Lenacil Degradation in the Environment and Its Metabolism in the Sugar Beets

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^{14}C-Lenacil photolysis and hydrolysis studies were conducted at 2 ppm in sterilized buffers at pH 5, 7, and 9 for up to 15 and 30 days, respectively. The degradation of ^{14}C-lenacil in three soils and in two sediments systems was monitored for up to 100 days. Residue level and metabolites were analyzed in sugar beets following the application of ^{14}C-lenacil at 4- and at 6-leaf stages at the rate of 204 g ai/ha and 321 g ai/ha, respectively. Lenacil was stable in the dark and at pH 5 and 7 under irradiation. At pH 9 under irradiation, the half-life (DT_{50}) was 41 days. Lenacil DT_{50} in three soils ranged from 81 to 150 days. The DT_{50} in two sediments ranged from 32 to 105 days. In mature sugar beets, the total radioactive residue was 0.16 ppm in the tops and <0.03 ppm in the roots. The majority of lenacil metabolites identified were hydroxylated or oxidized products and their conjugates.

Keywords: *Lenacil; environmental fate; metabolism; soil degradation; sugar beet*

INTRODUCTION

For decades lenacil (**1**) has been used for preemergence control of annual grass and broad-leaf weeds in sugar beets (Tomlin, 1994). Several studies were conducted to determine lenacil residue levels in various soil types and plants (Gabriella et al., 1985; Jarczyk, 1977; Neururer, 1972a, 1972b). Studies on microbial degradation of lenacil were reported (Blein, 1983; Stankiewicz, 1981). A preliminary study on sugar beet metabolism was reported by Sieber et al. (1973). However, the majority of lenacil metabolites remained to be identified. In this study using ^{14}C-label lenacil, the residue level and degradation rate of lenacil under different environmental conditions were determined. Major degradation products were isolated and identified. The residue level and metabolic pathway of lenacil in sugar beet plants were also determined.

MATERIALS AND METHODS

Chemicals. The ^{14}C-labeled lenacil was synthesized by NEN Products (Boston, MA) with a specific activity of 17.8 μCi/mg. Lenacil (3-cyclohexyl-6,7-dihydro-1*H*-cyclopentapyrimidine-2,4(3*H*, 5*H*)-dione, **1**) was synthesized by DuPont Agricultural Products, E. I. du Pont de Nemours and Company (Wilmington, DE).

Reference standards of lenacil metabolites (**2**−**4**) were synthesized from lenacil. Briefly, lenacil was reacted with a large excess of chromic anhydride (CrO_3) in 1:1 acetic acid−dichloromethane at ambient temperature for 4 h (yield 16%) to produce 5-oxolenacil (**3**), mp > 260 °C. MS (DCI/CH_4 probe): *m*/*z* 249 (95, MH^+), 167 (100). NMR (DMSO-d_6): δ 1.02−1.35 (2H, m), 1.49 (H, m), 1.61 (H, m), 1.77 (H, m), 2.27 (H, m), 2.41 (H, m), 2.76 (H, m), 4.57 (H, tt), 12.26 (H, br). The proton assignment was based on ^{1}H−^{1}H COSY. The 5-oxo position was confirmed by the observation of a NOE effect in the ^{1}H NMR between the 7-CH_2 proton and the NH ring proton.

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5-Hydroxylenacil (**2**) was obtained from 5-oxolenacil (**3**) by reduction with excess lithium aluminum hydride in tetrahydrofuran at ambient temperature for 2 h (yield 33%); mp 241−245 °C. MS (DCI/CH_4 probe): *m*/*z* 251 (35, MH^+), 233 (100, MH^+ - H_2O). NMR (DMSO-d_6): δ1.02−1.35 (2H, m), 1.46 (H, m), 1.55−1.82 (3H, m), 2.10−2.56 (3H, m), 2.76 (H, m), 4.62 (H, tt), 4.83 (H, m), 11.26 (H, s). The proton assignment was based on ^{1}H−^{1}H COSY.

7-Hydroxylenacil (**4**) was prepared by a three-step process starting from lenacil as follows: Lenacil was brominated at the 7-position using *N*-bromosuccinimide in tetrachloromethane and acetic acid with AIBN initiator at reflux for 4 h. This bromide product was converted to the 7-acetoxy derivative upon heating to 100 °C in acetic acid with sodium acetate for 8 h. Both intermediates were purified by chromatography on silica gel. Removal of the acetyl group using sodium methoxide in methanol (ambient temperature, 2 h) afforded **4**, mp 270−272 °C, in 47% yield overall from lenacil. MS (DCI/CH_4 probe): *m*/*z* 251 (89, MH^+), 169 (100). NMR (DMSO-d_6): δ1.03−1.34 (2H, m), 1.46 (H, m), 1.56−1.82 (3H, m), 2.21−2.40 (3H, m), 2.50 (H, m), 4.62 (H, tt), 4.80 (H, m), 5.40 (H, m), 11.17 (H, s). The proton assignment was based on ^{1}H−^{1}H COSY. The position of the hydroxy group was established via the observation of a NOE effect in the ^{1}H NMR between the 7-CHOH proton and the NH ring proton.

Organic solvents used were HPLC grade (EM Science, Gibbstown, NJ). Common chemicals were reagent grade (Fisher Scientific Co., Fair Lawn, NJ).

