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Toxaphene Composition and Toxicology

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TOXAPHENE COMPOSITION AND TOXICOLOGY

by

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

As part of its overall mission, the U.S. Environmental Protection Agency is concerned with the effects of pesticides on mammals including man. One area of specific concern is the composition and toxicology of toxaphene. The following report deals with analytical methodology for determining toxaphene composition, preparation and identification of major toxaphene components including those most toxic on an acute basis, and the metabolic fate and mutagenic activity of certain toxaphene components.

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PREFACE

The Federal Insecticide, Fungicide, and Rodenticide Act designates the Environmental Protection Agency as the governmental body responsible for the safety of all pesticides used in the United States. More recently, the Federal Environmental Pesticide Control Act (PL 92-516) strengthened EPA's regulatory responsibilities in the area of pesticides to include intra- as well as inter-state commerce.

To be federally registered, a pesticide must be determined to not be hazardous to health or to the environment when used according to its labeling restrictions. Thus, relative to the new law as well as to specific directives included in Public Law 93-135, 1973, EPA now is conducting a thorough review of the implications of using alternate chemicals for pest control, including older registered pesticides. The University of California at Berkeley is contributing to these goals through studies on toxaphene composition and toxicology. This report summarizes and discusses the findings on toxaphene.

2,5-endo,6-exo,8,9,10-hexachlorobornan-2,3-ene in the triphenyltin hydride and reduced hematin systems and in each of the organisms examined. Reduced hematin and the tin hydride system also convert the heptachlorobornane to 2,5-endo,8,9,10-pentachlorotricyclene. Fat from chickens and mammals treated orally with toxaphene contains products similar in GLC characteristics to toxaphene itself whereas liver and feces contain toxaphene-derived products of greatly altered GLC properties.

Toxaphene preparations and related chlorinated terpenes are mutagens in the histidine-requiring Salmonella typhimurium assay. The most potent mutagenic components, which are not identified, reside in the polar fractions on crystallization or column chromatography.

ABSTRACT

The composition and metabolism of toxaphene are examined to aid in understanding the conditions under which this major insecticide can be most effectively and safely used. The consistency of composition of toxaphene and related chlorinated terpenes are evaluated by open tubular column GLC analyses with an EC detector. Each of 8 toxaphene samples manufactured by Hercules from 1949 to 1975 shows the same 29 major peaks and in almost identical ratios. 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 5 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 IP and housefly topical LD_{50} values.

Five major toxaphene components [2,2,5-endo,6-exo,8,9,10-heptachlorobornane (I) and its 3-exo-chloro-, 8-chloro-, 9-chloro- and 10-chloro-derivatives] collectively account for up to 23% of the GLC-EC properties of chlorinated technical grade camphene (i.e., toxaphene insecticide) and up to 34% of those of chlorinated 2-exo,10-dichlorobornane. Chlorination of 2-exo, 10-dichlorobornane provides a convenient source of I, which on further chlorination gives the indicated octachlorobornanes and the 5-exo-chloro-derivative of I plus two nonachlorobornanes, one with the introduced chlorines at C-8 and C-10 and the other with these chlorines at the 3-exo-position and at C-10. On dehydrochlorination I yields two hexachlorobornenes and the 3-exo-chloro derivative of I gives one heptachlorobornene and one hexachlorobornadiene. The toxicity to mice, houseflies and goldfish of the octachlorobornanes formed by introducing chlorine substituents into I, relative to I itself, generally decreases in the order: 9-chloro > 8-chloro > no added chlorine (i.e. I) > 3-exo-chloro, 5-exo-chloro or 10-chloro.

Heptachlorobornane I undergoes reductive dechlorination at the geminal-dichloro group to yield 2-endo,5-endo,6-exo,8,9,10-hexachlorobornane and 2-exo,5-endo,6-exo,8,9,10-hexachlorobornane in the following systems: photolysis in hexane solution with UV light; triphenyltin hydride in hexane containing 2,2'-azobis(2-methylpropionitrile); reduced hematin in glacial acetic acid-N-methyl-2-pyrollidone; bovine rumen fluid; sewage primary effluent; rat liver microsomes under anaerobic conditions with NADPH as the critical co-factor; houseflies, chickens, guinea pigs, hamsters, rabbits, mice, rats and monkeys in vivo. This heptachlorobornane is also dehydrochlorinated to give

ABBREVIATIONS

CI-MS	chemical ionization-mass spectrometry
DMF	dimethylformamide
DMSO	dimethylsulfoxide
GLC	gas-liquid chromatography
GLC-CI-MS	GLC coupled with CI-MS
IP	intraperitoneal
LD_{50}	lethal dose for 50% of the animals
NMR	nuclear magnetic resonance
PB	piperonyl butoxide
R_f	ratio for distance moved by compound to that moved by developing solvent on TLC. ^3R_f refers to three TLC developments
TLC	thin-layer chromatography
T_r or t_R	GLC retention time
UV	ultraviolet light

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INTRODUCTION

Toxaphene is produced by chlorination of camphene to yield a chlorine content of 67-69% and an overall composition approximating $C_{10}H_{10}Cl_8$ (Buntin, 1951). In contrast to most pesticides, toxaphene is not a single major chemical entity but rather is a complex mixture of related chlorinated terpenes. About 60-70 million pounds of this insecticide are used each year, in large part combined with methyl parathion for cotton pest insect control. The cumulative use of toxaphene since it was introduced by Hercules Inc. in the late 1940's exceeds one billion pounds. Several other companies in the United States and abroad have produced and marketed similar insecticides prepared by chlorination of camphene and related terpenes. Knowledge of the metabolic and environmental fate of toxaphene has developed rapidly in recent years with associated advances in identification of components and improvements in analytical procedures.

The purpose of the present investigations is to define the composition, structure-activity relations, and the metabolic and environmental fate of toxaphene. The studies are described in four sections as indicated below.

Section I focuses on the composition of toxaphene and related chlorinated 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 since the 1940's and of related commercial materials. Toxaphene is a complex mixture of at least 177 components revealed by a combination of liquid adsorption column chromatography followed by GLC-CI-MS 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 section 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.

Section II deals with the relation of structure to biological activity of toxaphene components. Two of its most toxic components are

2,2,5-endo,6-exo,8,9,10-heptachlorobornane (compound I) and a mixture of the 8-chloro and 9-chloro derivatives of I (referred to here as 8-Cl-I and 9-Cl-I, respectively) (Casida et al., 1974; Khalifa et al., 1974; Matsumura et al., 1975; Palmer et al., 1975; Seiber et al., 1975; Turner et al., 1975). A probable precursor for these toxicants is 2-exo,10-dichlorobornane, a major product at an early stage in chlorination of camphene (Jennings and Herschbach, 1965; Richey et al., 1965). This dichlorobornane has been used to prepare trichlorobornanes as models for identification of toxaphene components (Parlar et al., 1977) and to obtain a product from which the mixture of 8-Cl-I and 9-Cl-I can be isolated by TLC and preparative GLC (Nelson and Matsumura, 1975). Section II gives a procedure for chlorination of 2-exo,10-dichlorobornane to prepare I on a gram scale and to obtain 3-exo-Cl-I. Additional octa- and nonachlorobornanes, hexa- and heptachlorobornenes, and a hexachlorobornadiene are obtained on chlorination or dehydrochlorination of I or dehydrochlorination of 3-exo-Cl-I. These products are identified by NMR and MS and used in comparative toxicity studies to determine the relationship of chemical structure and biological activity.

Section III examines the metabolism and environmental degradation of toxaphene and its components. Toxaphene undergoes rapid dechlorination in rats (Casida et al., 1974; Crowder and Dindal, 1974; Ohsawa et al., 1975) and is metabolized in houseflies (Hoffman and Lindquist, 1952) and in a cotton leafworm enzyme preparation (Abd El-Aziz et al., 1965, 1966). No toxaphene metabolite other than chloride ion was identified in these studies, in large part because of difficulties in examining such a complex mixture of polychlorobornanes and other materials (Holmstead et al., 1974). One toxaphene component, heptachlorobornane I, constitutes up to 8% of the technical grade insecticide (Palmer et al., 1975; see Section I) and four octachlorobornanes, each derivable by addition of one chlorine atom to I, make up an additional ~ 15% of toxaphene (Matsumura et al., 1975; Turner et al., 1975; see Sections I and II). Heptachlorobornane I has relatively high biological activity and is one of the most easily isolated components of toxaphene. It is therefore a suitable model compound for use in studies to gain an understanding of reactions involved in detoxication of several polychlorobornane components of toxaphene. An aqueous reduced hematin system degrades this heptachlorobornane to unidentified products by reductive dechlorination and dehydrochlorination, and it also dechlorinates many other toxaphene components (Khalifa et al., 1976). Section III considers the degradation and metabolic chemistry of heptachlorobornane I in several systems, selected to emphasize reductive dechlorination reactions, and the nature of toxaphene-derived products in a variety of organisms.

Section IV is a preliminary report on the mutagenic activity of toxaphene and some of its components. Toxaphene is a purported carcinogen in rats and mice (National Cancer Institute, 1977) and a related polychloropinene preparation is reported to give chromosomal abberations in humans (Samosh, 1974). The mutagenic activity of toxaphene and related insecticides and some of their components was therefore examined to expand the available knowledge on potential side effects from use of this insecticide.

SECTION I

COMPOSITION OF TOXAPHENE AND RELATED CHLORINATED TERPENES

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; two samples from Vicksburg Chemical Co. (Vicksburg, Miss.); two samples from Bison Chemical Co. and one from Sonford Chemical Co. (each at Fort Natchez, Texas); 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 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 of 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: I - 2,2,5-endo,6-exo,8,9,10-heptachlorobornane (Palmer et al., 1975); 8-Cl-I plus 9-Cl-I - 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); 2-endo,3,3,5-exo,6-exo,8,9,10,10-nonachlorobornane (for structure see Figure 2 given later) (Anagnostopoulos et al., 1974).

Chromatography

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. x 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 hexane. An on-line computer provided the t_R of each peak and its normalized area as 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 I and aldrin) to 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 x 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 DMF. Gel regions from the toxaphene chromatograms corresponding in ^3R_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 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 IP with the test sample dissolved in DMSO using 100 μl of DMSO 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-hr 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 1). The contribution of each of these peaks to the total peak area is highly reproducible on repeated analyses (Table 1).

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 I (TLC region b, ^3R_f 0.35-0.39, bifluorenylidene marker), of components 8-Cl-I plus 9-Cl-I (TLC region d, ^3R_f 0.45-0.49, benzylidenefluorene marker), and of regions above, below, and between those of components I and 8-Cl-I plus 9-Cl-I. GLC analysis of the TLC fractions

TABLE 1. 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						Flic &			[^{14}C]-	[^{36}Cl]-
		Standard	1949-1975	T-100	Vicksburg	Bison	Sonford	Hercasa	Procida	Fontaine	Melipax	East 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

(Continued)

TABLE 1. (Continued)

Analysis	Manufactured in United States: Hercules Standard	Hercules 1949-1975	Strobane T-100	Vicksburg	Bison	Sanford	Manufactured abroad: Hercasa	Procida	Flit & Fontaine	Melipax	East European	Radiolabeled: [^{14}C]-Toxaphene	[^{36}Cl]-Toxaphene
Elemental analysis, %													
Carbon	28.7[f]	29.2 ± 0.28	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 I, 8-Cl-I plus 9-Cl-I, and the nonachlorobornane structurally defined in Figure 2.

[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.

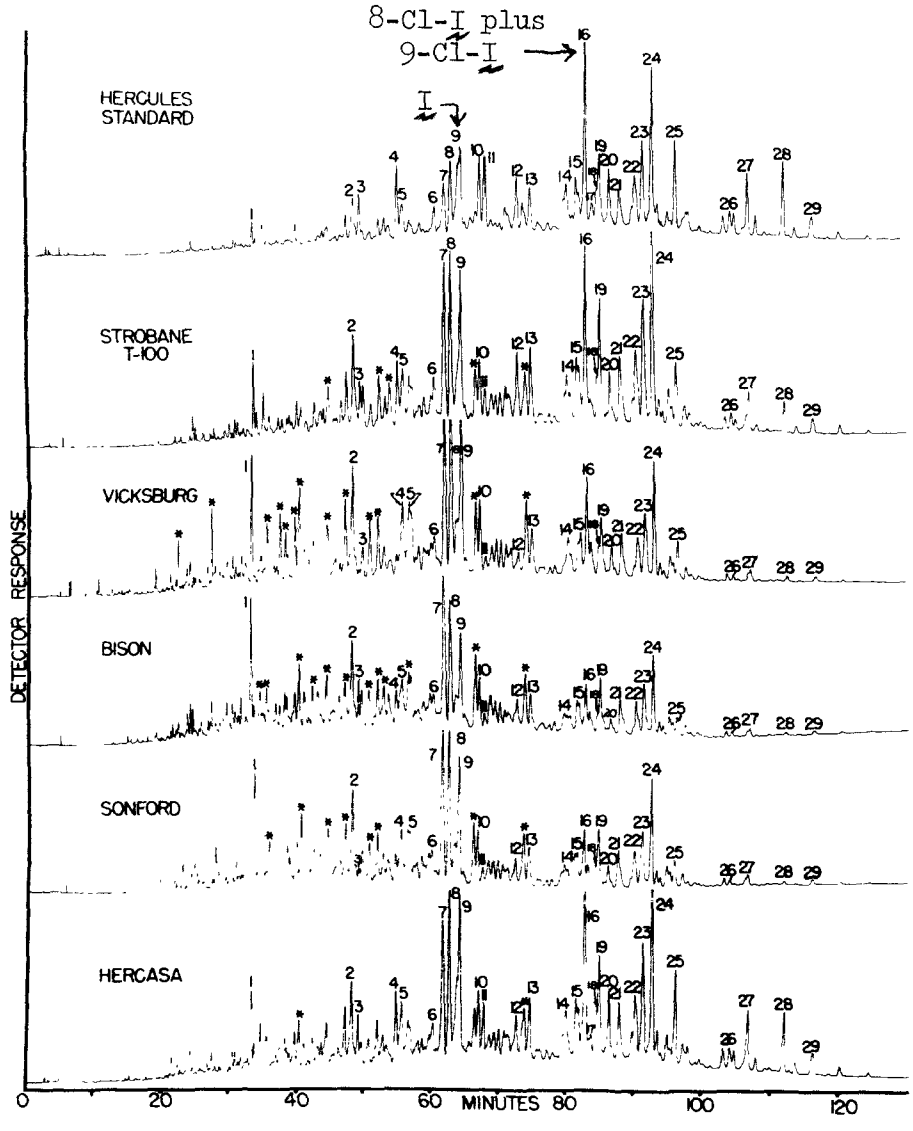

Figure 1. Open tubular column GLC analysis of the toxaphene standard and related chlorinated terpenes. The 29 peaks making up ≧ 1% of the total peak area in the toxaphene standard are designated by numbers as in Table 1. The same numbers designate peaks in the other samples with identical T_r values to those in the toxaphene standard. Additional peaks making up ≧ 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.

