Formation of *N*-Nitrosoterbuthylazine and *N*-Nitrosoterbutryn in a Model System of Soil Water

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The *s*-triazines terbutryn and terbuthylazine are currently used in Italy for weed control. A possible reaction of these compounds in the environment is *N*-nitrosation. Experiments performed in 10 mM CaCl₂ as a model of soil water indicated that *N*-nitrosation is favored only at low pH values and that the *N*-nitroso derivatives are fairly stable. In the presence of soil either parent compounds or *N*-nitroso derivatives are strongly adsorbed. These results seem to indicate that the possibility of formation of *N*-nitrosoterbuthylazine and *N*-nitrosoterbutryn in common agricultural soil is very remote.
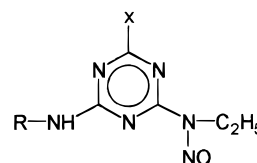
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INTRODUCTION

Triazine herbicides are among the most widely used pesticides all over the world: for example, atrazine [6-chloro-*N*-ethyl-*N*-(1-methylethyl)-1,3,5-triazine-2,4-diamine] is used alone or in combination with other herbicides for the control of broad-leaved weeds in corn. Its continuous use for many years produced widespread contamination of water beds in northern Italy, which led to a strict prohibition of its use in Italy. One possible substitute is terbuthylazine [6-chloro-*N*-(1,1-dimethylethyl)-*N*-ethyl-1,3,5-triazine-2,4-diamine], an analogue of atrazine in which a *tert*-butyl substituent is present instead of an isopropyl on one of the amino groups. Another herbicide with the same structural feature is currently used in Italy for vineyard weed control: terbutryn [*N*-(1,1-dimethylethyl)-*N*-ethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine], which has a methylthio group instead of a chlorine atom on the *s*-triazine ring.

In the environment a possible reaction of *s*-triazine herbicides containing secondary *N*-alkyl substituents is *N*-nitrosation (Zwickenpflug and Richter, 1994; Eisenbrand et al., 1975; Fine and Roundbehler, 1976). This reaction has been extensively studied for atrazine (Wolfe et al., 1976; Mirvish, 1975; Mirvish et al., 1991), but Kearney et al. (1977) demonstrated that if *N*-nitrosoatrazine is formed in the environment, it is also rapidly degraded, denitrosation being the primary degradation reaction. Recently, Zwickenpflug and Richter (1994) have studied the possible formation of *N*-nitrosocyanazine from cyanazine [2-[(4-chloro-6-(ethylamino)-*s*-triazin-2-yl)amino]-2-methylpropionitrile] in soil. They could isolate and fully characterize three *N*-nitroso

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Chart 1

compound	X	R
N-nitrosoatrazine	Cl	*iso*propyl
N-nitrosoterbuthylazine	Cl	*tert*butyl
N-nitrosoterbutryn	SCH₃	*tert*butyl

derivatives of cyanazine by chemical synthesis, the most abundant of which coeluted with a peak present also in extracts of soil contaminated with cyanazine, even if a quantitative evaluation was not possible.

Since several *N*-nitroso compounds have been shown to be carcinogenic in experimental animals (Druckrey et al., 1967; IARC, 1978), the complete lack of data on the formation of *N*-nitroso derivatives from terbuthylazine and terbutryn (Chart 1) prompted us to verify their possible formation in soil, because it was not known whether the structural differences, in particular the presence of an electron-releasing group such as the *tert*-butyl and the S—CH₃ in terbutryn, could favor the formation of toxic metabolites. The chemical synthesis, physicochemical characteristics, and kinetics of formation in human gastric juice of these *N*-nitroso metabolites have been reported previously (Cova et al., 1996).

MATERIALS AND METHODS

Materials. Standard samples of the *N*-nitroso derivatives were freshly prepared as described in Cova et al. (1996).

