Toxaphene Dissipation from Treated Cotton Field Environments: Component Residual Behavior on Leaves and in Air, Soil, and Sediments Determined by Capillary Gas Chromatography

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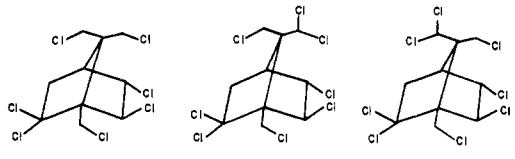
Residues of toxaphene were analyzed in leaf, air, and top soil samples taken up to 58 days following a commercial application at 9 kg/ha to a San Joaquin Valley, California, cotton field. Analyses were by packed column gas–liquid chromatography (GLC) for total toxaphene residues and by open-tubular (capillary) column GLC for enhanced component resolution. Leaf residues at 50 days following treatment (135 ppm) showed a regular trend toward greater loss of components of higher volatility when compared with the 0-day sample (661 ppm). This was matched by a corresponding enrichment of volatile components in air samples, most noticeable in an early (2-day) sample (1.8 $\mu g/m^3$) but still evident in a later (14-day) sample (1.9 $\mu g/m^3$). Vaporization was indicated to be the major route of loss from foliage, with no noticeable role played by chemical decomposition. Decline from aerated top soil (from 13.1 ppm at 0 days to 6.4 ppm at 58 days) was also primarily by vaporization, but at least one component was significantly degraded. Soil core and irrigation ditch residues sampled more than a year after treatment showed extensive toxaphene component degradation, resulting in selective decline of some components. The evidence indicated that anaerobic reduction occurred in these environmental compartments.

Toxaphene, chlorinated camphene, has been widely used as a contact insecticide on a variety of food, feed, and fiber crops since its introduction in 1947. In 1973, over 1.3 million kilograms of toxaphene was used in California alone (Pesticide Use Report, 1973). Usage since that year, particularly the major applications for the chemical to cotton and tomatoes (Table I), continued to be substantial in comparison with other insecticides.

The residual behavior of toxaphene is complicated by the multicomponent nature of the commercial product. Holmstead et al. (1974) showed technical toxaphene to consist of at least 177 separate compounds. Three components, 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (toxicant B, 1) (Palmer et al., 1975) and an isomer mixture



TOXICANT B TOXICANT A-1 TOXICANT A-2

1 2 3

of 2,2,5-*endo*,6-*exo*,8,8,9,10-octachlorobornane (toxicant A-1, 2) and 2,2,5-*endo*,6-*exo*,8,9,9,10-octachlorobornane (toxicant A-2, 3) (Turner et al., 1975; Matsumura et al., 1975), account for a significant proportion of the toxicity of technical toxaphene toward mice and houseflies (Khalifa et al., 1974). Several other components of toxaphene, for the most part polychlorinated bornanes (Anagnostopoulos et al., 1974; Turner et al., 1977) but including one dihydrocamphene (Landrum et al., 1976), have also been identified.

A reasonable expectation is that the environmental dissipation of individual toxaphene components will proceed at different rates and by different mechanisms. Gas–liquid chromatographic (GLC) profile changes in weathered foliage residues (Klein and Link, 1967), anaerobic soil and sediment residues (Parr and Smith, 1976; Williams and Bidleman, 1978), and elucidation of two pathways for the decomposition of 1 in a number of model environmental systems (Saleh et al., 1977) provide evidence for this supposition. GLC with packed columns, however, has insufficient resolution to differentiate component dissipation in environments treated with the technical insecticide.

Table I. Uses of Toxaphene in California, 1971–1977 (Pesticide Use Reports, 1971–1977)[a]

	cotton		tomatoes		all uses	
year	kg	ha	kg	ha	kg	ha
1971	425	80	237	46	1261	236
1972	229	47	153	33	811	167
1973	584	92	290	58	1317	239
1974	249	51	134	32	671	142
1975	93	23	187	46	455	114
1976	45	12	163	38	396	91
1977	214	54	353	81	832	199

[a] All figures are in thousands.

To more fully characterize toxaphene environmental dissipation, we have examined residues from the foliage, air, and soil of treated cotton fields. Packed column GLC was used to quantitate the total residue mixture during dissipation, and capillary column GLC provided sufficient resolution to characterize the major processes involved in component dissipation in general physical and chemical terms.

MATERIALS AND METHODS

Chemicals. Technical toxaphene standard was provided by Hercules, Inc., Wilmington, DE. Toxicants A (Khalifa et al., 1974) and B (Seiber et al., 1975) were isolated by published methods. Florisil (60/100 mesh, PR grade, Floridin Co., Pittsburgh, PA) was activated at 135 °C for at least 48 h before use. Solvents were either distilled in glass in this laboratory or were commercial equivalents.

Gas Chromatography. Two systems were employed for analytical packed column work. One consisted of a Dohrmann Instruments Co. Model G-100 gas chromatograph–combustion furnace, modified as described by Winterlin et al. (1967), with a T-300-S titration cell (halide mode) and Model C-200-B microcoulometer (MC). A 1.5

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m × 6 mm o.d. glass column, packed with 10% DC-200 on 60/80 mesh Gas-Chrom Q, was used. Injector, column, and combustion furnace temperatures were 230, 230, and 800 °C, respectively. Nitrogen carrier and oxygen combusion gas flow rates were 30 and 10 mL/min, respectively.

An alternate system consisted of a Varian Model 661B gas chromatograph interfaced with a Dohrmann microcoulometric detector similar to the one described above. A 1.8 m × 3.2 mm o.d. glass column, packed with 7.5% OV-101 on 100/120 mesh, acid-washed, silanized Chromosorb W, was used. Injector, column, and combustion furnace temperatures were 250, 210, and 840 °C, respectively. Nitrogen carrier, nitrogen makeup, and oxygen combustion gas flow rates were 23, 22, and 66 mL/min, respectively.

