Cyanazine Dissipation As Influenced by Soil Properties†

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A laboratory experiment was performed to study the aerobic degradation of cyanazine in six soils ranging in pH from 5.3 to 8.1. Atrazine degradation was evaluated in one soil to provide a reference for longevity values. Soil half-life values for cyanazine ranged from 5.2 to 29.7 days. Cyanazine was more persistent in the moderately acidic soils. The rapid degradation of cyanazine in the neutral to slightly basic soils was due to hydrolysis of the nitrile group. Cyanazine amide and cyanazine acid were the major degradation products. The soil half-life value for atrazine was approximately 3.4 times greater than that of cyanazine in a sandy loam soil. Dissipation of ring-labeled [^{14}C]cyanazine or ring-labeled [^{14}C]atrazine by volatilization or $^{14}CO_2$ evolution was not observed.

Cyanazine [2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropionitrile] and atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine] are selective herbicides used to control annual grass and broadleafed weeds in corn (*Zea mays* L.), grain sorghum [*Sorghum bicolor* (L.) Moench], and other crops. Atrazine is fairly persistent in the soil, and residues can persist into the following year at levels which are phytotoxic to crops such as soybeans (*Glycine max* L.). Cyanazine is less persistent than atrazine in the soil (Sirons et al., 1973) and can be used in situations where potential carry-over problems are a concern.

Atrazine degradation in soil has been studied by numerous researchers, and most studies have confirmed that the rate of degradation increases as soil pH decreases (Armstrong et al., 1967; Best and Weber, 1974; Muir and Baker, 1978). This has been attributed to acid-catalyzed chemical (nonbiological) hydroxylation to form hydroxyatrazine [6-hydroxy-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine]. The primary soil metabolites of cyanazine have been identified (Beynon, 1972; Beynon et al., 1972); however, there is little information available on the soil factors affecting cyanazine degradation. Therefore, the objectives of this study were to (a) determine the effect of soil factors on cyanazine longevity, (b) measure the relative contributions of volatilization, mineralization, and soil degradation to cyanazine and atrazine dissipation, and (c) compare the longevity of cyanazine to that of atrazine.

MATERIALS AND METHODS

Chemicals. Analytical grade cyanazine, cyanazine amide [2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropanamide], cyanazine acid [*N*-[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]-2-methylalanine], cyanazine hydroxy acid [*N*-[4-(ethylamino)-6-hydroxy-1,3,5-triazin-2-yl]-2-methylalanine] and ring-labeled [^{14}C]cyanazine (specific activity, 60.9 μCi/mg) were supplied by E. I. du Pont de Nemours and Co. Analytical grade atrazine, deethylatrazine [4-chloro-*N*-(1-methylethyl)-1,3,5-triazine-2,4-diamine], deisopropylatrazine (4-chloro-*N*-ethyl-1,3,5-triazine-2,4-diamine), hydroxyatrazine, and ring-labeled [^{14}C]-atrazine (specific activity, 20.6 μCi/mg) were supplied by Ciba-Geigy Corp. Radiochemical purity of both compounds was checked using thin-layer chromatography (TLC) and found to be greater than 98%.

Experimental Soils. Soils were collected from the A horizons of a Norfolk soil (typic paleudult; fine-loamy, siliceous, thermic) near Rocky Mount, NC; a Cape Fear soil (typic umbraquult; clayey, mixed, thermic), near Plymouth, NC; a Rion soil (typic hapludult; fine-loamy, mixed, thermic) near Reidsville, NC; a Haynie soil (mollic udifluvent; coarse-silty, mixed (calcareous), mesic) near Blair, NE; a Coolidge soil (typic calciorthid; coarse-loamy, mixed, hyperthermic), near Yuma, AZ; and a Judson soil (cumulic hapludoll; fine-silty, mixed, mesic) near Linwood, NE. Soils were sieved through a 2-mm screen and stored moist until used. Analyses to characterize the soils included determination of pH, humic matter content, organic matter content, cation-exchange capacity (CEC), texture, and estimation of microbial populations. Soil pH was measured in a 1:1 (w/w) soil/water mixture with a glass electrode pH meter. Humic matter content was determined using the NaOH/diethylenetriaminepentaacetic acid (DTPA)/alcohol extraction method (Mehlich, 1984). Organic matter content was determined using the chromic acid colorimetric method, cation-exchange capacity was measured using the summation of exchangeable cations procedure with 1.0 N ammonium acetate as buffer, and particle size analyses were performed using the hydrometer method (Reference Soil Test Methods for the Southern Region of the United States, 1983). Microbial populations were estimated using cultural methods outlined by Wollum (1982).