Nitrosation in CaCl₂. Standard solutions of each triazine in methanol were added to 100 mL of 10 mM CaCl₂. Different amounts of sodium nitrite (NaNO₂) were added to these solutions to obtain the desired concentration of these reagents (see figures). The pH of the solutions was adjusted with
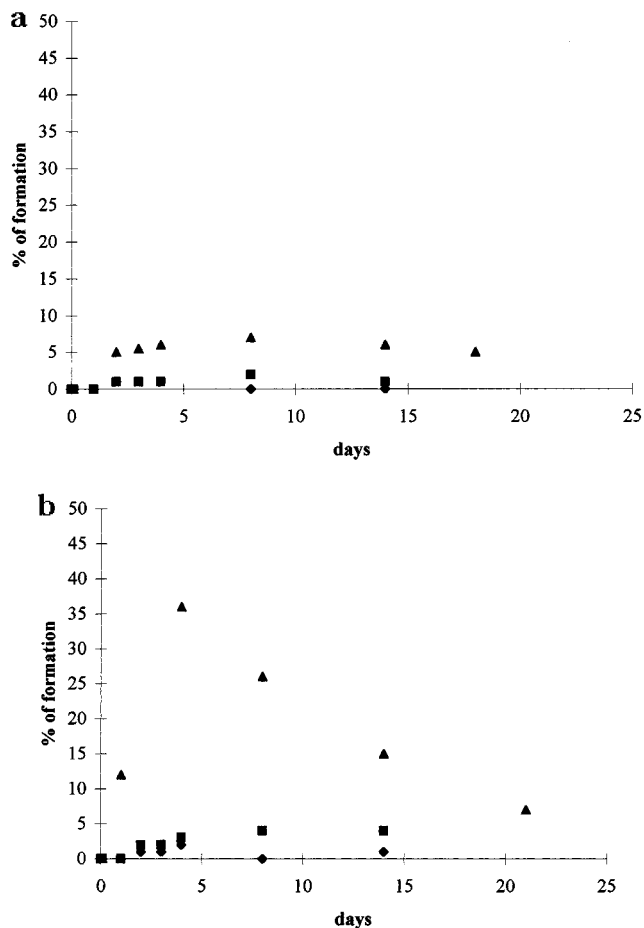
Figure 1. Effect of pH on the formation of N-nitrosoterbuthylazine from terbuthylazine (0.04 mM in 10 mM CaCl₂) in the presence of (a) 0.1 or (b) 20.0 mM NaNO₂: (◆) pH 5.5; (■) pH 4.0; (▲) pH 2.9. The concentration was determined by HPLC. Points represent the average of two experiments.

concentrated phosphoric acid. The solutions were incubated in the dark at 20 °C. Samples were directly injected in an HPLC apparatus. Every experiment was conducted in duplicate.

Nitrosation in Soil/Water Suspension. Soil (10 g) was treated with the solution indicated above. The soil characteristics were pH 7.8, 2% organic matter, sand 23%, silt 46%, clay 31%, CEC 20.1 mequiv/100 g, and 1.2% nitrogen. The pH of the suspensions was adjusted with phosphoric acid. The slurries were stirred at 20 °C. The formation of the N-nitroso derivative was checked by HPLC injection of the solution after centrifugation of the slurry. Every experiment was conducted in duplicate.

Determination of Stability of N-Nitroso Derivatives. These assays were prepared in the same way without sodium nitrite both with and without soil. Every experiment was conducted in duplicate.

HPLC Analysis. Analyses were conducted by HPLC on a Lichrospher 100 RP-18 column (5 μm, 4 × 125 mm) on a Hewlett-Packard 1090L instrument with automatic sampler. The eluent was acetonitrile/water 60:40 at a flow rate of 1 mL/min. Two different wavelengths were used for quantification: 226 nm for the parent triazines and 246 nm for the N-nitroso derivatives. Minimum detectable amounts were 0.08 mg/L for both the parent compounds and the nitroso derivatives.