Open tubular (capillary) column GLC was done on a Hewlett-Packard Model 5700A gas chromatograph fitted with a Model 18713A ^{63}Ni electron-capture (EC) detector and an all-glass injection splitter (Jennings, 1975). The system was similar to the one described previously by Seiber et al. (1975). A 65 m × 0.25 mm i.d. wall coated open tubular (WCOT) column, coated with OV-101 at 4 mg/mL containing 5% benzyltriphenylphosphonium chloride (BTPPC), was used. Injector–splitter and detector temperatures were 250 and 350 °C, respectively. The injection split ratio was held constant for series of injections and was on the order of 95:1. Column temperature was programmed from 150 to 240 °C at 1 °C/min and held at 240 °C for 4 min. Nitrogen carrier and 5% argon–methane makeup gas flows were 0.7 and 40 mL/min, respectively.

Cotton Leaves. The north 32 ha of a 200-ha mature cotton field (section 30) near Corcoran, CA, were commercially treated by aircraft with toxaphene emulsifiable concentrate at the rate of 9 kg of active ingredient (AI)/ha at 8 p.m. on August 20, 1975. Random leaf samples were taken from each of three 200-m^2 study plots located within the 32 ha treated area and also from similar plots in the untreated area to the south. Samples from each plot were composited and analyzed separately by the following method. A 100-g subsample of composited and finely chopped foliage was blended for 5 min with 400 mL of hexane in a Waring blender. The mixture was filtered through anhydrous sodium sulfate and a 100-mL aliquot was concentrated to 10 mL on a rotary evaporator at 30 °C. The residue was quantitatively transferred with 10 mL of hexane to a 20 × 2 cm chromatographic column containing 10 g of Florisil topped with 1 cm of anhydrous sodium sulfate and prewashed with 50 mL of hexane. The column was washed with 30 mL of hexane, which was discarded, and the toxaphene was eluted with 100 mL of 6% ether in hexane. The effluent volume was adjusted to an appropriate volume for GLC analysis (usually 50–100 μg/mL of toxaphene equivalent). Area measurements of the toxaphene envelope, made with a Leitz Model 236 planimeter, were compared to standard curves for quantitative analysis by packed column GLC. The results from similar study plots were averaged. The same samples were then analyzed by capillary GLC. Recoveries of toxaphene fortified at 10 and 100 ppm to untreated cotton leaf samples averaged 93.5%.

Soil and Sediment. A plywood plank, 0.5 × 4 m and covered with a polyethylene sheet, was laid between rows in the center of each study plot in the Section 30 cotton field. The planks were packed to a depth of 2.5 cm with top soil just before toxaphene application and left in place for the duration of the study. Samples consisted of 0.2 × 0.5 m strips of soil removed from the planks. The polyethylene sheet aided in complete removal of soil during sampling and prevented penetration of toxaphene into the plywood.

Twenty-five soil core samples, 7.5 cm in depth and 2.0 cm in diameter, were taken at random in each section 30 study plot and composited for analysis. Random core samples were also taken at 0–15, 15–30, and 30–45 cm depths from a second field (section 9) near Corcoran, CA, during 1974 and 1975. This field was treated with toxaphene at the rate of 4.5 kg of AI/ha on Aug 8, 1973, and 9 kg of AI/ha on Sept 19, 1973, as described for section 30. It was extensively cultivated prior to a 1974 planting of wheat and a 1975 planting of cotton, but it received no direct application of toxaphene after 1973.

Random sediment samples were taken on Aug 27, 1976, from an irrigation drainage ditch which ran parallel to the east end of section 30 by means of a glass jar scoop.

Wet soil samples were air dried 24 h at room temperature to remove the bulk of the moisture. Finely pulverized 100-g samples were Soxhlet extracted with 2:1 (v/v) isopropyl alcohol–benzene for 4 h (Bradley et al., 1972). The extract was filtered and concentrated just to dryness as a rotary evaporator before cleanup by Florisil column chromatography, as described for the foliage samples. The Florisil column effluent was treated with fuming nitric–sulfuric acid to remove DDT and its relatives from toxaphene essentially by the procedure of Klein and Link (1970). Hexane was used instead of methylene chloride to extract residue from the aqueous acid phase after nitration, and no Florisil cleanup of the acid phase extract was required prior to GLC analysis. Recoveries of toxaphene fortified at 1 ppm to untreated soil averaged 84%.

Air. Air was sampled at ca. 1 m^3/min through high volume air samplers each fitted to hold 30 g of XAD-4 macroreticular resin (Woodrow et al., 1977). Two samplers were placed 0.5 m above the crop canopy in the toxaphene treated portion of the field (section 30, north end), one about 30 m and the other about 100 m from the western edge. A third sampler was placed 1.5 m above the soil surface just outside the cotton field on the downwind, western edge. All samplers were operated for a known period of time, usually 1–2 h.

The resin from each sampler was transferred to a 500-mL Erlenmeyer flask, 250 mL of acetone was added, and the mixture was swirled for 1.5 h. The acetone was decanted, and the resin was rinsed with an additional 150 mL of acetone. The combined acetone extracts were evaporated just to dryness and taken up in 10 mL of hexane. The samples were cleaned up by Florisil column chromatography and analyzed by packed column GLC as described for foliage.

