The results of residue analyses from the apples showed that unchanged ethylene dibromide will desorb to low levels (below 0.1) in less than 4 weeks when the apples are held at both the treatment temperature and when placed in cold storage. The time required for desorption to a low level is partly dependent on temperature; when apples were treated and held at the lowest temperature likely to be used in field treatments (13°) at least 8 days were needed for desorption to take place. When similarly treated apples were placed in cold storage after a 3-day aeration at the treatment temperature the desorption time was extended to 3 weeks. Fumigant desorbed from different varieties of apples at somewhat similar rates; however, with Delicious, it was retained for a slightly longer time than in the McIntosh and Spy. Levels of inorganic bromide were of a low order even after treatment with fumigant at twice the required dosage.

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Metabolic Dechlorination of Toxaphene in Rats

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Rats treated orally with [^{36}Cl]toxaphene and each of seven fractions of [^{36}Cl]toxaphene of equal total chlorine content excrete about 50–60% of the ^{36}Cl in urine and 30–40% in feces within 14 days. In each case about half of the dose is excreted as chloride ion determined as phenylmercuric [^{36}Cl]chloride. Similar studies with ^{14}C-labeled preparations of toxaphene and one or both of two components of high mammalian toxicity, toxicants A and B, establish that the feces contain unmetabolized compound and that the metabolites probably include acidic materials, products formed by partial or complete dechlorination and [^{14}C]carbon dioxide. The tissues retain relatively low levels of ^{14}C several days after administration of [^{14}C]toxaphene or ^{14}C-labeled toxicant B. The structural features important for high toxicity to house flies and mice are present in only a few toxaphene components while those conferring biodegradability appear to be shared by most if not all components.

There is extensive knowledge on the metabolic fate of most chlorinated insecticides (Brooks, 1974) but this is not the case for toxaphene. Toxaphene is a very complex mixture obtained on chlorination of camphene. Analysis of toxaphene by gas chromatography (gc)–chemical ionization (CI)–mass spectroscopy (ms) reveals the presence of more than 177 C_{10} polychloro compounds (Holmstead *et al.*, 1974) only one of which has been identified, 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (Casida *et al.*, 1974; Palmer *et al.*, 1975). This component, referred to as toxicant B, and a $C_{10}H_{10}Cl_8$ material or materials of even higher biological activity, referred to as toxicant A, have only recently been isolated from the technical mixture (Khalifa *et al.*, 1974). Another advance important in metabolic fate studies is the preparation of [^{36}Cl]- and [^{14}C]toxaphene similar in composition to the technical insecticide (Hercules Inc., 1972).

Toxaphene appears to be less persistent in mammals than many other chlorinated insecticides (Guyer *et al.*, 1971). It is stated, without supporting evidence, that toxaphene is believed to be detoxified in the liver in as much as sulfate and glucuronide conjugates are found in the urine (Conley, 1952). The lack of specific information on toxaphene metabolism is not surprising in light of the complexity of the problem.

The present investigation concerns the metabolic fate of [^{36}Cl]- and [^{14}C]toxaphene administered orally to rats with particular attention to the metabolites formed on *in vivo* dechlorination. Seven fractions of [^{36}Cl]toxaphene and ^{14}C-labeled preparations of toxicants A and B are used to gain a preliminary concept of the variations in distribution, metabolism, and persistence among the various toxaphene components.

MATERIALS AND METHODS

Chemicals and Chromatography. Hercules Incorporated (Wilmington, Del.) provided the following samples of toxaphene: unlabeled standard (sample X-16189-49); [^{36}Cl]toxaphene from chlorination of camphene with $^{36}Cl_2$ (sample X-18306-27-1); [^{14}C]toxaphene from chlorination of [8-^{14}C]camphene (sample X-19098-4-2R). Toxicants A and B were isolated from toxaphene by the procedure of Khalifa *et al.* (1974). ^{14}C-Labeled preparations of purified toxicant A (1.5 mg) and pure toxicant B (0.8 mg) were obtained by chromatography of [^{14}C]toxaphene (100 mg) on a silica gel column followed directly by preparative gc; the chromatographic methods are those referred to as adsorption chromatography system I and preparative gc system I by Khalifa *et al.* (1974). Purified ^{14}C-labeled toxicant A consisted of 70% toxicant A, 14% of one impurity, and 16% of a second impurity. These components give gc R_t values of 10.5, 10.0, and 10.5 min, respectively, on the SE-30 column at 170° (conditions described below). Their thin-layer chromatography (tlc) R_f values on silica gel 60

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Table I. Composition and Toxicity of Toxaphene Compared with Seven Fractions of Equal Total Chlorine Content

Parameter	Toxaphene	Fractions of toxaphene: Overall av	I	II	III	IV	V	VI	VII
			Elemental Composition, %[a]						
Carbon	28.9	30.9	29.2	28.8	29.2	29.3	30.9	32.6	36.0
Hydrogen	2.4	3.1	2.7	2.4	2.5	2.5	2.4	3.1	6.2
Chlorine	68.6	60.8	68.1	68.3	68.8	68.1	66.8	64.1	21.2
			Number of Components of Indicated Type[b]						
$C_{10}H_{12}Cl_6$	3		1	0	0	0	0	0	2
$C_{10}H_{11}Cl_7$	29		2	2	6	6	6[c]	8[c]	7
$C_{10}H_{10}Cl_8$	64		11	9	11[d]	11[d]	14[d]	13	19
$C_{10}H_9Cl_9$	31		8	9	8	6	4	6	6
$C_{10}H_8Cl_{10}$	7		4	5	2	0	0	0	0
$C_{10}H_7Cl_{11}$	1		0	1	0	0	0	0	0
$C_{10}H_{10}Cl_6$	15		3	0	0	2	2	5	5
$C_{10}H_9Cl_7$	13		0	0	2	1	3	7	4
$C_{10}H_8Cl_8$	12		4	5	1	0	0	0	4
$C_{10}H_7Cl_9$	2		2	0	0	0	0	0	0
Total	177		35	31	30	26	29	39	47
				LD_{50}, mg/kg					
Mouse, ip	42	45[e]	252	110	16	25	52	63	194
House fly, topical	70	75[e]	316	139	42	33	76	101	171

[a] The per cent calculated composition for $C_{10}H_{10}Cl_8$ is: C, 29.0; H, 2.4; Cl, 68.5. [b] Adapted from Holmstead *et al.* (1974). Several of the components appear in two or three successive fractions from the silica gel column so this method of tabulation results in some duplication and more components in summating the fractions then the total for toxaphene where duplication is not involved. [c] Includes toxicant B. [d] Includes toxicant A. [e] Expected value calculated as harmonic mean, *i.e.* $\langle X\rangle_H = N/\Sigma(1/X_i)$ or LD_{50} of mixture = $7/(1/LD_{50}$ of I + $1/LD_{50}$ of II + . . . $1/LD_{50}$ of VII).

F-254 chromatoplates of 0.25 mm layer thickness impregnated with dimethylformamide (Khalifa *et al.*, 1974) are 0.55, 0.51, and 0.34, respectively, on development with pentane. $Na^{36}Cl$ was from the Radiochemical Centre, Amersham, England.