(Continued)

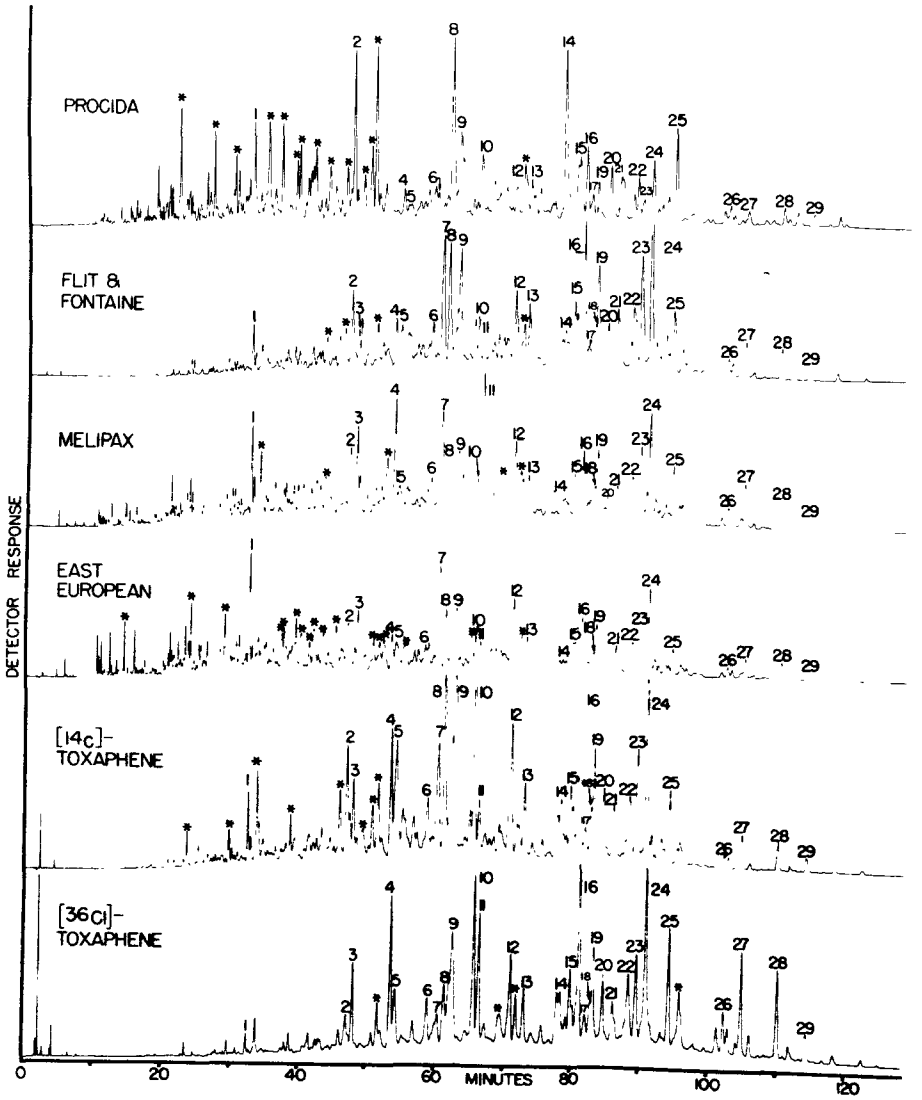

Figure 1. (Continued)

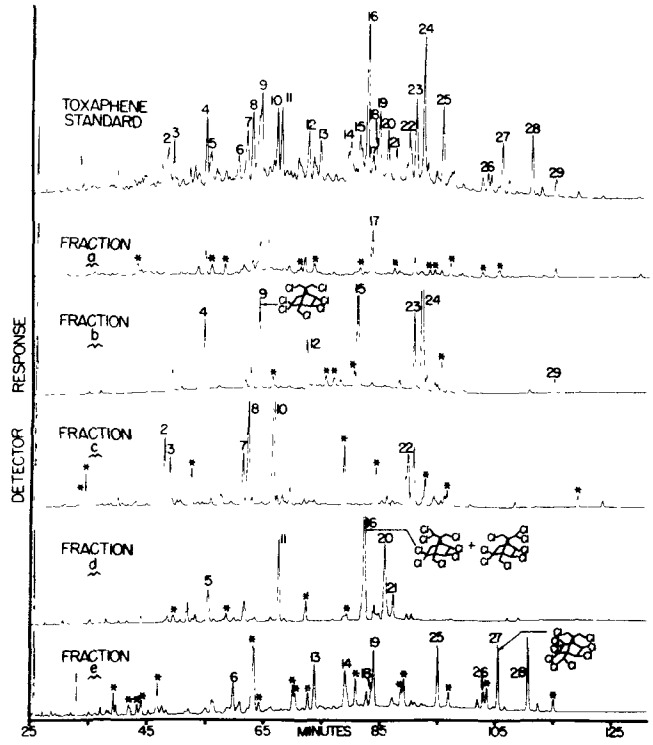

Figure 2. Open tubular column GLC analysis of the toxaphene standard and fractions a-e obtained from this standard by TLC. The 29 peaks making up ≧ 1% of the total peak area in the toxaphene standard are designated by numbers as in Table 1. The same numbers designate peaks in the TLC fractions with identical 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 8-Cl-I plus 9-Cl-I (peak 16), I (peak 9), and a nonachlorobornane (peak 27) (see also Table 1).

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 fairly similar radioactivity distribution for the five TLC regions 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 1).

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 1). Several GLC peaks appear in $\geq$ 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 1).

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 1). The other samples are less heavily chlorinated, 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 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 I and 8-Cl-I plus 9-Cl-I, respectively. Marker dyes were sought for the precise TLC positions of these components 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 components 8-Cl-I plus 9-Cl-I and bifluorenylidene for component I in the hexane-DMF system. The fact that the marker dyes cochromatograph with components 8-Cl-I plus 9-Cl-I and I 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 these components, individually or with these components in mixture with the normal toxaphene constituents. Although two-dimensional TLC (hexane x 3 and then hexane-DMF x 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 8-Cl-I plus 9-Cl-I and I.

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 I in toxaphene. This problem is not completely overcome by the TLC-GLC method in the case of component I. 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 I and 8-Cl-I plus 9-Cl-I) 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.

SECTION II

RELATION OF STRUCTURE TO BIOLOGICAL ACTIVITY OF TOXAPHENE COMPONENTS

MATERIALS AND METHODS

Chromatography. The composition of reaction mixtures and the purity of individual products were determined by open tubular column GLC (see Section I), assuming the same response for each component with the electron-capture detector. TLC utilized 20 x 20 cm silica gel 60 F-254 chromatoplates of 0.25 mm layer thickness (EM Laboratories) for analysis and silica gel F-254 chromatoplates of 0.5 mm layer thickness (EM Laboratories) for product isolation on a 5-10 mg scale. The products on the chromatoplates were detected by spraying with diphenylamine (10% w/v) in acetone and irradiating with UV light. Column chromatography utilized silicic acid (AR-100 mesh, Mallinckrodt Inc., St. Louis, Mo.; column length ~ 10 times the diameter) packed in technical grade hexane or hexane saturated with DMF and developed with the same solvent under 7-9 lb of N_2 pressure to increase the elution rate. In most cases, bifluorenylidene (~10 mg) was added as a marker dye (see Section I), since it elutes in almost the same position as I.

Spectroscopy and Elemental Analyses. 360 MHz NMR spectra were obtained on the Bruker HXS-360 spectrometer at the Stanford Magnetic Resonance Laboratory. 90 MHz spectra were run on the Perkin-Elmer R-32 spectrometer. CI-MS and GLC-CI-MS determinations utilized the Finnigan Model 1015D mass spectrometer as previously described (Holmstead et al., 1974) but with isobutane as the ionizing gas. Samples for elemental analyses were prepared by evaporating hexane solutions and drying at pump vacuum for 14 hr.

Bioassays. Procedures for determining the 24-hr LD_{50} values with male albino mice, adult female houseflies and goldfish were as previously reported (see Section I; Turner et al., 1975) except that the volume of water for the goldfish was 3 liters rather than 4 liters. In synergism studies, PB was applied topically to the houseflies at 250 µg/g 30 min prior to the chlorinated hydrocarbon or administered ip to the mice at 150 mg/kg one hr prior to the test compound. The carrier solvent for PB was 1 µl acetone for the houseflies and 50 µl DMSO for the mice.

EXPERIMENTAL PROCEDURES

Synthesis routes used to obtain the various chlorinated hydrocarbons are illustrated in Figure 3 which also gives the compound designations.

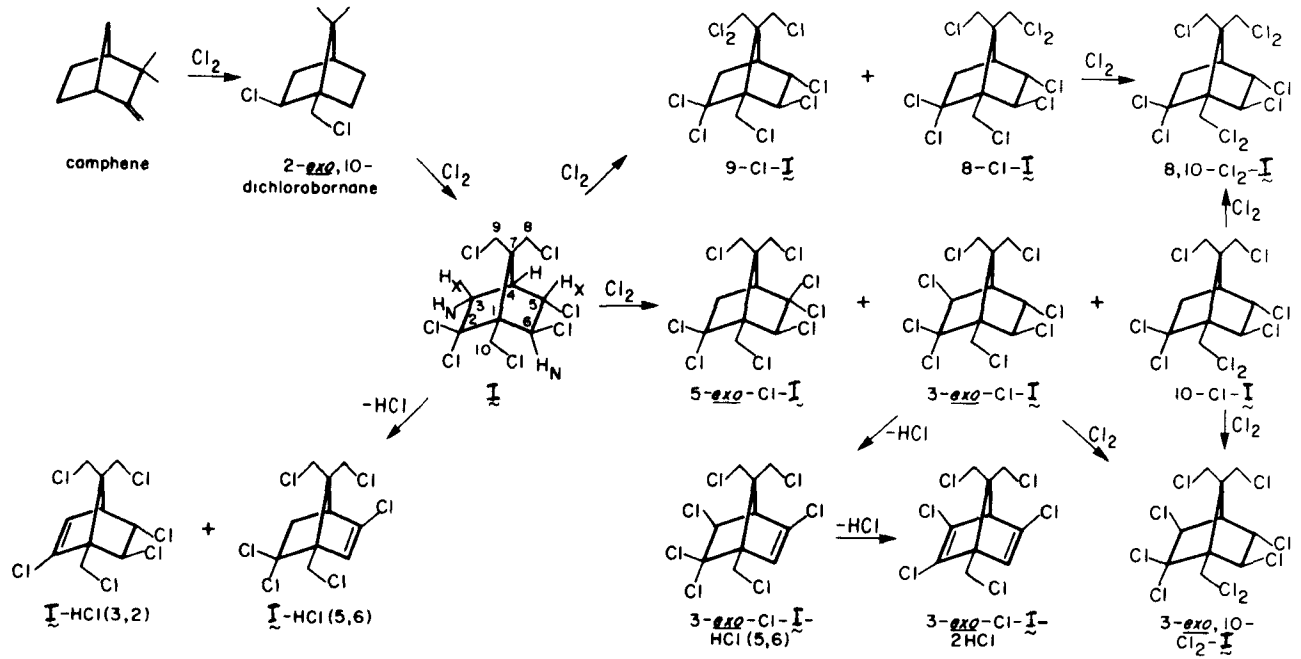

Figure 3. Conversion of camphene and 2-exo,10-dichlorobornane to 2,2,5-endo,6-exo,8,9,10-heptachlorobornane (I) and several octa- and nonachlorobornanes, hexa- and heptachlorobornenes, and a hexachlorobornadiene.

I and 3-exo-Cl-I from Chlorination of 2-exo,10-Dichlorobornane. 2-exo, 10-Dichlorobornane (52 g) (Richey et al., 1965) containing up to 25% 2-exo, 10,10-trichlorobornane (Parlar et al., 1976a, 1977) was dissolved in CCl_4 (1 liter) which was then heated to boiling to drive out O_2 and cooled under N_2. Chlorine was bubbled into the solution at ~ 0°C, the amount added being determined by weight gain. Stirring the solution under a sunlamp resulted in rapid HCl evolution (probably with some loss of Cl_2) and disappearance of the yellow color within a few min. GLC monitoring revealed that addition of 105.5 g Cl_2 gave the optimal yield of I (12%) (Table 2). Half of the product mixture was combined with the marker dye and chromatographed on a silicic acid column (1 kg) with hexane. Details on this column chromatographic procedure are given in Section III. Elution of the yellow dye and I began after 10.6 liters of hexane had been eluted and was essentially complete after an additional 3.8 liters of hexane. The crystals obtained on concentration of the fractions weighed 1.2 g (2.5% yield of I from 2-exo, 10-dichlorobornane) after washing with hexane and 0.7 g after recrystallization from hot acetone (> 98% pure I; mp 222°C).

In a second preparation, pure 2-exo,10-dichlorobornane (20 g) was chlorinated as above, using 52 g Cl_2, yielding 6% I, 4% 3-exo-Cl-I and 19% 8-Cl-I plus 9-Cl-I (Table 2). Fractions from chromatography as above containing 65-71% I were processed in the usual manner to obtain 220 mg I. Those containing 22-25% 3-exo-Cl-I were concentrated, and on standing yielded crystals which were washed with hexane and recrystallized from hot acetone to give 3-exo-Cl-I (220 mg, > 98% pure, mp > 240°C). Fractions containing 44-55% of 8-Cl-I plus 9-Cl-I were combined (7.2 g) and rechromatographed on a silicic acid column (265 g) with DMF-saturated hexane as eluent to yield 8-Cl-I plus 9-Cl-I in ~ 70% purity.

