an initial step for the removal of chloroolefin materials from water by ozone treatment, since the organically bound chlorine is completely removed as HCl with concomitant introduction of functional groups which render the molecules amenable to biological degradation. Recent unpublished work in our laboratory had demonstrated, that the ozonolysis of vinyl chloride type olefins in water indeed produces the corresponding carboxylic acid fragments: ozonolysis of 1-chlorocyclopentene and of 1-chlorocyclohexene in water–acetone afforded the corresponding diacids in approximately 85% yield.

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Reductive Dechlorination of the Toxaphene Component 2,2,5-*endo*,6-*exo*,8,9,10-Heptachlorobornane in Various Chemical, Photochemical, and Metabolic Systems

Mahmoud Abbas Saleh*[1] and John E. Casida

2,2,5-*endo*,6-*exo*,8,9,10-Heptachlorobornane, one of the most toxic components of toxaphene insecticide, 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 cofactor; rats and houseflies in vivo. This heptachlorobornane is also dehydrochlorinated to give 2,5-*endo*,6-*exo*,8,9,10-hexachloroborn-2,3-ene in the triphenyltin hydride and reduced hematin systems and in rats and houseflies in vivo. Reduced hematin and the tin hydride system also convert the heptachlorobornane to 2,5-*endo*,8,9,10-pentachlorotricyclene. Fat from rats 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.

Essential knowledge of the metabolic and environmental fate of toxaphene, the major chlorinated hydrocarbon insecticide used in the United States, has developed slowly in recent years with associated advances in identification of components and improvement of analytical procedures. Toxaphene undergoes rapid dechlorination in mammals (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, 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (I), constitutes up to 8% of the technical grade insecticide (Palmer et al., 1975; Saleh and Casida, 1977) and four octachlorobornanes, each derivable by addition of one chlorine atom to I, make up an additional ~15% of toxaphene (Matsumura et al., 1975; Saleh and Casida, 1977; Turner et al., 1975, 1977). 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). The present study and a preliminary report

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on this investigation (Saleh et al., 1977) consider the degradation and metabolic chemistry of the heptachlorobornane in several systems, selected to emphasize reductive dechlorination reactions, and the nature of toxaphene-derived products in rats.

MATERIALS AND METHODS

Chromatography. The composition of reaction mixtures and the purity of individual products were determined by open tubular column gas–liquid chromatography (GLC) with an electron capture (EC) detector. In studies on toxaphene and its metabolites all GLC conditions were identical with those reported by Saleh and Casida (1977) while 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–EC. The column (2 × 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 of 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 of hexane; 2 mL of biological extract in hexane containing 5 μg of mirex; three portions of 2 mL each of hexane; 100 mL of 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; however, the acid treatment decomposes the pentachlorotricyclene derivative.

Thin-layer chromatography (TLC) involved a reported procedure with bifluorenylidene and benzylidenefluorene as marker dyes (Saleh and Casida, 1977). Components or derivatives of [^{14}C]toxaphene and unlabeled toxaphene were detected by radioautography and the diphenylamine reagent, respectively (Turner et al., 1977).

Spectroscopy. 360 MHz nuclear magnetic resonance (NMR) spectra were recorded on the Bruker HXS-360 spectrometer at the Stanford Magnetic Resonance Laboratory (Stanford University, Stanford, Calif.) by arrangement with W. Conover and as supported by NSF Grant GR 23633 and NIH Grant RR 00711. Chemical ionization (CI)–mass spectrometry (MS) and GLC–CI–MS determinations utilized the Finnigan Model 1015D mass spectrometer with methane as the reagent gas (Holmstead et al., 1974).

Chemicals. Toxaphene and [^{14}C]toxaphene (Ohsawa et al., 1975) were used as received from Hercules Inc. (Wilmington, Del.), or the toxaphene was purified by recrystallization from isopropanol to obtain a white crystalline material (61% yield; referred to as crystallized toxaphene).

Heptachlorobornane I was obtained by chromatographing a mixture of crystallized toxaphene (35 g) and bifluorenylidine (10 mg) (a yellow marker dye for the elution position of I) in hexane (25 mL) on a 7 × 100 cm column containing 1 kg of silicic acid (AR-100 mesh, Mallinckrodt Inc., St. Louis, Mo.) packed with hexane.

