mixture of 20 g of phenylhydrazine hydrochloride and 30 g of sodium acetate was added. The solution was heated for an additional 3 hr on a steam bath.

The mixture was allowed to cool overnight in a refrigerator at 3–4°. The precipitated glucosazone was removed by filtration, washed several times with water, and recrystallized three times from a methanol–water mixture. Melting points were determined for each sample, and samples of the dried glucosazone from each recrystallization were combusted for assay of radioactivity.

Samples of glucosazone were prepared for infrared spectroscopy by pressing the sample into a potassium bromide pellet. A Perkin-Elmer RE infrared 137 spectrophotometer was used to determine the infrared spectra.

RESULTS AND DISCUSSION

Starch was isolated from wheat and rice grain and radioassayed by combustion followed by liquid scintillation counting. Preliminary experiments revealed that crude starch from nitrofen-^{14}C treated wheat did indeed contain radioactivity. To demonstrate that the radioactivity in the starch fraction was a part of the glucose unit, the starch was hydrolyzed; the resulting glucose was derivatized to the osazone and recrystallized several times. An infrared spectrum of the osazone was taken and appeared identical with the infrared spectrum of a standard of glucosazone.

Table I shows the results of typical experiments in which starch was isolated from wheat grain and hydrolyzed and the glucose derivatized to the glucosazone. The glucosazone was then recrystallized three times and radioassayed each time. The data in Table I indicate that a constant specific radioactivity was obtained for the wheat grain osazones. Similar experiments with rice gave radioactive starch, which after hydrolysis and derivatization yielded an osazone of constant specific radioactivity upon recrystallization. The radioactivity present in the purified glucosazones from both rice and wheat was significantly high enough to conclude that carbon atoms from nitrofen had entered the metabolic pool and had been channeled into glucose and subsequently into starch.

Table II shows that when the specific radioactivities of these glucosazones are used to calculate the percent of total radioactivity present as starch in wheat or rice grain, a large portion of the residue is accounted for. About 64–94% of the radioactive residues in nitrofen treated wheat or rice grain are in the starch 110 and 147 days, respectively, after planting and treatment. The question of which nitrofen ring is labeled or the species or variety of grain does not appear to make a difference in the extent to which ^{14}C residues appear as starch within the scope and experimental error of this work.

These data lead to the conclusion that the carbon atoms of nitrofen are channeled through at least three structural reorganizations during the metabolism of nitrofen to glucose. First, there must be a cleavage of the diphenyl ether bond during this process. As of now, no intermediate which would demonstrate conclusively that there has been such a cleavage has been isolated. However, Frear and coworkers have shown that the herbicide fluorodifen can be cleaved by extracts of *Pisum sativum* which documents the existence of such an enzymatic diphenyl ether cleaving system in plants (Frear and Swanson, 1973).

A second major metabolic occurrence appears to be ring opening eventually leading to glucose or simpler, presumably aliphatic moieties. Finally, a third anabolic process must then take place to convert these ^{14}C-containing materials to glucose and finally to starch.

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Characterization of Bound Residues of Nitrofen in Rice and Wheat Straw

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Crude lignin and cellulose isolated from rice and wheat straw after postemergence and preemergence treatment with nitrofen-^{14}C were found to contain radioactivity. The lignin was purified to a constant specific radioactivity. About 30% of the radioactive residues in rice and wheat straw was in lignin. The cellulose from rice and wheat straw was hydrolyzed to glucose and derivatized to the osazone with phenylhydrazine. The osazone was recrystallized to constant specific radioactivity. Very little of the radioactivity in the rice and wheat straw was found to be in cellulose.

The selective herbicide 2,4-dichloro-1-(4-nitrophenoxy)-benzene, sometimes referred to as nitrofen (I) has been used to control annual grasses and broadleafed weeds in many crops in the United States. Investigations into the metabolism of this herbicide have shown that the amine (II) as well as the acetamide (III) may be produced in vivo (Gutenmann and Lisk, 1967; Adler et al., 1971). Even though no direct evidence for cleavage of the diphenyl ether bond has been reported for nitrofen in plants, a similar herbicide, fluorodifen, has been shown to be cleaved at the diphenyl ether in peas (Frear and Swanson, 1973).

