Hydrolysis Kinetics of the Monophosphate Ester Triclofos Sodium

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Abstract □ The hydrolysis kinetics of the monophosphate ester triclofos sodium were studied in the pH 0.00–7.80 range, the 80.1–98.4° range, and ionic strengths of 0.02–1.50. The pK_1 and pK_2 values for triclofos were 1.00 ± 0.05 and 5.80 ± 0.05, respectively. The pH dependency observed was consistent with a theory in which the hydrolysis rates of the triclofos species follow the order: monoanion > unionized > dianion. The apparent first-order rate constants at 80.1° associated with each species were 6.4 ± 0.6 × 10^{-3}, 28.0 ± 0.5 × 10^{-3}, and 0.3 ± 0.1 hr^{-1}, respectively. Different Arrhenius activation energies were observed at pH 0.00, 3.50, and 7.80, at which values the unionized, monoanion, and dianion forms were the dominant species present, respectively. The reaction showed a modest positive kinetic salt effect at pH 3.50 and a modest negative effect at pH 7.80. Various mechanistic possibilities are discussed.

Keyphrases □ Triclofos sodium—hydrolysis kinetics, effect of pH, temperature, and ionic strength □ Hydrolysis kinetics—triclofos sodium, effect of pH, temperature, and ionic strength □ Kinetics—hydrolysis of triclofos sodium, effect of pH, temperature, and ionic strength □ Sedative-hypnotics—triclofos sodium, hydrolysis kinetics, effect of pH, temperature, and ionic strength

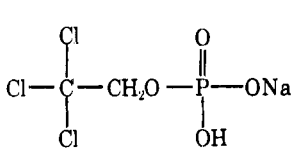
Triclofos sodium (2,2,2-trichloroethanol dihydrogen phosphate monosodium salt) (I) is marketed as a sedative–hypnotic whose pharmacological action is dependent on the *in vivo* liberation of trichloroethanol.

The only reported (1) *in vitro* hydrolysis of triclofos was conducted at 100° in 0.1 *N* HCl, 0.1 *N* NaOH, and 0.01 *M* formate buffer (pH 4.0); the hydrolysis was more rapid in the formate buffer than under the other two conditions studied. The pH dependencies of the hydrolysis rates of other phosphate monoesters were reported (2–13) to exhibit various patterns, and some pH–rate profiles indicated that the ester form most susceptible to hydrolysis was the monoanion. Since the one report (1) on triclofos hydrolysis did not establish any of the usual kinetic parameters, the purpose of this study was to determine such parameters to permit more precise stability predictions under various conditions.

EXPERIMENTAL

Materials—All chemicals were reagent grade except for pharmaceutical quality triclofos sodium[1]. Water was double distilled from a glass apparatus.

pKa Determinations—The pKa values of triclofos and maleic and acetic acids, used as part of the buffer systems, were determined by the Benet and Goyan (14) method as modified (15). For each acid, duplicate pK determinations were made at 80° in 0.75 *M* KCl in thermostated[2] vessels; pH measurements were made with glass and calomel electrodes, equilibrated and standardized at 80°, on the expanded scale of a pH meter[3]. The volumes of the solution being titrated and the titrant were measured at room temperature but corrected to 80° using literature values (16) for the volume dependency of pure water on temperature, with the assumption that these solutions would behave similarly. The titrant, a standardized sodium hydroxide solution, was 20-fold more concentrated than the titrated acid.



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The following results were obtained: 0.1 *M* maleic acid, pK_1 = 1.56 ± 0.01 and pK_2 = 5.63 ± 0.03; 0.1 *M* acetic acid, pK = 4.55 ± 0.04; and 0.01 *M* triclofos sodium, pK_2 = 5.80 ± 0.05. In addition to yielding a pK value of the titrated acid, this procedure gives a value of the actual amount of acid present in the titrated system and, hence, permits a calculation of acid purity. The purity calculated for triclofos sodium was 96%.

