Toxaphene Composition Analyzed by Combined Gas Chromatography–Chemical Ionization Mass Spectrometry

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Fractionation of toxaphene on a silica gel–hexane column and then analysis by combined gas chromatography–chemical ionization mass spectrometry reveal a complex mixture of at least 177 C_{10} polychlorinated derivatives. About two-thirds of these components are various $C_{10}H_{11}Cl_7$, $C_{10}H_{10}Cl_8$, and $C_{10}H_9Cl_9$ compounds. The remaining components are $C_{10}H_{10}Cl_6$, $C_{10}H_{12}Cl_6$, $C_{10}H_9Cl_7$, $C_{10}H_8Cl_8$, $C_{10}H_7Cl_9$, $C_{10}H_8Cl_{10}$, and $C_{10}H_7Cl_{11}$ derivatives. Based on the total ion current on mass spectrometry of fractionated toxaphene, 26 of these components, each present in 1.0–2.5% amounts, make up 40% of this insecticide. Reduction of toxaphene with triphenyltin hydride yields several mono- and polychloro derivatives but the major product is bornane, formed in about 20% yield. This study provides some of the information needed for analysis of toxaphene and toxaphene-derived residues by combined gas chromatography–mass spectrometry with the chemical ionization source.

Toxaphene is produced by chlorination of camphene using certain catalysts and ultraviolet irradiation. It has a chlorine content of 67–69% which corresponds to an average empirical formula $C_{10}H_{10}Cl_8$. About 40 million pounds of this insecticide are used each year, in large part combined with methylparathion for treatment of cotton. The cumulative use of toxaphene over the past 25 years approximates one billion pounds. It is therefore important to define the nature of the toxaphene components (Guyer *et al.*, 1971). Some chemical data on the nature of individual components have been published recently (Casida *et al.*, 1974; Khalifa *et al.*, 1974; Palmer *et al.*, 1974). Toxaphene is a very complex mixture so the combined gas chromatography (gc)–mass spectroscopy (ms) technique is likely to be the most suitable method for composition analysis.

This report gives the results of gc–ms analysis of toxaphene utilizing the chemical ionization (CI) procedure (Field, 1968). It also considers the products obtained on reduction of toxaphene with triphenyltin hydride (Ph_3SnH).

MATERIALS AND METHODS

Chemicals. Hercules Inc. (Wilmington, Del.) provided samples of toxaphene (reference standard, sample X-16189-49) and [^{14}C]toxaphene (1.35 mCi/g; from chlorination of [8-^{14}C]camphene; sample X-19098-4-2R). Toxicants A and B were isolated from toxaphene as described earlier (Khalifa *et al.*, 1974). Toxicant B is the $C_{10}H_{11}Cl_7$ compound, 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane, but the structure of toxicant A, a $C_{10}H_{10}Cl_8$ derivative, is not defined (Casida *et al.*, 1974; Khalifa *et al.*, 1974; Palmer *et al.*, 1974).

Analyses. *Electron Capture (EC)-Gc.* The Varian Aerograph Model 1400 instrument was used with a ^{63}Ni EC detector and a coiled glass column (2 m x 2 mm i.d.) containing 3% SE-30 on Gas Chrom Q (80–100 mesh) operated isothermally at a column temperature of 180° with a N_2 flow rate of 75 ml/min.

Routine Gc–Ms and Ms. The Finnigan Model 9500 gas chromatograph coupled to a Finnigan Model 1015D mass spectrometer with a CI source was used in combination with the System Industries Model 150 control system. A U-shaped column of 3 m length and 2 mm i.d. containing 3% Dexil-300 on Varaport-30 (100–120 mesh) was operated with temperature programming and a methane flow rate of 20 ml/min (methane pressure of 1–1.5 Torr in the CI source). The mass range of 125–600 amu was repeatedly scanned at the rate of 3–4 sec/scan. The first mass of the ion cluster, *i.e.* that due to the ^{35}Cl isotope, is used in all calculations of molecular composition.

Electron impact (EI) mass spectra were obtained at 70 eV with a CEC Model 21-110 mass spectrometer using the direct insertion technique and a sample volatilization temperature of 150°.

Preparative Gc. The Hewlett-Packard Model 5754 instrument equipped with a thermal conductivity detector and a coiled aluminum column (180 cm × 3 mm i.d.) containing 4% SF 96-50 on Gas Chrom G (80–100 mesh) was operated isothermally at a column temperature of 120° with a He flow rate of 60 ml/min. The eluting compound was obtained as white crystals on collection in a Teflon tube (20 cm × 1 mm i.d.) cooled with Dry Ice. It was recovered by rinsing the Teflon tube with carbon tetrachloride.

