Determination of Kinetics of Hydrolysis by High-Pressure Liquid Chromatography: Application to Hydrolysis of the Ethylene Glycol Butyl Ether Ester of Triclopyr

Sunny Y. Szeto

Agriculture Canada Research Station, 6660 Northwest Marine Drive, Vancouver, British Columbia, Canada V6T 1X2

Using a high-pressure liquid chromatographic (HPLC) method capable of determining 3,5,6-trichloro-2-pyridinol (P), triclopyr (T), and the ethylene glycol butyl ether ester of T (G) in aqueous media without extraction or cleanup, the pseudo-first-order rate constants for the hydrolysis of G were established at 35 °C in aqueous buffered solutions of pH 4.5–8.5. The reaction was base-catalyzed and the rate proportional to the pH ranging from 3.63×10^{-4} to 0.1596 h^{-1}. The major conversion product was T at pH 4.5–8.5, and P was an additional minor product at pH 4.5. Both products were stable in aqueous media. The hydrolysis of G was determined according to the same approach in two river waters of pH 6.6 and 7.6. Their kinetics were in agreement with those in aqueous buffers of the same pH with low ionic strength to reduce or eliminate buffer catalysis.

INTRODUCTION

To predict the fate and transport processes of pesticides in the aquatic environment requires a mathematical model based on the understanding of hydrolytic pathways and kinetic data over the normal pH ranges of the aquatic environment. In kinetic studies, a prescribed amount of pesticide is usually added to an aqueous medium to monitor the disappearance of reactants and the formation of any hydrolytic products under the given conditions. Many pesticides include a chromophore in their molecular structure. Therefore, reversed-phase high-pressure liquid chromatography and UV absorption (HPLC–UV) allows the hydrolytic reaction to be monitored easily by directly injecting an aliquot of the reaction mixture without extraction or cleanup or other tedious sample preparation. This approach has been successfully applied by Szeto *et al.* (1989) to determine the kinetics of hydrolysis of the dicarboximide fungicide vinclozolin. Their HPLC–UV methods could determine vinclozolin and its hydrolytic products simply by the injection of an aliquot of the reaction mixture without sample preparation.

Garlon 4, an emulsifiable concentrate [EPA (U.S.) Reg. No. 464554], is a broad spectrum herbicide manufactured and marketed by Dow Chemical U.S.A., Midland, MI 48667. It contains 61.6% of the ethylene glycol butyl ether ester of triclopyr (G) (Figure 1). G was selected for determining the kinetics of its hydrolysis by HPLC–UV because it includes a chromophore, the 3,5,6-trichloro-2-pyridinyl moiety. Furthermore, it is important to determine the rate of conversion of G to the active herbicidal ingredient, triclopyr [[(3,5,6-trichloro-2-pyridinyl)oxy]-acetic acid (T)], and whether 3,5,6-trichloro-2-pyridinol (P) would be produced upon hydrolysis (Figure 1). G, T, and P are known to be toxic to salmonids. The toxicities of G and P are similar, and T is less toxic than G and P (Wan *et al.*, 1987). Using an approach similar to that of Szeto *et al.* (1989), the kinetics of hydrolysis of G were examined in several buffers and two river waters. The results are reported here.

EXPERIMENTAL PROCEDURES

Reagents. G (99.1%), T (99.1%), and P (99%) were provided by Dow Chemical U.S.A. All solvents were of HPLC grade (Burdick & Jackson Laboratories, Inc., Muskegon, MI 49442)

