degradation or PH_3 oxidation. The most variable difference would appear to lie in the aerobic–anaerobic state of the closed system. Initially aerobic, the closed conditions would tend to become anaerobic from microorganism respiration, unless it were poisoned by PH_3. We assume that field conditions generally would be more aerobic. For all practical purposes we conclude that the amounts of Zn_3P_2 reaching field soils would convert rapidly to phosphate and zinc ions, without releasing detectable PH_3 into the atmosphere.

Zn_3P_2 in Water—the Influence of pH and Mineral Content. Zinc phosphide placed in neutral water at room temperature failed to produce more than trace quantities of PH_3 (Table VI and VII). Small amounts of PH_3 probably came from minor, easily hydrolyzed phosphide impurities—such as Ca_3P_2 or Mg_3P_2—in the commercial Zn_3P_2. Washed and dried Zn_3P_2 did not generate PH_3 when stored. Commercial Zn_3P_2 stored without water generated small easily detectable quantities of PH_3.

Hydrolysis of Zn_3P_2 to PH_3 occurred in acid or basic solutions at a rate dependent on pH and temperature (Robison, 1970). That the hydrolysis was not a simple pH-dependent phenomenon became evident from the anomalous hydrolysis of Zn_3P_2 at room temperature in a conventional buffer solution of pH 7.00. The buffer contained sodium and potassium phosphates; the experiments were repeated with fresh preparations. We did not pursue this investigation, but we do suggest that pH and ionic activity contribute to the hydrolysis of Zn_3P_2 to PH_3. This argument would help to explain the decomposition of Zn_3P_2 to PH_3 in soil, although the mechanism for oxidation to phosphate ion predominated in soil, whereas it was nearly absent in water. We did not determine whether the pH 7.00 buffer increased the rate of oxidation of PH_3.

Phosphine in air formed only traces of H_3PO_4 when stored over distilled water (Table V).

We concluded that Zn_3P_2 dropped or carried into streams or ocean water would not readily decompose. Bottom or suspended sediments would likely decompose Zn_3P_2, with the formation of PH_3 or H_3PO_4 in anaerobic or aerobic conditions, respectively.

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Fate of ^{14}C-Labeled Diazinon in Rice, Paddy Soil, and Pea Plants

Tapio L. Laanio, Gerard Dupuis, and Herbert O. Esser*

[2-^{14}C]Diazinon [*O,O*-diethyl *O*-(2-isopropyl-4-methylpyrimidin-6-yl) phosphorothioate] was rapidly absorbed by and translocated in rice plants. Loss of insecticide, of about 50% within 9 days, was due to volatilization from the paddy water and transpiration from the leaves. Less than 10% of the radioactivity remaining in the plants after 9 days was the parent compound. The metabolite fraction consisted of 2-isopropyl-4-methyl-6-hydroxypyrimidine (G 27550), 2-(1′-hydroxy-1′-methyl)ethyl-4-methyl-6-hydroxypyrimidine (GS 31144), the latter partly as a glucoside, and a small fraction of polar metabolites. The same metabolites and traces of diazoxon were found after stem injection of the insecticide. Hydroxydiazinon [*O,O*-diethyl *O*-[2-(1′-hydroxy-1′-methyl)-ethyl-4-methylpyrimidin-6-yl] phosphorothioate] if present represented only a minor metabolite. G 27550 and GS 31144 were also the main metabolites of paddy soil. Cleavage of the pyrimidine ring with the evolution of $^{14}CO_2$ proceeded at a low rate in rice plants and paddy soil. The degradative pathways found in rice also occurred in pea plants.

The behavior of the broad spectrum insecticide diazinon [*O,O*-diethyl *O*-(2-isopropyl-4-methylpyrimidin-6-yl) phosphorothioate] in plants has been followed thoroughly with the aid of chemical, radiochemical, and enzymatic methods (Aquino, 1970; Hirano and Yushima, 1969; Kansouh and Hopkins, 1968; Miles *et al.*, 1964; Onsager and Rusk, 1967; Randolph *et al.*, 1969).

After regular application of diazinon to a great variety of crops, diazoxon [*O,O*-diethyl *O*-(2-isopropyl-4-methylpyrimidin-6-yl) phosphate] was found, if present at all, in very small concentrations (Augustinsson and Jonsson, 1957; Coffin and McKinley, 1964; Eberle and Novak, 1969; Harding *et al.*, 1969; Ralls *et al.*, 1966). Another oxidation product, hydroxydiazinon (CGA 14128) [*O,O*-diethyl *O*-[2-(1′-hydroxy-1′-methyl)ethyl-4-methylpyrimidin-6-yl] phosphorothioate], has been reported to occur in small amounts in kale (Pardue, 1968; Pardue *et al.*, 1970) and in rice plants (Miyazaki *et al.*, 1969).

The initial product of hydrolysis of both diazinon and diazoxon, in addition to the phosphorus moiety, namely 2-isopropyl-4-methyl-6-hydroxypyrimidine (G 27550), has been identified in several plant species (Kansouh and Hopkins, 1968; Ralls *et al.*, 1966, 1967). No metabolites, but a loss of insecticide by transpiration, have been found in alfalfa grown in diazinon-treated soil (Nelson and Hamilton, 1970).