Capillary Column Gc. The Beckman Thermotrac instrument was used with a flame ionization (FI) detector and a coiled glass column (305 m × 0.76 mm i.d.) coated with OV-101 or Dexil-300. The oven temperature was 100° with the OV-101 column and 80° with the Dexil-300 column, in each case with a He flow rate of 50 ml/min.

Preparation and Analysis of Toxaphene Fractions 1–18. Preliminary studies indicated that toxaphene components are fractionated on a different basis by chromatography on a silica gel column developed with hexane than they are by gc. Composition analysis is therefore facilitated by adsorption chromatography followed by gc. To accomplish this, a small amount of [^{14}C]toxaphene was added to unlabeled toxaphene in order to monitor the elution from the adsorption column and also to aid in the systematic combination of the column effluent into fractions for gc–ms analysis. Details of the procedure used are given below.

A mixture of 99.0 mg of unlabeled toxaphene and 1.0 mg of [^{14}C]toxaphene was introduced into a column (1 m × 1.9 cm i.d.) of 160 g of silica gel 60 (30–70 mesh; EM Laboratories Inc., Elmsford, N. Y.) packed from a slurry in pesticide grade hexane (Fisher Scientific Co., Fair Lawn, N. J.) and topped with a filter paper disk and a 1-cm layer of white sand. Over 700 tubes containing ^{14}C-labeled compounds were obtained on developing this column with pesticide grade hexane and collection of 10-ml fractions at a rate of 0.5 ml/min. When the amount of ^{14}C eluting off the column with hexane had substantially decreased, a solvent mixture of hexane–ether (10:1) was used to remove residual ^{14}C from the column, resulting in a 94% overall recovery. Several steps were used in combining the eluted materials to obtain 18 fractions of relatively distinct gc properties. First, the tubes were combined in

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Table I. Components in Toxaphene Separated by Silica Gel Adsorption Chromatography and Then Gas

No.	Chrom. properties: Silica gel fraction	Chrom. properties: Gc R_t, min	Amt in toxaphene, %	No.	Chrom. properties: Silica gel fraction	Chrom. properties: Gc R_t, min	Amt in toxaphene, %	No.	Chrom. properties: Silica gel fraction	Chrom. properties: Gc R_t, min	Amt in toxaphene, %
$C_{10}H_{12}Cl_6$, $[M - Cl]^+ = m/e$ 307				31	13	17.7	0.30	61	8	17.4	0.08
1	2	9.1	0.02	32	17	21.1	0.65	62	14–16	17.4	2.40
2	16	15.6	0.92	$C_{10}H_{10}Cl_8$, $[M - Cl]^+ = m/e$ 375				63	2–4	17.5	1.42
3	16	15.8	0.52	33	4	11.1	0.07	64	11–12	17.5	0.55
$C_{10}H_{11}Cl_7$, $[M - Cl]^+ = m/e$ 341				34	2	11.3	0.03	65	5–8	17.7	2.00
4	17	9.9	0.47	35	4	11.3	0.07	66	17–18	17.7	1.85
5	1	10.7	0.60	36	1–2	12.0	0.24	67	5	17.9	0.59
6	1–2	11.8	0.45	37	2	12.4	0.13	68	9–14	17.9	2.39
7	12	12.1	0.06	38	1–2	12.7	0.32	69[a]	5–8	18.0	1.51
8	17	12.2	0.48	39	1–2	13.3	0.76	70	9–11	18.2	1.04
9	6	12.4	0.04	40	4	13.3	0.21	71	18	18.2	0.46
10	3–4	12.9	0.45	41	1	13.8	0.09	72	5	18.4	0.32
11	3	13.3	0.05	42	4	13.8	0.06	73	11–13	18.4	0.45
12	5	13.4	0.44	43	3–5	14.6	1.06	74	5–9	18.5	1.81
13	7–9	14.0	0.45	44	1	14.7	0.56	75	15	18.5	0.71
14	5–7	14.2	0.74	45	17–18	14.8	0.96	76	18	18.5	0.25
15	6–8	14.9	1.25	46	4–5	15.0	0.17	77	13–15	18.7	0.92
16	6	15.0	0.46	47	11	15.3	0.21	78	16–18	18.7	0.94
17	12	15.2	0.16	48	7	15.4	0.27	79	9–13	19.1	2.30
18	10	15.3	0.97	49	17–18	15.5	0.74	80	14–16	19.3	1.61
19	17	15.3	0.30	50	6–7	15.7	0.39	81	17–18	19.3	0.88
20	8	15.4	0.10	51	18	16.1	0.40	82	9–10	19.5	0.92
21	6	15.5	0.60	52	1–2	16.2	0.98	83	12–13	19.5	0.43
22	17	15.8	0.65	53	6–7	16.3	0.21	84	17–18	19.7	0.88
23	8–9	15.9	0.17	54	2–4	16.5	1.32	85	7–8	19.8	0.83
24	14	16.1	0.47	55	5–7	16.7	0.70	86	11	19.8	0.33
25	10–12	16.3	0.61	56	9	16.7	0.09	87	13–14	19.9	0.68
26	8–9	16.3	0.80	57	17–18	16.9	0.84	88	6–9	20.1	1.79
27	14–16	16.4	1.58	58	2–4	17.1	1.46	89	10–13	20.3	1.41
28[b]	10–14	16.7	2.48	59	8–11	17.1	0.56	90	15	20.3	0.46
29	15	16.9	1.05	60	14	17.1	0.26	91	18	20.3	0.74
30	12–13	17.3	0.90								

