Kinetics and Mechanism of Amitraz Hydrolysis

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As a precursor to the development of effective vat management and waste disposal strategies, the kinetics and basic mechanisms of amitraz, *N*′-(2,4-dimethylphenyl)-*N*-[[(2,4−dimethylphenyl)imino]-methyl]-*N*-methylmethanimidamide, hydrolysis were examined as was the effect of cosolvents and metal ions. Amitraz was readily hydrolyzed at low pH values, forming acid-stable 2,4-dimethylphenylformamide, which can be further hydrolyzed to 2,4-dimethylaniline. The hydrolysis of 2,4-dimethylphenylformamide was faster under basic conditions. Thus, the addition of lime, a management technique used to stabilize the amitraz, will enhance the hydrolysis of its degradation products to aniline.

Keywords: *Amitraz; hydrolysis; pesticide waste; dip-vats*

INTRODUCTION

Amitraz, *N*′-(2,4-dimethylphenyl)-*N*-[[(2,4-dimethylphenyl)imino]methyl]-*N*- methylmethanimidamide, a formamidine pesticide initially developed for use on deciduous fruit and citrus mites, is also effective against mange mites on livestock and ticks on cattle (Bonsall and Turnbull, 1983; Ware, 1989). Amitraz has moderate mammalian toxicity, is acutely toxic to fish, and may affect avian reproduction (Aziz and Knowles, 1973; Benezet and Knowles, 1976; Benezet *et al.*, 1978; Rieger *et al.*, 1980; Bonsall and Turnbull, 1983; Jones, 1990). Tactic EC, a formulated product of amitraz, is widely used in Puerto Rico to control ticks, specifically *Boophilus microplus* and *Amblyomma variegatum*. Mobile and stationary spray vats of up to 200 gal are used to apply the pesticide to cattle and livestock; however, large quantities of semiconcentrated (*ca.* 250 ppm) pesticide waste are generated. Amitraz is being evaluated as an alternative for tick eradication where coumaphos is currently used. Approximately 100 000 gal of pesticide waste is generated annually from 42 dip-vats on the Texas−Mexico border (Agricultural Research Service, 1996).

Amitraz can also be hydrolyzed, giving rise to 2,4-dimethylphenylformamide and *N*-2,4-dimethylphenyl-*N*-methylformamidine, both of which can be further hydrolyzed to 2,4-dimethylaniline (Figure 1) (U.S. Environmental Protection Agency, 1996). Thus, stoichiometrically, 1 mol of amitraz will give rise to 2 mol of 2,4-dimethylaniline. More importantly, 2,4-dimethylaniline is also toxic, with an acute oral LD_{50} of 467 mg/kg for rats, almost half that of the parent pesticide (Vernot *et al.*, 1977). To develop effective vat management and waste disposal strategies, the fate of amitraz in treatment vats must be studied. The purpose of this study was to examine and quantitate the kinetics and mechanisms of amitraz hydrolysis and the effect of cosolvents and metal ions on the hydrolysis of amitraz.

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MATERIALS AND METHODS

Standards and Reagents. Amitraz, 2,4-dimethylphenylformamide, and *N*′-(2,4-dimethylphenyl)-*N*-methylformamidine were obtained gratis from AgrEvo, Analytical Services (Wilmington, DE). 2,4-Dimethylaniline and salts (all of analytical grade) were purchased from Aldrich (Milwaukee, WI) and used without further purification. Tactic EC was purchased from Animal Medic, Inc. (Manchester, PA). Buffered solutions were prepared by combining appropriate volumes of 0.067 M potassium dihydrogen phosphate and 0.067 M disodium phosphate for pH values between 5 and 8 and 0.0125 M sodium tetraborate for pH values of 8−10 with high-purity water. Solutions were adjusted with hydrochloric acid (0.1 M) or sodium hydroxide (0.1 M) to the required pH.

Hydrolysis of Amitraz. Experiments were conducted three to five times. To determine the effects of cosolvents, solutions of 8 ppm of amitraz in 25, 30, 40, and 50% methanol or acetonitrile in buffered water were prepared and the loss of amitraz was monitored. A slight increase in the initial solution pH was observed when acetonitrile was used as a cosolvent. This was likely due to a cosolvent-induced increase in electrode liquid-junction potential, and, as such, the hydrolysis rates reported reflect the pH measured prior to acetonitrile addition.

