

Stability of 3-Chloro-*p*-toluidine Hydrochloride in Buffered Aqueous Solutions

Bruce A. Kimball* and Elizabeth A. Mishalanie†

United States Department of Agriculture, Animal and Plant Health Inspection Service, Denver Wildlife Research Center, Building 16, Denver Federal Center, Denver, Colorado 80225

The significance of hydrolysis as a dissipation mechanism for 3-chloro-*p*-toluidine hydrochloride was studied in aqueous solutions buffered at pH 5, 7, and 9. High-performance liquid chromatography with ultraviolet detection was used to assess the stability of the compound in the sample solutions at several intervals over 31 days. Experiments were conducted with polytetrafluoroethylene sample containers to circumvent adsorption of the free base (3-chloro-*p*-toluidine) on borosilicate glass. The results indicated that 3-chloro-*p*-toluidine hydrochloride does not significantly hydrolyze in pH 5, 7, or 9 buffer solutions at 25 °C over 31 days.

Introduction

Background. Approximately 1.2 billion lb of pesticides are sold each year in the United States (1). Many of these chemicals are used in terrestrial applications and may find their way into surface water and groundwater systems. Thus, information describing a pesticide's fate and persistence allows for the estimation of pesticide concentrations in natural wildlife habitats.

The compound of interest, 3-chloro-*p*-toluidine hydrochloride (CPT HCl), is a minor-use avicide which was initially registered for use in 1967 after several years of investigation by the U.S. Fish and Wildlife Service (2). It was found to be highly toxic to target species (starlings, blackbirds, ravens, crows, gulls, and pigeons) yet much less toxic to mammals and predacious birds. CPT HCl is rapidly metabolized and excreted and is not accumulated in the target species (3). Death to susceptible species is attributed primarily to nephrotoxicity. Only 200 lb of CPT HCl are used annually for the control of pest birds (4).

The U.S. EPA requires pesticide registrants to conduct laboratory and field studies as specified by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Conduct of environmental fate studies is defined by the EPA's Pesticide Assessment Guidelines (PAG), Subdivision N (5). Data generated from environmental fate studies are used by the EPA to assess potential hazards and risks associated with the application of a pesticide. The types of studies described in Subdivision N include the following: degradation, metabolism, mobility, dissipation, and accumulation studies. The nature of the studies required by the EPA is determined by the use pattern of the pesticide.

The EPA has determined that hydrolysis, aqueous photodegradation, aerobic soil metabolism, leaching/adsorption/desorption, and fish bioaccumulation studies are required to maintain the registration of CPT HCl for terrestrial, nonfood uses. In addition to the hydrolysis study, the aquatic photodegradation study has recently

been performed (6). Investigation of 1 ppm CPT HCl solutions in water buffered with 1 mM phosphate (pH 7) demonstrated that the first-order photolysis reaction has a minimum rate constant of 0.075 h⁻¹ and a maximum half-life of 9 h when exposed to conditions that simulate clear skies at 40° N latitude. The major hydrolysis product formed is the phenol generated by nucleophilic substitution of chlorine by hydroxide. The study was performed at 25 °C.

Hydrolysis. Terrestrial applications of agricultural chemicals rarely result in concentrations of the compound exceeding 10⁻⁶ M in environmental water systems (7). In addition, most natural waters have considerable buffering capacity, and therefore, the acid or base concentration remains quite constant during a hydrolysis reaction (8). These contributing factors result in hydrolytic degradation following pseudo-first-order reaction kinetics in the environment. Furthermore, most hydrolysis reactions are first order with respect to the chemical with few exceptions (9).

The hydrolysis experiments described in this paper were designed in accordance with general guidelines for performing environmental fate studies (5, 10, 11). A study duration of 30 days is prescribed in the PAGs and considered sufficient to establish if hydrolysis is a significant degradation pathway. If less than 10% of the pesticide hydrolyzes over the 30-day period, it is considered hydrolytically stable for the purposes of assessing environmental fate (5). To isolate hydrolytic degradation from other environmental dissipation mechanisms, hydrolysis experiments are performed under controlled laboratory conditions. Temperature and pH are strictly maintained to characterize the kinetics of the hydrolysis reaction. This study was conducted at 25 °C and at pH 5, 7, and 9. A total buffer concentration of 0.01 M and a CPT HCl concentration of 20.9 µg/mL were chosen to minimize the potential for buffer catalysis (7).