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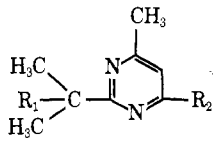
Table I. Structure of Diazinon and Related Compounds



Compound	R_1	R_2
Diazinon	H	$O-P(S)(OC_2H_5)_2$
G 24576	H	$O-P(O)(OC_2H_5)_2$
CGA 14128	HO	$O-P(S)(OC_2H_5)_2$
G 27550	H	OH
GS 31144	HO	OH
GS 29475	H	SH
G 31045	H	OC_2H_5

The rapid degradation of diazinon in dry and submerged soils (Aquino, 1970; Eberle and Novak, 1969; Malone *et al.*, 1967; Sethunathan, 1970) and by soil-born microorganisms (Gunner *et al.*, 1966a,b; Gunner and Zuckerman, 1968; Matsumura and Boush, 1968; Sethunathan and MacRae, 1969) has been demonstrated.

Cleavage of the aryl ester bond of diazinon in soil is mainly of a chemical nature (Bro-Rasmussen *et al.*, 1968; Getzin and Rosefield, 1966, 1968; Konrad *et al.*, 1967; Lichtenstein *et al.*, 1968; Mortland and Raman, 1967). Stimulation of this hydrolysis by microorganisms has been suggested to occur in submerged soil (Sethunathan and MacRae, 1969; Sethunathan and Yoshida, 1969) and has been observed when diazinon was incubated with isolated soil microorganisms (Trela *et al.*, 1968).

G 27550 has been identified as the primary hydrolysis product in different soils (Getzin, 1967; Konrad *et al.*, 1967; Sethunathan and Yoshida, 1969). Appreciable amounts of $^{14}CO_2$ have been liberated from soils treated with ethoxy and ring-labeled diazinon. However, this liberation was drastically reduced when submerged or sterilized soils were used (Getzin, 1967; Getzin and Rosefield, 1966; Sethunathan and MacRae, 1969).

As diazinon is effectively used for the control of the rice insect complex, studies were performed in our laboratories to follow its metabolic fate in the rice plant. Degradation of diazinon in pea plants was also studied to reinvestigate the presence of two metabolites reported to have the structures of 2-isopropyl-4-methyl-6-mercaptopyrimidine (G 29475) and 2-isopropyl-4-methyl-6-ethoxypyrimidine (G 31045) (Lichtenstein *et al.*, 1967).

MATERIALS AND METHODS

Materials. All studies were performed with the aid of ring 2-^{14}C-labeled diazinon with a specific radioactivity of 3.2 μCi/mg in the rice experiments and 4.4 μCi/mg in the pea experiments. The compound was synthesized by Dan Ryskiewich, Geigy Agricultural Chemicals, Division of Ciba-Geigy Corporation, Ardsley, N.Y. G 27550, GS 29475, and G 31045 (according to Margot and Gysin, 1957) and GS 31144 [*i.e.*, 2-(1'-hydroxy-1'-methyl)ethyl-4-methyl-6-hydroxypyrimidine (according to Ruefenacht, cited in Mücke *et al.*, 1970)] were synthesized by Kurt Ruefenacht. CGA 14128 was synthesized from GS 31144 and diethyl thionophosphoric acid chloride using known procedures (Lukaszczyk, 1971). The structures of diazinon and the related compounds mentioned in this paper are given in Table I.

Plants and Their Treatment. Rice plants (*Oryza sativa*) were cultivated in the laboratory in submerged soil of the following properties: clay loam, pH 6.2; organic matter, 5.4%; water holding capacity, 55.3%. The plants were kept at a temperature of 25 ± 1°, a relative humidity of 75 ± 3%, and were illuminated for 12 hr per day (Sylvania Gro-Lux fluorescent lamps).

Six plants per pot were cultivated in approximately 1 kg of submerged soil. For treatment the water above the soil surface of each pot was replaced by 160 ml of water containing 5.64 mg of ^{14}C-diazinon. Two pots were treated a second time, 43 days after the first application, with 2.82 mg of the labeled material. In a second experiment, 40-days-old rice plants were stem injected 3 cm above the paddy water level with 10 μl of a solution of 0.108 mg of ^{14}C-diazinon in ethanol–water, 1:1 (v/v) per plant. Five plants which had received a total of 0.54 mg of ^{14}C-diazinon corresponding to 3.9×10^6 dpm were analyzed per time interval.

After germination five pea seedlings were transplanted into beakers with 200 ml of quartz sand and 80 ml of Hewitt nutrient solution containing 3 mg of ^{14}C-diazinon, corresponding to 37.5 ppm. The following growth conditions were used: temperature, 21 ± 1°; relative humidity, 55 ± 3%; illumination as for rice plants.

Separation and Identification of Metabolites. At harvest the different parts of the plants were weighed and homogenized in an Omni-Mixer (Sorvall, Inc.) with methanol–water, 8:2 (v/v), and exhaustively extracted with this solvent (5–7 extractions). The nonextractable radioactivity of the plant material was determined by dry combustion according to Kalberer and Rutschmann (1961).

