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PESTICIDE RESIDUES

Identification of Polychlorinated Biphenyls in [illegible] Bald Eagles by Combined Gas-Liquid Chromatography-Mass Spectrometry

By G. E. BAGLEY, W. L. REICHEL, and E. CROMARTIE (Bureau of Sport Fisheries and Wildlife, Patuxent Wildlife Research Center, Laurel, Md. 20810)

Polychlorinated biphenyls are widely used industrial compounds marketed in the United States under the trade name Aroclor. They have appeared in fish and wildlife tissues in this country and in Europe. They are known to be toxic, but more importantly, their presence in samples along with the commonly occurring organochlorine pesticides complicates the analysis to a serious degree, making identification of pesticide residues by GLC without preliminary separation extremely unreliable. Residues of both polychlorinated biphenyls and organochlorine pesticides were present in eagle carcasses. Many of the polychlorinated biphenyl components had retention times identical with those of certain organochlorine pesticide residues. Identification was accomplished by preliminary separations on thin layer plates followed by combined GLC-mass spectrometry.

For several years this laboratory has been monitoring field-collected bald eagle (*Haliaeetus leucocephalus*) specimens for organochlorine pesticide residues by gas-liquid chromatography (GLC), utilizing [illegible] capture detection. Because of the complexity of the chromatograms a rather extensive separation technique has been employed, requiring [illegible] separation on silica gel thin layer plates [illegible] for identification on three columns, containing 10% DEGS, 3% OV-17, and 3% XE-60. This technique, although time-consuming, has proved satisfactory for most commonly occurring organochlorine pesticides; however, a number of [illegible] displaying appreciable electron capture capability would not be identified. Using [illegible] detection, these unknowns [illegible] chlorine-containing compounds.

The occurrence of polychlorinated biphenyls (PCB's) in wildlife tissues, reported by research workers in Europe (1, 2), led us to investigate the presence of these compounds in our wildlife samples.

Samples of Aroclors, a series of multiple-application industrial plasticizers comprising a complex mixture of closely related PCB's, were obtained from the manufacturer for investiga-

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tion. Gas-liquid chromatograms of some of these Aroclors demonstrated a striking resemblance to the chromatograms obtained from our wildlife samples. Results of investigations by combined GLC-mass spectrometry (GLC-MS) that led to the identification of unknown compounds in our field samples are reported here.

METHOD

Apparatus

The separation and identification of the unknown components were obtained with an LKB Model 9000 GLC-MS equipped with stainless steel molecule separator system of Ryhage (3). The spiral glass column (9' × 0.25") was packed with 1% SE-30 on 100–120 mesh Gas Chrom Q. Carrier gas was helium flowing at a rate of 35 ml/min. Operating temperatures (°C) were: flash heater 220, GLC oven 180, separator 240, and ion source 270. The ionization potential was 70 ev, trap current 60 μamp, and accelerating voltage 3.5 kv. Scan time was 12 sec from m/e 2–400.

Standard

Technical Aroclor 1254 (Monsanto) was dissolved in hexane to contain 0.5 μg/μl solution. Three μl of standard was used for GLC-MS analysis.

Preparation of Samples

Extracts from two bald eagle carcasses, 68-050 and 68-056, were used for this investigation. Sample 68-050 was an immature bird found dead in Arkansas and 68-056 was an adult found dead in Minnesota. The carcass, after removal of skin, wings, liver, feet, and gastrointestinal tract, was ground and mixed in a Hobart food cutter. A 20 g aliquot was mixed with sodium sulfate and extracted 7 hr with petroleum ether in a Soxhlet apparatus. The extract was cleaned up by acetonitrile partitioning and eluted on a Florisil column as previously described (4).

Thin Layer Chromatography

Silica gel-thin layer chromatography (TLC), employing zonal separation as described by Mulhern (5), was used to separate interfering organochlorine pesticides. For zone identification 5 μg of each pesticide was placed as one composite spot on the origin line at the far right end of the plate. The plates were placed in a lined tank and developed in a hexane-ethyl ether (98 + 2) mixture for a distance of 15 cm from the origin line. Developed plates were covered, except for the area containing the reference spots, sprayed with silver nitrate solutions, and exposed to UV light. The plate was divided into four horizontal zones, using the color developed reference spots as a guide. Zones III and IV of each sample were analyzed by GLC-MS. Practically all unknown components appear in these two zones, but only two of the commonly occurring pesticides (*p,p'*-DDT in zone III, *p,p'*-DDE in zone IV) are detected in these zones.

