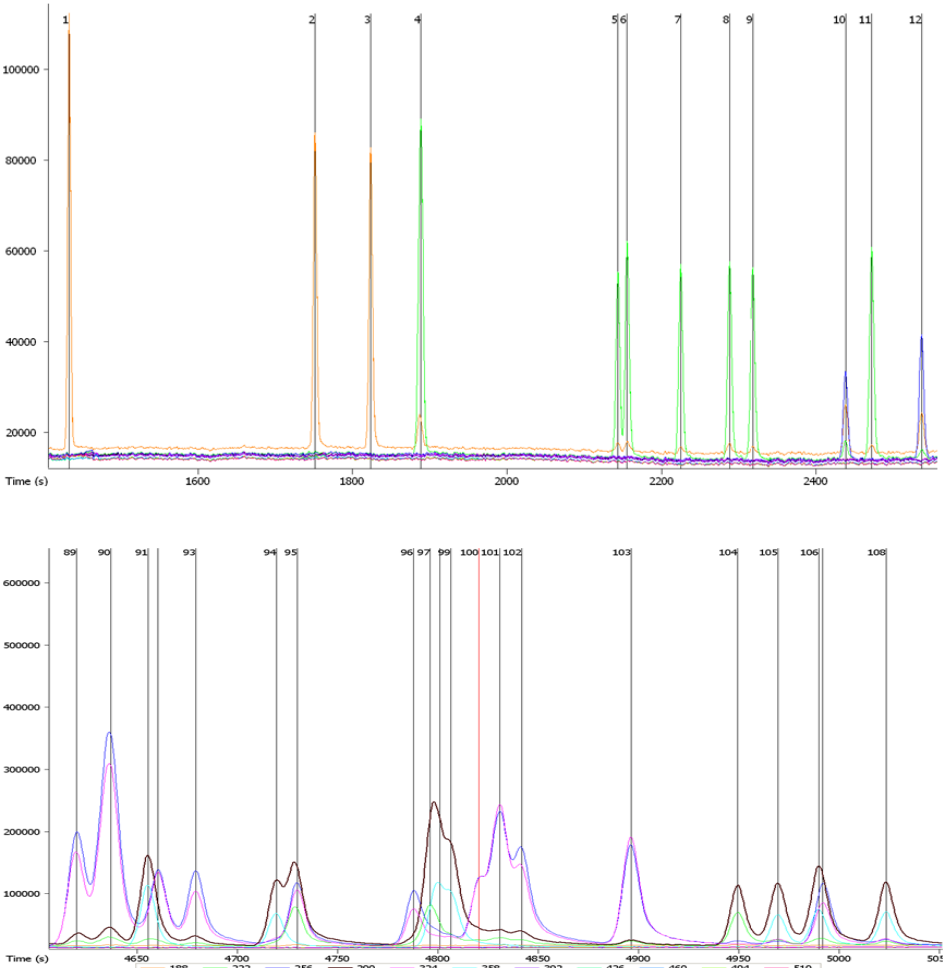
Analytical approach 1-D Vs. 2-D

- The magnitude of the number of PCB isomer-specific congeners in the environment have made the results of human and environmental health assessment inaccurate.
- The 1-D approach has been used over time but is not capable of distinguishing the 209 PCB congeners.
- The 2-D analytical approach is advantageous over the 1-D because of the high capacity of separation and better sensitivity.
- It may serve as an advance tool for separating difficult and complex samples.

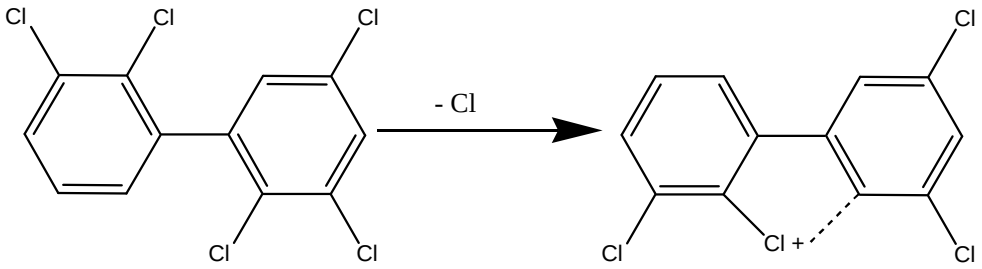
1-D Approach to 209 PCB Congeners Analyses



- The 1-D mode is a necessary preliminary step used to determine which PCB isomer-specific congeners need the 2-D mode of separation
- The upper figure from the same run shows separation of early eluters with relative ease.
- The lower figure does not; it shows several coeluting PCB congeners and therefore the need for 2-D mode of chromatographic separation.