Octa- and Nonachlorobornanes from Chlorination of I. A solution of I (1.63 g; 99% pure) in CCl_4 (100 ml) was heated to boiling, cooled and stirred under N_2. To this solution was added Cl_2 (0.2 g) in CCl_4 previously boiled and stored under N_2. On stirring the solution under a sunlamp, the Cl_2 color disappeared within 3 min. Conversion of I was 30% to the products shown in Table 2. Most of the unreacted I and a small portion of 3-exo-Cl-I were removed as crystals on recrystallization of the chlorination product mixture from hexane and then acetone. Chromatography of the supernatant from recrystallization on a silicic acid column (265 g) with hexane separated the octachlorobornanes and two of the nonachlorobornanes as shown in Figure 4. The fractions richest in each component were combined. The 5-exo-Cl-I obtained (25 mg; 70% purity containing 9% I, 2% 8-Cl-I plus 9-Cl-I and 14% 10-Cl-I) was not further purifiable by TLC or recrystallization. Early fractions of the mixture of 8-Cl-I and 9-Cl-I were found to be > 90% 8-Cl-I (NMR). Recrystallization of one fraction gave relatively pure 8-Cl-I (23 mg; < 2% 9-Cl-I; 5% 3-exo-Cl-I). The last fractions of the 8-Cl-I and 9-Cl-I mixture gave an 8-Cl-I:9-Cl-I ratio of 1:1.5 but they were already rich in 10-Cl-I. The next eluting portion was used to obtain 10-Cl-I after recrystallization from hot hexane (32 mg; 98% purity). The small amount of 3-exo,10-Cl_2-I obtained (12 mg) was > 86% pure (containing no I, 8-Cl-I or 9-Cl-I) and was not further purified. 8,10-Cl_2-I from this preparation (22 mg) was combined with that from another preparation and subjected to recrystallization from hot hexane, further

TABLE 2. PRODUCTS FROM CHLORINATION OF CAMPHENE, 2-exo,10-DICHLOROBORNANE AND HEPTACHLOROBORNANE I.

Compound or mixture analyzed and elemental composition of mixture, %	Hepta- and octachlorobornanes					
	I	3-*exo*-Cl-I	5-*exo*-Cl-I	8-Cl-I	9-Cl-I	10-Cl-I
	GLC t_R, min (peak no. designation)[a]					
Individual polychlorobornanes	63.8 (9)	79.4 (14)	95.8	82.2 (16)	82.2 (16)	90.7 (23)
	Composition, %[b]					
Chlorination of tech. grade camphene[c] C, 28.7; H, 2.4; Cl, 68.9 ($C_{10}H_{10.0}Cl_{8.1}$)	8	3	<1	5	3	4
Chlorination of 2-*exo*,10-dichlorobornane C, 30.90; H, 2.62; Cl, 66.68 ($C_{10}H_{10.1}Cl_{7.3}$)	12	2	<1	5		3
C, 27.43; H, 2.15; Cl, 70.27 ($C_{10}H_{9.3}Cl_{8.7}$)	6	4	<1	12	7	5
Chlorination of I C, 29.03; H, 2.67; Cl, 67.92 ($C_{10}H_{11.0}Cl_{7.9}$)	70	4	2	11	5	6
No elemental analysis	22[d]	12	1	27	13	13

[a] Procedure described in Section I. The identity of 3-exo-Cl-I with 14 and of 10-Cl-I with 23 is based on GLC cochromatography and the TLC-GLC method of Section I. Additional t_R values (min) for four nonachlorobornanes are 104.0 for 3-exo,10-Cl_2-I, 107.6 for 8,10-Cl_2-I and 102.7 and 104.4 for two unidentified compounds.

[b] Based on GLC analysis. The yields of I and possibly some other components include other materials not adequately separated by GLC, particularly in the chlorination products of tech. grade camphene and 2-exo,10-dichlorobornane. The 8-Cl-I plus 9-Cl-I content is based on GLC and their ratio on NMR (Turner et al., 1975 for toxaphene; this study for the chlorination products of 2-exo,10-dichlorobornane and I).

[c] Data for standard toxaphene from Section I.

[d] Additional components are 3-exo,10-Cl_2-I (2%), 8,10-Cl_2-I (5%) and two unidentified nonachlorobornanes (2 and 3%). These nonachlorobornanes also appear in small amounts on chlorination of I to 30% conversion but they are not detected (< 1%) in toxaphene or in the chlorination products of 2-exo,10-dichlorobornane.

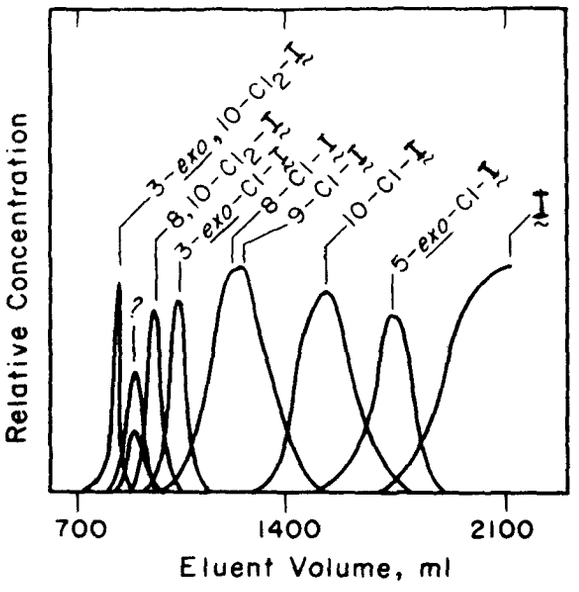

Figure 4. Chromatography of I and its chlorination products on a silicic acid column (265 g) developed with hexane. The elution position of two unidentified nonachlorobornanes is designated by ?.

purification by preparative TLC with hexane and another crystallization (> 75% purity containing no I, 8-Cl-I or 9-Cl-I).

Hexa- and Heptachlorobornenes and a Hexachlorobornadiene from Dehydrochlorination of I and 3-exo-Cl-I. A solution containing I (143 mg; > 90% purity) and KOH (~ 2 g) in ethanol (70 ml) was held at 25°C for 28 hr, yielding I (10%) and two dehydrochlorination products [90%; I-HCl(5,6) and I-HCl(3,2) in a 2.4:1 ratio]. The GLC t_R values (min) at 200°C isothermal are 20.2 for I, 11.6 for I-HCl(3,2) and 10.5 for I-HCl(5,6). Their TLC Rf values with hexane for development are 0.08 for I, 0.12 for I-HCl(3,2) and 0.19 for I-HCl(5,6). Water was added to the residue after ethanol evaporation, and the hexane-soluble products were purified by TLC as above or by chromatography on a silicic acid column (36 g) with hexane. The column separation yielded I-HCl(5,6) (39 mg) eluting in fractions from 370-480 ml total volume and I-HCl(3,2) (31 mg) in the 620-780 ml region. Recrystallization from hot hexane gave I-HCl(3,2) in 95% purity, while recrystallization from a small volume of hexane gave I-HCl(5,6) in > 98% purity. On dehydrochlorination of I by dissolving it in n-propylamine and holding for several days, the product mixture consisted of 10% I and 90% I-HCl(5,6) plus I-HCl(3,2) in a 6:1 ratio. Both of the dehydrochlorination products, when exposed to air and light, became discolored by a brown, hexane-insoluble material.

3-exo-Cl-I (51 mg) in ethanol (40 ml) containing KOH (250 mg) reacts rapidly (> 99% in 1 hr) to give 3-exo-Cl-I-HCl(5,6) (> 97%) and 3-exo-Cl-I-2HCl (1%). The GLC t_R values at 200°C isothermal are 25.1, 12.7, and 7.3 min, respectively, for 3-exo-Cl-I and its mono- and didehydrochlorination products. After preparative TLC with hexane (Rf 0.17 for 3-exo-Cl-I and 0.26 for both dehydrochlorination products), 3-exo-Cl-I-HCl(5,6) was recrystallized (> 99% purity) from a 2:1 mixture of tetramethylsilane and hexane, the tetramethylsilane serving to reduce the solubility in hexane. On treatment with more concentrated KOH, 3-exo-Cl-I-HCl(5,6) eliminates another HCl to give 3-exo-Cl-I-2HCl. This hexachlorobornadiene was also obtained directly from 3-exo-Cl-I (10 mg) on treatment with KOH (~ 100 mg) in ethanol (3 ml) for one hr (> 99% conversion). Several such preparations were combined and subjected to preparative TLC to obtain 3-exo-Cl-I-2HCl [17 mg; oil; 95% with 5% 3-exo-Cl-I-HCl(5,6)]. A brown impurity, removable by preparative TLC, was formed on exposure of its CCl_4 solutions to light and air.

RESULTS AND DISCUSSION

Preparation of Polychlorobornanes and Polychlorobornenes. On photochlorination, camphene and 2-exo,10-dichlorobornane yield relatively large amounts of I and its 3-exo-Cl,8-Cl, 9-Cl, and 10-Cl derivatives (Table 2). This is the first report of 3-exo-Cl-I and 10-Cl-I as toxaphene components. Since all these octachlorobornanes, along with 5-exo-Cl-I, are also formed on photochlorination of I (Table 2), it appears likely that I is their major precursor in both toxaphene and chlorinated 2-exo,10-dichlorobornane.

There is remarkable selectivity in chlorination of 2-exo,10-dichlorobornane to I, since this product is one of 943 isomers derivable by addition of five chlorine atoms to the dichlorobornane. Considerable site selectivity

also exists in photochlorination of I, i.e., C-8 > C-9 = C-10 ≧ C-3 > C-5. The two endo hydrogens (H-3N and H-6N) and the bridgehead hydrogen (H-4) appear not to be substituted at all. The greater resistance of position 5-exo to substitution relative to position 3-exo may be due to the deactivating effect of the 5-endo chlorine or to steric protection of this site by chlorines at positions 5-endo, 6-exo, and 8, as observed in comparison of a space-filling model with the crystal structure of I (Palmer et al., 1975). The 3-endo position of I is considerably protected by the two endo chlorine atoms, and the 6-endo position is almost completely occluded by these chlorine atoms and the one on C-10. Studies on norbornane also indicate that the bridgehead hydrogen is very unreactive, presumably because the dihedral angle at the bridgehead does not lend itself to stabilization of a radical, and that the endo positions are substituted less readily than the exo positions, possibly because of steric effects on chlorine approaching the radical (Kooyman and Vegter, 1958; Poutsma, 1969; Walling and Mayahi, 1959).

Chlorination of 2-exo,10-dichlorobornane with GLC monitoring of the product composition provides a convenient source of I on a gram scale. At an optimal I content of 12%, about one-fifth of this amount can be isolated pure by a single column chromatography, followed by recrystallization. The easiest products to isolate are I and 3-exo-Cl-I because of their low solubility in hexane, acetone, and some other solvents relative to most toxaphene components. On chlorination of the dichlorobornane to a higher chlorine content, the amount of I is reduced to 6% and that of 3-exo-Cl-I, 8-Cl-I, and 9-Cl-I is multiplied by a factor of 2 to 4 (Table 2). Chromatography of this product yields a mixture containing 70% 8-Cl-I and 9-Cl-I, but recrystallization does not provide further purification. Thus, chlorination of 2-exo,10-dichlorobornane provides convenient access to I and 3-exo-Cl-I but not to the other individual octachlorobornanes. Fortunately, direct chlorination of I yields 8-Cl-I and 9-Cl-I in a purity such that 8-Cl-I can be obtained by chromatography and recrystallization. In addition to the five identified octachlorobornanes and two identified nonachlorobornanes which are easily separated by column chromatography, two unidentified nonachlorobornanes are also formed, but they are not separated by this chromatographic technique (Table 2, Figure 4).

Dehydrochlorination of I and 3-exo-Cl-I with KOH or propylamine yields polychlorobornenes. An alternative dehydrochlorination of either compound via the equivalent of a Wagner-Meerwein rearrangement might give cis and trans isomers of two 8-chlorocamphene derivatives. An analogy for this rearrangement is the dehydrochlorination of 2-exo,10-dichlorobornane with dimethylaniline (Jennings and Herschbach, 1965). However, the NMR spectra of the dehydrochlorination products of I and 3-exo-Cl-I are not appropriate for the 8-chlorocamphene derivatives which would result from such a rearrangement. The preponderance of HCl elimination from the 5,6 positions of I over the 3,2 positions is expected from the greater acidity of the 5-exo-hydrogen over the 3-exo-hydrogen, due to the electron-withdrawing properties of the 5-endo-chlorine. The 5-exo-hydrogen is more easily removed than the 6-endo-hydrogen. This is predictable from the almost exclusive exo-cis dehydrochlorinations observed in trans-2,3-dihalonorbornanes (LeBel et al., 1964). Lacking an exo hydrogen in position 2 or 3,

3-exo-Cl-I can form a 2,3-olefin only by some mechanism other than exo-cis dehydrochlorination, and less than 1% of such a product is obtained in the initial dehydrochlorination. With more concentrated base, however, the monodehydrochlorination product from 3-exo-Cl-I undergoes further loss of HCl to form a hexachlorobornadiene.

Chromatographic Properties. Consistent chromatographic patterns related to the number of chlorine atoms are evident for I and its polychlorobornane derivatives on a silicic acid column (Figure 4) and on open tubular column GLC (Table 2). With the silicic acid column, the sequence of elution is four nonachlorobornanes first, then five octachlorobornanes, and finally the heptachlorobornane (I). The GLC t_R values of these bornane derivatives decrease in the same sequence. In addition, the GLC t_R values decrease further in the sequence of two heptachlorobornenes, two hexachlorobornenes, and one hexachlorobornadiene. These patterns are probably restricted to compounds within a closely related series, since many exceptions are evident in the variety of components in toxaphene (Casida et al., 1975; Holmstead et al., 1974).

