Kenaga, E. Predicted Bioconcentration Factors and Soil Sorption Coefficients of Pesticides and Other Chemicals. *Ecotox. Environ. Saf.* **1980**, *4*, 26-38.
Ku, C.; Hwang, S. The Preparation of Carbon-14 Labeled Avermectin B_{1a}. *J. Labelled Compd. Radiopharm.* **1985**, *22* (5), 451-459.
Lofgren, C.; Williams, D. Avermectin B_{1a}: Highly Potent Inhibitor of Reproduction by Queens of the Red Imported Fire Ant Solenopsis-Invicta Hymenoptera Formicidae. *J. Econ. Entomol.* **1982**, *75*, 798-803.
Putter, I.; MacConnell, J.; Preiser, F.; Haidri, A.; Ristich, S.; Dybas, R. Avermectins: Novel Insecticides, Acaricides and Nematicides from a Soil Microorganism. *Experientia* **1981**, *37*, 963-964.

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Environmental Fate of Ceftiofur Sodium, a Cephalosporin Antibiotic. Role of Animal Excreta in Its Decomposition

Terry J. Gilbertson,* Rex E. Hornish, Prem S. Jaglan, K. Thomas Koshy, John L. Nappier, Gerald L. Stahl, Alex R. Cazers, Jean M. Nappier, Marc F. Kubicek, Gregory A. Hoffman, and Philip J. Hamlow

Biochemistry and Residue Analysis, Agricultural Division, The Upjohn Company, Kalamazoo, Michigan 49001

The degradation of ceftiofur sodium, a wide-spectrum cephalosporin antibiotic, was studied in the urine and feces of cattle, in three soils, and in buffers of pH 5, 7, and 9. Photodegradation was also studied. Fortification of cattle feces with [^{14}C]ceftiofur showed that it was quickly degraded to microbiologically inactive metabolites. Sterilization of the cattle feces inhibited the degradation of ceftiofur, which suggests that microorganisms or heat-labile substances may be responsible for the degradation. The $t_{1/2}$ values of aerobic degradation of ceftiofur sodium in California, Florida, and Wisconsin soil were found to be 22.2, 49.0, and 41.4 days, respectively. Hydrolysis of ceftiofur, as measured by either HPLC or microbiological methods, was accelerated by increasing pH. The $t_{1/2}$ values at pH 5, 7, and 9 were 100.3, 8.0, and 4.2 days, respectively, at 22 °C and dramatically increased at 47 °C. The photodegradation of ceftiofur, as determined by HPLC and a microbiological method, showed that after initial degradation for several days the rate of degradation was minimal, probably due to a protective film formed from degradation products. A major role for feces in the degradation of ceftiofur was observed, although other pathways of degradation such as soil, light, and water were also important.

During the last 60 years, chemicals have dramatically improved the quality of life for humans. However, the world has witnessed a few cases of chemical hazard to the population that have changed our thinking about controlling pollution. Environmental contamination has become a topic of political debate. This is a justified concern, and environmental fate studies are a justifiable requirement for the registration of new chemicals. Animal health drugs intended for therapeutic use do not pose a threat similar to pesticides and other production chemicals due to their limited use in a rather controlled area. However, the question of environmental safety of an animal health drug has to be addressed because certain animal health drugs could have adverse effects on the environment as was recently demonstrated (Wall and Strong, 1987; Houston, 1987).

A recent symposium held at The Upjohn Co. (1988), Brook Lodge, Augusta, MI, highlighted the studies required to evaluate the safety of animal health drugs. Studies such as photolysis, hydrolysis, partition coefficients, and aerobic soil degradation similar to those used for pesticides are required.

This study was done to evaluate the environmental fate and impact of ceftiofur sodium (Figure 1; ^{14}C-labeled in the 2-position of the thiazole ring), a broad-spectrum antibiotic recently approved for the treatment of cattle for shipping fever (*Fed. Regist.* 1988). Ceftiofur sodium has been found to be quite effective against various pathogens of veterinary importance both in vitro and in vivo (Yancey et al. 1987).