In the course of investigations using nitrofen as a preemergence herbicide for wheat and rice, we obtained straw from these crops which contained radioactive residues. These residues could not be quantitatively removed by conventional organic solvent extraction techniques. Such

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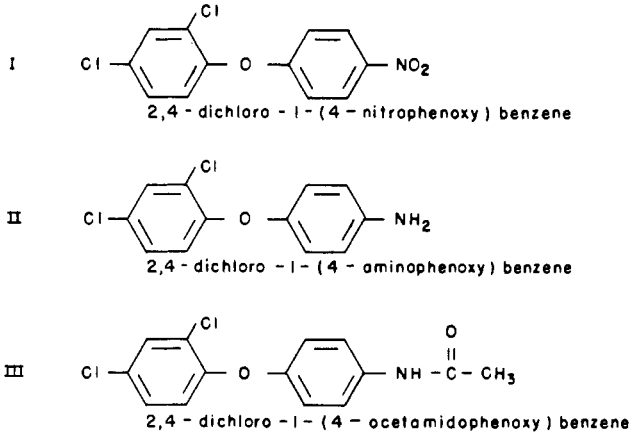
residues are commonly referred to as bound residues. Chin and coworkers (1964) have shown that in rice straw, bound residues of the herbicide SWEP [methyl *N*-(3,4-dichlorophenyl)carbamate] are in the form of a lignin–SWEP complex. Wilson et al. (1968) have reported that a major portion of the herbicide 3,4-dichloropropionanilide is metabolized to a dichloroanaline–lignin complex in rice and barnyard grass. Since rice and wheat straw are about 15% lignin (Stone et al., 1951) it appeared that lignin might well contain some of the bound residues that were associated with mature rice and wheat straw that had been treated with nitrofen-^{14}C.

Wargo et al. (1975) have shown that the ^{14}C from nitrofen-^{14}C can be reincorporated into starch in rice and wheat grain. Since glucose-^{14}C could lead to many major plant constituents, it was anticipated that cellulose isolated from nitrofen-^{14}C treated rice and wheat plants might also contain radioactivity.



MATERIALS AND METHODS

Chemicals. Nitrofen-^{14}C [2,4-dichloro-1-(4-nitrophenoxy)benzene] was prepared at Rohm and Haas Company, Bristol, Pa. (McLaughlin, 1969). Two ^{14}C-labeled preparations were used. One contained ^{14}C uniformly distributed about the dichlorophenyl ring. The other sample contained ^{14}C uniformly labeled about the nitrophenyl ring. For rice samples, nitrofen-^{14}C (dichlorophenyl ring labeled) was used at a specific radioactivity of 1.37 mCi/g and was applied preemergence at 3 lb/acre and postemergence at 6 lb/acre. Nitrofen-^{14}C was applied to wheat as a preemergence spray at 3.2 lb/acre with a specific radioactivity of 0.80 mCi/g for the nitrophenyl ring labeled material and 0.99 mCi/g for the dichlorophenyl ring labeled sample (4 lb/acre).

Ether, dioxane, and phenylhydrazine hydrochloride were purchased from J. T. Baker. Aquasol was purchased from Packard Instrument Company. Benzene was glass distilled before use. Soxhlet thimbles were obtained from Whatman.

Crops. Rice straw samples were from two small rice plots (4 ft × 4 ft) which had been sprayed preemergence or postemergence with nitrofen-^{14}C labeled uniformly in the dichlorophenyl ring. The straw samples from preemergence treatment were harvested 147 days after treatment and contained residues of 0.12 ppm calculated as nitrofen. The postemergence treated straw samples were harvested 147 days after planting, 96 days after treatment, and contained radioactive residues of 1.91 ppm calculated as nitrofen.

Wheat straw samples were from two small plots which had been treated preemergence with nitrofen-^{14}C labeled either uniformly in the nitrophenyl ring or in the dichlorophenyl ring. The straw samples were harvested 110 days after planting and treatment and contained residues of 1 ppm calculated as nitrofen.

Radioassay. Dry rice or wheat straw and samples at various stages of fractionation were weighed into zircon combustion boats for assay of radioactivity. The organic material was burned and the carbon dioxide was collected in a trap containing 10.0 ml of 5 *M* ethanolamine in methyl Cellosolve. An aliquot of this trap solution was then counted in 15 ml of a cocktail consisting of 2 l. of toluene, 1.5 l. of methanol, 7.5 g of 2,5-diphenyloxazole, and 2.1 g of 1,4-bis[2-(4-methyl-5-phenyloxyazolyl)]benzene. Aliquots of all other liquid samples were placed in polyethylene vials containing 15 ml of Aquasol. All samples were counted using a Packard Tri-Carb liquid scintillation spectrometer, either Model 3314 or 3320. Counting efficiency was determined by internal standardization.