Hydrolysis experiments were conducted using 8−16 ppm of amitraz in 25% acetonitrile/buffered or nonbuffered water and monitored by HPLC. The effect of metal ions was examined by adding one of the following metal sulfates or nitrates to pH 5 buffered acetonitrile/water: $ZnSO_4 \cdot 7H_2O$, $MgSO_4$, $Cu(NO_3)_2 \cdot 3H_2O$, $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, and $MnSO_4 \cdot H_2O$, for a final metal concentration of 10^{-4} M.

Some experiments were conducted with commercial product: 1 mL of Tactic EC was diluted in 500 mL of tap water (no acetonitrile) to achieve a concentration equivalent to the recommended amitraz treatment dosage of 250 ppm. The pH was determined and the reaction monitored. During the first 10 days of the experiment, a precipitate appeared, which was collected by filtering a 10 mL aliquot through a 5 μm filter. The filtered solid was dissolved in acetonitrile and analyzed by HPLC.

HPLC Analysis. Samples were analyzed directly by HPLC employing (1) two Gilson (Middleton, WI) Model 303 HPLC pumps equipped with a Model 715 controller, a Model 210 autosampler, and a Model 116 UV detector, monitoring at 288 and 240 nm, or (2) a Waters (Milford, MA) Model 616 LC and Millennium system equipped with two 510 pumps, a Model 717 autosampler, and a Model 996 photodiode array detector. Separations were achieved using a sequence of linear gradients, 40% (6 min), 40−85% (1 min), 85% (9 min) acetonitrile

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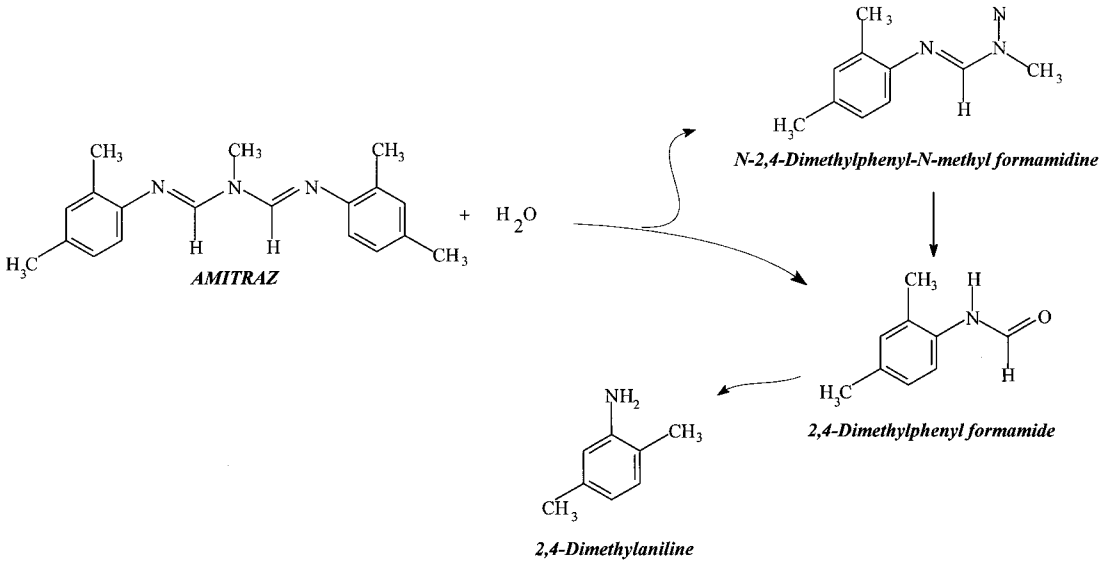

Figure 1. Hydrolysis pathway of amitraz in aqueous solutions.

