Metabolism of Cytrolane (Mephosfolan) Systemic Insecticide [(Diethoxyphosphinyl)dithioimidocarbonic Acid, Cyclic Propylene Ester] in a Simulated Rice Paddy

Chia C. Ku, Inder P. Kapoor,* and Joseph D. Rosen

The metabolic fate of [14C]mephosfolan systemic insecticide (Cytrolane, a registered trademark of American Cyanamid Company) in rice plants grown in a paddy environment has been studied. Of the 50% extractable radioactivity, more than 45% was identified as mephosfolan. The remaining 5% was due to a mixture of at least 17 metabolites. The unextractable radioactivity has been characterized as ^{14}C-labeled cellulose and possibly lignin. The results indicate that mephosfolan does not accumulate in the rice grain. The radioactive residue found in the rice grain was characterized as ^{14}C-labeled starch due to the incorporation of the ^{14}C-imido carbon atom of mephosfolan into glucose and, subsequently, into the starch. Although only mephosfolan was found in paddy water, its concentration in water decreased at a rapid rate due to its degradation by the rice plant, fish, and other living organisms in the paddy ecosystem.

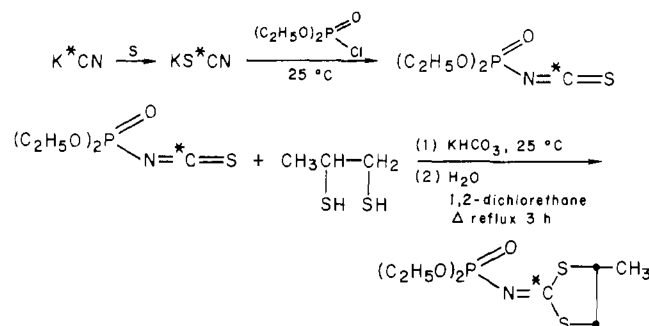
Cytrolane systemic insecticide [mephosfolan; (diethoxyphosphinyl)dithioimidocarbonic acid, cyclic propylene ester] is registered for the control of many lepidopterous and other pests of cotton, corn, rice, sorghum, sugarcane, etc., in several countries, especially in the Middle East and Asia. Among the prominent rice pests controlled are rice hispa (*Hispa armigera*), rice gall midge (*Pachydiplosis oryzae*), and all major rice stem borers like *Chilo suppressalis* and *Tryporyza incertulas*. This investigation of the metabolic fate of [^{14}C]mephosfolan labeled in the imido carbon position was initiated to study its uptake and metabolism in rice plants and in the paddy environment and to determine the nature of major metabolites to aid the toxicologist in evaluating their significance as crop residues.

MATERIALS AND METHODS.

Radiolabeled Mephosfolan. [^{14}C]Mephosfolan, labeled with carbon-14 in the imidocarbonate carbon atom, was synthesized as per reaction scheme given in Scheme I (the asterisk denotes ^{14}C).

Approximately 52.4 mg of potassium [^{14}C]cyanide (60.3 mCi/mmol, CFA.87, Batch 52, Amersham/Searle Corp., Arlington Heights, Ill.), 38 mg of sublimed sulfur (J. T. Baker Chemical Co., Phillipsburg, N.J.), and 5 mL of 1,2-dimethoxyethane (MC/B Manufacturing Chemicals, Norwood, Ohio) were added to a 100-mL round-bottom flask equipped with a condenser, a magnetic stirrer, and a heating mantle. The reaction mixture was refluxed for 1 h, then stirred at room temperature overnight. Five millimoles of diethylchlorophosphate (Aldrich Chemical Co., Inc., Milwaukee, Wis.) was next added to the reaction flask and stirred at room temperature for 1 h, followed by 409 mg of potassium thiocyanate as a "cold" carrier and stirred for an additional 1 h. To the reaction mixture, 540 mg of 1,2-propylenedithiol (Aldrich Chemical Co., Inc.) and *600 mg of potassium bicarbonate* (J. T. Baker Chemical Co.) together with 5 mL of water and 25 mL of 1,2-dichloroethane were added. This two-phase reaction mixture was refluxed for 3 h and stirred overnight at room temperature. The reaction mixture was transferred to a 125-mL separatory funnel. After the two phases had separated, the lower phase was removed and the aqueous

Scheme I



phase was further extracted twice with 50 mL of 1,2-dichloroethane. All the extracts were pooled and back-washed with 10 mL of 5% $KHCO_3$. The organic phase was dried with anhydrous magnesium sulfate. On evaporation of the solvent, a yellowish oil, crude product, was obtained.