Previous analysis of zone IV for pesticide residues by GLC electron capture detection showed 11.8 ppm *p,p'*-DDE in the carcass of 68-050 and 40 ppm in 68-056. Both quantities are based on wet weight. No *p,p'*-DDT or *o,p'*-DDT was detected in either sample, which, if present, would have been in zone III.

This Aroclor (one of 12) as reported in the manufacturer's bulletin (6) is composed of a series of chlorinated biphenyls containing approximately 54% chlorine. This one was selected as a reference because its chromatogram showed a striking resemblance to chromatograms of our wildlife specimens. As some components were not resolved on the GLC column, a solution containing 10 μg Aroclor 1254 was separated by the TLC zonal procedure as previously described and mass spectra were taken on major peaks.

GLC-MS Analysis

The major peaks as shown in the total ion current chromatogram for Aroclor 1254 (Fig. 1A) are numerically labeled for identification in the order of their retention time. The earliest eluter is No. 1 and subsequent compounds are numbered consecutively. Peaks in total ion current chromatograms for eagle samples (Figs. 1B-D) are labeled as they relate to the Aroclor standard. Peaks 1–16 have approximately the same retention time as identically numbered peaks in the Aroclor. The total ion current chromatogram of 68-050, zone III, is omitted because it is essentially the same as 68-056.

Mass spectra data were obtained on all major peaks in the total ion current chromatograms and peaks having similar retention times were compared. Figs. 2–16, unless otherwise indicated, show mass spectra for peaks in the Aroclor standard and corresponding peaks in eagle samples. These data, except for Fig. 15, represent identical compounds observed in the Aroclor standard and eagle samples. The compound represented by Fig. 15 was observed in both eagles but not in the Aroclor standard. Relative intensities were calculated by choosing as base peak the most intense peak in the spectrum and assigning it a value of 100. Doubly charged ions of odd mass values have been omitted. Peaks having a relative intensity of 1% or less and peaks less than m/e 100 are omitted for brevity and clarity. Background peaks were more apparent below m/e 100.

Presented in Table I are PCB's investigated in the

Reference to specific brand names is made to facilitate understanding and does not imply endorsement of such brands by the Bureau of Sport Fisheries and Wildlife.

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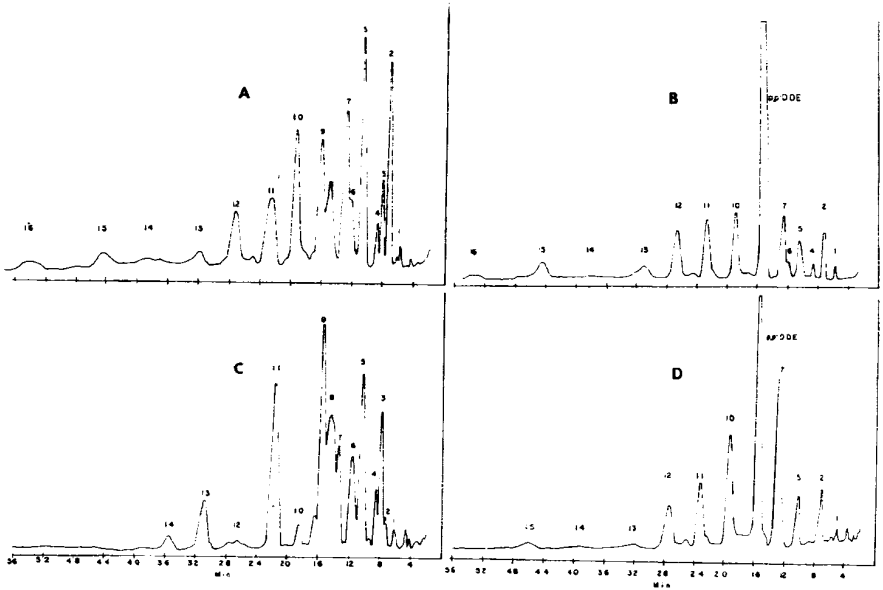

FIG. 1—Total ion current chromatogram of A, standard PCB mixture (Arochlor 1254, 1.5 μg); B, eagle carcass 68-056, TLC zone IV; C, eagle carcass 68-056, TLC zone III; and D, eagle carcass 68-058, TLC zone IV.
GLC conditions: 9' × 0.25" 1% SE-30 column, oven temperature 180°C, and helium flow 35 ml/min.