Seven fractions of [^{36}Cl]toxaphene were obtained by chromatographing a 200-mg sample of this material on a column (33 cm × 1.9 cm i.d.) of silica gel 60 (30–70 mesh; EM Laboratories Inc., Elmsford, N.Y.; 60 g) developed, as shown in Figure 1, with pesticide grade hexane (Fisher Scientific Co., Fair Lawn, N.J.) and then a hexane–ether (9:1) mixture collecting 10-ml fractions at 20 drops/min. This procedure gives two–four peaks of chlorine-containing materials but does not separate individual components. Accordingly, the eluted materials were combined into seven fractions of equal chlorine content, designated I–VII in their order of elution (Figure 1, Table I). Reproducible fractions of very similar composition to those obtained with [^{36}Cl]toxaphene were prepared from unlabeled toxaphene by fortifying it with tracer levels (5% w/w) of the ^{36}Cl compound and identical fractionation. The total weight of chlorine recovered from the column was 93–96% in all cases.

Intercomparisons by gc with the electron capture (EC) detector of unlabeled toxaphene and [^{36}Cl]toxaphene and the corresponding fractions obtained from these materials on silica gel column chromatography established that the same peaks are present in both preparations and in almost identical ratios. Thus, results obtained with [^{36}Cl]toxaphene should be applicable to unlabeled toxaphene. The gc pattern (see below) and toxicity to house flies are similar for [^{14}C]toxaphene and unlabeled toxaphene indicating that this sample of [^{14}C]toxaphene is appropriate for use in preliminary studies on the metabolic fate of the carbon skeleton.

Toxaphene and certain of its fractions or metabolites were examined by gc using a glass column (180 cm × 2 mm i.d.) containing 3% SE-30 on Gas-Chrom Q (80–100 mesh) at 170, 180, or 190° with a N_2 flow rate of 75 ml/min and an EC or flame ionization (FI) detector (Khalifa *et al.*, 1974). In some analyses of toxaphene and its metabolites, 3% Dexil-300 on Varaport-30 (100–120 mesh) was used under the same conditions except at column temperatures of 150 and 200° with the EC detector to supplement studies with the SE-30 column. The conditions for gc–CI–ms on the Dexil-300 column are given by Holmstead *et al.* (1974).

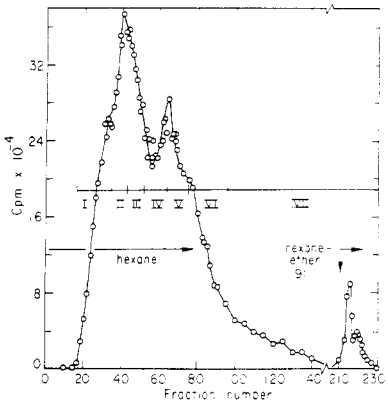

Figure 1. Chromatography of [^{36}Cl]toxaphene on a silica gel column to obtain seven fractions (I–VII) of equal total chlorine content.

Thin-layer chromatography involved the use of silica gel 60 F-254 chromatoplates (20 × 20 cm, 0.5 mm layer thickness, EM Laboratories Inc.) developed with pentane, benzene, chloroform–methanol, or ethyl acetate–methanol mixtures without special precautions to achieve saturation conditions. Chlorine-containing compounds were detected by use of the diphenylamine spray reagent followed by exposure to uv light (Khalifa *et al.*, 1974) while radioactive compounds were detected by autoradiography.

Treatment of Rats and Collection of Excreta. Individual male albino rats (Sprague-Dawley strain, Horton Lab-

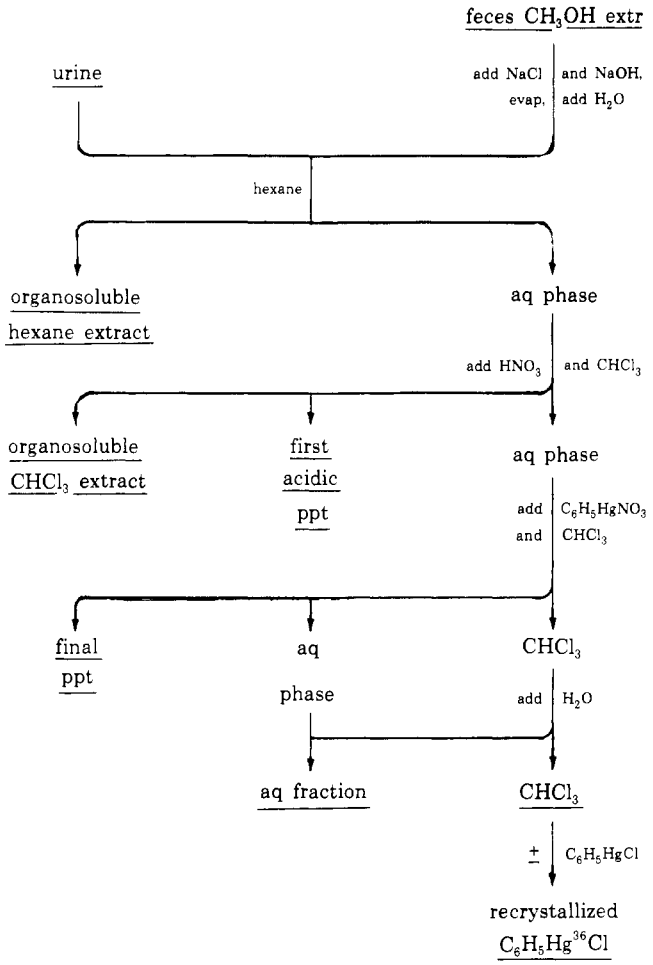

Figure 2. Procedure for fractionation of excreta of rats treated with [^{36}Cl]toxaphene, [^{36}Cl]toxaphene fractions, [^{14}C]toxaphene, and sodium [^{36}Cl]chloride. The fractions underlined are those quantitated. [^{36}Cl]Chloride is determined as phenylmercuric [^{36}Cl]chloride by isotope dilution (*i.e.*, ± unlabeled C_6H_5HgCl).

oratories Inc., Oakland, Calif.) were treated orally with each of the ^{36}Cl- and ^{14}C-labeled chlorinated hydrocarbons. Specific activities for these materials are given later in Table II. [^{36}Cl]Toxaphene and each of the seven fractions of [^{36}Cl]toxaphene were administered at about 13 mg/kg using rats weighing 250–290 g with 150 μl of corn oil as the vehicle and 100 μl of corn oil as a rinse for the stomach tube. [^{14}C]Toxaphene was administered at 8.5 and 19.0 mg/kg whereas purified ^{14}C-labeled toxicant A and pure ^{14}C-labeled toxicant B were administered at 0.84 and 2.6 mg/kg, respectively, in each case using rats weighing 200–225 g, 250 μl of corn oil as the administration vehicle, and 250 μl of corn oil as a rinse for the stomach tube. In parallel studies, rats were fed either corn oil lacking radiolabeled material or an aqueous solution of Na^{36}Cl (24 mg/kg). The rats receiving ^{36}Cl-labeled compounds were held in wire screen cages with glass collectors for urine and feces while those receiving ^{14}C-labeled compounds were held in all glass metabolism cages for collection not only of urine and feces but also of $^{14}CO_2$ and other volatile products utilizing the procedure of Krishna and Casida (1966). In all cases, the rats received Purina rat chow and water *ad libitum*.