Table 1. Pseudo-First-Order Rate Constants for Amitraz

pH	% cosolvent	k (h^{-1})/r^2	$t_{1/2}$ (h)
3.24	25	1.57/0.997	0.4
4.15	25	2.22×10^{-1}/0.982	3.1
5.09	25	8.09×10^{-2}/0.997	8.6
5.09	30	7.93×10^{-2}/0.993	8.7
5.09	40	7.47×10^{-2}/0.998	9.3
5.09	50	6.51×10^{-2}/0.997	11
5.63	25	5.42×10^{-2}/0.990	13
7.02	25	1.18×10^{-2}/0.987	59
7.57	25	9.39×10^{-3}/0.999	74
9.12	25	6.18×10^{-3}/0.992	112

in water, at a flow rate of 1.5 mL/min on a Beckman (Fullerton, CA) C_{18} (ODS 5 μm), end-capped 4.6 mm × 25 cm steel-jacketed column. Peak identification was established by comparison of the retention times and UV spectra with standards.

RESULTS AND DISCUSSION

Amitraz Hydrolysis and Product Fate. Solubility limitations of analytical grade amitraz required the use of a cosolvent to achieve suitable concentrations for analysis of the parent material and its products. Amitraz was unstable in pure methanol, rapidly hydrolyzing to form 2,4-dimethylphenylformamide, *N′*-(2,4-dimethylphenyl)-*N*-methylformamidine, and an unknown product (data not shown). However, amitraz was stable in acetonitrile. Negligible effects on the rate and product profile were observed at cosolvent concentrations of 25, 30, and 40%. A marked decrease was seen in the hydrolysis rate at 50%, probably due to protective dehydration of amitraz (Table 1). Reductions in cosolvent concentration below 25% resulted in parent compound precipitation.

The hydrolysis of amitraz was more rapid under acidic conditions and afforded 2,4-dimethylphenylformamide via cleavage of the carbon−nitrogen bond of the methanimidamide (Figure 2). After an initial delay of 22 h to 62 days, 2,4-dimethylaniline was also observed. The simultaneous formation of *N′*-(2,4-dimethylphenyl)-*N*-methylformamidine was contraindicated as the compound was not detected. It is likely that the instability of the formamidine, which can degrade to the formamide, precluded any detectable accumulation of formamidine.

2,4-Dimethylphenylformamide can further hydrolyze to yield 2,4-dimethylaniline. However, as Figure 2 illustrates, no changes in the concentrations of 2,4-dimethylphenylformamide and 2,4-dimethylaniline were observed after 175 days at pH 5.09 and 7.02. Formamide hydrolysis is base-catalyzed and is much slower than amitraz hydrolysis: $t_{1/2}$ at pH 9.12 *ca.* 300 days. Throughout all of the hydrolysis experiments, the total amount of 2,4-dimethylaniline and the formamide was approximately equal to twice the concentration of the amitraz lost.

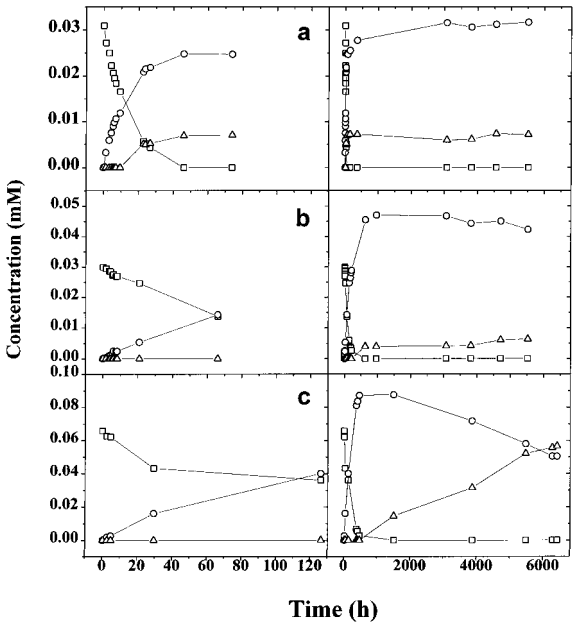

Figure 2. Product profile of amitraz hydrolysis: (a) pH 5.09, (b) pH 7.02, and (c) pH 9.12; (□) amitraz, (○) 2,4-dimethylphenylformamide, (△) 2,4-dimethylaniline.