Experimental Conditions. A laboratory experiment was designed to study the aerobic degradation of cyanazine in six soils. Soils varied in texture and organic content but were chosen specifically to provide a wide range of pH values. Atrazine degradation was evaluated in one soil to provide a reference for longevity values. Analytical grade and radiolabeled cyanazine or atrazine was added in 1 mL of methanol to 400 g (dry weight basis) of soil to provide a concentration of 5 ppm. Fortification solutions were incorporated with a spatula, and fortified soils were placed in 1-L Erlenmeyer flasks with two glass tube sidearms providing an inlet for water-saturated, compressed air and an outlet connected to a series of chambers containing 20 mL of ethylene glycol to trap volatile ^{14}C-labeled organic compounds and 15 mL of 1.0 M KOH to trap $^{14}CO_2$. Soil moisture content was adjusted to 80% of field capacity at 0.33 bar. Samples were maintained at 21 ± 2 °C in the dark. The experimental design was completely randomized with two replications.

Chemical Assays. Sampling intervals were 0, 4, 8, 12, 16, 24, 32, and 40 days after treatment. An additional sampling interval 48 days after treatment was included for the atrazine-treated samples. At each sampling interval, the amounts of trapped ^{14}C-labeled volatile products and $^{14}CO_2$ were determined by liquid scintillation counting, and fresh trapping solutions were provided. Methods for characterizing soil metabolites were modified from the procedure described by Beynon et al. (1972). A 20-g subsample of soil was taken from each treated sample at each sampling interval, placed in a Soxhlet apparatus, and extracted

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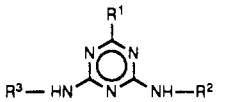
Table I. R_f Values of Some 1,3,5-Triazines on Silica GF TLC Plates Using Various Solvent Systems

chemical	chemical substituents[a]			solvent systems[b]		
	R^1	R^2	R^3	A	B	C
cyanazine	Cl	$C(CH_3)_2CN$	C_2H_5	0.57[c]	0.60	
cyanazine amide	Cl	$C(CH_3)_2CONH_2$	C_2H_5	0.11[c]	0.10	
cyanazine acid	Cl	$C(CH_3)_2COOH$	C_2H_5	0.29[c]	0.25	
cyanazine hydroxy acid	OH	$C(CH_3)_2COOH$	C_2H_5	0.00[c]	0.00	
atrazine	Cl	$CH(CH_3)_2$	C_2H_5	0.61		0.58
deethylatrazine	Cl	$CH(CH_3)_2$	H	0.43		0.32
deisopropylatrazine	Cl	H	C_2H_5	0.32		0.25
hydroxyatrazine	OH	$CH(CH_3)_2$	C_2H_5	0.00		0.00

[a] Parent structure of 1,3,5-triazines:



[b] Solvent systems: A, ethyl acetate/toluene (1/1 v/v); B, nitromethane/chloroform/acetic acid (60/40/1 v/v/v); C, benzene/acetone (8/2 v/v). [c] 1% acetic acid (v/v) added to solvent system.

Table II. Properties of the Soils

soil series	pH	humic matter, %	organic matter, %	clay, %	clay type[a]	CEC, mequiv/100 g	moisture content at 0.33 bar, %	bacteria,[b] cfu/g	fungi,[b] cfu/g	actinomycetes,[b] cfu/g
Norfolk (sl)[c]	5.9	0.2	1.1	6	K, V	2.7	15.8	1.2×10^6	2.1×10^4	2.1×10^6
Cape Fear (sl)	5.3	3.9	5.1	16	M, K	11.6	18.4	6.4×10^6	8.4×10^4	7.9×10^6
Rion (sl)	6.8	0.1	0.9	16	K	3.8	21.4	3.4×10^5	1.6×10^4	1.1×10^6
Haynie (sil)	7.4	0.5	2.6	18	M, K, I	12.6	31.4	1.3×10^6	3.1×10^4	1.3×10^6
Judson (sil)	7.4	0.7	3.2	22	M, K, I	13.4	32.7	1.4×10^6	4.2×10^4	6.0×10^6
Coolidge (sl)	8.1	0.0	0.7	14	M, K, I	14.8	13.9	1.9×10^5	9.0×10^3	4.2×10^5

[a] From X-ray diffraction analysis: K, kaolinite; V, vermiculite; M, montmorillonite; I, illite (hydrous mica). [b] Microbial populations (colony forming units per gram) enumerated by the plate count method using various media; bacteria were cultured on tryptic soy agar, fungi on peptone–glucose acid agar, and actinomycetes on starch–casein agar. [c] Sl, sandy loam; sil, silt loam.