We recently described the metabolism of [^{14}C]-ceftiofur sodium labeled in the 2-position of the thiazole ring in cattle and rats (Jaglan et al., 1989), which indicate that ceftiofur is metabolized and excreted. The drug is unusual in that after killing the bacteria in the cattle, it is readily degraded to microbiologically inactive products by substances or organisms in the feces, thus alleviating any environmental contamination concerns.

EXPERIMENTAL SECTION

Microbiological Assay. The standard cylinder plate method (AOAC, 1984) utilizing antibiotic medium 8 supplemented with 5.0 g/L agar, 0.45 g/L monobasic potassium phosphate, and 0.1% Tween 80 was used. *Micrococcus luteus* UC-130 (ATCC 9341) maintained as a frozen suspension over liquid nitrogen was the test organism. An inoculation of 0.08% (about 8×10^8

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Table I. Soil Characteristics and Dosage Data

	California	Florida	Wisconsin
soil type	clay loam	sand	silty clay loam
soil pH	8.02	6.96	7.37
cation-exchange capacity	16.5	1.1	21.4
organic matter, %	1.2	1.0	1.3
moisture content, %	10	13	23
moisture holding capacity for 50 g of dry soil, g of H_2O	26.5	15.5	23.3
70% moisture holding capacity, g	18.5	10.8	23.3
amt soil needed, g	57.14	55.37	62.11
amt H_2O in soil aliquot, g	7.14	5.37	12.11
amt H_2O in test soil added, g	12.3	5.5	4.2
glucose soln concn, mg/mL	2.032	4.546	5.952
total glucose added, mg	25.0	25.0	25.0
amt C added (40% C), mg	10	10	10
ceftiofur sodium soln concn, mg/mL	2.114	4.728	6.196
total CS added, mg	26.0	26.0	26.0
amt C added (39% C), mg	10	10	10

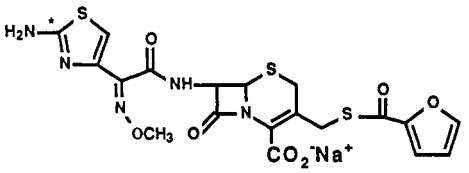

Figure 1. Structure of ceftiofur sodium.

viable cells) produced a zone of inhibition of about 10 mm when 5 ng/mL ceftiofur sodium was used. Standards and samples were plated in duplicate. Ceftiofur sodium standard solutions of 5, 10, 20, 40, and 80 ppb were used. The plates were incubated for 18 h at 30 °C, and concentrations were determined in samples from zone sizes compared to zone sizes for the standards. The limit of detection of the assay was 0.1 ppm, and the coefficient of variation was about 15%.

HPLC. *A. HPLC/RAM*: (1) pumps, Varian Model 5500 (Varian Associates, Walnut Creek, CA) (2) UV monitor, at 254 nm; (3) radioactive monitor (RAM), Radiomatic Flo-one (Radiomatic Instruments, Tampa, FL); (4) injector, Rheodyne Model 70-10 valve loop (Rheodyne, Inc., Cotati, CA).

B. HPLC gradient system I: column, Baker Bond wide-pore C_8; solvent A; 0.1% TFA in water; solvent B, 90:10 acetonitrile–water containing 0.1% TFA; gradient, isocratic at 0% B for 5 min and then linear increase to 16% B at 2%/min and to 19% at 0.1%/min.

C. HPLC gradient system II: Baker Bond wide-pore C_8; 5-μm column, 100 × 4.6 mm containing a guard column (4.6 × 30 mm, Aquapore RP-300); 1 mL/min flow of 0.01 M ammonium acetate–water solution (pH 5) (solvent A) and gradient of 29% of methanol–water (60:40) (solvent B) reached in 25 min; column flushed with 100% of solvent B after each run and equilibrated for 10 min with solvent A.

