lulose fraction of wheat and rice straw was actually in the carbon atoms of glucose, the cellulose isolated from the rice or wheat treated with nitrofen-^{14}C was hydrolyzed and the resulting glucose was derivatized to the osazone. The osazone was then recrystallized to a constant specific radioactivity and identified by infrared spectrophotometry.

The radioactivity present in the pure osazones from both rice and wheat was significantly high enough to conclude that a limited amount of conversion of the carbon atoms of nitrofen-^{14}C into glucose and cellulose had occurred.

By dividing the specific radioactivity of the cellulose by the specific radioactivity of the original rice or wheat straw and multiplying by the percent of crude fiber (cellulose) in the cereal straw, the percent of radioactive residues as cellulose at harvest was calculated. Table IV contains data from such calculations.

It can be seen that only preemergence nitrofen treated rice straw has any large amount of radioactive residue in the cellulose. Postemergence-treated rice straw as well as preemergence wheat straw contained little radioactivity in the cellulose.

SUMMARY

It is apparent that rice and wheat can metabolize nitrofen-^{14}C in such a way as to produce a lignin fraction containing radioactivity. This association is either in the form of a lignin–nitrofen conjugate or as an integral part of one or more of the molecules which comprise the lignin. There was no species variation with respect to the percentage of radioactive residue associated with the lignin, and the position of the ^{14}C label in the nitrofen molecule did not alter the percentage of radioactive residue associated with lignin.

Wheat and rice appear to metabolize nitrofen-^{14}C to cellulose to only a limited extent. Only preemergence nitrofen-^{14}C treated rice straw appeared to incorporate signifi-

cant amounts of nitrofen into cellulose. It has been shown that mature wheat and rice grain from nitrofen-^{14}C treated plants contain radioactive starch (Wargo et al., 1975). Apparently, the flow of carbon prefers a path from nitrofen-^{14}C into a starch of grain rather than into the cellulose of wheat or rice straw as the plants are maturing. The fact that the ^{14}C from nitrofen-^{14}C can be reincorporated into starch in cereal grains along with the fact that the ^{14}C can be reincorporated into cellulose in rice straw suggests that nitrofen goes through three major metabolic changes after being applied. First, there is cleavage of the diphenyl ether bond, followed by catabolism which includes a benzene ring opening, and finally reorganization of the carbon atoms into glucose, starch, or cellulose.

ACKNOWLEDGMENTS

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Heptachlor and Dieldrin Disappearance from a Field Soil Measured by Annual Residue Determinations

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In 1969 heptachlor was incorporated to 7.5 cm depth in an experimental field. Over 4.5 years the disappearance rate followed first-order reaction kinetics according to the equation $\log H = 0.063 - 0.33T$, where H is in parts per million and T in years, giving a half-life of 0.91 year. Heptachlor epoxide and hydroxychlordene were identified as degradation products. In a similar experiment with dieldrin, begun in 1966, the disappearance followed the equation $D = 2.72 - 0.20(\pm 0.09)T$, where the upper and lower values of the regression coefficient are the 80% confidence limits; the equation indicates a 95% disappearance time of 13 years. No dieldrin degradation products were identified. The results show that in experiments to measure persistence in the field, data must be obtained over the whole life of the pesticide. Sampling methods must permit determination of the variability inherent in data obtained in experiments using regular farm practices.

Measurements of the persistence of organochlorine insecticides in soil have been reported by a number of workers (Lichtenstein et al., 1971a,b; Nash and Woolson, 1967;

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Stewart and Fox, 1971; Young and Rawlins, 1958; Voerman and Besemer, 1970). Earlier data were summarized by Edwards (1966). In almost all cases measurements of disappearance were made on relatively small research plots using special incorporation methods to ensure good mixing of the pesticide into the soil; these were rarely representative of regular farming practice. The number of samples analyzed was often small, single laboratory samples being prepared by bulking a number of sample cores; this procedure precluded any estimate of sample variability. In many studies samples were only taken after extended periods of time so that the disappearance curve could not be fully es-

