Clomazone Fate in Soil As Affected by Microbial Activity, Temperature, and Soil Moisture

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Laboratory studies were conducted to investigate the fate of the herbicide clomazone [2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone] in soil. Effects of soil microbial activity, incubation temperature, and soil moisture on clomazone fate were determined up to 84 days following surface application of [^{14}C]clomazone uniformly labeled in aromatic rings ([AR-^{14}C]clomazone) to a Flanagan silty clay loam soil. On the basis of a comparison of clomazone fate in sterilized soil and reinoculated soil, clomazone degradation was biologically dependent. The greatest clomazone mineralization rate occurred at a lower temperature than did the greatest microbial respiration rate; respiration and clomazone mineralization increased with increasing soil moisture content. Clomazone volatilization increased with increasing temperature but was not significantly affected by soil moisture treatment. In all studies, 59% or more of applied clomazone was extracted from soil as parent clomazone 84 days after application. Unextractable radioactivity accounted for 12% or less of that applied. A single detectable metabolite, persistent only under conditions of low temperature or low soil moisture content, accounted for less than 5% of applied radioactivity at any sampling time. The metabolite did not contain the carbonyl carbon of clomazone. These data suggest the carbonyl carbon is converted to CO_2 during formation of the clomazone metabolite.

Keywords: *Clomazone; biodegradation; volatilization; sorption*

INTRODUCTION

Clomazone [2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone] (Figure 1), a soil-applied herbicide, selectively controls many grass and broadleaf weeds in soybeans, cotton, and some vegetable crops (*Crop Protection Chemicals Reference*, 1994). Clomazone inhibits synthesis of chlorophyll and carotenoids in sensitive plants, resulting in foliage devoid of pigmentation (Weed Sci. Soc. Am., 1989). The solubility of clomazone in water is 1100 mg/L and its vapor pressure at 25 °C is 1.92×10^{-2} Pa (Weed Sci. Soc. Am., 1989).

To avoid off-site injury to sensitive plants from volatilized clomazone, the herbicide must either be incorporated into the soil, applied in early spring before sensitive plants develop foliage, or applied at specified minimum distances away from sensitive plants (*Crop Protection Chemicals Reference*, 1994). The limited data available suggest that clomazone volatilization is favored by wet soil conditions and crop residue cover (Halstead and Harvey, 1985, 1986; Thelen et al., 1988).

FMC researchers reported that under aerobic conditions, clomazone was primarily converted to bound soil residues and CO_2; in flooded soils, the compound was rapidly converted to a reductive metabolite, *N*-[(2′-chlorophenyl)methyl]-3-hydroxy-2,2-dimethylpropanamide (Froelich et al., 1984). Clomazone is not subject to significant photochemical or thermal decomposition (Weed Sci. Soc. Am., 1989). The mechanism of degradation in aerobic soil has not been reported, but some data are available on the effects of soil type. More rapid clomazone degradation occurred in soils at pH 6.5 than at pH 5.5 and in sandy loam than in silt loam or clay loams. The half-life of clomazone, determined from field studies in several soil types, ranged from 28 to 84 days (Weed Sci. Soc. Am., 1989). Clomazone half-life in a loam and a silty clay loam soil was 33 and 37 days, respectively, as estimated by an oat bioassay (Gallandt et al., 1989). On the basis of HPLC analysis of soil extracts, the half-life of clomazone applied to a silt loam averaged 26 and 11 days in conventional and no-till systems, respectively (Mills et al., 1989), due in part to volatilization from surface wheat straw in the no-till soil. Both adsorption and persistence of clomazone were greater in a Drummer silty clay loam (5.8% organic matter) than in a Cisne silt loam (1.3% organic matter) (Loux et al., 1989a). The half-life of clomazone in a Flanagan silt loam ranged from 52 to 117 days (Curran et al., 1992). Clomazone persistence in soil can result in injury to rotational crops such as wheat or corn (Ahrens and Fuerst, 1990; Curran et al., 1992). As a result, rotational crop guidelines prohibit the planting of many crops for 9–12 months after clomazone application to a field (*Crop Protection Chemicals Reference*, 1994).

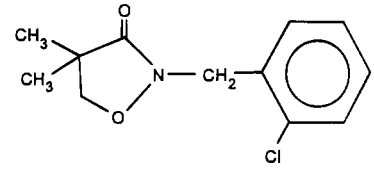

Figure 1. Clomazone [2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone].

The distribution adsorption constant (K_d) for clomazone was linearly correlated with the organic carbon content of 19 soils and sediments (Loux et al., 1989b). Values for K_d ranged from 0.47 to 5.30 mL/g, and the organic carbon partition coefficient, K_{oc}, for clomazone averaged 150 mL/g. Loux et al. (1989a,b) proposed

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hydrophobic bonding to organic matter to be the primary mechanism of clomazone sorption and that bioavailability and dissipation of clomazone in soil are determined by adsorption properties.

No studies of clomazone fate in soils under controlled laboratory conditions have been published in the peer-reviewed literature. Environmental effects on clomazone volatilization and degradation and the mechanism of its aerobic degradation in soils are not well understood. The objectives of this study were to determine the effects of biological activity, temperature, and soil moisture on the fate of clomazone in soil and to gain information about the clomazone aerobic degradation process.

