made significant contributions to the initial design of this project.

LITERATURE CITED

Abou-Donia, M. B., *Appl. Spectrosc.* **29**, 261 (1975).
Boyle, P. H., *Chem. Soc. Rev.* **25**, 323 (1971).
Forrest, J., Stephenson, O., Waters, W. A., *J. Chem. Soc.*, 333 (1946).
Haller, H. L., Bartlett, P. D., Drake, N. L., Newman, M. S., Cristol, S. J., Eaker, C. M., Hayes, R. A., Kilmer, G. W., Magerlein, B., Mueller, G. P., Schneider, A., Wheatley, W., *J. Am. Chem. Soc.* **67**, 1591 (1945).
Kupfer, D., *Crit. Rev. Toxicol.* 4, 83 (1975).
McBlain, W. A., Lewin, V., Wolfe, F. H., *Can. J. Physiol. Pharmacol.*, **54**, 629 (1976).
McBlain, W. A., Wolfe, F. H., *Tetrahedron Lett.* **49**, 4351 (1975).
McKinney, J. D., Wilson, N. K., Keith, L. H., Alford, A. L., "Mass Spectrometry and NMR Spectroscopy in Pesticide Chemistry", Plenum Press, New York, N.Y., 1974, pp 139–160.
Sagar, W. C., Monroe, R. E., Zabik, M. J., *J. Agric. Food Chem.* **20**, 1176 (1972).
Smith, R. A., Bennett, M. J., *Acta Crystallogr.*, in press (1977).
Sparks, A. K., *J. Org. Chem.* **31**, 2299 (1966).
Straw, J. A., Hart, M. M., *Handb. Exp. Pharmakol.* **38**(2), 808–819 (1975).
Vogel, A. I., "A Text-book of Practical Organic Chemistry", Longmans, London, 1967, pp 559–564.
Wilen, S. H., *Top. Stereochem.* **6**, 107–176 (1971).

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Consistency of Toxaphene Composition Analyzed by Open Tubular Column Gas–Liquid Chromatography

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Each of 8 toxaphene samples manufactured by Hercules from 1949 to 1975 shows the same 29 major peaks in almost identical ratios based on analyses by open tubular column gas–liquid chromatography (GLC) with a linear electron capture detector. About 85% of the total peak area is accounted for by these 29 peaks which individually vary from 1 to 8% of the total. The 8 toxaphene samples from Hercules are easily differentiated by open tubular column GLC from 12 samples of related chlorinated terpenes from other manufacturers in the United States and abroad and from [^{14}C]- and [^{36}Cl]toxaphene prepared by Hercules. A more detailed analysis of toxaphene composition is provided by open tubular column GLC of toxaphene components in each of five TLC regions which are precisely defined by the use of selected fluorene marker dyes. Despite large composition differences between some of the samples, there is surprisingly little variation in their mouse intraperitoneal and housefly topical LD_{50} values.

Following the introduction of toxaphene by Hercules in the late 1940's (Buntin, 1951), several other companies in the United States and abroad have produced and marketed similar insecticides prepared by the chlorination of camphene and related terpenes. Food and feed containing residues of toxaphene and related materials are regulated on the basis of tolerances derived from analytical data using methods developed for toxaphene (Guyer et al., 1971; Zweig and Sherma, 1972) and from dietary no-effect levels in chronic feeding studies with toxaphene from Hercules (Lehman, 1965). These residue methods and toxicology data are only suitable for use with materials that closely approximate the composition of Hercules toxaphene. It is therefore important to intercompare the composition of toxaphene samples manufactured by Hercules over the past 26 years and of related commercial materials.

Toxaphene with an overall average molecular formula of $C_{10}H_{10}Cl_8$ is a complex mixture of at least 177 components revealed by a combination of liquid adsorption column chromatography followed by GLC–CIMS analysis on a packed column of the resulting fractions (Holmstead et al., 1974). An improved procedure for separation and quantitative analysis of toxaphene components is needed to critically intercompare the composition of toxaphene samples and related materials. This report gives an open tubular column GLC method for toxaphene analysis and applies this procedure to 8 samples of toxaphene manufactured by Hercules from 1949 until 1975, to 12 samples of toxaphene-like materials from other manufacturers, and to samples of [^{14}C]- and [^{36}Cl]toxaphene. It also evaluates a TLC–GLC method for more complete separation and analysis of toxaphene components and the effect of composition on the acute toxicity of toxaphene-like materials.