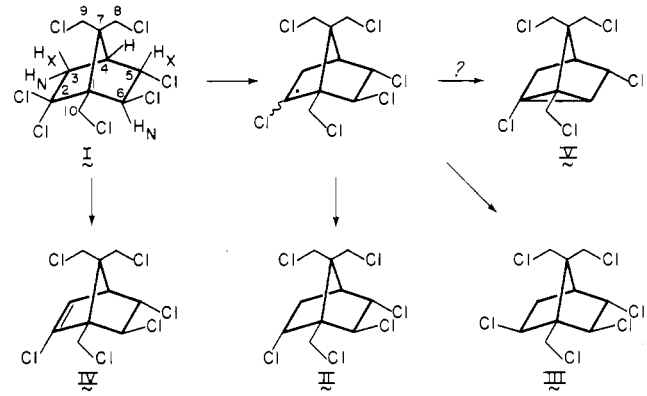

Figure 1. Conversion of heptachlorobornane I to various hexachlorobornane, hexachlorobornene, and pentachlorotricyclene derivatives.

The column was developed with hexane under 20 psi N_2 pressure. Elution of the yellow dye and I (GLC monitoring) began after 10 L of hexane had been eluted and was essentially complete after an additional 1.25 L of hexane. Heptachlorobornane I (360 mg, 1.03% yield; >99% purity) was obtained on evaporation of this 1.25 L 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 (Figure 1) from dehydrochlorination of heptachlorobornane I with ethanolic KOH (Turner et al., 1977) and the 8-chloro derivative of I (VII) were provided by W. V. Turner of this laboratory.

Reactions of Heptachlorobornane I. *Photolysis.* Heptachlorobornane I at 1.3×10^{-3} M in hexane was irradiated with UV light (λ >220 nm; 450-W 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 h. 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 1-L round-bottom flask. Argon was flushed through the flask for a few minutes to displace the air and the mixture was then subjected to magnetic stirring for 1 h, 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 h at 25 °C. The products were extracted into hexane (500 mL × 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 of crude product (~86% yield considering the degree of dechlo-

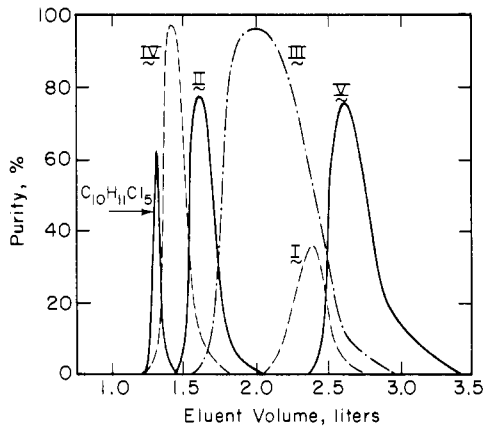

Figure 2. Chromatography of I and its reaction products with reduced hematin on a silicic acid column developed with hexane. Conditions are given in the text.

rination) which was chromatographed on a silicic acid column (2.5 × 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 (Figure 2). 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 of heptachlorobornane I, 15 mg of hematin, 2 mg of iron powder, and 25 mL of 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 ($MgSO_4$) and 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 0.1 M, pH 7.4 phosphate buffer (2 mL) consisted of rat liver microsomes (4 mg of protein), NADPH (0 or 3 mg), and heptachlorobornane I (10 μg) added last in ethanol (50 μL). After 1 h incubation at 37 °C in air or argon with shaking, each mixture was extracted with hexane (5 mL × 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 Rats and Analysis of Their Tissues and Feces.* Male albino rats (Sprague-Dawley strain, Simonsen Laboratories, Inc., Gilroy, Calif.) were treated orally with toxaphene (13 mg/kg), compound I, II, or III (3.1 mg/kg each) or a mixture of compounds II and III (0.95 and 0.52 mg/kg, respectively) using soybean oil as the administration vehicle (150 μL) and rinse (100 μL) for the stomach tube. Animals treated with toxaphene and heptachlorobornane I were sacrificed for removal of the liver and a sample of fat at 7 and 72 h. Feces were collected from the 72-h experiments. The fat, liver, and feces (0–72 h composite samples) were extracted with acetone (10 mL/g; containing a total of 5 μg of mirex as internal standard), the acetone was evaporated, and the products, in hexane solution, were subjected to cleanup on the sulfuric acid–Celite column and GLC analysis.

In a separate study, similar rats were treated orally with [^{14}C]toxaphene (1.5 mg/kg), and the cumulative feces at 72 h were extracted with acetone as above. The acetone-extractable compounds were analyzed by TLC before and after the sulfuric acid–Celite cleanup column and by GLC and the TLC–GLC procedure of Saleh and Casida (1977) after cleanup.