Table I. Physical Characteristics, Crops, and Management of Experimental Watersheds

	Year of dieldrin treatment		
	1966	1968	1969
Watershed no.	128	106	109
Area (hectares)	1.09	0.63	0.68
Av slope (%)	13.6	14.3	12.7
Soil type	Berks Silt Loam	Berks Silt Loam	Rayne Silt Loam
Organic matter content (Topsoil % dry basis)	2.1	1.6	2.2

Year	Crop rotations		
1966	Maize	(Meadow)	(Wheat)
1967	Wheat	(Meadow)	(Meadow)
1968	Meadow	Maize	(Meadow)
1969	Meadow	Wheat	Maize
1970	Maize	Meadow	Maize
1971	Wheat	Meadow	Maize
1972	Meadow	Maize	Maize

	Dates of operations[a]		
Pesticide application	5/20/66	5/3/68	4/30/69
	Oct 1966 (T)	Oct 1968 (T)	Oct 1969 (T)
	April 1970 (P)	April 1972 (P)	April 1970 (P)
	Sept 1970 (T)	Oct 1972 (T)	April 1971 (P)
			April 1972 (P)

[a] T = tillage to 7.5 cm; P = plowed to 17.7 cm.

tablished, although Nash and Woolson (1967) were able to conclude that the disappearance of several insecticides from small undisturbed plots appeared to follow first-order kinetics.

A series of field experiments was begun in 1966 to measure the amounts of organochlorine insecticides lost from field soils under normal agricultural management. These offered the opportunity to examine how far increased sampling frequency could improve the accuracy of measurement of disappearance rate and to assess the impact of sample variability on the confidence limits placed on the result. Heptachlor disappearance was measured in a single experiment begun in 1969; dieldrin data were obtained from experiments begun in 1966, 1968, and 1969.

EXPERIMENTAL SECTION

General Description. The experiments were performed on three small watersheds at the North Appalachian Experimental Watershed at Coshocton, Ohio. Since the objectives included measurements of the amounts of insecticides lost in runoff and sediment, the entire area of each watershed was uniformly treated with insecticide and no plot replications were possible. Earlier results of other parts of the work have been presented elsewhere (Caro et al., 1972; Caro and Taylor, 1971).

The crop sequences and management of each watershed are described in Table I. A single application of the insecticide was made on each watershed in May immediately before maize planting. The 1969 watershed was maintained in continuous maize and was plowed to 17.7 cm depth in March of each year, with subsequent cultivation to 7.5 cm before maize planting. The 1966 and 1968 watersheds were kept in a 4-year rotation of maize, winter wheat, and 2 years of grass–legume meadow. In the first October after the maize crop the soil was cultivated to 7.5 cm to prepare the seedbed for winter wheat; in April of the second year a mixture of alsike clover and timothy was seeded into the

ankle-high wheat without cultivation. The same sequence of cultivations was repeated in the second rotation.

Insecticides were applied to the bare soil as a uniform spray of aqueous emulsion from a 20-ft tractor-mounted boom at a rate of 5.6 kg/ha of active material. The pesticide was always disked into the soil to 7.5 cm depth within 30 min of application using a conventional two-gang disk with depth control wheels. In 1969 heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-$endo$,exo-5,8-dimethanonaphthalene) were applied together in the same spray. In 1966 and 1968 dieldrin was applied alone. The heptachlor and dieldrin used were regular commercial formulations supplied by the courtesy of the Velsicol and Shell Chemical Corporations, respectively.

Sampling Program. Each watershed was subdivided into sections as illustrated in Figure 1. The actual field sampling procedures varied slightly during the experiments. In 1966, 1967, and 1968, 12 randomly spaced holes were dug in each section and a vertical slice was cut from the side of each with a spade. This slice was then cut horizontally to give samples at the 0–7.5, 7.5–17.7, and 17.7–25 cm depths. Each of the 12 samples was then composited to give a single sample to each depth for each section.

In 1969, 75 cores, each 21 mm in diameter and 23 cm long, were taken from each section and composited to make the bulk sample. This modification was made in an attempt to reduce the variability of the data (Taylor et al., 1971).