The soil–paddy water system was separated into paddy water, an upper (5 cm), and a lower soil layer. Each layer was extracted with water and finally with methanol in a Soxhlet apparatus for 7 hr. Radioactivity still remaining in the soil was determined by wet combustion using a mixture of 32 ml of phosphorus acid (85%, d = 1.71), 68 ml of sulfuric acid (with 25% of SO_3), and 14 g of potassium dichromate (70 ml of solution per 2 g of soil; 20 min at 240°).

Any parent compound and hydroxydiazinon present in the methanol–water extracts of plants were extracted by hexane. Radioactivity of extracts and solutions was characterized by thin-layer chromatography (tlc, silica gel G with a fluorescent indicator, layer thickness 0.25 mm; Merck, Darmstadt, Germany), using the following solvent systems.

1.	Ethyl acetate–ethanol–ammonia	16:3:1
2.	Benzene–ethanol	9:1
3.	Benzene–chloroform–ethyl acetate	2:2:1
4.	Chloroform–acetone	7:3
5.	Benzene–chloroform–ethanol–formic acid	4:4:1:1
6.	Ethyl acetate–benzene–chloroform–propanol	2:2:1:1
7.	Isopropyl alcohol–ammonia–water	20:1:2
8.	Acetone–chloroform–acetic acid	3:5:1.5
9.	Chloroform–acetonitrile	8:2
10.	Chloroform	

The R_f values for the synthetic reference materials used in this study are summarized in Table II. Nonlabeled compounds were located by their quenching of the fluorescence of the silica gel indicator. Diazinon and G 27550 were also characterized by gas–liquid chromatography (glc). Retention times of 1.5 min for diazinon and 2 min for G 27550 were obtained using columns of 1 m × 2 mm (i.d.) packed with FFAP, 2% on Chromosorb G. The operating temperature was 200° for the column and 240° for the injection port. Helium was

Table II. R_f Values for Diazinon and Related Compounds in tlc on Silica Gel

Compound	Solvent system 1	2	3	4	5	6	7	8	9	10
Diazinon	0.95	0.95	0.82	0.95	0.78	0.94	0.86	0.69	0.91	0.82
CGA 14128	0.91	0.86	0.70	0.94	0.75	0.92	0.86	0.69	0.88	0.52
G 24576	0.87	0.56	0.22	0.83	0.55	0.80	0.80	0.64	0.63	0.32
G 27550	0.50	0.25	0.05	0.25	0.47	0.53	0.81	0.59	0.18	0.08
GS 31144	0.25	0.20	0.03	0.18	0.37	0.40	0.70	0.45	0.15	0.05
G 31045	0.95	0.75	0.65	0.95	0.52	0.95	0.89	0.59	0.85	0.65
GS 29475	0.45	0.22	0.35	0.90	0.65	0.95	0.74	0.69	0.81	0.46

Table III. Distribution of Radioactivity in Rice Plants and Nutrient Medium After Application of [2-^{14}C]Diazinon into the Paddy Water

Days after first treatment	Fresh weight, g[a] Sprouts	Roots	Radioactivity (in percent of the dose applied)[b] Rice plants: Extractable Sprouts	Roots	Nonextractable Sprouts	Roots	Nutrient medium: Paddy water	Soil layers Upper	Lower	Recovery
First treatment										
3	9.4	15.0	1.4	0.9	0.1	0.1	16.9	48.1	1.7	69.1
9	11.9	15.3	4.7	0.6	0.3	0.3	3.3	41.8	3.4	54.4
21	15.4	18.0	8.9	0.6	1.1	0.7	0.3	31.1	6.5	49.2
39	18.6	24.4	8.6	0.5	2.5	1.7	0.4	20.8	5.1	39.6
52	23.8	26.4	10.1	0.4	3.0	2.3	0.2	20.3	5.4	41.7
80	27.8	22.0	10.8	0.6	2.0	2.3	0.1	12.9	4.5	33.2
Second treatment										
52	22.1	24.5	7.9	0.7	2.2	1.5	1.0	24.3	3.9	41.5
80	27.4	15.4	11.2	0.6	2.6	1.3	0.2	17.8	4.4	38.1

[a] Six plants analyzed each time. [b] 1. Treatment: 40.0 × 10^6 dpm (= 5.64 mg) of [2-^{14}C]diazinon into the paddy water of each pot. 2. Treatment: 20.0 × 10^6 dpm (= 2.82 mg) of [2-^{14}C]diazinon into the paddy water; 43 days after the first treatment.

used as a carrier gas at a flow rate of 30 ml per min. All determinations were made on an Aerograph Model 1200 gas chromatograph using an electrolytic conductivity detector (Coulson, 1966).

The possible presence of diazoxon and other cholinesterase inhibiting substances in plant extracts was examined by the method described by Eberle and Novak (1969).

Enzymatic cleavage of the conjugate found in rice plants was performed with β-glucosidase (Fluka, Buchs, Switzerland) at pH 6.2 in 0.02 *M* sodium borate buffer for 6 hr at 37°.