Experimental Section

Reagents. Analytical-grade CPT HCl (98%) was provided by Purina Mills Inc. (St. Louis, MO). Preformulated buffers were used to prepare two of the three 0.01 M buffer solutions: The pH 7 solution was prepared from a 0.05 M potassium phosphate monobasic/sodium hydroxide solution, and the pH 9 solution was prepared from a 0.1 M boric acid/potassium chloride/sodium hydroxide solution (Fisher Scientific, Pittsburgh, PA). These buffers were diluted to 0.01 M with HPLC-grade water. The pH 5 buffer solution was prepared with sodium acetate (6.6 mM) and acetic acid (3.3 mM). Formalin was added to this solution at a concentration of 0.05% (v/v) to minimize microbial growth. The pH of the 0.01 M buffer solutions was determined after preparation and adjusted, if necessary, to within 0.01 pH unit of the desired value.

Apparatus. Test and control solutions were maintained at 25 °C in a refrigerated, circulating water bath (Model 2325, Forma Scientific Inc., Marietta, OH) and

* Present Address: United States Environmental Protection Agency, Office of Enforcement, National Enforcement Investigations Center, Building 45, Denver Federal Center, Denver, Colorado 80225.

were protected from light to minimize photochemical degradation. Borosilicate glass screw-cap culture tubes and 40-mL PTFE (Teflon) screw-cap culture tubes were used to contain the solutions. Special precautions were not taken to trap volatile hydrolysis products.

Preparation of Test Solutions and Controls. A 1.00 mg/mL solution of CPT HCl in water was prepared prior to the initiation of the experiments. This concentrated solution was tightly sealed, refrigerated, and maintained in the dark when not in use. Triplicate 20.9 $\mu\text{g/mL}$ test solutions were prepared at each pH by diluting the concentrated CPT HCl solution with the pH 5, 7, or 9 buffer. Triplicate control solutions (pH 5, 7, or 9 buffer only) were prepared and maintained under conditions identical to the test solutions. Test solutions and controls were placed in 50-mL screw-cap culture tubes.

The headspace in the tubes was less than 2 mL at the beginning of the experiments. The tubes were capped, sealed with Parafilm around the neck of the tube, and placed in plastic test tube racks in the constant temperature bath. The water level in the bath was maintained above the level of the solutions in the tubes. A calibrated thermometer was placed in the bath to monitor the temperature throughout the study period and the bath was covered to protect the samples from light.

Preparation of Working Standards. Working standards were freshly prepared from the concentrated CPT HCl solution at each sampling interval. The working standard concentration was 20.9 $\mu\text{g/mL}$ CPT HCl in each buffer.

Chromatographic Analysis. A Hewlett-Packard Model 1090M HPLC equipped with an ultraviolet (UV) diode-array detector (DAD), autosampler, and computer workstation (Hewlett-Packard Co., Palo Alto, CA) was used throughout the study. The HPLC column was a 4.6 mm \times 25 cm, 5 μm octadecylsilane (ODS) analytical column (Alltech Associates, Deerfield, IL).

Reversed-phase high-performance liquid chromatography (RP-HPLC) was used to determine the CPT HCl concentration in the test solutions (measured as the free base, 3-chloro-*p*-toluidine or CPT). The mobile phase consisted of 80% acetonitrile and 20% water with a flow rate of 1.0 mL/min. The injection volume was 10 μL . Chromatographic data for the quantitation of CPT were acquired at 241 nm. Additional data were obtained at 220, 280, 320, and 365 nm to screen for the appearance of UV-absorbing hydrolysis products. A typical chromatogram is shown in Figure 1.