RESULTS

Foliage and Air. Results from the analysis of cotton leaf samples (Table II) showed 59% toxaphene loss at 28 days after application. The loss rate was considerably less than that observed by Klein and Link (1967) from kale 21 days after application of 2.2 kg/ha of a water suspension of toxaphene. Differences in the formulation, application rate, foliage texture, and perhaps meteorological conditions may account for the observed differences in dissipation rates. Of particular interest to the present study are the GLC profiles of cotton leaf extracts from the weathered samples. Packed column GLC with a chlorine-selective MC detector for a 14-day leaf residue registered a substantial depletion in peaks of shorter retention time (A) relative to those of longer retention time (B) and (C) when compared to toxaphene standard or 0-day residue (Figure 1). The chromatogram of 50-day cotton leaf extract gave

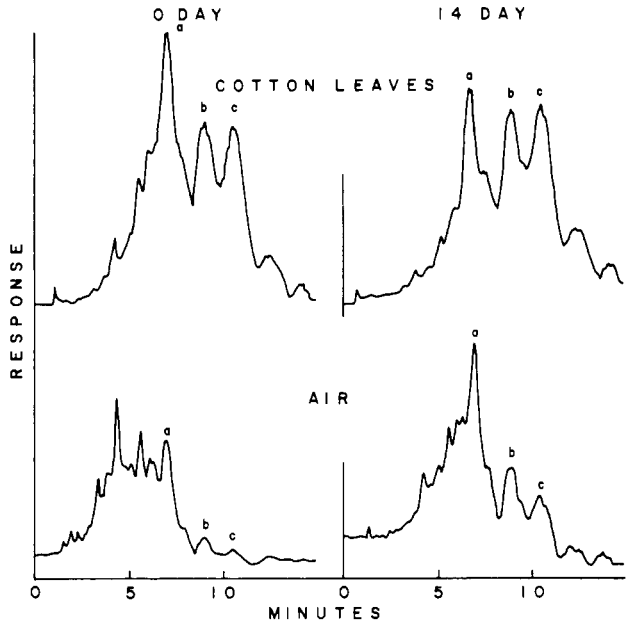

Figure 1. Packed column gas chromatograms of toxaphene residue in (top) 0- and 14-day cotton leaf extracts and (bottom) 0- and 14-day air samples taken from within the same field.

Table II. Total Toxaphene Residues from Leaf, Air, and Top Soil Samples from Section 30 Cotton Field Measured by Packed Column GLC[a]

days treatment to sampling	leaf, ppm	air, μg/m³ inside	air, μg/m³ downwind outside	soil, ppm top 2.5 cm soil from plank	soil, ppm top 7.5 cm soil from core
−1	1	0.13	*b*	*b*	*b*
0	661 (7.2)	4.2	*b*	13.1	*b*
1	*b*	*b*	*b*	*b*	4.6 (1.6)
2	396 (5.5)	1.8	1.4	9.1 (2.8)	4.3 (1.8)
7	464	*b*	*b*	12.4	4.4
14	420 (2.1)	1.9	1.7	10.0 (2.4)	*b*
28	253	1.3	1.2	9.3 (2.8)	3.9
50	135	*b*	*b*	*b*	*b*
58	*b*	*b*	*b*	6.4	3.2

[a] Numbers in parentheses are corresponding residues from an adjacent untreated (control) cotton field. [b] Not analyzed.

a similar but slightly more pronounced depletion. The cotton leaf residue profiles were very similar to those observed by Klein and Link (1967) on kale.

The poorly defined "peaks" obtained from toxaphene with packed column GLC each represent many components. An open tubular (capillary) GLC column provided a substantial improvement in resolution, and when interfaced with an EC detector maintained the required sensitivity and selectivity for a closer examination of toxaphene components in these environmental samples (Seiber et al., 1975). Capillary GLC of residues from 0- and 50-day cotton leaf extracts (Figure 2) showed a significant depletion of components of shorter retention times in the weathered residue. Peak-by-peak inspection showed that no dramatic changes occurred since all of the peaks present at 0 day were still there after 50 days. These data were placed on quantitative terms by computing relative heights of peaks to one arbitrarily chosen reference peak (peak 6) in the capillary GLC profile for the 0-, 7-, and 50-day cotton leaf extracts (Table III). The peaks for which data were obtained are relatively abundant ones and included those corresponding to toxicant B (1) (peak 3) and toxicant A isomer mixture (2 and 3) (peak 5). Percentage change in relative peak heights for each numbered peak are presented in Figure 3 for 0- and 50-day cotton leaf extracts. The graphical data show that for most of

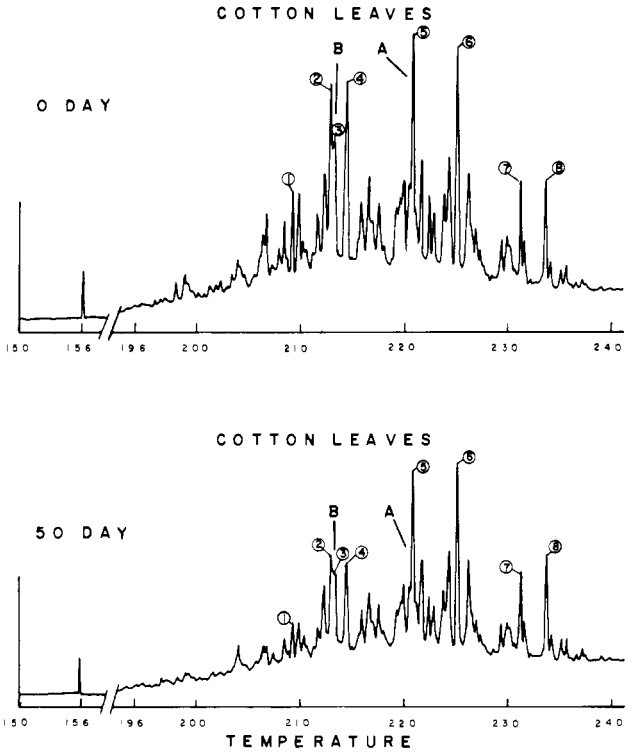

Figure 2. Capillary gas chromatograms of toxaphene residue in 0- and 50-day cotton leaf extracts. Peaks corresponding in retention to toxicants B and A isomer mixture are indicated.