MATERIALS AND METHODS

Samples. Charles L. Dunn (Hercules Inc., Wilmington, Del.) provided the following samples: standard toxaphene and toxaphene batches manufactured by Hercules in 1949, 1954, 1957, 1960, 1963, 1970, and 1975; [^{14}C]toxaphene (1.35 mCi/g) and [^{36}Cl]toxaphene (43.6 μCi/g) prepared by Hercules; Hercasa product from the Hercules owned plant at Managua, Nicaragua. He also supplied additional samples believed to originate from the following sources: two samples from Vicksburg Chemical Co. (Vicksburg, Miss.); two samples from Bison Chemical Co. and one from Sonford Chemical Co. (both at Fort Natchez, Tex.); one sample from Procida (Paris, France). Strobane T-100 was supplied by Roy T. Gottesman (Tenneco Chemicals, Piscataway, N.J.). Kenneth R. Hill (Agricultural Environmental Quality Institute, United States Department of Agriculture, Beltsville, Md.) provided four samples: Flit & Fontaine manufactured in South Africa; Melipax

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manufactured in the German Democratic Republic; two East European samples (light and dark) from one producer in Eastern Europe but of different manufacturing periods. One of the Vicksburg samples and the Bison and Sonford samples were obtained as 90% solutions in xylene, from which the solvent was removed under reduced pressure. The Melipax sample was provided as a 9–10% dust from which the desired material was recovered in 7% yield on extraction with hexane. The physical properties of the samples were as follows: yellow viscous liquid, Flit & Fontaine; yellow-brown and black viscous liquids, East European light and dark, respectively; white waxy solid, [^{14}C]toxaphene; yellow or yellow-brown waxy solids, the remaining samples. Elemental analyses on these samples were carried out by the Department of Chemistry, University of California, Berkeley, Calif.

The following three toxaphene components were used as chromatographic standards: (A) mixture of 2,2,5-*endo*,6-*exo*,8,8,9,10-octachlorobornane and 2,2,5-*endo*,6-*exo*,8,9,9,10-octachlorobornane (Matsumura et al., 1975; Turner et al., 1975); (B) 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (Palmer et al., 1975); (C) 2-*endo*,3,3,5-*exo*,6-*exo*,8,9,10,10-nonachlorobornane (Anagnostopoulos et al., 1974).

Open Tubular Column GLC. The Hewlett-Packard Model 5830A gas chromatograph was used with a linear electron capture ^{63}Ni detector with extended dynamic range and an open tubular column (0.25 mm i.d. × 30 m) coated with SE 30 (4 μg/ml). The operating conditions were: injection temperature, 210 °C; oven temperature, maintained at 170 °C for 60 min followed by programming from 170 to 200 °C at 0.5 °C/min and finally a constant temperature of 200 °C for 30 min; detector temperature, 255 °C; split ratio, 1:120; helium carrier gas and argon–methane (95:5) makeup gas for the detector; 1-μg sample injected in 2 μl of hexane. An on-line computer provided the T_r of each peak and its normalized area as a percentage of the total peak area from the chromatogram.

The linear electron capture detector used provided excellent proportionality of the amount of compound injected (examined with component B and aldrin) to the peak area over the entire range of peak areas involved in the present study. This linear response also appears to hold for most if not all of the other GLC peaks on chromatography of toxaphene.

TLC and TLC–GLC. Silica gel 60 chromatoplates (20 × 20 cm, 0.25 mm layer thickness, EM Laboratories, Inc., Elmsford, N.Y.) were spotted with 500 μg of standard toxaphene divided equally among 11 spots and, in additional spots, with 1 μg/spot of the appropriate fluorene marker dyes. The chromatograms were developed three times in the same direction with hexane saturated with dimethylformamide (DMF). Gel regions from the toxaphene chromatograms corresponding in $^{3}R_f$ values (Stahl, 1969) to the appropriate marker dyes (detected by their yellow color or UV-absorbing property) were scraped free from the glass support and extracted with acetone. The acetone was evaporated to dryness, the residue redissolved in 300 μl of acetone, and a 2-μl aliquot of the extract, fortified with aldrin as the GLC marker, was analyzed by open tubular column GLC.

Bioassays. Male albino mice (18–20 g, Horton Laboratories Inc., Oakland, Calif.) were treated by the intraperitoneal (ip) route with the test sample dissolved in dimethyl sulfoxide using 100 μl of dimethyl sulfoxide per mouse. Adult female houseflies (*Musca domestica* L., SCR susceptible strain, 3–4 days after emergence, 18–20 mg) were treated topically on the dorsum of the abdomen with acetone solutions of the test sample, using 1 μl of acetone per fly. The 24-h LD_{50} values are based on 8–12 mice for each dose and a 1.4-fold dose differential and 70 flies per dose and a 2-fold dose differential.