Table III. Dissipation Equations, Coefficients of Determination (R^2), and Half-Life Values for Atrazine and Cyanazine in Soils

herbicide	soil series	equation[a]	R^2	half-life, days
atrazine	Norfolk	$\ln C_0/C_i = (-0.0116)t$	0.927	59.6
cyanazine	Norfolk	$\ln C_0/C_i = (-0.0399)t$	0.978	17.4
cyanazine	Cape Fear	$\ln C_0/C_i = (-0.0234)t$	0.949	29.7
cyanazine	Rion	$\ln C_0/C_i = (-0.0778)t$	0.992	8.9
cyanazine	Haynie	$\ln C_0/C_i = (-0.0758)t$	0.988	9.2
cyanazine	Judson	$\ln C_0/C_i = (-0.1177)t$	0.982	5.9
cyanazine	Coolidge	$\ln C_0/C_i = (-0.1330)t$	0.984	5.2
				$LSD_{0.05}$ 1.7

[a] C_i, initial concentration; C_0, concentration at time t (days).

with 200 mL of methanol/water (9/1 v/v) for 4 h. Aliquots of the methanol/water extracts were radioassayed to determine ^{14}C extraction levels, and the methanol was removed using rotary evaporation. The remaining aqueous solutions were partitioned twice with methylene chloride. Cyanazine-treated samples were acidified (pH 3) prior to methylene chloride extraction to partition cyanazine acid into the organic phase. The aqueous phases were radioassayed, and the organic phases were radioassayed, concentrated, and analyzed using TLC. Identification of radiolabeled compounds was performed by comparison of R_f values with those of authentic standards using two solvent systems. Solvent systems for atrazine were ethyl acetate/toluene (1/1) and benzene/acetone (4/1). Solvent systems for cyanazine were ethyl acetate/toluene/acetic acid (50/50/1) and nitromethane/chloroform/acetic acid (60/40/1). Chemical structures and R_f values of the analytical standards are given in Table I. After the plates were developed, cyanazine, atrazine, and their respective metabolite standards were visualized using ultraviolet light (254 nm). The entire sample lanes were scraped, and areas corresponding to the R_f values of the standards and nonidentified products were radioassayed. The data were transformed and subjected to analysis of variance.

RESULTS AND DISCUSSION

Soil Properties. Soil properties are presented in Table II. Soil pH levels ranged from a moderately acidic value of 5.3 to a slightly basic value of 8.1. Soil organic contents ranged from 0.0 to 3.9% humic matter and from 0.7 to 5.1% organic matter. Humic matter is considered to be the most active binding fraction of soil organic matter (Kozak et al., 1983). Cation-exchange capacities (CEC) were a function of the organic content, and the clay content and type and ranged from 2.7 to 14.8 mequiv/100 g. The high CEC of the Coolidge soil (14.8 mequiv/100 g) is due primarily to the high level of calcite ($CaCO_3$) present in this soil. Soil microbial populations were greatest in the Cape Fear soil and least in the Coolidge soil.

Volatilization and Mineralization. Dissipation of ^{14}C residues via volatilization or mineralization was not detected from soils treated with either ring-labeled atrazine or cyanazine. Volatilization of 1,3,5-triazines from solid surfaces has been observed (Foy, 1964); however, little information is available on the relative importance of volatilization of 1,3,5-triazines from soil under agricultural conditions. Glotfelty et al. (1989) reported minor losses of atrazine due to volatilization from fallow soil under field conditions (2.4% of the applied material). Lack of detectable $^{14}CO_2$ evolution 48 days after application is not surprising. Several researchers (Beynon et al., 1972; Kaufman and Kearney, 1970) have noted the 1,3,5-triazine ring is quite resistant to complete mineralization.

Atrazine Degradation. Atrazine degradation was evaluated in the Norfolk soil, and the results are presented in Figure 1. Atrazine was fairly persistent in this soil, with only minor levels of extractable metabolites detected 48 days after treatment. These products primarily were the N-dealkylated metabolites of atrazine. The decrease noted in total ^{14}C extractable levels over time (sum of shaded areas) suggests that bound residue formation was a significant route of atrazine dissipation. Best and Weber (1974) observed similar results with atrazine-treated soil and noted that bound residue formation was more rapid in acidic soils and related to hydroxyatrazine formation.