[a] Toxicant A. [b] Toxicant B.

the order of elution in such a manner as to produce 100 fractions, each containing *ca.* 1.0% of the recovered ^{14}C. Second, each of the 100 fractions was examined by gc–EC. Fractions observed to be identical or with only slight differences were combined giving 18 fractions. Finally, the solvent was removed from the 18 fractions using a gentle stream of dry N_2 after which time they were concentrated to a constant weight, redissolved in hexane, and subjected to gc–ms analysis. The sample was injected at an oven temperature of 180° with this temperature being maintained for 5 min and then programmed at a rate of 4°/min until a temperature of 270° was reached.

The mass spectral data from each of the 18 fractions were analyzed in the following manner. The ion current data accumulated on a disk storage unit were used to obtain a reconstructed gas chromatogram for the total 125–600-amu range scanned. This procedure was than repeated seven times by having the computer search for and plot only those peaks which correspond to mass ranges for components containing 11 down through 5 chlorine atoms. The mass spectrum of each peak from each mass chromatogram was then systematically examined starting at the highest mass isomers. This technique was combined with consideration of the chlorine cluster patterns to recognize fragments from components of high mass which appear in lower mass ranges and therefore must be accounted for and eliminated when appropriate. The data were assembled in tabular form listing for each fraction the retention time (R_t) and the molecular composition of each isomer. An intercomparison of the data for all fractions then served to eliminate duplication and to assign an average R_t value when the same component appeared in two or more successive fractions.

Quantitation of the individual toxaphene components was accomplished with the 125–600-amu plots by determining the peak area for each component with a compensating polar planimeter and then repeating this procedure for each of the 18 fractions. Using the proportionate area for each component relative to the total area for all components in a fraction, and the weight of that fraction relative to the total amount of toxaphene examined, a per cent by weight was assigned to each component. If a component appeared in more than one fraction, the percentage values were summated for the successive fractions in which the component was found.

Reduction of Toxaphene with Ph_3SnH and Analysis of Products. A solution of toxaphene (0.25 g, 0.61 mmol) in hexane (5 ml) was added to each of six Pyrex tubes. Four of the tubes were treated with various amounts of Ph_3SnH (Kuivila and Beumel, 1961) as follows: 0.64 g (3 mol equiv); 1.07 g (5 mol equiv); 1.7 g (8 mol equiv); 2.1 g (10 mol equiv). The fifth tube contained the toxaphene solution but no organotin compound. To the sixth tube was added 0.71 g (3 mol equiv) of triphenyltin chloride (Ph_3SnCl). 2,2′-Azobis(2-methylpropionitrile) (AIBN) (*ca.* 1 mg) was then added to each tube and they were irradiat-