RESULTS

Open Tubular Column GLC and TLC–GLC Analysis of Hercules Toxaphene Standard. Open tubular column GLC of standard toxaphene reveals 29 peaks (Figure 1) that individually make up 1.0 to 8.4% of the total peak area and collectively account for about 88% of the total peak area (Table I). The contribution of each of these peaks to the total peak area is highly reproducible on repeated analyses (Table I).

Several of these open tubular column GLC peaks consist of multiple components as shown most clearly by combined TLC–GLC analysis (Figure 2). The five TLC fractions were selected with marker dyes to recover the precise TLC regions of component A (TLC region d, $^{3}R_f$ 0.45–0.49, benzylidenefluorene marker), of component B (TLC region b, $^{3}R_f$ 0.35–0.39, bifluorenylidene marker), and of regions above, below, and between those of components A and B (Table II). GLC analysis of the TLC fractions reveals that several peaks from the toxaphene standard consist of two or more components since GLC peaks of similar or identical T_r values appear in different TLC regions which are not adjacent to each other (Figure 2). Some of these multicomponent peaks are as follows: 1, 5, 9, 12, 14, 15, 19, 21, 22, 25, and 29. On an overall basis, the TLC–GLC analysis of the toxaphene standard serves to detect more than 74 components as unique and relatively major peaks (designated by numbers and asterisks in Figure 2).

The proportion of the standard toxaphene components that appear in the five TLC regions is not known. However, studies with [^{14}C]- and [^{36}Cl]toxaphene establish a radioactivity distribution for the five TLC regions as shown in Table II and that the acetone extraction procedure recovers 99–100% of the radioactivity from the gel in each of the TLC regions.

Open Tubular Column GLC Analysis of Various Hercules Toxaphene Samples. The seven samples manufactured from 1949 to 1975 are not distinguishable by open tubular column GLC, each showing the same 29 major peaks and in almost identical ratios to those observed in the toxaphene standard (Table I).

Open Tubular Column GLC Analysis of Related Materials. Very similar results were obtained with each of the two samples designated as Vicksburg, Bison, and East European so only the average results are presented for these materials. Some of the samples (Strobane T-100, Hercasa, and [^{36}Cl]toxaphene) are very similar to Hercules toxaphene while others (Procida, Melipax, East European, and [^{14}C]toxaphene) are considerably different (Figure 1, Table I). Several GLC peaks appear in ≥1% amount in samples other than those manufactured by Hercules in the United States but are minor or almost absent in Hercules toxaphene. These components, designated by asterisks in Figure 1, are useful in recognizing samples originating from a particular manufacturer. Another criterion which is adequately reproducible and characteristic in comparing various samples is the time required for 25 and 50% of the total peak area to elute. On this basis, the [^{36}Cl]toxaphene most closely reproduces the Hercules toxaphene samples (Table I).

Other Criteria for Intercomparison of Samples. An almost identical elemental composition, approximating $C_{10}H_{10}Cl_8$, is obtained with each of the Hercules toxaphene samples, and with Strobane T-100 and the Hercasa sample (Table I). The other samples are less heavily chlorinated,