MATERIALS AND METHODS

Soil. The soil used in all studies was a Flanagan silty clay loam (fine, montmorillonitic, mesic, aquic argiudoll) containing 31% clay, 53% silt, and 16% sand size fractions; organic carbon content was 2.6%, and pH in water was 5.9 (A & L Laboratories, Fort Wayne, IN). Moist soil was collected in October 1992 and March 1993 near Champaign, IL, from the top 10 cm of a field in which soybeans had been grown in 1992. All soil used in a given study was collected on the same date. Clomazone had not been applied to the field for at least 3 years. Soil was sieved through a 2 mm screen, mixed, and stored moist at 5 °C in thin-walled polyethylene bags for less than 6 months before use.

Chemicals. Three separate sources of [^{14}C]clomazone were radiolabeled in one of three molecular sites (Figure 1): uniformly in aromatic ring carbons [AR-^{14}C] (1.04×10^9 Bq/mmol, 98.6% purity), the methylene carbon [$^{14}CH_2$] (9.92×10^8 Bq/mmol, 99.2% purity), or the carbonyl carbon [$^{14}C{=}O$] (1.17×10^9 Bq/mmol, 99.5% purity). Formulated clomazone (emulsifiable concentrate) contained 46.7% clomazone in xylene range aromatic solvents. All labeled and unlabeled forms of clomazone were obtained from FMC Corp., Princeton, NJ.

Clomazone Fate Studies. The following procedures, unless otherwise noted, applied to all studies. A soil moisture characteristic curve for the Flanagan soil was generated using a pressure plate procedure (Klute, 1986). Soil moisture was adjusted to the water content at a tension of 300 kPa (210 g of H_2O/kg of dry soil) at the beginning of a study. Soil samples (25 g dry wt basis) were transferred to 1 pint (480 mL) Mason jars. Formulated clomazone was diluted in distilled water and supplemented with [AR-^{14}C]clomazone. A 0.2 mL aliquot of this [^{14}C]clomazone solution, containing 0.5 mg of clomazone and approximately 4670 Bq, was applied to the soil surface with a 0.25 mL syringe. This was equivalent to an application rate of 1.12 kg/ha on an area basis. Soil was then incubated in the dark at 25 °C for 0, 3, 7, 14, 28, 56, or 84 days after clomazone application, at which time soil was frozen until analyzed. Studies were conducted using triplicate experimental units, except for the biological dependence study which was unreplicated. A 20 mL scintillation vial containing 10 mL of 0.2 N KOH, used to trap evolved CO_2, was suspended from the lid above the soil. A polyurethane foam (PUF) plug loosely filling the mouth of the vial trapped volatilized clomazone and prevented it from contacting the KOH solution (confirmed in preliminary evaluations). Prior to conducting studies, the experimental system was evaluated for $^{14}CO_2$ trapping efficiency using $NaH^{14}CO_3$ (500 Bq). At 24 h, an average of 101% of the ^{14}C had been trapped in the KOH and none was in the PUF.

At 3, 7, 14, 28, and 56 days after clomazone application, jars were opened and vials containing KOH and PUF were removed for analysis and then replaced with new materials in all remaining jars. Each jar that was incubated for more than one week was opened weekly to provide aeration. Replicates of KOH and PUF samples contributing to a mean for the 0–3, 3–7, 7–14, 14–28, 28–56, and 56–84 day intervals were 18, 15, 12, 9, 6, and 3, respectively.

Biological Dependence Study. Soil samples (25 g dry wt basis) in 40 mL borosilicate vials were sterilized with 5 Mrad gamma radiation from a ^{60}Co source. Henceforth, all procedures were conducted using aseptic technique. Soil samples were transferred to autoclaved Mason jars. Soil sterility at the beginning of the study was confirmed by a lack of colony development on nutrient agar. One jar was reinoculated with 0.2 g of nonsterile soil 4 days before clomazone was introduced. Following clomazone application, sterilized samples were incubated for 0, 7, 14, 28, or 56 days at 15, 25, or 35 °C. The reinoculated sample was incubated for 56 days at 25 °C, and 2 g subsamples were removed 7, 14, and 28 days after application. Vials containing KOH and PUF were removed for analysis and replaced in all remaining jars 7, 14, and 28 days after application.

Temperature Dependence Study. Soil was incubated at 5, 15, 25, or 35 °C for 4 days prior to clomazone application, and at these same temperatures for up to 84 days after clomazone application.

Soil Moisture Dependence Study. Soil was dried to slightly below the water content at a tension of 1500 Pa. Soil was remoistened to the water contents at tensions of 100, 300, 700, and 1500 kPa (247, 210, 176, and 134 g of H_2O/kg of dry soil, respectively) 4 days prior to clomazone application. Soil was incubated at 25 °C at these same moistures for up to 84 days after clomazone application.

^{14}C Label Site Study. The purpose of this study was to obtain information about the clomazone degradation process by following the fate of three radiolabeled carbon sites in the clomazone molecule. [^{14}C]Clomazone radiolabeled in either the aromatic ring carbons, the methylene carbon, or the carbonyl carbon (Figure 1) was applied to the soil as described before.