Photodegradation in pH 5, 7, and 9 Buffers under Simulated Sunlight. Three buffer solutions were prepared for photolyses: 0.02 M sodium acetate, pH 5; 0.02 M sodium phosphate, pH 7; and 0.5 M sodium borate, pH 9. The 250-mL sterilized photolysis vessels consisted of sterilized water-jacketed beakers covered with borosilicate lids. The 100-mL filter-sterilized buffers in the vessels were maintained at ∼25 °C in circulating water from a controlled Neslab RTE-8 water circulating bath (Neslab Instruments, Inc., Newington, NH). Lenacil was added to the buffers to a final concentration of 2 ppm (about 4 μCi). The solutions were irradiated continuously for up to 15 days in a Suntest accelerated exposure apparatus (Heraeus Instrument, Hanau, Germany) at 32 W-h/(m^2/h) exposure rate. Aliquot of 5-mL samples were collected periodically and analyzed by liquid scintillation counting (LSC) and by HPLC.

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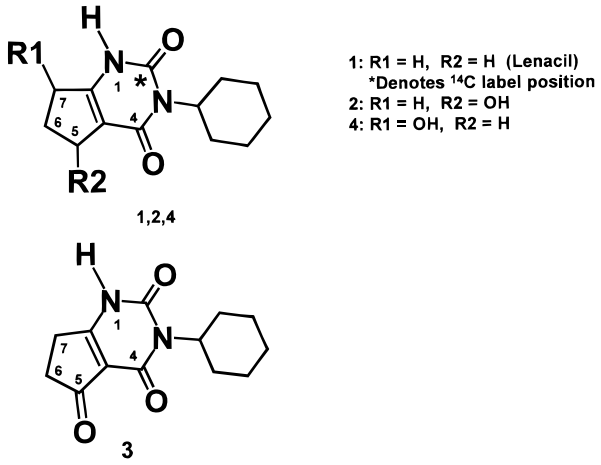


Hydrolyses in pH 5, 7 and 9 Buffers. The test sample and the buffer solutions were prepared as described in the photolysis study. The hydrolysis studies under these three pH conditions were conducted in the dark under sterilized conditions in culture tubes for 30 days. Aliquots of 5-mL samples were collected periodically and analyzed by LSC and by HPLC.

Degradation in Soil. The degradation rates of lenacil in three soil types were determined under aerobic conditions. Soils used were Sassafras sandy loam (Carney's Point, NJ), Hillsdale sandy loam (Quincy, MI) and Tama silt loam (Carrollton, IL). The characterization of these soils is summarized in Table 1. The soils were passed through a 2-mm sieve and allowed to air dry overnight. Aliquots of soil samples were heated at 100 °C for 12 h. The weight differences before and after heating were used to determine the moisture content. Aliquots of soil samples were also heated at 360 °C for 2 h. The differences of the sample weights before and after heating were used to determine the percentage of organic matter content. Each air-dried soil sample equivalent to 50 g dry weight was transferred to a flask. The soil samples were hydrated with 3.1−9.8 mL of water to a moisture content of pF 2.5 (0.33 bar). An aliquot of lenacil in methylene chloride (250 μL containing 0.15 mg of lenacil, ~3 μCi) was added to each flask and mixed thoroughly with the soil. The final concentration of lenacil in the soils was ~3 ppm. This concentration was equivalent to a field application rate of 2 kg ai/ha in the top 5 cm of soil. Sixteen flasks were set up for each soil type. The flasks were incubated at 25 °C in the dark. Humidified air was allowed to pass through each flask, and the $^{14}CO_2$ evolved was collected in the 1 N NaOH solution.

At 0, 1, 3, 7, 14, 30, 60, and 100-day sampling points, two flasks were removed for the analyses of lenacil and its metabolites. Each soil sample was extracted three times with 100 mL of 90% methanol. The combined methanol extracts were concentrated to near dryness and resuspended with methanol to 10 mL. The concentrated extracts were analyzed by LSC and by HPLC. Aliquots of NaOH from the CO_2 traps were analyzed by LSC to detect $^{14}CO_2$.

Degradation in Water/Sediment Systems. Water/sediment samples (Bickenbach and Unter Widdersheim) were collected in the summer from Pfungstadt and Wetterau, Germany, respectively. The characterization of these sediments is summarized in Table 2. Each 500 mL flask was filled with 200 g of moist sediment and 6.0 cm of the corresponding sediment water. Humidified air was allowed to flow through each flask. A total of 22 flasks was set up for each sediment system. At the first and last sampling points (day 100), sediment biomass was determined with the method by Anderson et al. (1978).

After 2 weeks of acclimatization, 250 μL of DMSO containing 0.67 mg of ^{14}C-lenacil (about 12 μCi) was added to each sediment system. This concentration corresponds to a maximum recommended field rate of 2 kg ai/ha. Test flasks were incubated at 20 °C in the dark. The $^{14}CO_2$ from the mineralization of ^{14}C-lenacil was trapped in 50 mL of 2 N NaOH. Organic volatiles were trapped in 50 mL of ethylene glycol. Flasks were removed periodically for analysis. Once each flask was removed, the water in the flask was separated from the sediment. An aliquot of water was analyzed by LSC and by HPLC. The sediments were extracted four times with 200 mL of 90% methanol. The water and sediment extracts were filtered and analyzed by LSC. An aliquot of 200 mL of sediment extracts was evaporated to about 1 mL. The concentrated extracts were further dried under a N_2 stream. Each dried extract was dissolved in 1 mL of methanol using an ultrasonic bath. An aliquot of the concentrated extract was analyzed by HPLC.