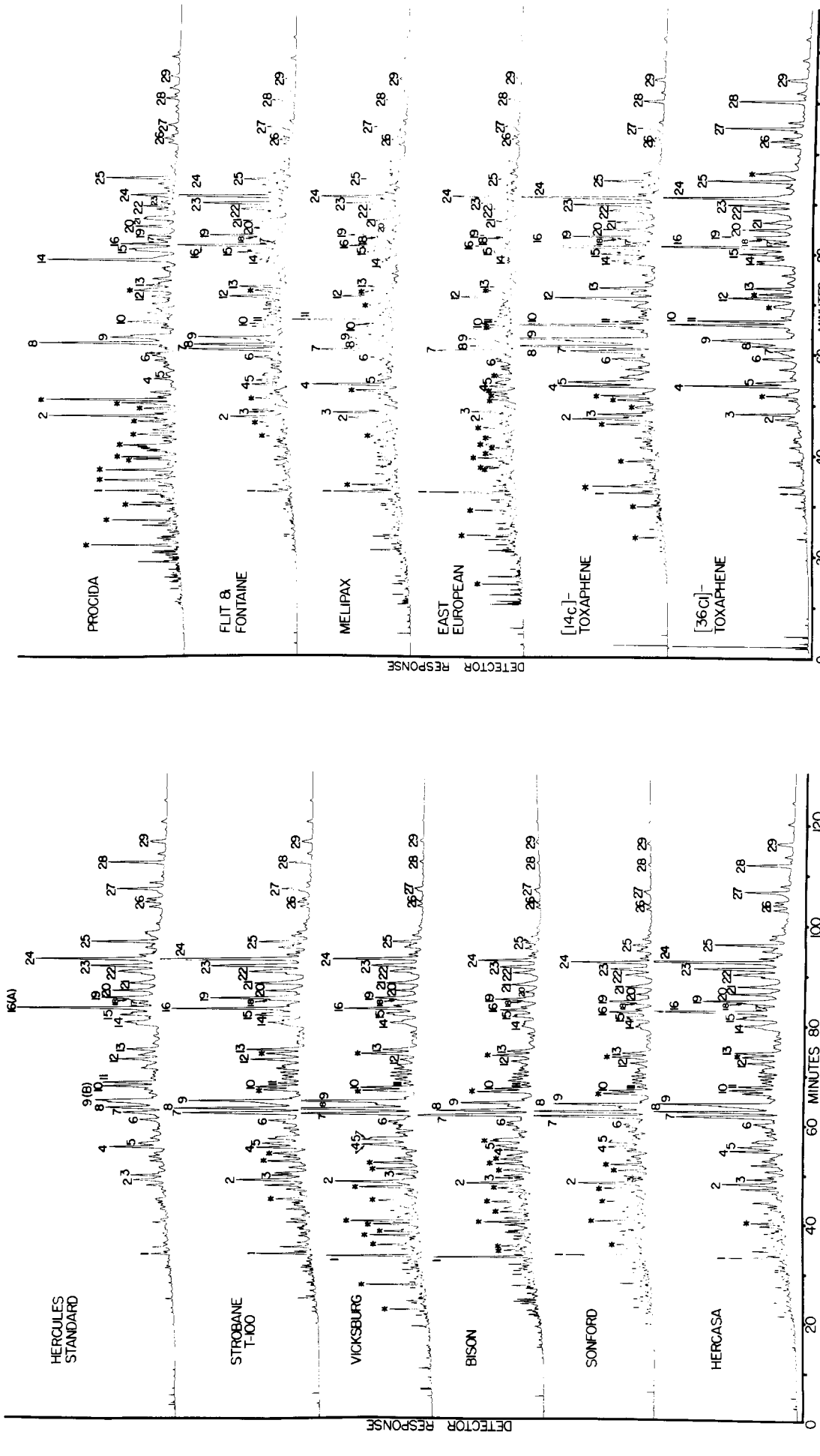

Figure 1. Open tubular column GLC analysis of the toxaphene standard and related chlorinated terpenes. The 29 peaks making up $\geq 1\%$ of the total peak area in the toxaphene standard are designated by numbers as in Table I. The same numbers designate peaks in the other samples with identical T_r values to those in the toxaphene standard. Additional peaks making up $\geq 1\%$ of the total peak area in the related chlorinated terpenes are designated by asterisks. No additional components are eluted at times later than those indicated.

Table I. Open Tubular Column GLC Analysis, Elemental Composition, and Biological Activity of Various Samples of Toxaphene and Related Chlorinated Terpenes