Table III. Heights of Capillary GLC Peaks, Calculated Relative to GLC Peak 6, in Toxaphene Environmental Residues

sample	time treatment to sampling	relative heights of numbered peaks 1	2	3	4	5	6	7	8
leaves	0 day	41.9	87.3	59.0	87.6	110.8	(100.0)	47.0	49.6
	7	31.9	71.2	50.5	67.0	96.4	(100.0)	46.1	49.7
	50	19.3	50.9	39.4	46.1	89.4	(100.0)	49.1	60.0
air	2	118.7	200.0	112.5	195.8	129.2	(100.0)	31.2	28.1
	14	61.6	123.1	75.4	125.4	126.4	(100.0)	32.6	32.6
top soil	0	44.4	75.6	46.9	84.4	93.7	(100.0)	39.4	43.1
	58	40.0	58.9	37.9	70.5	90.5	(100.0)	21.0	44.2
top soil[a]	0	50.5	111.8[b]		122.6	132.2	(100.0)	53.8	49.5
0–15 cm soil section 9[a]	13 month	64.1	105.1[b]		146.1	110.3	(100.0)	33.3	35.9
ditch sediment[a]	12 month	107.1	128.6[b]		239.3	78.6	(100.0)	17.8	21.4

[a] From chromatograms run at different time as, and under slightly different GLC conditions than the first seven entries in the table. [b] Peaks 2 and 3 were unresolved in chromatograms from these three samples.

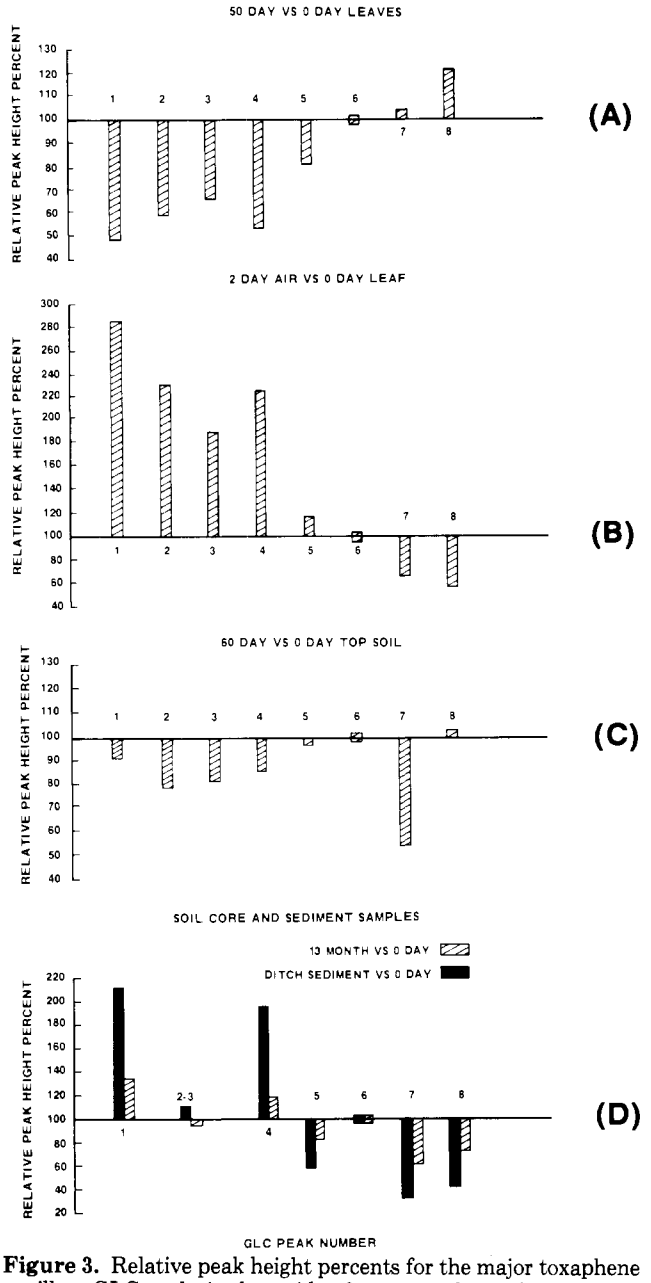

Figure 3. Relative peak height percents for the major toxaphene capillary GLC peaks in the residue from several sample pairs. For example, the top graph (A) shows the heights (relative to peak 6) of peaks from the 50-day leaf extract expressed as percents of corresponding peaks from the 0-day leaf extract using Table III data.

the numbered peaks the trend was quite regular in that peaks of shorter retention times decreased and those of longer retention times increased in relative abundance on weathering. Only peak 4 was out of line in regularity, exhibiting a greater decrease than expected for its GLC retention position relative to the other components.

Analysis of air samples gave vapor concentrations of toxaphene which exhibited regular decline with time inside the treated cotton field and showed comparable and fairly invariant levels just downwind from the field (Table II). Comparison of packed column chromatograms of a few air samples with those of leaf extracts obtained on the same days (Figure 1) showed a higher proportion of early eluting peaks in the air samples than in the foliage. This was most striking for the 0-day sample, in which the profile bore little resemblance to the leaf residue, but was still evident for the 14-day air sample. The same trend was seen in capillary chromatograms (Figure 4) but with much more detail apparent in the behavior of individual peaks. Using the peak ratio technique described above for one early (2 day) and one later (14 day) air sample (Table III), the trend was toward an enrichment of components in the air in proportion to component volatility as signalled by GLC retention times. Applying the graphical treatment to a comparison of percentage change in relative peak heights for the 2-day air sample and 0-day leaf sample (Figure 3B), the trend was once again regular but the inverse of that observed for weathered vs. fresh foliage residue (Figure 3A). That is, comparison of the two graphs showed that those components most rapidly lost from the leaf surface were proportionately enriched in the air. The anomolously greater rate of loss of peak 4 in the leaf residue was matched by a correspondingly greater enrichment of this peak in the air residue. That is, peak 4 simply vaporized from leaf surfaces more rapidly than its GLC retention position indicated that it should. Taken collectively, the data clearly indicated differential vaporization as the primary mode of toxaphene loss from leaf surfaces and gave no indication that chemical reactivity played even a minor role.

