Degradation of Aldrin and Heptachlor in Field Soils

During a Ten-Year Period

Translocation into Crops

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Data are presented relative to the accumulation following treatment and the subsequent decline of aldrin or heptachlor residues in loam soils during a 10-year period (1958–1968). Loam soils treated with aldrin or heptachlor at 25 pounds per five-inch acre over the five-year period of 1958 through 1962 contained in the fall of 1968, 4 to 5% of the applied dosages primarily in the form of dieldrin and heptachlor epoxide. Aldrin treated soils also contained photo-dieldrin, which amounted to 1.5% of the recovered dieldrin. In addition, three unidentified, more polar compounds were detected in these soils, but they were nontoxic to both vinegar flies and houseflies. In addition to gamma chlordane and nonachlor which were present in the original heptachlor formulation, two toxic metabolites (heptachlor epoxide and alpha-chlordane) and three unidentified, nontoxic compounds were detected, thus indicating the breakdown in soils of heptachlor and related compounds. All crops grown in these soils contained some insecticidal compounds. Potatoes from aldrin treated soils contained dieldrin (0.047 p.p.m.) and photo-dieldrin (0.0006 p.p.m.), while those grown in heptachlor treated soils contained heptachlor (0.002 p.p.m.), heptachlor epoxide (0.054 p.p.m.), gamma-chlordane (0.015 p.p.m.), alpha-chlordane (0.004 p.p.m.), and nonachlor (0.002 p.p.m.).

During the first 10 to 15 years after the introduction of organic synthetic pesticides, long term residual properties were regarded as desirable. It was thought to be a remarkable feature when it was found that an insecticide like DDT could be applied to mud huts and result in mosquito control over an extended period of time. In fact, it was regarded as desirable to make insecticide residues "last longer and look better" through the addition of "polychlorinated polyphenyls for improving lindane residues" (Hornstein and Sullivan, 1953). This attitude has changed considerably when it was found that some insecticidal residues are persistent and widely distributed and are found in materials where their presence is undesirable. The synthetic organochlorine insecticides degrade in soil, although the rate of this degradation is different for each compound, depending on the nature of the chemical itself and on a variety of environmental factors. Some of these chemicals are more persistent, or less degradable, while others are less persistent and more susceptible to the effects of biological, chemical, and physical factors.

In this study, long term field experiments are described in which the insecticides aldrin or heptachlor were applied to agricultural loam soils. The fate of these insecticides in the soil and their translocation into crops during the ten-year period of 1958–68 are discussed.

PROCEDURE

Soil Treatments at Abnormally High Dosages and Soil Sampling. In May 1958, duplicate 30- × 40-foot Carrington silt loam plots near Madison, Wis., were treated with emulsions of aldrin and heptachlor at 5 or 25 pounds per acre (Lichtenstein, 1960). The soils were then rototilled to a depth of 4 to 5 inches.

Those soils treated at 5 pounds per 5-inch acre were retreated at the same rate each May from 1959 through 1962. At the end of the five years, all plots had been treated in either one or five yearly applications with a total of 25 pounds of insecticide per 5-inch acre (5 × 5 or 25 pounds). These abnormally high treatment rates were chosen, because at the time of the first insecticidal application (1958) colorimetric methods had to be used for analyses. It was also felt that for the reliable detection of potential metabolites higher insecticidal application rates would be desirable.

For soil residue studies, it was intended to determine if and to what extent the insecticides would accumulate in the soil following yearly applications of 5 pounds per 5-inch acre and how fast they would disappear in a subsequent five-year period during which no further insecticidal application was made. These data were compared with data from soils which had received the total 25-pound dosage in one massive application. Insecticide translocation into crops grown in these soils, and of the metabolism of insecticides in soils and crops were also investigated.

Six-inch soil samples were collected as described (Lichtenstein, 1960) immediately after treatment in 1958 and in October of each year. A final soil sample was collected in October of 1968.

Crop Growth and Crop Sampling. During the years 1958–1962 various crops were grown on the insecticide treated plots as previously described (Lichtenstein and Schulz, 1965). From 1963 through 1967, however, only carrots (Red Cored Chantenay) and potatoes (Russet Sebago) were grown as indicator crops, while during the 11th growing season (1968) radishes (Early Scarlet Globe), beets (Detroit Dark Red), and cucumbers (Straight Eight) were grown in addition to carrots and potatoes for translocation studies. Crop sampling and processing was performed according to a previously described procedure (Lichtenstein and Schulz, 1965).

Analytical Methods. Various analytical methods were employed during the 10-year (11 growing seasons) duration of this experiment. Soil and crop samples obtained through 1961 were extracted, cleaned up, and analyzed colorimetrically as described (Lichtenstein, 1960). Samples that were obtained

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in 1962 were analyzed by both colorimetric and gas-liquid chromatographic (GLC) methods (Lichtenstein *et al.*, 1964). Data secured by those two methods showed good agreement. Soil and crop samples obtained during the remainder of the period (1963 through 1968) were extracted and analyzed by GLC as described by Lichtenstein *et al.* (1967). In addition, soils and crops grown in 1963 on heptachlor treated plots were analyzed for γ-chlordane. All crops that were grown in 1968 were also tested for the presence of toxic substances by exposing vinegar flies (*Drosophilia melanogaster* Meig) to the dry residue obtained from crop extracts (Edwards *et al.*, 1957).