Analysis		Manufactured in United States						Manufactured abroad					Radiolabeled	
		Hercules		Strobane						Flit &		East	[^{14}C]-	[^{36}Cl]-
		Standard	1949-1975	T-100	Vicksburg	Bison	Sonford	Hercasa	Procida	Fontaine	Melipax	European	Toxaphene	Toxaphene
		GLC peak area, % of total												
Peak No.	T_r, min													
1	33.1	1.0 ± 0.1[a]	1.1 ± 0.1[b]	1.4[c]	2.8[d]	4.7[d]	3.1[c]	1.6[c]	2.1[c]	1.2[c]	2.6[c]	4.3[d]	2.2[c]	1.2[c]
2	48.0	2.2 ± 0.1	2.0 ± 0.3	3.1	3.7	3.1	3.6	2.5	4.4	2.9	3.0	1.9	2.5	0.8
3	48.9	1.4 ± 0.1	1.1 ± 0.4	1.0	0.8	1.6	0.2	1.0	0.0	1.0	2.5	0.5	1.7	2.7
4	54.5	3.1 ± 0.3	2.4 ± 0.8	1.8	2.6	1.2	1.0	2.1	0.6	1.5	3.8	1.1	4.3	5.7
5	55.3	1.9 ± 0.1	2.3 ± 0.6	2.1	3.6	2.6	2.7	2.3	0.5	1.8	0.1	0.3	4.7	2.7
6	60.0	1.2 ± 0.1	1.0 ± 0.1	1.7	0.6	1.2	0.8	1.1	0.4	1.3	0.6	0.6	1.3	1.6
7	61.5	3.2 ± 0.3	4.1 ± 0.4	5.5	5.5	8.5	7.2	5.1	0.0	5.5	4.9	5.8	4.4	0.5
8	62.4	3.9 ± 0.4	4.7 ± 0.2	5.3	7.3	4.8	6.5	5.7	5.3	5.0	2.4	2.4	6.0	1.8
9[e]	63.8	7.8 ± 0.6	7.9 ± 0.7	6.2	8.5	2.8	4.1	7.7	3.6	6.5	4.1	2.9	9.9	6.4
10	66.7	3.0 ± 0.1	2.9 ± 1.0	1.8	2.0	1.9	2.0	2.0	1.9	1.4	1.2	1.0	5.8	5.4
11	67.4	3.1 ± 0.2	1.9 ± 0.4	0.8	0.4	0.3	0.5	1.4	0.0	0.8	5.3	0.7	1.3	5.0
12	72.1	2.6 ± 0.1	2.4 ± 0.7	2.1	0.4	0.4	1.1	1.2	0.8	2.9	2.8	2.7	4.8	3.3
13	74.1	2.0 ± 0.1	2.0 ± 0.2	2.4	1.8	1.4	1.8	1.9	1.1	2.4	1.4	1.4	2.1	1.7
14	79.4	3.3 ± 0.1	3.0 ± 0.3	1.3	1.4	0.4	0.1	0.9	6.4	0.6	0.5	0.2	2.7	3.7
15	81.0	3.3 ± 0.1	3.0 ± 0.6	3.1	2.0	0.8	2.0	2.5	3.5	3.2	0.9	0.9	2.4	3.6
16[e]	82.2	8.4 ± 0.2	7.7 ± 1.0	4.5	3.5	0.9	1.4	5.9	1.9	5.0	1.9	1.4	6.8	7.7
17	83.2	1.3 ± 0.2	0.6 ± 0.1	0.0	0.0	0.0	0.0	<0.1	0.9	0.1	0.0	0.0	0.7	1.1
18	84.0	1.3 ± 0.1	1.0 ± 0.1	0.9	0.5	0.4	0.5	0.6	0.0	0.2	0.8	0.1	0.9	0.9
19	84.3	3.5 ± 0.3	3.8 ± 0.8	4.1	2.6	1.4	2.7	3.1	1.0	4.3	2.8	1.5	2.9	3.9
20	85.8	2.3 ± 0.3	1.9 ± 0.4	1.1	0.8	0.1	0.5	1.5	1.2	1.0	0.3	0.0	1.9	2.0
21	87.2	1.7 ± 0.3	1.9 ± 0.4	2.6	1.5	0.9	1.8	1.9	2.3	2.8	1.0	0.6	1.4	1.7
22	89.9	3.2 ± 0.5	2.8 ± 0.4	2.8	1.4	0.9	1.5	2.4	0.9	3.8	2.4	0.7	2.3	3.2
23	90.7	4.3 ± 0.1	4.4 ± 0.3	4.6	2.7	2.0	2.4	3.9	0.7	5.0	3.0	2.3	3.9	3.4
24	92.1	7.0 ± 0.3	7.6 ± 0.6	7.2	3.5	2.7	3.6	6.4	2.0	7.3	3.5	2.9	5.8	6.8
25	95.5	4.2 ± 0.2	3.4 ± 0.6	2.1	1.3	0.3	0.8	2.7	2.8	2.2	2.2	0.9	2.1	3.9
26	103.4	1.1 ± 0.3	0.9 ± 0.2	0.6	0.1	0.2	0.2	0.6	0.7	0.7	0.8	0.4	0.5	1.5
27[e]	106.0	2.6 ± 0.2	2.2 ± 0.4	1.0	0.4	< 0.1	0.2	1.5	0.3	1.2	1.6	0.4	0.8	3.3
28	111.3	3.1 ± 0.3	2.5 ± 0.5	0.9	0.2	< 0.1	0.1	1.7	0.5	1.0	0.9	0.5	0.7	2.9
29	115.4	1.2 ± 0.1	1.0 ± 0.2	0.5	0.2	< 0.1	0.2	0.8	0.3	0.6	0.6	0.2	0.5	1.3
Other		11.8 ± 0.3	16.5 ± 1.7	27.5	37.9	54.5	47.4	28.0	53.9	26.8	42.1	61.4	12.7	10.3
		GLC elution time, min for indicated % of total peak area												
%														
25		63.0 ±0.2[a]	62.2 ± 1.3[b]	55.4[c]	42.3[d]	36.8[d]	42.8[c]	53.4[c]	36.0[c]	54.5[c]	42.7[c]	29.0[d]	56.4[c]	64.1[c]
50		81.3 ±0.1	78.0 ± 5.1	67.8	61.8	51.4	61.3	63.8	51.6	72.5	62.3	43.7	66.1	80.6
		Elemental analysis, %												
Carbon		28.7[f]	29.2 ± 0.2[g]	29.7[f]	31.6[h]	32.2[h]	31.1[f]	29.9[f]	32.3[f]	33.6[f]	32.7[f]	32.4[h]		
Hydrogen		2.4	2.4 ± 0.0	2.7	3.4	2.9	2.8	2.5	3.2	2.9	3.3	3.0		
Chlorine		68.9	68.3 ± 0.4	67.6	64.9	64.4	64.7	67.8	64.7	63.2	61.0	64.6		
Total		100.0	99.9 ± 0.2	100.0	99.8	99.4	98.6	100.2	100.1	99.7	96.9	99.9		
		LD_{50}, mg/kg												
Mouse		48	47 ± 4[i]	42	33	42	40	43	138	42	125	64		
Housefly		26	24 ± 3[i]	25	19	28	19	24	33	22	48	29		