D. HPLC system III: 254 nm; Zorbax C_8 bonded 5 μM spherical packing 25 cm × 4.6 mm (i.d.) column; mobile phase, 70% aqueous (0.05 M ammonium acetate containing 1.95% of 40% tetrabutylammonium hydroxide adjusted to pH 6.7 with glacial acetic acid), 20% methanol, and 10% tetrahydrofuran; flow, 1 mL/min. The coefficient of variation of all HPLC methods was about 10%.

Degradation of Ceftiofur in Urine and Feces of Cattle, Swine, and Chickens. Ten-gram aliquots of urine, feces slurry, and urine and feces slurry mixture (1:1) obtained at 24 h after im injection of 2.2 mg of [^{14}C]ceftiofur free acid equivalents/kg of body weight in cattle were incubated in five replicates in the dark in beakers covered with aluminum foil at room temperature for various intervals of time. The specific activity of the dose was about 3000 dpm/μg, the counting efficiency of samples was 95%, and the limit of detection was 0.02 ppm. The samples were then shaken with 100 mL of 0.1 M phosphate buffer (pH 6) for 15 min and centrifuged at 2500 rpm for 5 min. A 1-mL aliquot of the supernatant was diluted with an equal volume of methanol (or further diluted if necessary) and

Table II. Concentration (ppm [^{14}C]Ceftiofur Equivalents)[a] in the Urine and Feces of Animals Used in Incubations To Determine Microbiological Activity

	animal no.					
	1	2	3	4	5	6
sex of animal	F	M	F	M	F	M
sample urine	11.31	16.33	43.88	216.09	110.77	67.05
sample feces (slurry)	4.87	3.32	3.32	5.44	3.88	3.25
sample (urine + feces, 1:1)[b]	8.09	9.83	23.60	110.77	57.33	35.15

[a] ppm found in 24-h samples after the last treatment. ppm calculated.

analyzed by the standard plate assay for microbiological activity. Control feces samples were also fortified with 102 ppm solution of [^{14}C]ceftiofur sodium (specific activity 675 dpm/μg) and analyzed as above at 0-, 1-, 2-, 4-, 6-, 8- and 24-h intervals. The supernatant was also analyzed by HPLC (system I).

Control cattle feces (sterilized and nonsterilized) were incubated at room temperature with 100 ppm of [^{14}C]ceftiofur sodium. These were extracted with 10 volumes of methanol at various intervals of incubation. An aliquot of methanol extract was analyzed by HPLC/radioactive monitor (system II), and the amount of ceftiofur (radioactivity in the peak of ceftiofur) was determined in both sterilized and nonsterilized feces extracts. Similar studies were also done in chicken and swine excreta.

Aerobic Degradation of Ceftiofur in Soils. Soils (50-g samples) from three locations, viz. California, Florida, and Wisconsin, were incubated with ceftiofur in biometer flasks (Bartha and Pramer, 1965). For each soil type there were three controls (water only), three glucose samples as a positive reference, and three ceftiofur sodium samples. The characteristic of the soils and the doses used for glucose and ceftiofur sodium were based on supplying 10 mg of carbon for 50 g of soil at a 70% moisture-holding capacity (see Table I).

Photolysis of Ceftiofur Sodium. An exact amount of ceftiofur sodium (~10 mg) was weighed into glass vials 1.5 cm in diameter and 1.2 cm in height, spread evenly over the glass surface by gentle tapping, and then exposed to a UV light source, RUL 3000-0 UV lamp from a Rayonet RPR-208 photochemical reactor (Southern New England Ultraviolet Co., Middletown, CT), mounted 4 in. above the samples for several weeks, until degradation reached a plateau. A set of controls were similarly positioned but covered with aluminum foil to subtract the effect of localized heat. Control and treated samples were dissolved in ammonium acetate and analyzed by cylinder plate microbiological assay and by HPLC system III. Ceftiofur sodium has λ_{max} at 293 m_u and molar absorptivity of 27 800.