Urine and feces were separately collected at 24-hr intervals for 14 days except with ^{14}C-labeled toxicant B where the experimental period was 9 days. After the daily collections, all glassware contacted by the urine was rinsed with 50% aqueous methanol, recovering urinary products remaining on the glass surfaces; these washings were combined for the entire experimental period (220–450 ml final volume, 4.4 ± 3.6% of the administered radioactivity for the series of compounds studied) and are tabulated as part of the urinary products. Composite representative samples of the 0–14 day cumulative urine and feces extracts (described below) were prepared for each rat receiving a ^{36}Cl-labeled material or [^{14}C]toxaphene by taking appropriate aliquots from each daily sample. Except when specifically noted otherwise, each experiment was made with two–four rats and the average results are presented.

In an attempt to obtain large amounts of fecal metabolites for analysis, male rats (160–180 g) were treated every 48 hr by the intraperitoneal (ip) route with 50 mg of toxaphene in 100 μl of methoxytriglycol. The feces were collected up until the time of death. The LD_{50} value for the cumulative toxaphene dose was 700–2000 mg/kg administered over a 10–12-day period. On sacrifice, the rats were found to contain unusual lumps of material associated with the omentum and the abdominal wall. Analysis of this material by gc–EC established that it was unmetabolized toxaphene so a portion of the administered compound was probably deposited there shortly after the injections. The feces of these rats were mixed prior to metabolite analysis.

Standard Procedure for Fractionation of Metabolites in Urine and Feces of Rats Receiving [^{36}Cl]Toxaphene, Each of Seven Fractions of [^{36}Cl]Toxaphene, and [^{14}C]Toxaphene. In preliminary studies, designed to analyze [^{36}Cl]chloride as a possible metabolite of [^{36}Cl]toxaphene, the excreta fractions in aqueous acetone solution were treated with $AgNO_3$ to precipitate Ag^{36}Cl, a procedure found subsequently to lack the desired specificity in that it precipitated labeled materials in addition to Ag^{36}Cl. Accordingly, a procedure was needed for [^{36}Cl]chloride analysis that would fit the following specifications: specific for [^{36}Cl]chloride without interference from other urinary and fecal metabolites of toxaphene; applicable to very low levels of [^{36}Cl]chloride in the excreta; involving mild conditions so that toxaphene and its metabolites are not degraded to [^{36}Cl]chloride during analysis. These specifications were met by modifying the procedure used by Belcher *et al.* (1971), designed for recovery of chloride from aqueous solutions as phenylmercuric chloride (C_6H_5HgCl) for gc analysis, in order to obtain an isotope dilution method of analysis for [^{36}Cl]chloride. The isotope dilution procedure is specific for [^{36}Cl]chloride since no urinary or fecal metabolites of [^{14}C]toxaphene appear in the recrystallized C_6H_5HgCl fraction. It is suitable for low levels of radioactivity since large amounts of C_6H_5HgCl are soluble in the scintillation mixture and do not quench significantly on liquid scintillation counting (LSC). The procedure requires neutralization of any acidic samples prior to evaporation to prevent loss of [^{36}Cl]chloride as H^{36}Cl. Under the mild alkaline conditions required for this neutralization there is no degradation of [^{36}Cl]toxaphene to yield [^{36}Cl]chloride and it is also unlikely that the [^{36}Cl]toxaphene metabolites are degraded under these conditions.

The standard procedure used for analysis of the urine and methanol extracts of feces for toxaphene metabolites is given in Figure 2 and detailed below. In fortification studies with urine and feces extracts of animals receiving only corn oil, it was established that [^{36}Cl]toxaphene appears to the extent of 99.1% in the hexane extract and 0.9% in the chloroform extract. On fortification of normal urine with Na^{36}Cl, 99% of the radioactivity appears in the C_6H_5HgCl fraction, with no significant change in specific activity on recrystallization; only 1% appears in the final aqueous fraction and the various precipitates.

The cumulative 0–14-day urine and feces samples from each of the animals receiving [^{36}Cl]toxaphene and fractions of [^{36}Cl]toxaphene were subjected to the same extraction and fractionation procedure as shown in Figure 2. Essentially the same technique was also used for the ex-

creta of rats treated with [^{14}C]toxaphene. This procedure was applied directly to urine but a preliminary extraction and work-up was necessary with the feces to obtain the methanol-soluble portion, which was the only part fractionated. The feces (average weight, 126 g per animal) were steeped in methanol (3 ml/g) for 18 hr and then homogenized for 2 min in a Virtis "45" homogenizer. The mixture was filtered under suction followed by reextraction of the residue by holding it in methanol (3.5 ml/g) for 18 hr and then homogenization before filtration. The methanol extracts were combined. A portion (45–65 ml) of the combined methanol fraction was mixed with unlabeled NaCl (60 mg), raised to an apparent pH of 8.0–9.6 by addition of 1 *N* NaOH (0.5–2.2 ml), and evaporated under reduced pressure to 1–2 ml at <40°, and water was added to make the volume to 7 ml. (It is not necessary to add NaCl to the urine because the normal chloride content in urine is adequate for the fractionation and derivatization procedures.) Extractions in all subsequent steps were carried out by shaking for 2 min followed by centrifugation at 12,000*g* for 10 min unless otherwise indicated.

The first step in the fractionation procedure (Figure 2) involved extraction of the urine (5 ml) and the feces extract (7 ml) with hexane (5 ml × 2 for the urine and 7 ml × 3 for the feces) to obtain the organosoluble hexane extract. The aqueous phase (pH 5.7–9.3) was then acidified to pH 0.6–1.1 by addition of 4 *N* HNO_3 (1.5–8.5 ml) followed by extraction with chloroform (8 ml × 3 for urine and 5 ml × 3 for feces) yielding, after centrifugation, the organosoluble chloroform fraction, the first acidic precipitate, and the aqueous fraction. The aqueous fraction was then poured into 1.1 l. of 0.09% (w/v) phenylmercuric nitrate ($C_6H_5HgNO_3$) aqueous solution prepared by dissolving the nitrate in boiling water and magnetic stirring while cooling. This mixture in a 2-l. separatory funnel was shaken for 2 min, let stand for 15–30 min, and the shaking and standing repeated once more to complete the precipitation of C_6H_5HgCl. Extraction with chloroform (250, 100, and 50 ml in separate extractions) yielded the aqueous phase, the chloroform extract which was backwashed with water (40 ml) for addition to the aqueous phase, and the final precipitate. Ten per cent of the chloroform phase was used directly for determination of radioactivity content. Ninety per cent of the chloroform phase was divided into two equal portions, each of which was evaporated to dryness yielding a residue characterized as follows: average weight 230 mg, range 120–370 mg; average melting characteristic on heating the sample in a sealed capillary tube with a bath of concentrated H_2SO_4, starting at 236°, ending at 244°, range 118–252°, with darkening. To one of the duplicate samples unlabeled C_6H_5HgCl (80 mg) was added; then each sample was recrystallized twice from absolute ethanol (1 ml/10 mg) (64% average recovery) to constant mp (ranging from 249 to 254°; the mp of authentic C_6H_5HgCl was 250–252°) and constant specific activity (4–200 cpm/mg). The specific activities of the recrystallized $C_6H_5Hg^{36}Cl$ were compared for analyses made with and without fortification with unlabeled C_6H_5HgCl in order to determine the [^{36}Cl]chloride content of the excreta by appropriate calculations from the isotope dilution data.