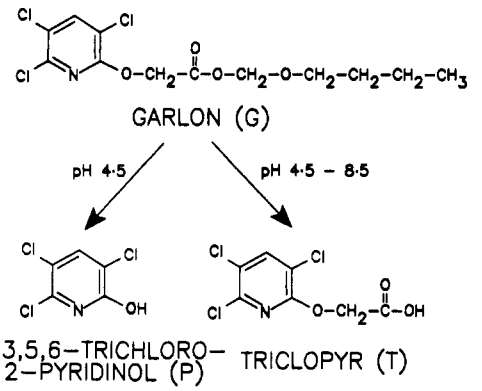

Figure 1. Proposed degradation pathway for the hydrolysis of the ethylene glycol butyl ether ester of triclopyr (G) leading to the formation of triclopyr (T) and 3,5,6-trichloro-2-pyridinol (P).

and all other chemicals of reagent grade (Fisher Scientific Co., Fair Lawn, NJ 07410). Two river waters of mountain origin were used in this study, one from the Capilano River (pH 6.6) in North Vancouver, BC, and the other from Tamihi Creek (pH 7.6), a tributary of the Fraser River about 150 km east of Vancouver.

Preparation of Aqueous Buffers. Buffered solutions of 0.01 M were prepared with sterilized, deionized water by adjusting the pH with the following solutions: mixing 1 L of 0.01 M NaH_2PO_4 with dropwise addition of 0.01 M H_3PO_4 to pH 4.5,; mixing appropriate volumes of 0.01 M NaH_2PO_4 and 0.01 M Na_2HPO_4 for pH 5.5, 6.5, 6.6, 7.0, 7.5, 7.6, and 8.0 according to the method of Dawson *et al.* (1969); mixing appropriate volumes of 0.01 M $Na_2B_4O_7$ and 0.04 M HCl for pH 8.5 (Dawson *et al.*, 1969). The 0.01 M buffered solutions of pH 6.6 and 7.6 were appropriately diluted with sterilized, deionized water to 0.001 and 0.002 M, respectively.

Incubation of G in Aqueous Buffers and Natural Waters. Aliquots of 1 mL of a stock solution of G at 1000 μg/mL in acetonitrile were thoroughly mixed with the aqueous buffers and natural waters to produce final concentrations of G at 10 μg/mL. The controls were similarly prepared to ensure that no UV response was produced during incubation in the absence of G. Aliquots of approximately 2 mL of the solutions were transferred to 2-mL brown ampules and sealed under nitrogen. The sealed ampules were incubated at 35 °C in a water bath in darkness; those of pH 7.0 were also incubated in darkness at 40, 45, 52, and 54 °C.

Analysis by High-Pressure Liquid Chromatography (HPLC). Concentrations of G and its conversion products, T

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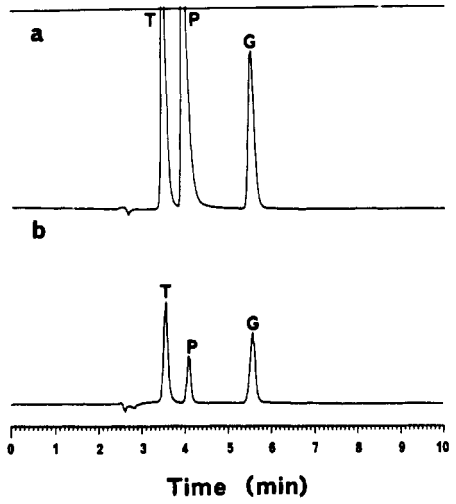

Figure 2. High-pressure liquid chromatograms of UV absorption at 300 nm: (a) triclopyr (T, 3.52 min), 3,5,6-trichloro-2-pyridinol (P, 4.06 min), and the ethylene glycol butyl ether ester of triclopyr (G, 5.54 min); (b) 20-μL aliquot of 10 μg/mL of G incubated in 0.01 M phosphate buffer of pH 4.5 at 35 °C for 110 days.

and P, present in the incubation solutions at various intervals were determined by HPLC with a Varian Model 5000 high-pressure liquid chromatograph equipped with a Hewlett-Packard Model 1040A high-speed spectrophotometric detector. The operating parameters were as follows: column, Beckman Ultrasphere ODS 5 μm, 4.6 (i.d.) × 250 mm; mobile solvent system, 85% methanol and 15% 0.05 M phosphate buffer of pH 2.4, isocratic at 1 mL/min; UV detector wavelength, 300 nm ± 2 nm. Aliquots of 20 μL of the incubated solutions were injected directly into the high-pressure liquid chromatograph for determination of G, T, and P.