Hydrolysis Rate of Ceftiofur. The hydrolysis of ceftiofur sodium was carried out in 0.2 M acetate buffer (pH 5), 0.2 M phosphate buffer (pH 7), and 0.2 M borate buffer (pH 9) at 22 and 47 °C. One-hundred-milliliter samples of each buffer containing ceftiofur sodium at 2–7 ppm were covered with aluminum foil and incubated for various intervals of time. A 2-mL

Table III. Microbiological Activity (ppm Ceftiofur Equivalents)[a] in the Urine[b] of Bovine Treated with [^{14}C]Ceftiofur at Various Intervals of Incubation

time after incubn, h	animal no. 1	2	3	4	5	6	mean ± SD
0	7.82	2.62	2.07	11.21	6.60	6.51	6.14 ± 3.40
24	4.99	1.69	2.12	6.61	3.88	4.03	3.89 ± 1.82
48	3.22	NS	1.53	4.77	2.47	3.45	2.59 ± 1.63
72	1.74	NS	1.05	2.85	0.96	1.79	1.42 ± 0.94
144	0.16	NS	0.12	0.12	NS	NS	0.12 ± 0.0

[a] Average of five replicates. [b] See Table II for ppm [^{14}C]ceftiofur equivalents. NS = <0.1 ppm.

Table IV. Microbiological Activity (ppm Ceftiofur Equivalents)[a] in the Urine and Feces (1:1 Mixture)[b] at Various Intervals of Incubation at Room Temperature

time after incubn, h	animal no. 1	2	3	4	5	6	mean ± SD
0	2.19	0.53	2.25	2.57	2.03	1.69	1.88 ± 0.72
24	1.27	NS	1.17	1.53	0.35	0.37	0.80 ± 0.60
48	0.46	NS	0.63	0.38	0.14	0.13	0.31 ± 0.22
72	NS	NS	0.35	0.14	NS	NS	0.15 ± 0.10

[a] Average of five replicates. [b] See Table II for ppm [^{14}C]ceftiofur equivalents. NS = <0.1 ppm.

Table V. Relative Hydrolytic Stability of Ceftiofur Sodium at 22 and 47 °C Expressed in Terms of $t_{1/2}$ (Days)

temp, °C	buffer pH 5	7	9
22	100.3	8.0	4.2
47	5.5	2.9	

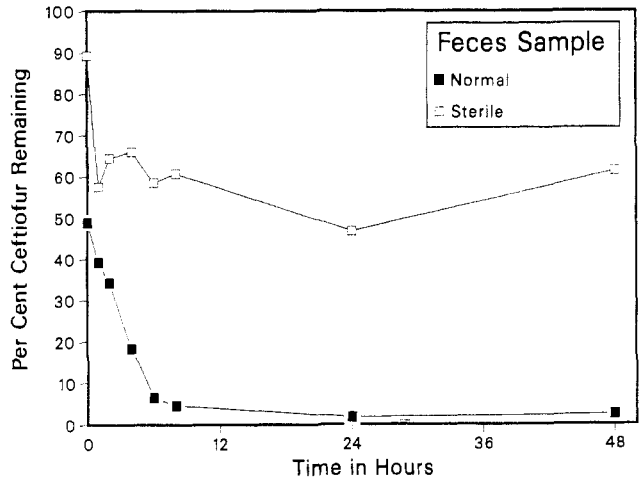

Figure 2. Degradation of ceftiofur in the sterilized and normal feces of cattle.

aliquot was taken and diluted to 50 mL with 0.05 M ammonium acetate containing the internal standard acetophenone and analyzed by HPLC system III. An aliquot of each sample was also analyzed by the microbiological method.