Metabolite Studies in 1968. CHEMICALS USED. Soil and crop extracts from aldrin treated soils were compared with analytical grade aldrin, dieldrin, photo-aldrin or the photo isomer of aldrin (1,1,2,3,3*a*,7*a*-hexachloro-2,3,3*a*,3*b*,4,-6*a*,7,7*a*-octahydro-2,4,7-metheno-1*H*-cyclopenta(*a*)-pentalene), photo-dieldrin or the photo isomer of dieldrin (1,1,2,3,-3*a*,7*a*-hexachloro-5,6-epoxydecahydro-2,4,7-metheno-1*H*-cyclopenta(*a*)-pentalene), "aldrin-OH" (6,7-*trans*-dihydroxydihydro-aldrin or *trans*-aldrin diol) a metabolite obtained by Korte and Arent (1965) from rabbit urine after oral administration of dieldrin, and dicarboxyl aldrin (1,2,3,4,10,10-hexachloro-6,7-dicarboxyl-1,4 endo-5,8-exodimethano-1,4,4*a*,-5,6,7,8,8*a*-octahydronaphthalene.) These chemicals were obtained through the courtesy of the Shell Chemical Company.

Soil and crop extracts from heptachlor treated soils were compared with analytical grade heptachlor, heptachlor epoxide, chlordene (4,5,6,7,8,8-hexachloro-3*a*,4,7,7*a*-tetra hydro-4,7 endomethanoindene), chlordane (α and γ isomer) (2,3,4,5,6,7,8,8-octachloro-2,3,3*a*,4,7,7*a*-hexahydro-4,7-endomethanoindene), nonachlor (delta-trichloro-chlordene) (1,2,3,-4,5,6,7,8,8-nonachloro-2,3,3*a*,4,7,7*a*-hexahydro-4,7-endomethanoindene). All these compounds were obtained through the courtesy of the Velsicol Chemical Corporation.

To determine the toxicity of these various compounds, 50 vinegar flies were exposed in each of two bioassay jars to the dry residue of 20 μg of each of these chemicals. Approximate 50% mortalities were obtained with aldrin or dieldrin in 2.5 hours, photo-aldrin in 1.5 hours, photo-dieldrin in 2.5 hours, heptachlor or heptachlor epoxide in 1 hour, α-chlordane or γ-chlordane in 3 hours, nonachlor in 16 hours, and chlordene in 24 hours. No toxicity effects were obtained during a 48-hour exposure period with "aldrin-OH," dicarboxyl aldrin, and "1-OH chlordene" (1-hydroxy-4,5,6,7,8,8-hexachloro-3*a*,-4,7,7*a*-tetrahydro-4,7-endomethanoindene).

SOILS, QUALITATIVE MEASUREMENTS. Soil samples collected in October of 1968 were examined for the presence of metabolites that could have been produced in addition to dieldrin or heptachlor epoxide. Tests were also conducted to determine the presence of compounds that could have remained in the soil after application as impurities in the original insecticide formulation. Soils were extracted in a homogenizer with redistilled acetonitrile (2 ml per gram of wet soil), followed by concentration of the extract to 0.2 ml at 25° C. in a flash evaporator. One fifth of this concentrate, usually representing 40 grams of wet soil, was then analyzed by thin-layer chromatography (TLC). The concentrate was spotted on aluminum oxide G coated glass plates (5 × 20 cm), 2.5 cm above the lower edge. Chromatograms from aldrin treated soils were developed with isooctane-pyridine (7 to 3) or with isooctane-diethyl ether (7 to 3), while those from heptachlor treated soils were developed with isooctane or with cyclohexane-ethylacetate (7 to 3). The separated compounds were visualized by spraying with reagents as described by Mitchell (1957) and subsequent exposure to ultraviolet light for 10 minutes.

After the first thin-layer chromatograms had been developed, the same spotting procedure was repeated except that two portions of the concentrate were spotted side by side. After development of the chromatogram, one half of the plate was covered with aluminum foil and the other half sprayed and visualized as described. The foil-covered unsprayed portions of the aluminum oxide G layers corresponding to each of the different spots observed on the sprayed side of the plate were then scraped off and extracted with a 1 to 12 mixture of acetonitrile and acetone. Aliquots of these extracts (approximately 0.5 ml) were then evaporated to dryness, the residue was redissolved in 0.5 ml of hexane followed by analyses by GLC.