Sample Analyses. PUF plugs were placed in 15 mL of Bio-Safe II (Research Products International Corp., Mt. Prospect, IL) scintillation cocktail plus 1 mL of methanol and pressed to remove trapped air bubbles. [^{14}C]Clomazone in PUF was quantified using a Packard 1900TR liquid scintillation analyzer. When all trapped air was removed, this technique provided a counting efficiency of greater than 95%. $^{14}CO_2$ evolution was quantified by liquid scintillation spectrometry (LSS) of a 2 mL aliquot of the CO_2 trap in 15 mL of cocktail plus 1 mL of methanol. Microbial respiration in soil was determined by titrating 1 mL of the CO_2 trap, after adding 0.5 mL of 3 N $BaCl_2$, with 0.2 N H_2SO_4 using phenolpthalein as an indicator (Anderson, 1982). Total ^{14}C content of soil was determined by combusting two 1 g soil subsamples from each replicate in a Harvey OX-500 biological oxidizer and trapping CO_2 in Carbon 14 cocktail (R. J. Harvey Instrument Corp., Hillsdale, NJ). Data were adjusted for the 92.3 ± 0.6% ^{14}C recovery efficiency of the oxidizer when [^{14}C]clomazone-treated soil was combusted. Twenty grams of soil were added to a 250 mL high-density polyethylene bottle and shaken for 12 h in 100 mL of ethyl acetate plus 20 mL of 0.01 M $CaCl_2$. Following a 5 min centrifugation at 2000g, aqueous and ethyl acetate phases were separated. The ^{14}C content of a 2 mL aliquot of the aqueous phase was quantified by LSS to facilitate mass balance. The ethyl acetate phase was rotary evaporated to dryness at 30 °C, and the extract was resuspended in 1 mL water/acetonitrile (70:30) and filtered through a 0.45 μm PTFE filter (Alltech Associates Inc., Deerfield, IL). Radioactivity was then quantified by LSS of a 0.2 mL aliquot of the concentrated extract. Total extracted radioactivity was corrected for the extraction efficiency of 84.9 ± 2.1%. [^{14}C]-Clomazone and metabolites were analyzed using a high-performance liquid chromatography (HPLC) system (Waters Chromatography, Milford, MA) equipped with a UV detector and a Radiomatic Flo-One Beta (Packard Instrument Co., Meriden, CT) radioactivity flow detector. Operating parameters were as follows: injection volume, 0.2 mL; mobile phase flow rate, 1.5 mL/min; reverse-phase C-18 column (Beckman Ultrasphere ODS, 4.6 × 250 mm, 5-μm particle size); detector wavelength, 220 nm; Ultima-Flo M (Packard Instrument) scintillation fluid flow rate, 4.2 mL/min. The mobile phase gradient (% water/% acetonitrile) was as follows (gradient change was linear during unspecified time intervals): 0 to 5 min, 70/30; 6 to 20 min, 60/40; 22 to 24 min, 0/100; 28 to 30 min, 100/0; 32 to 35 min, 70/30. Retention times for

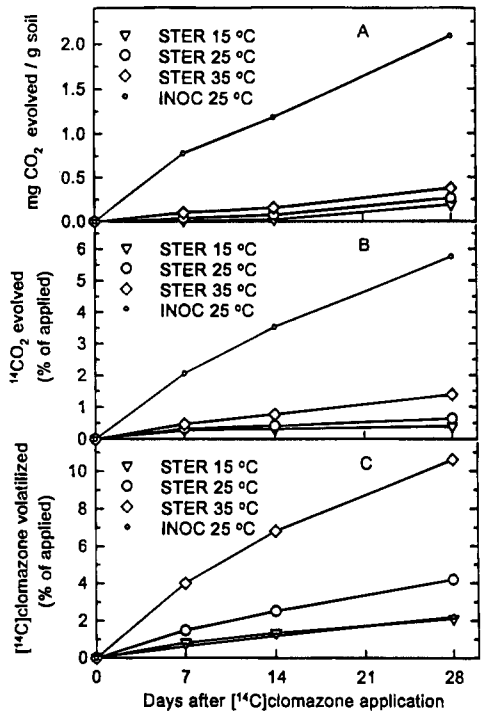

Figure 2. Effects of sterilization and reinoculation of Flanagan silty clay loam soil on cumulative microbial respiration (A), [^{14}C]clomazone mineralization (B), and [^{14}C]clomazone volatilization (C) up to 28 days after [^{14}C]clomazone application.

clomazone and its single significant metabolite were approximately 17 and 3 min, respectively.

Data Analyses. Analyses of variance were conducted by the general linear models procedure of SAS Release 6.08 (SAS Institute Inc., Cary, NC). Fisher's protected least significant difference (LSD) values at an α level of 0.05 were generated for treatment, day, and treatment by day interaction effects. Figures expressing cumulative respiration, clomazone mineralization, and clomazone volatilization data contain cumulative LSD(0.05) bars (for effects with significant F tests). For all other data, LSD(0.05) values derived from the treatment by day interaction are expressed. Equations for clomazone dissipation data were fit with the program TableCurve (Jandel Scientific, San Rafael, CA).