Identification of New Compounds. Mass spectrometry confirmed that the major products of chlorination of I are $C_{10}H_{10}Cl_8$ and $C_{10}H_9Cl_9$ compounds and that base treatment of I and 3-exo-Cl-I leads initially to $C_{10}H_{10}Cl_6$ and $C_{10}H_9Cl_7$ derivatives, respectively. In CI-MS with methane as the ionizing gas, toxaphene components generally give no M + 1 ions, but instead have their highest masses at M-Cl (Holmstead et al., 1974). In the present study using isobutane as the ionizing gas, the identified products from chlorination of I conform to this rule, as do I-HCl(5,6) and 3-exo-Cl-I-HCl(5,6). However, I-HCl(3,2) gives a weak molecular ion (m/e 340) and a weak M-1 ion (m/e 339) as well as the base peak at m/e 305 (M-Cl). The fragmentation patterns of I-HCl(5,6) and I-HCl(3,2) are almost identical in other respects, with major fragment ions at m/e 305, 304, 269, 244, 209, 195, 173, 159, and 125. 3-exo-Cl-I-2HCl has a molecular ion at m/e 338 and an M+1 ion at m/e 339, as well as prominent fragment ions at m/e 303, 302, 267, 266, 231, and 193.

NMR spectroscopy, employed previously to assign the structures of 8-Cl-I and 9-Cl-I (Matsumura et al., 1975; Turner et al., 1975), also proved useful in structural assignments for the new compounds in the present study (Table 3). The structure of each compound, except the dehydrochlorination products of 3-exo-Cl-I, is readily evident from the number and coupling patterns of protons at low and high fields. However, some difficulty is encountered in resolving and assigning all the resonances, particularly the chloromethyl and chloromethylene protons. Determining the spectra in several different solvents and in some cases at 360 MHz allowed the chemical shifts of protons incompletely resolved in CCl_4 solution to be estimated.

These structural assignments proceeded from the assumption that the bornane skeleton of 2-exo,10-dichlorobornane and I does not undergo any rearrangement on chlorination. While rearrangement during photochlorination is reported for bicyclic compounds with considerably strained three- and four-membered rings, it is not expected in less strained systems (Poutsma, 1969) such as bornane derivatives. The resemblance of observed

TABLE 3. NMR SPECTRA OF HEPTA-, OCTA- AND NONACHLOROBORNANES, HEXA- AND HEPTACHLOROBORNENES, AND A HEXACHLOROBORNADIENE

Compound	Protons										
	3N	3(X)	4	5X	6(N)	8a	8b	9a	9b	10a	10b
	Chemical Shifts[a]										
3-exo-Cl-I	5.20		2.73	4.67	5.24	4.10[b]	4.75[c]	4.16[b]	4.54[c]	3.76[b]	4.67[b]
5-exo-Cl-I	3.58	3.22	3.15		5.78	4.06[d]	4.26[d]	4.26[d]	4.31[d]	4.43[d]	4.51[d]
10-Cl-I	3.36	3.05	2.65	4.66	5.32	4.15[e]	4.28[e]	4.44[e]	4.66[e]	6.81	
3-exo,10-Cl$_2$-I	5.22		2.77	4.73	5.24	3.99[c]	4.73[c,e]	4.45[e]	4.57[e]	6.88	
8,10-Cl$_2$-I	3.54	~3.10	~3.15	5.04	5.52	6.94		4.54	5.15	6.70	
I-HCl(3,4)		6.32	3.28	4.59	4.00	3.97[c,e]	4.30[e]	3.79[e]	4.29[c,e]	4.08	4.31
I-HCl(5,6)	2.62	3.00	2.03		6.05	4.14[c,e]	4.24[e]	4.03[e]	4.16[c,e]	3.98	4.51
3-exo-Cl-I-HCl(5,6)	4.57		3.14		6.15	4.02[c,e]	4.35[e]	4.72[c,e]	4.10[e]	3.96	4.58
3-exo-Cl-I-2HCl[f]			3.08		5.79	3.57[e]	3.76[c,e]	3.65[c,e]	3.83[e]	3.46	3.46
	Coupling Constants[g]										
3-exo-Cl-I	s		4.6	4.6, 4.6	4.6	12.5	13, 2.5	12	12, 2.5	13	12
5-exo-Cl-I	16.6	16.6, 4.6	4.6		s	~12	~12	~12	~12	~12	~12
10-Cl-I	16.6	16.6, 5,2	5,6	5, 5, 2	5[h]	i	~12[h]	13	*j*	*s*[h]	
3-exo,10-Cl$_2$-I	s		5	5, 5	5	~13[h]	~13, 2[h]	13	~13[h]	*s*[h]	
8,10-Cl$_2$-I	17	17, 4.5, 2	4.5, 4.5	4.5, 4.5, 2	4.5	2		14	14, 2	s	
I-HCl(3,4)		d	4,4	4,4	3.5	12,2	12.5	12.5	12.5, 1.5	12.5	11.8
I-HCl(5,6)	15	15, 3	3[h]		*s*[h]	11.8, 2.5	11.5	12.5	12.5, 2.5	12	12
3-exo-Cl-I-HCl(5,6)	s		1.2[h]		1.2	11.5, 2.5	12[h]	13, 2.5	12[h]	12.5	12
3-exo-Cl-I-2HCl			1.7		1.7	*k*	*c,k*	*c,k*	*k*	*l*	*l*

[a] Ppm downfield from tetramethylsilane in CCl_4, except for 3-exo-Cl-I-2HCl which was run in C_6D_6.

[b] These assignments are arbitrary.

[c] These appear as distinct doublets of doublets (geminal and four-bond coupling) and are therefore assigned to C-8 or C-9.

[d] These are not assigned because impurities interfered with measurement of relative line intensities. The downfield pair, however, is coupled to each other.

[e] It could not be ascertained which set of protons is on C-8 and which on C-9.

[f] The data tabulated are in C_6D_6. In CCl_4, H-4 appears at δ3.33 and H-6 at δ6.53. The other resonances, except for a doublet at δ4.28 (J=13 Hz), are incompletely resolved at δ3.85-4.2.

[g] Except for H-3N, H-3X, and H-4 for 5-exo-Cl-I, for which coupling constants were obtained by computer simulation, these numbers represent observed line separations, not calculated or averaged coupling constants. s = singlet.

(Continued)

TABLE 3. (Continued)

[h] Broad.

[i] Singlet but broad enough to include 12-13 Hz coupling.

[j] Obscured by H-5X.

[k] The resonances at δ3.57 and 3.76 are coupled to each other (J~13 Hz) as are those at δ3.65 and 3.83. The 360 MHz spectrum was not expanded to allow accurate measurement of coupling constants.

[l] Presumably if these were not isochronous they would show ~13 Hz coupling to each other.

coupling constants in the new compounds to analogous coupling constants in I (Palmer et al., 1975) supports their proposed bornane skeletons. A departure from the coupling patterns of I, however, is the absence of some long-range coupling between protons on the bridge chloromethyl groups C-8 and C-9. In I, each of the chloromethyl group protons, in addition to geminal coupling, shows ~ 1.8 Hz four-bond coupling across the bridge to one other proton. The fact that none of these protons shows long-range coupling to two protons suggests the importance of the relative conformations of the chloromethyl groups in I (Palmer et al., 1975). It is thus not surprising that addition of chlorine to the molecules should alter these conformations and thus the long-range coupling. Addition of chlorine is less likely to affect angles of protons attached to the relatively rigid six-membered ring of bornane, and thus four-bond coupling of H-3X to H-5X, as observed in I, is expected in related compounds which contain both of these protons.

10-Cl-I has a singlet at δ 6.81, indicating the presence of one dichloromethyl group. Two of the three possible isomers with one dichloromethyl group are already identified (8-Cl-I and 9-Cl-I), so the dichloromethyl group in the third isomer must be C-10. The absence of long-range coupling to this dichloromethyl proton is in accord with this structure. The coupling constants of the ring protons are essentially the same as in I. Although H-5X is isochronous with chloromethyl group proton 9b, its distinctive coupling pattern can be observed in the 360 MHz spectrum, and it can be decoupled from H-3X, H-4, and H-6N. Several resonances of this compound are broad, probably reflecting long-range coupling: 6N and 8b are broadened doublets, while 8a (and probably the obscured 9b) appears as a broad singlet.

3-exo-Cl-I was examined in several solvents and solvent mixtures, but complete resolution of the resonances was not achieved in any individual spectrum; a benzene-$CHCl_3$ mixture (2:1) gave the best resolution. The C-3 methylene group is not present, since there is only a single proton resonating at high field (a doublet, H-4), compared with three in I. If the remaining H-3 were exo, H-4 would be an apparent triplet (overlapping doublet of doublets), rather than a simple doublet, since it would be coupled to both H-3X and H-5X; the absence of long-range coupling to H-5X also indicates that H-3X is lacking. Irradiation of H-5X collapsed the doublets for H-4 and H-6N to singlets. In this compound, only one proton on C-8 is coupled to one on C-9.

5-exo-Cl-I was the most difficult of these octachlorobornanes to identify, because neither the three protons at higher field nor the six chloromethyl group protons are fully resolved, even at 360 MHz. After identification of 3-exo-Cl-I, 8-Cl-I, 9-Cl-I, and 10-Cl-I, however, there remain just four positions (3-endo, 4,5-exo, and 6-endo) to which a chlorine can be added to I. Only addition of chlorine to position 5-exo would yield a compound with three protons at higher field (H-3N, H-3X, and H-4) and an isolated singlet at δ 5.78 (H-6N). Support for the structure was obtained by computer simulation of the complex multiplets for the three upfield protons as they appear in two different solvents, CCl_4 and benzene, using the same set of coupling constants, but different chemical shifts. As expected, H-3X lacks the four-bond coupling to H-5X anticipated if the latter proton remained in the molecule.

8,10-Cl_2-I has resonances of two dichloromethyl group protons, one of which has 2 Hz coupling to one of the protons of the remaining chloromethyl group. This must be four-bond coupling, indicating that the chloromethyl group and coupled dichloromethyl group are on the bridge. Thus, 8,10-Cl_2-I is related to 10-Cl-I in the same way in which 8-Cl-I or 9-Cl-I is related to I, so the assignment of the dichloromethyl group to C-8 or C-9 is similar to its earlier assignment in 8-Cl-I and 9-Cl-I. In the latter two compounds, relative to I, there is significant deshielding of the 3-exo or 5-exo proton to which the dichloromethyl group is syn, without significant change in the chemical shift of the exo proton to which it is anti (Matsumura et al., 1975; Turner et al., 1975). In benzene solution, where this effect is seen most dramatically, protons 3X and 5X of 8,10-Cl_2-I are shifted downfield 0.05 and ≧ 0.58 ppm, respectively, relative to their chemical shifts in 10-Cl-I; thus, the dichloromethyl group must be C-8, i.e., syn to the 5-exo proton. In 8,10-Cl_2-I, H-4 resonates 0.75 ppm downfield of its chemical shift in 10-Cl-I, and this effect also has an analogy in the chemical shift of H-4 of 8-Cl-I and 9-Cl-I relative to I.

3-exo,10-Cl_2-I has an NMR spectrum similar to that of 3-exo-Cl-I in that both have a doublet for H-4 as the single proton at high field, indicating that chlorine has been added to position 3X. The broadness of the dichloromethyl proton might suggest that it is on C-8 or C-9 with small four-bond coupling, but this is not likely, since H-4 is not significantly shifted downfield relative to H-4 of 3-exo-Cl-I as noted above in compounds with C-8 and C-9 dichloromethyl groups. As in 10-Cl-I, other resonances (H-8a and H-9b) are broad. The resonances of H-5X, although isochronous with those of chloromethyl proton 8b at δ 4.73, are apparent in the 360 MHz spectrum; absence of coupling of H-5X to a 3-exo proton is confirmed by an INDOR experiment involving monitoring the resonances of H-4 or H-6N.

The dehydrochlorination products of I have either one proton [I-HCl-(3,2)] or three protons [I-HCl(5,6)] at high field. Thus the methylene group at C-3 of I is preserved in I-HCl(5,6) but not in I-HCl(3,2). The vinyl proton of I-HCl(5,6) appears as a singlet. If it were at C-5, it would be expected to show 3 to 4 Hz coupling to H-4, as H-3 does in I-HCl-(3,2). The broadness of H-4 and H-6 suggests small allylic coupling in I-HCl(5,6). In comparison with I, protons 3N in I-HCl(5,6) and 6N in I-HCl(3,2) are shifted considerably to higher field. This effect is probably due to decreased deshielding by chlorine atoms on C-2 and C-5 in I, which are lost or repositioned on formation of the double bond; this chemical shift change is not expected to arise from mere introduction of the double bond into the bornane system (Jackman and Sternhell, 1969).

3-exo-Cl-I-HCl(5,6) has a vinyl proton, indicating that the double bond is in position 5,6 rather than 2,3. Although this vinyl proton is coupled to H-4, the observed coupling of 1.2 Hz (confirmed by a decoupling experiment) is more likely to be allylic $J_{4,6}$ than vicinal $J_{4,5}$ (see above). The broadness of the resonances for H-4 and H-6 in the analogous I-HCl(5,6) is attributed to small allylic coupling. The remaining endo proton (H-3N), like those of the dehydrochlorination products of I, resonates at higher field than in its saturated precursor.

3-exo-Cl-I-2HCl also has a single vinyl proton coupled to the bridgehead proton, and here the coupling constant is increased to 1.7 Hz. The protons of the three chloromethyl groups fall in the range of $\delta < 0.4$. Although a 360 MHz spectrum in C_6D_6 separated the protons on C-8 and C-9, those on C-10 were isochronous and thus appeared as a singlet.

Relationship of Chemical Structure and Biological Activity. Toxaphene is moderately toxic to mice and houseflies and highly toxic to goldfish (Table 4). The synergist PB increases its housefly toxicity but not its mouse toxicity. Compound I (> 98% purity) is less toxic to mice than toxaphene, but with PB-treated mice it is more toxic than toxaphene, as a result of the 7.9-fold synergism. The previous finding that I is more toxic than toxaphene to mice (Khalifa et al., 1974) suggests the presence of a minor impurity (< 10%) of high toxicity in the sample used in the earlier studies. This impurity is not removed by column chromatography or preparative GLC, but is minimized on recrystallization. With houseflies, in the presence or absence of PB, and with goldfish, I is 1.6 to 7 times as toxic as toxaphene. Addition of a chlorine atom at the 3-exo or 5-exo position generally reduces the toxicity of I to each species. A large toxicity increase results in introducing the 8-chloro substituent into I, except with houseflies in the presence of PB. The mixture of 8-Cl-I and 9-Cl-I is 1.2 to 2.0 times as toxic as 8-Cl-I alone, so 9-Cl-I is as much as four times as toxic as 8-Cl-I. Introduction of chlorine at C-10 greatly reduces the toxicity of I and its 3-exo-chloro- and 8-chloro derivatives. The toxicity of I and 3-exo-Cl-I is reduced by dehydrochlorination, particularly when the olefin is formed at the 5,6 position. Three of the samples assayed (5-exo-Cl-I, 3-exo,10-Cl_2-I, and 8,10-Cl_2-I) were of only moderate purity (70 - > 86%). It is not known to what extent the reported potency values for these compounds are due to the assigned structures as opposed to impurities. However, it is clear that each of these compounds is of low toxicity relative to I, 8-Cl-I, and 9-Cl-I.