[a] Mean ± standard deviation of three analyses. [b] Mean ± standard deviation of average values from three analyses on each of seven samples manufactured in the period of 1949-1975. [c] Mean of three analyses for which the standard deviation values relative to the mean are similar to those in footnote *a* above. [d] Mean of average values from three analyses on each of two samples from the same manufacturer. [e] The major component of peaks 9, 16, and 27 cochromatographs with toxaphene components **B**, **A**, and C, respectively. [f] Mean of two analyses. [g] Mean ± standard deviation of average values from two analyses on each of seven samples manufactured in the period of 1949-1975. [h] Mean of average values from two analyses on each of two samples from the same manufacturer. [i] Mean ± standard deviation of analyses on each of seven samples manufactured in the period of 1949-1975.

ranging from 61.0 to 64.9% in chlorine content.

The bioassay results are of little value in differentiating between the various samples. However, the East European samples are slightly less toxic and the Melipax and Procida samples are distinctly less toxic than the others.

DISCUSSION

Open tubular column GLC has been used previously for determination of the purity of toxaphene components (Khalifa et al., 1974) and for qualitative analysis of toxaphene composition (Seiber et al., 1975). In the present investigation, this method is optimized for quantitative analysis of toxaphene and related materials. Particular attention is given to five factors: a suitable column as to length and liquid phase; the lowest possible temperature to minimize thermal decomposition of the components; a suitable temperature program to maintain a constant baseline and reasonable overall time for analysis; an

Table II. TLC Properties of Toxaphene Components and of Marker Dyes

Property	TLC regions a	b	c	d	e
^3R_f range for region	0.00-0.35	0.35-0.39	0.39-0.45	0.45-0.49	0.49-1.00
Dye marker					
Name	9,9,9-Trifluorene	Bifluorenylidene	5-Diphenylmethylenefluorene	Benzylidenefluorene	9-(2-Ethylhexyl)-fluorene
^3R_f	0.18	0.37	0.40	0.47	0.57
Individual toxaphene components					
Designation[a]		B		A	C
^3R_f		0.37		0.47	0.81
No. of unique components by GLC[b]	13	12	14	9	26
Recovery of radioact., %					
[^{14}C]Toxaphene	21.5	19.6	17.3	17.3	24.3
[^{36}Cl]Toxaphene	30.3	15.5	13.2	19.9	21.1

[a] See Figure 2 for chemical structures of identified components. [b] See Figure 2 for components with unique chromatographic properties (TLC and GLC). The numbers refer to components that make up ≥1% of the total peak area in the standard toxaphene sample and the asterisks refer to additional components that constitute ≥1% of the total peak area for the individual TLC regions.