Instrument Limits of Detection. The instrument limit of detection for CPT HCl in each buffered solution was also determined during the study. The limit of detection is defined as the analyte concentration required to produce a chromatographic peak response equal to three times the peak-to-peak noise. The CPT HCl limits of detection were determined from the injection of 0.1 $\mu\text{g/mL}$ of CPT HCl solutions prepared with the appropriate buffer at each pH. The limit of detection was determined to be 0.07 $\mu\text{g/mL}$ CPT HCl in each of the three buffer solutions.

Procedure. The CPT HCl concentration was determined in each solution at nine intervals over 31 days. Approximately 1–2 mL of each test and control solution was removed from the screw-cap tubes and placed in HPLC autosampler vials for qualitative and quantitative analysis by HPLC. At each interval the pH of one of the triplicate

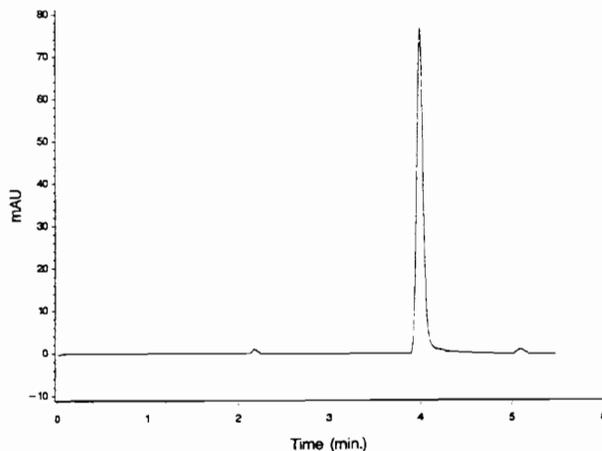


Figure 1. Chromatogram of a pH 7 test solution. Column, 4.6 mm \times 25 cm octadecylsilane; flow, 1.0 mL/min; mobile phase, 80:20 ACN: water; injection volume, 10 μL ; detection, UV 241 nm.

test solutions and one of the triplicate control solutions was also measured. The replicate solutions were alternated for subsequent pH measurements so that each individual solution was measured three times during the 31-day study. All test and control solutions were returned to the constant temperature bath immediately after sampling.

Results and Discussion

Storage Stability of Concentrated CPT HCl Solution. The storage stability of the concentrated solution was demonstrated by performing CPT HCl analyses on a refrigerated 1.00 mg/mL aqueous solution of CPT HCl over a period of 4 months. The mean value of percent CPT HCl present (relative to initial concentration) was 99.8% ($n = 14$, $\text{SD} = 2.1\%$). The data indicate that the refrigerated solution could be used throughout the experiment to prepare fresh working standard solutions.

CPT HCl Solubility in Buffer Solutions. The solubility of CPT HCl in the test solutions was evaluated from solutions with CPT HCl concentrations near 100 $\mu\text{g/mL}$, which is five times the test solution concentration. Working standards at 73.5, 99.8, and 126 $\mu\text{g/mL}$ were prepared in each buffer system and each solution was analyzed for CPT HCl in duplicate by HPLC. Linear regression analysis of the data demonstrated that the relationship between CPT HCl concentration and detector response was linear for each buffer solution according to Beer's law. Since Beer's law was obeyed over this range, CPT HCl would be expected to be completely soluble in each buffer at the lower test concentration of 20.9 $\mu\text{g/mL}$. Additionally, work in this laboratory has shown the solubility of CPT HCl in water to be approximately 9% (w/v).

Adsorption of CPT to Borosilicate Glass. The experiments were originally initiated with borosilicate glass screw-cap tubes as sample containers. Approximately 16 days into the experimental period, significant loss (approaching 10%) of CPT HCl was observed at each pH. The data were fit to the first-order reaction model hypothesized for hydrolysis. The data indicated that the degradation was not first order with respect to CPT HCl. Therefore, hydrolysis was not considered a likely mechanism for the observed loss, and irreversible adsorption of the compound to the glassware was suspected to be the cause of this behavior.