RESULTS AND DISCUSSION

Biological Dependence of Clomazone Degradation. This study was terminated 28 days after [^{14}C]-clomazone application because of regrowth in the sterilized treatments, as indicated by slightly increasing CO_2 evolution over time (Figure 2A) and low level colony growth on nutrient agar (data not shown). However, CO_2 evolution from the reinoculated soil, incubated at 25 °C, far exceeded that from any of the sterilized soils (Figure 2A).

Greater [^{14}C]clomazone mineralization occurred in the reinoculated soil than in any of the sterilized soils (Figure 2B). In 28 days, less than 1.5% of the clomazone was mineralized in sterilized soil, but 5.8% mineralized in the reinoculated soil. At 25 °C eight times more CO_2 and nine times more $^{14}CO_2$ were evolved in 28 days from reinoculated than from sterilized soils (Figure 2, parts A and B). The similar responses of microbial respiration and clomazone mineralization provide evidence for the dependence of clomazone mineralization on soil microbial activity. In addition, no significant metabolites of clomazone were detected in extracts of sterilized soil (data not shown).

Table 1. Incubation Temperature Effects on [AR-^{14}C] Distribution 84 Days after [^{14}C]Clomazone Application to Soil

	^{14}C recovered as % of applied [^{14}C]clomazone[a]				
	incubation temperature (°C)				
	5	15	25	35	LSD[b]
volatilized	1.4	2.1	3.3	7.2	0.8
mineralized	2.8	9.8	15.4	15.0	0.5
total extracted from soil[c]	92.8	78.4	68.4	60.0	4.4
in aqueous phase	1.3	1.2	1.1	0.8	0.2
in ethyl acetate phase	91.5	77.2	67.3	59.2	4.5
as clomazone	87.1	76.3	67.3	59.1	4.3
as metabolite	3.7	0.5	0.0	0.1	0.8
unextractable (bound)[d]	1.1	5.5	9.7	10.8	4.5
total recovered	98.1	95.7	96.8	93.0	NS

[a] Results are means of triplicate tests. [b] Least significant differences at α level of 0.05; nonsignificant F tests for incubation temperature are indicated by NS (based on ANOVA of data from all sampling dates). [c] Corrected for extraction efficiency of 84.9%. [d] Difference between ^{14}C oxidized (corrected for 92.3% oxidizer efficiency) and total ^{14}C extracted from soil.

In the sterilized soil, clomazone volatilization increased with increasing temperature (Figure 2C). For the 35 °C treatment, greater than 10% of applied [^{14}C]-clomazone was volatilized in 28 days. Less clomazone was volatilized from the reinoculated soil than from the sterilized soil at 25 °C. Because more clomazone was degraded in the reinoculated soil, less clomazone may have remained available in solution for volatilization.

Temperature Dependence of Clomazone Fate. At the end of the temperature study, total recovery of applied radioactivity averaged 95.7% (Table 1). Most of the activity was recovered as clomazone, metabolites, and carbon dioxide. Bound residue levels (approximately 10% of applied clomazone) at the highest temperature were low compared to the 10–70% typically reported for studies of pesticide fate in soils (Calderbank et al., 1989). Bound residues accounted for 35–45% of radioactivity 100 days after [^{14}C]flumetsulam application (Lehmann et al., 1992) and approximately 50% of radioactivity 70 days after [^{14}C]fluometuron application (Mueller et al., 1992).

Soil respiration increased as incubation temperature increased from 5 to 35 °C (Figure 3A). Respiration rates decreased over the 84 day period. Within the biologically active temperature range, microbial respiration is expected to approximately double with each 10 °C increase (Stanier et al., 1976). An Arrhenius plot of respiration rates from 0 to 7 days [ln(mg of CO_2 evolved/(g of soil)$^{-1}$ day^{-1}] vs temperature [1/K] yielded a line with $r^2 = 0.976$ from which a Q_{10} of 1.75 was calculated.

The effect of temperature on [^{14}C]clomazone mineralization differed from the effect on microbial respiration. $^{14}CO_2$ evolution increased as temperature increased from 5 to 15 to 25 °C, but did not increase further at 35 °C (Figure 3B). In soil that was incubated at either 25 or 35 °C for 84 days, approximately 15% of applied clomazone was mineralized. An Arrhenius plot of initial (0–7 days) clomazone mineralization rates [ln(% $^{14}CO_2$ evolved/day)] vs temperature [1/K] yielded an r^2 of 0.860 and a Q_{10} of 2.23. On the basis of respiration data, clomazone mineralization was much greater than expected at 15 °C and much lower than expected at 35 °C. The specific organisms responsible for mineralizing clomazone in this soil may have been more active at cooler temperatures than the overall microbial population. Choi et al. (1988) observed a