Determination of pK_a Values. Acid dissociation constants were determined by UV−visible spectroscopy (Battistuzzi et al., 1994). Spectra of the compounds were obtained at different pH values by addition of very small amounts of HCl (37%) or NaOH (32%) to the sample. Spectral changes are due to the equilibrium between the amine and the
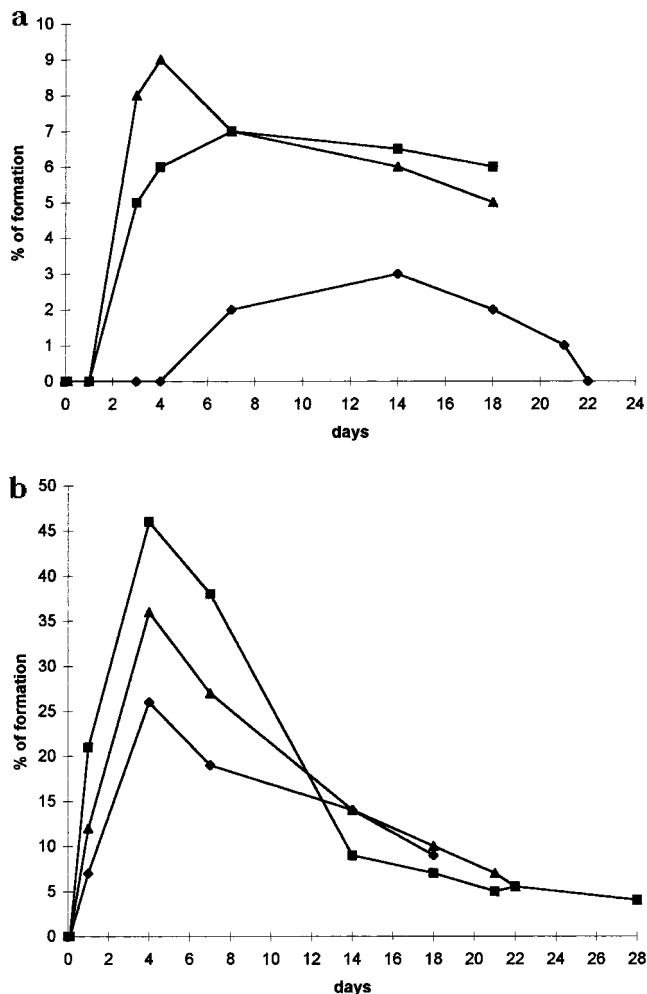

Figure 2. Effect of NaNO₂ concentration on the formation of N-nitrosoterbuthylazine from terbuthylazine (0.04 mM in 10 mM CaCl₂) at pH 2.9: (a) (◆) 0.01, (■) 0.1, and (▲) 0.2 mM; (b) (◆) 1, (■) 2, and (▲) 20 mM. The concentration was determined by HPLC. Points represent the average of two experiments.

ammonium salt, and pK_a values were derived from the isosbestic points. The values for atrazine (pK_a = 1.76) and terbutryn (pK_a = 4.36) matched very well with the values (pK_a = 1.68 and 4.3, respectively) reported by Wauchope et al. (1992).

RESULTS

Experiments were conducted in 10 mM CaCl₂ as a model system of soil water. A solution containing terbuthylazine (0.04 mM) was incubated at pH 2.9, 4.0, and 5.5 in the presence of NaNO₂ (0.1 or 20.0 mM). Low pH values favor N-nitrosation: after 3 days at pH 2.9, a 10-fold greater amount of N-nitrosoterbuthylazine is formed with respect to that at pH 5.5 with either 0.1 or 20.0 mM NaNO₂ (Figure 1). After this time, especially at pH 2.9 and in the presence of 20.0 mM NaNO₂, the N-nitroso derivative concentration slowly decreases.

The effect of the nitrite concentration was investigated at pH 2.9 (Figure 2). The percentage formation depends on the nitrite concentration (at least 10-fold higher than the parent compound). The maximum amounts are formed in the first days; afterward, the N-nitroso derivative is slowly destroyed through conversion to the starting material.

The stability of N-nitrosoterbuthylazine (concentration = 0.04 mM) was studied at pH 3.6, 5.6, and 6.3. At
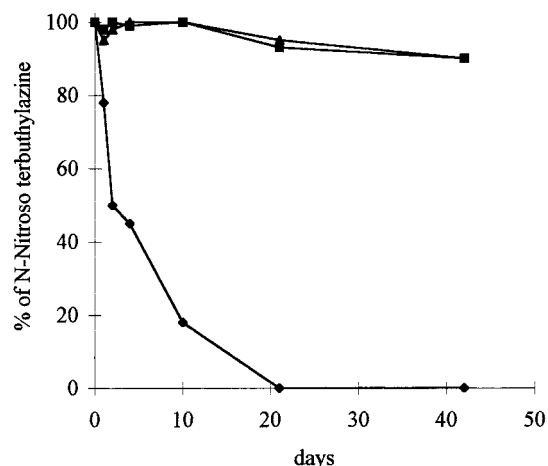
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Figure 3. Degradation of *N*-nitrosoterbuthylazine (0.04 mM) in 10 mM CaCl$_2$: (◆) pH 3.6; (■) pH 6.3; (▲) pH 5.6. The concentration was determined by HPLC. Points represent the average of two experiments.

high pH values the compound is quite stable (at pH 5.6 or 6.3 after 1000 h, 90% of the compound is recovered), while at pH 3.6 the half-life time is ∼56 h and after 500 h only the parent compound could be detected (Figure 3).