The same acetonitrile-acetone extracts were also used for toxicity tests with vinegar flies (Edwards *et al.*, 1957) and houseflies (*Musca domestica*, C.S.M.A., 1948 strain). With vinegar flies, aliquots of the extracts representing 36 grams of soil were pipetted into bioassay jars and the solvents were evaporated in a fume hood. To test toxic effects resulting from contact with the residue or by vapors emanating from this residue, 50 flies were introduced into each of two bioassay jars. Mortality counts were performed at intervals during a 45-hour exposure period. When houseflies were used, aliquots of the acetonitrile-acetone extracts were evaporated to dryness, then redissolved in 0.5 ml of acetone. One microliter of this acetone solution representing 100 mg or 300 mg of soil was then applied topically to the ventral portion of the abdomen of female houseflies. Mortality counts were performed 45 hours later.

For the qualitative determination of potential water soluble metabolites, soil samples were extracted with acetonitrile (2 ml per gram of soil). The resulting solution was then concentrated at 45° C. in a flash evaporator to approximately 10 ml, to which 100 ml of water was added. This mixture was then re-extracted with three 50-ml portions of hexane. The water-acetonitrile phase was evaporated to dryness at 45° C., the residue was redissolved in small amounts of acetone and spotted on an aluminum oxide G coated glass plate. The chromatogram was then developed with isooctane-pyridine (7 to 3) and sprayed as described. Aliquots of the hexane phase were handled in the same way. Finally, isolates were prepared from these plates as described and also analyzed by GLC.

SOILS, QUANTITATIVE MEASUREMENTS. To measure actual amounts of the various metabolites, soil samples were extracted with acetonitrile (2 ml per gram of soil), followed by diluting the extract with water (5 ml per gram of soil) and partitioning of the insecticidal residues into hexane. After the hexane had been dried over anhydrous sodium sulfate, it was adjusted to volume and appropriate aliquots were used for analyses by GLC. Added amounts of aldrin, dieldrin, heptachlor, or heptachlor epoxide were recovered to an extent of 90–95%.

POTATOES, QUALITATIVE AND QUANTITATIVE MEASUREMENTS. Because of minimal analytical interference, potatoes were used as the primary test plant to determine the presence of aldrin or heptachlor metabolites in a crop. Potatoes and some samples of carrots were extracted with acetonitrile as described for soils (quantatitive measurements) and partitioned into hexane. The hexane fraction was then concentrated and cleaned up by passing it through a 10-gram Florisil (PR grade, 60- to 80-mesh) column using 150 ml of 15% diethylether in hexane as the eluting solvents. This

Table I. Recoveries of Aldrin (*A*) and Dieldrin (*D*) Residues from Soils and Crops Grown in 1968 on Aldrin-Treated Field Plots

	Aldrin Applied to Soil, Lb/5-Inch Acre					
	5 × 5[a]			25[b]		
	Recovered in Fall of 1968, P.P.M.[c]					
	A + *D*	%*D*[d]	CR%S[e]	*A* + *D*	%*D*	CR%S
Soil	0.860	99	...	0.690	98	...
Carrots	0.129	100	15.0	0.176	100	25.4
Potatoes	0.044	100	5.1	0.046	100	6.6
Beets	0.048	100	5.6	0.053	100	7.7
Radish	0.085	100	10.0	0.078	100	11.4
Cucumber	0.102	100	12.8	0.122	100	17.8

[a] Aldrin applied at 5 lb/5-inch acre in May of each year (1958 through 1962). Total application: 25 lb/acre (15.6 ppm) over 5-year period.
[b] Aldrin applied at 25 lb/5-inch acre (15.6 ppm) in May 1958 only.
[c] Results are averages of duplicate field plots.
[d] Dieldrin in per cent of total residue recovered (*A* + *D*).
[e] CR%S = crop residue in % of soil residues.

cleaned up extract was concentrated to volume and analyzed by GLC and TLC.

Extracts from potatoes that were grown on aldrin treated soils were qualitatively analyzed by TLC using aluminum oxide G as the coating and isooctane-pyridine (7 to 3) as the moving solvent system. Areas corresponding to R_f values obtained with dieldrin and photo-dieldrin were scraped off the plate and tested by GLC as described. For quantitative residue determinations aliquots of the cleaned up diethylether-hexane extract were also analyzed directly by GLC.