Treatment and Analysis of Houseflies. Adult 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 h 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-h LD_{50} values using adult female houseflies [with or without pretreatment with piperonyl butoxide (PB)] and goldfish were as previously reported (Turner et al., 1975, 1977).

RESULTS

Identification of New Compounds. The products under consideration and their designations are shown in Figure 1. Compounds I–V are crystalline materials that are partially resolved by TLC and completely by GLC (Table I). 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 I).

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 (Turner et al., 1977) so its structure is not considered further here.

The CI–MS data provide the elemental compositions of compounds II–V (Table I). Under CI conditions saturated chlorobornanes give no $[M + 1]^+$ peak but instead give $[M - Cl]^+$ as the base peak (Holmstead et al., 1974; Turner et al., 1977). 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 II. 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

Table I. 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 three developments; see Saleh and Casida, 1977. [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.

Table II. 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.

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 (Turner et al., 1977).

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 (Turner et al., 1977) 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 pentachlorotricyclene 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 (Turner et al., 1977) using GLC cochromatography in each case.

Table III. Percent of Heptachlorobornane I and Its Reaction Products in Various Chemical, Photochemical, and Metabolic Systems

Reaction variable	Compound, % of initial or administered amount I	II	III	IV	V	Other	Product ratio, III/II
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	
Hour			Rat Feces				
0-72	0.2 ± 0.1[b]	2.1 ± 0.6[b]	5.3 ± 1.6[b]	1.0 ± 0.3[b]	[a]		2.5
			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 five experiments.

Table IV. Amount of Heptachlorobornane I and Its Metabolites in Fat and Liver at 7 and 72 h after Oral Administration of I to Rats at 3.1 mg/kg

Tissue	Time, h	Ppb[a] I	II	III	IV	Metabolite ratio, III/II
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 three rats at 7 h and five rats at 72 h.

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 III. 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 h) 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 in Rats and Houseflies. Metabolite yields in feces at 72 h and levels in tissues at 7 and 72 h after treatment of rats are given in Tables III and IV, respectively. Heptachlorobornane I appears at moderate levels in the fat and low levels in the liver and feces. Metabolites II-IV are detected at low levels in the fat and liver and are in surprisingly high amounts in the feces. 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 h contains 45-47% of the unmetabolized compounds and no metabolites are detected by GLC-EC. At least 7.4% and possibly twice this amount of the heptachlorobornane I dose is metabolized in rats by reductive dechlorination at the geminal dichloro group.

Houseflies treated with heptachlorobornane I contain more hexachlorobornane II than its isomer III (Table III).