RESULTS AND DISCUSSION

Microbiological Activity in the Urine and Feces. Table II gives the concentrations of [^{14}C]ceftiofur present in the samples of urine, feces, and a 1:1 urine–feces mixture of the six cattle used in the study. No microbiological activity was found in any of the feces samples. Microbiological activity in urine samples (Table III) was also lower compared to the radioactivity data in Table II, indicating the ceftiofur metabolites that are present have less microbiological activity compared to ceftiofur. Previous metabolism studies in bovine have demonstrated that no ceftiofur was present in urine (Jaglan et al., 1989) and give credence to the above observation. All the urine samples lost their microbiological activity during incubation for various intervals of time, and only traces of

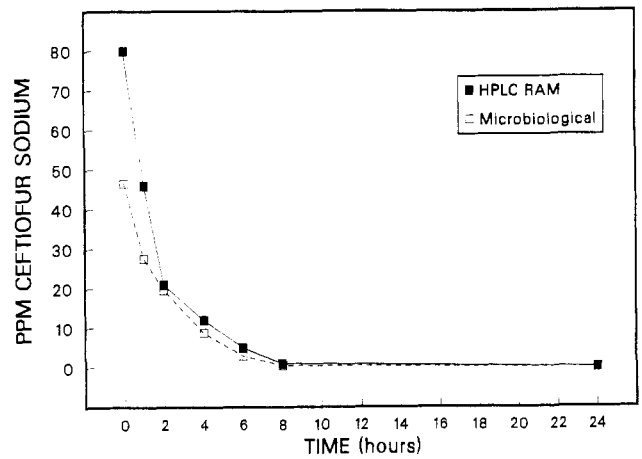

Figure 3. Degradation of ceftiofur spiked in the feces of cattle.

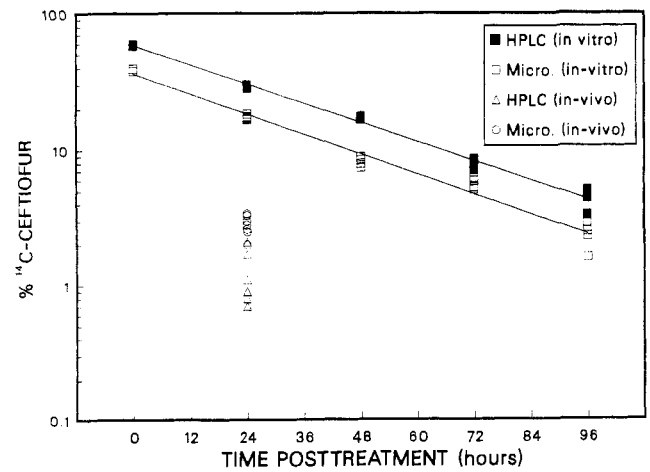

Figure 4. Degradation of ceftiofur in the excreta of chickens, in vivo versus in vitro.

microbiological activity were present at 144 h of incubation (Table III). The mean half-life for the six samples was 23 h. When the incubations were carried out on urine samples mixed with an equal amount of feces, the microbiological activity declined even faster than in urine alone and the mean half-life for the samples was reduced to 17 h (Table IV), indicating that feces have some component catalyzing this degradation. In order to clarify

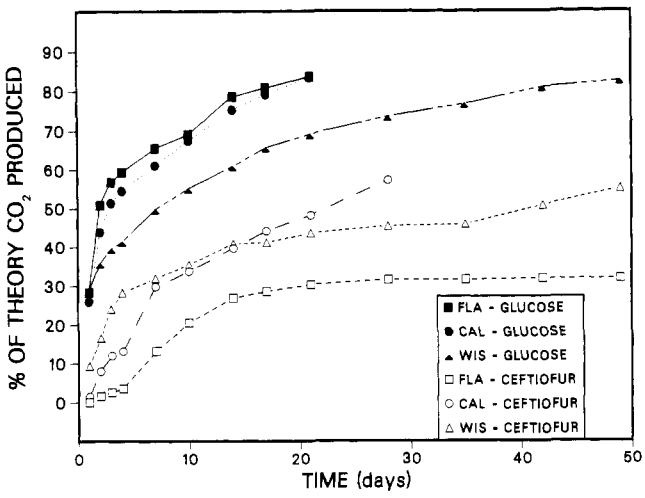

Figure 5. Aerobic degradation of glucose and ceftiofur in soils.