The ^{36}Cl and ^{14}C levels were determined by LSC using 10 ml of methyl Cellosolve–toluene mixture containing 0.55% (w/v) 2,5-diphenyloxazole as the scintillation fluid; the methyl Cellosolve–toluene ratio was 3.2:1 for ^{36}Cl and 1:2 for ^{14}C determinations. The Packard Tri-Carb liquid scintillation spectrometer was used with Na^{36}Cl and [^{14}C]toluene as internal standards. The background level used for calculations was that obtained with the corresponding sample or extract of excreta from a rat treated only with corn oil in the same experiment. The samples for counting were: 0.5 ml of urine and 1 ml of feces methanol extract; the entire hexane extract of urine and 10% of the hexane extract of feces, after solvent evaporation; one-fourth and one-tenth of the urine and feces chloroform extracts, respectively, after solvent evaporation; all of the first acidic precipitate from urine and one-tenth of this fraction from feces in the scintillation mixture, in the former case using Cab-O-Sil (0.7 g) to obtain a suspension; one-tenth of the aqueous fraction (pH 2.0–2.7) from treatment with $C_6H_5HgNO_3$ and chloroform extraction, after addition of 1 *N* NaOH (0.6–3.7 ml) to pH 8.6–10.8 and then evaporation to dryness prior to addition of scintillation mixture using the entire fraction for LSC of the urine and one-third of the sample for feces; all of the final precipitate for urine and one-tenth of it for feces, using Cab-O-Sil in each case for suspension; one-tenth of the chloroform extract containing C_6H_5HgCl; 30–50-mg amounts of each crystalline material in the final analytical stages. The counting of many of these fractions is complicated by intense chemiluminescence giving high spurious counts, a difficulty overcome by storing the samples in the dark for decay of the chemiluminescence until a constant counting rate is obtained; this procedure is critical with samples containing low [^{36}Cl]chloride levels.

Other Procedures Used in Fractionation and Analysis of Urinary and Fecal Metabolites. The possible presence of unmetabolized [^{14}C]toxaphene, ^{14}C-labeled toxicant A and ^{14}C-labeled toxicant B in feces of rats treated with each of these compounds was examined by subjecting the methanol-soluble ^{14}C products to tlc development with benzene, recovery of the region corresponding to unmetabolized material (R_f 0.73 region) and gc-EC analysis on the SE-30 column at 170° or the Dexil-300 column at 200°.

The urine and feces from rats 14 days after treatment with [^{14}C]toxaphene and 1 day after treatment with ^{14}C-labeled toxicant B were used for analysis of acidic ^{14}C-labeled compounds. This involved determination of the extent of ether-extractable ^{14}C from neutral and acidic urine, and two-dimensional tlc of the ether-extractable products from ^{14}C-labeled toxicant B. It also involved treatment of the methanol-soluble fraction of [^{14}C]toxaphene feces with diazomethane and tlc analysis to detect changes in the chromatographic behavior of metabolites resulting from diazomethane treatment.

Several steps were used to purify one fecal metabolite of [^{14}C]toxaphene for gc analysis, as follows: tlc development with benzene (R_f <0.37); treatment with diazomethane; tlc development with chloroform–methanol (10:1) mixture (R_f *ca.* 0.7); two-dimensional tlc in the first direction with hexane and then with benzene (R_f 0.57) followed by a second direction of development with chloroform–methanol (100:1) mixture (R_f 0.57).

Metabolites in the feces of rats receiving large ip doses of unlabeled toxaphene were fractionated by evaporation of the methanol extract equivalent to 5.7 g of feces onto silica gel (3 g) which was then added to a column (32 cm × 1 cm i.d.) of MN silica gel (70–120 mesh, Machery, Nagel and Co., Düren, Germany) (20 g) prepared with benzene. This column was developed in sequence with about 100 ml of each of benzene, benzene–ethyl acetate (1:1) mixture, ethyl acetate, and ethyl acetate–methanol (2:1) mixture with tlc monitoring of the fractions using the diphenylamine–uv method. Fractions containing chloro compounds were then subjected to tlc purification and gc analysis.

Other Analyses. Combustion analyses were used for ^{36}Cl- and ^{14}C-labeled compounds in the feces after methanol extraction and then lyophilization and in the tissues after lyophilization. The combustion procedure was that of Krishna and Casida (1966) with samples weighing up to 100 mg and trapping solutions of 6 ml of methyl Cellosolve–ethanolamine (2:1) mixture for liberated $^{14}CO_2$ and 7.6 ml of methyl Cellosolve for liberated ^{36}Cl-labeled compounds. For LSC of the trapped $^{14}CO_2$ a 2-ml aliquot

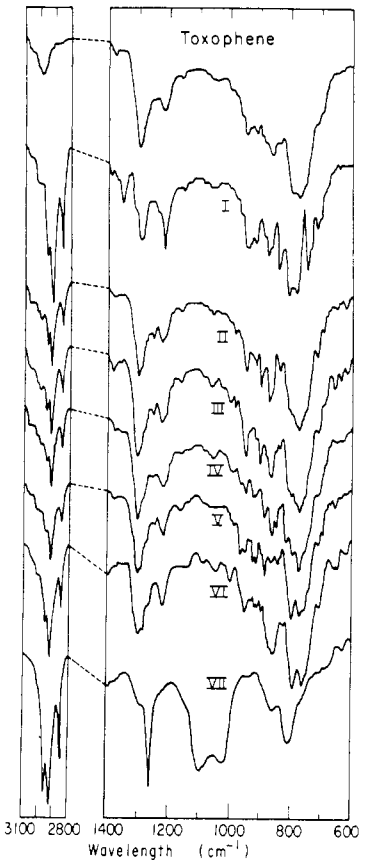

Figure 3. Infrared spectra of toxaphene and fractions I–VII as carbon disulfide solutions.

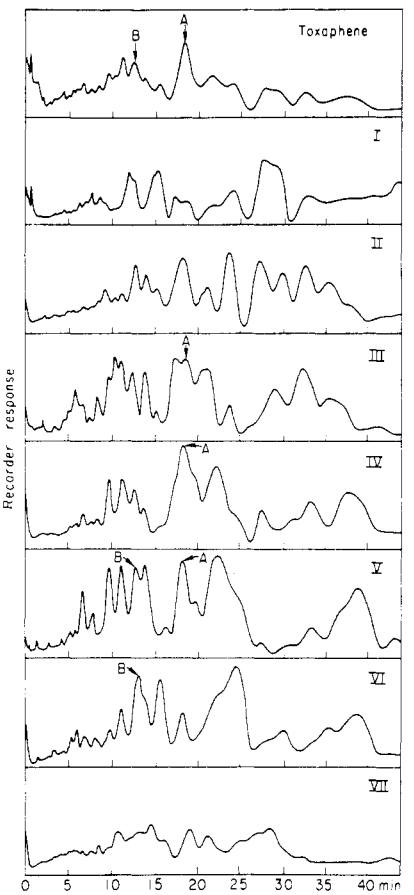

Figure 4. Gas chromatographic patterns of technical toxaphene and fractions I–VII on the SE-30 column at 170° indicating the chromatographic positions of toxicants A and B.