As the best conditions for nitrosating these compounds appeared to be low pH (<2.5) and a nitrite concentration 100-fold higher than the triazine concentration, the subsequent experiments, dedicated to the comparison of the behavior of terbutryn and terbuthylazine, were performed at pH 1.5 and 2.0 and with 0.6 or 6.0 mM nitrite concentration (Figure 4). With 0.6 mM NaNO$_2$ (Figure 4a) low conversions to *N*-nitroso derivatives are reached very slowly: for example, at pH 2 the maximum amount of *N*-nitrosoterbuthylazine (40%) is reached in 24 h and the maximum amount of *N*-nitrosoterbutryn (15%) in 96 h. With 6.0 mM NaNO$_2$ (Figure 4b) at pH 1.5 the maximum nitrosation, reached in 2−3 h, is practically quantitative in the case of terbutryn, and after this time only a weak decrease was observed. Also, terbuthylazine is nitrosated very quickly, reaching 87% in 2 h, but its decomposition is much more extensive. At pH 2 the differences between the compounds are similar but the nitrosation is much slower, the maximum being around 12 h instead of 2−3 h. The decomposition of the two compounds is favored also, but *N*-nitrosoterbutryn appears to be more stable and begins to decompose significantly only after 96 h.

The concentration observed in the presence of NaNO$_2$ is therefore the balance between the formation and the decomposition rates. The nitrosating agent is actually HONO, which is known to decompose slowly in acidic water solution. The concentration reduction of this reagent is responsible for the decaying of the *N*-nitroso derivatives during this time.

Experiments were carried out in the presence of silty loam soil at several pH values with atrazine as a reference compound. At pH >2.0 no *N*-nitroso derivative could be detected; at pH 2.0 atrazine and terbuthylazine (Table 1) were quickly nitrosated (maximum reached after 1 h). Terbutryn was completely adsorbed by soil, and after 30 min, either parent or *N*-nitroso compound had disappeared. This behavior appears to be related to the pK_a: atrazine = 1.76, terbuthylazine = 2.08, terbutryn = 4.36. The easier protonation of the last compound facilitates soil absorption. This was
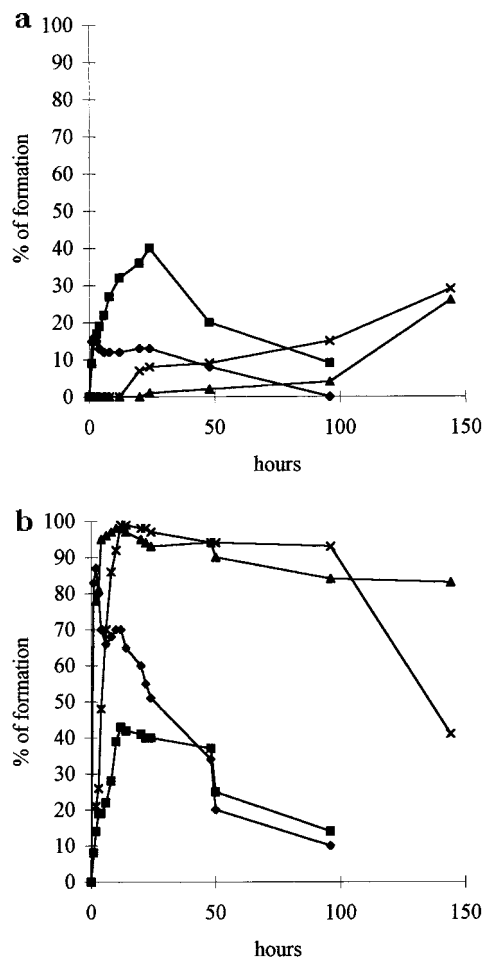

Figure 4. Comparison of the formation of *N*-nitroso derivatives from 0.04 mM terbuthylazine (TBA) and terbutryn (TBR) in 10 mM CaCl$_2$ at pH 1.5 and 2.0 in the presence of (a) 0.6 or (b) 6.0 mM NaNO$_2$: (◆) TBA, pH 1.5; (■) TBA, pH 2.0; (▲) TBR, pH 1.5; (×) TBR, pH 2.0. The concentration was determined by HPLC. Points represent the average of two experiments.