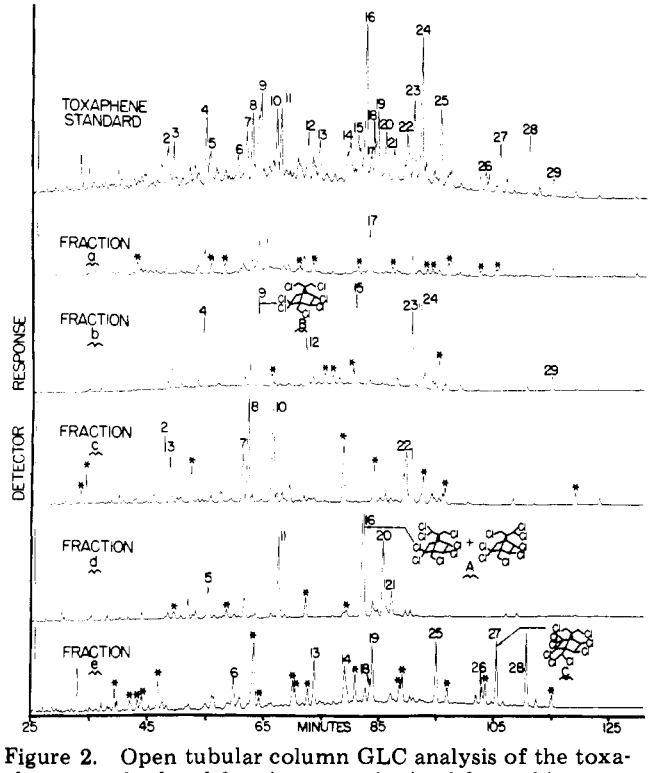

Figure 2. Open tubular column GLC analysis of the toxaphene standard and fractions a-e obtained from this standard by TLC. Table II gives the R_f regions of the TLC fractions. The 29 peaks making up ≥1% of the total peak area in the toxaphene standard are designated by numbers as in Table I. The same numbers designate peaks in the TLC fractions with identical T_r values to those in the toxaphene standard. Additional peaks making up ≥1% of the total peak area in the TLC fractions are designated by asterisks. Each peak is designated only for the TLC fraction in which it appears in maximum amount. The first major peak in each chromatogram (T_r 25.9 min) is aldrin used as an internal standard. Structures are given for toxaphene components A (peak 16), B (peak 9), and C (peak 27) (see also Table I).

electron capture detector linear over the range of amount of individual peaks to be analyzed; an on-line computer to normalize the peak areas and provide precise T_r values. The standard toxaphene reveals the following numbers of peaks exceeding the indicated percentages of the total peak area: 29 peaks at the 1% discriminating level, 51 peaks at 0.3%, 66 peaks at 0.1%, and 104 peaks at 0.03%.

The TLC–GLC method provides a more complete analysis than GLC alone of components in toxaphene and related materials. The two major GLC peaks (9 and 16) in Hercules toxaphene contain components B and A, respectively. Marker dyes were sought for the precise TLC positions of components A and B in a TLC system that provides near optimal separation of toxaphene components. After examining many TLC systems (mostly based on Khalifa et al., 1974) and potential marker dyes, benzylidenefluorene was selected as the marker for component A and bifluorenylidene for component B in the hexane–DMF system. Marker dyes for other TLC regions are also reported since they may be useful in later studies with other toxaphene components. The fact that the marker dyes cochromatograph with components A and B in the hexane–DMF system does not mean that they are necessarily useful on the same basis with other chromatographic conditions. Thus, on three TLC developments with hexane, the corresponding markers fall 0.05–0.15 ^3R_f units below the positions of components A and B, individually or with these components in mixture with the normal toxaphene constituents. Although two-dimensional TLC (hexane × 3 and then hexane–DMF × 3) provides better separation of toxaphene components than one-dimensional development, this two-dimensional procedure negates the use of the marker dyes to exactly locate components A and B.

The present methodology is not adequate for quantitative analysis of each individual component in toxaphene and related materials. Thus, some of the GLC peaks including 9 contain multiple components so quantitative analysis by open tubular column GLC alone overestimates the amount of component B in toxaphene. This problem is not completely overcome by the TLC–GLC method in the case of component B. Also, there is no evidence that the same components are present in each GLC peak over the wide range of samples analyzed although TLC–GLC analyses should be suitable to evaluate this point.

Criteria useful in critically intercomparing toxaphene and related materials are the GLC peaks exceeding 1% of the total peak area, the percent of GLC peaks 9 and 16 since they reflect major components that vary considerably among the samples, and the time required for elution of 25 and 50% of the total GLC peak area. Based on each of these criteria, the samples of Hercules toxaphene are essentially identical with each other even though they were manufactured at intervals over a period of 26 years. Thus, residue and toxicology data obtained with any one of the Hercules samples are probably applicable to any of the

other Hercules samples but not necessarily to certain of the remaining chlorinated terpenes examined.