Quantifications of G, T, and P were based on an external standard. Detector response was calibrated for each analysis with authentic reference standards and calculated from average peak areas of these external standards, which were injected before and after each sample.

RESULTS AND DISCUSSION

Hydrolysis of G in Buffered Solutions. Under the chromatographic conditions described, G, T, and P could be simultaneously determined by directly injecting a 20-μL aliquot of the incubation solution without extraction or cleanup. The absolute retention times were 3.52, 4.06, and 5.54 min for T, P, and G, respectively (Figure 2a). These results show that the HPLC approach is extremely efficient for determining the kinetic data of hydrolysis of pesticides which include a chromophore in their molecular structure.

In the range pH 4.5–8.5, which includes most natural water, G underwent hydrolysis readily. All data for the disappearance of G at 35 °C in aqueous buffers of this pH range and in the two river waters of pH 6.6 and 7.6 followed simple pseudo-first-order kinetics. Table I summarizes these data. On hydrolysis, G was converted to its acid, T (Figure 1). The rate of conversion was pH dependent, fast at basic pH but very slow at acidic pH. At pH 8.5 the half-life was 4.3 h, whereas at pH 4.5 it was about 1900 h (Table I). These findings suggest that Garlon 4, a commercial formulation of G, may readily be hydrolyzed in water, especially in slightly basic water. A linear relationship was indicated between the logarithm of the observed rate and the pH from 4.5 to 8.5 (Figure 3). By a least-squares method a linear regression was calculated as follows: $\log k_{obsd} = 0.7174\ pH - 6.9757$ ($n = 9$, $r = 0.9827$*, significant at $p = 0.05$). It was evident that the rate of disappearance of G was dependent on hydroxide ion concentration. The second-order rate constants (k_{OH^-}) were calculated from $k_{obsd}/[OH^-]$ and are also given in Table I.

To study the influence of temperature on the rate of hydrolysis, the rate constants at 35, 40, 45, 52, and 54 °C were determined for the hydrolysis of G in 0.01 M phosphate buffer of pH 7.0. The Arrhenius plot was established from the kinetic data generated (Table II). The linear regression of the Arrhenius plot of $\log k_{obsd}$ vs $1/T$ (Figure 4) was as follows: $\log k_{obsd} = -4114[1/T] + 11.3$ ($n = 5$, $r = 0.9953$*, significant at $p = 0.05$). The energy of activation and the frequency factor A for the hydrolysis of G at pH 7.0 were calculated to be 78.8 kJ mol^{-1} and 2.05×10^{11} h^{-1}, respectively.

McCall and Gavit (1986), citing the unpublished data of Bidlack, reported that hydrolysis of the ester G to the acid T in water is pH-dependent, with half-lives of 84, 8.7, and 0.5 days at 25 °C and pH 5, 7, and 9, respectively. Using the linear regression of the Arrhenius plot (Figure 4), the calculated half-life at pH 7 and 25 °C of 9.2 days is in close agreement with that (8.7 days) cited by McCall and Gavit (1986).

The susceptibility of G to hydrolysis was compared with that of the various esters of 2,4-D, a common herbicide long used in weed management. According to Zepp *et al.* (1975), 2,4-D esters were also hydrolyzed more readily at basic than acidic pH. The calculated hydrolytic half-lives of the methyl, isopropyl, *n*-butyl, *n*-octyl, and isooctyl esters for 2,4-D esters in aqueous solutions at 28 °C were 1.1, 17.0, 5.2, 5.2, and 37.0 h, respectively, at pH 9.0 and 44, 710, 220, 220, and 1500 days at pH 6.0. Using the Arrhenius plot (Figure 4), the hydrolytic half-life of G in aqueous solutions at 28 °C and pH 7.0 was calculated to be 6.6 days. Because G is more susceptible to hydrolysis at basic than acidic pH (Figure 3), its calculated hydrolytic half-life at 28 °C and pH 6.0 will be longer than 6.6 days. It is apparent that the susceptibility of G to hydrolysis is comparable to that of the methyl ester of 2,4-D but higher than that of the isopropyl, *n*-butyl, *n*-octyl and isooctyl esters.