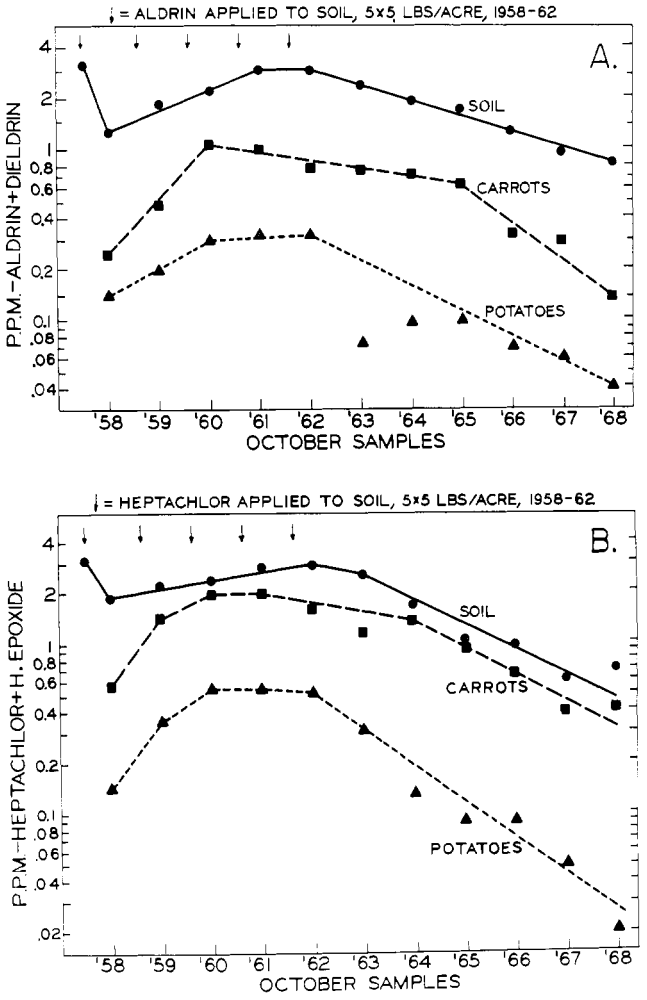

Figure 1. Aldrin plus dieldrin and heptachlor plus heptachlor epoxide residues in soils and their translocation into crops, after 5 yearly soil applications (1958–62) of aldrin or heptachlor at 5 lbs/5-inch acre

Extracts from potatoes that were grown on heptachlor treated soil were analyzed qualitatively and quantitatively as described. However, isooctane was used as the moving solvent for TLC and nonsprayed areas that corresponded to all the visualized spots (Figure 4) obtained from potato extracts were scraped off the plate and tested by GLC and *Drosophilia* bioassay.

RESULTS AND DISCUSSION

Insecticide residue levels in soils treated with aldrin or heptachlor at 5 pounds per acre per year from 1958 through 1962 are presented in Figure 1 for the 10-year period 1958–68. Data for soils, carrots, and potatoes represent the total of aldrin plus dieldrin or of heptachlor plus its epoxide. Dieldrin was produced within the soil from aldrin and amounted to 50 and 90% of the total aldrin plus dieldrin recovered in 1959 and 1963, respectively. Heptachlor epoxide was produced from heptachlor at a slower rate and reached the 50% level in the fall of 1964 and the 90% level in the fall of 1968.

During the period of annual insecticide soil treatments residue levels increased steadily through 1962, when their concentrations in soil amounted to 19% of the totally applied insecticide dosage of 25 (5 × 5) pounds per acre. In subsequent years, when no further soil treatments were performed, residue levels declined at a relatively slow rate. In fall of 1968, 5.3% (aldrin plus dieldrin) and 4.6% (heptachlor plus heptachlor epoxide) of the applied aldrin or heptachlor were detected in these soils.

Insecticide residues were also absorbed by crops grown in these soils, with carrots absorbing the largest amounts (Figure 1). Although residue levels in soils increased up to 1962, the residue concentration in both carrots and potatoes reached its peak during the 1960 growing season. During that year, the concentration of aldrin plus dieldrin in carrots was 1.08 ppm and of heptachlor plus heptachlor epoxide 1.90 ppm. Residue levels in potatoes never exceeded 0.30 to 0.32 ppm (1960–62) of aldrin plus dieldrin or 0.54 to 0.51 (1960–62) ppm of heptachlor plus its epoxide. Apparently a threshold had been reached beyond which the content of insecticidal residues remained constant in these two crops. If more residues were absorbed, they could have been metabolized by the plant tissue into compounds that were not detected at that time. When insecticide residue levels in soils started to decline (1963), both carrots and potatoes also contained proportionally smaller amounts of residue.

For analyses of samples obtained in 1968, both soils and some of the crops were extracted by two procedures as described. Results obtained after samples had been extracted with a 1 to 1 mixture of hexane and acetone and analyzed by GLC for aldrin and dieldrin are summarized in Table I.

Table II. Recoveries of Heptachlor (H), Heptachlor Epoxide (HO), and γ-Chlordane (γ-Ch) Residues from Soils and Crops Grown in 1968 on Heptachlor-Treated Field Plots

	Heptachlor[a] Applied to Soil, Lb/5-Inch Acre									
	5 × 5[b]					25[c]				
	Recovered in Fall of 1968, P.P.M.[d]									
	H + HO	%HO[e]	CR%S[f]	γ-CH	CR%S	H + HO	%HO	CR%S	γ-CH	CR%S
Soil	0.701	89	...	0.817	...	0.719	93	...	0.925	...
Carrots	0.413	92	58.0	0.136	16.6	0.223	98	31.2	0.075	8.1
Potatoes	0.070	98	9.5	0.016	2.0	0.064	100	8.8	0.023	2.5
Beets	0.057	100	8.1	0.015	2.0	0.052	100	7.2	0.013	1.4
Radish	0.139	100	19.8	0.027	3.3	0.130	100	18.1	0.031	3.3
Cucumber	0.085	95	12.0	0.022	2.7	0.068	100	9.4	0.024	2.6

[a] Formulation contained in addition to one pound of actual heptachlor 0.25–0.3 pounds of γ-chlordane.
[b] Heptachlor applied at 5 lb/5-inch acre in May of each year (1958 through 1962). Total application: 25 lb/acre (15.6 ppm) over 5-year period.
[c] Heptachlor applied at 25 lb/5-inch acre (15.6 ppm) in May 1958 only.
[d] Results are averages of duplicate field plots.
[e] Heptachlor epoxide in per cent of total residues recovered (H + HO).
[f] CR%S = crop residue in % of soil residue.