Top Soil. Samples of the top 2.5 cm of soil, isolated in the cotton field from the bulk of the soil by means of a plywood partition, were obtained at intervals up to 2 months following application. Core samples to a 7.5-cm depth were obtained from the same field at some of the sampling intervals. All soil samples required cleanup by nitration (Klein and Link, 1970) prior to determination to remove residues of DDT and its relatives (primarily DDD) apparently remaining from DDT applications made more than 2 years before the sampling dates. Without this precaution toxaphene residues at low levels could be obscured by the greater GLC response of DDT-related residues. Residues in the partitioned 2.5 cm top soil samples declined regularly, but at a slower rate than for leaves (Table II). GLC profiles on the capillary column (Figure 5) were quite similar for both the 0- and 58-day samples, although a trend was toward slightly greater loss among the peaks of earlier GLC retention. Using the peak ratio technique (Table III) and graphical data treatment (Figure 3C), a more or less regular trend was observed in the loss rate of peaks 2, 3, 4, 5, 6, and 8 when the 0- and 58-day soil samples were compared. Peak 1 was lost at a lesser rate than its GLC retention time indicated it should be if volatilization was the major dissipation force. Peak 7 disappeared at a much greater rate relative to peaks in the same retention time vicinity, perhaps signalling chemical breakdown of this component(s). It is possible that the peak 7 component(s) was transformed to peak 1 component(s) or to a product with the same GLC retention time as peak 1. Neglecting these anomalies in peaks 1 and 7, differential vaporization was indicated as the major loss mechanism for toxaphene in this relatively dry, aerobic top soil. The deeper soil cores (7.5-cm samples) showed slower toxaphene decline with time than did the 2.5 cm top soil samples, further supporting vaporization as a plausible route of loss at least over the relatively short period of time during which these samples were collected. Capillary GLC peak ratios in the core samples followed the same trends noted in the top soil. Fairly substantial toxaphene residues in the control soil samples may have been due to drift during application or carry over from previous applications.

Soil and Sediment Cores. Core soil samples were obtained from a second field (Section 9) in the same vicinity as the first over 1 year after two applications of toxaphene totalling 13.5 kg/ha. This field was in wheat

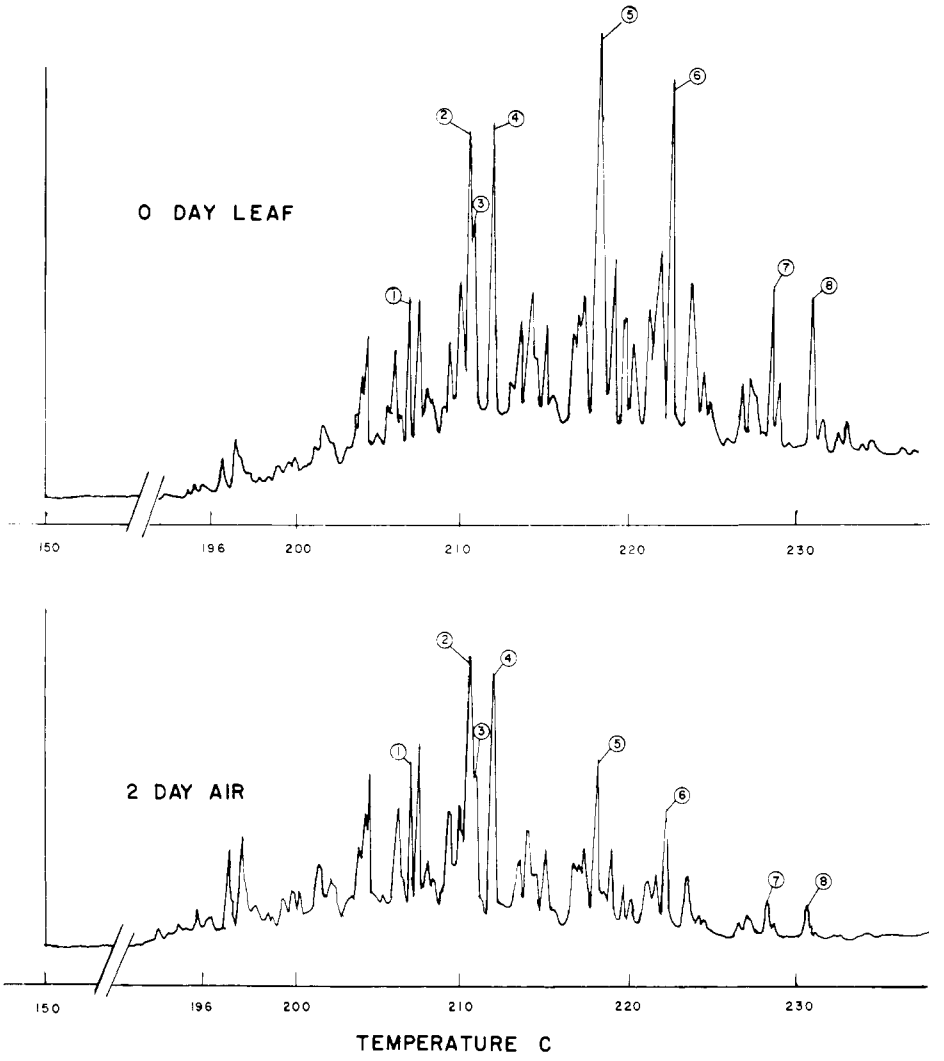

Figure 4. Capillary gas chromatograms of toxaphene residue in 0-day cotton leaf and 2-day air samples taken from within the same field.

during the summer season intervening between toxaphene application and sampling and had been extensively cultivated at least twice during this period. Total toxaphene average residues—3.9, 4.0, and 4.0 ppm for 0–15, 15–30, and 30–45 cm cores, respectively, at 13 months after the second application—were quite high. Lack of significant variation with depth was very likely due more to mixing during deep cultivation than movement of toxaphene through the soil profile.