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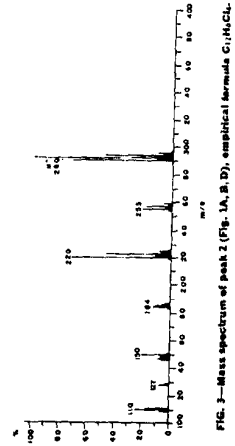

FIG. 3—Mass spectrum of peak 2 (Fig. 1A, B, D), empirical formula $C_{12}H_6Cl_4$.

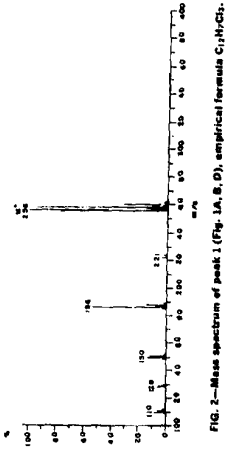

FIG. 2—Mass spectrum of peak 1 (Fig. 1A, B, D), empirical formula $C_{12}H_7Cl_3$.

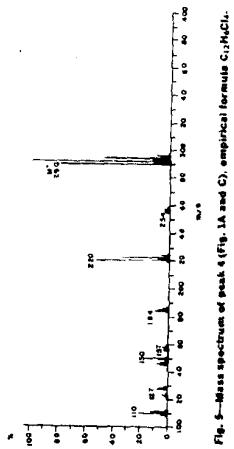

Fig. 5—Mass spectrum of peak 4 (Fig. 1A and C), empirical formula $C_{12}H_6Cl_4$.

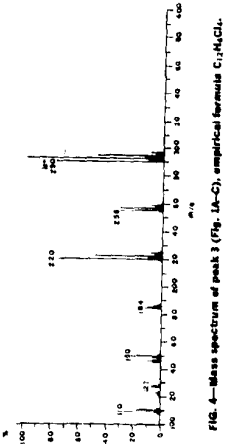

FIG. 4—Mass spectrum of peak 3 (Fig. 1A–C), empirical formula $C_{12}H_6Cl_4$.

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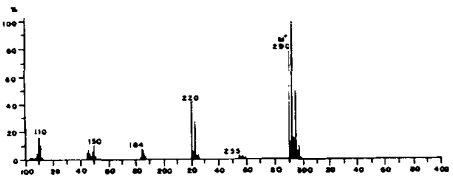

FIG. 6—Mass spectrum of peak 5 (Fig. 1A, B, and D), empirical formula $C_{12}H_4Cl_4$.

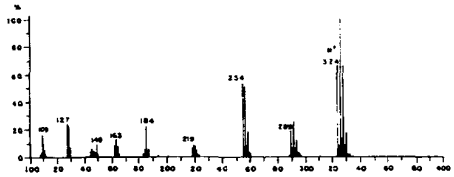

FIG. 7—Mass spectrum of peak 5 (Fig. 1C; 1254-III (total ion current chromatogram not shown for Aroclor 1254, TLC zone III)), empirical formula $C_{12}H_5Cl_5$.

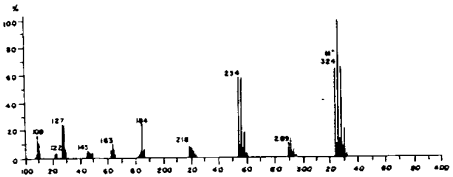

FIG. 8—Mass spectrum of peak 7 (Fig. 1A, B, and D), empirical formula $C_{12}H_5Cl_5$.