In general, the potency of compounds formed on introducing one chlorine substituent into I decreases in the order: 9-chloro > 8-chloro > none > 3-exo-chloro or 5-exo-chloro or 10-chloro. It appears that PB-sensitive mechanisms detoxify I more readily than its 8-chloro- and 9-chloro derivatives.

TABLE 4. BIOLOGICAL ACTIVITY OF HEPTACHLOROBORNANE I AND RELATED OCTA- AND NONACHLOROBORNANES, HEXA- AND HEPTACHLOROBORNENES, AND A HEXACHLOROBORNADIENE

Compound	Purity, %	LD_{50}, 24 h						
		Mouse ip, mg/kg			Housefly topical, μg/g			Goldfish, ppb
		−PB	+PB	−PB/+PB	−PB	+PB	−PB/+PB	
Comparison Standard								
Toxaphene		47	42	1.1	18.0	9.5	1.9	20
Heptachlorobornane								
I	>98	75	9.5	7.9	11.5	2.4	4.8	2.9
Octachlorobornanes								
3-*exo*-Cl-I	>98	>100	>100		18.5	3.2	5.8	43
5-*exo*-Cl-I	70	~24	~28	~0.9	26	7.5	3.5	13
8-Cl-I	>93	3.3	3.1	1.1	5.5	2.2	2.5	1.1
8-Cl-I (57%) + 9-Cl-I (43%)	>92	2.5	1.9	1.3	3.1	1.9	1.6	0.55
10-Cl-I	98	>100	48	>2.1	80	34	2.4	36
Nonachlorobornanes								
3-*exo*,10-Cl_2-I	>86				95	65	1.5	>100
8,10-Cl_2-I	>75				60	22	2.7	44
Hexachlorobornenes								
I-HCl(3,2)	95				36	11	3.3	27
I-HCl(5,6)	>98	~65	~50	~1.3	225	85	2.6	>100
Heptachlorobornene								
3-*exo*-Cl-I-HCl(5,6)	>99	>100	>100		105	35	3.0	>100
Hexachlorobornadiene								
3-*exo*-Cl-I-2HCl	95				105	29	3.6	>100

SECTION III

METABOLISM AND ENVIRONMENTAL DEGRADATION OF TOXAPHENE AND ITS COMPONENTS

MATERIALS AND METHODS

Analyses

The composition of reaction mixtures and the purity of individual products were determined by open tubular column GLC using conditions identical to those in Section I for studies on toxaphene and its metabolites whereas in analyses of heptachlorobornane I and its reaction products the column temperature was isothermal at 200°C. To assist in quantitation in the latter investigations, mirex was used as an internal standard with corrections for differences in detector response for mirex and other products under consideration.

The sulfuric acid-celite column procedure of Zweig and Sherma (1972) was used for cleanup of biological samples prior to GLC. The column (2 x 20 cm) was packed with 2 g celite 545 powder (Sargent-Welch Scientific Co., Anaheim, Calif.), then with a sulfuric acid-celite mixture prepared by thorough blending with a mortar and pestle of 10 g celite with 10 ml of a 1:1 (v/v) mixture of concentrated sulfuric acid (98.6%) and fuming sulfuric acid (115%). The following solutions were then added to the column in sequence, allowing the solvent each time to completely enter the column: 10 ml hexane; 2 ml biological extract in hexane containing 5 µg mirex; three portions of 2 ml each of hexane; 100 ml hexane. Once the biological extract completely entered the celite column, the total eluate was collected up to a volume of 100 ml. This procedure elutes all toxaphene components without detectable alteration in their ratios (GLC). It provides > 98% recovery with [^{14}C]toxaphene and essentially quantitative recoveries of the heptachlorobornane, hexachlorobornane and hexachlorobornene derivatives discussed later (Figure 5); however, the acid treatment decomposes the pentachlorotricyclene derivative.

TLC involved the use of bifluorenylidene and benzylidenefluorene as marker dyes (See Section I). Components or derivatives of [^{14}C]toxaphene and unlabeled toxaphene were detected by radioautography and the diphenylamine reagent (See Section II), respectively.

Spectroscopy and elemental analyses were carried out as in Section II.

Chemicals

Sources for the chemicals used were the same as in Sections I and II except as noted below.

Heptachlorobornane I was obtained by chromatographing a mixture of crystallized toxaphene (35 g, obtained in 61% yield as a white crystalline material on crystallization from isopropanol) and bifluorenylidine (10 mg) (a yellow marker dye for the elution position of I) in hexane (25 ml) on a 7 x 100 cm column containing 1 kg of silicic acid packed with hexane. The column was developed with hexane under 20 psi N_2 pressure. Elution of the yellow dye and I (GLC monitoring) began after 10 liters of hexane had been eluted and was essentially complete after an additional 1.25 liter of hexane. Heptachlorobornane I (360 mg, 1.03% yield; > 99% purity) was obtained on evaporation of this 1.25 liter of eluent to 2 ml, washing the resulting crystals several times with ice cold hexane to remove traces of the yellow dye and recrystallization twice from hexane.

Hexachlorobornene IV [the same as I-HCl(3,2) in Figure 3] from dehydrochlorination of heptachlorobornane I with ethanolic KOH and 8-Cl-I (see Section II) from chlorination of I were obtained as reported in Section II.

Reactions of Heptachlorobornane I

Photolysis. Heptachlorobornane I at 1.3×10^{-3}M in hexane was irradiated with UV light ($\lambda > 220$ nm; 450 watt medium pressure lamp with quartz filter; Conrad-Hanovia, Inc., Newark, N.J.) using GLC to monitor the reaction. The major product was isolated as with the hematin reaction (see below) for examination by NMR and GLC-CI-MS.

Triphenyltin hydride. A mixture of heptachlorobornane I (80 mg, 0.21 mmol), triphenyltin hydride (120 mg, 0.34 mmol; prepared from triphenyltin chloride and $LiAlH_4$ according to Kuivila and Beumel, 1961) and 2,2'-azobis-(2-methylpropionitrile) (AIBN) (2 mg) in hexane solution (100 ml) was refluxed for 3 hr. The products were analyzed by GLC, then isolated by column chromatography (see below) for examination of the three major components by NMR and GLC-CI-MS. Each of these products was contaminated with ~ 10% triphenyltin chloride even after chromatographic purification.

Reduced hematin. A preparative scale reaction was carried out by the general procedure of Wade and Castro (1973) as follows. A solution of hematin (500 mg, 0.79 mmol) in 500 ml of glacial acetic acid-N-methyl-2-pyrrolidone (1:1) was mixed with washed (glacial acetic acid and ether) iron powder (50 mg) in a one liter round bottom flask. Argon was flushed through the flask for a few min to displace the air and the mixture was then subjected to magnetic stirring for 1 hr, resulting in a color change for the solution from brown to red indicating the presence of reduced hematin. Heptachlorobornane I (280 mg, 0.74 mmol) in 250 ml of glacial acetic acid-N-methyl-2-pyrrolidone (1:1) was then added through a dropping funnel and the reaction was allowed to proceed under argon with continuous stirring for 72 hr at 25° C. The products were extracted into hexane (500 ml x 4) which was then washed twice with each of water, saturated $NaHCO_3$ and saturated

NaCl and dried over anhydrous $MgSO_4$. Evaporation of the hexane gave 220 mg crude product (~ 86% yield considering the degree of dechlorination) which was chromatographed on a silicic acid column (2.5 x 40 cm) with hexane as the eluting solvent and pressure as above. GLC analysis of relevant fractions revealed heptachlorobornane I, four major products (II-V) and an unknown (Figures 5 and 6). The first eluting compound is an unidentified $C_{10}H_{11}Cl_5$ derivative (GLC-CI-MS) which, although in very minor amount, is not separable by GLC from compound V. Compounds III and IV are easily obtained pure by evaporating the hexane from fractions of > 80% purity and recrystallizing from hexane. Compound V is essentially pure in the last fractions eluted. Compounds I and III when present in the same fractions are separated by first crystallizing I from hexane and then crystallizing III. When compounds II and III are present in mixtures, compound III is removed first on crystallization from hexane then II is recrystallized from hexane. Selective crystallization is not appropriate to separate mixtures of II and IV. The amount and purity of each product were as follows: II-25 mg, > 90%; III-106 mg, > 99%; IV-28 mg, > 99%; V-10 mg, > 99%. Structures of these compounds were assigned by NMR and CI-MS.

The reaction rate was monitored (GLC) in a small scale reaction involving 3 mg heptachlorobornane I, 15 mg hematin, 2 mg iron powder and 25 ml total reaction volume but otherwise as above.

Bovine rumen fluid. Rumen fluid from a fistulated cow at the University of California at Davis was used immediately after filtration through four layers of cheese cloth to remove large particles. Heptachlorobornane I (66 µg) in ethanol (1 ml) was added to the fresh fluid (~ 500 ml) completely filling a flask which was then stoppered and incubated at 37°C. The reaction mixture was acidified to pH ~ 1 by adding sulfuric acid and extracted with ether containing mirex (5 µg, internal standard). The ether was dried ($MgSO_4$), evaporated and the resulting residue was dissolved in hexane (2 ml) and subjected to cleanup on the fuming sulfuric acid-celite column prior to GLC analysis.

Sewage primary effluent. The incubation and analysis procedures used for the rumen fluid were also employed with the primary effluent (anaerobic) from the sewage treatment process (Richmond Field Station, University of California, Richmond).

Rat liver microsome-NADPH system. Reaction mixtures in 0.1M pH 7.4 phosphate buffer (2 ml) consisted of rat liver microsomes (4 mg protein), NADPH (0 or 3 mg) and heptachlorobornane I (10 µg) added last in ethanol (50 µl). After one hr incubation at 37°C in air or argon with shaking, each mixture was extracted with hexane (5 ml x 3) containing mirex (5 µg, internal standard) and the extract was subjected to cleanup and analysis as in the rumen fluid studies.

In Vivo Studies

Treatment of chickens and mammals and analyses of their feces and tissues. The following test animals were used: female white leghorn chickens (1.1-1.5 kg) and male rabbits (478-610 g) from Western Scientific Supply Co.

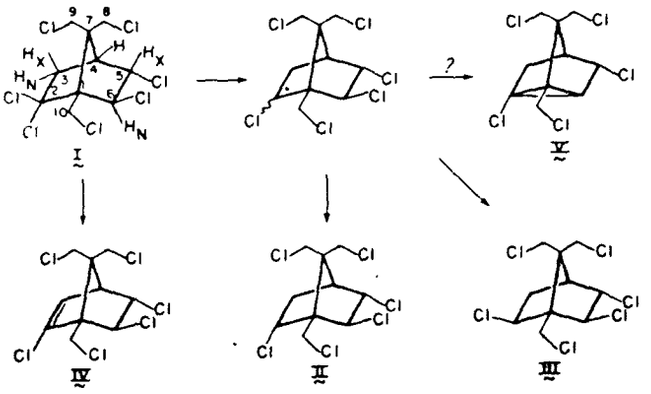

Figure 5. Conversion of heptachlorobornane I to various hexachlorobornane, hexachlorobornene, and pentachlorotricyclene derivatives.

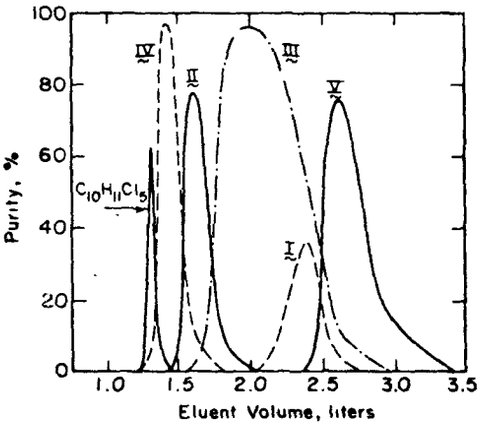

Figure 6. Chromatography of I and its reaction products with reduced hematin on a silicic acid column developed with hexane. Conditions are given in the text.

(West Sacramento, Calif.); male Swiss-Webster mice (18-20 g), male albino Sprague-Dawley rats (150-165 g), male Hartley guinea pigs (224-260 g), and male hamsters (70-85 g) from Simonsen Laboratories, Inc. (Gilroy, Calif.); male long-tailed monkeys (Macaca fascicularis) (~ 8 kg) (colony born and handled at the Primate Research Center, University of California, Davis).

Chickens, mice, rats and monkeys received either toxaphene (~ 13 mg/kg) or heptachlorobornane I (~ 3 mg/kg) using soybean oil as the administration vehicle and rinse for the stomach tube. Hamsters, guinea pigs and rabbits received the same mg/kg dose as above but the compounds in soybean oil were applied to lettuce which was quickly consumed by the animals. Feces were collected in each case for 72 hr then the toxaphene-treated animals, except the monkeys, were sacrificed for removal of the liver and a sample of fat. Liver and fat samples were removed in separate experiments from rats 7 hr after treatment with heptachlorobornane I. These tissues were also obtained by biopsy at 72 hr after treatment of monkeys with toxaphene and heptachlorobornane I.

An additional study with rats involved administration of compounds II and III (3.1 mg/kg each) or a mixture of compounds II and III (0.95 and 0.52 mg/kg, respectively) as above, collecting the 0-72 hr feces.

The fat, liver and feces, as appropriate, were extracted with acetone (~ 10 ml/g; containing a total of 5 µg mirex as internal standard in studies with heptachlorobornane I), the acetone evaporated and the products in hexane solution were subjected to cleanup on the sulfuric acid-celite column and GLC analysis.