The overall yield of the crude product was approximately 59%. The preparation was purified by chromatography on a 1.5 × 15-in. column of Florisil F101 (Fisher Scientific Company, Pittsburgh, Pa.), 100/120 mesh, equilibrated with 10% acetone in methylene chloride. The crude product was applied to the column in a small volume of acetone. The column was eluted using an acetone gradient in methylene chloride, the concentration of acetone being increased stepwise through the sequence 2, 5, and 10%. The column effluent was monitored by detecting the radioactivity and ultraviolet absorption on multichannel thin-layer chromatography (TLC) plates which were developed in ethyl acetate. After the mephosfolan peak was located, all the fractions comprising the peak were pooled together and evaporated to a volume of approximately 3 mL. Since there was a trace amount of impurity found in the column effluent, the concentrated solution was streaked on two preparative PQ1F, 1000 TLC plates (Quanta Industries, Fairfield, N.J.) and developed by using a programmed multiple development (Perry et al., 1975) technique with ethyl acetate. After the radioactive zone was located by autoradiography, the zone was scraped from the TLC plate and eluted with methylene chloride, followed by ethyl acetate. The eluate was concentrated and mephosfolan was quantitated by a gas-liquid chromatography (GLC) method as described in the latter part of this article. The specific activity of the preparation was found to be 28.8 μCi/mg. The radiopurity of this purified imido-labeled mephosfolan was determined

Agricultural Division, American Cyanamid Company, Princeton, New Jersey 08540 (C.C.K., I.P.K.) and the Department of Food Science, Rutgers–The State University, New Brunswick, New Jersey 08903 (J.D.R.).

0021-8561/78/1426-1352$01.00/0 © 1978 American Chemical Society

by two-dimensional TLC in solvent systems A (30% acetone in methylene chloride) and B (ethyl acetate) and the preparation was found to be 99.9% radiopure. The identity was confirmed by exact cochromatograph with standard mephosfolan by TLC and by GLC (6 ft 5% OV1 at 200 °C) using both flame ionization and radioactivity detectors.

Carbon-13 Labeled Mephosfolan. Mephosfolan, labeled with carbon-13 in the imidocarbonate carbon atom, was synthesized by using carbon-13 KCN as per procedure described for carbon-14.

Granular Formulation. All metabolism studies were carried out with a mixture of approximately one part mass-labeled (carbon-13) mephosfolan and one part unlabeled (carbon-12) containing sufficient carbon-14 mephosfolan to allow ready detection and measurement of the mephosfolan and its metabolites by conventional radiotracer techniques. By this means, a doublet ion peak is provided in the mass spectra of the mephosfolan or its metabolites due to the carbon-12/carbon-13 mixture. These doublets provided a means for distinguishing mass peaks due to the metabolites from the multitude of single peaks from interfering nonlabeled compounds that were always present, even after extensive and elaborate sequences of purifications. Mephosfolan was formulated into granules according to the following procedures: 3.8 mL of methylene chloride solution containing 14.58 mg of mephosfolan and 1 mL of a solution containing 18.6 mg of Glycol Deactivator E were pipetted into a 25-mL beaker and mixed well. To this solution was added 467 mg of Attaclay (25/50 mesh) with gentle stirring. The beaker was left in a well-ventilated hood until all the solvent had evaporated and dry granules remained in the beaker. This granular formulation, containing 3% of active ingredient, was used for the treatment of the rice paddy.

Experimental Design. Four rectangular polyethylene tanks (24 × 12 × 12 in.) were filled with a silt loam soil (sand 27.2%, silt 56.4%, clay 16.4%, organic matter 4.6%; pH 6.2) to a depth of 8 in. and flooded with water to maintain a level about 2 in. above the soil surface.

Rice seedlings (approximately 3 to 4 in. tall) were obtained by germinating IR-22 rice seeds in two 8 × 16-in. rectangular vermiculite flats for 2 weeks. Six to eight seedlings were transplanted as bundles into each tank (three rows and six columns) with approximately a 4-in. space between the bundles. The tanks were kept in the greenhouse under artificial light (GTE Sylvania M1000 B-HOR) on a 12-h day (Figure 1). Three weeks after transplanting, three of the four tanks were treated with mephosfolan granules by broadcasting the formulated mephosfolan at the rate of 0.75 kg of active ingredient/ha (or 14 mg/2 ft^2). The fourth tank was untreated and served as the control. All tanks were kept flooded to a depth of about 2 in. for 4 months by adding water as needed. At the end of the 4-month period when the paddy grains started to turn yellow, the flood water was allowed to evaporate, leaving the plants in moistened soil until the plants were fully matured.

Fifteen *Carassius auratus* (goldfish), a member of the carp family, 1.5 to 2 in. in length, were obtained from a local pet store. The fish were held in the nontreated control rice paddy for 2 days. Eleven fish were transferred to mephosfolan-treated rice paddy tanks 1 week after the pesticide treatment. The remaining fish were left in the control rice paddy. No fish food was added to the rice paddy during the course of this study.