Conversion Products of G. When G hydrolyzed in aqueous buffers at 35 °C in the range pH 4.5–8.5, the major conversion product was the acid T, except at pH 4.5 where P was a minor conversion product in addition to T (Figures 1 and 2b). After incubation for 252 days, the concentrations of G, T, and P were 10.8%, 67.1%, and 22.1% of the sum of all three compounds, respectively. To ascertain that P was converted directly from G rather than by way of T as an intermediate, an aqueous solution of 10 μg/mL of T at pH 4.5 was incubated at 35 °C for 199 days. P was never detected in the incubation mixture during the study, and the concentration of T remained unchanged. The results clearly indicate that P was converted directly from G.

At the range pH 4.5–8.5 and incubation periods of 205–252 days, the sum of the concentrations of G + T + P remained relatively unchanged, indicating that both T and P were stable in water and their hydrolysis was rather slow. The degradation of T and P in the aquatic environment would therefore be dependent mostly on mechanisms other than hydrolysis, such as photolysis and microbial degradation. The fact that G and its T and P conversion products were relatively stable in aqueous media is of toxicological importance because all three compounds are toxic to salmonids. The 96-h LC_{50} values of 0.7, 7.9, and 2.1 mg/L to salmonids for G, T, and P, respectively, in a static bioassay were reported by Wan *et al.* (1987).

Table I. Kinetic Data of Hydrolysis of G at 35 °C in Buffered Solutions and Natural Waters

medium	pH	no. of readings	corrln coeff	k_{obsd},[a] × 10^{-4} h^{-1}	k_{OH^-},[b] × 10^{-4} M^{-1} h^{-1}	half-life,[c] h
0.01 M phosphate buffer	4.5	14	0.9964	3.63	115	1,909
0.01 M phosphate buffer	5.5	12	0.9990	5.28	16.7	1,313
0.01 M phosphate buffer	6.5	9	0.9992	34.7	11.0	200
0.01 M phosphate buffer	6.6	13	0.9998	40.9	10.3	169
0.001 M phosphate buffer	6.6	13	0.9980	28.0	7.0	248
Capilano River water	6.6	17	0.9987	24.7	6.0	281
0.01 M phosphate buffer	7.0	11	0.9997	95.4	9.5	72.7
0.01 M phosphate buffer	7.5	9	0.9984	283	8.9	24.5
0.01 M phosphate buffer	7.6	7	0.9953	403	10.1	17.2
0.002 M phosphate buffer	7.6	11	0.9962	144	3.6	48.1
Tamihi Creek water	7.6	16	0.9931	173	4.3	40.1
0.01 M phosphate buffer	8.0	10	0.9990	639	6.4	10.8
0.01 M borate buffer	8.5	7	0.9980	1596	5.0	4.3

[a] K_{obsd} = pseudo-first-order rate constant. [b] K_{OH^-} = second-order rate constant. [c] Half-life was calculated from the corresponding k_{obsd}.