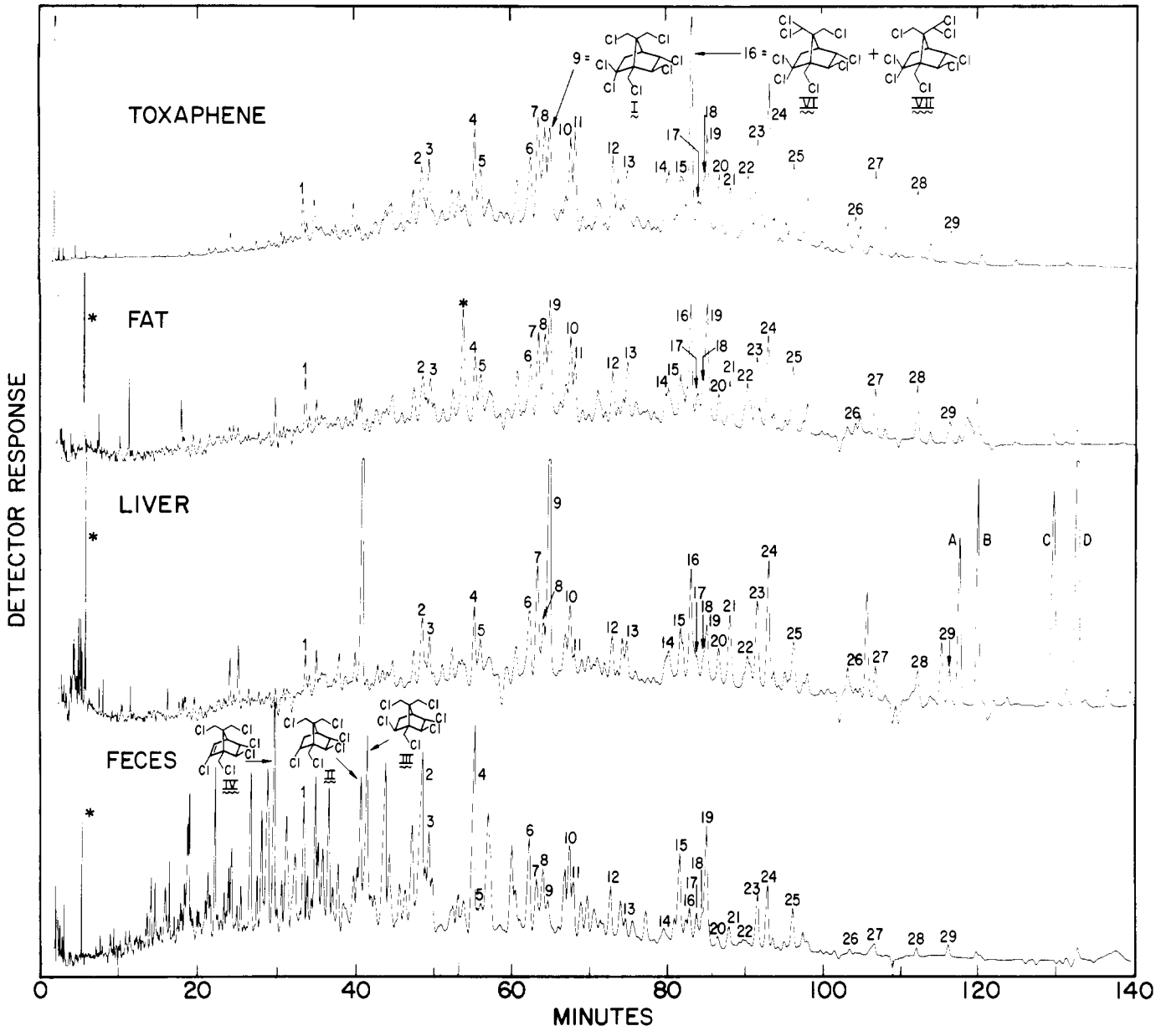

Figure 3. Open tubular column GLC analysis of toxaphene and of toxaphene-derived products in fat, liver, and feces of rats 72 h after oral administration of toxaphene. The 29 arabic numerals refer to toxaphene components present in greater than 1% amounts as designated by Saleh and Casida (1977). The chromatographic positions of toxaphene components I (peak 9) and VI + VII (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.

The relatively high yield of hexachlorobornene IV suggests that dehydrochlorination may be more important in housefly than in rat metabolism of I.

Products Derived from Toxaphene in Fat, Liver, and Feces of Rats. Figure 3 compares the GLC–EC pattern of toxaphene with those of toxaphene-derived products in fat, liver, and feces 72 h 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 (VI + VII) 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 h are intermediate between those of toxaphene and the corresponding 72-h 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 I; Saleh and Casida, 1977).

Degradation of 2,2,5-*endo*,6-*exo*,8,8,9,10-Octachlorobornane (VII) 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 I). 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 1 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., 1976). 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., 1976) 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). Tricyclene 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 VII 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–chloride 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 a combined yield of 7.4% in the feces of rats orally administered heptachlorobornane I. The hexachlorobornane ratio (III/II) is similar in the fat, liver, and feces 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. 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 contains an unusual proportion of toxaphene-derived products of very high t_R values appropriate for heavily chlorinated compounds. The chromatographic pattern of the 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 fecal products appear to include metabolites II–IV of heptachlorobornane I. 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.

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Pyrethroid Photodecomposition: Permethrin

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Photolysis of 3-phenoxybenzyl (1*RS*)-*trans*- or (1*RS*)-*cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (permethrin) in various solvents (hexane, methanol, water, and water–acetone) with artificial light (λ >290 nm) and on soil in sunlight results primarily in cyclopropane ring isomerization and ester cleavage to 3-phenoxybenzyl alcohol and the dichlorovinyl acids. Other photoproducts, often in trace amounts, from *trans*- and *cis*-permethrin are: monochloropermethrin formed by reductive dechlorination and its monochlorovinyl acid cleavage product; 3-phenoxybenzyl 3,3-dimethylacrylate; 3-phenoxybenzaldehyde and the corresponding acid; benzyl and 3-hydroxybenzyl alcohols and the corresponding aldehydes and acids. Permethrin and monochloropermethrin do not undergo epoxidation at the dichlorovinyl or chlorovinyl substituent under normal photooxidative conditions.