Identification of water soluble ^{14}C residues was not

Table IV. Coefficients of the Correlation Matrix

	humic matter, %	organic matter, %	clay, %	CEC, mequiv/100 g	bacteria, cfu/g	fungi, cfu/g	actinomycetes, cfu/g	cyanazine half-life, days
pH	−0.69	−0.50	0.41	0.52	−0.74	−0.65	−0.57	−0.93[a]
humic matter, %		0.90[a]	0.17	0.23	0.99[a]	0.96[a]	0.84	0.86
organic matter, %			0.46	0.40	0.89[a]	0.98[a]	0.91[a]	0.65
clay, %				0.64	0.10	0.31	0.36	−0.32
CEC, mequiv/100 g					0.17	0.26	0.25	−0.21
bacteria, cfu/g						0.96[a]	0.84	0.90[a]
fungi, cfu/g							0.92[a]	0.78
actinomycetes, cfu/g								0.63

[a] Significant correlation at $\alpha = 0.01$ level.

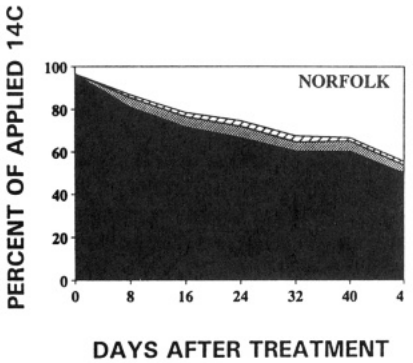


Figure 1. Atrazine dissipation in Norfolk soil (black, atrazine; heavy shading, deethylatrazine plus deisopropyl atrazine; light shading, nonidentified compounds; no shading, nonextracted ^{14}C).

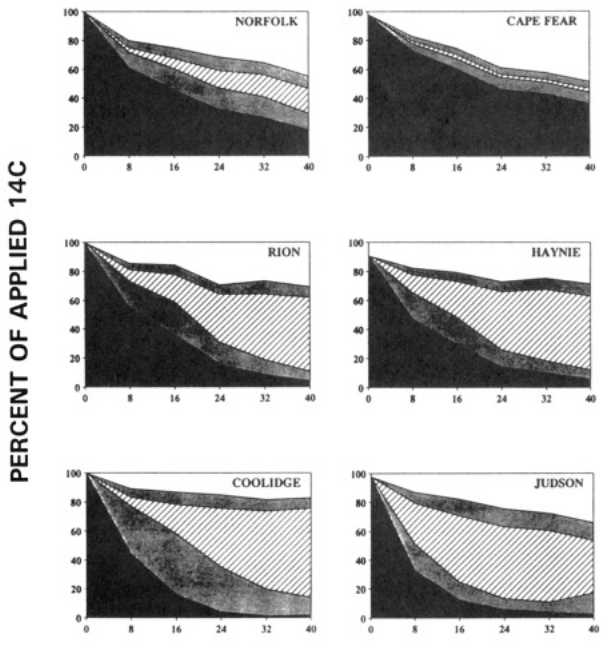


Figure 2. Cyanazine dissipation in various soils (black, cyanazine; lower heavy shading, cyanazine amide; light shading, cyanazine acid; upper heavy shading, nonidentified compounds; no shading nonextracted ^{14}C).

performed. Areas of the degradation graphs corresponding to nonidentified compounds include water-soluble ^{14}C residues and organosoluble ^{14}C residues which did not chromatograph with available reference standards. ^{14}C residues that remained at the origin were considered to be nonidentified.

Cyanazine Degradation. Cyanazine degradation results from the Norfolk soil are presented in Figure 2. As with atrazine, ^{14}C extraction levels decreased steadily over time. Similar results were obtained with cyanazine in the Cape Fear soil. Both the Norfolk and Cape Fear soils are moderately acidic; therefore, a contributing factor to the accumulation of nonextractable residues may have been protonation of the triazine ring and resultant binding to the negatively charged soil constituents. Weber (1970) noted that weakly basic 1,3,5-triazine molecules could be protonated in acidic soil environments, resulting in increased and possibly irreversible adsorption. Cyanazine levels were consistently greater in the Norfolk and Cape Fear soils compared to those in the other four soils even though total ^{14}C extractable levels were lower. In the Rion and Haynie soils, cyanazine degradation resulted in a rapid accumulation of hydrolyzed metabolites (Figure 2). The initial degradation product was cyanazine amide, but the concentration of this product decreased with time as it underwent further hydrolysis to cyanazine acid. Forty days after treatment, greater than 50% of the applied cyanazine had been converted to cyanazine acid in both of these soils, and less than 6% of the applied cyanazine remained at this time. Cyanazine degradation in the Coolidge and Judson soils was similar to that in the Rion and Haynie soils. Forty days after treatment, greater than 84% of the applied ^{14}C was extracted from the Coolidge soil, but cyanazine accounted for less than 3% of the total.