The toxicity of the toxaphene samples and related materials to mice and houseflies does not clearly correlate with their chlorine content, with the amount of components (including B and A) appearing in GLC peaks 9 or 16, or with the amount of any individual GLC peak. This suggests that the toxicity of such diverse samples may be due to many components which could vary with the manufacturing method or that it is due to relatively minor components not easily differentiated on examining such complex mixtures.

Methodology is now available to distinguish between toxaphene and related materials. These procedures may be useful in evaluating the chemical and environmental degradation of these insecticides and their residues.

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LITERATURE CITED

Anagnostopoulos, M. L., Parlar, H., Korte, F., *Chemosphere* **2**, 65 (1974).
Buntin, G. A., U.S. Patent 2565471 (1951).
Guyer, G. E., Adkisson, P. L., DuBois, K., Menzie, C., Nicholson, H. P., Zweig, G., Dunn, C. L., "Toxaphene Status Report", Environmental Protection Agency, Washington, D.C., Nov 1971.
Holmstead, R. L., Khalifa, S., Casida, J. E., *J. Agric. Food Chem.* **22**, 939 (1974).
Khalifa, S., Mon, T. R., Engel, J. L., Casida, J. E., *J. Agric. Food Chem.* **22**, 653 (1974).
Lehman, A. J., "Summaries of Pesticide Toxicity", Association of Food and Drug Officials, U.S., Topeka, Kan., 1965, p 36.
Matsumura, F., Howard, R. W., Nelson, J. O., *Chemosphere* **5**, 271 (1975).
Palmer, K. J., Wong, R. Y., Lundin, R. E., Khalifa, S., Casida, J. E., *J. Am. Chem. Soc.* **97**, 408 (1975).
Seiber, J. N., Landrum, P. F., Madden, S. C., Nugent, K. D., Winterlin, W. L., *J. Chromatogr.* **114**, 361 (1975).
Stahl, E., Ed., "Thin-Layer Chromatography", 2nd ed, Springer-Verlag, New York, N.Y., 1969, p 86.
Turner, W. V., Khalifa, S., Casida, J. E., *J. Agric. Food Chem.* **23**, 991 (1975).
Zweig, G., Sherma, J., *Anal. Methods Pestic. Plant Growth Regulat.* **6**, 514 (1972).

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A Contribution to the Structure of the Toxaphene Components. Spectroscopic Studies on Chlorinated Bornane Derivatives

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The ir, MS, ^{1}H NMR, and ^{13}C NMR spectroscopic behavior of the synthetically produced low chlorinated bornane derivatives was studied. The obtained data were used for the spectral analysis of compound I, a substance isolated from technical toxaphene. It was further attempted to characterize the composition of technical toxaphene on the basis of spectroscopic data from defined toxaphene fractions.

Technical toxaphene is an insecticidal mixture of C_{10} components with 5–12 chlorine atoms, produced by chlorinating camphene in carbon tetrachloride under uv light. Toxaphene contains up to 70% chlorine and consequently conforms to the empirical formula $C_{10}H_{10}Cl_8$. So far only a few compounds have been isolated from the toxaphene mixture in pure form and studied in regard to their toxicological properties (Anagnostopoulos et al., 1974; Casida et al., 1974; Khalifa et al., 1974; Matsumura et al., 1975). In addition nearly 177 substances have been detected with the aid of GC–MS (chemical ionization) (Holmstead et al., 1974). Detailed reports have also been given on analytical methods for the determination of toxaphene components in environmental samples (Dolan et al., 1974). In the present work, the ir, ^{1}H NMR, ^{13}C NMR, and mass spectroscopic behavior of synthetically produced chlorinated bornane derivatives was investigated and compared with that of compound I, isolated from technical toxaphene (Anagnostopoulos et al., 1974). In addition, attempts were made to characterize the composition of the mixture on the basis of spectroscopic data

Table I. Physical Data of Chlorinated Bornane Derivatives

Compound	R_f	t_R	Mp, °C
2-*exo*,10,10-Trichlorobornane (**4**)	0.70	2.05	141.5
2-*exo*,10-Dichlorobornane (**5**)	0.60	1.23	122.5
2-*exo*,9,10-Trichlorobornane (**7A** or **7B**)	0.41	3.02	120
2-*exo*,3-*endo*,10-Trichlorobornane (**8**)	0.58	3.30	130 dec
2-*exo*,6-*endo*,10-Trichlorobornane (**9**)	0.72	1.64	18
2-*endo*,6-*endo*-Dichlorobornane (**10**)	0.40	1.00	177
2-*endo*,3-*exo*,5-*exo*,6-*endo*-Tetrachlorobornane (**11**)	0.51	3.00	115 dec

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