Measurement of Radioactivity. Radioactivity was determined as described by Mücke *et al.* (1970). Scintillation cocktail 1 mentioned in that paper was used for the determination of radioactivity of plant and soil extracts as well as of paddy water. Limits of detection were found to be 0.02% for soil and 0.005% for plants of the dose applied into the paddy water corresponding to 0.005 and 0.02 ppm of diazinon equivalents, respectively.

RESULTS AND DISCUSSION

Metabolism of Diazinon in Rice Plants. Uptake of radioactivity and its distribution within the rice plant was followed after one and two applications of ^{14}C-labeled diazinon to the paddy water. The results are summarized in Table III, which demonstrates that most of the radioactivity added to the paddy water was absorbed by the upper soil layer. The radioactivity of the roots remained constant over the experimental period, whereas a rapid translocation of the radioactivity into the shoots took place. This was supported by autoradiography, demonstrating a strong concentration of radioactivity in the tips of the leaves.

Poor recoveries were obtained in these experiments. Since an open experimental system had been used, the possible loss of radioactivity in the form of CO_2 was checked. After stem injection of 0.108 mg of diazinon per rice plant, 1.9% of the radioactivity applied was found as $^{14}CO_2$ within 39 days. When ^{14}C-diazinon was applied to paddy soil without plants, 1.4% of the added radioactivity was recovered as $^{14}CO_2$ in 52 days. Control experiments showed that, within 3 days, volatilized [2-^{14}C]diazinon is not degraded to $^{14}CO_2$ in the 2 *N* sodium hydroxide used as an absorption solution.

These control experiments indicate that loss of radioactivity by CO_2 liberation was unlikely and that disappearance of the parent compound and/or metabolites by volatilization might be the main reason for the poor recoveries obtained. In the first days of the experiment, loss of radioactivity was primarily due to direct volatilization of radioactivity from the water surface as checked by a control experiment. When an aqueous solution of ^{14}C-diazinon was kept under the conditions used in the plant experiments, 50% of the insecticide was volatilized within 5 days.

Later in the experiment, when the bulk of radioactivity was adsorbed to the soil, losses of label were mainly due to excretion of radioactivity from the leaves. This was demonstrated by means of a biotest with flies. A pot with five rice plants, stem injected with ^{14}C-diazinon, was placed in a closed chamber and protected with a wire-gauze. Three days after the injection, 50 *Aedes aegypti* and 50 *Drosophila melanogaster* were placed in the chamber. Within 48 hr all of the flies were killed, indicating the presence of an active insecticide. When the same amount of diazinon which had been applied to the plants was placed on a glass plate in the chamber without plants, all insects were killed within 24 hr. In a control experiment with untreated plants, all flies were alive after 48 hr.

Characterization of the radioactivity excreted by the leaves was achieved by tlc analysis in solvent systems 1, 2, and 3 of the guttation water of plants growing in ^{14}C-diazinon fortified nutrient solution. Diazinon was found to be the main radioactive constituent during the first days of the experiment.

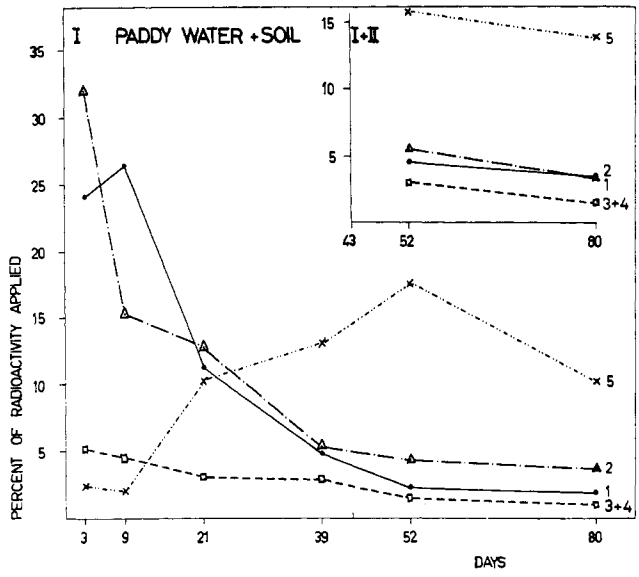


Figure 1. Distribution of radioactivity in paddy water plus soil after application of ^{14}C-diazinon into the paddy water. 1 = diazinon. 2 = G 27550. 3 = GS 31144. 4 = polar metabolite fraction. 5 = nonextractable radioactivity. I = data after one treatment. I + II = data after two treatments