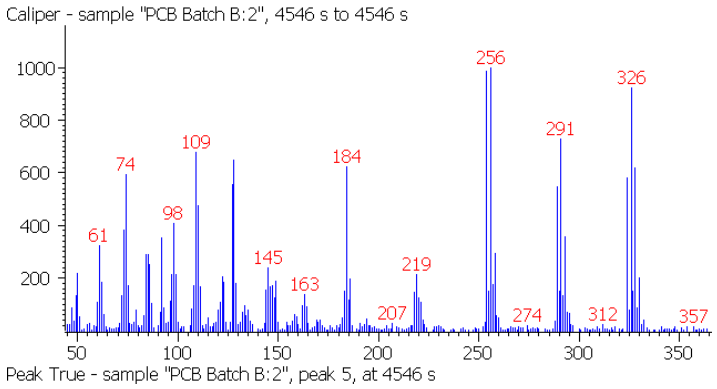
PCB Congener Identification by Ortho Effect

PCB 83 (IUPAC)

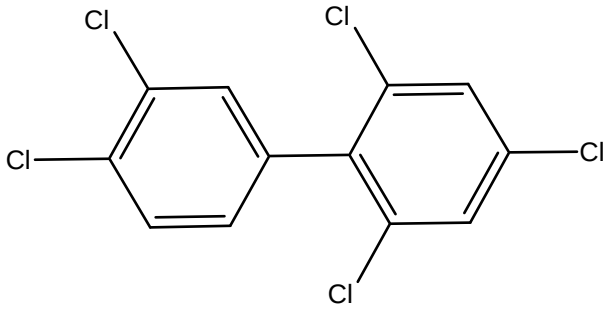


Chloronium ion intermediate

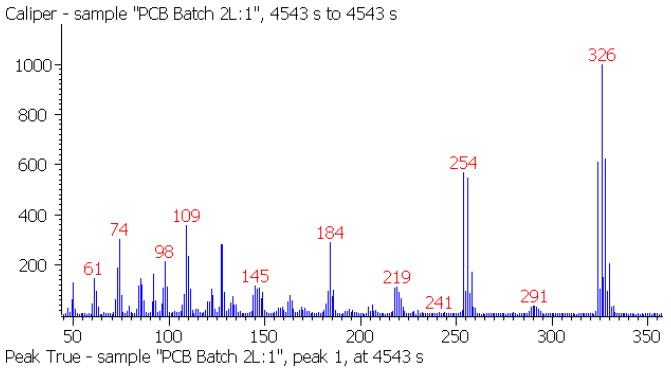
Ortho effect



PCB 119 (IUPAC)