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No.	Chrom. properties: Silica gel fraction	Chrom. properties: Gc R_t, min	Amt in toxaphene, %
92	15–16	20.7	1.00
93	18	20.7	0.54
94	13–14	21.1	0.17
95	18	21.1	0.69
96	12	21.3	0.09
	$C_{10}H_9Cl_9$, $[M - Cl]^+ = m/e$ 409		
97	17	13.7	0.30
98	1	16.9	0.47
99	1	17.4	0.33
100	1–2	17.9	0.14
101	1–4	18.0	1.64
102	4	18.5	0.93
103	1	18.9	0.04
104	3–4	19.0	0.37
105	6–7	19.1	0.22
106	3	19.5	0.33
107	4–6	19.7	0.61
108	1–2	20.1	0.97
109	3	20.1	0.35
110	4–5	20.1	0.47
111	1	20.5	0.41
112	5–7	20.7	0.69
113	14	20.7	0.20
114	17	20.7	0.23
115	3–8	20.9	1.56
116	10–12	20.9	0.33
117	1–5	21.3	0.82
118	7–8	21.3	0.31
119	18	21.3	0.18
120	5	21.5	0.07
121	11	21.5	0.31
122	13–16	21.5	0.84
123	6–10	21.7	0.64
124	7–11	22.1	0.61
125	14–17	22.1	0.64
126	14	22.4	0.54
127	14–18	23.3	1.00
	$C_{10}H_8Cl_{10}$, $[M - Cl]^+ = m/e$ 443		
128	3–5	19.3	1.09
129	2–3	21.9	0.13
130	5	21.9	0.08
131	1–3	22.4	0.37
132	2–4	23.3	0.14
133	3	23.7	0.03
134	1–2	24.0	0.21
	$C_{10}H_7Cl_{11}$, $[M - Cl]^+ = m/e$ 477		
135	4	25.4	0.02
	$C_{10}H_{10}Cl_6$, $[M - Cl]^+ = m/e$ 305		
136	1–2	7.9	0.05
137	1	9.0	0.13
138	8	10.1	0.46
139	2	10.5	0.09
140	14	10.9	0.10
141	8–9	11.3	0.53
142	11–12	11.8	0.28
143	14	11.9	0.16
144	18	12.7	0.42
145	15	12.8	0.06
146	15–16	13.3	0.25
147	18	13.3	0.42
148	13	13.5	0.10
149	18	14.0	0.03
150	13	14.9	0.06
	$C_{10}H_9Cl_7$, $[M - Cl]^+ = m/e$ 339		
151	11	12.7	0.08
152	14	12.8	0.08
153	6	13.1	0.23
154	17	13.1	0.22
155	8–12	13.5	1.03
156	6	13.6	0.13
157	14	14.3	0.59
158	11–12	14.7	0.81
159	14	14.9	0.29
160	16	14.9	0.15
161	16	15.2	0.37
162	14–15	15.5	0.58
163	12–13	16.1	0.60
	$C_{10}H_8Cl_8$, $[M - Cl]^+ = m/e$ 373		
164	2–3	13.8	0.13
165	2	14.2	0.20
166	16–17	14.2	0.47
167	1–3	15.0	1.47
168	18	15.2	0.42
169	5	15.3	0.10
170	3–4	15.6	0.46
171	1	15.8	0.04
172	3	15.8	0.30
173	18	15.8	0.59
174	3	16.2	0.08
175	15	17.9	0.29
	$C_{10}H_7Cl_9$, $[M - Cl]^+ = m/e$ 407		
176	1	16.5	0.36
177	2	18.9	0.14

ed for 3 hr at 55 ± 5° using a General Electric sunlamp. The reduction results in formation of large amounts of Ph_3SnCl which is removed by cooling the reaction mixture and then filtration. The filtrate was combined with several washings of the solid with cold hexane and then additional hexane was added as appropriate to obtain the same final volume for each of the six mixtures. The products were analyzed by gc-EC and gc-ms, in each case with sample injection at an oven temperature of 110° and temperature programming at 10°/min until 180° was reached. The fifth and sixth control tubes produced the normal gc pattern of toxaphene so Ph_3SnCl and AIBN do not result in decomposition of toxaphene or interference with the analyses.

One component of reduced toxaphene recovered by preparative gc (R_t *ca.* 2 min) was compared with authentic 1,7,7-trimethylbicyclo[2.2.1]heptane (bornane) in respect to their infrared spectra as carbon tetrachloride solutions, mass spectra, and their gc behavior on capillary columns (R_t for bornane, 26.5 min on the OV-101 column and 28.5 min on the Dexil-300 column).