Potentiometric titrations to determine the pK_1 for triclofos at 80° were performed with 4.053 *N* HCl on solutions that were 0.125, 0.2, 0.3, 0.4, and 0.5 *M* in triclofos sodium and 0.625, 0.55, 0.45, 0.35, and 0.25 *M*, respectively, in potassium chloride to produce an initial ionic strength of 0.75. The pK_1 for triclofos also was determined in 0.2 and 0.3 *M* triclofos sodium solutions with 1.3 and 1.2 *M* KCl added, respectively, to produce an initial ionic strength of 1.5. Since the present approach for the pK_1 determination differed from that used in the other pK determinations reported here, a discussion of the findings is given in the *Results and Discussion* section.

The pKa of tromethamine at 90° was determined by preparing three sets of solutions. Each was 0.1 *M* in tromethamine (primary standard grade base[4]) and 0.75 *M* in potassium chloride, and sufficient standardized hydrochloric acid was added to neutralize 0.25, 0.5, and 0.75 of the tromethamine base present. The pH values of these solutions were taken at 90°, and the pKa was calculated from the pH readings using the Henderson–Hasselbalch equation. The pKa of tromethamine obtained at 90° was 6.95 ± 0.01.

Mean Ionic Activity Coefficient of Hydrogen Ion—To determine the mean ionic activity coefficient for the hydrogen ion under the experimental conditions, solutions of 0.75, 0.875, 1.0, 1.125, 1.25, and 1.5 *M* KCl in carbon dioxide-free water were titrated at 80° with 4.053 *N* HCl. The pairs of volume *versus* pH data so obtained were converted to hydrogen-ion molarities and activities. Then the activity in each data pair was divided by its molarity to give a set of mean ionic activity coefficients for the hydrogen ion: 0.820 ± 0.006 in 0.75 *M* KCl, 0.840 ± 0.008 in 0.875 *M* KCl, 0.880 ± 0.01 in 1.0 *M* KCl, 0.900 ± 0.006 in 1.125 *M* KCl, 0.920 ± 0.006 in 1.25 *M* KCl, and 0.985 ± 0.024 in 1.5 *M* KCl. The increase in the activity coefficient with ionic strength in the potassium chloride concentration range used is consistent with a trend reported elsewhere for this type of system (17).

Triclofos Hydrolysis Studies—The hydrolysis of triclofos at 80.1° over the pH 0.00–6.89 range was studied by preparing triclofos sodium solutions in 0.001 *M* initial concentration in the following buffer systems: perchloric acid (pH 0.00–1.31), 0.1 *M* maleic acid–potassium hydrogen maleate (pH 1.81–3.04), 0.1 *M* acetic acid–sodium acetate (pH 3.50–5.05), and 0.1 *M* potassium hydrogen maleate–potassium maleate (pH 5.52–6.89). Potassium chloride was used to adjust the ionic strength to 0.75 in each buffer.

The solutions, in stoppered volumetric flasks, were brought to temperature equilibrium in a constant-temperature oil bath and then sampled by pipet in appropriate frequency and number to permit monitoring of the reaction from essentially zero time to attainment of equilibrium conditions. Reaction samples were quenched by chilling in an ice bath and then kept frozen in stoppered vials until all samples for a given kinetic run were assembled; they were then assayed for inorganic phosphate content. The pH of each reaction solution was measured at the end of the

[1] Lakeside Laboratories, Milwaukee, Wis.
[2] Haake model FK-10, Polyscience Corp., Evanston, Ill.
[3] Model 7415-EO, Leeds and Northrup Co., North Wales, Pa.
[4] Trizma Base, Sigma Chemical Co., St. Louis, Mo.

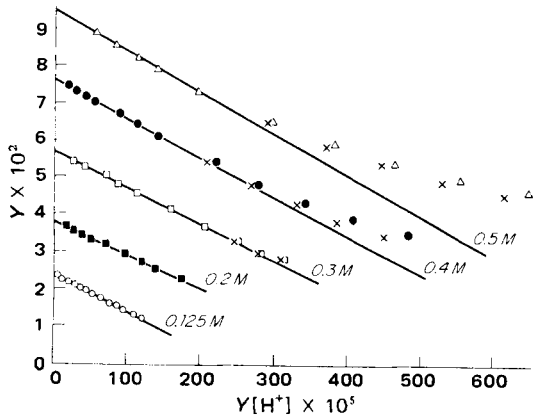


Figure 1—*Potentiometric titration data for 0.125–0.5* M *triclofos sodium plotted according to Eq. 1; ×'s are data points corrected for ionic strength changes during titration.*

run at room temperature, and no change from initial pH was observed greater than ±0.10.