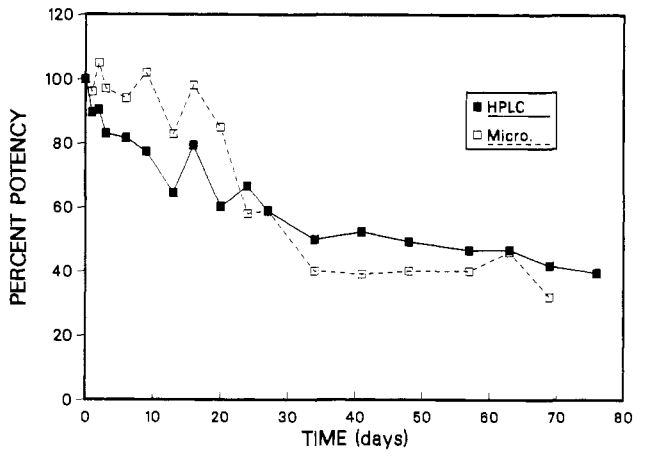

Figure 6. Photodegradation of ceftiofur.

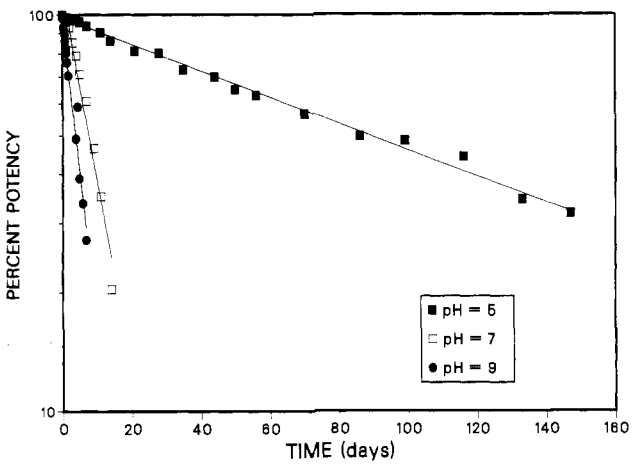

Figure 7. Hydrolysis of ceftiofur at 22 °C.

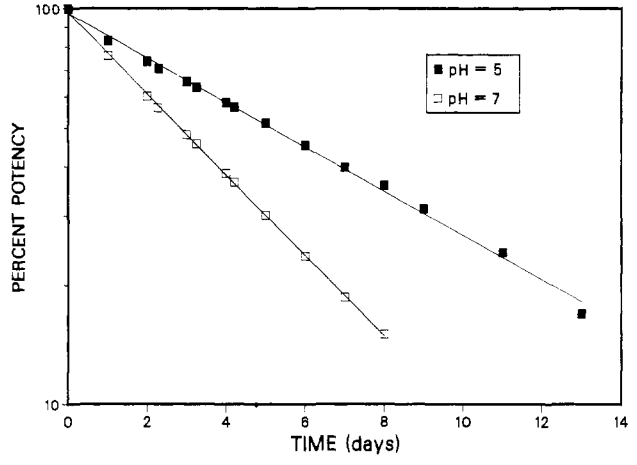

Figure 8. Hydrolysis of ceftiofur at 47 °C.