Core samples of sediment were also taken from the bottom of a drainage ditch adjacent to section 30 12 months after the last toxaphene application. This ditch had flowing or brackish standing water through much of the year. Due to its proximity to section 30 it is likely that a substantial part of the toxaphene residue was derived from drift during the 1975 applications to section 30 and through runoff in irrigation and winter rain water drainage from the same field subsequent to the 1975 application.

Packed column GLC with chlorine selective MC detection showed an enhancement in early eluting peaks for both the section 9 soil and section 30 ditch sediment when compared with the GLC profile for a 0-day soil sample. This profile change bore some resemblance to those reported for toxaphene residues from anaerobic incubation in moist soil amended with alfalfa meal (Parr and Smith, 1976) and from anoxic salt marsh sediments (Williams and Bidleman, 1978). Capillary column GLC (Figure 6) accentuated the appearance of early eluting components in the toxaphene residue profile, and showed that extensive changes within the major components of toxaphene had occurred as well. Peaks 5, 7, and 8 declined more rapidly than the reference peak (peak 6); for all three the extent of decline decreased in the order section 30 drainage ditch sediment > section 9 soil core > section 30 top soil. For peak 5 this was most dramatic since it declined much more rapidly than adjacent peaks in the chromatogram (Figure 3) and was perhaps of most significance since peak 5 encompasses the retention time of toxicant A (**2** and **3**). Peaks 1 and 4 increased in proportion relative to the reference peak, in the order section 30 drainage ditch sediment > section 9 soil core > section 30 top soil. Peaks 2 and 3, unresolved under the capillary GLC conditions used for this set of samples, bore roughly the same relationship to the reference peak in all three samples. At least six early eluting peaks—most notably those eluting at 191 and 194 °C (Figure 6)—were of substantially greater relative importance in section 9 soil and, particularly, section 30 drainage ditch sediment when compared with the 0-day soil sample. The results argue strongly for extensive degradation in the weathered soil and sediment residues, leading to formation of products of lower molecular weight than exist in technical toxaphene.

DISCUSSION

The use of capillary column GLC has provided new information on the behavior of toxaphene residues during