The dependency of the 0.001 *M* triclofos hydrolysis rate on ionic strength at 80.1° was studied by preparing solutions in acetate buffers (0.05–0.10 *M*) at pH 3.50 and varying the ionic strength from 0.019 to 1.5 by adding potassium chloride. The ionic strength dependency of the reaction at 98.4° and pH 7.80 was studied in 0.1 *M* tromethamine buffers whose ionic strength was varied from 0.055 to 1.434 by adding potassium chloride.

The temperature dependency of the 0.001 *M* triclofos hydrolysis rate was followed at pH 0.00 at 80.1, 88.0, and 96.6° in perchloric acid buffers; at pH 3.50 at 80.1, 89.8, and 98.4° in 0.1 *M* acetate buffers; and at pH 7.80 at 90.0, 94.0, and 98.4° in 0.1 *M* tromethamine buffers. The tromethamine buffers were individually adjusted to pH 7.80 at each temperature because of the significant dependency of pH on temperature of basic buffers.

Separately prepared, duplicate runs were made of all kinetic experiments performed at each condition described.

Inorganic Phosphate Assay—Triclofos hydrolysis was followed by assaying for liberated inorganic phosphate by adaptation of the phosphomolybdenum blue assay (18). Aliquots of samples from the kinetic runs, representing an inorganic phosphate concentration of 0.1–0.6 μg/ml, were placed in 50-ml volumetric flasks. Then 2 ml of 2% (w/v) ammonium molybdate in 25% (v/v) sulfuric acid and 1 ml of 5% (w/v) stannous chloride in 6 *N* HCl were added. Each flask was brought to volume with water and allowed to stand 15 min.

The absorbance was measured at 720 nm in a 1-cm cell in a spectrophotometer[5] containing water in the reference cell. The absorbance of a blank, identical in composition to the sample cell except for the absence of the triclofos reaction mixture, was subtracted from sample readings. The net absorbance of each sample was converted to concentration using a standard curve prepared for each assay at the beginning of the assay. The standard curve was constructed using monobasic potassium phosphate in known concentrations of 0.2–0.6 μg/ml.

RESULTS AND DISCUSSION

Determination of pK_1 for Triclofos—Preliminary studies found that the pK_1 for triclofos was apparently less than 2.0, and efforts to determine its first dissociation constant in solutions of 0.01 *M* failed. This behavior agrees with results of Albert and Serjeant (19) who stated that ordinary potentiometric titration methods are unreliable for pK determinations on solutions where the negative logarithm of the concentration of the acid being titrated is greater than its pK value. It was, therefore, decided to determine the pK_1 for triclofos at several higher concentrations than those used in the kinetic runs and either to use such values or to extrapolate them to lower concentrations if the data seemed to justify this approach.

Since only the monosodium salt of triclofos was available rather than the free acid, this salt was used for pK_1 determinations. The method of Leeson and Brown (15) for pK determinations requires some modification

[5] Acta T. M. II UV–visible spectrophotometer, Beckman Instruments, Fullerton, Calif.

Table I—Values of pK_1 for Various Molarities of Triclofos Determined at Two Ionic Strengths at 80°