The first samples from each watershed were taken within 24 hr of the application.

Analytical Methods. All samples were transported from the field to the laboratory in hermetically sealed cans; before analysis the moist samples were sieved through a 4-mesh screen to remove small stones. The fraction retained by the screen was measured and included in the total dry weight used in calculating the results. Analyses of this frac-
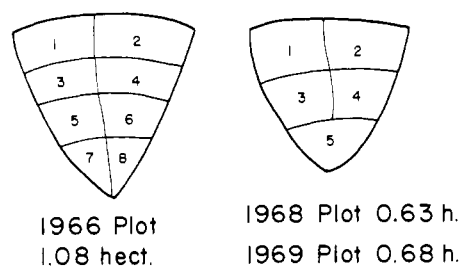
Figure 1. Distribution of sampling sections and total areas of experimental watersheds.

Table II. Change in Distribution of Dieldrin between Three Soil Layers with Time

	% fraction of dieldrin (means of 8 samples) at age (yr)			
	0		2.42	
Depth, cm	Mean	Std dev	Mean	Std dev
0–7.5	75.7	±12	80.7	±9
7.5–17.7	22.2	±12	16.9	±8
17.7–30.0	2.1	±3.4	2.4	±1.7

tion showed that it never carried significant amounts of insecticide. After screening, a 10–20-g subsample was taken and the moisture tension adjusted to between 1 and 10 bars with a pressure plate. The sample was then extracted for 3 hr with a 3:1 mixture of hexane and 2-propanol in a Soxhlet apparatus. The extract was passed through a short column of powdered alumina to break any emulsion and the column eluate was washed with water to remove the 2-propanol. The residual hexane was diluted to a convenient volume (10 to 250 ml, depending upon anticipated insecticide concentration), and a 2–8-μl aliquot was injected into a gas chromatograph.

The GLC column used was either a 2 mm i.d. × 165 cm glass column packed with a 1:1 mixture of 10% DC-200 and 15% QF-1 on Gas-Chrom Q or a 4 mm × 165 cm column packed with 3% DC-200 on Gas-Chrom Q. The temperatures of the [63]Ni detector and injection port were 275 and 234 °C, respectively. The column temperature and flow rate were 190 °C and 100 ml/min for the 2 mm column and 220 °C and 70 ml/min for the 4 mm column. The carrier gas was a 95:5 mixture of argon and methane. In tests with soils spiked in the laboratory, the recovery of the method was found to be very close to 95%.

RESULTS

Downward Movement of Dieldrin. The distribution of the dieldrin in the samples from the 0–7.5, 7.5–17.7, and below 17.7 cm depths in the 1966 watershed is presented in Table II. Statistical analysis reveals that the changes in the mean concentrations are not significant in light of the variability of the data. Thus, there was no evidence of dieldrin movement in the profile during 2.5 years after application. The presence of about 20% of the dieldrin in the 7.5–17.7 cm layer in both sets of data probably resulted from inadvertent incorporation to below 7.5 cm during the initial mixing. The amounts below 17.7 cm appear to represent contamination at the time of sampling, when it is extremely difficult to prevent small amounts of topsoil from contaminating the lower layers. The mean value of 2.1% in the lowest layer on the date of initial sampling is almost entirely due to a single sample from one of the eight sites.

In view of the difficulties of sampling, coupled with the problems of locating the original layers in the soil after 2 years when the watershed was in meadow, samples were not separated for depth studies after 1969. The data in Table II show that long-term studies would be necessary to obtain reliable results. In view of the soil disturbances due to the subsequent plowing and cultivations, such work was considered impractical. Very slow rates of downward movement of dieldrin over longer periods of time have been demonstrated by Lichtenstein et al., (1971a,b) and by Nash and Woolson (1968).