Sample Processing. Rice plants were harvested by cutting the stems 2 in. above the water level at intervals

Figure 1. Simulated rice paddy treated with [^{14}C]mephosfolan at 0.75 kg/ha.

of 1, 2, 4, 8, and 18 weeks after treatment. For the 18-week samples (harvest time), the paddy seeds were separated from the foliage, and a portion of these seeds was hulled for analysis of carbon-14 content in both hulls and the edible seeds (rice). Rice plants were extracted twice with absolute methanol by blending with 10 mL/g in a Waring blender for 10 min. The resultant homogenate was filtered with suction through a Buchner-type sintered-glass funnel (coarse porosity). The resultant extracts were combined and evaporated in a rotary evaporator under vacuum at 40 °C. The plant residue (marc) was dried in a well-ventilated hood before determination of residual carbon-14 content.

Water samples were taken from each tank at 3, 7, 14, and 45 days after the granular mephosfolan was applied to the tanks. One-milliliter aliquots of water samples from each tank were used for counting. Five milliliters of 3- and 7-day samples were freeze-dried using a VirTis freeze drier and the residues were dissolved in a small volume of methanol for analysis by TLC.

Fish were collected at intervals of 1, 3, 5, 7, and 9 weeks after the fish were introduced into the mephosfolan-treated water. Two fish were arbitrarily taken from the treated tanks at each sampling time. The fish were homogenized in a Potter-Elvehjem tissue grinder with 10 mL/g of acetonitrile. The resultant homogenate was separated into extract and residue (marc) by centrifugation. The residue was extracted again with absolute methanol by the same procedures. The resultant extracts were combined and evaporated to a small volume in a rotary evaporator under reduced pressure at 40 °C. The fish residue (marc) was dried in a well-ventilated hood before further determination of residual carbon-14 content.

After the mature rice plants were harvested, the paddy soil was dried in the air at room temperature for 4 days. It was observed that the original 8-in. depth of soil had swelled and become fluffy due to ingrowth of the root system to give a 10-in. depth. Samples of the dry soil were taken from each tank by driving a cylindrical sampler (2 in. i.d. × 12 in.) into the soil to the bottom of the tank. The soil samples from each tank were separated into two portions according to their depth; the 0 to 5 and 5 to 10 in. samples from all the tanks were pooled. Each pooled sample was transferred to a 0.5-gal bottle and extracted with a methanol–water mixture (2 mL of methanol and 0.2 mL of water per gram of soil) on a reciprocal shaker for 16 h. The resultant soil suspension was filtered with suction through a Buchner-type sintered-glass funnel (coarse porosity). The extraction was repeated once, the combined extracts were evaporated in a rotary evaporator

under reduced pressure at 40 °C, and the residue was dissolved in a small volume of methanol for analysis. The portion of radioactivity that remained in the marc after extraction with the methanol–water mixture was reextracted twice with 2% HCl in methanol. The soil marc was dried in a well-ventilated hood at room temperature and analyzed for residual radioactivity.

Characterization of Bound Residues. Cellulose and lignin were isolated from rice straw by the procedure reported by Honeycutt and Adler (1975). Lignin was purified by the method of Brauns (1945) and the cellulose fraction was hydrolyzed to glucose by the procedure of Adams and Castagne (1949). Starch from rice grain was isolated and hydrolyzed to glucose by the method of Wargo et al. (1975). The glucose from rice grain and straw was derivatized to glucosazone and the precipitated glucosazone was removed by filtration, washed several times with water, and recrystallized three times from a 60% aqueous ethanol solution. Melting points were determined for each crystallization sample and samples of the dried glucosazone from each recrystallization were assayed for radioactivity. The structure of the recrystallized glucosazone was confirmed by infrared and carbon-13 nuclear magnetic resonance spectroscopy. Soil bound residues were characterized as per the procedure of Schnitzer and Khan (1972).

Radioassay. Radioactivity was assayed using an Intertechnique Multi-Mat system consisting of a Model SL 300 scintillation spectrometer and a Microdata 1600 computer (Intertechnique, Westwood, N.J.). The extracted radioactivity was assayed by counting aliquots of the extracts in Aquasol-2 scintillation cocktail (New England Nuclear, Boston, Mass.). All solid samples were assayed by combustion in a Model 306 Tricarb sample oxidizer (Packard Instrument Company, Downers Grove, Ill.). Aliquots of approximately 300 mg were placed in a Combusto-Cone sample holder (Packard Instrument Company) and combusted in the instrument which automatically prepared the sample for liquid scintillation counting. The counting solutions were Oxisorb-2 and Oxiprep-2 (New England Nuclear). Scrapings from thin-layer plates were radioassayed directly in a gelled scintillation cocktail consisting of 4 mL of water and 11 mL of Aquasol (New England Nuclear).