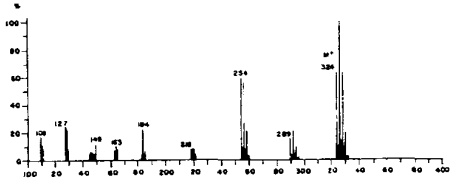

FIG. 9—Mass spectrum of peak 8 (Fig. 1A and C), empirical formula $C_{12}H_5Cl_5$.

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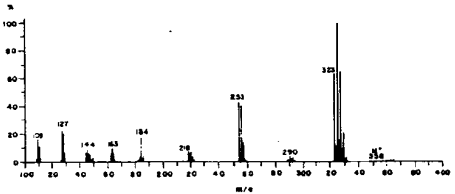

FIG. 10—Mass spectrum of peak 9 (Fig. 1A and C), empirical formula $C_{12}H_4Cl_6$.

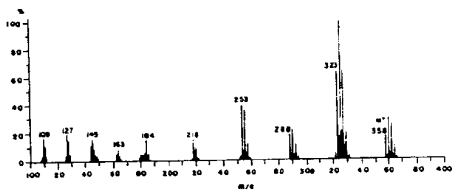

FIG. 11—Mass spectrum of peak 10 (Fig. 1A, B, and D), empirical formula $C_{12}H_4Cl_6$.

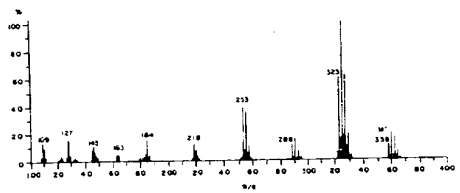

FIG. 12—Mass spectrum of peak 11 (Fig. 1C; 1254-III (total ion current chromatogram not shown for Aroclor 1254, TLC zone III)), empirical formula $C_{12}H_4Cl_6$.

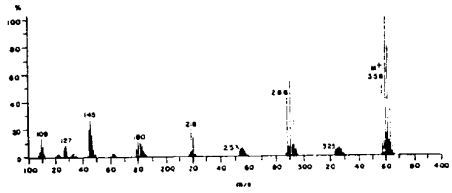

FIG. 13—Mass spectrum of peak 11 (Fig. 1A, B, and D), empirical formula $C_{12}H_4Cl_6$.

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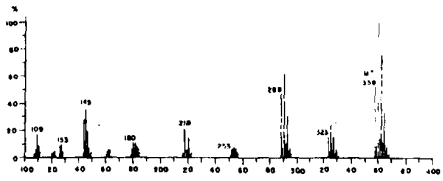

FIG. 14—Mass spectrum of peak 12 (Fig. 1A, B, and D), empirical formula $C_{12}H_4Cl_6$.

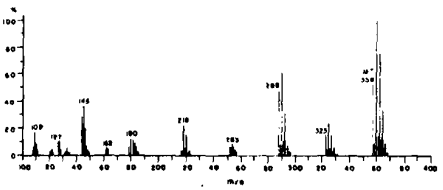

FIG. 15—Mass spectrum of peak 13 (Fig. 1B and D), empirical formula $C_{12}H_4Cl_6$.

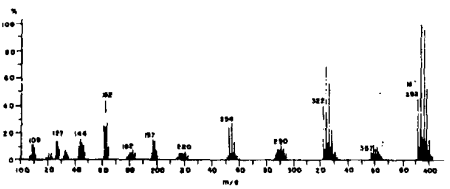

FIG. 16—Mass spectrum of peak 15 (Fig. 1A, B, and D), empirical formula $C_{12}H_3Cl_7$.

Aroclor standard, their molecular weight, and the number of chlorine atoms per molecule. The relative retention times (*DDE = 1*) of these compounds and some organochlorine pesticides are also shown. A 1% SE-30 column was used for all determinations. These data obviously demonstrate that the PCB's and the commonly occurring organochlorine pesticides have similar retention times, therefore prohibiting identification by GLC alone.

Results and Discussion

The GLC-MS spectra data show conclusively that most chemicals in the eagle samples are identical to components of Aroclor 1254. The two specimens collected from widely separated areas contain essentially identical PCB's varying slightly in apparent concentration of specific compounds.