Table 1. Characterization of Three Soil Types

parameter	soil type		
	Sassafras	Hillsdale	Tama
soil texture	sandy loam	sandy loam	silt loam
pH	6.2	6.3	6.6
sand (%)	64.4	62.4	16.4
silt (%)	30.4	34.4	70.4
clay (%)	5.2	3.2	13.2
organic content (%)	1.3	2.0	2.3
cation exchange capacity (mequiv/100 g of dry wt)	5.2	7.7	14.4
maximum water capacity (%)	12.1	17.5	28.2

Table 2. Characterization of Two Soil Sediments

parameter	soil sediment	
	Bickenbach	Unter Weddersheim
sediment texture	silty sand	clayish loam
pH	7.8	7.8
redox potential (mV)	+110	+60
sand (%)	65.4	14.0
silt (%)	26.9	41.0
clay (%)	7.7	45.0
carbon content (% dry matter)	0.77	4.16
organic content (% dry matter)	1.33	7.17
total P (mg/kg of dry wt)	273	1567
total N (mg/kg of dry wt)	186	954
cation exchange capacity (mval/kg of dry wt)	180	426

Generation of ^{14}C-Lenacil Metabolites from Excised Sugar Beet Plants. Sugar beet plants at the 7−12 leaf stage were collected, and the roots were removed from the foliage tops. The excised sugar beet top was incubated on 100 mL of water in a 8-oz jar at 23 °C. Before the incubation 1 mg of ^{14}C-lenacil (18 μCi) in 50 μL of DMSO was added to the water. Water was added each day to replace that lost due to transpiration. After 3−8 days of incubation, water solutions were collected for analyses. An aliquot of 50 mL of water solution was loaded on a 60-mL C_{18} Mega Bond-Elut cartridge (Varian, Palo Alto, CA). The cartridge was washed with 30 mL of water and then eluted in order with 50 mL of 15%, 30%, and 100% aqueous acetonitrile. Samples eluted by 30% acetonitrile contained the majority of ^{14}C-metabolites. This fraction was concentrated and the metabolites were isolated by HPLC equipped with a Zorbax SB-Phenyl (9.4 × 250 mm) column: solvent A, acetonitrile; solvent B, water; flow rate, 2 mL/min; gradient, 0−5 min 15%A, 5−25 min 15%A−60%A, 25−30 min 60%A−100%A. The UV peaks at 20.4, 21.5, and 22.1 min were collected. These three UV peaks were designated as metabolites **2**, **3**, and **4**, respectively. Metabolite **4** was further purified on a Zorbax Rx-CN (4.6 × 250 mm) column: solvent A, acetonitrile; solvent B, water; gradients, 0−15 min 15%A, 15−18 min 15%A−50%A, 18−19 min 50%A; flow rate, 1 mL/min. Structures of these lenacil metabolites were confirmed by LC−MS and NMR analyses in comparison with the synthetic standards. These purified radioactive metabolites together with the synthetic standards were used as references for metabolite identification in the sugar beet metabolism study.

Metabolism in Sugar Beets. Sugar beets were grown in nine 10-gallon pots in the green house. At the 4-leaf stage,

Table 3. Percentage of ^{14}C-Lenacil Remaining and Degradate Formating in Solutions under Irradiation

		pH 7		pH 9	
day	pH 5[a] lenacil	lenacil	metabolite **2**	lenacil[c]	metabolite **2**
0	93	96	ND[b]	98	ND
1	99	98	ND		
2	98	98	ND	98	ND
6	98	97	1	88	7
8	97	95	2		
10	98	93	2	81	10
15	97	92	4	78	13

[a] Metabolite **2** was not detected. [b] ND, not detectable. [c] The time for 50% lenacil degradation (DT_{50}) was estimated to be 41 days. The degradation rate followed first-order reaction kinetics: $C_t = 0.987e^{-kt}$ (r^2=0.954) where $k = 0.017$ is the degradation rate constant and t is time in days. $DT_{50} = \ln(2)/0.017 = 41$ days.

Table 4. Distribution of Lenacil and Metabolites in Sassafras Soil

	distribution (%)			
day	lenacil	metabolite **3**	unextractable	CO_2
0	103	ND[a]	0.2	ND
1	105	ND	0.4	ND
3	106	ND	0.9	ND
7	105	ND	1.6	<0.1
14	103	ND	2.2	<0.1
30	84	4.6	9.4	0.2
60	81	5.1	9.8	1.0
100	67	6.5	14.4	2.6

[a] ND: not detectable.