Chromatography and Analysis. Thin-layer chromatography was performed on commerical precoated silica gel plates (Merck F-254). Development of chromatograms was accomplished by two different sets of two-dimensional systems: (C) ethyl acetate–1-propanol–formic acid–water (90:150:15:45) vs. ethyl acetate–1-propanol–ammonium hydroxide–water (90:150:15:45); (D) acetone–methylene chloride (30:70) vs. absolute ethanol. The radioactive extracts obtained from the rice plant, fish tissue, paddy water, or from the paddy soil were spotted on thin-layer plates along with standard reference nonradiolabeled mephosfolan and developed by the described two-dimensional systems. Radiolabeled compounds were visualized in a Birchover Instrument radiochromatogram spark chamber (Model 986-010) or by radioautography on Kodak SB-54 single-coated blue-sensitive medical X-ray film (Eastman Kodak Co., Rochester, N.Y.). The nonradiolabeled mephosfolan was visualized with ultraviolet light. Identification of mephosfolan was afforded by demonstration of exact cochromatography of the unlabeled compound with the radioactive spot.

A Tracor 550 gas chromatograph (Tracor, Inc., Austin, Tex.) equipped with a flame-ionization detector and a Packard radioactivity monitor (Model 894 gas proportional counter, Packard Instrument Company) was used to identify [^{14}C]mephosfolan in the plant extracts. The column packing was 5% OV-1 on Chromosorb W-HP (80/90 mesh) packed in 4 mm i.d., 6-ft long glass column. The conditions were: column temperature, 200 °C (isothermal); injector temperature, 230 °C; detector temperature, 260 °C; air flow rate, 14 mL/min; hydrogen flow rate, 50 mL/min; and helium flow rate, 60 mL/min. The flame ionization detector was used to detect the nonradiolabeled mephosfolan and the gas proportional counter was used to detect the radiolabeled mephosfolan.

Table I. Residual Radioactivity (ppm)[a] in Rice Plants Treated with [^{14}C]Mephosfolan[b] in a Greenhouse

weeks after treatment	concentration, ppm			
	methanol extract			
	total	mephosfolan	unextracted	total
1	2.4	2.3	1.7	4.1
2	3.2	3.0	3.9	7.1
4	3.4	3.1	3.8	7.2
8	2.2	2.1	2.4	4.6
18	1.3	1.2	1.3	2.6

[a] Calculated as mephosfolan. [b] Paddy water treated with [^{14}C]mephosfolan at the rate of 0.75 kg of AI/ha.

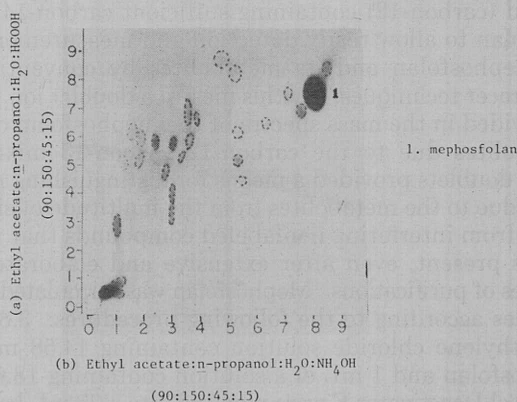


Figure 2. Thin-layer chromatography of radioactivity recovered from rice plant extract 4 weeks after mephosfolan treatment.

RESULTS AND DISCUSSION

Uptake and Metabolism in Plants and Rice Seeds. Gross radioactivity levels and contribution of mephosfolan residues to the total radioactivity in the rice plants attributable to treatment with [^{14}C]mephosfolan are shown in Table I. The concentration of radioactivity recovered in the plants calculated as mephosfolan increased from 4.1 ppm after 1 week to 7.2 ppm at the end of 4 weeks after treatment. Thereafter, as the plants grew and gained in size and mass, the levels of radioactivity showed a steady decline to 2.6 ppm at the end of 18 weeks. About half of the radioactivity in the plants was extractable into methanol. Thin-layer chromatographic analyses of the plant extracts showed that the only major component in the rice plant extracts was mephosfolan, constituting from 91.0 to 96.5% of the total extractable radioactivity. None of the minor radioactive components comprised more than 2% of the total. Thus, no further attempt was made to identify these minor components. The thin-layer chromatographic pattern of the 4-week plant extract, which is typical of the extracts at all the sampling time intervals, is shown in Figure 2. Identification of the parent compound was afforded by demonstration of exact cochromatography of the unlabeled compound with the radioactive spot on TLC in two different two-dimensional solvent systems and by the same retention time by gas