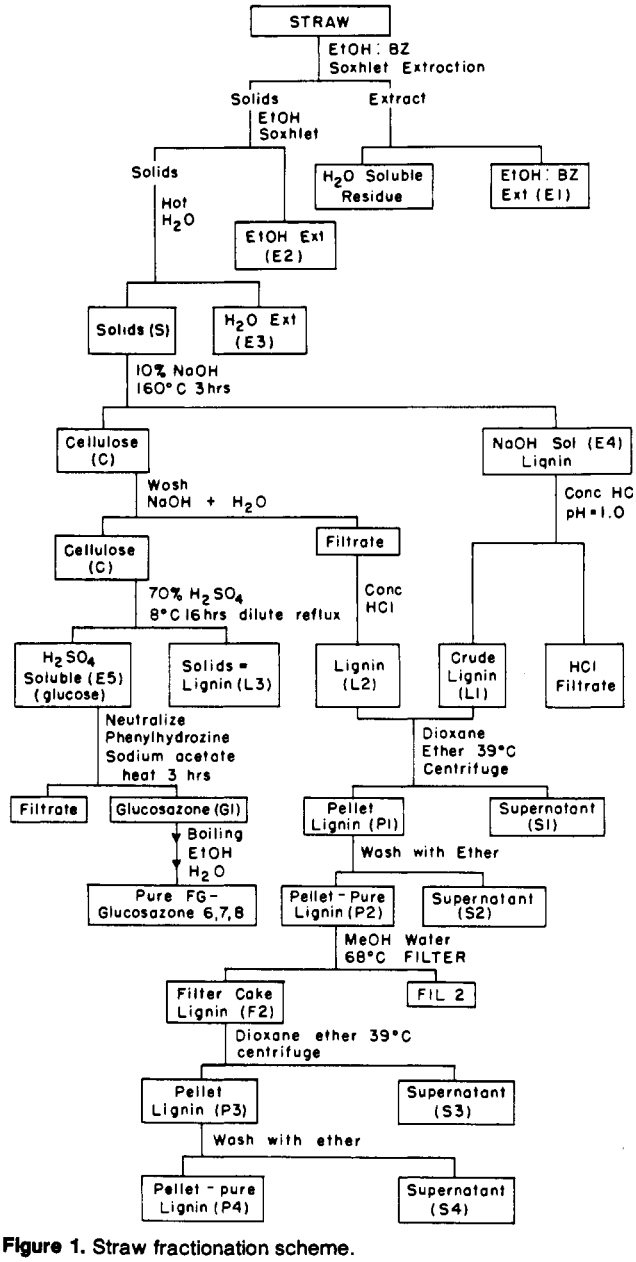


Figure 1. Straw fractionation scheme.

Soxhlet Extraction. To remove moisture, waxes, and lipids from the straw, a Soxhlet extraction using various solvents was undertaken. A flow chart of the extraction scheme is shown in Figure 1. The extraction procedure is a modification of a method reported by Stone et al. (1951).

About 60 g of rice or wheat straw was weighed into a Soxhlet thimble and extracted for 7.5 hr in a Soxhlet extractor using 2000 ml of a solvent composed of anhydrous ethanol and glass distilled benzene in a ratio of 1:2 (v/v). Aliquots of the extract (E1, Figure 1) were taken for liquid scintillation counting. The solid residue was extracted with

Table I. Material Balance of Radioactivity from Rice Straw

Fraction of straw containing radioact.[a]	Preemergence rice straw		Postemergence rice straw	
	dpm recovered	% of original ^{14}C act.	dpm recovered	% of original ^{14}C act.
Whole straw	14,300	100	232,000	100
EtOH–benzene extract (E1)[b]	3,430	24	123,000	53
EtOH extract (E2)	0	0	6,300	3
H_2O extract (E3)	0	0	9,000	4
Crude cellulose (C)	4,120	29	22,730	10
Crude lignin (L1 + L2)	1,808	13	31,500	14
Crude lignin (L3)	844	6	8,602	4

[a] All values are averages of duplicate samples and are corrected for background radioactivity determined by taking aliquots from a sample of nontreated control straw which had been processed in the same way. [b] The water-soluble portion of the EtOH–benzene extract contained about 3% of the original ^{14}C activity.

2000 ml of anhydrous ethanol in a Soxhlet extractor for 16 hr. Aliquots of this extract (E2, Figure 1) were taken for radioassay. The remaining straw residue was placed in 1500 ml of deionized water and stirred at 70–90° for 3 hr. The mixture was filtered with suction through a cotton pad and aliquots of the filtrate (E3, Figure 1) were taken for radioassay. The filter cake excluding the cotton pad (S, Figure 1) was dried in a vacuum oven at 95–100° for 2 days to remove moisture. The solids were then homogenized by dry blending in a Waring Blendor for 2 min at full speed. Aliquots of the solid material (S) were taken for combustion and radioassay.