Treatment and analysis of houseflies. Adult female houseflies (Musca domestica L., SCR susceptible strain, 3-4 days after emergence, 18-20 mg) were treated topically on the abdomen with heptachlorobornane I (4.5 µg/g) in acetone. After 24 hr the flies and their feces were extracted with acetone containing mirex (5 µg) and subjected to cleanup and GLC analysis.

Bioassays

Procedures for determining the 24-hr LD_{50} values using adult female houseflies (with and without PB) and goldfish were as in Section II.

RESULTS

Identification of New Compounds. The products under consideration and their designations are shown in Figure 5. Compounds I-V are crystalline materials that are partially resolved by TLC and completely by GLC (Table 5). They vary in sensitivity of detection by EC depending on the number of chlorines and, with II and III, the configuration of the chloro substituent at C-2 (Table 5).

Each of compounds II-V was isolated from the reduced hematin system in sufficient amount for identification by NMR and CI-MS. Compound IV was identical in all respects with an authentic standard [I-HCl(3,2) of Section II)] so its structure is not considered further here.

TABLE 5. PROPERTIES OF HEPTACHLOROBORNANE I AND ITS REACTION PRODUCTS IN VARIOUS CHEMICAL, PHOTOCHEMICAL AND METABOLIC SYSTEMS

Property	Compound				
	I	II	III	IV	V
Mp, °C	221-222	146-148	155-156	107-108	100-101
TLC, R_f[a]	0.37	0.48	0.40	0.53	0.35
GLC t_R at 200 °C, min[b]	20.41	13.33	14.00	11.15	8.45
EC response per unit weight relative to mirex (=100)	51	13	23	23	11
Molecular formula, CI-MS	$C_{10}H_{11}Cl_7$	$C_{10}H_{12}Cl_6$	$C_{10}H_{12}Cl_6$	$C_{10}H_{10}Cl_6$	$C_{10}H_{11}Cl_5$
CI-MS(m/e, rel intensity)[c]					
$[M + 1]^+$	377 (0)	343 (0)	343 (0)	341 (10)	307 (4)
$[M - Cl]^+$	341 (53)	307 (62)	307 (61)	305 (60)	272 (10)
$[M - HCl]^+$	340 (35)	306 (17)	306 (19)	304 (13)	271 (77)
$[M - Cl, -HCl]^+$	305 (37)	271 (55)	271 (56)	269 (14)	236 (0)
LD_{50}					
Housefly topical, µg/g					
-PB	11.5	250	36	36	255
+PB	2.4	175	10	11	68
Goldfish, ppb	2.9	85	4.8	27	>100

[a] R_f with 3 developments; see Section I.

[b] The t_R value for mirex is 45.50 min.

[c] ^{35}Chlorine isotope peak only. The isotope clusters appear in appropriate ratios for the designated compositions. Methane as reagent gas.

The CI-MS data provide the elemental compositions of compounds II-V (Table 5). Under CI conditions saturated chlorobornanes give no $[M+1]^+$ peak but instead give $[M-Cl]^+$ as the base peak (Holmstead et al., 1974; see Section II). Hexachlorobornanes II and III conform to this relationship. In contrast, hexachlorobornene IV and pentachlorotricyclene V give small $[M+1]^+$ peaks with $[M-Cl]^+$ and $[M-HCl]^+$ as the base peaks, respectively.

The structural assignments for compounds II, III, and V are based on NMR spectral data given in Table 6. Each compound contains six chloromethyl group protons with the typical geminal coupling of ~ 12 Hz observed with heptachlorobornane I.

The assignment of the bornane skeleton for compounds II and III is based on the resemblence of their observed coupling constants to the analagous coupling constants of heptachlorobornane I. Compounds II and III each give signals for six ring protons, establishing that they are formed by reductive dechlorination. The similarity of coupling patterns of protons 4, 5 and 6 in compounds I-III reveals that I undergoes reductive dechlorination at the 2 position to form II and III. The new proton on C-2 of II and III can be assigned as endo or exo by its coupling with the protons on C-3. In this ring system, syn coupling is typically larger than anti (Williamson, 1963). The C-2 proton in II is assigned to the exo position since it is coupled with the 3-endo proton with a coupling constant of 5.0 Hz and with the 3-exo proton with a coupling constant of 10.5 Hz. In compound III, the coupling constant of the proton on C-2 with H-3N (9.0 Hz) is larger than that with H-3X (4.7 Hz), so this isomer must have H-2 in the endo position. The relative chemical shifts of H-5X and H-6N in compounds I-III are consistent with these assignments. In II, as in I, H-6N is farther downfield than H-5X whereas in III H-6N has been shifted to considerably higher field by introduction of the endo proton at C-2. Changes in the long-range coupling of protons on the geminal chloromethyl groups with addition or removal of chlorine atoms on the ring have been observed before (see Section II).

Compound V does not retain the bornane skeleton and instead is assigned a tricyclene structure to accommodate the $C_{10}H_{11}Cl_5$ composition and the NMR spectral features. There are five ring protons, as in I, but two chlorines are removed. The absence of any vinylic proton (see Section II) and the presence of a single proton at higher field (δ 1.94) are consistent with a cyclopropane ring formed on elimination of chlorine atoms at positions 2 and 6. Suitable literature data do not appear to be available for the coupling patterns in tricyclene derivatives, and unfortunately the spectrum of V is inadequate for accurate measurements of the small coupling constants involved (~ 1.3 Hz). However, the coupling constants of the ring protons are considerably different from those of bornane derivatives I-III. The geminal coupling of the protons on C-3 is reduced from 16.2 Hz in I to 12.0 Hz in V, and long-range coupling appears to be introduced between H-4 and H-6. The geminal protons on C-3 have similar small (~ 1.3 Hz) coupling with H-4, as expected in a tricyclene derivative, where they are symmetrical with regard to H-4. The same small coupling is also evident for H-4 with H-5.

The structures of hexachlorobornanes II and III are confirmed by X-ray crystallography (Wong et al., 1978) but suitable crystals of penta-

TABLE 6. NMR SPECTRA OF HEPTACHLOROBORNANE I AND ITS REACTION PRODUCTS WITH REDUCED HEMATIN

Protons[b]	Chemical shifts (coupling constants, Hz)[a]			
	I[c]	II	III	V
2N			4.22 (9.0, 4.7)[d]	
2X		4.98 (10.5, 5.0)		
3N	3.36 [16.2 (0.6)]	2.43 (14.8, 5.0)	2.89 (16.0, 9.0)	2.68 (12.0, 1.3)
3X	3.01 (16.2, 4.5, 1.8)	2.52 (14.8, 10.5, 4.6, 1.9)	2.07 (16.0, 4.7, 4.7, 2.4)	2.17 (12.0, 1.3)
4	2.59 [4.5, 4.5 (0.6)]	2.59 (4.6, 4.6)	2.56 (4.7, 4.7)	2.30 (1.3, 1.3, 1.3, 1.3)
5X	4.68 (4.6, 4.5, 1.8)	4.54 (4.6, 4.4, 1.9)	4.46 (4.7, 4.3, 2.4)	4.34 (1.3, 1.3)
6N	5.33 (4.6)	4.84 (4.4)	3.87 (4.3)	1.94 (1.3, 1.3)
8a	4.18 (12.4, 1.8)[e]	3.58 (12.0)	3.83 (11.5)[f]	3.74 (12.0)[f]
8b	4.61 (12.4, 1.8)[e]	4.43 (12.0, 2.5)[e]	4.21 (11.5, 1.1)[e]	4.01 (12.0)[f]
9a	4.18 (12.5, 1.8)[e]	3.68 (12.2, 2.5)[e]	4.07 (11.5)[f]	3.89 (12.0)[f]
9b	4.35 (12.5, 1.8)[e]	4.45 (12.2)[f]	4.18 (11.5, 1.1)[e]	4.01 (12.0)[f]
10a	3.83 (12.5)	4.08 (12.2)[f]	4.07 (11.5)[f]	3.67 (12.0)[f]
10b	4.53 (12.5)	4.35 (12.2)[f]	4.12 (11.5)[f]	4.04 (12.0)[f]

[a] Ppm downfield from tetramethylsilane in CCl_4. The 1.3 Hz coupling constants are estimated.

[b] Endo(N) and exo(X) in V refer to the positions of the corresponding protons in I.

[c] Palmer et al., 1975.

[d] All four lines of the doublet of doublets for this proton were located by an INDOR experiment involving monitoring the resonances of H-3N.

[e] These appear as distinct doublets of doublets (geminal and four-bond coupling) and are therefore assigned to C-8 or C-9. It could not be ascertained which set of protons is on C-8 and which on C-9.

[f] These assignments are arbitrary.

chlorotricyclene V have not been obtained for X-ray examination.

Products from reaction of heptachlorobornane I in other systems are identified or tentatively identified by comparison with the standards from the reduced hematin system and with authentic hexachlorobornene IV (see Section II) using GLC cochromatography in each case. Supporting NMR and GLC-CI-MS evidence is available on identifications in the following cases: II from photolysis; II-IV from the triphenyltin hydride system.

Reaction Products of Heptachlorobornane I in Various Chemical, Photochemical, and Metabolic Systems. Quantitative data on the products from reaction of heptachlorobornane I in various systems are given in Table 7. These data are based on at least three analyses in each case and standard errors are reported when three to five independent experiments were involved.

Photolysis of heptachlorobornane I in hexane irradiated with UV light proceeds rapidly and gives only two products detected by GLC-EC, hexachlorobornanes III and II in a ~ 0.12 ratio. The major products with triphenyltin hydride are hexachlorobornanes III and II in similar yields, and there are small amounts of hexachlorobornene IV and a compound tentatively identified as pentachlorotricyclene V. Reduced hematin gives excellent yields of products II-V with minimal difficulty in their isolation. The reaction proceeds rapidly at 25°C ($t_{1/2}$= ~10 min) and the product ratio does not significantly change during the course of the reaction, with hexachlorobornanes III and II appearing in a ~ 3.8 ratio. Bovine rumen fluid and sewage primary effluent form only two products detected by GLC-EC after cleanup, i.e., hexachlorobornanes III and II in a ~ 2.4 ratio. The conversion rate is rapid in rumen fluid ($t_{1/2}$= ~ 2 hr)and significant in sewage primary effluent considering that it lacks the more potent degrading organisms of sewage sludge.

Rat liver microsomes do not metabolize heptachlorobornane I unless fortified with NADPH. They apparently carry out different reactions under aerobic and anaerobic conditions in the presence of cofactor. The identified products under anaerobic conditions are hexachlorobornanes III and II in a 2.0 ratio, but these compounds are not detected on incubation in air where metabolism of I proceeds at a greater rate.

Metabolites of Heptachlorobornane I. Rats treated with this heptachlorobornane contain moderate levels of the parent compound and low levels of metabolites II-IV in the fat and low levels of each of these compounds in the liver (Table 8). A monkey treated with heptachlorobornane I at 3.0 mg/kg contains the following ppb levels of I, II, III and IV, respectively, in tissues at 72 hr: 255, 0, 0 and 0 in fat; 50, 0, 450 and 0 in liver.

Metabolite yields in feces are given in Table 9. Each species examined excretes metabolites II-IV. Chickens excrete large amounts of I whereas mice, guinea pigs and rabbits excrete intermediate amounts and rats, hamsters and monkeys excrete little or no unmetabolized compound. Metabolite IV is minor relative to II and III in each species. The yields of II + III are highest with rabbits and monkeys, intermediate with chickens, rats and guinea pigs, and lowest with mice and hamsters. The ratio of metabolites III/II

TABLE 7. PERCENT OF HEPTACHLOROBORNANE I AND ITS REACTION PRODUCTS IN VARIOUS CHEMICAL, PHOTOCHEMICAL, AND METABOLIC SYSTEMS

Reaction variable	Compound, % of initial or administered amount						Product ratio, III/II
	I	II	III	IV	V	Other	
Min			Photolysis in Hexane				
10	8.8	16.8	2.4	0.0	0.0	72.0	0.14
20	3.8	19.0	2.9	0.0	0.0	74.3	0.15
30	0.2	14.3	1.3	0.0	0.0	84.2	0.09
40-60	0.2	13.6	1.3	0.0	0.0	84.9	0.10
			Triphenyltin Hydride (AIBN)				
180	28.4	24.4	32.2	1.0	0.9	13.1	1.3
			Reduced Hematin				
10	59.9	7.5	24.5	4.1	3.9	0.1	3.3
24	22.3	13.5	52.7	5.5	5.8	0.2	3.9
40	6.5	15.9	65.7	6.0	5.8	0.1	4.1
60-120	<0.2	17.5	69.0	6.5	6.0	0.8	3.9
Hour			Bovine Rumen Fluid				
0.4	62.9	10.5	26.6	0.0	0.0	0.0	2.5
2	50.2	15.8	34.0	0.0	0.0	0.0	2.2
4	6.6	29.4	64.0	0.0	0.0	0.0	2.2
24	0.0	30.2	69.8	0.0	0.0	0.0	2.3
			Sewage Primary Effluent				
24	67.3	9.5	23.2	0.0	0.0	0.0	2.4
Components			Rat Liver Microsome System				
+NADPH, argon	69.1	9.2	18.4	0.0	[a]	3.3	2.0
+NADPH, air	40.0	0.0	0.0	0.0	[a]	60.0	
-NADPH, argon	100.0	0.0	0.0	0.0	[a]	0.0	
Hr			Houseflies				
24	23 ± 5[b]	11 ± 1[b]	6 ± 1[b]	7 ± 1[b]	[a]		0.55

[a] Not analyzed.

[b] Average and standard error based on 5 experiments.

TABLE 8. AMOUNT OF HEPTACHLOROBORNANE I AND ITS METABOLITES IN FAT AND LIVER AT 7 AND 72 HR AFTER ORAL ADMINISTRATION OF I TO RATS AT 3.1 mg/kg

Tissue	Time, h	Ppb[a]				Metabolite ratio, III/II
		I	II	III	IV	
Fat	7	453 ± 285	8 ± 7	14 ± 13	15 ± 9	1.8
	72	335 ± 44	13 ± 5	34 ± 8	14 ± 2	2.6
Liver	7	8.6 ± 4.1	5.9 ± 1.9	10.2 ± 2.3	3.4 ± 0.9	1.7
	72	17.3 ± 11.7	2.0 ± 0.6	9.2 ± 7.0	0.8 ± 0.6	4.6

[a] Average and standard error based on experiments with 3 rats at 7 hr and 5 rats at 72 hr.