Table 5. Distribution of Lenacil and Metabolites in Hillsdale Soil

	distribution (%)			
day	lenacil	metabolite **3**	unextractable	CO_2
0	106	ND[a]	0.3	ND
1	101	ND	0.9	ND
3	108	N	2.2	ND
7	102	N	3.2	<0.1
14	103	ND	4.8	0.2
30	93	3.3	4.8	1.5
60	57	10.0	22.1	4.6
100	50	7.9	18.6	9.4

[a] ND: not detectable.

each pot with the sugar beets was sprayed with 2 mL of ^{14}C-lenacil (2.18 mg, 18 μCi) formulated as a 50% wettable powder. This application rate was equivalent to 204 g ai/ha. At the 6-leaf stage each pot was sprayed again with 3 mL of formulated ^{14}C-lenacil (3.42 mg, 29 μCi), equivalent to 321 g ai/ha. Two untreated sugar beet pots were used as controls. One sugar beet plant was removed from each pot at 0, 15, 32, 47, 74, and 99 days after the first treatment. A final harvest was made for the mature plants at 130 days.

At harvest, sugar beet roots were separated from the foliage tops. The roots and tops were cut into small pieces and chilled in liquid nitrogen. Aliquots of chilled samples were homogenized in a dry ice prechilled blender. Aliquots of 0.2-g homogenized samples were combusted in an Oximet OX300 Biological Oxidizer (R. J. Harvey Instrument, Hillsdale, NJ). The evolved $^{14}CO_2$ collected in Harveysm ^{14}C II Solution (R. J. Harvey Instrument, Hillsdale, NJ) was analyzed by LSC to determine the total radioactive residue (TRR).

To extract lenacil metabolites from foliage and root samples, a 25-g aliquot of frozen homogenized sample was added to 50 mL of acetonitrile/water (2:1), sonicated briefly for 2 min, and incubated at room temperature for 30 min with gentle shaking. The solvent extracts were separated from solid residues by centrifugation. The extraction was repeated two more times. The radioactivity of the combined extracts was analyzed by LSC to determine the extractable ^{14}C residue. Extracts were evaporated at 30 °C in vacuo to dryness and redissolved in 20 mL of 15% aqueous acetonitrile. An aliquot of concentrated extract was analyzed by HPLC. The remaining solid after extraction was dried under a N_2 stream and combusted to determine the unextractable ^{14}C residues.

Table 6. Distribution of Lenacil and Metabolites in Tama Soil

	distribution (%)			
day	lenacil	metabolite **3**	unextractable	CO_2
0	100	ND[a]	0.4	ND
1	93	ND	0.8	ND
3	103	ND	1.8	ND
7	102	ND	3.0	<0.1
14	98	ND	3.9	0.2
30	82	3.5	8.8	0.7
60	60	6.1	15.6	2.3
100	44	8.9	19.2	5.2

[a] ND: not detectable.

Table 7. Degradation Rate of Lenacil in Three Soil Types

soil type	organic content (%)	kinetics of lenacil degradation[a]	k	DT50[b] (days)	r^2
Sassafras sandy loam	1.3	$C_t = 1.054e^{-kt}$	0.0046	150	0.933
Hillsdale sandy loam	2.0	$C_t = 1.083e^{-kt}$	0.0082	84	0.929
Tama silt loam	2.3	$C_t = 1.034e^{-kt}$	0.0086	81	0.949

[a] Kinetics of lenacil degradation determined by the best fitting line to the first-order reaction kinetics using data points from Tables 4−6. Key: k, degradation rate constant; t, time in days. [b] $DT_{50} = \ln(2)/k$, time for 50% lenacil degradation.

Enzyme Hydrolysis. β-Glucosidase (Sigma, St. Louis, MO) was used to hydrolyze the foliage extract or HPLC-purified fractions. The hydrolysis was conducted at 37 °C for 24−48 h in 0.01 M sodium acetate buffer, pH 5. β-Glucosidase was added to initiate the hydrolysis. Controls were incubated under the same conditions except that enzyme was not added. After hydrolysis, the control and enzyme-treated samples were analyzed by HPLC.

Analysis. Generally, reverse phase HPLC columns were used to analyze lenacil metabolites. Half-minute fractions were collected and were mixed with 5 mL of Formula-989 LSC cocktail (Packard, Meriden, CT).The radioactivity of the collected fractions was analyzed by LSC. HPLC conditions are presented under each chromatogram. In other experiments, the fractions collected were concentrated under a N_2 stream and subjected to β-glucosidase hydrolyses.

Mass spectra were obtained on a Finnigan MAT TSQ 700 mass spectrometer with electrospray ionization and other ionization mode (ThermoQuest, San Jose, CA). The mass spectrometer was interfaced with HP1090 II HPLC (Hewlett-Packard, Wilmington, DE). The HPLC column used for LC−MS was Hypersil ODS (2.1 × 200 mm): flow rate, 0.3 mL/min; solvent A, acetonitrile; solvent B, water with 0.05% formic acid; gradient, 0−5 min 0%A, 5.0−5.1 min 15%A, 5.1−25 min 40%A. NMR analyses were made in DMSO-d_6 using a Unity-Inova 500 NMR spectrometer (Varian, Palo Alto, CA).