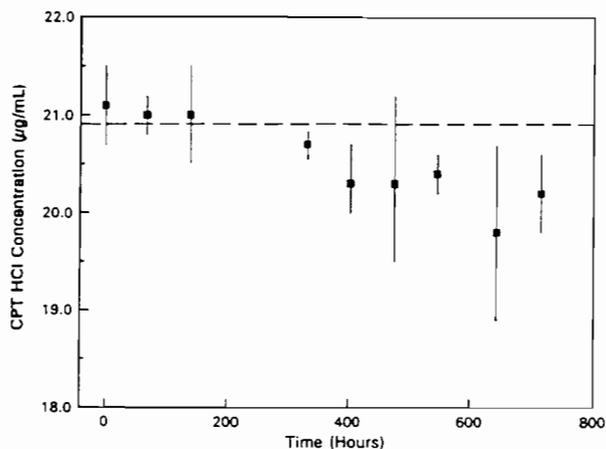


Figure 2. Results of 31-day CPT HCl hydrolysis study at 25 °C. Concentration vs time at pH 5 ($p = 0.0001$, slope = -0.00157).

Table 1. Test and Control Solution pH Data ($n = 9$)

nominal pH	test solutions			control solutions		
	mean	SD	RSD (%)	mean	SD	RSD (%)
5	5.08	0.01	0.2	5.10	0.02	0.4
7	7.02	0.01	0.1	7.05	0.02	0.3
9	9.00	0.02	0.2	8.99	0.02	0.2

Aromatic amines are known to display great affinities for adsorption to glass surfaces. Since the acid dissociation constant of $CPTH^+$ is 2×10^{-4} ($pK = 3.7$), the free base (CPT) is the main species present in each buffer solution. Previous work in this laboratory indicated that CPT is adsorbed on glass. The experiment was repeated using PTFE sample tubes to minimize CPT adsorption to sample container surfaces. Only data obtained from conducting the hydrolysis experiment in PTFE sample tubes are presented here.

Determination of Hydrolysis Rate. The H_3O^+ concentration was carefully controlled during the experiment. The pH of the test and control solutions was measured at each sampling interval to ensure that the H_3O^+ concentration did not vary. The mean, standard deviation (SD), and relative standard deviation (RSD) of the pH data are provided in Table 1. The data indicate that experimental pH was maintained and no changes in test solution pH occurred due to sample handling. The water bath maintained the temperature of the solutions at a mean temperature of 25.1 °C and ranged from 25.0 to 25.5 °C.

The CPT HCl concentration in the test solutions was plotted as a function of time to assess the stability of the compound at each pH. These data are shown in Figures 2-4 for pH 5, 7, and 9 test solutions, respectively. The dashed line at 20.9 µg/mL represents the calculated initial test solution concentration. The mean concentration of three replicate test solutions is plotted with error bars indicating the 95% confidence interval. The relative standard deviations obtained from the analyses of the replicates were typically less than 1%.

A linear regression was performed on the test solution concentration data to test the null hypothesis that the slope of the zero-order reaction model is equal to zero. If the slope is zero, no correlation exists between concentration and time and no degradation occurred. The data indicate a negative correlation between CPT HCl concentration and time for the experiment conducted at pH 5 ($p = 0.0001$, slope = -0.00157 , $r^2 = 0.731$). The first-

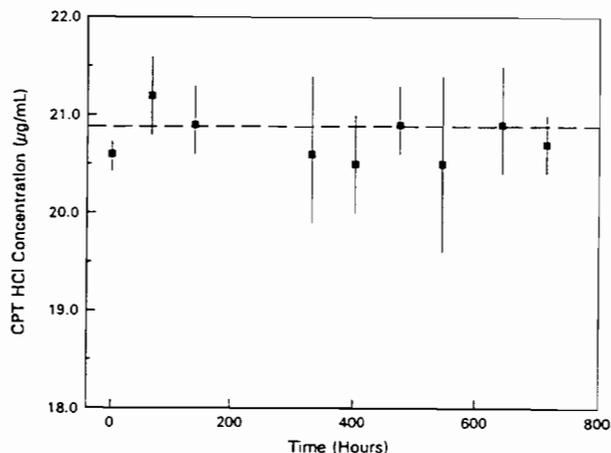


Figure 3. Results of 31-day CPT HCl hydrolysis study at 25 °C. Concentration vs time at pH 7 ($p = 0.449$).