Soils that had been treated with aldrin at five yearly dosages of 5 pounds per acre contained in the fall of 1962 more aldrin plus dieldrin residues than those that had been treated with one 25-pound-per-acre dose in 1958 (Lichtenstein and Schulz, 1965). By 1968, these differences had nearly disappeared: 5.3 and 4.4% of the total aldrin applied were recovered from these soils in the form of aldrin and dieldrin. Residues in crops were all in the form of dieldrin, but varied in their concentration according to the particular crop. The highest dieldrin concentration was found in carrots, followed by cucumbers, radishes, beets, and potatoes.

Table II summarizes data from analyses of samples obtained in 1968 from heptachlor treated soils and from crops grown therein. The commercial formulations of heptachlor used contained in addition to 1 pound of actual heptachlor 0.25 to 0.3 pound of γ-chlordane and 0.04 to 0.1 pound of "other compounds" which are largely in the form of nonachlor (Velsicol Chemical Corp., 1967). During the five-year soil treatment, a total of 25 pounds of actual heptachlor had been applied which also resulted in an application of 6.25 to 7.5 pounds of γ-chlordane and 1.0 to 2.5 pounds of nonachlor. Since chlordane is more persistent than heptachlor (Lichtenstein and Polivka, 1959), more γ-chlordane than heptachlor and heptachlor epoxide was present in the soil after 10 years.

In the fall of 1968 heptachlor plus heptachlor epoxide concentrations in soils amounted to 4.5% of the total heptachlor applied (Table II). They were similar to concentrations of aldrin plus dieldrin in aldrin treated soils. In fall of 1968, 18.5 and 21% of the total γ-chlordane applied was still in the soil. A total of 32 pounds of heptachlor and γ-chlordane had been applied to these soils and close to 8% of that combined total was recovered from the soil in the form of heptachlor, heptachlor epoxide, and γ-chlordane.

Crops grown in 1968 on these soils primarily contained heptachlor epoxide and γ-chlordane (Table II). Although more γ-chlordane than heptachlor epoxide was present in the soil, the amounts of γ-chlordane in crops were only one fourth of the heptachlor epoxide concentration. Proportionally more heptachlor epoxide than γ-chlordane had been absorbed by these vegetables.

Bioassay procedures with vinegar flies showed that all crop extracts from both aldrin and heptachlor treated soils caused appreciable insect mortalities during a 48-hour exposure period. No mortalities, though, were observed with extracts from crops that were grown as controls on insecticide free soil.

Metabolite Studies in 1968 of Aldrin-Treated Soils and Crops Grown Therein. SOILS, QUALITATIVE MEASUREMENTS. Photographs of thin-layer chromatograms obtained with extracts of aldrin treated soils are presented in Figure 2. They show the presence of five to seven spots depending on the solvent system used. When eluates from the area corresponding to aldrin (R_f 0.72) were analyzed by GLC, small peaks were obtained with retention times identical to aldrin. In addition to the originally applied insecticide, dieldrin and photo-dieldrin were detected by both TLC and GLC. The presence of photo-aldrin could not be confirmed by GLC but its area contained traces of dieldrin with the isooctane-pyridine system. Eluates from areas corresponding to "aldrin-OH" (*trans*-aldrin diol) were silanized (Ludwig and

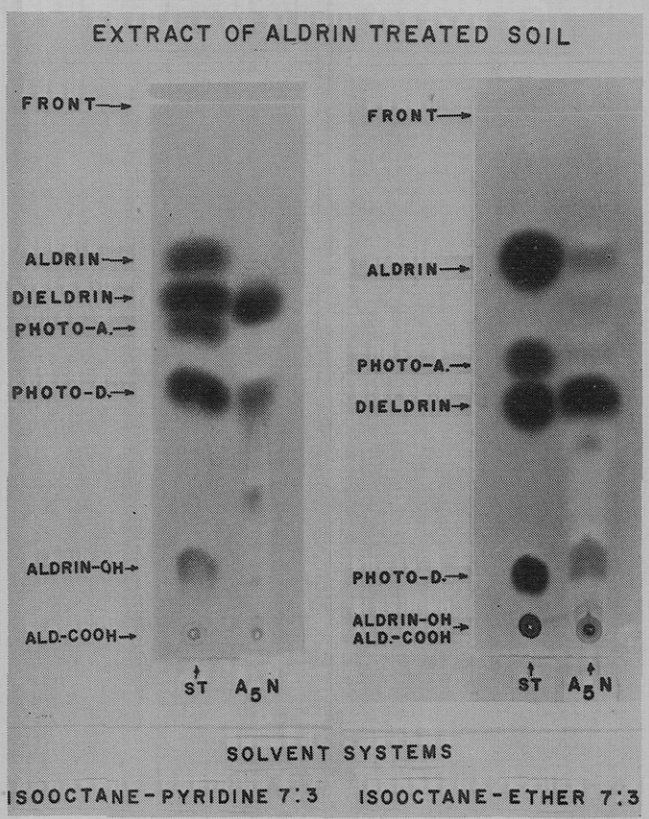

Figure 2. Thin-layer chromatogram of extracts from soil (A_5N), *treated with aldrin at 5 × 5 lbs/5-inch acre (1958–62) and sampled in 1968*