Molarity of Triclofos Sodium	Molarity of Potassium Chloride	Ionic Strength at Time Zero	pK_1
0.125	0.625	0.75	0.98
0.125	0.625	0.75	0.99
0.200	0.550	0.75	0.95
0.200	0.550	0.75	0.95
0.200	0.550	0.75	0.99
0.300	0.450	0.75	0.99
0.400	0.350	0.75	1.02
0.500	0.250	0.75	1.04
0.200	1.300	1.50	0.99
0.300	1.200	1.50	1.02

in the final equation when the conjugate base form (or salt) of the acid is titrated with strong acid. But by methods similar to those used earlier (14, 15), an equation of the following form can be developed:

$$Y = HA_o^- - \frac{1}{K_1} Y[H^+] \qquad \text{(Eq. 1)}$$

where:

$$Y = H^+ + Na_o^+ - Cl^- \qquad \text{(Eq. 2)}$$

and HA_o^- is the absolute number of moles of triclofos sodium added to the solution being titrated, H^+ is the absolute number of moles of the hydrogen ion present, Na_o^+ is the absolute number of moles of the sodium ion present due to the addition of triclofos sodium, Cl^- is the absolute number of moles of the chloride ion present due to the addition of hydrochloric acid titrant, $[H^+]$ is the molar concentration of the hydrogen ion present, and K_1 is the apparent first dissociation constant for triclofos.

To plot Y *versus* $Y[H^+]$ in accordance with Eq. 1 to obtain a linear relationship (the negative reciprocal of whose slope should give K_1), it is necessary to evaluate the terms in Y. Both H^+ and Cl^- can be obtained from calculations based on pH readings, titrant volume, normality data, and the experimentally determined mean ionic activity coefficient for the hydrogen ion. The value of Na_o^+ can be obtained by correcting the known amount of triclofos sodium added by its purity factor of 96% (obtained in the separate, independent determination of K_2 for triclofos as reported under *Experimental*).

Figure 1 gives five plots of titration data obtained at five different molarities of triclofos sodium as titrated with hydrochloric acid and plotted according to Eq. 1. The curves tend to be linear at values of $Y[H^+]$ less than 200×10^{-5} but show increasingly pronounced curvature for titrations of triclofos sodium of 0.3 *M* and higher where completion of the titration leads to values of $Y[H^+]$ greater than 200×10^{-5}. When the Y *versus* $Y[H^+]$ data for values of $Y[H^+]$ less than 200×10^{-5} are fitted by linear regression to a straight line, the straight lines shown in Fig. 1 result, permitting the calculation of pK_1 values (Table I). At ionic strengths of 0.75 and 1.5, the pK_1 can reasonably be assigned a value of 1.00 ± 0.05 under these limitations.

Since the actual kinetic runs were done with an initial triclofos sodium concentration of 0.001 *M*, the question of whether a pK_1 value of 1.0 is appropriate for application to the kinetic data must be considered. Even though the ionic strength in both the kinetic runs and the pK_1 determinations (at least at the beginning of the titrations used in the determinations) was either 0.75 or 1.5, the species contributing to this ionic strength were different in each case. The question of interest then becomes whether the first acid dissociation step of triclofos will be detectably different in an environment where the triclofos itself is contributing 0.125–0.5 of the total ionic strength of 0.75. Although a difference might be expected in the extent of acid dissociation occurring under such different environments, the data presented in Table I do not show any particular trend to higher or lower values of pK_1 as the triclofos sodium concentration drops from 0.5 to 0.125 *M*. Consequently, the use of a value of 1.00 ± 0.05 for pK_1 in the kinetic data seems justified.

The nonlinear behavior of parts of several curves in Fig. 1 could be due to several causes, including pH meter reading error due to faulty standardization (14). But this possibility seems unlikely here because careful attention was paid to this detail. Lack of constancy in the mean ionic activity coefficient of the hydrogen ion during the titration could also produce the observed curvatures. That this activity coefficient is a rather sensitive function of ionic strength was shown under *Experimental*. Assuming that triclofos has a pK_1 value of 1.0 permits one to approximate