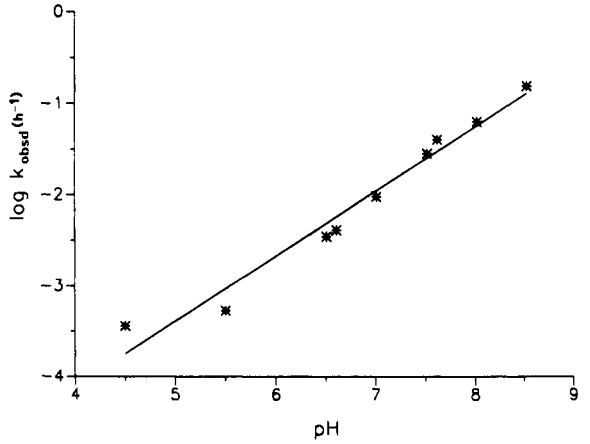

Figure 3. Profile of log k_{obsd} vs pH for the hydrolysis of the ethylene glycol butyl ether ester of triclopyr (G) at 35 °C.

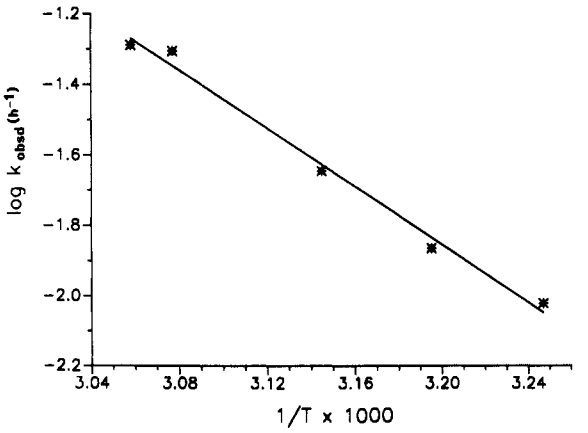

Figure 4. Arrhenius plot of log k_{obsd} vs $1/T \times 1000$ for the hydrolysis of the ethylene glycol butyl ether ester of triclopyr (G) at pH 7.0.

Table II. Kinetic Data of Hydrolysis of G in 0.01 M Phosphate Buffer of pH 7.0 at Five Temperatures

temp, °C	k_{obsd}, × 10^{-3} h^{-1}	half-life, h	no. of readings	corrln coeff
35	9.54	72.7	11	0.9997
40	13.7	50.6	11	0.9993
45	22.6	30.7	9	0.9984
52	49.4	14.0	9	0.9976
54	51.3	13.2	8	0.9995

Hydrolysis of G in River Waters. Two river waters were studied. The pH, hardness (total concentrations of Ca^{2+} and Mg^{2+} in micrograms per milliliter) and conductivity (microohms) were, respectively, 6.6, 6, and 14 for the Capilano River water and 7.6, 24, and 53 for Tamihi Creek water. Using the approach described here, the conversion of G to its hydrolytic conversion products T and P in river waters can be readily determined and quantified by injecting a 20-μL aliquot of the incubation mixture without extraction and sample cleanup (Figures 5 and 6). T was the only conversion product present in the two river waters. On the basis of the conversion of G in buffered solutions of range pH 4.5–8.5, P was found only in a buffered solution of pH 4.5. The findings on the conversion of G in the two river waters were consistent with those in buffered solutions (Table I).

The disappearance of G in the river waters at 35 °C also followed simple pseudo-first-order kinetics as in buffered solutions (Table I). G was converted to the acid T more rapidly in Tamihi Creek water (pH 7.6) than in the Capilano River water (pH 6.6) because the former was slightly basic and the latter slightly acidic. The calculated half-lives of G were 40.1 h in Tamihi Creek water and 281 h in the Capilano River water.

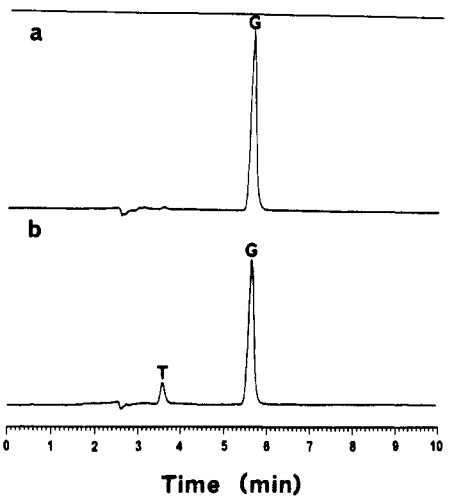

Figure 5. High-pressure liquid chromatograms of UV absorption at 300 nm of a 20-μL aliquot of 10 μg/mL of the ethylene glycol butyl ether ester of triclopyr (G) in the Capilano River water (pH 6.6) at 35 °C: (a) at zero time of incubation; (b) after incubation for 2 days.