Isolation of Crude Cellulose and Crude Lignin from Straw. Cellulose and lignin were isolated from rice and wheat straw by a modification of a procedure reported by Powell (Powell and Whittaker, 1925). Forty grams of the extracted solids fraction from wheat or rice straw obtained as described above was placed in a stainless steel high-pressure reaction vessel containing 500 ml of 10% NaOH. The top of the vessel was bolted on, and the mixture was heated with agitation in an oil bath at 140–160° for 3 hr. The vessel was allowed to cool to 90° before it was opened. The brown mixture was filtered while hot with suction through glass wool to obtain the fastest possible filtering process. A filter cake (C) and a filtrate (E4) resulted.

The crude cellulose filter cake (C) was washed first with 400 ml of 10% NaOH (80°) and then with 200 ml of water at 25° and dried in an oven at 50°. The wash (filtrate) was acidified with concentrated HCl to produce a lignin (L2) precipitate.

Concentrated HCl was added to the brown filtrate above (E4, Figure 1) with stirring until the mixture reached pH 1.0 and/or until a dark brown flocculent precipitate (L1) appeared.

Preparation of an Osazone from Crude Cellulose. The cellulose fraction was hydrolyzed to glucose by a procedure described by Adams and Castagne (1949). The crude cellulose was placed in a cold mortar with 150 ml of 70% H_2SO_4 (v/v) previously cooled to 8°. About 15 g of crude cellulose was used for every 100 ml of 70% H_2SO_4. The mixture was stirred for 3 min and then washed into a stoppered flask with 50 ml of cold 70% H_2SO_4. The stoppered flask was placed in a refrigerator at 10° for 1 hr at which time it was shaken for 3 min. The mixture was then left in the refrigerator at 10° for 16 hr. There was a minimal amount of charring of the cellulose when cold temperatures were adhered to during this hydrolysis. The contents of the flask were poured with vigorous stirring into 2 l. of deionized water previously cooled to 8°. The mixture was refluxed for 4 hr and filtered with suction. A dark brown residue of lignin remained on the filter paper (L3).

The osazone derivative of the resulting cellulose (C) hydrolysate was prepared using the method described below. The yellow filtrate of the H_2SO_4 hydrolysate (E5) was neutralized with 240 ml of 50% NaOH. Ninety grams of phenylhydrazine hydrochloride and 135 g of sodium acetate were premixed and added to the neutralized filtrate which was at 70–80°. The mixture was heated at 95–100° for 3 hr in a water bath with constant stirring. After 3 hr, the mixture was placed in a refrigerator to cool overnight; then 1–2 l. of deionized water was added. The precipitated osazone (G1) was filtered, washed with water, washed at room temperature with 1 l. of anhydrous ethanol, and recrystallized three times from aqueous ethanol. The final yield (FG8) was about 4.5 g. Melting points were determined after each recrystallization, and subsamples were weighed into combustion boats for radioassay after drying the samples in a steam oven at 70° for 5 days.

Purification of Lignin. Lignin isolated from straw was purified to constant specific radioactivity to confirm that the ^{14}C activity found was actually associated with the lignin. A method described by Brauns (1945) was used for the purification of lignin. The crude lignin precipitates (L1 + L2) were ground with a mortar and pestle with 800 ml of *p*-dioxane and insoluble impurities filtered out with suction. The filter cake was washed by resuspending it in 200 ml of *p*-dioxane. This mixture was filtered into the previous dioxane filtrate. The combined filtrates were reduced in volume to 25 ml by flash evaporation and then added dropwise to 200 ml of anhydrous ethyl ether while vigorously stirring the mixture with a glass stirring rod. The mixture was heated to 39° for 1 min and allowed to sit 15 min before centrifuging at 900*g* for 10 min. The supernatant (S1) was decanted and 150 ml of anhydrous ether was added to the centrifuge bottle, and the pellet (P1) was suspended by shaking the bottle vigorously. The mixture was allowed to settle 15 min and the supernatant (S2) was decanted. The precipitate (P2) was poured onto a watch glass to dry.

After combustion of an aliquot sample, the remaining portion of P2 was added to absolute methanol to make a 10% solution (w/v). This solution was added to distilled water (100 ml) dropwise with vigorous stirring. The mixture was heated to 68° and cooled at room temperature for 5–10 min. The brown flocculent precipitate was filtered warm through No. 1 Whatman filter paper using suction and a No. 26-P Buchner funnel. The filter cake (F2) was dried in an oven at 90° overnight. After taking an aliquot sample for combustion, the remaining F2 was dissolved in 200 ml of dioxane which was flash evaporated to 25 ml and then added dropwise to ether as described above to complete the purification of the lignin.