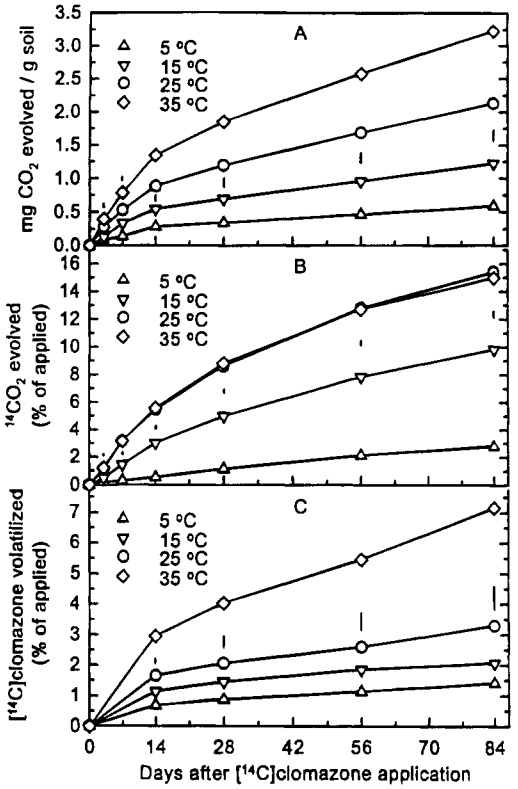

Figure 3. Incubation temperature effects on cumulative microbial respiration (A), [^{14}C]clomazone mineralization (B), and [^{14}C]clomazone volatilization (C) up to 84 days after [^{14}C]-clomazone application to a Flanagan silty clay loam. Vertical bars are cumulative Fisher's protected LSD values at α level of 0.05.

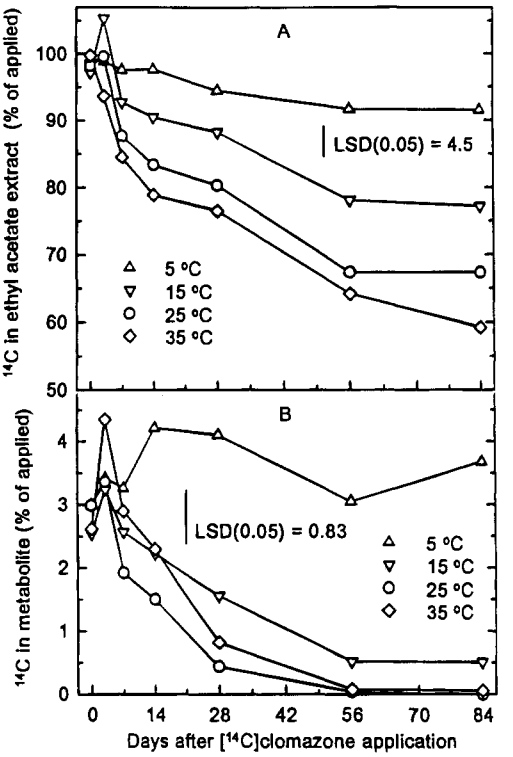

Figure 4. Incubation temperature effects on extractable ^{14}C (A) and [^{14}C]clomazone metabolite levels (B) up to 84 days after [^{14}C]clomazone application to a Flanagan silty clay loam.

similar temperature response in a Flanagan silt loam for DCPA (dimethyl tetrachloroterephthalate) degradation, which reached a maximum rate between 25 and 30 °C, and then decreased at 35 °C.

As expected, clomazone volatilization increased as temperature increased (Figure 3C), probably due to temperature dependence of fugacity (Taylor and Spencer, 1990). By 84 days after application, 7.2% of clomazone had been volatilized. The greater volatilization loss of clomazone from the soil at 35 °C may also be a factor in the lower than expected mineralization of clomazone at 35 °C (Figure 3B).

The decrease in extractable radioactivity remaining in the soil was clearly a function of incubation temperature, likely due to greater ^{14}C losses from soil via volatilization and mineralization and from higher levels of unextractable (bound) soil residues (Figure 4A). At 84 days after clomazone application, 91.5 and 59.2% of applied radioactivity were recovered in ethyl acetate extracts of soil incubated at 5 and 35 °C, respectively. Thus, even at elevated temperature, well over 50% of the clomazone still remained in the soil after an 84-day incubation (Table 1).

For all soil samples, the predominant component in the extractable radioactivity was parent clomazone. A single detectable metabolite accumulated to no more than 5% of applied ^{14}C at any sampling time (Figure 4B). Initial metabolite formation was rapid, reaching a maximum within 3 days. As mentioned previously, no metabolites were detected in extracts of sterilized soil, thus assuming sterilization did not alter the mechanism, metabolite formation occurred via a biological process. The metabolite persisted over time at 3–4% of that applied only in soil incubated at 5 °C (Figure 4B). Above 5 °C, metabolite levels began to fall after 3 days. The low level of $^{14}CO_2$ evolution from soil incubated at 5 °C (Figure 3B) may have resulted from slow mineralization of this metabolite in cold soil.