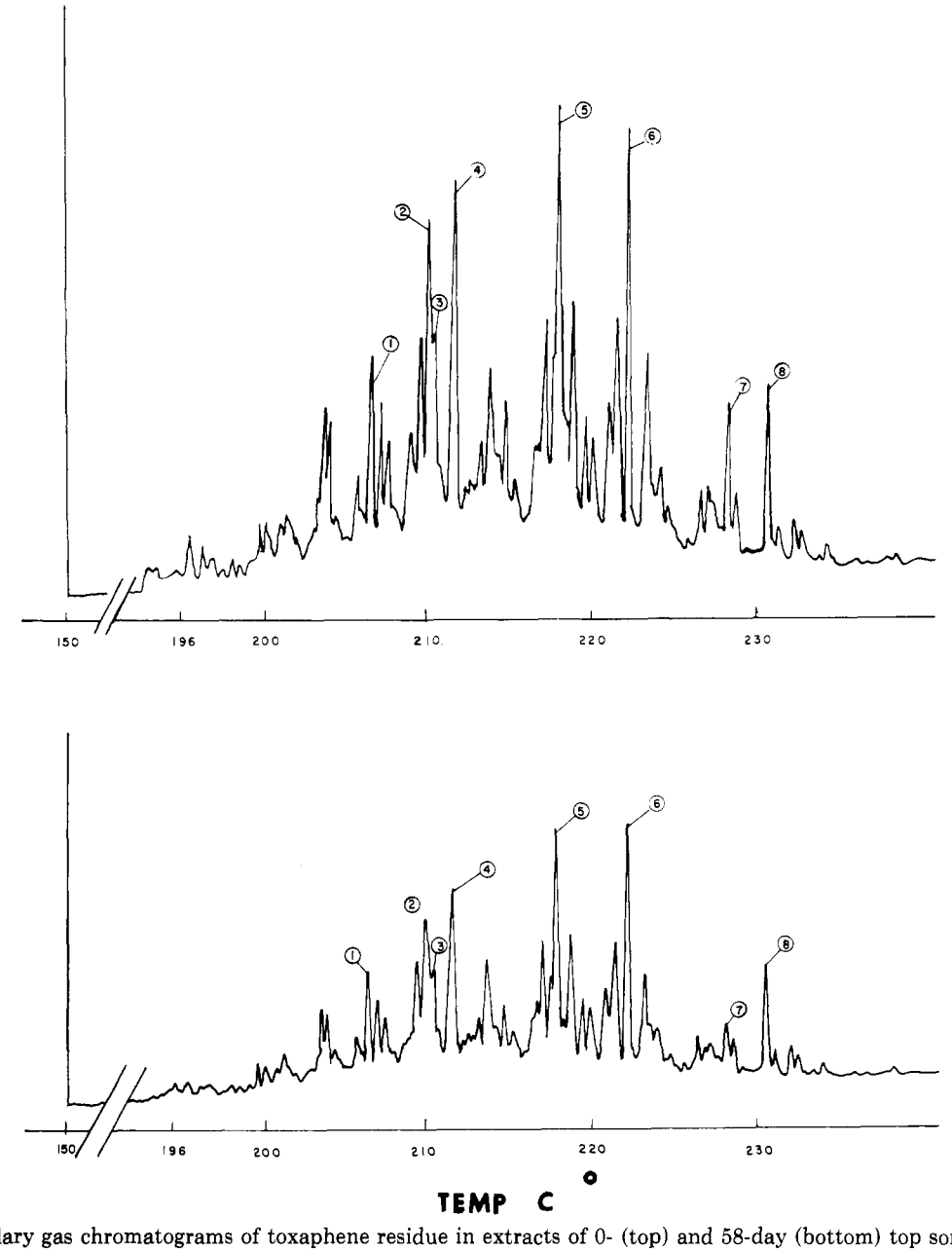

Figure 5. Capillary gas chromatograms of toxaphene residue in extracts of 0- (top) and 58-day (bottom) top soil samples.

weathering. In an agricultural environment typical of those treated with substantial qualities of toxaphene in California, residues were shown to decline from foliage by vaporization of components in proportion to their volatilities. There was no evidence for any contribution by chemical degradation during foliage decline, even though sunlight, oxygen, and atmospheric oxidant were in abundance during the period of study. The foliage dissipation of toxaphene is thus similar to that for many other chemicals possessing moderate volatility, low surface reactivity, and little tendency toward absorption by or translocation within plant tissues (Spencer et al., 1973). The consequence of volatilization of foliage residues of toxaphene and their stability is apparently reflected in relatively high toxaphene air levels in use areas (Stanley et al., 1971; Arthur et al., 1976) and significant levels in air samples from the western North Atlantic (Bidleman and Olney, 1975). Close examination of published packed column GLC profiles from the latter study shows relatively more of the early eluting components than are present in the standard, as would be expected from the results reported here for collections made near a treated field.

Toxaphene has been considered to be one of the more persistent pesticides in soil (Edwards, 1964; Swoboda et al., 1971). Indeed, we found that short-term decline in aerated top soil was slow, with half-lives on the order of 2 months (top 2.5 cm) and 4 months (top 7.5 cm). While vaporization was a major loss route, leading once again to selectively greater loss among the more volatile components, there was evidence for chemical decomposition of at least one component. Considering the possibilities of dehydrochlorination or reductive dechlorination shown for toxicant B in a variety of chemical and biological systems (Saleh et al., 1977), reduction would appear the less likely in this instance since the soil was quite dry and exposed to air during the period of study. However, no direct evidence was obtained on this point since neither the component(s) undergoing decomposition nor the products were identified.

Several of the major components of toxaphene, including toxicant B, underwent substantial decomposition in soil core and sediment samples taken a year or more after the last application. Since the soil samples were from a field that was well irrigated during the intervening growing

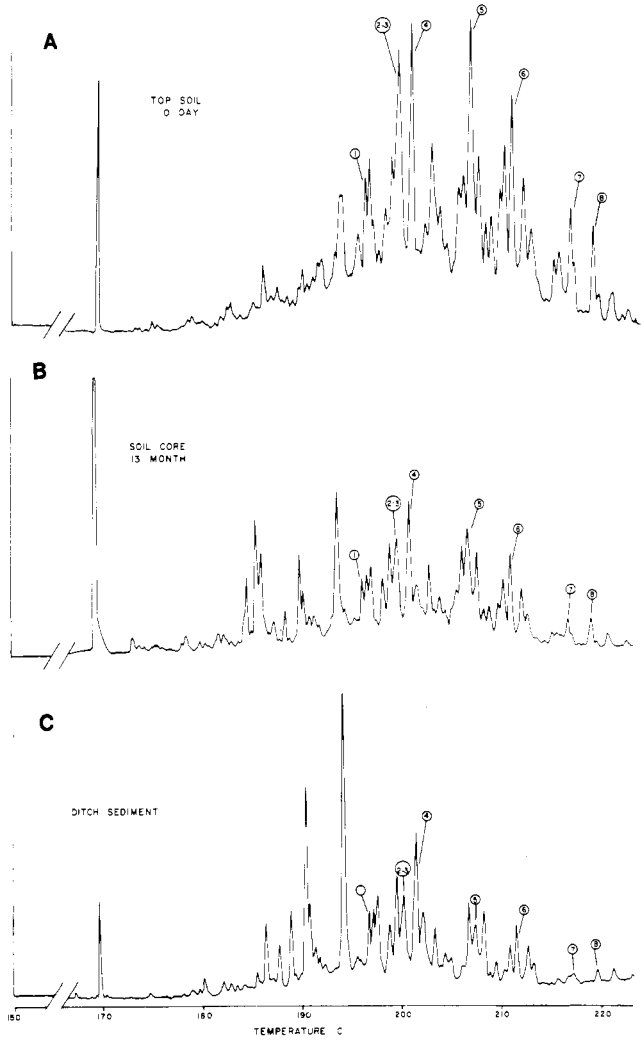

Figure 6. Capillary column gas chromatograms of toxaphene residue in extracts of (A) 0-day section 30 top soil, (B) 13-month section 9 soil core, and (C) 12-month section 30 drainage ditch sediment.

season and subjected to winter rains, it is to be expected that anaerobic conditions existed during a part of the time period between last treatment and sampling. This condition certainly existed in the sediment, which was underwater through most of the year. Coupled with the similarity in GLC profiles, particularly the marked appearance of early eluting GLC peaks, with those reported for residues from anaerobic soil incubations (Parr and Smith, 1976) and estuarine sediments (Williams and Bidleman, 1978), and the presence of DDD as the only major DDT degradation product observed in our samples, reductive dechlorination was indicated to be a major degradation pathway. This leads to formation of products of lower molecular weight than exist in technical toxaphene, at least some of which must be relatively stable from their enrichment in the environmental samples we examined. Thus, even though extensive degradation had occurred, the possibility that the products formed may have some environmental and toxicological significance needs to be determined.