Permethrin (also known as NRDC 143) (Elliott et al., 1973) combines high insecticidal activity and low mammalian toxicity with enhanced photostability as compared with earlier synthetic pyrethroids (Elliott, 1977). The available knowledge on pyrethroid photochemistry is based primarily on chrysanthemates (Elliott and Janes, 1973; Ueda et al., 1974) rather than on compounds, such as permethrin, where the photolabile isobutenyl group is replaced with the more photostable dichlorovinyl substituent. We examined the photodecomposition of (1*RS*)-*trans*- and (1*RS*)-*cis*-permethrin in various solvents and on soil utilizing artificial and natural light sources. Some of the results were discussed in our recent review on pyrethroid photochemistry (Holmstead et al., 1977).

MATERIALS AND METHODS

Chemicals. The compounds are designated as shown in Figure 1, which also gives the ^{14}C positions in acid-labeled (1*RS*)-*trans*- and (1*RS*)-*cis*-permethrin (*t*- and *c*-per, respectively) (Ac* −58.2 mCi/mmol) and alcohol-labeled *t*- and *c*-per (Alc* −55.9 mCi/mmol). The individual ^{14}C *t*- and *c*-per isomers (>99% radiochemical purity) were separated from labeled (1*RS*)-*trans*,*cis*-per by thin-layer chromatography (TLC) using solvent system CBX2 described below.

FMC Corp. (Middleport, N.Y.) provided unlabeled *t*- and *c*-per (>99% purity), the corresponding dichlorovinyl acids (*t*- and *c*-Cl_2CA; >99% purity) and (1*RS*)-*trans*-monochloro-per (*E*:*Z* = 1:1): proton magnetic resonance (^1H NMR) ($CDCl_3$, internal Me_4Si) δ 1.15 (s, 3 H), 1.22 (s, 1.5 H), 1.28 (s, 1.5 H), 1.63 (d, 1 H, J = 5.5 Hz), 2.07 (dd, 0.5 H, J = 5.5, 8 Hz, cyclopropyl, *E*), 2.45 (dd, 0.5 H, J = 5.5, 8 Hz, cyclopropyl, *Z*), 5.09 (s, 2 H, benzyl), 5.48 (apparent triplet, 0.5 H, J = ca. 8 Hz, vinyl, *Z*), 5.65 (dd, 0.5 H, J = 8, 13.5 Hz, vinyl, *E*), 6.08 (d, 0.5 H, J = 13.5 Hz, vinyl *E*), 6.13 (brd, 0.5 H, J = 7 Hz, vinyl, *Z*), and 6.85–7.5 ppm (bm, 9 H, aromatic).

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Other standards for comparison with per photoproducts were obtained from Aldrich Chemical Co. (Milwaukee, Wis.) and used without further purification. [C=O]2,4-Dichlorophenoxyacetic acid (2,4-D) (10.2 mCi/mmol) from New England Nuclear Corp. (Boston, Mass.) was purified by TLC in solvent system CFEX2 (see below). Solvents of pesticide grade quality were deaerated before use in photodecomposition studies.

Chromatography and Analysis. Photolysis products of unlabeled *t*- and *c*-per were analyzed by gas–liquid chromatography (GLC) utilizing a Varian Aerograph Model 1400 instrument with a flame ionization detector and a glass column (1.8 m × 2 mm i.d.) with either 3% OV-101 on Chromosorb W (60–80 mesh) A/W (DCMS treated) or 3% Dexil 300 on Varaport-30 (100–120 mesh). The column temperature was maintained at 250 °C with a N_2 flow rate of 75 mL/min.

Chromatoplates (20 × 20 cm) precoated with silica gel F-254 (fluorescent indicator) (0.25 mm) (EM Laboratories, Inc., Elmsford, N.Y.) or with silica gel GF (1 mm) (Analtech, Inc., Newark, Del.) were used for analytical and preparative TLC, respectively in the following solvent systems: carbon tetrachloride–benzene (4:1) (CB); benzene (saturated with formic acid)–ether (10:3) (BFE); chloroform (saturated with formic acid)–ether (10:3) (CFE). The resolved products were detected by their quenching of gel fluorescence under short wavelength UV light. Radioactive gel regions detected by radioautography were scraped free from the glass support for liquid scintillation counting (LSC) or for product recovery by extraction of the gel with ethyl acetate, methanol, or other solvents as required followed by cochromatography with unlabeled standards.

Spectroscopy. ^1H NMR spectra were obtained with the Perkin Elmer R32B 90 MHz spectrometer using samples dissolved in $CDCl_3$ containing 3% tetramethylsilane. Mass spectra (MS) and combined GLC–MS were obtained on a Finnigan Model 1015D chemical ionization mass spectrometer with a System Industries Model 150 control system. The gas chromatograph (Finnigan Model 9500) was operated with temperature programming (120–300 °C, 10 °C/min) and with helium at 30 mL/min

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