Table II. Specific Activity Determination and Recrystallization of Glucosazone from Rice Grain Starch and Straw Cellulose

	specific activity, dpm/g	
[^{14}C]glucosazone	from starch	from cellulose
washed precipitate	2670	2160
1st recrystallization	2730	2120
2nd recrystallization	2810	2180
3rd recrystallization	2700	2170

chromatography. The radioactivity level in the whole seed (paddy) was 0.57 ppm, with 56.4% of the radioactivity being in the hull or seed covering and the remaining 43.6% in the rice grain. No mephosfolan was found in the rice-grain extract.

Characterization of Bound Residue in Rice Grian and Straw. Since the rice grain consists of about 70% starch, starch was isolated from the rice grain and radioassayed by combustion. Preliminary experiments revealed that crude starch from [^{14}C]mephosfolan treated rice did, indeed, contain radioactivity. To determine whether the radioactivity in the starch fraction was a part of the glucose unit, the starch was hydrolyzed and the resulting glucose was derivatized to the glucosazone by reaction with phenylhydrazine and recrystallized several times. Infrared and ^{13}C nuclear magnetic resonance spectroscopy confirmed the structure of glucosazone synthesized from rice starch.

In order to explore the identity of the bound residue in the rice straw, the plant material was fractionated into crude cellulose and crude lignin. The preliminary experiments revealed that both the crude cellulose and the crude lignin fractions did, indeed, contain radioactivity. To determine if the radioactivity found in the crude cellulose fraction of the rice straw was actually due to the incorporation of radioactivity into the glucose and subsequently into cellulose, the crude cellulose was hydrolyzed and the resulting glucose was derivatized to glucosazone.

In Table II, data are presented which show that a constant specific activity was obtained for the rice grain starch and straw glucosazones after three recrystallization procedures. The radioactivity present in the recrystallized glucosazones was converted to the specific activity of starch by using a factor of 2.27 (Wargo et al., 1975). This value was multiplied by 0.70 (since rice grain contains about 70% starch) and divided by the original specific activity to obtain 42.6% distribution of radioactivity in the rice starch. This value was sufficiently high to conclude that the imido carbon atom of mephosfolan is incorporated into the glucose pool and, subsequently, into the rice starch.

Efforts to show that the radioactivity isolated in the crude lignin fraction of the rice straw was actually associated with lignin were only partially successful due to inability to crystallize or cleanly precipitate the isolate and also due to the low yield of lignin achieved during fractionation. However, on analysis it was found that the isolated lignin fraction had significant amounts of radioactivity associated with it.

Degradation in Water and Soil. Data on the concentrations of mephosfolan found in paddy water at various time intervals after treatment is presented in Figure 3. The radioactivity at 3 days after granular application was 0.79 ppm. Thereafter, there was a rapid decline in the concentration to 0.16 ppm after 7 days, 0.03 ppm after 14 days, and 0.004 ppm after 45 days.

The thin-layer chromatographic pattern of the freeze-dried water samples taken 7 days after treatment is shown in Figure 4A. Mephosfolan was the only radioactive spot

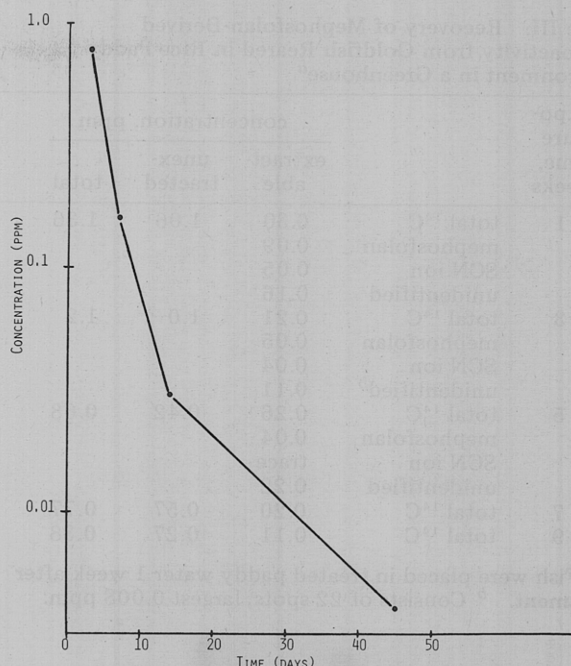


Figure 3. Concentration of radioactivity recovered from paddy water from a rice paddy treated with [^{14}C]mephosfolan.