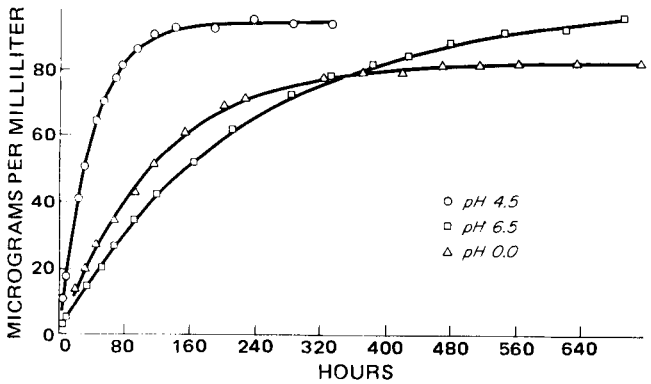


Figure 2—*Concentration of liberated inorganic phosphate* versus *time during hydrolysis of triclofos at 80.1° at three pH conditions.*

a more realistic ionic strength throughout the titration as it increases from its initial value of 0.75 because of the strong acid character of triclofos. Then, with the available data, estimates of the increasing mean ionic activity coefficient of the hydrogen ion at each pH during the titration were made, yielding finally the new points shown as ×'s in Fig. 1. While these points bring the data points closer to linearity, there is still significant curvature.

Since correction of hydrogen-ion activity coefficients does not fully eliminate the curvature in Fig. 1 plots, an additional cause of some or all of the nonlinearity may be that K_1 of Eq. 1 is an *apparent* dissociation constant rather than the thermodynamic dissociation constant. An apparent dissociation constant will vary with ionic strength to the extent that the activity coefficients (or their ratio) of the species involved in the equilibrium vary. In this case, where both ionic and nonionic species are involved in the equilibrium, one could probably expect a differing response to change in ionic strength by the charged and uncharged species. Whatever the exact cause of the nonlinearity, no further attempt was made to delineate this phenomenon because the main purpose of this part of the study—to obtain a value of pK_1 appropriately applicable to the treatment of the kinetic data—was satisfied adequately by using the limiting, linear parts of the curves since the conditions that yielded these data most closely duplicated the conditions of the kinetic experiments.

pH Dependency of Triclofos Hydrolysis—The simplest representation for the studied reaction would be as shown in Scheme I:

$$\text{triclofos} \underset{k_e}{\overset{k_h}{\rightleftharpoons}} \text{trichloroethanol} + \text{phosphoric acid}$$

Scheme I

where k_h is the observed hydrolysis rate constant and k_e is the observed esterification rate constant. Such a reaction would be first and second order in its hydrolysis and esterification steps, respectively. When the k_h/k_e ratio was expressed in terms of initial and equilibrium concentrations of triclofos (20), k_h was at least 300 times larger than k_e under all reaction conditions. Since $k_h \gg k_e$ and the reaction was always 65% or more complete at equilibrium, the data should conform reasonably well to a mathematical model for first-order hydrolysis.

When the concentration of inorganic phosphate, an expected hydrolysis product, is plotted *versus* time, the data exhibit the behavior shown in Fig. 2. The concentration *versus* time data for inorganic phosphate were processed by a computer program[6] designed to produce a nonlinear, least-squares fit to the following first-order equation for product appearance:

$$C = C_\infty(1 - e^{-k't}) \qquad \text{(Eq. 3)}$$

where C represents product concentration at time t and k' is the observed first-order rate constant for product appearance as it goes to a final concentration of C_∞. The computer program produces an estimate for both k' and C_∞. The solid lines in Fig. 2 are computer-drawn curves based on these computer estimates of k' and C.

In Fig. 3, the computer-generated values of k' are plotted as a function of pH, with the range of values from the duplicate runs at each pH indicated by the I-bars. The value of k' shown at pH 7.80 is an estimate of its value at 80.1° obtained by extrapolating values obtained at higher temperatures, using the Arrhenius equation. Examination of the pH dependency of k' reveals a pattern with the maximum rate between pH 1.8 and 4.0, suggesting, based on the values found for pK_1 and pK_2, a reaction rate largely dependent on the triclofos monoanion concentration with possible lesser contributions from the hydrolysis of the unionized and dianionic forms. If k_{H_2A}, k_{HA^-}, and $k_{A^{2-}}$ are the specific rate constants associated with hydrolysis of the unionized, monoanionic, and dianionic forms of triclofos, respectively, then the following relationship can be developed:

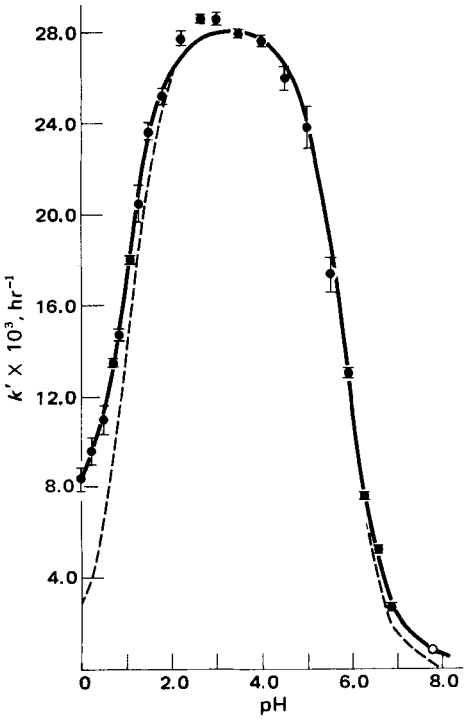


Figure 3—*Observed rate constant,* k', *for triclofos hydrolysis at 80.1° (●), or extrapolated to 80.1° (○),* versus *pH. Key (theoretical curves): – –, hydrolysis of triclofos monoanion form only; and —, combined hydrolysis of triclofos in unionized, monoanion, and dianion forms.*

$$k' = f_{H_2A}k_{H_2A} + f_{HA^-}k_{HA^-} + f_{A^{2-}}k_{A^{2-}} \qquad \text{(Eq. 4)}$$

where the terms f_{H_2A}, f_{HA^-}, and $f_{A^{2-}}$ represent the fraction of total triclofos in the system at any pH present in the forms indicated by the subscripts.

At pH 3.5, f_{H_2A} and $f_{A^{2-}}$ are approximately equal to zero and f_{HA^-} approaches 1.0 in value. At pH 7.80, $f_{H_2A} \cong 0.00$ while $f_{HA^-} \cong 0.01$ and $f_{A^{2-}} \cong 0.99$. If the average experimental value of k' at pH 3.50, *i.e.*, $28 \pm 0.5 \times 10^{-3}$ hr^{-1}, is equated to the specific rate constant, k_{HA^-}, then this value can be combined with experimental values of k' at pH 0.00 and 7.80 to calculate k_{H_2A} and $k_{A^{2-}}$. At pH 0.00:

$$k' \cong k_{H_2A}f_{H_2A} + k_{HA^-}f_{HA^-} \qquad \text{(Eq. 5}a\text{)}$$

At pH 7.80:

$$k' \cong k_{HA^-}f_{HA^-} + k_{A^{2-}}f_{A^{2-}} \qquad \text{(Eq. 5}b\text{)}$$

The values of f_{H_2A}, f_{HA^-}, and $f_{A^{2-}}$ needed for Eqs. 5*a* and 5*b* can be readily calculated from the separately determined values of K_1 and K_2 for triclofos. The results of this approach are: $k_{H_2A} = 6.4 \pm 0.6 \times 10^{-3}$ hr^{-1} and $k_{A^{2-}} = 0.3 \pm 0.1 \times 10^{-3}$ hr^{-1}.

When the calculated values for the specified rate constants are used to evaluate Eq. 4 over the pH 0.00–7.80 range, the data give the solid curve (hereafter designated as *k*th) shown in Fig. 3. The broken-line curve represents a theoretical curve based only on the product $k_{HA^-}f_{HA^-}$; this curve deviates significantly from the experimental results below pH 2.0 and to a more modest extent above pH 6.0. The solid curve defined by *k*th affords a reasonably good fit to the experimental data except that the experimental results all exceed the values predicted by *k*th at pH 2.25, 2.65, and 3.04. However, the difference between *k*th and the average value of the experimental data at these three pH values is ~3% or less.

[6] NONLIN program, The Upjohn Co., Kalamazoo, Mich., as adapted for use by Dr. Richard Hunt onto the CDC CYBER 74 system, University of Minnesota Computer Center.