TABLE 9. PERCENT OF HEPTACHLOROBORNANE I AND ITS METABOLITES IN FECES WITHIN 72 HR AFTER ORAL ADMINISTRATION OF I AT ~ 3 MG/KG

Species	Compound, % of administered dose[a]				Product ratio, III/II
	I	II	III	IV	
Chicken[b]	17.7±2.7	3.8±0.7	5.2±0.9	0.9±0.2	1.4
Guinea pig	2.3±0.5	3.6±0.5	4.0±0.7	0.7±0.1	1.1
Hamster	0.6±0.1	1.3±0.1	1.4±0.1	0.2±0.0	1.1
Rabbit	2.5±0.0	10.1±0.1	10.1±0.1	1.5±0.2	1.0
Mouse[c]	2.8±0.3	1.8±0.1	2.2±0.3	0.3±0.0	1.2
Rat	0.2±0.1	2.1±0.6	5.3±1.6	1.0±0.3	2.5
Monkey[d]	0.0	8.8	10.7	0.9	1.2

[a]Average and standard error based on three experiments except with rats where five experiments were involved and monkeys where a single animal was used. For structures of compounds see Figure 5.

[b]Feces combined with urine.

[c]Comparable values for mice pretreated with PB are (% of administered dose): I - 7.5±0.9, II - 4.3±0.1, III - 5.5±0.8, and IV - 0.8±0.0. The product ratio III/II is 1.3.

[d]The percentages of the total II + III + IV excreted at intervals up to 72 hr were as follows: 0-12 hr - 0%, 12-24 hr - 7.3%, 24-48 hr - 10.2%, 48-72 hr - 2.9%. The metabolite ratio was the same at each of these time intervals.

is nearly constant (1.0-1.4) for all species except rats where metabolite III definitely predominates.

Studies with rats reveal that the yields noted for metabolites II and III are minimal values. Thus, when rats are administered either hexachlorobornane II or III their feces contain the administered compound as the only GLC-EC detectable product. On administering a mixture of hexachlorobornanes II and III, the feces excreted within 72 hr contains 45-47% of the unmetabolized compounds and no metabolites are detected by GLC-EC. These findings clearly establish that reductive dechlorination at a geminal dichloro group is a major pathway in metabolism of heptachlorobornane I in rats, other mammals and chickens.

Houseflies treated with heptachlorobornane I contain more hexachlorobornane II than its isomer III (Table 7). The relatively high yield of hexachlorobornene IV suggests that dehydrochlorination may be more important in housefly than in mammalian or chicken metabolism of I.

Products Derived from Toxaphene in Fat, Liver, and Feces of Rats. Figure 7 compares the GLC-EC pattern of toxaphene with those of toxaphene-derived products in fat, liver, and feces 72 hr after oral administration of toxaphene. The pattern of products in fat is similar to that of toxaphene itself although there are minor changes, e.g., reduced importance of peaks 16 (8-Cl-I + 9-Cl-I) and 24 relative to 9 (containing I). The liver chromatogram is characterized by: peaks corresponding to each of the 29 designated toxaphene components, the major one appearing at the position of 9 (other criteria not used to confirm identity as I); a large change in ratio for peaks 9 and 16; four major late-eluting peaks (A-D), some or all of which are possibly toxaphene components undergoing slow metabolism and selective concentration; appearance of a new peak between those designated as 26 and 27; a major peak at ~ 40 min (the general position of hexachlorobornane II or III - possible identity with this hexachlorobornane not further examined). The patterns of fat and liver chromatograms at 7 hr are intermediate between those of toxaphene and the corresponding 72-hr samples. The feces shows peaks corresponding to each of the 29 designated toxaphene components but in contrast to the tissues there is a predominance of short t_R compounds. Peaks 9 and 16 are barely detectable with feces and three of the major peaks correspond to compounds II-IV (metabolites of heptachlorobornane I).

TLC-GLC evidence is available that I-IV are present in feces of rats receiving [^{14}C]toxaphene orally. The acetone-soluble metabolites include compounds at the origin on TLC as well as materials spread over the normal broad TLC region for toxaphene. Cleanup on the sulfuric acid-celite column removes the materials at the origin on TLC and subsequent TLC-GLC reveals compounds I-IV each at its expected TLC position (Table 5; see Section I).

Products Derived from Toxaphene in Fat, Liver and Feces of Other Mammals and Chickens. The fat of each species gives a GLC product pattern very similar to that of toxaphene with each of the 29 designated toxaphene components clearly evident although with some alterations in peak ratios (Figure 8). The liver chromatogram patterns differ with each species and

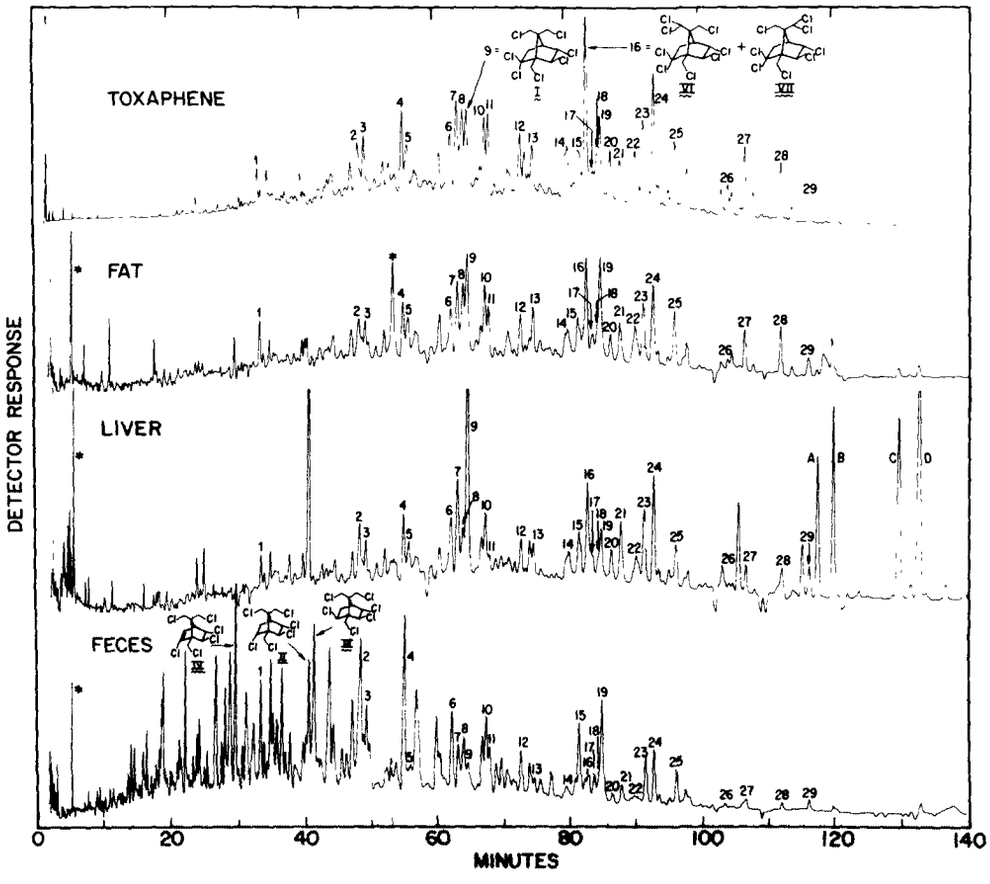

Figure 7. Open tubular column GLC analysis of toxaphene and of toxaphene-derived products in fat, liver, and feces of rats 72 hr after oral administration of toxaphene. The 29 arabic numerals refer to toxaphene components present in greater than 1% amounts as designated in Section I. The chromatographic positions of toxaphene components I (peak 9) and 8-Cl-I + 9-Cl-I (peak 16) and of metabolites II-IV of heptachlorobornane I are designated by structural formulae. Letter designations (A-D) refer to toxaphene-derived products in liver, some or all of which may be toxaphene components. Asterisks designate interfering materials of biological origin.

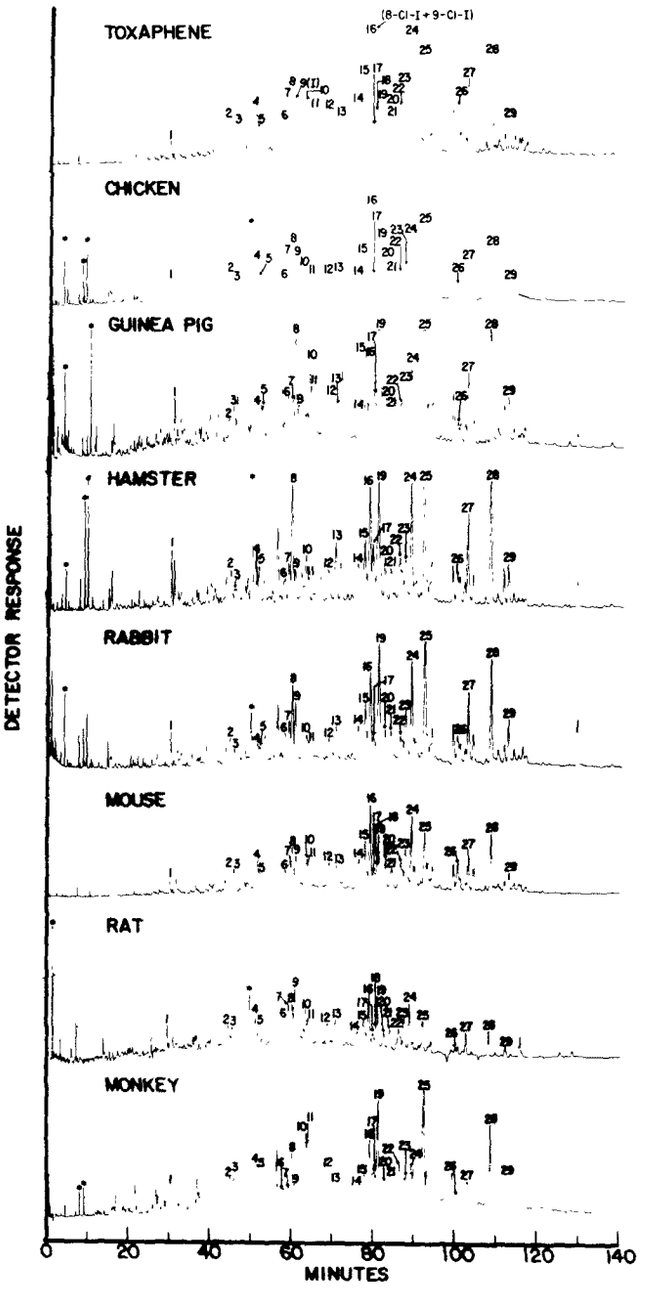

Figure 8. Open tubular column GLC analysis of toxaphene and of toxaphene-derived products in the fat of chickens and mammals 72 hr after oral administration of toxaphene. The 29 arabic numerals refer to toxaphene components present in ≧ 1% amounts as designated in Section I. The chromatographic positions of toxaphene components I (peak 9) and 8-Cl-I plus 9-Cl-I (peak 16) are indicated. Asterisks designate interfering materials of biological origin.

vary from relatively few major peaks (chicken, rat and monkey) to many major peaks (the other species) (Figure 9). Several products retained for 72 hr in liver give similar t_R values in two or more species (A - U). It is not established which of the designated liver peaks are toxaphene components and which are metabolites. The feces chromatograms differ greatly depending on the species with chickens and guinea pigs showing the closest similarity to toxaphene and rats and monkeys the largest difference (Figure 10). This relationship parallels in the most part the amount of unmetabolized heptachlorobornane I excreted by each species (Table 9). Fecal products chromatographing in the positions of metabolites II, III and IV are detected with monkey and several of the other species (Figure 10) but their identity is not confirmed by other analytical methods.

Degradation of 2,2,5-endo,6-exo,8,8,9,10-Octachlorobornane (8-Cl-I) by Reduced Hematin. This octachlorobornane reacts rapidly with reduced hematin in a small scale reaction to give five major products. Two of these have t_R values similar to hexachlorobornane III and hexachlorobornene IV and a third chromatographs as anticipated for a heptachlorobornane. The others have shorter t_R values. Heptachlorobornane I is found in < 1% amount.

Biological Activity. Heptachlorobornane I is more toxic than its metabolites or derivatives II-V to houseflies and goldfish (Table 5). A greater loss in toxicity occurs on tricyclene formation (V) or removing the exo chlorine at C-2 to form II than on removing the endo chlorine or on dehydrochlorination to give III and IV, respectively. Each of the products is probably metabolized by a microsomal cytochrome P-450 system in houseflies since they are synergized by PB.

DISCUSSION

Figure 5 gives the metabolic and chemical pathways established for heptachlorobornane I. In most cases examined, the major reaction is reductive dechlorination at the geminal dichloro group yielding isomeric hexachlorobornanes II and III, but in some systems there is also dehydrochlorination to hexachlorobornene IV and formation of pentachlorotricyclene V.

Photochemical reductive dechlorination of I leads preferentially to the product with chlorine at the 2-endo position, i.e., hexachlorobornane II. However, there are large amounts of unidentified products which are not detected by the usual GLC-EC method. Similar findings are reported in the photochemistry of 2-endo,3,3,5-exo,6-exo,8,9,10,10-nonachlorobornane (Parlar et al., 1976b). Triphenyltin hydride gives more III than II and also yields some hexachlorobornene IV and possibly pentachlorotricyclene V. It is likely that a radical intermediate is involved in these photochemical (Parlar et al.,1976b) and tin hydride (Kuivila, 1968) reductive dechlorinations of I to II and III.