No Ortho effect



Selection of PCB Isomers for 2-D Analysis

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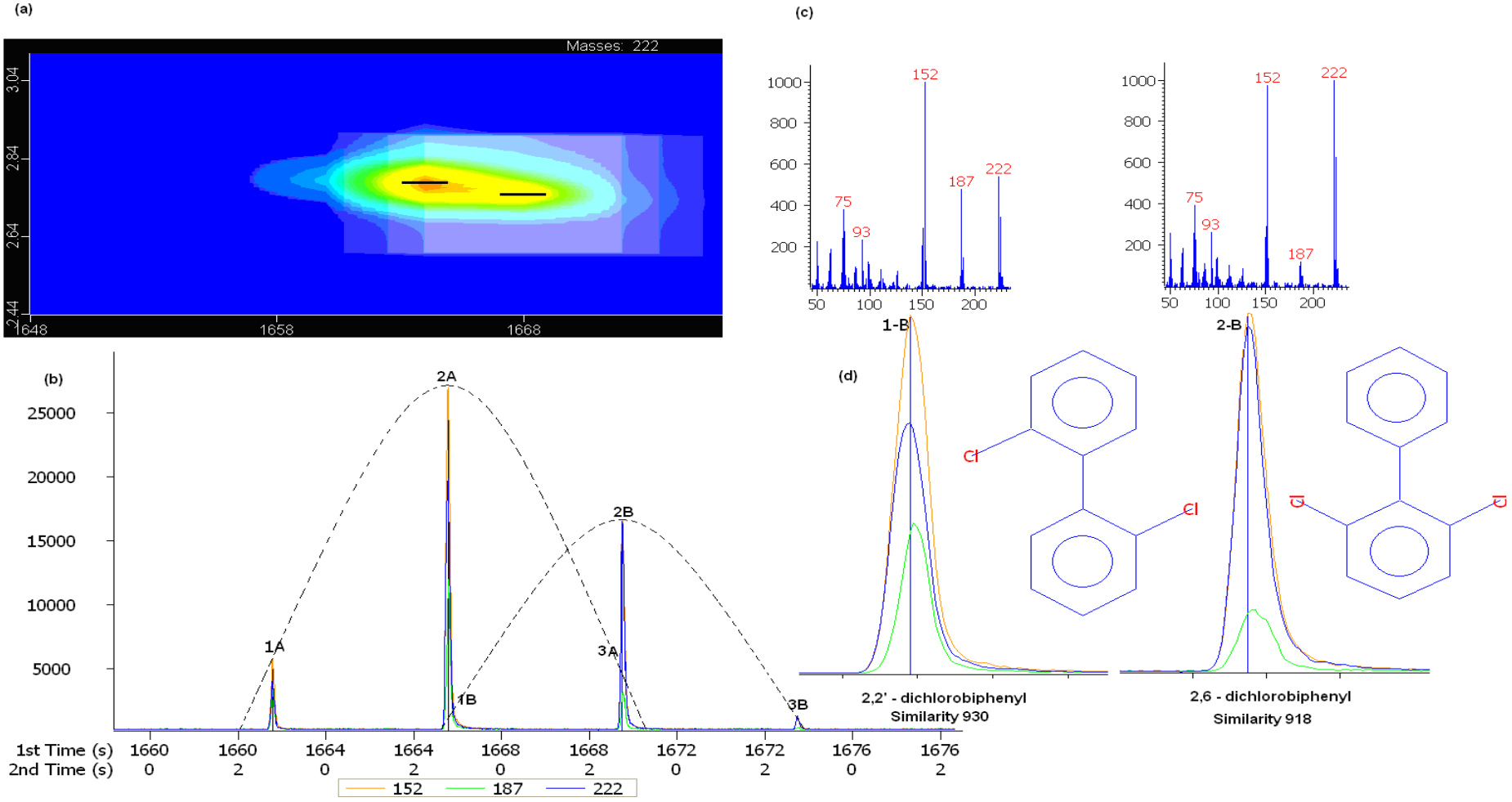
- a = Values rounded off
- b = Largest abundance components of isotope clusters of 100([M-Cl]/[M])
- c, d, e, f, g = Groups of isomers with separations of 5 sec. or less. Need 2D.
- h = "*Ortho effect*" used with the elution order can distinguish these nearest neighbors
- **h** = From groups c, d, e, f, g, these coeluters can be distinguished using "*ortho effect.*"

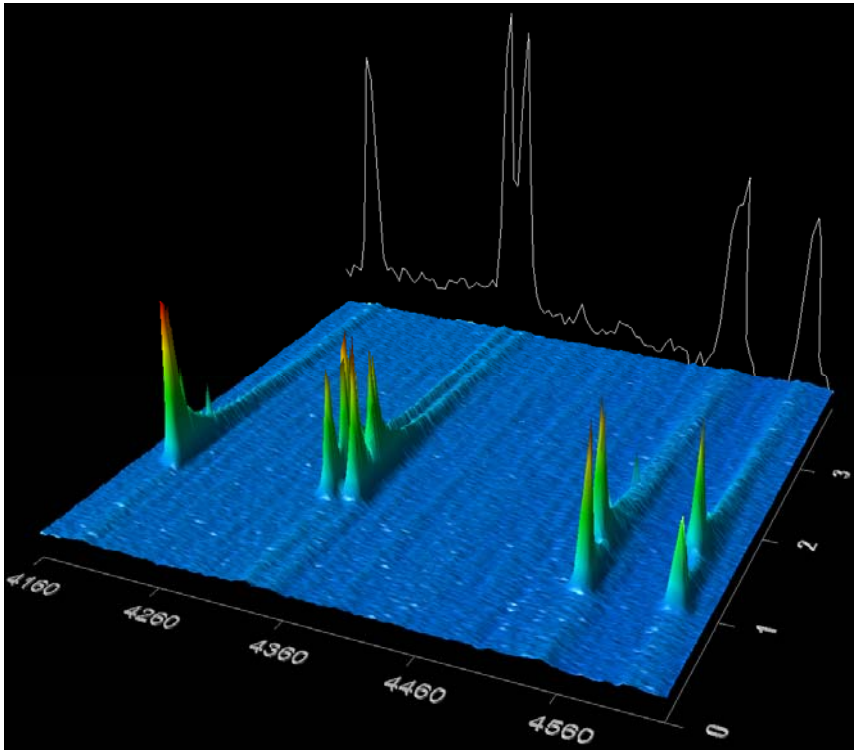
GC Elution and MS "*Ortho Effect*" of the Forty-six Penta CBs