RESULTS

Fragmentation Patterns of Toxicants A and B by EI- and CI-Ms. The mass spectral data provide little interpretable information for structural elucidation on toxaphene components. However, the EI and particularly the CI data are useful in determining the molecular formula as illustrated in Figure 1 with toxicants A and B. The EI spectra of A ($C_{10}H_{10}Cl_8$) and B ($C_{10}H_{11}Cl_7$) show the ions at high mass to be in very low abundance relative to the ions at lower mass, whereas the opposite relationship is observed with the CI spectra. The molecular ions are not readily observed in EI and normal "quasi" molecular ions are nonexistent in CI with methane. The $[M - Cl]^+$, $[M - Cl - HCl]^+$, and $[M - Cl - 2HCl]^+$ ion clusters are of relatively low intensity in the EI spectra but these clusters are the major fragments with CI, the $[M - Cl]^+$ ion presumably forming first and then undergoing successive losses of HCl (or Cl). The large $[M - Cl]^+$ ion in the methane CI spectra is probably formed on rapid loss of Cl from the parent molecule as HCl resulting from protonation by the reagent gas ions (CH_5^+); analogous findings are obtained with other polycyclic chlorinated pesticides such as aldrin, chlordane and isodrin (Biros *et al.*, 1972). The CI fragmentation patterns for toxicants A and B appear to be typical of those for each of the individual toxaphene components examined either in a mixture or in pure form. Thus, with CI, the highest ion cluster which is the $[M - Cl]^+$ ion is used in determining the molecular composition of the toxaphene component (see Figure 1).

Composition of Toxaphene Based on Analysis of 18 Fractions. Direct examination of toxaphene by gc-ms is not appropriate for composition analysis because of the complexity of the mixture and the overlapping of the numerous components on gc. However, preliminary fraction-

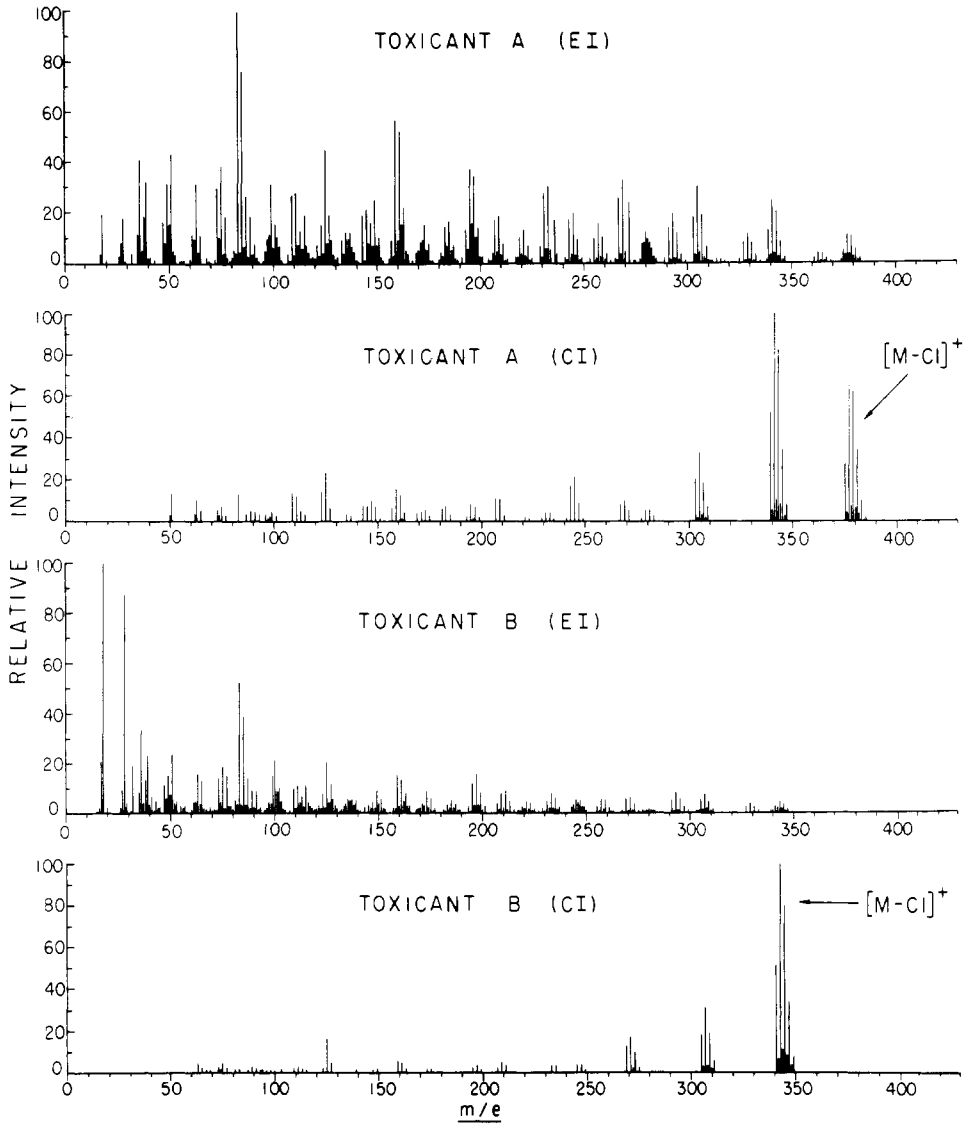

Figure 1. Comparison between the electron impact (EI) and chemical ionization (CI) mass spectra of toxicant A ($C_{10}H_{10}Cl_8$) and toxicant B ($C_{10}H_{11}Cl_7$).