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Table 1. Relative retention time of PCB's and some commonly occurring organochlorine pesticides (*p,p'*-DDE = 1)

PCB Peak No.	Mol. Wt	Relative Retention Time	Compound	Relative Retention Time
1	256 (3)*	0.39	Heptachlor	0.43
2	290 (4)	0.49		
3	290 (4)	0.53	Aldrin	0.53
4	290 (4)	0.58		
5	290 (4),324 (5)	0.70	Heptachlor epoxide	0.66
6	324 (5)	0.79		
7	324 (5)	0.83	Dieldrin	0.96
8	324 (5)	0.97	*o,p'*-DDD	1.02
9	358 (6)	1.04	Endrin	1.08
10	358 (6)	1.23	*p,p'*-DDD	1.27
11	358 (6)	1.46	*o,p'*-DDT	1.35
12	358 (6)	1.72	*p,p'*-DDT	1.69
13	358 (6),392 (7)	2.01		
14	392 (7)	2.32		
15	392 (7)	2.86		
16	392 (7)	3.38		

* Number in parentheses following molecular weight is the number of chlorine atoms in the molecule.

In the Aroclor standard, peaks 5 and 11 (Fig. 1A) each contain two compounds. The compounds were separated on a TLC plate. The two compounds in peak 5 differed in number of chlorines (Figs. 6 and 7); the compounds in peak 11 had the same number of chlorines but differed in spectral characteristics due to the position of chlorine substitution on the ring (Figs. 12 and 13). The four compounds were detected in the samples.

Mass spectra data of peaks 13, 14, and 16 in the bird samples varied with the corresponding peaks in the Aroclor standard. A difference in the number of chlorines (6 for samples, 7 for standard) was observed for peak 13. Peaks 14 and 16 differed from the standard in spectral characteristics due to the position of chlorine substitution on the ring.

Two or more components are uniformly distributed throughout peak 6 in both field samples; therefore a mass spectrum could not be obtained.

Table 2 shows spectral data on some PCB's observed either in the Aroclor standard or the eagle samples. Identically numbered peaks have similar retention times. Even though some peaks have similar retention times mass spectral data show them not to be the same compound but isomers. Data on the ability of animals to alter this class of compounds are meager. An explanation of this may hold the solution to compounds observed in the eagle samples but not in the Aroclor.

Discussions of the fragmentation processes of spectra shown in Figs. 2–16 and Table 2 have been omitted for future presentation. The spectra for all compounds investigated are qualitatively similar and the molecular ions in nearly all cases are major peaks. With exception of the position of ring substitution, information necessary to determine molecular structure is readily obtained from the identity of the molecular ion and the most intense ion in the mass spectrum (7).

Tabulations from research workers in Sweden (8) show that as many as 114 different PCB's are theoretically possible, assuming that the compounds contain 3–7 chlorine atoms and the number of chlorine substitution on each of the two benzene rings differs by no more than one. They have shown that at least 24 of these compounds are present in their field samples. We have obtained mass spectra for 19 PCB's in these two field samples.

In this laboratory PCB's have been detected in tissues and eggs of other avian species and also in mussel and fish samples. Field samples that contain residues of PCB's and organochlorine pesticides usually have what appears to be proportionate amounts of each (9). This has not yet been explained.

Positive identification by GLC-MS of organochlorine components in two typical field samples have shown that they are PCB's and that most are identical to PCB's found in Aroclor 1254. These organochlorine compounds are known to have toxic properties (10) and their distribution appears to be now a part of the global ecosystem (11, 12), but aside from their toxicity, significant interferences occur in the analysis of organochlorine pesticide residues.

References

(1) Holmes, D. C., Simmons, J. H., and Tatton, J. O'G., *Nature* **216**, 227–229 (1967).
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(3) Ryhage, R., *Anal. Chem.* **36**, 759–764 (1964).
(4) Reichel, W. L., Lamont, T. G., Cromartie, E., and Locke, L. N., *Bull. Environ. Contamin. Toxicol.* **4**, 24–30 (1969).
(5) Mulhern, B. M., *J. Chromatog.* **34**, 556–558 (1968).
(6) Technical Bulletin O/PL-306, Monsanto Co., St. Louis, Mo.