ST = reference compounds

Table III. Toxicity to Vinegar Flies and Houseflies of Aldrin Metabolites Isolated from Soil Extracts by Thin-Layer Chromatography

TLC Solvent System: Isooctane-Pyridine 7:3

		Per Cent Mortality/45 Hours			
		Drosophila[a]		Musca[b]	
R_f	Same as	Contact	Vapor	100 mg	300 mg
0.72	Aldrin[c]	100[d]	28	100	...
0.63	Dieldrin[c]				
0.57	Photo-A[e]	100[f]	16	73	...
0.46	Photo-D[c]	100[g]	20	20	42
0.26	?	0	0	0	0
0.12	Aldrin-OH	0	0	0	0
0.04	?	0	0	0	0

[a] Vinegar flies exposed directly (contact) or to vapors of the dry residue of isolates from thin-layer plates, representing 36 grams of soil each.
[b] One μl of acetone containing the residue from 100 mg or 300 mg of soil isolated from thin-layer plates, was applied topically to the ventral portion of the abdomen of each housefly.
[c] Confirmed by GLC.
[d] 50% in 6 hours.
[e] Area contained dieldrin, as determined by GLC, but no photo-aldrin.
[f] 56% in 6 hours.
[g] 36% in 6 hours.

Korte, 1965) prior to analyses by GLC but no "aldrin-OH" could be detected. "Aldrin-COOH" (dicarboxyl aldrin) did not move with either solvent system and was not detectable by GLC under the described conditions. Results thus indicated that the major metabolites detected in the soil were dieldrin and photo-dieldrin. With isooctane-pyridine as the solvent system, three unknown compounds were found in addition to dieldrin and photo-dieldrin. Their R_f values were 0.26, 0.12, and 0.04, thus indicating more polar properties than those of aldrin, dieldrin, or photo-dieldrin.

Table III summarizes the results obtained after vinegar flies or houseflies had been exposed to isolates from the thin-layer plates which had been developed with isooctane-pyridine (7 to 3). Spots containing dieldrin were most toxic to the insects because of the presence of relatively large amounts of this insecticide. The eluate from the area corresponding to photo-aldrin (R_f 0.57) exhibited toxicity, although this compound could not be confirmed by GLC. As mentioned previously, dieldrin was also found in this area, thus accounting for the toxicity. The isolated photo-dieldrin was least toxic because of its much lower concentration in the soil. The three unknown compounds of increasing polarity (R_f 0.26, 0.12, and 0.04) found in aldrin treated soils could not be detected in control soils. They were nontoxic to insects under the described conditions.

To detect potential water soluble metabolites, the water phase obtained from an acetonitrile extract was investigated by TLC and GLC as described. Traces of aldrin and dieldrin were found in the water, plus two peaks whose retention times were 3.0 and 3.4 in relation to the retention time of aldrin. No attempt was made to further characterize these two unknowns.

Soils, Quantitative Measurements. Samples obtained in 1968 from soil that had been treated with aldrin at five yearly dosages of 5 pounds per acre from 1958–62 were extrated with acetonitrile and contained 0.005 ppm of aldrin, 0.930 ppm of dieldrin, and 0.015 ppm of photo-dieldrin (1.6% of dieldrin). These latter compounds represent the two major toxic metabolites in addition to the three un-