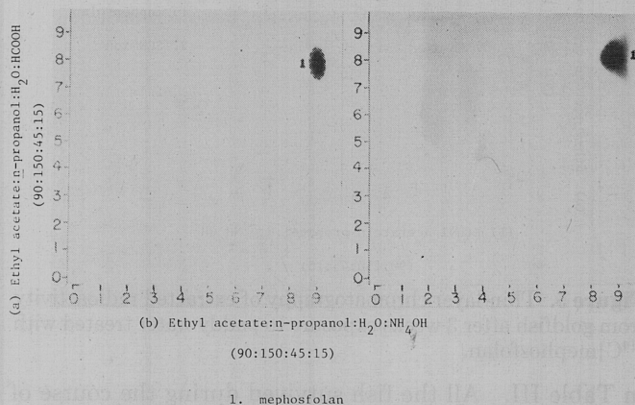


Figure 4. Thin-layer chromatography of radioactivity recovered from (a) paddy water 7 days after treatment and (b) paddy soil extracts 19 weeks after treatment.

found in that water sample. Due to the very low levels of radioactivity in 14- and 45-day samples, it was not possible to analyze them by TLC.

Recovery of radioactivity remaining in the paddy soil after harvesting of the rice indicated that 20.3% of the applied radioactivity remained in the soil, with 15.3% in the methanol extracts and the remaining 5% unextracted in the residue (marc). About 13.9% of the applied radioactivity remained in the top 0 to 5 in. level of the soil profile. The thin-layer chromatographic pattern of methanol extracts of the soil is presented in Figure 4B. The chromatogram shows only one radioactive spot which was identified as mephosfolan.

The unextracted residue was fractionated into various components. Humin, humic acid, and fulvic acid fractions contained 38.1, 18.9, and 43.0%, respectively, of the unextracted residues. The high radioactivity content of the fulvic acid fraction may be attributed to incorporation of fragments of the pesticide molecule into this natural product as suggested by Meikle et al. (1976).

Uptake and Metabolism in Fish in the Rice Paddy Environment. The radioactive residue levels found at various time intervals in the fish kept in the [^{14}C]mephosfolan treated rice-paddy environment are presented

Table III. Recovery of Mephosfolan-Derived Radioactivity from Goldfish Reared in Rice Paddy Environment in a Greenhouse[a]

exposure time, weeks		concentration, ppm		
		extractable	unextracted	total
1	total ^{14}C	0.30	1.06	1.36
	mephosfolan	0.09		
	SCN ion	0.05		
	unidentified	0.16		
3	total ^{14}C	0.21	1.0	1.2
	mephosfolan	0.05		
	SCN ion	0.04		
	unidentified[b]	0.11		
5	total ^{14}C	0.26	0.42	0.68
	mephosfolan	0.04		
	SCN ion	trace		
	unidentified	0.22		
7	total ^{14}C	0.20	0.57	0.77
9	total ^{14}C	0.11	0.27	0.38

[a] Fish were placed in treated paddy water 1 week after treatment. [b] Consists of 22 spots; largest 0.008 ppm.

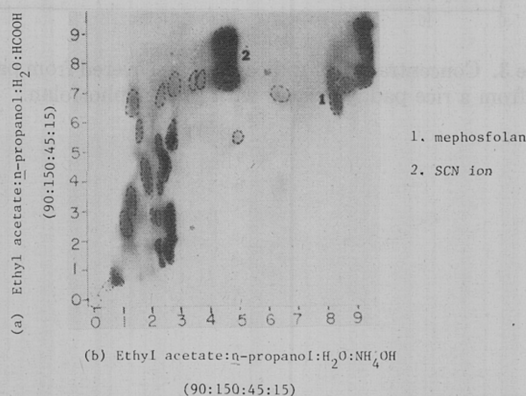
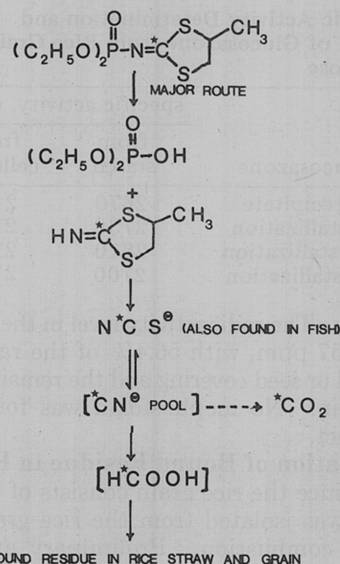


Figure 5. Thin-layer chromatography of extracted radioactivity from goldfish after 3 weeks exposure to paddy water treated with [^{14}C]mephosfolan.