Persistence of Heptachlor. Heptachlor concentrations in samples from each section of the 1969 watershed are presented in Table III together with mean values and standard deviations for the whole area. Comparison of the correlation coefficients for the two regression equations shows the results are best described by regression of the logarithm of

the concentrations upon time, indicating that the heptachlor disappearance follows first-order kinetics. The best estimate of the half-life is 0.91 year, with a 90% confidence that the true value is between 0.86 and 0.97 year: this corresponds to a best estimate of 3.94 years for 95% disappearance. This estimate of the disappearance rate must be regarded as an "average" value taken over the 4.5 year period of the experiment. Even if further refinement were not precluded by the variability of the data, it is unlikely that any better estimate would be meaningful, since the rate would be expected to show seasonal variations due to changes in soil temperature and soil conditions due to the cultivations that took place. Examination of a plot of the results does suggest that the rate of disappearance was faster in the summer than winter months.

Heptachlor Conversion Products. The samples from the 1969 watershed also contained heptachlor epoxide (1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindene) and hydroxychlordene (4,5,6,7,8,8-hexachloro-1-hydroxy-3a,4,7,7a-tetrahydro-4,7-methanoindene). The average amounts for the whole watershed, together with the standard deviations, are presented in Table IV. The amount of heptachlor epoxide increased during the first year and then remained essentially constant. Insufficient time has elapsed to permit an estimate of the disappearance rate, but the compound appears to be persistent, as demonstrated by Lichtenstein et al. (1970). The hydroxychlordene concentration reached a maximum of 95 ppb after about 1 year. The decrease to very low levels (below 10 ppb) at the end of the second year suggests that hydroxychlordene disappears rapidly with a half-life of several weeks as shown by Bonderman and Slack (1972).

Volatilization measurements on the 1969 watershed showed that 383 g/ha was lost to the air between May and October 1969, or about 6.8% of that applied. Volatilization was not measured in later years, but was probably much less (Taylor et al., 1972). No significant sediment was lost from the watershed and runoff losses were small; comparison with earlier dieldrin observations indicates that heptachlor losses by these pathways were not likely to be more than 0.1–0.3% (Caro and Taylor, 1971). Calculation of the heptachlor epoxide and hydroxychlordene in terms of "heptachlor" equivalents indicates that the formation of these products and the loss by volatilization can account for nearly all the heptachlor disappearance in the first year. An increasing fraction of the parent material could not be traced. After 4.5 years only 25% of the heptachlor could be accounted for, of which about four-fifths remained in the soil as heptachlor epoxide. Other undetected reaction products must therefore have been formed.

Persistence of Dieldrin. Dieldrin concentrations in the sections of the 1969 watershed are presented in Table III. The rate of disappearance is best described by the equation $D = 1.404 - 0.134(\pm0.042)T$ with a correlation coefficient of 0.88. Calculation of the regression in terms of a logarithmic relationship gives no improvement in the correlation coefficient and it is evident that, in view of the variability

Table III. Insecticide Contents on the 1969 Watershed, with Means and Regressions upon Time

Time after application, years	Insecticide concn (ppm to 23 cm depth) in field sections					Mean	Std dev
	1	2	3	4	5		
			Heptachlor				
0	1.38	1.51	2.10	1.01	1.31	1.46	0.40
0.47	0.80	0.72	0.91	0.56	0.98	0.79	0.16
0.92	0.76	0.64	0.85	0.53	0.69	0.69	0.12
1.51	0.27	0.42	0.33	0.16	0.24	0.28	0.10
1.93	0.23	0.26	0.22	0.22	0.20	0.23	0.02
2.43	0.17	0.15	0.13	0.14	0.18	0.15	0.02
3.49	0.08	0.09	0.08	0.07	0.06	0.08	0.01
4.45	0.07	0.05	0.05	0.03	0.03	0.05	0.02
			Dieldrin				
0	1.37	1.52	2.12	0.96	1.24	1.44	0.43
0.47	1.40	1.24	1.67	1.04	1.65	1.40	0.27
0.92	1.47	1.24	1.54	1.25	1.38	1.38	0.13
1.51	1.11	1.30	1.12	0.68	0.93	1.03	0.23
1.93	1.14	1.20	1.04	1.06	0.68	1.02	0.20
2.43	1.20	0.98	1.28	0.82	0.97	1.05	0.19
3.49	1.12	1.17	1.11	1.14	0.85	1.08	0.13
4.45	1.06	0.87	0.79	0.56	0.72	0.80	0.18