Soil Moisture Effects on Clomazone Fate. Initially, respiration was unaffected by soil moisture treatment, but 7 days after clomazone application, respiration increased with increasing soil moisture content (Figure 5A). Clomazone mineralization rates were also higher in more moist soils (Figure 5B). In contrast to respiration data, soil moisture effects on clomazone mineralization were apparent within the first 7 days, but mineralization rates were nearly equal by 56 days after clomazone application. Soil moisture differences should not only affect microbial activity, but also alter the distribution of clomazone between solution and sorbed phases. The partitioning of a herbicide between solution and sorbed phases affects its availability to microorganisms and, consequently, its mineralization kinetics (Ogram et al., 1985; Shelton and Parkin, 1991; Greer and Shelton, 1992). Thus, increased clomazone mineralization at higher soil moisture contents may have resulted from both greater microbial activity and an increased ratio of clomazone in solution to clomazone sorbed.

Clomazone volatilization was not effected by soil moisture treatment (Figure 5C), a result that differs from previous field studies in which more clomazone was volatilized from wet than dry soils (Halstead and Harvey, 1985, 1986; Thelen et al., 1988). Because volatilization occurs primarily from the solution phase, volatility of any pesticide is expected to increase with increasing water content. However, volatilization of some pesticides is not greatly affected by soil moisture until the moisture content is reduced to a level at which only a monomolecular layer of water exists on the soil surfaces (Taylor and Spencer, 1990). Below this moisture content, greater pesticide sorption occurs, and thus

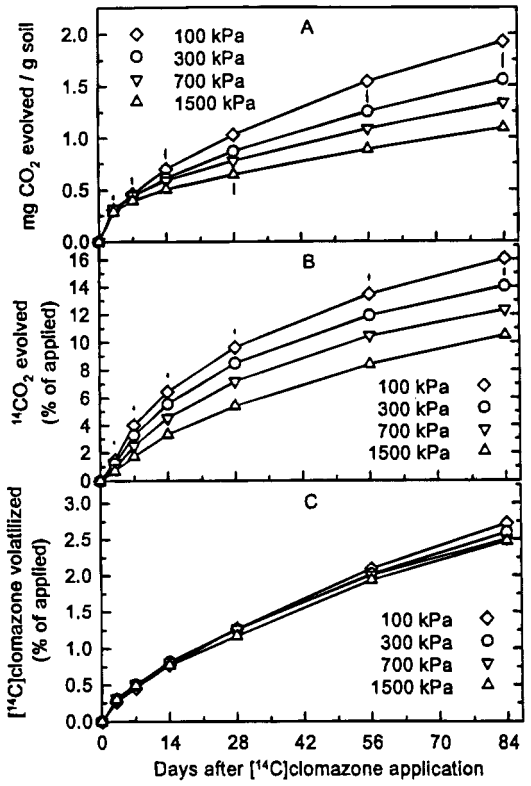

Figure 5. Soil moisture effects on cumulative microbial respiration (A), [^{14}C]clomazone mineralization (B), and [^{14}C]-clomazone volatilization (C) up to 84 days after [^{14}C]clomazone application to a Flanagan silty clay loam. Vertical bars are cumulative Fisher's protected LSD values at α level of 0.05 (no bars are shown for effects with nonsignificant F tests).

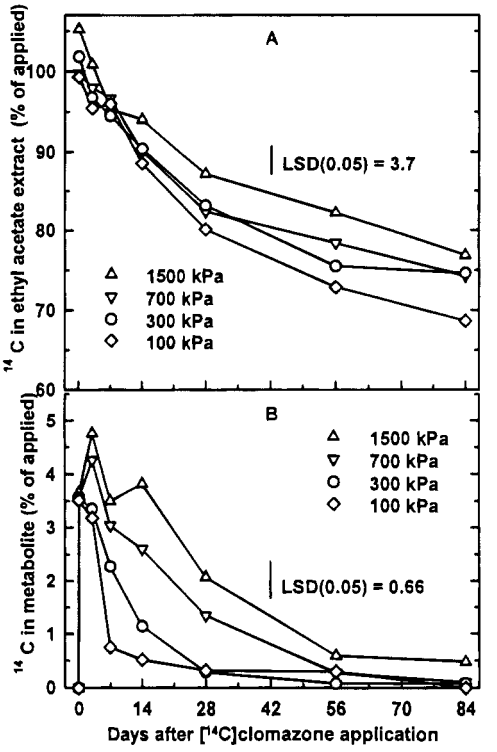

Figure 6. Soil moisture effects on extractable ^{14}C (A) and [^{14}C]clomazone metabolite levels (B) up to 84 days after [^{14}C]clomazone application to a Flanagan silty clay loam.