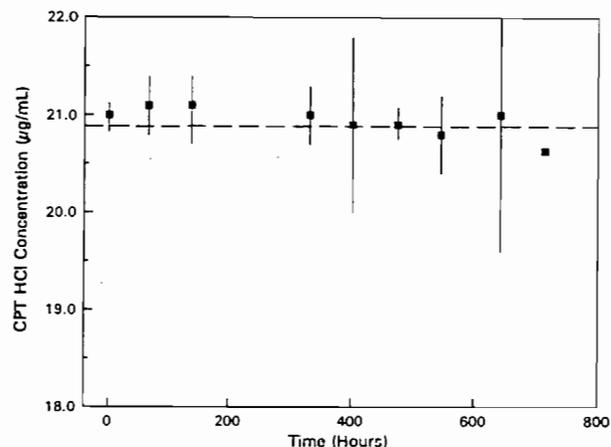


Figure 4. Results of 31-day CPT HCl hydrolysis study at 25 °C. Concentration vs time at pH 9 ($p = 0.0287$, slope = 0.000424).

order model was subsequently tested using the pH 5 data. Plotting $\ln([A]/[A]_0)$ vs time demonstrated that these data did not fit the first-order reaction model (slope = -0.0000765 , $r^2 = 0.725$). While it is possible that a first-order reaction may be occurring, sufficient degradation did not occur in 30 days. To fully characterize the kinetics of this degradation, the experiment must be conducted for a period extending at least two half-lives.

Similarly, the data did not fit second- or third-order reaction models with respect to CPT HCl. This observed loss of CPT HCl may be attributed to slow hydrolysis and/or buffer catalysis. Microbial activity was not visually evident in any of the test or control solutions. This loss is not considered significant according to the parameters dictated for conducting this type of study since the degradation did not exceed 10% over the 31-day period.

Data for the hydrolysis experiment conducted at pH 7 and 9 were also subjected to the zero-order model. The linear regression data indicated no detectable correlation between CPT HCl concentration and time at pH 7 ($p = 0.449$). Therefore, no degradation occurred. A negligible correlation was noted for the pH 9 data ($p = 0.0287$, slope = -0.000424 , $r^2 = 0.177$). Since the correlation is even less significant than that observed with the pH 5 data, fitting the data to a reaction model does not yield any useful information concerning the kinetics of the degradation. Again, more data are required to accurately determine the reaction kinetics.

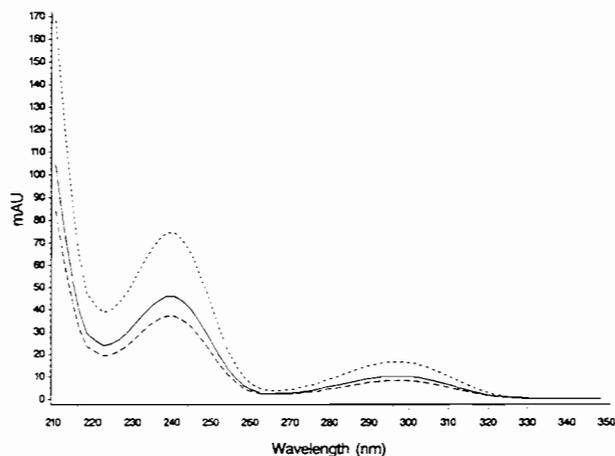


Figure 5. Peak purity spectra of CPT in pH 7 test solution (chromatogram shown in Figure 1). (—) UV spectrum at retention time of 3.956 min; (---) UV spectrum at retention time of 3.995 min; (- -) UV spectrum at retention time of 4.022 min.