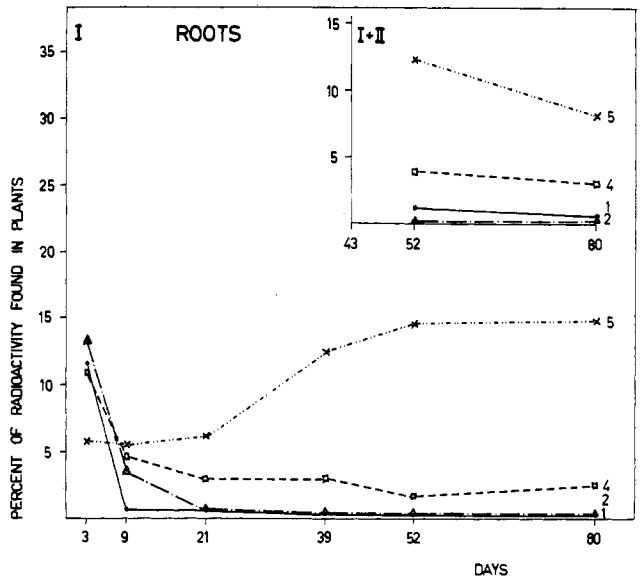


Figure 2. Distribution of radioactivity in the roots of rice plants after application of ^{14}C-diazinon into the paddy water. See Figure 1 for symbol legend

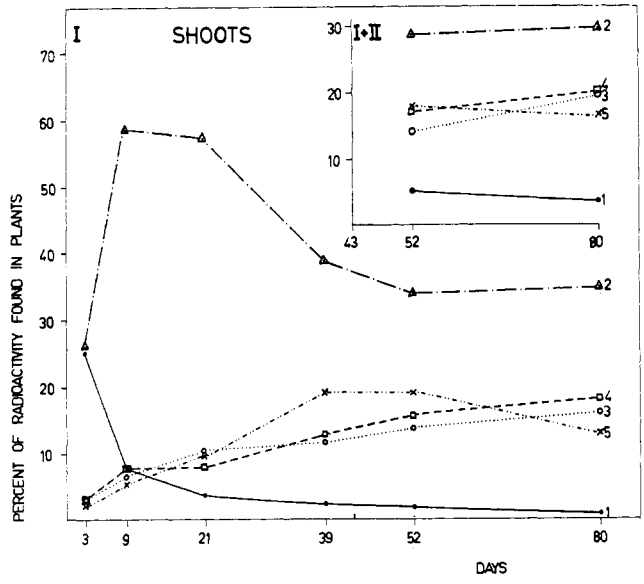


Figure 3. Distribution of radioactivity in the shoots of rice plants after application of ^{14}C-diazinon into the paddy water. See Figure 1 for symbol legend

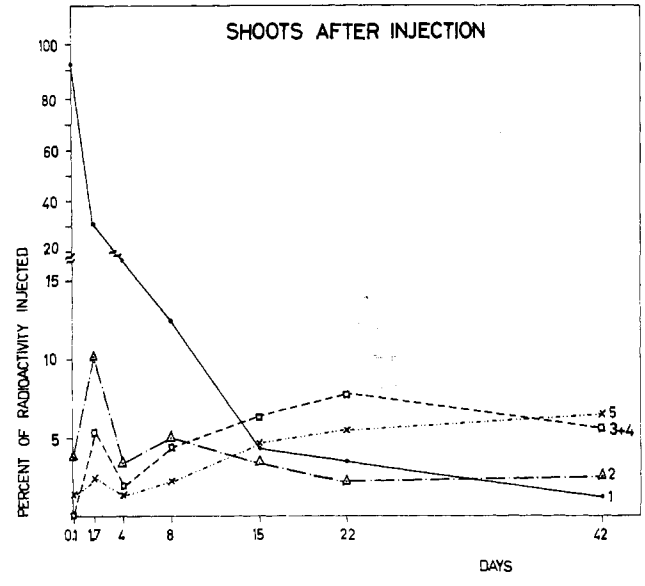


Figure 4. Distribution of radioactivity in the shoots of rice plants stem injected with ^{14}C-diazinon. See Figure 1 for symbol legend

Similar loss of unchanged diazinon from alfalfa plants by transpiration has been reported (Nelson and Hamilton, 1970).

The extent of the translocation of radioactivity and its excretion by the leaves was determined after stem injection of diazinon. Approximately 2 hr after injection, the applied radioactivity was completely recovered. But already 2 days after application 50% of the applied dose had disappeared. Of the remaining radioactivity another 50% was lost during the next 2 days. Then radioactivity decreased only slowly, reaching 15.6% of the injected radioactivity after 6 weeks.

Radioactivity of the roots was found to be below 0.1% of the injected dose at all time intervals, demonstrating a negligible basipetal transport.

Independent of the mode of application of diazinon (paddy soil or stem injection) four radioactive fractions were separated by tlc in solvent system 1 in the case of plant shoots and soil extracts. These fractions behaved identical to diazinon at R_f 0.95, G 27550 at R_f 0.50, GS 31144 at R_f 0.25, and a fraction of polar metabolites that remained at the origin. GS 31144 was absent in the extracts of the plant roots.

Confirmation of the identity of the compounds was obtained by rechromatography with the corresponding reference materials in solvent systems 1, 2, 4, and 6 for diazinon, 1, 5, and 6 for G 27550, and 1, 7, 8, and 9 for GS 31144. Isolated diazinon and G 27550 were also characterized by glc.