Table 2. Soil Moisture Effects on [AR-^{14}C] Distribution 84 Days after [^{14}C]Clomazone Application to Soil

	14C recovered as % of applied [^{14}C]clomazone[a]				
	soil water tension (kPa)				
	1500	700	300	100	LSD[b]
volatilized	2.5	2.5	2.6	2.7	NS
mineralized	10.5	12.3	14.0	16.0	0.4
total extracted from soil[c]	76.8	74.3	74.6	68.7	3.7
in aqueous phase	1.0	0.9	0.9	0.8	0.2
in ethyl acetate phase	75.8	73.0	73.7	67.8	3.7
as clomazone	74.8	72.8	72.3	66.6	4.0
as metabolite	0.5	0.1	0.1	0.0	0.7
unextractable (bound)[d]	8.2	9.0	8.7	12.1	4.9
total recovered	98.0	98.1	100.0	99.5	NS

[a] Results are means of triplicate tests. [b] Least significant differences at α level of 0.05; nonsignificant F tests for soil moisture are indicated by NS (based on ANOVA of data from all sampling dates). [c] Corrected for extraction efficiency of 84.9%. [d] Difference between ^{14}C oxidized (corrected for 92.3% oxidizer efficiency) and total ^{14}C extracted from soil.

the fugacity of the pesticide decreases. In the present study, the range in soil water content may not have been large enough to yield differences in clomazone volatilization, because the thickness of water surfaces within the soil at the lowest moisture content (134 g of H_2O/kg of dry soil) probably exceeded a monomolecular layer. Also, due to soil compression, the pressure plate procedure used to develop the soil characteristic curve probably overestimated the necessary water content to achieve higher tension values used in the incubations. This inherent problem with batch studies probably resulted in a narrower water tension range than expected.

At 84 days after [^{14}C]clomazone application, total ^{14}C content of soil extracts ranged from 68.7% (100 kPa) to 76.8% (1500 kPa) of applied (Table 1). As observed in the temperature study (Figure 4B), approximately 3.5% of applied ^{14}C was present in the metabolite in 3-day samples (Figure 6B). The metabolite dissipated more rapidly as soil moisture content increased.

Moisture treatment had little effect on formation of bound residues, which accounted for 8–12% of applied radioactivity 84 days after clomazone application (Table 2). Recovery of applied ^{14}C averaged 98.9% across all moisture treatments.

Fate of ^{14}C-Labeled Carbons. From 0 to 3 days after [^{14}C]clomazone application, a greater percentage of the carbonyl carbon was evolved as $^{14}CO_2$ than either the methylene carbon or the aromatic ring carbons (Figure 7). After 3 days, differences among ^{14}C labels in $^{14}CO_2$ evolution rates were minimal or not significant. Overall, the effect of the ^{14}C label site on clomazone mineralization was nonsignificant (Table 3), but a significant ^{14}C label site by day interaction existed, due to more rapid initial mineralization of the carbonyl carbon.

Radiochromatograms of soil extracts were analyzed to determine the presence or absence of radiolabeled carbons in the metabolite molecule. As observed previously (Figures 4B and 6B), the metabolite contained aromatic ring carbons and represented up to 4% of applied ^{14}C before it began to dissipate (Figure 8B). In contrast, no metabolite peak was detected for extracts from soil treated with [^{14}C=O]clomazone, indicating that the metabolite lacks the carbonyl carbon of the parent compound. Absence of the carbonyl carbon in the metabolite (Figure 8B) in addition to more rapid initial mineralization of the carbonyl carbon than the methylene or aromatic ring carbons (Figure 7) suggests that clomazone metabolism in this soil initially involved

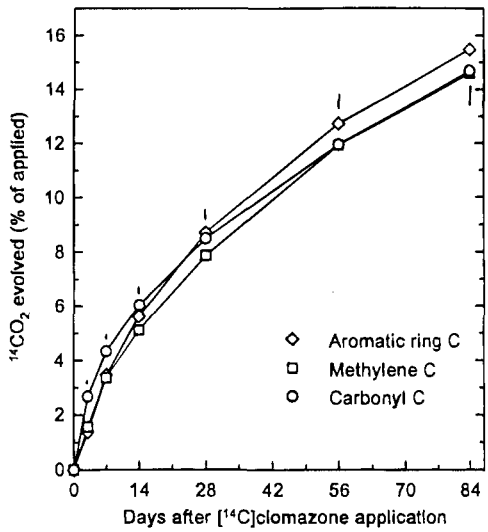

Figure 7. ^{14}C mineralization up to 84 days after [^{14}C]clomazone application to a Flanagan silty clay loam as affected by [^{14}C]label position. Vertical bars are cumulative Fisher's protected LSD values at α level of 0.05.

Table 3. ^{14}C Distribution 84 Days after [^{14}C]Clomazone Application to Soil as Affected by ^{14}C Position in Clomazone Molecule

	14C recovered as % of applied [^{14}C]clomazone[a]			
	position of ^{14}C in clomazone molecule			
	aromatic	methylene	carbonyl	LSD[b]
volatilized	2.6	2.7	2.6	NS
mineralized	15.5	14.6	14.7	0.7
total extracted from soil[c]	75.5	77.8	79.9	3.3
in aqueous phase	1.1	0.6	0.5	0.1
in ethyl acetate phase	74.5	77.3	79.5	3.3
as clomazone	73.1	76.6	79.2	3.2
as metabolite	0.2	0.0	0.0	0.6
unextractable (bound)[d]	5.6	3.5	3.6	NS
total recovered	99.3	98.6	100.8	NS

[a] Results are means of triplicate tests. [b] Least significant differences at α level of 0.05; nonsignificant F tests for ^{14}C label position are indicated by NS (based on ANOVA of data from all sampling dates). [c] Corrected for extraction efficiency of 84.9%. [d] Difference between ^{14}C oxidized (corrected for 92.3% oxidizer efficiency) and total ^{14}C extracted from soil.

cleavage of the isoxazolidinone ring and subsequent loss of the carbonyl carbon as CO_2.