of the methyl Cellosolve–ethanolamine solution was added to 15 ml of the ^{14}C scintillation fluid described above. For ^{36}Cl-labeled compounds, the entire methyl Cellosolve trapping solution was added to the other scintillation fluid ingredients as appropriate to obtain 15 ml of the normal ^{36}Cl scintillation fluid plus 1 ml of water. The small amount of ash, which contained some ^{36}Cl, was washed first with 7.6 ml of methyl Cellosolve and then with 1 ml of water; the washings were combined for LSC as with the trapping solution for volatile ^{36}Cl-labeled compounds. Analyses of fortified feces and tissue samples gave recovery values of 81 ± 6% for the ^{14}C from [^{14}C]toxaphene and 101 ± 5% for the ^{36}Cl from [^{36}Cl]toxaphene or $Na^{36}Cl$. The results reported are corrected for these recovery values and involve two or more determinations in each case.

The bioassay procedures to determine house fly topical and mouse ip LD_{50} values are given by Khalifa *et al.* (1974). Elemental analyses were carried out at the Department of Chemistry, University of California, Berkeley, after the samples were held 2 hr at 80° under reduced pressure (0.1 mm). Infrared (ir) spectra were obtained on 10% (w/v) solutions in carbon disulfide using a Perkin-Elmer Model 457 ir grating spectrophotometer.

RESULTS

Properties of Seven Toxaphene Fractions. Seven toxaphene fractions are used in the metabolism studies so it is important to define their nature, composition, and toxicity.

The first six of the seven fractions approximate an overall elemental composition of $C_{10}H_{10}Cl_8$ as does toxaphene (Table I). The last fraction (VII) has a much lower percentage chlorine content and the average chlorine content of the fractions is less than that for toxaphene itself; this may result from a small degree of dechlorination during chromatography or the presence in fraction VII of impurities from the silica gel or solvent. The ir spectra of fractions II–VI show the possible presence of chloromethyl groups (1305 cm^{-1}) (Figure 3) which are also evident in technical toxaphene and toxicants A and B (Khalifa *et al.*, 1974). These spectra also indicate that fractions I–VII are complex mixtures when compared with the much simpler spectra of toxicants A and B (Khalifa *et al.*, 1974). Each of the seven toxaphene fractions contains many components based on analyses involving tlc (Khalifa *et al.*, 1974), gc–EC (Figure 4), and gc–CI–ms (Table I). The number of apparent components in toxaphene depends on the resolving capability of the overall analytical procedure. Thus, about 25 distinct gc peaks are evident with toxaphene itself but this number is increased to about 65 when partial separation is achieved on the adsorption column prior to gc. More than 177 components are detected when CI–ms analysis is added to the other procedures (Table I; Holmstead *et al.*, 1974). Toxicant A appears in fractions III, IV, and V while toxicant B is in fractions V and VI (Table I, Figure 4). Several other components of toxaphene also appear in two or three adjacent fractions from the silica gel column. It is clear that each of the toxaphene fractions used in the metabolism studies consists of a very complex mixture of related compounds.

The mouse ip and house fly topical potency of the fractions varies from 1.7- to 2.6-fold more toxic than toxaphene (fractions III and IV) to much less toxic than toxaphene (fractions I, II, VI, and VII) (Table I). The toxicity is about ten-fold different for two fractions (I and IV) of identical chlorine content, indicating that the toxicity is not related to the total chlorine content but rather to the specific compounds making up the mixture. Even though toxicants A and B are 14- and 6-fold more toxic, respec-

Table II. Excretion of Radiolabeled Products in the Urine and Feces of Rats within 14 Days after Oral Administration of [^{36}Cl]Toxaphene, Seven Fractions of [^{36}Cl]Toxaphene of Equal Total Chlorine Content, Sodium [^{36}Cl]Chloride, and [^{14}C]Toxaphene

Property or fraction	[^{36}Cl]-Toxaphene	Fractions of [^{36}Cl]toxaphene								Na^{36}Cl	[^{14}C]-Toxaphene
		Overall av	I	II	III	IV	V	VI	VII		
					Specific Activity						
μCi/g	43.6	40.4	40.8	43.1	46.1	45.7	45.0	41.2	21.0	23.6	1350
					Average Oral Dose						
mg/kg	14.2	13.4	8.4	14.3	15.2	10.3	13.5	14.9	17.1	24	12.7
					Radioact. in Excreta Fractions, % of Administered Dose at 14 Days						
Urine											
Organosoluble											
Hexane extr	0.5	0.7	0.7	0.6	0.8	0.6	0.8	0.5	1.0	0.0	0.7
Chloroform extr	2.8	3.5	3.0	4.0	3.3	3.6	3.2	4.2	3.4	0.1	14.6
C_6H_5HgCl fraction	44.0[a]	51.2[a]	56.9[a]	54.3[a]	42.2[a]	56.5[a]	46.2[a]	52.9[a]	49.5[a]	90.1[a]	3.4[b]
Other fractions											
First acidic ppt	0.6	0.3	0.5	0.4	0.5	0.3	0.1	0.2	0.2	0.7	0.1
Final ppt	0.7	0.3	0.0	0.3	1.0	0.0	0.2	0.3	0.3	0.2	0.9
Aq fraction	0.5	0.4	0.0	0.0	0.0	1.6	0.9	0.2	0.0	0.2	5.7
Total	49.1	56.4	61.1	59.6	47.8	62.6	51.4	58.3	54.4	91.3	25.4
Feces											
Organosoluble											
Hexane extr	11.9	11.2	11.6	12.6	10.2	14.0	11.4	10.3	8.7	0.1	8.0
Chloroform extr	8.4	7.7	11.9	6.7	7.0	8.2	7.5	6.6	5.7	0.0	12.4
C_6H_5HgCl fraction	6.3[b]	4.6	4.7	4.7	3.9	8.2	3.8	3.2	3.4	3.7	3.2[b]
Other fractions											
First acidic ppt	0.2	0.5	0.6	0.5	0.4	0.6	0.6	0.4	0.6	0.0	1.4
Final ppt	0.1	0.3	0.2	0.6	0.0	0.4	0.4	0.3	0.4	0.1	1.0
Aq fraction	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.7
Total	26.9	24.3	29.0	25.1	21.5	31.4	23.7	20.8	18.8	3.9	31.7
Total excreta[c]	76.0	80.7	90.1	84.7	69.3	94.0	75.1	79.1	73.2	95.2	57.1

[a] At least 95% of the radioactivity is attributable to $C_6H_5Hg^{36}Cl$ based on isotope dilution analysis. [b] Radioactivity is not retained in the recrystallized C_6H_5HgCl. [c] Urine plus methanol-soluble products from feces. Combustion analyses showed that the methanol-insoluble fraction of feces contained 9.8% of the administered radioactivity as an overall average for the [^{36}Cl]toxaphene fractions, 1.3% with Na^{36}Cl, and 29.1% with [^{14}C]toxaphene. Thus, the total recoveries in the excreta averaged 90.5% with the [^{36}Cl]toxaphene fractions, 96.5% with Na^{36}Cl, and 86.2% with [^{14}C]toxaphene.

tively, than toxaphene to mice they are relatively minor components of toxaphene (Khalifa *et al.*, 1974) and of fractions III–VI (Table I). Most of the remaining components of toxaphene present in each of fractions I–VII must be of low toxicity to mice, relative either to toxaphene or to toxicants A and B. There is no indication of interactions in the toxicity of the seven fractions, based on comparing the harmonic mean of their LD_{50} values with the LD_{50} of toxaphene under the same conditions (Table I).