G disappeared much more slowly in river waters than in 0.01 M buffered solutions of corresponding pHs (Table I), probably due to buffer catalysis. According to Perdue and Wolfe (1983), buffer catalysis is predicted to be potentially significant in laboratory studies that use buffers at concentrations above 0.001 M to maintain constant pH. The maximum concentration of buffer that can be used without significant catalytic activity is about 0.001 M. Because the potential buffer concentrations in most aquatic environments seldom exceed 0.001 M, buffer

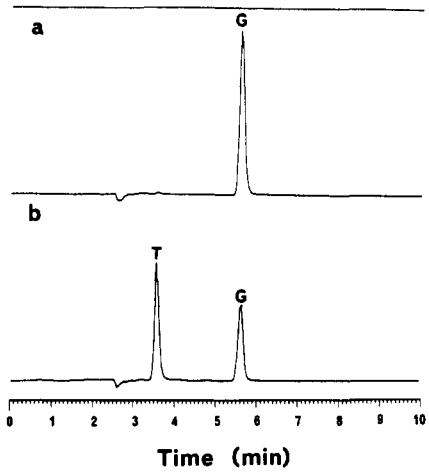

Figure 6. High-pressure liquid chromatograms of UV absorption at 300 nm of a 20-μL aliquot of 10 μg/mL of the ethylene glycol butyl ether ester of triclopyr (G) in Tamihi Creek water (pH 7.6) at 35 °C: (a) at zero time of incubation; (b) after incubation for 2 days.

catalysis is not expected to be significant. To confirm the accuracy of the kinetic data generated by the approach described here, the disappearance of G was determined in 0.001 M phosphate buffer of pH 6.6 and 0.002 M phosphate buffer of pH 7.6. The results are given in Table I. The ionic strength of 0.002 M was the minimun to maintain a constant pH at 7.6 throughout incubation. The kinetic data generated from these two buffers were in agreement with those from the river waters (Table I). The calculated half-lives at 35 °C were 281 h in the Capilano River water (pH 6.6), 248 h in 0.001 M phosphate buffer of pH 6.6, 40.1 h in Tamihi Creek water (pH 7.6), and 48.1 h in 0.002 M phosphate buffer of pH 7.6. It is evident that the effect of buffer catalysis of hydrolysis must be considered when rate constants of hydrolysis are determined under laboratory conditions as recommended by Perdue and Wolfe (1983).

The results of this study indicate that Garlon is readily hydrolyzed to triclopyr in aqueous buffers of pH 4.5–8.5 and in natural waters, but triclopyr itself is quite resistant to hydrolysis. To understand the fate of Garlon in aquatic environment, other factors such as photolysis and microbial degradation must be considered. According to the study of McCall and Gavit (1986), both triclopyr and Garlon were readily photodecomposed in water. The rate of photodecomposition of triclopyr was approximately 6 times faster than that of Garlon. At 40° N latitute, the calculated midday, mid-summer half-lives at the water's surface were 2.1 h for triclopyr and 12.5 h for Garlon. Furthermore, the rapid dissipation of triclopyr from water has also been demonstrated by Solomon *et al.* (1988) in a field study carried out in a bog lake in northeastern Ontario. After application of Garlon at 0.3 and 3 kg/ha, less than 5% of the initial triclopyr concentration remained in water after 15 days and the chemical was no longer detected after 42 days.

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