ation on an adsorption column followed by gc–ms analysis of the fractions permit a more accurate assessment of the number of components in toxaphene and the molecular formulas since the gc patterns are then greatly simplified (Table I). The silica gel adsorption column provides little or no separation of components on the basis of chlorine content. Thus, components with six–nine chlorines appear almost as frequently in the early fractions as in the later fractions. On the other hand, gc separation has some relationship to the number of chlorine atoms, the components of low chlorine content generally eluting earlier. These differences in the chromatographic properties for the toxaphene components are convenient for composition analysis.

Examination of the 18 fractions by gc–ms indicates that the toxaphene sample used in this study contains at least 177 components (Table I). Isomeric $C_{10}H_{10}Cl_8$ compounds account for about one-third of the toxaphene components and another third is made up of $C_{10}H_{11}Cl_7$ and $C_{10}H_9Cl_9$ derivatives. One of these components (toxicant B) is known to be a heptachlorobornane so it seems likely that the major toxaphene components are hepta-, octa-, and nonachlorobornanes. Most of the remaining components, by number, correspond in molecular formulas to unsaturated Cl_6, Cl_7, and Cl_8 bicyclic derivatives, but it is also possible that some of them may be tricyclic compounds.

Characterization of the toxaphene components was greatly aided by the use of the on-line computer to search for specific mass ranges, as illustrated in Figure 2 for the first of the 18 fractions. The most difficult part of the analysis was the integration of the mass spectral data assembled for each of the individual fractions into a composite representing the total 18 fractions. In order to do this, the total ion current trace was compared with the corresponding EC trace for each of the fractions. The integration was also aided by the presence of phthalates ($[M + 1]^+ = m/e$ 149 and 279) as contaminants (possibly from contact of the fractions with plastic vial caps) in each of the 18 fractions. These phthalates were used as internal standards (R_t 20.2 and 10.0 min, respectively) in integrating the 18 fractions.

The quantitative values for the amounts of individual toxaphene components listed in Table I are based on the total ion current and are therefore only approximate in nature.

Products from Reduction of Toxaphene with Ph_3SnH. The more heavily chlorinated toxaphene components appear to react most readily with Ph_3SnH at 55° in the presence of light. Thus, the EC-sensitive components of higher R_t values tend to disappear first on reduction (Figure 3). The gc–EC patterns become progressively simplified as the amount of Ph_3SnH is increased up to but not beyond the point where 8 molar equivalents of Ph_3SnH are used. A few of the components are resistant to this type of re-