Soil extraction validation was not performed for all of the compounds listed in Table I. Zero-time analyses indicated the method provided acceptable recovery of the parent herbicides, and later results from the neutral to slightly basic soils indicated the method also provided acceptable recovery of cyanazine amide and cyanazine acid. The method appears to be ineffective, however, at extracting the hydroxylated metabolites. Extensive extraction procedures were not developed for the hydroxylated metabolites due to the difficulties associated with these analyses (Muir and Baker, 1978; Sirons et al., 1973). In addition, Sirons et al. (1973) questioned the involvement of ring hydroxylation in cyanazine degradation, noting that the analytical procedures used by Beynon (1972) and Beynon et al. (1972) induced substitution of a hydroxyl group for chlorine in the 2-position.

Regression analyses were used to calculate cyanazine and atrazine half-lives in soils. Data from individual replicates were tested using zero-order and first-order models, and the model achieving the highest degree of fit was used for calculation purposes. The first-order model, $\ln C_0/C_i = kt$, in which C_i is the initial concentration, C_0 is the concentration at time t (days), and k is the rate constant, was found to closely fit the data for atrazine and cyanazine degradation in all samples. Calculated half-life values were subjected to analysis of variance. Degradation data between replicates were pooled (Table III), and mean half-life values were correlated to various soil properties (Table IV).

A soil half-life value of 59.6 days for atrazine compared favorably with previously reported values in the literature (Muir and Baker, 1978; Sirons et al., 1973). Atrazine

longevity in the Norfolk soil was approximately 3.4 times greater than that of cyanazine (Table III). The isopropyl group in atrazine is less susceptible to degradative processes than the propanenitrile moiety in cyanazine, and this is the primary reason for the greater soil persistence of atrazine compared to that of cyanazine. Soil half-life values for cyanazine ranged from 5.2 to 9.2 days in the neutral to slightly basic soils and from 17.4 to 29.7 days in the moderately acidic soils (Table III). The significant negative correlation between soil pH and cyanazine half-life and lack of interaction between soil pH and other soil properties (Table IV) indicated that soil pH was an important factor influencing cyanazine degradation rate, possibly due to its effect on cyanazine bioavailability. In fact, soil pH was the only soil property that showed a significant negative correlation to cyanazine half-life (cyanazine half-life decreased as soil pH increased). The positive correlations between cyanazine half-life and soil microbial populations indicate that cyanazine degradation rate was not simply a function of microorganism numbers. Significant correlations were noted between (a) humic matter and organic matter content, (b) bacteria/fungi/actinomycetes populations, and (c) microbial populations and humic/organic matter content. Correlations a and b would be expected and correlation c is not surprising since microbial populations generally increase with increasing soil organic matter content (Atlas, 1984).

Increased cyanazine longevity in moderately acidic soils contrasts with results obtained with most 1,3,5-triazines (Best and Weber, 1974; Sheets, 1970). Increased degradation of 1,3,5-triazines at low soil pH values is generally associated with increased chemical hydroxylation. The relative importance of chemical vs microbial degradation of cyanazine has not been reported, however. It is unlikely that soil pH levels of 6.8–7.4 catalyzed a chemical hydrolysis reaction. Instead, lower extractable ^{14}C residues but higher cyanazine levels in the moderately acidic soils suggest that these soils may have offered some "protection" from degradative processes, possibly due to greater adsorption. Cyanazine may persist longer in acidic soils but in an adsorbed, and potentially unavailable, state.

Enhanced microbial degradation of herbicides has been observed in situations where repeated annual applications of a herbicide were made (Skipper et al., 1986; Wilson, 1984). However, none of the soils used in this experiment had a 2-year prior use history of 1,3,5-triazine herbicides. Therefore, accelerated biodegradation of cyanazine in this study due to the buildup of an effective herbicide-degrading microbial population was unlikely. Further research is needed to characterize the nature of the rapid degradation of cyanazine.

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