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Table 2. Mass spectra of polychlorinated biphenyls[a]

m/e	1254 III 6 324 (5)	68-050 III 9 326 (5)	68-050 III 10 358 (6)	1254 14 392 (7)	68-056 IV 14 392 (7)	1254 16 392 (7)	68-056 IV 16 392 (7)	1254 13 392 (7)
98	10.2	5.4	6.3	8.2	10.9	10.9	7.6	10.8
99	7.0	3.7	2.8	4.1	5.4	5.0	4.1	4.4
107	3.9	1.0	2.1	4.1			3.4	4.6
108	4.5	2.2	5.3	11.8	15.9	13.6	10.7	11.9
109	20.2	12.5	16.0	15.3	20.0	16.4	10.0	22.8
110	17.0	8.3	11.3	13.8	14.1	14.5	8.3	16.1
111	9.5	3.3	4.0	5.3	6.4	6.8	4.1	5.8
119			1.2		4.1	4.5	3.1	2.8
120	3.2	1.3	2.5	5.9	6.4	6.4	5.2	5.5
121	3.9	1.5	2.8	5.3	5.4	7.3	4.8	5.3
122	6.4	3.4	5.7	7.1	8.2	6.8	5.9	7.1
123	6.6	3.0	3.1		5.9		4.1	3.3
125	3.6							
126		1.0	9.1	17.1	18.9	19.1	15.2	19.2
127	29.5	22.9	11.2	22.3	20.7	25.4	16.2	21.9
128	27.3	21.2	6.0	14.1	9.5	14.5	6.7	9.7
129	9.3	5.6	1.8			5.4		
131			1.7		5.9		3.4	4.2
132		1.6	3.8	8.8	10.0	10.0	7.2	8.3
133	4.8	2.4	3.7	8.2	9.1	10.0	5.9	7.2
134		1.4	2.1	4.4	5.9		3.4	3.9
135	3.9	1.6	1.8	2.9	4.5		3.1	3.2
136								
137	2.7							
138	5.2							
143				10.3	17.3	11.8	10.3	8.8
144	6.6	4.6	27.4	18.2	29.5	20.9	11.7	37.5
145	9.8	5.8	34.0	16.2	26.4	17.3	11.0	38.9
146	6.8	4.2	18.9	14.4	16.8	13.5	9.3	23.6
147	7.3	3.7	8.5	6.5	8.6	10.0	5.2	11.4
148	5.0	2.9	3.2	3.2			2.1	3.3
149	13.4	5.0	2.8	2.9			3.1	2.8
150	5.7			3.5				2.5
156			1.5	4.4			4.5	4.2
157			2.3	2.9			2.8	3.6
158			1.7	2.3				2.8
161			2.5	25.3	29.1	27.3	26.7	17.5
162	9.3		3.8	42.9	47.7	43.6	48.3	29.4
163	13.2		3.7	30.6	31.4	30.0	31.0	21.0
164	9.3		1.9	12.3	11.4	10.9	9.8	7.8
165	3.2							
167	3.2							2.2
168								2.2
169								3.6
170								1.4
171								1.9
179			9.4	6.9	9.1	8.2	6.0	8.1
180			16.0	7.1	13.2	7.7	6.9	14.2
181			15.1	8.2	10.9	9.1	6.5	13.3
182	2.9	1.5	11.3	8.8	12.7	10.0	9.5	12.9
183	6.1	4.6	8.5	5.6	6.4	7.3		7.2
184	25.2	17.5	5.7	9.1	5.9	10.0	4.3	7.5
185	6.8	3.7	3.8	3.2		5.4		3.0
186	9.1	5.8	1.1	3.2				1.9
196				8.8	9.1	8.2	7.6	5.0
197				17.6	19.5	12.7	13.8	11.4
198				15.6	17.3	13.6	13.4	11.7
199				9.1	8.6	6.4	9.0	5.1
216			1.2	6.5	7.7	5.4	5.5	5.0
217	7.9		3.7	8.2	8.6	7.3	6.5	7.5
218	9.6	6.7	23.6	11.5	16.4	10.9	6.0	29.8
219	16.6	8.2	8.6	7.1	7.7	8.2	5.5	8.3
220	7.3	5.8	16.0	12.3	11.8	15.4	4.6	19.2