Reduced hematin provides a convenient system to study the various dechlorination reactions since it rapidly gives II-V in high yields and the products are more resistant to reaction than starting heptachlorobornane I. A radical intermediate is likely to be involved in reductive dechlorination of I to hexachlorobornanes II and III (Wade and Castro, 1973). Tri-

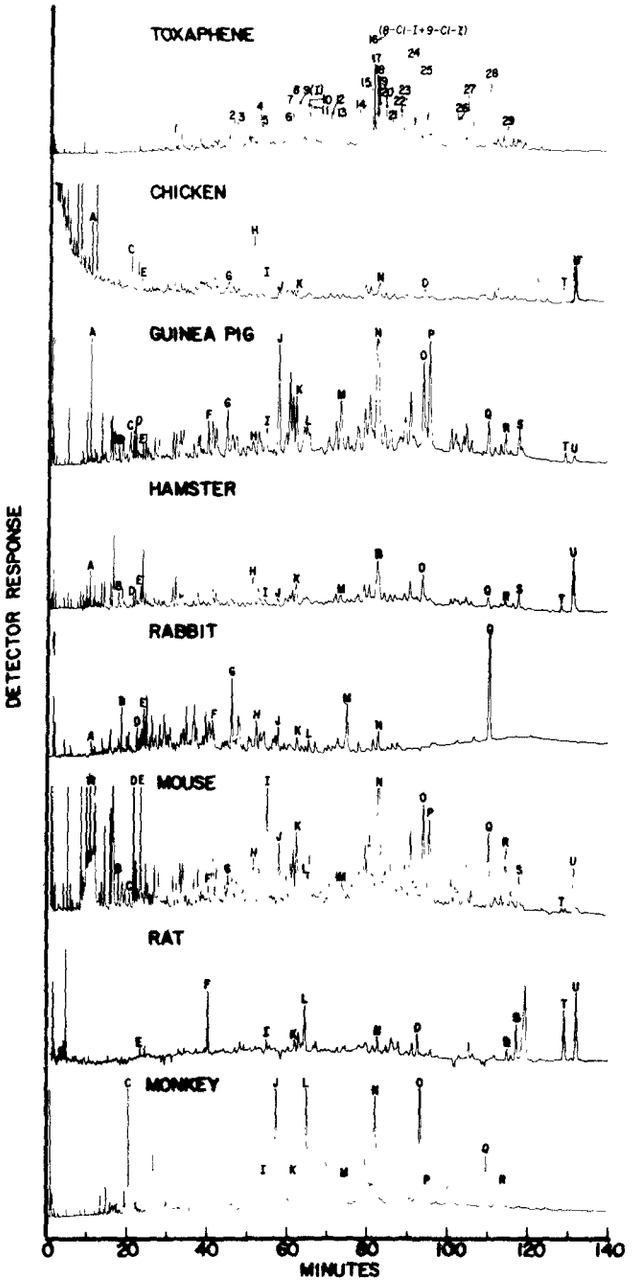

Figure 9. Open tubular column GLC analysis of toxaphene and of toxaphene-derived products in the liver of chickens and mammals 72 hr after oral administration of toxaphene. The 29 arabic numerals refer to toxaphene components present in ≧ 1% amounts as designated in Section I. The chromatographic positions of toxaphene components I (peak 9) and 8-Cl-I plus 9-Cl-I (peak 16) are indicated. Letter designations (A-U) refer to toxaphene-derived products in liver, some of which may be toxaphene components. Asterisks designate interfering materials of biological origin.

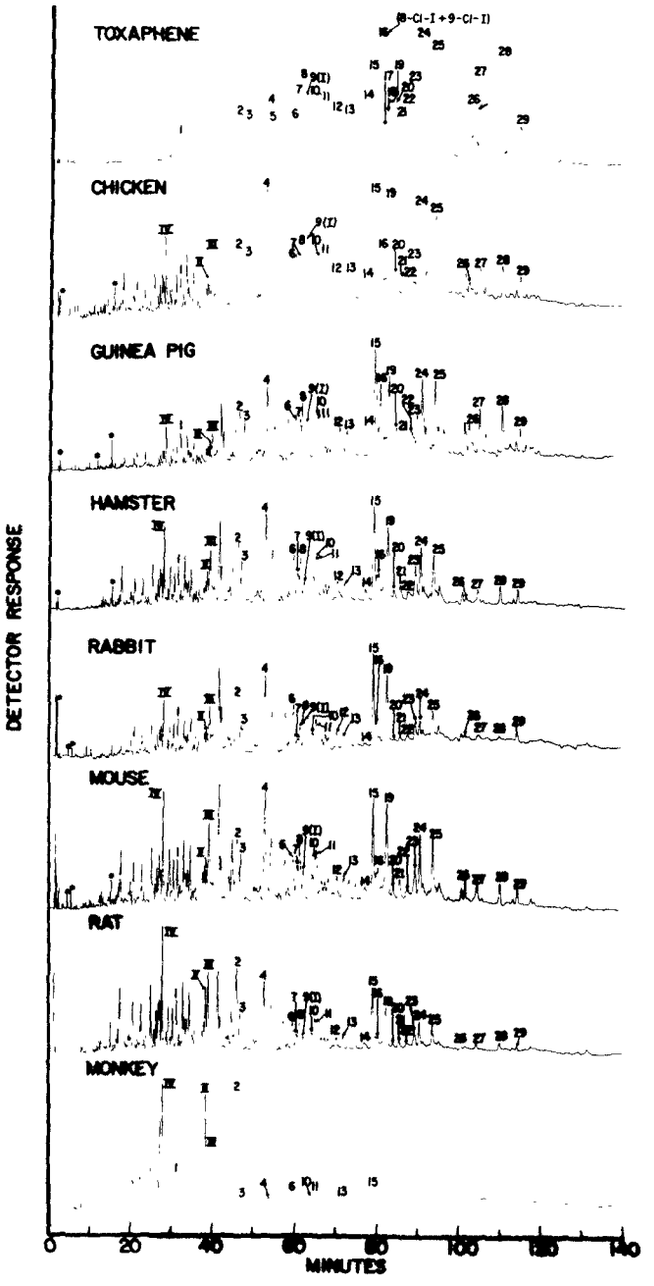

Figure 10. Open tubular column GLC analysis of toxaphene and of toxaphene-derived products in the feces of chickens and mammals 72 hr after oral administration of toxaphene. The 29 arabic numerals refer to toxaphene components present in ≧ 1% amounts as designated in Section I. The chromatographic positions of toxaphene components I (peak 9) and 8-Cl-I plus 9-Cl-I (peak 16) and of metabolites II-IV of heptachlorobornane I are indicated. Asterisks designate interfering materials of biological origin.

cyclene formation to give V may also proceed via the same radical intermediate whereas dehydrochlorination to IV probably involves a different pathway. Reduced hematin reacts with octachlorobornane 8-Cl-I to give products formed by both reductive dechlorination and dehydrochlorination (this study; Khalifa et al., 1976). It also acts in aqueous medium to cleave about half of the carbon-chlorine bonds in toxaphene (Khalifa et al., 1976).

The finding of extensive reductive dechlorination of heptachlorobornane I in bovine rumen fluid and sewage primary effluent suggests that this and other toxaphene components may undergo significant reductive dechlorination in the bovine rumen prior to absorption and in microbial systems under anaerobic conditions.

Metabolism of heptachlorobornane I by rat liver microsomes requires NADPH but proceeds by a different mechanism in air, where the products are not identified, than in an inert atmosphere, where hexachlorobornanes II and III are the major products. It was therefore of considerable interest to find hexachlorobornanes II and III in the feces of chickens and six mammalian species orally administered heptachlorobornane I. The hexachlorobornane ratio (III/II) is similar in the fat, liver and feces of rats to that found in the microsomal system, indicating that reductive dechlorination in vivo may occur in the liver microsomes. This ratio also is similar to those found in the hematin, bovine rumen fluid, and sewage primary effluent reactions, suggesting similar mechanisms of reductive dechlorination in each case on reaction with reduced porphyrins. Chickens, guinea pigs, hamsters, rabbits, mice and monkeys give more similar amounts of II and III than observed in rats and these in vitro systems. In contrast, houseflies give a greatly different ratio of hexachlorobornanes III and II, possibly due to varying rates in their further metabolism rather than to different mechanisms in their formation since synergist studies suggest the involvement of cytochrome P-450 in detoxification of heptachlorobornane I and its derivatives.

Metabolite identification is more difficult following administration of toxaphene compared to an individual toxaphene constituent because of the likelihood that many toxaphene components undergo reductive dechlorination and dehydrochlorination to products that fall within the same GLC t_R range. However, some findings with toxaphene itself are of interest. The liver of several species contains an unusual proportion of toxaphene-derived products of very high t_R values appropriate for heavily chlorinated compounds. The chromatographic pattern of the rat fecal products is characterized by short t_R compounds, suggesting extensive dechlorination, a conclusion supporting previous studies with [^{14}C]- and [^{36}Cl]toxaphene (Ohsawa et al., 1975). The rat fecal products appear to include metabolites II-IV of heptachlorobornane I. Similar observations are available for five other mammalian species and chickens although the proportion of fecal products vary greatly with some of the species. It is desirable to develop a GLC-EC system for monitoring toxaphene exposure by analysis of tissues and excreta, but this requires a better understanding than currently available on the changes in component ratios and introduction of new compounds on metabolism.

SECTION IV

MUTAGENIC ACTIVITY OF TOXAPHENE AND SOME OF ITS COMPONENTS

MATERIALS AND METHODS

Samples of Hercules toxaphene manufactured in various years (see Section I) or of toxaphene components or fractions and samples of related chlorinated terpenes from other manufacturers (see Section I) were dissolved in DMSO and added to cultures of the TA100 histidine-requiring mutant strain of Salmonella typhimurium (Ames et al., 1975). Potency is expressed as revertants per mg of test chemical.

RESULTS AND DISCUSSION

Mutagenic Activity of Toxaphene and Related Chlorinated Terpenes. Toxaphene manufactured by Hercules from 1949 to 1975 has a mutagenic potency averaging 728 revertants/mg and ranging from 310 to 1270 revertants/mg (Table 10). This potency range is similar to that for other samples of related chlorinated terpenes from various manufacturers (Table 10).

Other Observations. Preliminary studies were made on the nature of the mutagenic components in standard Hercules toxaphene (Table 11). Heptachlorobornane I has no significant mutagenic activity. Crystallization of toxaphene from isopropanol concentrates the mutagenic activity in the mother liquor fraction but does not completely remove mutagenic agents from the crystalline portion. Passage of toxaphene in hexane through a celite column, with or without fuming sulfuric acid, removes some of the mutagenic activity, thereby decreasing its overall potency. A highly mutagenic fraction (> 17,000 revertants/mg) is obtained on chromatographing toxaphene on celite or silicic acid columns using various solvents, with methanol for final elution of the mutagenic fraction. The identity of the mutagens in toxaphene has not been established.

TABLE 10. MUTAGENIC ACTIVITY OF HERCULES TOXAPHENE AND RELATED CHLORINATED TERPENES IN THE TA100 HISTIDINE-REQUIRING MUTANT STRAIN OF SALMONELLA TYPHIMURIUM

Sample	Revertants/mg	Sample	Revertants/mg
Hercules toxaphene by year		Related chlorinated terpenes	
1954	310	Procida	230
1969	530	Vicksburg A	380
1973	575	Vicksburg B	620
1957	685	Hercasa	394
1949	725	Flit & Fontaine	440
1970	745	East European dark	465
1960	750	East European light	580
1975	760	Strobane T-100	515
1974	930	Melipax	635
1963	1270	Sonford	945
		Bison A	1530
		Bison B	420

TABLE 11. MUTAGENIC ACTIVITY OF HERCULES STANDARD TOXAPHENE AND ITS FRACTIONS IN THE TA100 HISTIDINE-REQUIRING MUTANT STRAIN OF SALMONELLA TYPHIMURIUM

Sample	Revertants/mg
Heptachlorobornane I	< 30
Standard toxaphene	550
Crystallization of toxaphene	
Crystalline fraction	116
Mother liquor	950
Column chromatography of toxaphene, methanol fraction	> 17,000

RECOMMENDATIONS

The findings provide a portion of the needed information on toxaphene composition and toxicology but there are continuing research needs in several aspects of the overall problem. The open tubular column GLC method can be further perfected by coupling it with CI-MS for peak analysis. It is evident that further toxaphene components can be prepared and identified for toxicological evaluation. The metabolism studies deal with only one major toxaphene component and provide only partial information in this case on its metabolism and environmental fate. These studies should be continued using mammals, plants, soils and various environmental systems. Mutagenic components in toxaphene should be further defined and possibly identified and ways and means sought to remove them from the commercial insecticide.

Difficulties in defining the composition and toxicology of toxaphene illustrate the dilemma created when complex and poorly-defined mixtures of polychlorohydrocarbons are introduced into the environment in enormous amounts. While alternatives are being developed to minimize dependence on chemicals for pest management, it is preferable where possible to use pesticides whose structures and metabolites are well characterized and which have short-half lives and target organism specificity.

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

The composition and metabolism of Toxaphene have been examined to aid in understanding the conditions under which this insecticide can be most effectively and safely used. Each of 8 Toxaphene samples manufactured by Hercules Chemical Co. from 1949 to 1975 shows the same 29 major peaks and in almost identical ratios. 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 were easily differentiated from 12 samples of chlorinated terpenes from other manufacturers in the United States and abroad. There is surprisingly little variation in the acute toxicity of any sample.

Five major Toxaphene components (2,2,5-endo,6-exo,8,9,10-heptachlorobornane (I) and its 3-exo-chloro-, 8-chloro-, 9-chloro- and 10-chloro-derivatives) collectively account for up to 23% of the technical grade Toxaphene and up to 34% of those of chlorinated 2-exo,10-dichlorobornane. Chlorination of 2-exo,10-dichlorobornane provides a convenient source of I and other chlorinated bornanes. The toxicity to mice, houseflies and goldfish of the octachlorobornanes formed by introducing chlorine substituents into I, relative to I itself, generally decreases in the order: 9-chloro > 8-chloro > no added chlorine (i.e. I) > 3-exo-chloro, 5-exo-chloro or 10-chloro.

Fat from chickens and mammals treated orally with Toxaphene contains products similar in GLC characteristics to Toxaphene itself whereas liver and feces contain Toxaphene-derived products of greatly altered GLC properties. Toxaphene preparations and related chlorinated terpenes are mutagens in the histidine-requiring Salmonella typhimurium assay. The most potent mutagenic components, which are not identified, reside in the polar fractions on crystallization or solumn chromatography.

17. KEY WORDS AND DOCUMENT ANALYSIS

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insecticides metabolism composition(property) toxicity	Toxaphene	07 C 06 A, T

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