Use of one capillary GLC peak as a reference materially assisted both qualitative and quantitative examination of chromatographic data upon which our conclusions were based. This reference peak was originally chosen based on its resolution and appearance in the central part of the chromatographic profile of toxaphene standard. However, its relative stability in the samples examined recommends its use as a marker in environmental analyses in which toxaphene is suspected to be present but interpretation is obscured by substantial profile changes. It is further recommended that analyses of soil and sediments of environmental origin include nitration as a part of the cleanup. We observed severe overlap even in the capillary chromatograms with DDE, DDD, and DDT, all of which may be removed by nitration. This procedure did not alter the toxaphene profile in either fresh or weathered residues.

The use of capillary GLC coupled with electron-capture detection substantially increased the amount of information obtained from environmental analyses. Column lifetime was equivalent to several months of near-continuous operation, although some deterioration occurred which necessitated frequent recalibration. Prescreening samples on a packed column GLC column before capillary analysis helped to avoid contamination and/or deterioration from excessively large injections of unknown samples.

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Determination of Naphthaleneacetic Acid (NAA) in Oranges, Tangerines, and Processed Products: High-Performance Liquid Chromatography with Fluorometric Detection

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A rapid procedure involving high-performance liquid chromatography with fluorometric detection is described which is capable of analyzing tangerines and oranges, as well as their processed fractions, for residues of NAA as low as 0.008 ppm. Sulfuric acid is used to release conjugates for extraction with methylene chloride, followed by partitioning between ethyl ether and potassium hydrogen phosphate for cleanup. The insensitivity of the fluorometer to pulsatile flow allows the use of an economical single piston solvent pump for chromatography. Residues were measured in 'Dancy' tangerines and processed fractions in one disappearance experiment and in 'Pineapple' orange in another.

The inherently high fluorescent quantum yield for naphthaleneacetic acid (NAA) was utilized by Jolliffe and Coggins (1970) in the development of a sensitive and selective analytical procedure for the analysis of residues in citrus as low as 0.1 ppm. Their report summarizes the approaches of previous methods as well.

As has been reported for California citrus (Hield et al., 1966), NAA has been found to be effective in Florida for improving fruit size and reducing alternation of heavy and light crops of 'Dancy' tangerines (Wheaton and Stewart, 1973), as well as for preventing sprouting of 'Bearss' lemon trees when applied directly to tree trunks following pruning (Lundberg and Smith, 1974).

Metabolism studies of NAA, applied as a dip to fruiting 'Kinnow' mandarin branches, by Shindy et al. (1973) showed that major metabolites, appearing between 6 h and 4 days after application, were naphthaleneacetylaspartic acid and 1-β-D-glucose-α-naphthaleneacetate. Together, these two metabolites represented, at 4 days after application, 59% of total radioactivity in the whole fruit. Both of these metabolites were readily hydrolyzed by hydrochloric acid to give free NAA. Coggins et al. (1972) studied the disappearance of NAA in Kinnow mandarin fruit as well as determining residues in eight other types of citrus. Residues became immeasurable (<0.07 ppm) in fruit harvested 3 to 6 weeks after application.

This paper describes the residues of NAA in 'Dancy' tangerines and various types of fractions resulting from a typical commercial processor as well as in fresh 'Pineapple' oranges after a single application to trees containing immature fruit. Additionally, a high-performance liquid chromatographic procedure is described which provides for the simple, rapid, highly sensitive (0.008 ppm limit of detection) and highly selective analysis of fresh fruit as well as many types of processed products.

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EXPERIMENTAL SECTION

Apparatus. An American Instrument Co. (Silver Spring, MD) spectrophotofluorometer, Model 4-8202, equipped with a 150-W mercury–xenon lamp, Model B16-63019 flow cell (300-μL volume), and an A363-62140 adapter, was used as the fluorescence monitor for the eluant of a liquid chromatographic column. Wavelengths used were 288-nm excitation and 340-nm emission, with a slit program of 3, 3, 3, 3, 3, and 5 mm. Chromatograms were recorded on a Sargent Model MR recorder.

Separations were performed by reverse phase either on a 2 mm i.d. × 50 cm ETH (Dupont) column or on a 4 mm i.d. × 25 cm μ Bondapak CN (Waters Associates). Injection on the ETH column was by syringe (10 μL) via a Chromatronix Model 107B25 sample injection tee; a Chromatronix Model HPSV-20 sample injection valve with a 25-μL loop was used in conjunction with the μ Bondapak CN column. Mobile phase for the ETH column was 0.1 M pH 4.3 citrate at 1.3 mL/min (pH 4.7 was used for the molasses samples); a 0.1 M pH 7.0 phosphate buffer was used for the μ Bondapak CN column at 1.0 mL/min.

Mobile phase was pumped by a Waters Associates Model 6000 solvent delivery system or a Milton Roy Model 196-0042-028 single-piston pump.

Blending of the samples was accomplished with a Lourdes Model VM blender.

Concentration of extracts was performed on a steam bath by use of a 500-mL Kuderna-Danish flask fitted with a three-ball Snyder column and a 10-mL ampule.

Procedure for Fresh or Dried Fruit. Twenty-five grams of peel, pulp, whole, or dried fruit was placed in a 1-qt Mason jar along with 200 mL of methylene chloride and 5 mL of 18 N H_2SO_4; the sample was blended at medium speed for 4 min. It was then filtered under vacuum through a no. 2 porcelain Buchner funnel containing no. 1 filter paper. The Mason jar was rinsed with an additional 50 mL of methylene chloride which was poured through the filter cake. The filtrate was then

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