In the extracts of plants and soil, a second compound was frequently found in small amounts in the diazinon peak when chromatographed in solvent system 1. According to R_f values this compound could be hydroxydiazinon, *i.e.*, CGA

Table IV. Distribution of Radioactivity in Pea Plants and Nutrient Solution After Root Absorption of [2-^{14}C]Diazinon

	Fresh weight, g[a]			Radioactivity (% of initial radioactivity in nutrient solution)[b]							
				Pea plants							
				Extractable			Nonextractable				
Weeks	Sprouts	Cotyl	Roots	Sprouts	Cotyl	Roots	Sprouts	Cotyl	Roots	Nutrient solution[c]	Recovery
1	5.05	2.6	2.3	4.3	1.7	14.0	<0.1	1.8	0.4	46.7	69.0
2	7.88	1.68	3.79	13.4	1.3	24.5	0.1	1.8	0.8	25.6	67.5
3	9.11	1.43	5.57	20.1	0.8	20.1	0.3	2.6	0.5	21.5	65.9
4	6.4	1.3	4.95	22.3	0.6	15.8	0.6	1.6	1.3	23.6	65.8

[a] Five plants analyzed each time. [b] Initial radioactivity: 29.3×10^6 dpm (= 3 mg) of diazinon in 80 ml of solution per five plants. [c] Including sand washings.

14128. Since the synthetic reference material became available only after termination of the work, it was not possible to identify this metabolite definitely. However, considering the low concentration of unchanged diazinon, especially from the second week on, then hydroxydiazinon could obviously be only a minor metabolite. Even during the first time intervals where the concentration of diazinon was relatively high, the amount of hydroxydiazinon could not exceed 2% of the radioactivity totally present in the plants. The cholinesterase inhibitory activity of hydroxydiazinon, determined directly and after its chemical oxidation by bromine water to hydroxydiazoxon, was found to be of the same order of magnitude as diazinon and diazoxon, respectively. Using an enzyme preparation of bovine erythrocytes and an automated procedure (Voss, 1969), beginning inhibition of the enzyme by diazinon and hydroxydiazinon was found at a concentration of 10^{-5} *M*. The i.d. 50 values of diazoxon and hydroxydiazoxon were found to be 2.8×10^{-7} *M* and 1.2×10^{-7} *M*, respectively.

G 31045 and GS 29475, two metabolites reported to be formed in pea plants (Lichtenstein *et al.*, 1967), were found to be absent in the extracts of both rice plants and soil when checked in tlc with the solvent systems described for pea plants. Traces of diazoxon not exceeding 0.01% of the radioactivity in the plants were found in the leaf extracts of rice plants 3 days after stem injection of diazinon. Other cholinesterase-inhibiting substances were not detected.

The polar metabolite fractions obtained by tlc of the extracts of both plant and soil in solvent system 1 were found to be inhomogeneous. In the case of plant shoots (paddy water application of diazinon), this fraction was incubated with β-glucosidase. After incubation one-third of the original radioactivity of each time interval was characterized as GS 31144 by tlc in solvent systems 1 and 6. The rest of the radioactivity still remained at the origin in these solvent systems.

The pattern of metabolites as a function of time in paddy soil, roots, and shoots after application of the insecticide to the paddy water is presented in Figures 1, 2, and 3 after one (I) and two (I + II) applications. In Figure 4 the distribution in shoots, after stem injection of the insecticide, is shown.

In addition to the remarkable volatilization already mentioned, extensive degradation in all plant parts was responsible for the rapid disappearance of the insecticide, as shown in Figures 1 through 4. G 27550 increased drastically during the first days, reaching approximately 40 and 60% of the radioactivity of the plant shoots in 3 and 9 days, respectively. Then the concentration of G 27550 decreased relatively rapidly in all parts up to the third week. Thereafter the dissipation of G 27550 in the plants was slowed down, possibly owing to a favored uptake of this compound from the paddy soil. This uptake was facilitated by the moderate adsorption of G 27550 to the soil. In contrast, diazinon was present in the paddy soil mainly in an adsorbed form.

Already at the third day, water extracts of the soil contained less than 2% of the extractable diazinon in contrast to about 50% of the metabolites. Residual diazinon and metabolites were extracted by methanol.

GS 31144 and the fraction of polar metabolites (accounted for in a single fraction in Figures 1 and 4) reached a maximum in the shoots 3 weeks after injection of diazinon. In contrast, a slightly increasing concentration of both fractions found after root absorption of diazinon is probably also due to the uptake of these fractions from the paddy soil as discussed for G 27550. Nonextractable radioactivity was found to reach a maximum after 6 to 7 weeks in all parts investigated.

A second dose of diazinon applied to the paddy soil was degraded according to the same pattern of metabolites within the same time interval found for the first application, as shown by the curves marked I + II of Figures 1, 2, and 3.

Metabolism of Diazinon in Pea Plants. G 31045 and GS 29475 were reported to be formed in pea plants grown in nutrient solution in the presence of nonlabeled diazinon. To check the formation of these metabolites, the behavior of diazinon in pea plants was reinvestigated by means of ^{14}C-labeled material. The distribution of radioactivity in plants and nutrient solution was determined 1, 2, 3, and 4 weeks after the application of the insecticide. The result is presented in Table IV.