The rate of amitraz hydrolysis is pseudo-first-order as shown in the linear plots of ln([amitraz]/[amitraz]$_0$) versus time, eq 1 (Figure 3). The pseudo-first-order rate

$$\ln[\text{amitraz}]/[\text{amitraz}]_0 = -k_{obs}t \qquad (1)$$

constants (k_{obs} in h^{-1}) and r^2 values are shown in Table 1. The observed rate constant, k_{obs}, is described in eq 2. Since no base-catalyzed hydrolysis was observed for amitraz, the term $k_{OH^-}[OH^-]$ can be neglected. Performing a least-squares fit of the data, k_{H^+} (acid-

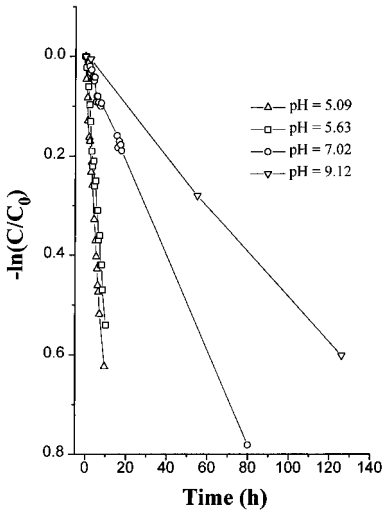

Figure 3. First-order fit for amitraz hydrolysis at pH 5.09, 5.63, 7.02, and 9.12.

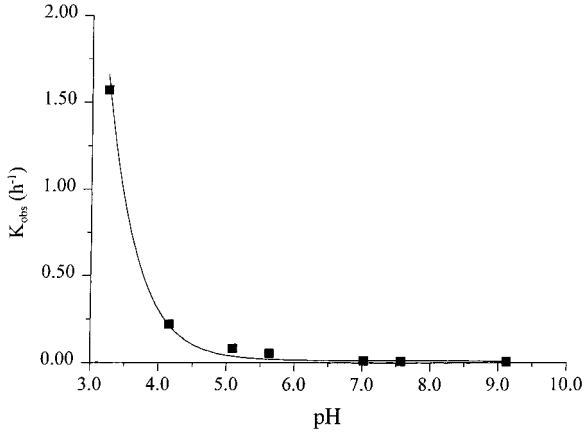

Figure 4. Least-squares fit of k_{obs} values at pH 3.24, 4.15, 5.09, 5.63, 7.02, 7.57, and 9.12.

catalyzed hydrolysis) equals 2862 ± 553 M^{-1} h^{-1} and k_n (unassisted or neutral hydrolysis) equals (1.13 × 10^{-2}) ± 0.006 h^{-1}. Figure 4 shows the least-squares fit curve with the observed values.

$$k_{obs} = k_{H^+}[H^+] + k_n + k_{OH^-}[OH^-] \qquad (2)$$

The addition of 10^{-4} M Zn^{2+}, Mg^{2+}, Cu^{2+}, Al^{3+}, Ni^{2+}, and Mn^{2+} did not have a significant effect on the hydrolysis of amitraz, at 10^{-5} M, or on the degradation of its products (data not shown). Further study is necessary to determine the fate of amitraz following treatment of cattle and livestock, since spray-dip and dip-vat conditions, *i.e.* suspended solids, organic matter, soil, etc., would also affect hydrolysis.

Finally, the hydrolysis rate of amitraz in the diluted formulated product, Tactic EC, was substantially slower than predicted: $t_{1/2}$ = 80 days at pH 8.3. A precipitate formed during the reaction, which was found to be amitraz. Precipitation of amitraz during the reaction and possible dehydration of the active ingredient by formulation components, such as organic cosolvents and surfactants, may have been responsible for the decreased hydrolysis rate.

Significance. Amitraz is readily hydrolyzed at low pH values, forming acid-stable 2,4-dimethylphenylformamide. A common management method for dip-vats is the addition of lime, which neutralizes uric acid, increases pH, and stabilizes the parent compound. Unfortunately, hydrolysis of the formamide is base-catalyzed, forming 2,4-dimethylaniline, which is stable in aqueous systems. Thus, despite the decreased hydrolysis rate of amitraz associated with the formulation components and possible lime addition, an environmental impact or risk may remain due to the presence 2,4-dimethylphenylformamide and 2,4-dimethylaniline. Additional studies are necessary to identify management strategies and develop disposal options for spray-dip and dip-vat waste.

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