Appearance of Hydrolysis Products. Qualitative assessment of the chromatographic data obtained with the DAD at 220, 241, 280, 320, and 365 nm indicated that no UV-absorbing compounds could be detected in the test solutions that were not present in the freshly prepared working standards. Additionally, peak purity data obtained from the recorded spectral data at the upslope, apex, and downslope of the CPT chromatographic peak indicated that CPT was the primary UV absorbing species producing the chromatographic response. Hydrolysis products with significant molar absorptivities, which were retained by the chromatographic column under these conditions could have been detected by a chromatographic response at one or more of the multiple wavelengths. Products with significantly different spectra than that of CPT would have been detected by the peak purity test. Spectra obtained from the peak purity assessment are provided (Figure 5) for a pH 7 test solution from the last analysis interval (31 days or 730 h).

Conclusion

The hydrolysis data generated by this study indicate that the compound is stable in water under dark conditions for the 30-day period required to assess the significance of hydrolysis as an environmental dissipation mechanism. Therefore, simple hydrolysis would not be considered an important degradation route for CPT HCl in the environment, particularly groundwater. However, our observations of the adsorptive behavior of CPT HCl with glass indicate that adsorption of this compound to siliceous matter would constitute a significant pathway for the elimination from aqueous systems. Aquatic photolysis

would probably be the primary degradation pathway of CPT HCl in surface water.

Acknowledgments

The authors wish to thank Doreen McHugh for providing the storage stability data for the concentrated CPT HCl solutions and N. Paige Groninger for her assistance with the figures. Mention of commercial products is for identification only and does not constitute endorsement by the United States Department of Agriculture or the United States Environmental Protection Agency.

Literature Cited

- (1) Swanson, R. G. *Endangered Species Tech. Bull.* 1990, 15 (1), 8-12.
- (2) Schafer, E. W. *Proc. Vertebr. Pest Conf.* 1984, 11, 217-222.
- (3) Schafer, E. W. In *CRC Handbook of Pest Management in Agriculture*; Pimentel, D., Ed.; CRC Press: Boca Raton, FL, 1991; Vol II, pp 599-610.
- (4) Dell'Orco, L. PM Resources, personal communication, 1993.
- (5) *Pesticide Assessment Guidelines Subdivision N, Chemistry: Environmental Fate*; U.S. Environmental Protection Agency, Office of Pesticide Programs; U.S. Government Printing Office: Washington, DC, 1982; EPA-540/9-82-021.
- (6) Yao, D.; Mill, T. *Aquatic Photolysis Study of 3-Chloro-p-toluidine Hydrochloride*. Unpublished Report-QA-268. SRI: Menlo Park/Denver Wildlife Research Center, Denver, CO, 1993.
- (7) Maby, W.; Mill, T. *J. Phys. Chem. Ref. Data* 1978, 7, 383-415.
- (8) Wolfe, N. L.; Zepp, R. G.; Baughman, G. L.; Fincher, R. C.; Gordon, J. A. *Chemical and Photochemical Transformation of Selected Pesticides in Aquatic Systems*; U.S. Environmental Protection Agency, Ecological Research Series; U.S. Government Printing Office: Washington, DC, 1976; EPA-600/3-76-067.
- (9) Smith, J. H.; Mabey, W. R.; Bohonos, N.; Holt, B. R.; Lee, S. S.; Chou, T. W.; Bomberger, D. C.; Mill, T. *Environmental Pathways of Selected Chemicals in Freshwater Systems, Part I: Background and Experimental Procedures*; U.S. Environmental Protection Agency, Interagency Energy-Environment Research and Development Report, U.S. Government Printing Office: Washington, DC, 1977; EPA-600/7-77-113.
- (10) *Environmental Fate Data Requirements*; Code of Federal Regulations, 40, Part 158.290; National Archives and Records Administration, Office of the Federal Register, U.S. Government Printing Office: Washington, DC, 1991.
- (11) *Hazard Evaluation Division, Standard Evaluation Procedure: Hydrolysis Studies*; U.S. Environmental Protection Agency, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC, 1985; EPA-540/9-85-013.

Received for review May 17, 1993. Revised manuscript received November 29, 1993. Accepted December 13, 1993.*

* Abstract published in *Advance ACS Abstracts*, January 15, 1994.