(Continued)

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Table 2. (*Continued*)

m/e	1254 III 8 324 (5)	68-050 III 9 324 (5)	68-050 III 10 358 (6)	1254 14 392 (7)	68-056 IV 14 392 (7)	1254 16 392 (7)	68-056 IV 16 392 (7)	1254 13 392 (7)
221	8.6	4.2	2.9	3.8		4.5		4.4
222	2.7	1.4	2.8	6.8	4.5	7.3		5.5
251								2.1
252			5.7	25.9	28.2	25.4	25.5	20.8
253			6.6	8.2	9.8	9.1	6.9	9.3
254	59.1	54.2	11.3	40.0	32.7	40.0	25.5	28.6
255	10.2	7.5	7.5	8.8	7.7	9.1	5.7	10.4
256	61.4	53.7	8.5	21.8	14.1	25.4	10.7	15.5
257	9.3	7.3	3.4	5.3	4.5	6.4	2.2	5.3
258	20.4	15.8	2.7	7.1	3.6	9.1	2.4	4.7
259	4.1	3.3	1.4					
260	4.2	2.7						
261	1.8	1.1						
262	1.9							
286				3.6	4.5	4.5	4.6	5.3
287				5.9	7.3	7.3	5.9	52.8
288			46.2	12.3	24.5	13.6	9.0	13.6
289	29.5	14.2	7.5	12.3	13.2	11.8	9.3	69.4
290	20.4	3.7	59.4	23.5	34.1	26.4	10.7	14.2
291	43.2	17.9	10.4	10.0	10.0	10.9	5.5	43.0
292	23.9	3.7	3.0	18.8	20.9	23.6	7.1	8.9
293	22.7	0.9	5.7	5.9	5.7	6.8	3.1	12.2
294	11.4	1.5	8.0	9.4	7.0	10.0	2.8	3.6
295	5.7	2.1	2.6	2.3				3.0
296	2.9		1.7	2.9				
297	0.7							
322				38.8	40.9	40.9	40.3	22.5
323			12.3	9.7	9.1	10.4	8.6	18.6
324	63.6	62.5	13.2	82.3	68.2	82.3	69.0	52.8
325	10.4	9.2	20.7	17.6	13.6	17.3	13.6	33.3
326	100.0	100.0	19.8	66.5	50.4	74.5	48.3	44.4
327	13.9	12.9	15.1	14.7	10.0	16.4	9.0	19.7
328	63.6	62.5	12.3	32.9	20.9	36.4	17.9	21.4
329	8.9	7.9	5.7	7.1	5.0	9.1	4.0	8.6
330	21.6	18.3	3.8	10.6	6.4	12.3	5.2	6.4
331	2.7	2.6	1.2	3.5				
332	3.4	2.7		2.9				
333	0.6							
357				14.1	38.6	13.6	9.0	55.5
358			52.8	11.2	35.2	11.8	5.9	21.1
359			9.4	27.6	72.7	24.5	17.9	100.0
360			100.0	20.6	32.7	19.1	10.2	23.6
361			16.0	22.9	54.1	20.0	16.2	81.9
362			80.2	15.9	17.0	17.3	7.6	15.8
363			12.3	10.6	23.6	10.0	7.2	36.1
364			34.0	7.1	6.8	7.3	3.4	6.1
365			4.7	3.2	6.4	3.6	1.7	8.6
366			8.5	2.1				
367			1.1					
368			1.2					
392				47.1	43.2	47.3	51.7	26.7
393				10.6	10.4	13.6	10.7	6.4
394				100.0	100.0	100.0	100.0	61.1
395				17.6	17.3	20.0	17.6	10.8
396				97.1	95.4	90.9	96.5	58.3
397				14.7	13.9	16.4	15.2	8.9
398				52.9	46.8	51.2	53.4	28.9
399				7.6	7.7	10.0	7.9	4.4
400				15.9	16.6	17.3	16.5	10.5
401				2.6	2.5	2.7	2.4	1.4
402				3.5	3.2	3.6	2.9	1.9

* The first line in the heading is sample, the second peak number, and the third molecular weight with the number of chlorine atoms in the molecule in parentheses.

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