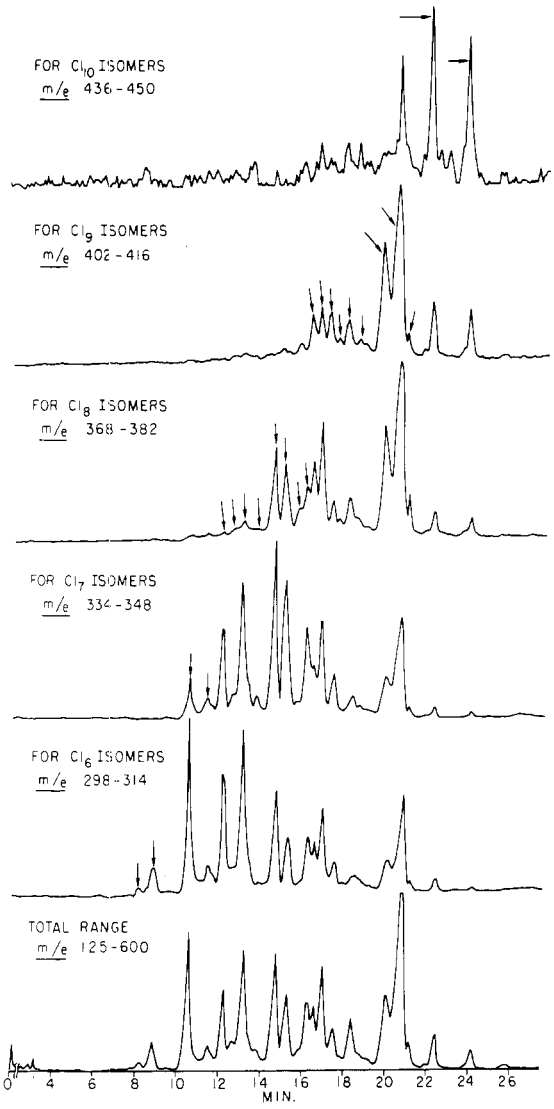

Figure 2. Reconstructed gas chromatograms illustrating the mass ranges searched by the computer in determining the type and number of ions in fraction 1 obtained by chromatography of toxaphene on a silica gel column. The arrows designate the positions of the indicated type of isomer.

duction, even with excess Ph_3SnH. Gc–ms examination of the extensively reduced toxaphene establishes that one component is a $C_{10}H_{18}$ material, four or more components are $C_{10}H_{15}Cl$ and $C_{10}H_{17}Cl$ compounds, and an additional three or more derivatives contain four or five chlorine atoms. The $C_{10}H_{18}$ material is identical with an authentic sample of bornane in respect to its retention times on two different capillary columns, mass spectrum and infrared spectrum. The yield of bornane on reacting toxaphene with 8 molar equivalents of Ph_3SnH is about 20% of the theoretical value based on gc comparison with authentic bornane (conditions same as for preparative gc).

Reaction of toxaphene with a large excess of Ph_3SnH at 105° using heptane as the solvent results in even more extensive reduction of the chlorine-containing components without an increase in the yield of bornane. Several hydrocarbons are formed under these more vigorous conditions that are not detected in reductions carried out at 55°.

DISCUSSION

Toxaphene contains at least 177 components based on gc–CI–ms analysis. This value is probably a minimal one since it does not include minor components that separate

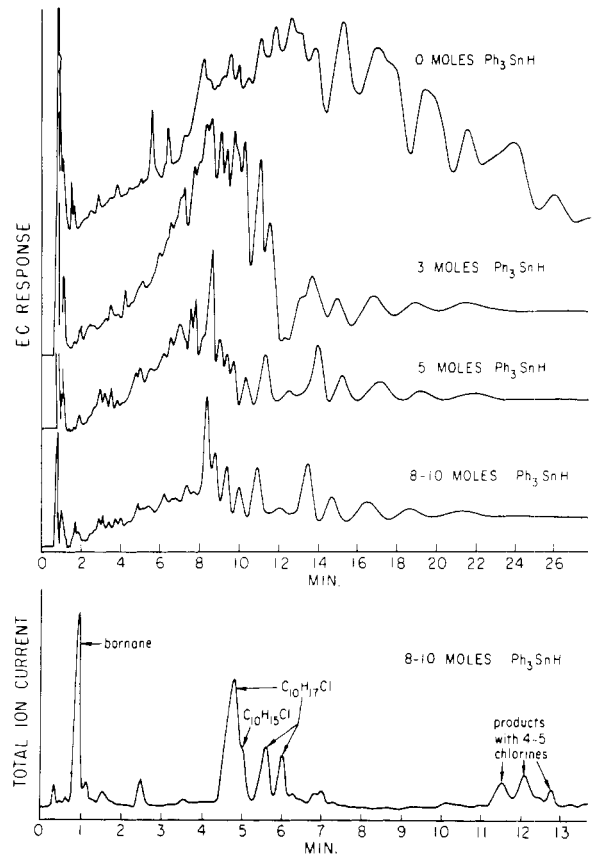

Figure 3. Gas chromatograms illustrating the products obtained on reduction of toxaphene with various molar amounts of triphenyltin hydride (Ph_3SnH).

chromatographically but are in insufficient amounts for obtaining unambiguous mass spectra. Other components may also be overlooked due to inadequate resolution by the two chromatographic procedures used. While it is possible that some of the recorded components may be artifacts resulting from decomposition during analysis, it is likely that most of them are present in the original toxaphene. Thus, toxicants A and B are not decomposed by normal gc procedures of isolation (Khalifa *et al.*, 1974).