whether organisms are involved, sterilized and nonsterilized normal bovine feces were fortified with [^{14}C]-ceftiofur and analyzed for ceftiofur with the HPLC/radioactive monitor. The data demonstrate that the degradation of ceftiofur in sterile feces is slower compared to normal feces, suggesting the role microorganisms play in this degradation (Figure 2). Figure 3 shows that degradation of ceftiofur by feces was rapid and the amounts found by HPLC or by the microbiological assay were nearly the same. It should be pointed out that the nature of the degradation products could not be determined, although the degradation did not progress all the way to [^{14}C]CO_2 since all the radioactivity was still present. These metabolites were polar in nature. Similar results of the degradation of [^{14}C]ceftiofur were observed in chicken excreta from in vitro incubations (Figure 4), and a linear relationship between HPLC and microbiological assay was observed. When the excreta samples collected from the treated animals containing 10–16 ppm [^{14}C]ceftiofur equivalents were analyzed by HPLC and the microbiological method, less than 0.5 ppm ceftiofur equivalents was observed as active compounds, indicating degradation products. Similar results were observed for pig feces. The above data suggest that fecal materials contain microorganisms capable of degrading ceftiofur to nonmicrobiologically active materials. This may occur through fission of the β-lactam ring and hydrolysis of the peptide bond of ceftiofur. The above observations showed that the drug, after exercising its effect on the target microorganism, will leave the body and be detoxified on a continuous basis right in the excreta, leaving no environmental problems or concerns. These detoxifying enzymes or microorganisms are present in the excreta of cattle, pigs, and chickens as described above. A similar population of microorganism is also likely to be present in the excreta of humans, since fecal metabolites of ceftriazone were also found to be biologically inactive (Patel and Kaplan, 1984).

Aerobic Degradation of Ceftiofur Sodium. The principle of the soil biodegradation test is that as the test compound is degraded to CO_2, the liberated CO_2 consumes hydroxide in the alkali solution in the biometer flask to form carbonate, which is precipitated out by the addition of barium chloride (Bartha and Pramer, 1965). The remaining hydroxide is quantitated by titration with dilute hydrochloric acid. The amount of carbon dioxide produced is calculated from the hydroxide consumed in the flask. The CO_2 found in control soil samples is subtracted from treated samples since some endogenous organic matter in soil is degraded to CO_2. The amount of CO_2 liberated from soils into the biometer flask is controlled by the pH of the soil.

Aerobic degradation of ceftiofur sodium in three soils is shown in Figure 5. The reference compound, glucose, was degraded to CO_2 to the extent of about 85% of the theoretical amount added in all three soils. The degradation of ceftiofur sodium was slower compared to glucose in all soils. The $t_{1/2}$ values at 50% for glucose in Florida, California, and Wisconsin soil were 2.0, 2.8, and 7.6 days respectively, whereas for ceftiofur sodium these were >49.0, 22.2, and 41.4 days, respectively.

Photolysis of Ceftiofur Sodium. Figure 6 com-

pares the photodegradation as determined by HPLC and microbiological assay. The data obtained by both methods are comparable. About 50% of the drug was degraded in less than 1 month. Thereafter, the rate of degradation became minimal, probably due to a protective film of degradation products formed around the unreacted drug. The degradation products could not be characterized and are believed to be polymeric. Small amounts of the *anti*-oxime of ceftiofur sodium were also observed, indicating inversion of the *syn*-oxime form of ceftiofur sodium used in this study.

Hydrolysis of Ceftiofur Sodium. The hydrolysis of ceftiofur sodium in pH 5, 7, and 9 buffers at 22 °C is shown in Figure 7. The half-life for the hydrolytic degradation is given in Table V. These data are based on the HPLC analysis of ceftiofur sodium. The potencies as a percent of initial concentration (μg/mL) of ceftiofur sodium by HPLC and microbiological assay were similar. The data at 47 °C for HPLC and microbiological assay were also similar (Figure 8) except that degradation was faster compared to 22 °C as expected (Table V). The two methods gave results that were linearly related with $y = 0.96$. The expected hydrolysis products, desfuroyl ceftiofur and furoic acid, were not present, and there was no indication of the appearance of products that could be detected by HPLC. These products are thought to be polymers that do not elute from the HPLC column until the end of the gradient.