Regression of means (T in years)	% confidence of slope	r
$H = 0.98 - 0.270(\pm 0.102)T$	80	0.84
$\log H = 0.063 - 0.330(\pm 0.021)T$	90	0.985
$D = 1.404 - 0.134(\pm 0.042)T$	80	0.88

Table IV. Conversion Products of Heptachlor in Samples from 1969 Watershed

Time after application, years	Mean concn, ppm ($\pm$ std dev), sections of watershed	
	Heptachlor epoxide	Hydroxychlordene
0	0	0
0.47	0.18 $\pm$ 0.03	0.011 $\pm$ 0.006
0.92	0.25 $\pm$ 0.02	0.095 $\pm$ 0.030
1.51	0.25 $\pm$ 0.06	0.022 $\bullet$ 0.020
1.93	0.24 $\pm$ 0.02	<0.01
2.43	0.28 $\pm$ 0.02	0.01
3.49	0.30 $\pm$ 0.03	0.01
4.45	0.20 $\pm$ 0.06	0.01

of the data, the sampling period was too short to shed light on the kinetic characteristics of the dieldrin disappearance. The linear regression leads to a "best estimate" of 10.0 years for 95% disappearance with an 80% probability that the "true value" lies between 8.1 and 13.6 years.

Data from the 1966 watershed are presented in Table V. The best estimate of the 95% disappearance time is 12.9 years, with an 80% confidence that it lies between 10.0 and 20.5 years. The wider spread of this estimate beyond the 1969 result is due to the greater uncertainty of the slope and the poorer correlation coefficient. Logarithmic regression on the 1966 data shows no improvement. Calculation of the coefficient of variation shows that the variability of the 1966 data was greater than the 1969 data, so that the confidence limits that can be placed on the regression are wider despite the longer sampling period. The lower variability of the 1969 data reflects the improved sampling technique introduced in that year. Statistical analysis of

the regressions for the eight separate sections of the 1966 watershed (Table IV) indicates that there were no significant differences at the 5% level between the slopes or intercepts of the separate regressions.

Data from the 1968 watershed were similar to the 1966 data, giving a regression equation $D = 2.22 - 0.208(\pm 0.135)T$ and $r = 0.67$. This corresponds to a best estimate of 10.1 years for the 95% disappearance with 80% confidence limits of 6.1 and 29 years. Although these results are consistent with the other experiments the sampling period of 5.4 years is clearly too short for meaningful extrapolation.

The differences between the intercepts of the 1969 and the 1966 and 1968 equations reflect, in large degree, the differences in sampling depth in the two experiments.

No conversion products of dieldrin were identified in any of the experiments.

DISCUSSION

Comparison of the usefulness of the results from the single heptachlor experiment with the three experiments on dieldrin in terms of their value for making accurate measurements of the disappearance rate of the two insecticides reveals the importance of taking measurements over the whole life of each compound under field conditions. Owing to the inherent variability of the data, extrapolations made from samples taken over a fraction of the insecticide life give very uncertain estimates of the persistence times of the materials. The need for extended sampling is exemplified in the heptachlor results, where data were obtained over a period longer than the 95% disappearance time. It may be noted that if the analysis of these data is confined to the first 2.5 years, excluding the results obtained after 3.5 and 4.45 years, the persistence of the chemical is seriously underestimated and the statistical analysis does not permit a clear distinction between a linear degradation rate and first-order kinetics. Despite the larger amount of data for dieldrin, this distinction cannot be drawn because the

Table V. Dieldrin Content of Samples from Sections of 1966 Watershed, with Mean Values, and Regressions of Concentration upon Time