RESULTS

Photolysis and Hydrolysis of Lenacil. Lenacil is stable in pH 5, pH 7, and pH 9 solutions in the dark. At the end of the 30-day incubation, >95% of the lenacil was recovered. In the solution under irradiation for 15 days, >90% of the parent remained at pH 5 and pH 7 (Table 3). Lenacil was less stable at pH 9 under irradiation. The estimated half-life (DT_{50}) of lenacil at

Table 8. Lenacil Degradation and Metabolite Formation in Two Water/Sediment Systems

	% distribution in Bickenbach system				% distribution in Unter Widdersheim system			
days	lenacil	CO_2	other metabolites[a]	bound residue	lenacil	CO_2	other metabolites[a]	bound residue
0	101.3		ND[b]	1.2	100.3		ND	1.7
0.25	101.9	<0.1	ND	1.2	99.9	<0.1	ND	2.7
1	97.9	<0.1	ND	1.5	99.1	<0.1	ND	1.5
2	97.9	0.1	ND	1.2	98.3	0.1	ND	2.0
7	97.4	0.3	ND	2.1	96.5	0.2	ND	3.0
14	96.1	0.2	ND	3.0	94.3	0.2	ND	4.6
30	84.9	1.7	8.6	6.8	86.5	0.7	5.6	9.3
61	45.8	8.7	27.3	16.0	59.5	2.2	24.0	13.8
100	9.2	21.6	52.9	15.2	54.6	3.0	21.8	17.9

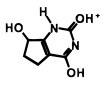
[a] Contained multiple polar metabolites. [b] ND: not detectable.

Table 9. Degradation Rate of Lenacil in Two Water/Sediment Systems

	biomass					
sediment system	initial (mg of C/100 g of dry wt)	end[a] (mg of C/100 g of dry wt)	kinetics of lenacil degradation[b]	k	DT50[c] (days)	r^2
Bichenbach	15.3	13.3	$C_t = 1.149e^{-kt}$	0.0216	32	0.869
Unter Widdersheim	16.7	4.8	$C_t = 1.004e^{-kt}$	0.0066	105	0.964

[a] Biomass at the end of 100-day incubation. [b] Kinetics of lenacil degradation determined by the best fitting line to the first-order reaction kinetics using data points from Table 8. k, degradation rate constant; t, time in days. [c] $DT_{50} = \ln(2)/k$, time for 50% lenacil degradation.

Table 10. LC−MS Analyses of Lenacil Metabolites

Lenacil Metabolites	LC-MS[a] Retention (Min)	Fragmentation			
2	14:46	m/z 251 (35)	m/z 233 (100)	m/z 151 (10)	
		MH^+	MH^+-H_2O		
3	15:10	m/z 271 (15)	m/z 249 (100)	m/z 167 (70)	
		$[M+Na]^+$	MH^+		
4	16:19	m/z 251 (62)	m/z 233 (5)	m/z 169 (100)	m/z 151 (35)
		MH^+	MH^+-H_2O		

[a] LC conditions: see Materials and Methods. Mass spectra conditions: ionization mode, positive; Electrospray voltage, 4.2 kv; capillary temperature, 220 °C; auxiliary gas, 20 psi; sheath gas, 60 psi.

pH 9 under irradiation was 41 days. A major photolysis product was confirmed to be metabolite **2**.

Degradation of Lenacil in Soils. The lenacil degradation and metabolite formation in aerobic soils are shown in Tables 4−6. The estimated DT_{50} of lenacil in the soils is summarized in Table 7. The majority (44%−67%) of the radioactivity recovered in 100-day samples was lenacil. Metabolite **3** was the major lenacil degradate (7%−9%). Several minor polar degradates were detected. The mineralization of lenacil to CO_2 was detected, but it was <10%. Bound radioactivity increased with time. In the 100-day samples, 14%−19% of the applied radioactivity was bound to the soils (Tables 4−6). Organic contents in these three soil types were different. Organic matter was much less in Sassafras soil than in Hillsdale and Tama soils (Table 7). Correspondingly, the DT_{50} of lenacil in Sassafras soil (150 days) is longer than in Hillsdale (84 days) or Tama soils (81 days).

Table 11. Total Radioactive Residue in Sugar Beets

	foliage		root	
day after first application	TRR[a] (ppm)	extractable (% TRR)	TRR (ppm)	extractable (% TRR)
0	7.35	97	0.02	NA[b]
15	4.71	98	0.02	NA
32	1.06	100	0.02	NA
47	1.04	100	0.03	79
74	0.69	96	0.01	67
99	0.30	96	0.03	80
130	0.16	94	<0.01	NA

[a] TRR: total radioactive residues in lenacil equivalent on a fresh weight basis; numbers were the average results from duplicate solvent extraction analyses. [b] NA, not extracted for analysis.

Degradation of Lenacil in Water/Sediment Systems. Lenacil degradation in two water/sediment systems is summarized in Table 8. In the Bickenbach water phase, recovery of the applied radioactivity decreased from 76% in 0-day samples to 43% in 100-day samples, while in the sediment, the recovery increased from 27% in 0-day samples to 46% in 30-day samples. It decreased to 34% in 100-day samples. In the Unter Widdersheim water phase, recovery of the applied radioactivity decreased from 64% at day 0 to 22% at day 100, while radioactivity in the sediment increased from 38% to 74%. After 100 days, the multiple polar lenacil degradates accounted for 53% in the Bickenbach water/sediment system and 22% in the Unter Widdersheim water/sediment system. The structures of these polar degradates remain to be further determined. Bound residue in both sediments increased over time (Table 8). The rate of lenacil degradation followed first-order kinetics (Table 9). Lenacil degraded faster in the Bichenbach water/sediment system than in the Unter Widdersheim water/sediment system. In addition, more polar degradates were generated in the Bickenbach water/sediment system (Table 8). Organic volatile levels in both systems were very low (<0.4%) while the CO_2 quantity was significant in the Bickenbach water/