Dechlorination of [^{36}Cl]Toxaphene and Seven [^{36}Cl]Toxaphene Fractions following Their Oral Administration to Rats. [^{36}Cl]Toxaphene and each of the seven fractions of [^{36}Cl]toxaphene give essentially the same pattern for excretion of radioactive products in urine and the methanol extracts of feces (Table II, Figure 5). In addition, there is no significant difference between [^{36}Cl]toxaphene and any of its fractions in regard to the quantitative data on fractionation of their urinary and fecal products (Table II). On this basis, the results obtained with [^{36}Cl]toxaphene and the average of the results obtained with the ^{36}Cl fractions are considered together in interpreting the nature of the toxaphene metabolites.

The only identified metabolite of [^{36}Cl]toxaphene is the [^{36}Cl]chloride ion which appears almost entirely in urine and accounts for about half of the administered radioactivity (Table II, Figure 5). [^{36}Cl]Chloride ion from metabolism of [^{36}Cl]toxaphene is excreted at almost the same rate as that resulting from the direct administration of Na^{36}Cl, with a half-time for elimination of about 2–3 days

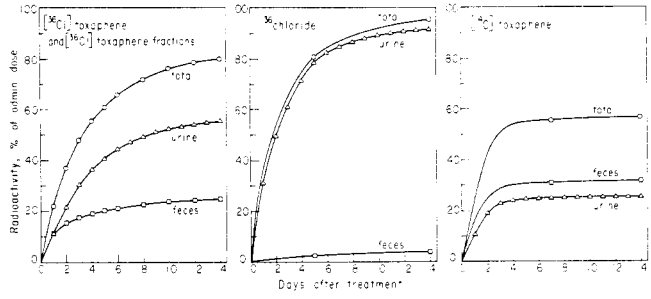

Figure 5. Excretion of radioactive products by rats treated orally with [^{36}Cl]toxaphene, [^{36}Cl]toxaphene fractions, sodium [^{36}Cl]chloride, and [^{14}C]toxaphene. Only the methanol-soluble products in the feces are shown (see footnote *c* of Table II).

in each case. Only 5–6% of the radioactivity from Na^{36}Cl administration appears in feces relative to the amount in urine. Although it is possible that the ^{36}Cl in the C_6H_5HgCl fraction of feces following Na^{36}Cl administration may be an artifact resulting from contamination of the feces by urine during collection, this does not appear to be involved for this fraction from [^{36}Cl]toxaphene administration since in this case the radioactivity is lost on recrystallization of the C_6H_5HgCl. In any case, the results indicate that the dechlorination of toxaphene, to the extent it occurs, takes place largely within the first day or two after toxaphene administration. Although about half

Table III. Excretion and Expiration of Radiolabeled Products by Rats after Oral Administration of ^{14}C-Labeled Preparations of Toxaphene and Toxicants A and B

Sample and days after treatment	Radiocarbon excreted or expired, % of administered dose: Toxaphene 8.5 mg/kg, 14 days, 3 rats	Toxaphene 19.0 mg/kg, 14 days, 2 rats	Toxicant A, 0.84 mg/kg, 14 days, 1 rat	Toxicant B, 2.6 mg/kg, 9 days, 1 rat
Urine 0–1	10.6	11.3	18.9	15.7
1–2	6.3	11.8	6.0	6.2
2–sacrifice	4.4	8.7	3.4	4.8
Feces[a] 0–1			27.4	32.2
1–2	34.7	27.1	6.0	9.7
2–sacrifice			5.0	5.9
$^{14}CO_2$ 0–1	0.8		0.8	0.5
2–sacrifice	0.4		1.0	0.2
Total	57.2	58.9	68.5	75.2

[a] Methanol-soluble fraction only.

of the carbon–chlorine bonds are cleaved on metabolism of toxaphene and each of the seven fractions, this value is an average one since some of the metabolites are probably formed by partial dechlorination and others by complete dechlorination as discussed below.

Nature of Urinary and Fecal Metabolites Based on Comparative Studies with [^{36}Cl]- and [^{14}C]Toxaphene and ^{14}C-Labeled Toxicants A and B. The data shown in Table II for [^{14}C]toxaphene at an oral dose of 12.7 mg/kg are the average of the results found with three rats at 8.5 mg/kg and two rats at 19.0 mg/kg since no dose-related difference was found in the metabolite distribution among various urine and feces fractions.

The results obtained with the ^{36}Cl and ^{14}C preparations of toxaphene (Table II) differ in several respects that are interpretable relative to the nature of the excreted metabolites. With either labeled preparation, the hexane-soluble fraction, which would contain unmetabolized components of toxaphene, is in very small amount with urine and accounts for 8–12% of the administered dose with feces. Very little of the total excreted material consists of unmetabolized toxaphene components as discussed later. On considering both the urine and feces, the chloroform-soluble fraction contains a much higher proportion of the administered ^{14}C (27%) than of the ^{36}Cl (11.2%) so this fraction consists of partially dechlorinated metabolites; on an individual basis, the $^{14}C/^{36}Cl$ ratio for the chloroform-soluble fraction indicates a greater degree of dechlorination for the products in urine than for those in the feces. A portion of the urinary and fecal radioactivity from [^{14}C]toxaphene appears in the crude C_6H_5HgCl fraction but this radioactivity is not retained in the C_6H_5HgCl on repeated recrystallization. Thus, the radioactivity of the crude C_6H_5HgCl fraction from urine consists almost entirely of $C_6H_5Hg^{36}Cl$ following [^{36}Cl]toxaphene administration and yet metabolites of [^{14}C]toxaphene accounting for 3.4% of the administered dose also appear in this fraction prior to recrystallization; this apparent anomaly suggests that the nonchloride components of the crude C_6H_5HgCl fraction consist of metabolites of high $^{14}C/^{36}Cl$ ratio, *i.e.*, metabolites formed by partial to extensive dechlorination. The precipitate fractions contain little radioactivity in any case. The final aqueous fraction with 11.4% of the ^{14}C dose and only about 0.5% of the ^{36}Cl dose must contain almost entirely metabolites formed by processes involving complete dechlorination. After methanol extraction of the feces, the residue contains 29.1% of the ^{14}C and 9.8% of the ^{36}Cl dose indicating that these metabolites are partially dechlorinated compounds. Thus, the excreted products from toxaphene administration include a small amount of unmetabolized material, larger amounts of partially dechlorinated products, and some compounds that appear to be completely dechlorinated.