Since toxaphene is produced by chlorination of a bicyclic hydrocarbon, camphene, it is assumed that the majority of the chlorinated components are bicyclic in nature, which is indeed the case for toxicant B. It was therefore of interest to determine to what extent the bicyclic structure would be retained or altered on reduction of toxaphene to hydrocarbon products. Ph_3SnH is an active halogen-reducing substance known to proceed through a free-radical mechanism (Kuivila, 1968). Conditions in using this reagent can be very mild and the reagent is very selective. Perhaps the halogens in the toxaphene components found to be resistant to reduction are either aromatic or vinylic in nature since Ph_3SnH is very unreactive with these types of chlorines. Bornane is the major single product of Ph_3SnH reduction of toxaphene. The yield of bornane, *ca.* 20% under the specified reduction conditions, establishes that a large number of the toxaphene components are polychlorobornanes or are bicyclic compounds that rearrange to bornane on reduction.

Analysis of toxaphene and its residues is normally carried out by gc–EC with or without alkaline pretreatment (Guyer *et al.*, 1971; Zweig and Sherma, 1972). Thus, it is important to compare the composition of toxaphene by gc–EC with that found by the total ion current on gc–CI–ms. The conditions described for gc–ms were duplicated in the gc–EC analysis except that a coiled glass column replaced the U-shaped column and N_2 was used instead of

methane as the carrier gas. Comparison of the same fractions from the silica gel column by both analytical methods shows large variations in the relative peak intensities. The percentage composition of several components (10, 43, 116, 139, and 165; Table I) was found by EC to be 70–130% of the corresponding values with CI total ion current. Some smaller components (*i.e.*, 1, 133, and 135) appeared to be in >fourfold higher amounts when analyzed by EC than by CI-ms. It is clear that the EC response is not directly proportional to that of CI-ms. The EC response is known to vary with isomeric compounds; for example, the α, β, γ, and δ isomers of hexachlorocyclohexane differ by up to fourfold in their apparent ionization efficiencies (Ishida and Dahm, 1965). Similarly, the CI-ms response will differ with isomeric materials since the total ion current depends on the relative intensities of the ions generated which in turn varies with different isomers. Attempts to quantitate using the FI detector proved fruitless due to its insensitivity with these types of compounds.

The approximate composition values for toxaphene take on additional significance when one multiplies these percentage values by one billion (the number of pounds of toxaphene already used) to obtain the number of pounds of each component introduced into the environment over the past 25 years. It is cautioned, however, that the composition data are only approximate and are directly related to the CI-ms technique used in the quantitation. It should also be noted that one batch of toxaphene may differ from another so the present composition values for individual components may vary over a small range with different batches.

Toxaphene is a very complex mixture which is difficult to analyze as to composition even under idealized conditions. The complexity will be even greater on considering a mixture of these components with their metabolites and photoproducts such as may be the case under environmental conditions. It is fortunate that, at least with toxaphene itself, most of the components undergo rapid metabolism in mammals (Casida *et al.*, 1974; Ohsawa *et al.*, 1974).

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Gas Chromatographic Analysis of Urethan (Ethyl Carbamate) in Wine

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An analytical method is described for the determination of urethan (ethyl carbamate) in wines. The quantitative method involves an extraction with chloroform followed by a cleanup with Florisil and detection by gas–liquid chromatography. A Coulson electrolytic conductivity detector is capable of detecting levels of urethan at <100 ppb. Confirmation of identity is carried out with trifluoroacetic anhydride derivatized urethan, by gas chromatography using an alkali flame ionization detector.

Diethyl pyrocarbonate (DEP) has been widely used as a food additive for controlling microbiological activity in alcoholic and nonalcoholic beverages (Pauli and Genth, 1966; Fischer, 1970; Gejvall and Löfroth, 1971). Recently, however, Löfroth and Gejvall (1971) were able to show, by use of isotope dilution analysis with tritium-labeled DEP, that the DEP can result in the formation of urethan (ethyl carbamate), a known carcinogen (Nettleship *et al.*, 1943; Mirvish, 1968). The experiments performed by Löfroth and Gejvall (1971) showed that, under laboratory conditions, white wine and beer which normally contain about 5–128 mg/l. of ammonia (Muth and Malsch, 1934; Bishop, 1943) can react with DEP added in the amounts of 280–560 mg/l. to form 1.3–2.6 mg, respectively, of urethan. The reaction is pH dependent. As a result of these studies, the beverage industry has withheld any further use of DEP pending studies to see what levels of urethan can be and are produced under actual food processing conditions. Since urethan is considered a carcinogen, any levels detected could fall within the Delaney Clause, preventing the use of DEP in foods.

The purpose of this study was to develop an analytical method for low levels of urethan useful for routine monitoring applications and for quantitatively studying formation of urethan under varying conditions (Ough, 1974).

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