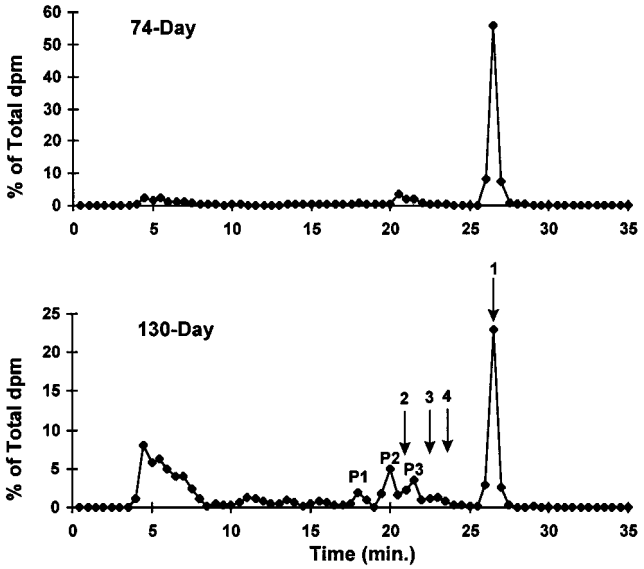


Figure 1. Radio-HPLC analyses of sugar beet foliage extracts from 74-day and 130-day samples. HPLC conditions: Zorbax Rx-C8 column (4.6 mm × 25 cm); solvent A, acetonitrile with 0.1% formic acid; solvent B, Milli-Q purified water with 0.1% formic acid; flow rate, 1 mL/min; gradient, 0−5 min 15%A, 5−25 min 15%A−80%A, 25−30 min 80%A−100%A. The 0.5-min fractions were collected and analyzed by LSC. Arrows indicate the retention time of the references: **1**, lenacil; **2**−**4**, metabolites **2**, **3**, and **4**.

sediment (Table 8). Biomasses in these two water/sediment systems were different at the end of the study period. A larger biomass quantity was recovered in the Bickenbach sediment at the of end the study (Table 9). Results also showed that the lenacil DT_{50} in the Bichenbach sediment was over three times shorter than that in the Unter Widdersheim sediment (Table 9).

Major ^{14}C-Lenacil Metabolites from Excised Sugar Beet Incubation. The purified lenacil metabolites (**2**−**4**) were analyzed by LC−MS (Table 10). Their spectral data were comparable to those of the synthetic standards. Metabolite **2** was the major product in the incubation medium. Of the minor metabolites, the structures of metabolites **3** and **4** were also confirmed. Those metabolites were used as references in radio-HPLC analysis of sugar beet extracts.

Metabolism of ^{14}C-Lenacil in Sugar Beet Plants. The total ^{14}C residue in sugar beet foliage is presented (Table 11) as micrograms of lenacil equivalents per gram (ppm) on a fresh weight basis. Radioactive residue in the foliage declined from 7.35 ppm at day 0 after treatment to 0.16 ppm in the final harvest foliage samples at day 130. In the foliage, >94% of the ^{14}C residue was extractable. The ^{14}C residue in the roots was low throughout the sugar beet growth period (≲0.01−0.03 ppm), and no significant time-dependent ^{14}C residue accumulation in the roots was observed.

In early harvested samples (up to 47-day samples), the majority of the extractable radioactivity was identified as due to lenacil. In later harvests, polar metabolites (with HPLC retention time at 4−8 min) were observed (Figure 1). Besides the polar metabolite peaks, there were three major metabolites (P1, P2, and P3) in 130-day foliage extracts (Figure 1). When these metabolites were treated with β-glucosidase, the HPLC retention time of these three peaks changed and the new retention times matched those of metabolites **2** and **4**, respectively (Figure 2). The polar peaks were also changed after β-glucosidase hydrolysis, suggesting that