Radioactivity from ^{14}C-labeled toxicants A and B is excreted as rapidly and completely or more so than that from [^{14}C]toxaphene (Table III). A small portion of the dose is expired as $^{14}CO_2$ or other volatile products, *i.e.* 1.2, 1.8, and 0.7% of the administered ^{14}C with toxaphene, toxicant A, and toxicant B, respectively. In a comparable study of 48-hr duration with male mice, 1.4% of the radioactivity was found in the $^{14}CO_2$ or other volatile products following ip administration of [^{14}C]toxaphene at a dose of 0.29 mg/kg using methoxytriglycol as the vehicle.

The feces of rats 14 days after treatment with [^{14}C]toxaphene and one day after treatment with ^{14}C-labeled toxicants A and B contain 3.4, 8.1, and 2.6%, respectively, of the administered dose as labeled materials chromatographing on tlc with benzene development in the region of the unmetabolized compound. This radioactive region from toxaphene administration gave a very similar gc–EC pattern on both the SE-30 and Dexil-300 columns to that obtained with standard toxaphene. This material with toxicants A and B yielded the following EC-sensitive peaks on the SE-30 column at 180°: R_t 4.7, 5.7, 6.7, 8.4, and 10.9 min with toxicant A; R_t 4.7 and 7.6 min with toxicant B. The peak of longest retention time is attributable to the unmetabolized material and it is the major peak in each case. These findings indicate that a portion of the administered compound is excreted without metabolism and that the feces also contain metabolites with a tlc polarity similar to that of the unmetabolized compound but of shorter gc retention time.

The urinary and fecal metabolites of [^{14}C]toxaphene and the urinary metabolites of ^{14}C-labeled toxicant B probably include acidic compounds based on three types of evidence. First, after all toxaphene and some neutral urinary and fecal metabolites of [^{14}C]toxaphene are removed by extracting the neutral aqueous solution with hexane, subsequent acidification and extraction with chloroform recovers 14.6 and 12.4% of the administered ^{14}C from the urine and feces, respectively (Table II). This additional recovery might result from either or both of the acidifications and the change to a better extracting solvent. Second, the urinary metabolites of either toxaphene or toxicant B recovered by ether extraction at pH 6.6–9.3 account for only 2–3% of the administered ^{14}C, whereas a subsequent ether extraction after acidification to pH 0.9–2.0 recovers an additional 8% of the administered ^{14}C. The products in the ether extracts of toxicant B urine were subjected to two-dimensional tlc development, first with an ethyl acetate–methanol (10:1) mixture and then with an ethyl acetate–methanol (2:1) mixture. Almost all of the metabolites move free from the origin and the eight or more spots obtained with the extract at neutral pH differ in chromatographic behavior from a comparable number of spots obtained with the extract at acidic pH. The products recovered only after acidification are likely to be acidic compounds. Third, treatment of the methanol-extractable products of [^{14}C]toxaphene feces with diazomethane changes the properties of the ^{14}C components as follows: the ether-soluble ^{14}C is 35 and 60–70% of the total ^{14}C without and with diazomethane treatment, respectively; tlc development with a chloroform–methanol (5:1) mixture reveals four major bands (R_f 0.00, 0.08, 0.17, and 0.60) without diazomethane treatment and only one major band (R_f 0.54) after this treatment.

Several metabolites appear in the 0–24-hr feces of rats treated with ^{14}C-labeled toxicants A and B based on tlc development first with benzene, which moves unmetabo-

lized material and some metabolites to R_f 0.73, and then two-thirds of the way up the chromatoplate with a more polar solvent system. The polar metabolites collectively account for 19 and 30% of the administered dose, respectively, and appear at the following R_f values for the second tlc development: 0.00, 0.43, 0.57, and 0.76 with toxicant A and development with a chloroform–methanol (1:1) mixture; 0.00, 0.49, 0.71, and 0.80 with toxicant B and development with an ethyl acetate–methanol (10:1) mixture.

One fecal metabolite of [^{14}C]toxaphene representing 3–6% of the administered ^{14}C was purified by a multistep procedure including tlc and treatment with diazomethane. Analysis by gc on a Dexil-300 column at 150° revealed a single product (R_t 10 min) with an EC detector whereas three major peaks (R_t 6.3, 12.5, and 14.1 min) appeared with the SE-30 column at 190° with a FI detector. When examined by gc–CI–ms (Dexil-300, temperature program), two major and two minor peaks are detected, only one of the minor peaks containing chlorine. The CI mass spectrum of this chlorine-containing material gives as a major fragment an ion corresponding to $[C_6H_5Cl_4]^+$. This is not only the major fragment but it is also the highest mass fragment obtained. The molecular composition of the product is likely to be $C_6H_5Cl_4X$ where the X group, although unidentified, may be Cl, OH, or a sulfur-containing substituent which is particularly labile under the CI conditions involved. The polarity of the compound on tlc tends to favor an X group other than chlorine. It is not likely that the diazomethane treatment during isolation resulted in any modification of its structure unless a portion of the sulfur-containing substituent underwent methylation.

Methanol extracts of feces from rats receiving large ip doses of unlabeled toxaphene contain toxaphene and at least four other compounds that give a blue-green color with the diphenylamine–uv detection method after silica gel column chromatography and then tlc resolution with an ethyl acetate–methanol (5:1) mixture. Toxaphene appears in the benzene eluate (gc pattern on SE-30 at 180° similar to the standard), an R_f 0.65 metabolite in the benzene–ethyl acetate (1:1) eluate, and three metabolites (R_f 0.22 with rapid color development and R_f 0.06 and 0.41 with very slow color development) in the ethyl acetate–methanol (2:1) eluate. Purification by tlc with ethyl acetate–methanol (10:1) and then analysis by gc–EC (SE-30, 170°) gave the following results: benzene–ethyl acetate fraction, peaks at R_t 13.5, 16.1, and 19.7 min; ethyl acetate–methanol fraction, major peaks at 5.4, 13.5, and 16.4 min plus many minor peaks. It is possible that either the 16.1- or 16.4-min peak, respectively, is the $C_6H_5Cl_4X$ metabolite noted above with [^{14}C]toxaphene since it gives R_t 16.1 min under the same conditions. This suggests that the feces of rats treated with large ip doses of unlabeled toxaphene may be a convenient source of metabolites for structure determination.

Distribution of Radiocarbon in Tissues of Rats Administered [^{14}C]Toxaphene and ^{14}C-Labeled Toxicant B. The parts per million levels of toxaphene and toxicant B or their metabolites in tissues based on total radiocarbon analyses were as follows: 0.78 ppm in fat and 0.30 ppm in liver 14 days after a 19.0 mg/kg dose of [^{14}C]toxaphene; 0.52 ppm in fat, 0.12 ppm in liver, 0.17 ppm in kidney, 0.14 ppm in blood, and 0.02–0.09 ppm in each of bone, brain, heart, lung, muscle, spleen, and testes 14 days after an 8.5 mg/kg dose of [^{14}C]toxaphene; 0.12 ppm in liver, 0.09 ppm in kidney, 0.07 ppm in blood, and <0.03 ppm in other tissues 9 days after a 2.6 mg/kg dose of ^{14}C-labeled toxicant B. A fat sample was not available for analysis with the ^{14}C-labeled toxicant B rat. These findings indicate that toxaphene and toxicant B and their metabolites are not persistent in orally treated rats. Attempts to obtain meaningful data on tissue levels of ^{36}Cl-labeled compounds in rats 14 days after treatment with [^{36}Cl]toxaphene and its seven fractions proved fruitless due to the low specific activity of the administered materials.