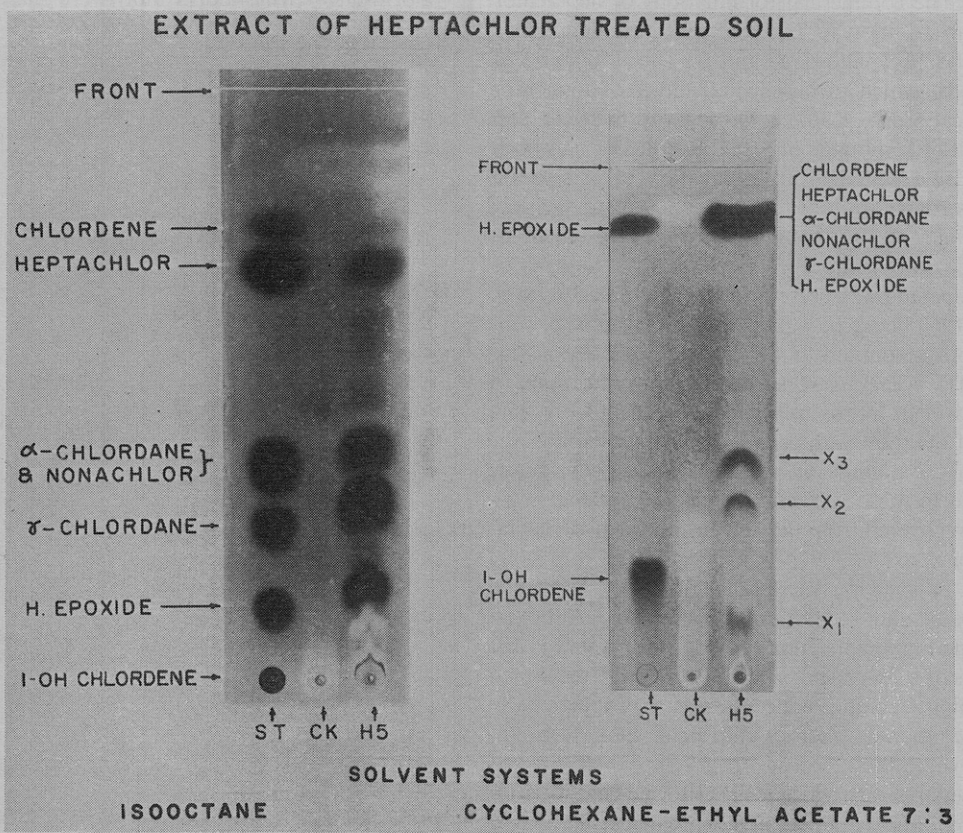

Figure 3. Thin-layer chromatogram of extracts from soil (H_5), treated with a heptachlor formulation at 5 × 5 lbs/5-inch acre (1958–62) and sampled in 1968

ST = reference compounds, CK = extract from insecticide free soil, X_1, X_2, X_3 = unknown compounds

identified and nontoxic compounds which appeared in soil because of the original aldrin application.

POTATOES AND CARROTS, QUALITATIVE AND QUANTITATIVE MEASUREMENTS. Potatoes contained dieldrin (0.047 ppm) and photo-dieldrin (0.0006 ppm) which amounted to 1.3% of the dieldrin concentration. "Aldrin-OH" and "aldrin-COOH" did not pass through the florisil column with 15% diethylether in hexane and could therefore not be detected. Analyses of carrots by TLC was inconclusive because of the presence of interfering substances. Analyses by GLC, though, showed the presence of dieldrin (0.133 ppm) and photo-dieldrin (0.002 ppm), which amounted to 1.5% of the dieldrin concentration.

Metabolite Studies in 1968 of Heptachlor-Treated Soils and Crops Grown Therein. SOILS, QUALITATIVE MEASUREMENTS. Results obtained by TLC with extracts from heptachlor treated soils are presented in Figure 3. With isooctane as the solvent, spots were obtained whose R_f values were similar to those secured with reference grade chlordene, heptachlor, α-chlordane plus nonachlor, γ-chlordane and heptachlor epoxide. In addition, three unknown spots (R_f 0.08, 0.48, and 0.60) were observed. With cyclohexane-ethylacetate (7 to 3) as the solvent system 1–OH-chlordene had an R_f value of 0.21 while all the other compounds did not separate, yielding one spot (R_f 0.89) that corresponded to heptachlor epoxide reference compound in Figure 3. Three unidentified spots (X_1, X_2, and X_3 at R_f 0.11, 0.34, and 0.43) were visualized with this solvent system although they may not have been identical to those obtained with isooctane as the solvent. To separate α-chlordane from nonachlor, an additional thin layer chromatogram was prepared from heptachlor-treated soil. The chromatogram was also developed with isooctane but the unsprayed area corresponding to α-chlordane plus nonachlor was removed from the plate, extracted with acetonitrile and re-spotted onto a second thin-layer plate. This chromatogram was then developed with 2% diethyl ether in isooctane and resulted in a clear separation of α-chlordane and nonachlor, thus confirming the presnce of these two compounds in the soil.

Analyses by GLC of extracts from isooctane developed thin-layer plates confirmed the presence of heptachlor, nonachlor, α-chlordane, γ-chlordane, and heptachlor epoxide (Table IV). These extracts were also toxic when tested with vinegar flies and houseflies. The lower mortalities observed with houseflies probably resulted from the fact that equivalents of only 100 mg of soil were applied per fly. Extracts obtained from the areas of the unknown compounds were nontoxic to the insects. This data, therefore, indicates that after the application of a heptachlor formulation to soil two toxic metabolites (heptachlor epoxide and α-chlordane) and three nontoxic compounds were formed, indicating the breakdown in the soil of heptachlor and related compounds.

TLC of the water-acetonitrile phase, obtained by an acetonitrile extraction of soils as described, revealed the presence of one spot (R_f 0.00) which was not comparable to spots obtained with any of the reference materials used. The hexane phase, though, contained all the other previously described compounds.

SOILS, QUANTITATIVE MEASUREMENTS. Soil samples from plots that had been treated with heptachlor at 5 pounds per acre per year over the five-year period 1958–62 were also extracted with acetonitrile and analyzed quantitatively as described. Concentrations of 0.105 ppm of heptachlor, 0.511 ppm of heptachlor epoxide, 0.769 ppm of γ-chlordane, 0.092 ppm of α-chlordane, and 0.047 ppm of nonachlor were found.