Elution Order	IUPAC #	Structure Ring1:Ring 2	RT a sec	"*Ortho Effect*" b %
1	104	246:26	3550	10
2	96	236:26	3814	13 h
3	103	246:25	3864	38 h
4	100	246:24	3939	31
5	94	235:26	3952	50
6	102	245:26	4030	36
7	98	246:23	4077	51
8	93	2356:2	4093	59
9	88	2346:2	4127	48
10	95	236:25	4136	46 h
11	121	246:35	4155	6 h
12	91	236:24	4217	42 h
13	92	235:25	4343	49
14	89	234:26	4353	51
15	84	236:23	4360	63
16	90	235:24	4404 c	42
17	101	245:25	4407 c	29
18	113	236:35	4421	7 h
19	99	245:24	4460	20 h
20	119	246:34	4543 d	3 **h**
21	83	235:23	4546 d	69 **h**
22	125	345:26	4558 e	7 **h**
23	86	2345:2	4560 e	44 **h**
24	112	2356:3	4562 e	6 **h**
25	108	2346:3	4584	4
26	97	245:23	4603	38 h
27	116	23456	4653	6 h
28	87	234:25	4712	41 h
29	117	2356:4	4744	4 h
30	115	2346:4	4752 f	3
31	111	235:35	4757 f	3
32	85	234:24	4767	33 h
33	110	236:34	4818 g	3 h
34	120	245:35	4822 g	3
35	82	234:23	4915	54 h
36	124	345:25	5068	5 h
37	107	234:35	5106	3

Use of Chromatographic and Mass Spectrometric Data in a Case of Isomer Coelution



Separation of Coeluting Pentachlorobiphenyl Isomers using GCxGC-TOFMS in the 2-D Mode

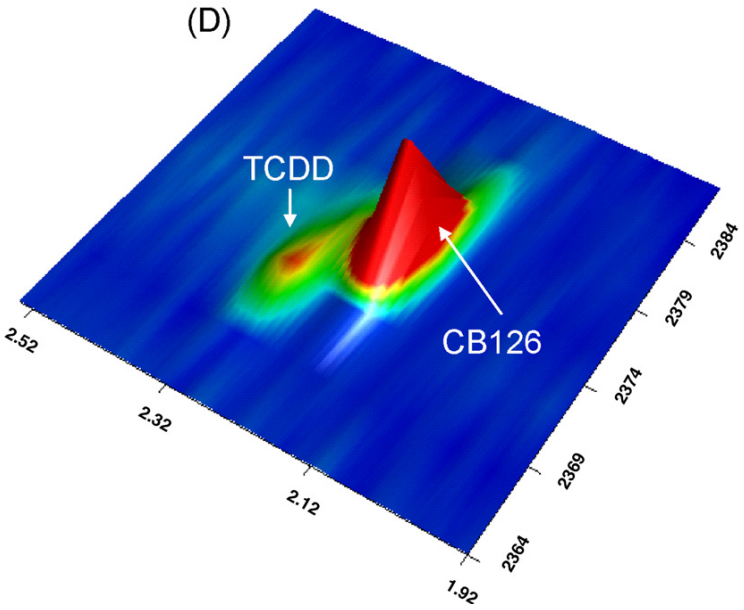


Separation of four pairs of pentachlorinated biphenyl isomers with IUPAC numbers beginning from the right (110+120), (115+111), (86+112), and (83+119).

The left pair of penta-isomers, number (90+101), were inseparable under the chromatographic conditions used.

196 PCB congeners were distinguished when the 1-D mode, 2-D mode, and the use of “ortho-effect” were employed.

PCB Ions Interference in Mass Quantification



(adapted from E. Hoh et al., 2007)

- Most columns used in the 1-D approach have inherent interferences of ions from PCB congeners with IUPAC numbers 81, 123, and 169 in environmental samples.
- Ions 324 and 326 from 2,3,7,8-tetrachlorodibenzo-p-dioxin interfere in the quantification of PCB congener 126 and vice versa
- With the right modulation and hot/pulse setting, GCxGC/TOF-MS easily separates these compounds chromatographically in the 2-D mode

Summary

- By using GCxGC-TOFMS in the 1-D mode of separation, followed by the 2-D mode, including the use of “ortho-effect,” we were able to distinguish 196 PCB congeners.

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Conclusion

- The GCxGC-TOFMS did not distinguish all of the 209 PCB isomer-specific congeners but it did distinguish many and with further optimization it may distinguish more.
- It may be regarded as a tool that permits customized chromatographic separation of recalcitrant chemicals using the 2-D mode.

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- Dr. Bill Brumley; U.S EPA, NERL, ESD, ECB, Las Vegas, Nevada.
- Dr. Mark Liberdoni; LECO Corporation, 3000 Lakeview Ave, St Joseph MI 49085.

2009 EPA's PCB Research

- PCBs in Caulk (1950 -78 buildings)
- Source migration path - air, dust, and soil in school buildings
- http://www.epa.gov/pcbsincaulk/caulkresearch.htm