DISCUSSION

Knowledge of the metabolic fate of a pesticide is important in defining the mechanism of residue dissipation, the nature of persisting residues, and the requirements for an appropriate method of residue analysis. This information is most difficult to obtain with multicomponent pesticides such as toxaphene. In the present study, particular emphasis is given to determining the extent of metabolic dechlorination because of the concern on persistence of organochlorine compounds. The extent of dechlorination is compared for toxaphene, a mixture of more than 177 C_{10} polychloro compounds, and for each of seven fractions of toxaphene which are still complex mixtures containing, at a minimum, 26 to 47 compounds in each case. This is a convenient number of fractions for metabolic studies but it is also an arbitrary number so these fractions from a silica gel–hexane column are defined as to their nature, composition, and toxicity to evaluate if the ease of dechlorination is correlated with the toxicity of the mixtures. The metabolic fate is then compared for [^{36}Cl]- and [^{14}C]toxaphene in different animals by analysis of the isotope distribution in various solvent extracts and crystalline derivatives prepared from the urine and feces. Fractions containing the same ^{36}Cl/^{14}C ratio as that administered might contain unmetabolized compound whereas fractions with almost no ^{36}Cl and yet large amounts of ^{14}C must consist of partially or completely dechlorinated toxaphene metabolites. Purified or pure ^{14}C-labeled toxicants A and B are then examined to evaluate the persistence of the toxaphene components of highest acute toxicity to mice treated by the ip route and possibly to mammals in general.

[^{36}Cl]Toxaphene and the seven fractions of this material undergo essentially the same extent of dechlorination; about half of the carbon–chlorine bonds, on an average, are cleaved prior to excretion of the metabolites. Within the limitations of the analytical methods used, the metabolites other than chloride ion also appear to be similar in composition and amount with each of the seven fractions. There are large toxicity differences between the [^{36}Cl]toxaphene fractions but this is not reflected in their extent of metabolism. The toxicity is attributable, in the most part, to a few toxaphene components whereas the ease of metabolic dechlorination is shared by most if not all of the components and is probably independent of the structural features that confer high toxicity.

The fact that half of the carbon–chlorine bonds in toxaphene undergo cleavage suggests the possibility that either all of the components have in common certain substituent groups that are easily dechlorinated and others that are not or that about half of the toxaphene components undergo complete dechlorination while the remainder are relatively resistant to dechlorination. However, neither of these alternatives is consistent with the available metabolic data comparing [^{36}Cl]- and [^{14}C]toxaphene preparations. A small portion of the dose, up to about 3%, is excreted in feces as unmetabolized material. One minor toxaphene metabolite appears to be a $C_6H_5Cl_4X$ compound; even though the X group is not defined it is likely that a multistep metabolic sequence is involved in formation of this material. Twenty-seven per cent of the [^{14}C]toxaphene dose appears in the chloroform fraction from urine and feces and this probably consists of partially dechlorinated metabolites. More than 5% and possibly 10% of the dose is excreted in the urine and feces as completely dechlorinated metabolites that appear in the aqueous fraction. Some of the metabolites are acidic compounds for which possible structures are mercapturic

acids, terpene carboxylic acids and their glucuronides, and terpene alcohols as their glucuronides or sulfates. About 2% of the ^{14}C appears as expired products, probably $^{14}CO_2$, and this portion arises from methyl, chloromethyl, or dichloromethyl substituents in the original toxaphene components. On the assumption that most of the toxaphene components are polychlorobornanes related to the identified 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane and in light of the extensive or even complete metabolic dechlorination of some components, a portion of the terminal metabolites may be monocyclic or acyclic compounds.

Even though most of the toxaphene components undergo rapid metabolism in mammals, there are probably large rate differences for metabolism of the various components. The components of highest toxicity are presumed to be those that combine appropriate configurations for disruption of nerve activity with some degree of resistance to metabolism. However, limited evidence from studies with toxicants A and B indicates that they are no more persistent in rats than most of the other toxaphene components. A portion of the dose of these compounds is excreted in feces without metabolism. Some of the metabolites have gc properties similar to those of other toxaphene components. Thus, it is likely that tissue residues will include metabolites formed by dechlorination that fall within the normal gc range of the toxaphene components, a complication in gc residue analyses. Tissue analyses for total radioactivity indicate that toxaphene components are not persistent materials in rats. However, further studies are needed to define the chemical nature of the tissue residues resulting from administration of toxaphene and of individual toxaphene components, such as toxicants A and B. The gc–CI–ms technique appears to be an appropriate method for these investigations. There is also a need for information on the biological activity of the toxaphene metabolites.

Toxaphene is a complex mixture of related C_{10} polychloro compounds but most if not all of these components undergo extensive metabolic dechlorination in rats. Toxaphene differs in this respect from many other chlorinated hydrocarbon insecticides and environmental pollutants.

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Metabolism of (+)-*trans*- and (+)-*cis*-Resmethrin in Rats

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Rats treated orally at about 1 mg/kg with either 5-benzyl-3-furylmethyl (+)-*trans*-chrysanthemate [(+)-*trans*-resmethrin] or the corresponding (+)-cis compound excrete the following metabolites: no esters with (+)-*trans*-resmethrin; two unidentified esters with (+)-*cis*-resmethrin; 5-benzyl-3-furoic, 5-(α-hydroxybenzyl)-3-furoic, and 5-(4′-hydroxybenzyl)-3-furoic acids; chrysanthemic acid, chrysanthemumdicarboxylic acid, and the intermediate alcohol and aldehyde oxidation products; conjugates of each of these acids. The metabolite(s) persisting longest in the body is derived from the alcohol moiety of (+)-*trans*-resmethrin. The isobutenyl moiety is oxidized at either the cis or trans methyl group with (+)-*cis*-resmethrin but only at the trans methyl group with (+)-*trans*-resmethrin. An unanticipated metabolic pathway involves epimerization at C-3 of the cyclopropane group leading to excretion of isomerized forms of chrysanthemumdicarboxylic and hydroxylated chrysanthemic acids. Three of the (+)-*trans*-resmethrin metabolites are much more toxic than the parent compound.

Resmethrin consists of two highly insecticidal components, the (+)-trans isomer (bioresmethrin) and the (+)-cis isomer, and two noninsecticidal components, the corresponding (−) isomers, in an approximate 40:10:40:10 ratio, respectively (Elliott, 1971; Elliott *et al.*, 1967; Jao and Casida, 1975). The acute oral toxicity to rats is more than 48-fold greater for (+)-*cis*-resmethrin than for the (+)-trans compound (Verschoyle and Barnes, 1972). (±)-*trans*-Resmethrin undergoes rapid hydrolysis in orally treated rats followed by oxidation of the alcohol moiety to yield benzylfuroic acid and several oxidized derivatives of this acid which are excreted in free or conjugated form (Miyamoto *et al.*, 1971). These studies did not define the fate of the alcohol moiety from *cis*-resmethrin or the chrysanthemate moiety from either *trans*- or *cis*-resmethrin. Mouse liver microsomal esterases hydrolyze (+)-*trans*-resmethrin 8- to 14-fold more rapidly than (+)-*cis*-

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