Radiochromatograms for soil treated with [*methylene*-^{14}C]clomazone contained a peak that was smaller but at the same retention time as that of the metabolite containing aromatic ring carbons (Figure 8B). If the metabolite contains both the methylene carbon and the aromatic ring carbons, one would expect equivalent peaks (area and retention time) from both [*methylene*-^{14}C]clomazone and [AR-^{14}C]clomazone treatments. If the metabolite contains only the aromatic ring carbons, no peak should appear in radiochromatograms from the [*methylene*-^{14}C]clomazone treatment. The presence of two metabolites having similar retention times, both containing aromatic ring carbons and one containing the methylene carbon, may be an explanation. However, a modified HPLC protocol, which delayed elution of the compound(s) to 11 min, yielded a single peak.

The most commonly used kinetic model for describing pesticide biodegradation in soils is the first-order equation. However, the dissipation of many chemicals is not accurately described by this expression (Alexander and Scow, 1989). Biodegradation rates in soils often become slower than the rates predicted by first-order kinetics at later sampling times, and thus the time required for disappearance of half the remaining chemical continues to increase during the degradation process (Hamaker, 1972). Although Loux et al. (1989a) reported a half-life of 49–58 days for clomazone in a Drummer silty clay loam, they were able to detect the compound in soil 3 years after application. If the dissipation were a first-order process, the clomazone concentration 3 years after application would have been only 5×10^{-7} the initial concentration, almost certainly undetectable by HPLC. Many other kinetic models have been proposed for the biodegradation of organic chemicals in soil, including a three-half-order model (Brunner and Focht, 1984) and a two-compartment model (Scow et al., 1986), which provided the best fit for mineralization of low concentrations of phenol and aniline in soil.

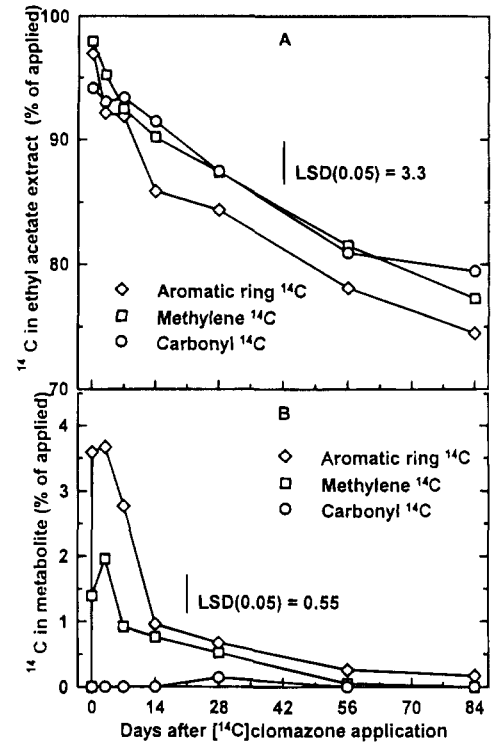

Figure 8. Extractable ^{14}C (A) and ^{14}C content of [^{14}C]clomazone metabolite (B) as affected by site of radiolabeled carbon in [^{14}C]clomazone molecule.

First-order kinetics fit (r^2 = 0.897) clomazone dissipation in the present studies (Figures 4A, 6A, and 8A). An equation with a linear form of $\ln(C) = \ln(C_o) + kt^{0.5}$ provided a better fit (r^2 = 0.986), suggesting diffusion involvement due to the dependence on $t^{0.5}$. Although this equation does not have a readily apparent theoretical basis, it more accurately describes the decreasing rate of clomazone dissipation observed at later sampling times. For the 35 °C treatment, a first-order equation predicts a half-life for clomazone of 104 days; the non-first-order equation predicts a first half-life of 154 days, which, on the basis of data through 84 days, appears to be a more reasonable estimate. Regardless of temperature or soil moisture conditions in this study, a half-life for clomazone was not reached within an 84-day incubation period. The only other report of a half-life greater than 84 days was in a field study during a drought year (Curran et al., 1992).

In summary, clomazone degradation in a Flanagan silty clay loam was biologically dependent, but cloma-

zone mineralization was not always correlated with microbial respiration. The processes of degradation, sorption, and volatilization appeared to be competitive in this experimental system. Our data did not support previous findings that clomazone volatilization is enhanced by increasing soil moisture. Within the ranges examined, temperature affected clomazone degradation and volatilization more than soil moisture did. For all studies, more than half the applied clomazone could be extracted from the soil as parent clomazone 84 days after application. One detectable metabolite, which lacked the carbonyl carbon of clomazone, appeared rapidly after application, but accumulated to no more than 5% of applied radioactivity at any sampling time.

The studies presented here provide overall mass balance data for the fate of clomazone during a 12 week incubation in a soil under specific temperature and soil moisture conditions. Data of this type may be generally useful for understanding the fate of any pesticide, and may assist in the development of models capable of predicting pesticide fate as a function of environmental conditions.

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