Time after application, years	Dieldrin content of samples from section, ppm (0–17.7 cm depth, dry basis)								Mean	Std dev
	1	2	3	4	5	6	7	8		
0	3.85	8.76	3.69	3.15	2.55	2.36	1.98	2.43	3.60	2.19
0.34	3.41	2.32	4.37	0.66	1.47	2.34	0.94	2.68	2.27	1.24
1.31	0.77	1.97	1.48	2.59	1.29	2.05	1.41	1.75	1.66	0.55
2.42	1.14	4.42	2.19	2.61	3.53	2.50	2.24	2.75	2.67	0.97
3.43	0.04	0.40	3.28	1.13	2.15	3.99	2.71	1.46	1.89	1.39
3.87	1.43	2.55	1.15	2.19	1.66	2.46	1.12	1.08	1.71	0.61
4.46[a]	0.81	1.94	1.88	2.40	2.16	2.40	1.05	1.25	1.74	0.62
5.51[a]	0.80	1.96	1.63	2.34	2.64	2.37	1.07	1.21	1.75	0.68
6.40[a]	0.84	1.72	1.71	2.12	2.51	1.84	0.99	1.04	1.60	0.59
7.41[a]	0.71	1.45	1.07	1.31	1.63	2.24	0.77	0.89	1.26	0.51

Regression equation	% confidence of slope	r
$D = 2.72 - 0.200(\pm 0.090)T$	80	0.74

[a] Values normalized to 0–17.7 cm depth.

longest sampling period is only about three-quarters of the best estimate of the 95% disappearance time. While all three sets of data indicate that this is between 10 and 13 years, based on linear regressions, this must be regarded as a minimum estimate. If later data indicate that first-order kinetics apply the 95% disappearance time may be much greater.

These experiments illustrate several considerations that must be applied in the design of experiments to measure degradation rates of soil-applied pesticides under field conditions reflecting actual farm practice. First, sampling must be extended over a period comparable to the lifetime of the pesticide. Extrapolation of shorter period data is very uncertain. Second, sampling procedures must be designed to compare data from several samples representative of the treated area so that variability can be assessed. Third, in the interest of economy, sampling times should be scheduled at intervals such that statistically meaningful differences are to be expected between successive samplings. Where the number of analyses that can be made is limited, it is clearly preferable to obtain several representative samples on each of fewer dates than to take too few too frequently. The design of the experiment should therefore take into account the variability, measured at the first sampling, and the expected approximate rate of degradation.

The degree of variability that can be expected between replicate samples taken from a plot or field area depends upon the uniformity of mixing of the pesticide in the soil and the sampling techniques employed. A detailed study on the dieldrin distribution in the 1968 watershed showed that this was highly irregular over small distances, with up to sixfold variations over distances of less than 1 m (Taylor et al., 1971). Analysis of these results showed that the coefficients of variation between duplicate bulk samples could be reduced to 20% at sampling densities as high as 1 per 3 m². This is impracticably high for most field studies, and higher coefficients of variation must often be accepted, further emphasizing the need for replicate sampling to assess variability. Some indication of the improvement that can be expected from improved techniques may be seen in the reduction in coefficients of variability between the 1966 and 1969 experiments, after an improved sampling technique was used.

Variability due to errors in analytical procedures is likely to be small in comparison with that due to sampling. In the 1969 experiment, heptachlor and dieldrin were applied in the same spray and determined in the same sample extracts. Calculation of the heptachlor/dieldrin ratios from the initial sampling data in Table III gave a mean value of 1.02 with a standard deviation of 0.04. This is very small in comparison with the standard deviations of the mean concentrations themselves. Although the ratio decreased steadily with time, its variability remained small throughout the experiment.

The need for critical sampling procedures is less important in small plot or greenhouse experiments where increased precautions can be taken to ensure very thorough initial mixing of pesticides in the soil. Such experiments do not, however, often reproduce field conditions, where poorer mixing may produce localized pesticide concentrations or the uniformity of the soil itself is much less on a microscale. While the results of more highly controlled experiments provide essential data for estimating field persistence, comparative studies made under actual use conditions are equally necessary for a full understanding of environmental behavior.

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