Table 1. Percent Formation of *N*-Nitrosoatrazine and *N*-Nitrosoterbuthylazine from Atrazine and Terbuthylazine (0.04 mM) in 10 mM CaCl$_2$ at pH 2.0 in the Presence of Silty Clay Loam Soil

time, h	percentage of *N*-nitrosoatrazine		percentage of *N*-nitrosoterbuthylazine	
	0.06 mM[a]	6.0 mM[a]	0.06 mM[a]	6.0 mM[a]
0	0	0	0	0
1	0	34	4	29
2	0	32	0	17
6	0	30	0	12
24	0	16	0	5

[a] Sodium nitrite concentration.

checked by adding soil to *N*-nitrosotriazines at pH 2.0. For example, after 10 min, only 15% of *N*-nitrosoterbutryn was recovered, while after 24 h neither parent nor *N*-nitroso compound was found. No trial on behavior in soil was performed.

DISCUSSION

In a preceding paper (Cova et al., 1996), we have reported the synthesis of the *N*-nitroso derivatives of terbuthylazine and terbutryn and have demonstrated that, owing to the presence of a large *tert*-butyl group, only one *N*-nitroso derivative is formed and that nitro-

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sation occurs on the amino group bearing the ethyl substituent. This behavior is identical with that of atrazine (Mirvish et al., 1991), while cyanazine is nitrosated on the most hindered group (Zwickenpflug and Richter, 1994) for the presence of the cyano group.

The formation of *N*-nitroso derivatives from *s*-triazines in aqueous media, without any catalyst such as NaSCN (Zwickenpflug and Richter, 1994), is generally very low. For example, Eisenbrand et al. (1975) obtained only 1–7% of *N*-nitrososimazine and *N*-nitrosoatrazine. In the present work high yields were reached only in the presence of a large excess of nitrite: Figure 2 emphasizes the very important role of nitrite concentration.

Another important factor is pH. Although the significance of chemical formation of *N*-nitroso derivatives in very acidic solutions is probably academic, since major crops cannot grow in acidic soils, the data indicate clearly that a decrease in pH increases the formation of *N*-nitroso derivatives. The formation of *N*-nitrosoterbuthylazine and *N*-nitrosoterbutryn was studied as a function of time: at low pH their concentration increases very rapidly (for example, at pH 1.5 after a few hours *N*-nitrosoterbutryn is formed almost quantitatively), but subsequently their concentrations decrease. At high pH the formation is quite limited. The actual concentration appears to be the result of the balance between the formation and decomposition rates. At low pH both the formation and the decomposition are fast; at high pH the compounds are stable, but their formation is scarce: a low environmental impact can be envisaged.

The structural difference of the compounds consists only in the substituent on the heterocyclic ring: a less electron-withdrawing group increases the maximum concentration obtained ($\sigma_{\text{meta}} = 0.37$ for Cl and 0.12 for SCH$_3$; Hansch and Leo, 1979).

The soil study indicates that soil binding of the parent compounds competes with the formation of *N*-nitrosotriazines. This was already observed with atrazine (Kearney et al., 1977), but it is more important here because the compounds considered are stronger bases, which, on the one hand, can give higher amounts of *N*-nitroso derivatives, but, on the other, are more intensely bound to soil (Mortland, 1970), especially at low pH.

As nitrates already present in soil or added as fertilizers are converted to nitrites by soil microorganisms (Manahan, 1991), the formation of *N*-nitrosoamines from pesticides is considered a risk for the environment. The results of the present paper on the possible formation and stability of *N*-nitrosoterbuthylazine and *N*-nitrosoterbutryn, compared with the data collected in the past on *N*-nitrosoatrazine (Kearney et al., 1977), seem to indicate that their formation in common agricultural soils (pH 5.0–7.0) is more remote than in the case of cyanazine (Zwickenpflug and Richter, 1994). Therefore, the use of these herbicides seems not to be an environmental threat from this point of view. However, in very acidic soils these herbicides could undergo nitrosation, giving rise to transient *N*-nitroso derivatives that could be the precursor of metabolites which could interact with biological materials such as proteins or DNA. Although soil could immobilize the *N*-nitroso derivatives, further work is needed to establish the complete safety of these herbicides.

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