Table II. Material Balance of Radioactivity from Wheat Straw

Fraction of straw containing radioact.	NO_2 ring labeled		Cl_2 ring labeled	
	dpm[a] recovered	% of original ^{14}C act.	dpm[a] recovered	% of original ^{14}C act.
Whole straw	104,400	100	157,974	100
EtOH–benzene extract (E1)	24,769	23	36,313	22
H_2O extract (E3)	10,673	10	19,557	12
Solids (S)	42,953	41	69,873	44
Crude cellulose (C)	3,252	3	4,123	3
Crude lignin (L1 + L2)	18,148	17	33,455	21

[a] All values are averages of duplicate samples and are corrected for background radioactivity determined by taking aliquots from a sample of nontreated control straw which had been processed in the same way.

Table III. Radioactivity in Rice and Wheat Lignins

Sample	Treatment	Site of label	Specific radioact. of straw, dpm/g	Av specific radioact. of lignin, dpm/g[a]	% of radioact. at harvest in lignin
Rice	Preemergence	Cl_2 ring	929	2,025	33
Rice	Postemergence	Cl_2 ring	18,550	30,735	25
Wheat	Preemergence	NO_2 ring	1,740	3,645	31
Wheat	Preemergence	Cl_2 ring	2,468	4,835	30

[a] These values are averages of the specific radioactivities of lignin from several purification steps. All values were corrected for background radiation from lignin samples of nontreated control plants put through the same process.

Table IV. Radioactivity in Cellulose of Rice and Wheat Straw

Sample	Treatment	Site of label	Specific radioact. of straw, dpm/g	Av specific radioact. of cellulose, dpm/g[a]	% of radioact. at harvest in cellulose[b]
Rice	Preemergence	Cl_2 ring	929	688	25 ± 0.8
Rice	Postemergence	Cl_2 ring	18,550	869	2 ± 0.2
Wheat	Preemergence	NO_2 ring	1,740	228	5 ± 0.5
Wheat	Preemergence	Cl_2 ring	2,468	258	4 ± 0.8

[a] Samples from several recrystallizations. The average specific activity of the cellulose was calculated by multiplying the average specific radioactivity of the glucosazones by 2.27. [b] Rice straw is 34% cellulose ("Food and Life", 1939); wheat straw is 37% cellulose.

RESULTS AND DISCUSSION

Fractionation of Rice Straw. Table I presents a typical material balance for the fractionation of rice straw from rice plants treated either pre- or postemergence with nitrofen-^{14}C. When the postemergence treated rice straw was Soxhlet extracted, a total of 60% of the original radioactive residue was removed. The solid material was subjected to alkaline hydrolysis and 18% of the original radioactivity was found in the crude lignin fraction while 10% of the original radioactivity was found in crude cellulose. The rice straw from rice plants treated preemergence with nitrofen-^{14}C retained more of its radioactive residues after Soxhlet extraction and more radioactivity was found in the crude cellulose fraction of these plants.

The material balance for a wheat straw fractionation is similar to that of rice (Table II).

Purification of Lignin-^{14}C. To show that the radioactivity isolated in the lignin fraction of rice and wheat straw was actually associated with lignin, the lignin was purified to a constant specific radioactivity as described above. Infrared spectroscopy was used to confirm the identity of the isolated lignin.

One may calculate the percent of the radioactive residues that are actually in the plant lignin fraction by dividing the average specific radioactivity of the isolated lignin by the specific radioactivity of the original straw and multiplying by the percentage of lignin in straw. Table III reveals that approximately 30% of the radioactive residue in rice straw or wheat straw treated pre- or postemergence with nitrofen-^{14}C is in lignin at harvest. Apparently, neither the site of label nor the type of application of nitrofen-^{14}C affects the percentage of radioactive residue in the lignin. The difference between the calculated percent of radioactivity in lignin and the observed percent of original radioactivity found in the isolated fraction of the material balance is due to the low yield of lignin (16%) during fractionation.

Whether the radioactive residue in rice or wheat straw lignin is in a nitrofen–lignin complex or in the constituent carbon atoms of lignin remains to be elucidated.

To determine if the radioactivity found in the crude cel-

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 significant 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.

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

Horatio P. Freeman, Alan W. Taylor,* and William M. Edwards

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; 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-

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