Table IV demonstrates that the uptake of diazinon from the nutrient solution was rapid during the first 2 weeks and then tended to level off. A pronounced transport of radioactivity from the roots to the leaves was observed. Autoradiography showed that, unlike in rice plants, an even distribution of radioactivity with some accumulation at the edges of the leaves had taken place.

The recovery of radioactivity (plants plus nutrient solution) dropped after 1 week to 70% but then remained practically constant up to 4 weeks. The loss during the first days probably is the result of a volatilization from the nutrient solution as found with rice plants. Loss of parent compound by exudation from the leaves was unlikely, because diazinon was present in the leaves, if at all, only in small concentrations.

Radioactivity of the various extracts was separated by tlc with solvent systems 1 and 4 into diazinon, G 27550, GS 31144, and a fraction of very polar metabolites. The identity of the compounds was confirmed by rechromatography with reference materials in solvent systems 1, 2, 3, and 4 for diazinon, in systems 1, 5, and 6 for G 27550, and in systems 1, 2, 7, 8, and 9 for GS 31144. Diazinon and G 27550 were also characterized by glc. The distribution of metabolites as a function of time is presented in Figures 5 and 6.

Diazinon represented the main fraction of radioactivity of the roots, although its concentration decreased constantly. Rapid translocation of diazinon and/or low degradative capacity of the roots was indicated by the absence of substantial

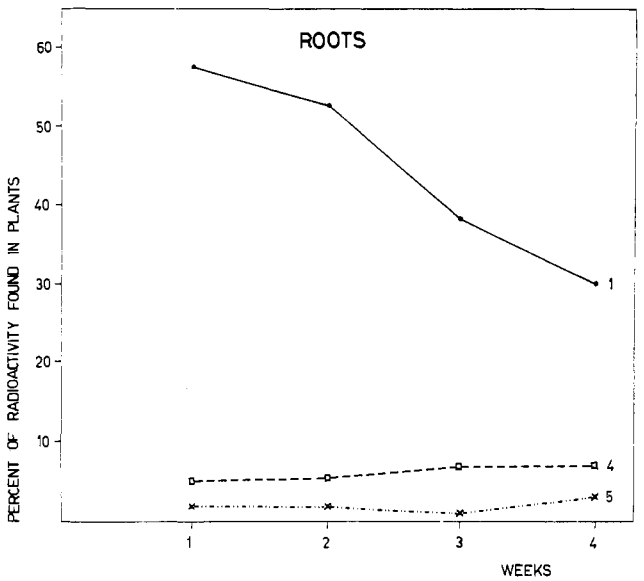


Figure 5. Distribution of radioactivity in the roots of pea plants grown in nutrient solution with ^{14}C-diazinon. See Figure 1 for symbol legend

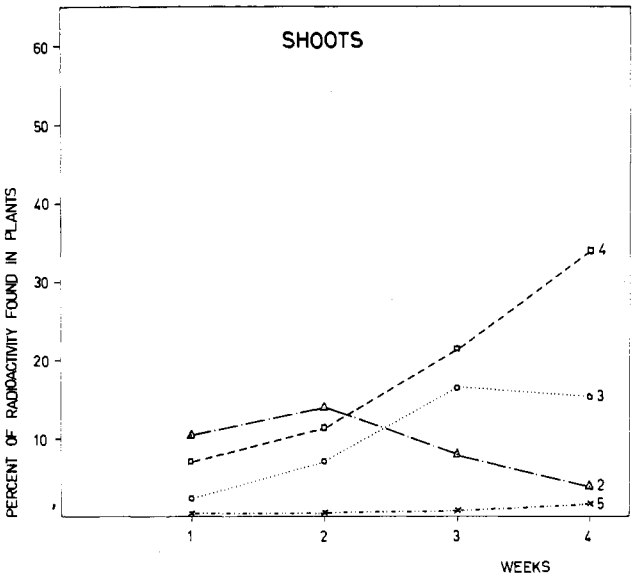


Figure 6. Distribution of radioactivity in the shoots of pea plants grown in nutrient solution with ^{14}C-diazinon. See Figure 1 for symbol legend

amounts of G 27550 and GS 31144. This result is surprising in view of the fact that G 27550 in the early phase and GS 31144 in the later phase of the experiment represented the main metabolites of the shoots. In addition, high concentrations of G 27550 in the nutrient solution were available for the roots throughout the experiment. The concentration of both compounds in the shoots showed the typical time dependency of transient metabolites.

Efficient degradation of the insecticide took place in the shoots, since trace amounts of diazinon were found only after 1 week. The fraction of polar metabolites was low and practically constant in the roots. The contrary was found in shoots where a steady increase of this fraction was observed reaching one-third of the total radioactivity present in the plants at the end of the experiment. Nonextractable radioactivity was of no importance either in roots or in shoots.

The same pattern of metabolites as in the leaves was found in the extracts of the cotyledons. Diazinon, G 27550, and GS 31144 each represented approximately one-fourth to one-sixth of the extractable radioactivity. The remainder was in the form of a very polar fraction. The cotyledons which had been in contact with the nutrient solution contained increased amounts of metabolites and especially of G 27550, indicating these parts to have either a high retention capacity or an efficient metabolic activity.