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 14th ed.; AOAC: Arlington, VA, 1984; Sections 42.203, 42.204, 42.205, 42.206, 42.207, 42.208, pp 813–815.

Bartha, R.; Pramer, D. Features of a Flask and Method for Measuring the Persistence and Biological Effects of Pesticides in Soil. *Soil Sci.* **1965**, *100*, 68–70.

Food and Drug Administration. Animal Drugs, Foods and Related Products, Ceftiofur Sterile Powder. *Fed. Regist.* **1988**, *53*, 5369–5370.

Houston, I. K. Effects of Invermectin on Fauna in Dung. *Aust. Vet. J.* **1987**, *64*, 4.

Jaglan, P. S.; Kubicek, M. F.; Arnold, T. S.; Cox, B. L.; Robins, R. H.; Johnson, D. B.; Gilbertson, T. J. Metabolism of Ceftiofur. The Nature of Urinary and Plasma Metabolites in Rats and Cattle. *J. Agric. Food Chem.* **1989**, *37*, 1112–1118.

Patel, I. H.; Kaplan, S. A. Pharmacokinetic Profile of Ceftriaxone in Man. *Am. J. Med.* **1984**, *77* (4C), 17–25.

UpJohn Co. Environmental Studies Related to Approval of New Animal Health Drugs. Symposium, Brook Lodge, Augusta, MI, May 26, 1988.

Wall, R.; Strong, L. Environmental Consequences of Treating Cattle with the Anti-Parasitic Drug Ivermectin. **1987**, *327*, 418–421.

Yancey, R. J.; Kinney, M. L.; Roberts, B. J.; Goodenough, K. R.; Hamel, J. C.; Ford, C. W. Ceftiofur Sodium, a Broad Spectrum Cephalosporin. Evaluation In Vitro and In Vivo in Mice. *Am. J. Vet. Res.* **1987**, *48*, 1050–1053.

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Effect of Endomycorrhizae on the Bioavailability of Bound ^{14}C Residues to Onion Plants from an Organic Soil Treated with [^{14}C]Fonofos†

Sherman D. Nelson* and Shahamat U. Khan

Land Resource Research Centre, Research Branch, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6

Uptake of bound ^{14}C residues from an organic soil treated with radiolabeled fonofos (*O*-ethyl *S*-phenyl ethylphosphonodithioate) by selected *Glomus* endomycorrhiza and onion roots was studied. The hyphae of endomycorrhizal associations were capable of removing ^{14}C residues from the soil and transporting them to onion plants. Bioavailability of soil-bound ^{14}C residues, as measured by ^{14}C residue content in onion, was increased 32 and 40% over that of nonmycorrhizal plants by hyphae of *Glomus intraradices* and *Glomus vesiculiferium*, respectively. The data suggest that under field conditions endomycorrhizal infection may greatly increase the bioavailability of soil-bound pesticide residues to plants.

It is now well established that many commonly used pesticides form bound residues when applied to agricultural soils (Khan, 1982; Katan et al., 1976). These residues may constitute an environmental hazard as their bioavailability and ultimate fate is generally unknown. Recent studies using radiotracer techniques have indicated that bound pesticide residues in soil may become bioavailable to plants (Racke and Lichtenstein, 1985; Khan, 1980; Helling and Krivonak, 1978) and microbes (Khan and Ivarson, 1981, 1982). In general, plants grown in soil containing only ^{14}C-bound residues of pesticides were found to take up 0.1–1.0% of these residues (Khan, 1982). The role of plant roots and soil microbes and the mechanisms of release and removal of these bound pesticide residues remain unclear.

† LRRC Contribution No. 89-105.

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