in Table III. All the fish survived during the course of this study. The total concentration of radioactivity recovered in the fish tissue decreased from 1.4 ppm at 2 weeks (1 week of exposure) to 0.38 ppm at 10 weeks (9 weeks of exposure) after the treatment. From the two-dimensional thin-layer chromatogram of the fish tissue extract (Figure 5), it is evident that mephosfolan is metabolized extensively into many polar metabolites. The predominant metabolite was identified as thiocyanate ion by cochromatography with unlabeled thiocyanate ion. The relative abundance and concentrations of mephosfolan and its metabolites in the fish extracts at 1, 3, and 5 weeks of exposure to the paddy environment are also shown in Table III. With these data and the data obtained from paddy water analyses, it is appropriate to evaluate the significance of the relatively minor pesticide uptake by the fish when reared in the paddy environment. Metcalf et al. (1971) and Kapoor et al. (1973) have defined the ecological magnification constant as the ratio of the concentration of parent compound in the organism vs. the concentration of parent compound in water. The bioaccumulation constant is defined as the ratio of the total radioactivity concentration in the organism vs. the total radioactivity concentration in water. The authors obtained data for DDT and methoxychlor in a terrestrial-aquatic model ecosystem where the pesticide was applied to terrestrial foliage only. The fish were kept in this system for 3 days and the highest value for ecological magnification constant for DDT was 84 500 and that for bioaccumulation was 13 500. The comparative figures for methoxychlor, a biodegradable pesticide, were 1545 and 206, respectively. Although it is difficult to compare this study with those using the Metcalf system, due to the differences in the two systems, we can examine and compare their data with the mephosfolan study in which fish were exposed for 1 week in the paddy environment. From a starting concentration of 0.16 ppm, an ecological magnification of 0.56 and a bioaccumulation of 8.5 were found for fish exposed to mephosfolan. Since very low values of ecologial magnification (0.56 vs. 84 500 for DDT) and bioaccumulation (8.5 vs. 13 500 for DDT) were obtained, it is evident that mephosfolan is not likely to cause any ecological or bioaccumulation hazards when it is used in a rice-paddy environment.



* DENOTES CARBON-14

Figure 6. Proposed pathways of metabolism of [^{14}C]mephosfolan in rice paddy.

CONCLUSION

The metabolic fate of mephosfolan in rice plants grown in a paddy environment was studied. The distribution of extractable and unextractable residual radioactivity was found to be approximately equal in the rice plants. Of the extractable radioactivity, more than 91% was identified as mephosfolan. The remaining 9% was due to a mixture of at least 17 or more metabolites. The unextractable radioactivity has been characterized as ^{14}C-labeled cellulose and possibly lignin, due to the incorporation of imido ^{14}C-carbon atom into these natural products.

Although only mephosfolan was found in paddy water, its concentration in water decreased at a rapid rate, presumably due to its uptake and degradation by the rice plant, fish, and other living organisms in the paddy ecosystem.

The results indicate that while mephosfolan is found in the rice plant, it does not accumulate in the rice grain. The radioactive residues found in the rice grain were characterized as ^{14}C-labeled starch due to the incorporation of the ^{14}C-imido carbon atom of mephosfolan into glucose and subsequently into the starch.

A metabolic pathway of mephosfolan in rice paddy is proposed in Figure 6. The mechanism of the formation of 2-imino-1,3-dithiolanes as an intermediate step in the formation of thiocyanate was discussed in an earlier paper (Kapoor and Blinn, 1977).

ACKNOWLEDGMENT

We thank Milon W. Bullock for synthesis of carbon-13 mephosfolan and Karen Miller for assistance rendered in the conduct of the experiment.

LITERATURE CITED

Adams, G., Castagne, A., *Can. J. Res., Sect. B* **27**, 915 (1949).
Brauns, F., *J. Org. Chem.* **10**, 211 (1945).
Honeycutt, R. C., Adler, I. L., *J. Agric. Food Chem.* **23**, 1097 (1975).
Kapoor, I. P., Blinn, R. C., *J. Agric. Food Chem.* **25**, 413 (1977).
Kapoor, I. P., Metcalf, R. L., Hirwe, A. S., Coats, J. R., Khalsa, M. S., *J. Agric. Food Chem.* **21**, 310 (1973).
Meikle, R. W., Regoli, A. J., Kurihara, N. H., *ACS Symp. Ser.* **29**, 272 (1976).
Metcalf, R. L., Sangha, G. K., Kapoor, I. P., *Environ. Sci. Technol.* **5**, 709 (1971).
Perry, J. A., Jupille, T. H., Glunz, L. J., *Anal. Chem.* **47**, 65A (1975).
Schnitzer, M., Kahn, S. U., "Humic Substances in the Environment", Dekker Marcel, New York, N.Y., 1972, p 17.
Wargo, J. P., Honeycutt, R. C., Adler, I. L., *J. Agric. Food Chem.* **23**, 1095 (1975).