The analysis of the nutrient solution after 1, 2, 3, and 4 weeks showed 65, 38.9, 15.4, and 15.2% of the radioactivity, respectively, to be still in the form of diazinon. G 27550 represented the main degradation product (up to 75.2% after 4 weeks) besides small amounts of polar substances (approximately 10% after 4 weeks). No substantial amounts of GS 31144 were found at any time.

A similar degradation pattern, but at a lower rate, developed in the control nutrient solution without plants, yielding 62.9% of G 27550 after 4 weeks. These results indicate hydrolysis to be the primary degradation reaction occurring in the nutrient solution. The question of the chemical and/or microbial nature of this hydrolysis was not examined.

No evidence was found for the presence of either G 31045 or GS 29475. Tlc of the plant extracts in solvent system 4 separated a nonpolar zone (R_f 0.9) possibly containing diazinon, G 31045, and GS 29475. This zone was eluted and rechromatographed with reference substances. Tlc in solvent systems 1, 2, 3, and 5 in the case of G 31045 and 2, 3, and 10 in the case of GS 29475 unequivocally excluded the presence of these compounds and demonstrated the homogeneity of the diazinon peak.

In conclusion, the results reported in this paper demonstrate hydrolysis of the ester bond and oxidation of the isopropyl side chain to be the primary degradation mechanisms common to plants, soil, and, as already reported, to the rat (Mücke *et al.*, 1970).

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Determination of Residues of Fensulfothion and its Sulfone in Muck Soil

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A method is described for determining fensulfothion {*O,O*-diethyl *O*-[*p*-(methylsulfinyl)phenyl] phosphorothioate} and its sulfone in muck soils, using Soxhlet extraction with a 9:1 chloroform–ethanol mixture. Cleanup and fractionation was on an alumina–silica gel column and determination was by gas chromatography with flame-photometric detection. Optimum recoveries were obtained when the moisture content of the soil was between 20 and 60%. Recoveries from soils fortified at 0.1, 1.0, and 10.0 ppm were 80% or greater. Residues in soils field-treated with fensulfothion for protection against carrot rust fly ranged from 19.9 to 90.5 ppm for the parent compound and from 5.9 to 21.8 ppm for its sulfone.

Fensulfothion {*O,O*-diethyl *O*-[*p*-(methylsulfinyl)phenyl] phosphorothioate} is an insecticide showing considerable promise for the control of carrot rust fly, *Psila rosae* (F.) (Finlayson *et al.*, 1964, 1966). Residues of this insecticide in carrots treated at various rates have been determined (Finlayson *et al.*, 1970) and it was desirable to relate these results to residues of fensulfothion in the muck soil in which the carrots were grown.

Pesticide residues in soils high in organic matter are not easily extracted because of the strong adsorptive properties of such soils. Fensulfothion and its sulfone, being relatively polar compounds, are particularly difficult to extract. Cleanup of the extract for gas chromatographic (gc) analysis also presents a problem because high concentrations of extraneous matter are coextracted and these, if not removed, would quickly foul the gc column.

A number of methods of extracting organophosphate residues from organic soils have been reported. Beynon *et al.* (1966) used a 20% acetone–hexane mixture to extract Birlane from a peat soil, and obtained maximum recovery after 2 hr of tumbling. However, the mixture was fortified by adding Birlane to the solvent just prior to extraction so that adsorption was minimal. Suett (1971) used a 9:1 chloroform–methanol mixture with 2 hr of tumbling to extract phorate from a peaty loam. Fortification was by the addition of an acetone solution of the pesticide to the soil 1 hr before extraction. Recovery at the 0.1-ppm level averaged 85%. Getzin and Rosefield (1966) extracted C_{14}-labeled diazinon and Zinophos from an organic soil by shaking it for 15-min periods with three 50-ml portions of a 1:1 mixture of 0.05 *N* calcium chloride and acetone. A recovery of 98% was obtained from soil freshly fortified with Zinophos, but after 2 weeks 12% was nonextractable. Similar results were obtained for diazinon.

In this laboratory a number of extraction procedures were tested. These included Soxhlet extraction with a 1:1 hexane–acetone mixture, dichloromethane, and a 9:1 chloroform–methanol mixture. Extraction by prolonged shaking with dichloromethane was also tried. The final method of choice was Soxhlet extraction with chloroform–methanol.

EXPERIMENTAL SECTION

Materials. Two field-treated muck soils designated S and K and their corresponding untreated checks were used. The chemical and physical analyses of these soils are shown in Table I.

The silica gel and eluting solutions A and B used in the cleanup and fractionation step have been previously described (Williams *et al.*, 1971).

Alumina, Woelm, Grade W200 acid (Waters Associates, Framingham, Mass.), deactivated to activity II by the addition of 4% water, was also used in the cleanup step.

Solvents were redistilled from reagent grade material.

Apparatus. A 50-mm i.d. Soxhlet extractor with a 300-ml receiving flask was used. A pad of glass wool at the base of the extraction tube took the place of the normal filter thimble.

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