Table IV. Toxicity to Vinegar Flies and Houseflies of Heptachlor Metabolites Isolated from Soil Extracts by Thin-Layer Chromatography

TLC Solvent System: Isooctane

		Per cent Mortality/45 Hours		
		Drosophila[a]		Musca[b]
R_f	Similar to	Contact	Vapors	100 mg
0.77	Chlordene	0	0	7
0.70	Heptachlor[c]	36	22	22
0.61	?	0	0	0
0.48	?	0	0	0
0.39	Nonachlor[c]	85	...	0
	α-Chlordane[c]	94	...	11
0.30	γ-Chlordane[c]	100[d]	100	11
0.16	H. epoxide[c]	100[e]	100	69
0.05	1-OH-Chlordene	0	0	0

[a] Vinegar flies exposed directly (contact) or to vapors of the dry residue of isolates from thin-layer plates, representing 36 grams of soil each.
[b] One μl of acetone containing the residue from 100 mg of soil isolated from thin-layer plates, was applied topically to the ventral portion of the abdomen of each housefly.
[c] Confirmed by GLC.
[d] 73% in 6 hours.
[e] 100% in 2 hours.

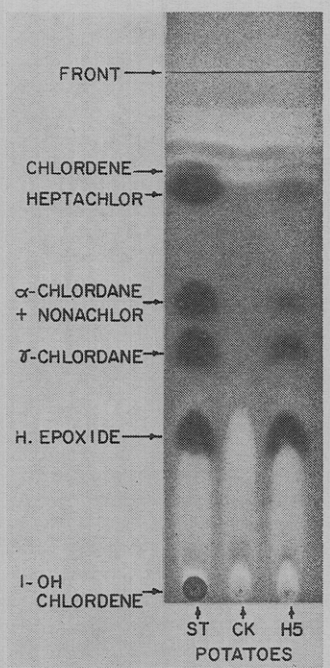

Figure 4. Thin-layer chromatogram of extracts from potatoes grown in heptachlor treated soil (H_5). Solvent: isooctane

ST = reference compounds,
CK = extract from potatoes grown in insecticide free soil

POTATOES, QUALITATIVE AND QUANTITATIVE MEASUREMENTS. Figure 4 is a photograph of a thin-layer chromatogram obtained with an extract from 72 grams of potato tissue. Accordingly, four spots were found which had R_f values identical with those obtained with reference grade heptachlor, α-chlordane plus nonachlor, γ-chlordane, and heptachlor epoxide. Isolation of these spots and analyses by GLC as described resulted in peaks that were identical with reference

grade heptachlor, γ-chlordane, and heptachlor epoxide. Small peaks, indicating trace amounts of both α-chlordane and nonachlor were also found. These results are qualitatively similar to those obtained with soils, except that the three unknown compounds recovered from soils were not found in potatoes.

To test the biological activity of these compounds, vinegar flies were exposed to isolates from the thin-layer plates. All four spots exhibited some toxicity. After a 48-hour exposure period, mortalities amounted to 8% with isolates from the heptachlor spot, 52% with isolates from the α-chlordane plus nonachlor spot, 64% with isolates from the γ-chlordane spot, and 100% with isolates from the heptachlor epoxide spot. Exposure of flies to isolates corresponding to R_f values obtained with chlordene and "1-OH chlordene" did not result in insect mortality.

Quantitative analyses of potatoes showed the presence of 0.002 ppm of heptachlor, 0.004 ppm of α-chlordane, 0.002 ppm of nonachlor, 0.015 ppm of γ-chlordane, and 0.054 ppm of heptachlor epoxide.

LITERATURE CITED

Edwards, C. A., Beck, S. D., Lichtenstein, E. P., *J. Econ. Entomol.* **50,** 622 (1957).
Hornstein, I., Sullivan, W. N., *J. Econ. Entomol.* **46,** 937 (1953).
Korte, F., Arent, H., *Life Sciences* **4,** 2017 (1965).
Lichtenstein, E. P., J. AGR. FOOD CHEM. **8,** 448 (1960).
Lichtenstein, E. P., Fuhremann, T. W., Scopes, N. E. A., Skrentny, R. F., J. AGR. FOOD CHEM. **15,** 864–9 (1967).
Lichtenstein, E. P., Myrdal, G. R., Schulz, K. R., *J. Econ. Entomol.* **57,** 133 (1964).
Lichtenstein, E. P., Polivka, J. B., *J. Econ. Entomol.* **52,** 289 (1959).
Lichtenstein, E. P., Schulz, K. R., J. AGR. FOOD CHEM. **13,** 57 (1965).
Ludwig, G., Korte, F., *Life Sciences* **4,** 2027 (1965).
Mitchell, L. C., *J. Assoc. Offic. Agr. Chemists* **40,** 999 (1957).
Velsicol Chemical Corp., Chicago, Ill., private communication, 1967.

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