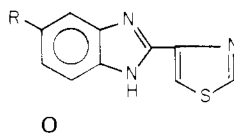
Received for review June 12, 1978. Accepted August 21, 1978. From a thesis submitted by the senior author to the Graduate School, Rutgers–The State University, New Brunswick, N.J., in partial fulfillment of the requirements for the Ph.D. degree. Presented at the Division of Pesticide Chemistry, 174th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1977.

Urinary Metabolites of Cambendazole

William J. A. VandenHeuvel,* Donald E. Wolf, Byron H. Arison, Rudolf P. Buhs, Josephine R. Carlin, Robert L. Ellsworth, Theodore A. Jacob, Frank R. Koniuszy, Jack L. Smith, Nelson R. Trenner, Robert W. Walker, and Frank J. Wolf

Metabolism of orally administered cambendazole-^{14}C [isopropyl 2-(4-thiazolyl)-5-benzimidazolecarbamate] in cattle, swine, and sheep leads to urinary excretion of 20–40% of the dose as biotransformation products. Fourteen urinary metabolites were identified, primarily by mass spectrometric techniques, and eleven of these were synthesized. Metabolic attack on the thiazole ring is the major route of structural transformation, resulting in a series of 11 identified metabolites. The carbamate side chain is also involved in several metabolic changes, and hydroxylation of the benzene ring yielded a phenolic metabolite.

Cambendazole, isopropyl 2-[4-thiazolyl]-5-benzimidazolecarbamate (CBZ), is a broad-spectrum anthel-



CBZ, R = $(CH_3)_2CHOC(=O)HN$; TBZ, R = H; 5-HTBZ, R = OH

mintic active in cattle, swine, and sheep against a wide variety of mature and immature parasites (Hoff et al., 1970; Campbell and Yakstis, 1970; Egerton and Campbell, 1970; Egerton et al., 1970a,b). This drug is a substituted thiabendazole (TBZ) (Brown et al., 1961). TBZ is also an anthelmintic and antifungal agent (Campbell, 1961; Cuckler, 1961; Robinson et al., 1964). Both drugs are rapidly metabolized after oral administration to test animals. Tocco et al. (1964) reported on the metabolic fate of TBZ in the sheep and indicated that the phenol 5-hydroxy-TBZ and its glucuronide and sulfate conjugates were urinary metabolites in this species. Hydroxylation at this position of the benzimidazole ring system followed by conjugation is a metabolic pathway for 2-(2-furyl)-benzimidazole (Frank, 1971) and benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] (Gardiner et al., 1974). Tyler et al. (1976) have demonstrated that in the rat, hydroxylation at the 5-position is the preferred route of metabolism for benzimidazole itself. When the 5-position of the benzimidazole nucleus of a drug is substituted, metabolic reactions may occur elsewhere on the molecule. Parbendazole (methyl 5-butyl-2-benzimidazolecarbamate), for example, is metabolized by sheep and cattle to form a series of oxidative products of the butyl side chain (Dunn et al., 1973) and we (VandenHeuvel et al., 1972) have reported that CBZ is transformed to several side chain metabolites in various species. It was thus of interest to further investigate the urinary metabolite pattern for CBZ so as to ascertain the positions of metabolic attack in this 5-substituted benzimidazole, especially with respect to the thiazole ring.

Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065.

EXPERIMENTAL SECTION

Radiochemical Procedures.. *Tracers Employed.* Radioactively labeled CBZ was employed in the metabolism studies; for the syntheses of the labeled compounds [CBZ-^{14}C (benzene ring, 0.15–1.2 μCi/mg) and CBZ-*t* (isopropyl methyl, 6.0 μCi/mg)], see Ellsworth et al. (1976). Each compound exhibited (by thin-layer chromatography) >99% radiochemical purity.

Liquid Scintillation Counting. Measurements of radioactivity were made using either a Packard Tri-Carb or an Intertechnique liquid scintillation spectrometer operated on optimal setting for ^{14}C or ^{3}H. Scintillation solutions were made from either Omnifluor or Liquifluor (Bio-Rad) in toluene or a 70:30 mixture of toluene–ethanol. When required, quenching corrections were made by the internal standard method using the appropriate standard. Samples insoluble in the phosphor were analyzed by combustion to $^{14}CO_2$ or tritium water using the Schoniger method (Kelly, 1961) or the more rapid Peterson (1969) modification.

Animal Studies. Large animals were kept in metabolism stalls or cages so that feces and urine could be collected separately. The single dose of drug was administered either as a dry blend (or gelatin capsule) or in

0021-8561/78/1426-1357$01.00/0 © 1978 American Chemical Society