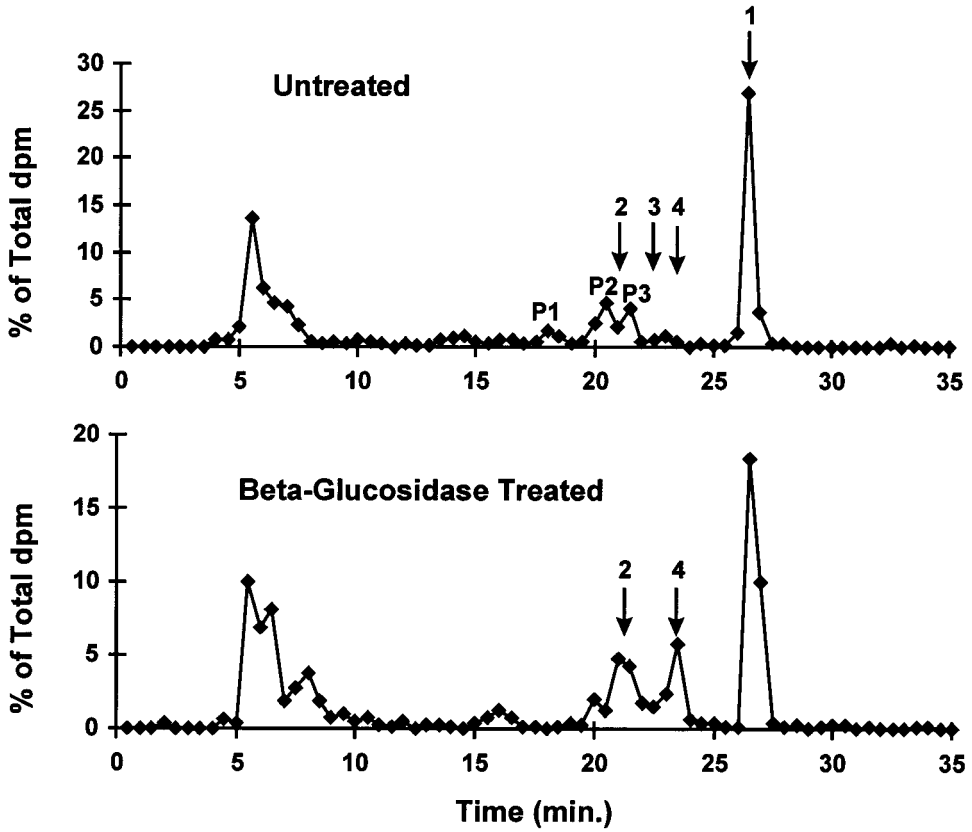


Figure 2. Radio-HPLC analysis of sugar beet foliage extracts before and after β-glucosidase treatment. Extracts were obtained from 130-day foliage samples. HPLC conditions: see Figure 1. Arrows indicate the retention time of the references: **1**, lenacil; **2**−**4**, metabolites **2**, **3**, and **4**.

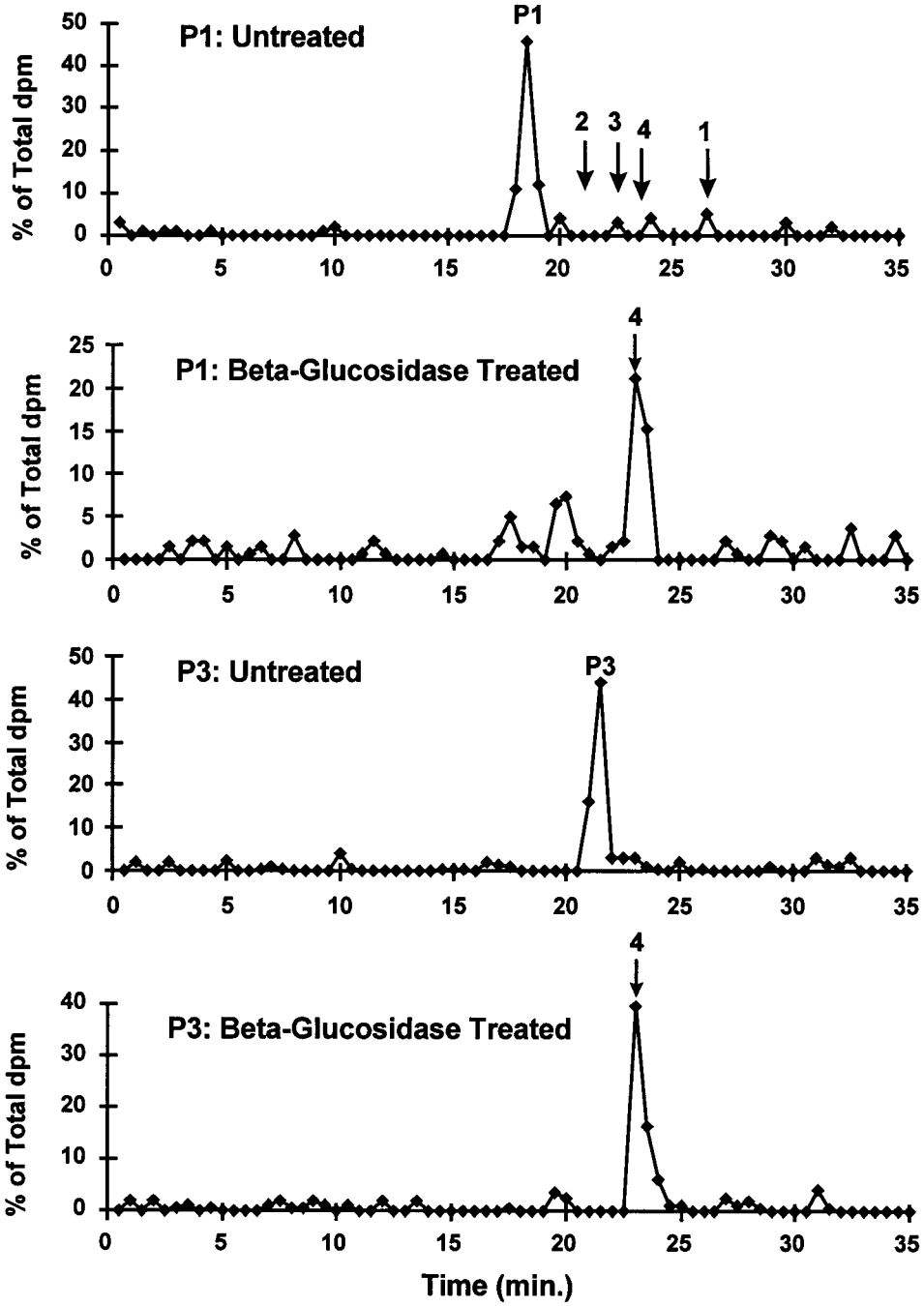


Figure 3. Radio-HPLC analysis of metabolites P1 and P3 before and after β-glucosidase treatments. HPLC conditions: see Figure 1. Arrows indicate the retention time of the references: **1**, lenacil; **2**−**4**, metabolites **2**, **3**, and **4**.

Table 12. Estimated Composition of ^{14}C Residues in the Foliage Extracts

	residue level in ppm (% TRR) in the foliage samples[a]		
lenacil and metabolites	74-day	99-day	130-day
lenacil (**1**)	0.47(67.9)	0.16(52.0)	0.04(28.4)
metabolite **2**	<0.01(<0.1)	<0.01(1.6)	<0.01(3.1)
P3	0.03(3.9)	0.02(5.2)	0.01(7.7)
P2	0.03(4.5)	0.02(5.0)	0.01(7.5)
P1	<0.01(1.4)	0.01(3.6)	<0.01(3.0)
polar	0.07(10.5)	0.05(18.0)	0.06(37.9)

[a] Residue level (ppm) in lenacil equivalents.

these polar peaks contained some glucose conjugates of lenacil metabolites. No further analyses were made on these polar metabolites. The residue levels of these metabolites are summarized in Table 12.

Efforts were made to identified P1, P2, and P3 from HPLC fractions. Fractions P1 and P3 were collected from HPLC. However, we were unable to obtain intact P2. When incubated with β-glucosidase, both P1 and P3 were hydrolyzed to metabolite **4** (Figure 3). As shown in Figure 2, metabolite **2** was detected in crude foliage extracts after β-glucosidase treatment. However, the glucose conjugate of metabolite **2** has not been isolated and identified.

DISCUSSION

Under sterilized conditions, lenacil was relatively stable at pH 5, 7 and 9 in the dark; however at pH 9 under irradiation, it was less stable. Lenacil half-life (DT_{50}) in soils ranged from 81 to 150 days. The majority of lenacil metabolites were polar products and signifi-

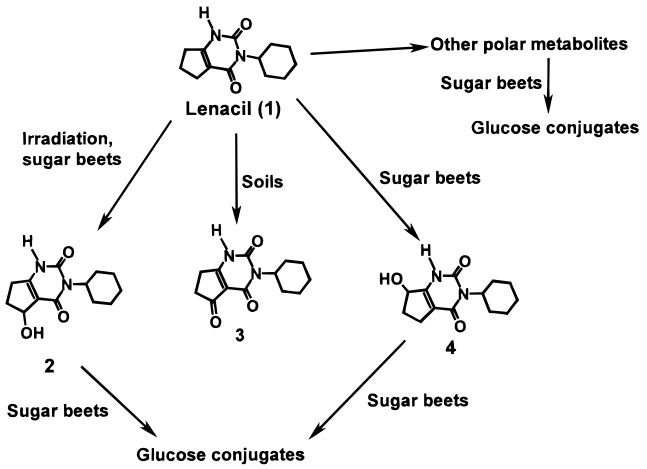


Figure 4. Proposed metabolic fate of lenacil in the sugar beets and in the environment.

cant quantity was mineralized to CO_2 . Soil and water sediment metabolism studies indicate that microorganisms played important role in lenacil degradation in the environment. The organic matter in the soil or biomass in the sediment might have great effect on the half-life of lenacil in these systems. In vitro microorganism metabolism studies have demonstrated that lenacil was quickly degraded by microorganisms (Blein, 1983; Stankiewicz, 1981). Since only single soil and sediment sample time point was used for the current studies, the microorganism may not reflect the total population in different seasons. It is difficult to determine which and how the soil microorganism played important role in the degradation of lenacil under the field condition.

In sugar beet roots ^{14}C residue was very low even at early harvests (~0.02 ppm). The results indicate that translocation of lenacil from leaves to roots was insignificant. The actual residue in the roots might be lower since some of the residues might have come from soil attached to the root. The residue in the foliage tops decreased to 0.16 ppm in the mature (130-day) samples. The residue levels in sugar beet roots and tops were comparable to a previous study by Sieber et al. (1973). The majority of the ^{14}C residue in the tops was polar metabolites. These polar metabolites included glucose conjugates.

Conjugation is an important pathway for lenacil metabolism in sugar beets. While glucose conjugate of **2** has not been isolated, glucose conjugates appeared as the major form of metabolites **2** and **4** in the sugar beet, since **2** and **4** were detected only after β-glucosidase hydrolysis.

The proposed metabolic fate of lenacil is summarized in Figure 4. Similar lenacil metabolites were observed in hydrolysis, aqueous photolysis, water sediment, and sugar beet metabolism studies. Though hydroxylation and oxidation of the cyclohexyl ring were expected, in this study the majority of hydroxylation and oxidation was found in cyclopentyl ring. The study demonstrates that lenacil is readily biodegradable and that, under the suggested application rate, it is unlikely to accumulate in the environment.

ABBREVIATIONS USED

AIBN, azobisisobutyronitrile; DMSO, dimethyl sulfoxide; LSC, liquid scintillation counting; COSY, Correlated spectroscopy; mp, melting point; NOE, nuclear overhauser effect; TRR, total radioactive residue.

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