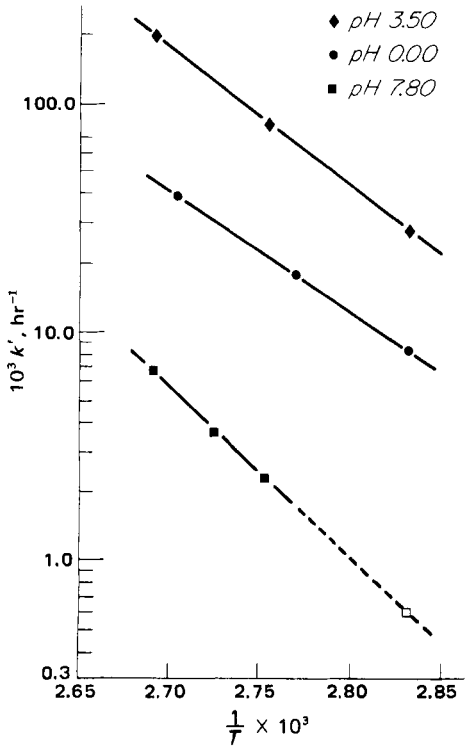


Figure 4—*Semilogarithmic plot of observed triclofos hydrolysis rate constant,* k′, *at three pH conditions* versus *reciprocal Kelvin temperature. Key:* □, *extrapolated value of* k′ *to 80.1° at pH 7.80.*

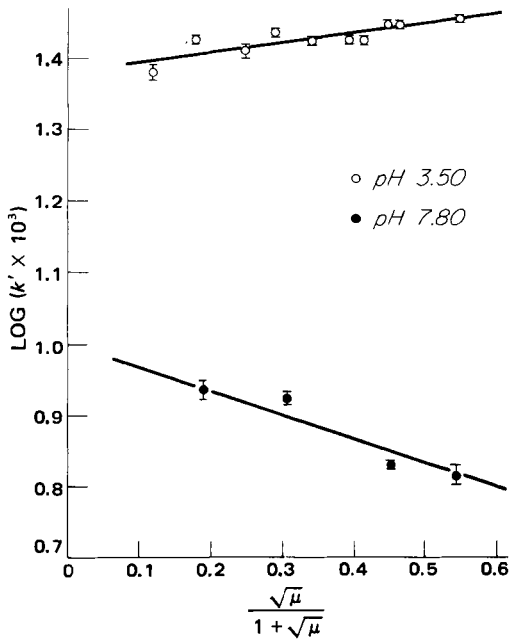


Figure 5—*Logarithm of* k′, *the observed triclofos hydrolysis rate constant,* versus $\mu^{1/2}/(1 + \mu^{1/2})$; μ *is ionic strength.*

The buffer system used at each of these three pH conditions (as well as at pH 1.81 where *k*th agrees well with the experimental data) depends upon the half-salt of maleic acid. Such half-salt species have been shown to exhibit strong catalytic effects upon hydrolysis of certain amides (21). Although general acid and base catalysis of esters is not common, it has been demonstrated (22, 23) in some cases, so the kinetic data in these particular maleate buffers may be exhibiting a buffer catalysis effect. The possibility that buffer catalysis might be the source of these deviations was not explored. To establish such apparently modest buffer effects would require use of significantly more concentrated buffers and, in the pH range involved, the maleate buffers were near the limits of their solubility as used.

Temperature Dependency of Triclofos Hydrolysis—Figure 4 gives the results of the temperature dependency studies at pH 0.00, 3.50, and 7.80 plotted as log *k′ versus* reciprocal temperature in accordance with the Arrhenius equation. Linear regression treatment of the data at each pH gives values for the activation energy, E_a, as follows: 24.1 kcal/mole at pH 0.00, 28.1 kcal/mole at pH 3.50, and 34.1 kcal/mole at pH 7.80.

At pH 3.50, about 100% of the total triclofos present is in the monoanion form, so that the activation energy at pH 3.50 probably can be properly identified as almost exclusively associated with monoanion hydrolysis. Evaluation of Eq. 5*a* at pH 0.00 leads to the conclusion that approximately 70% of the value of *k′* is due to unionized triclofos hydrolysis and the balance to monoanion hydrolysis. Consequently, the activation energy observed at pH 0.00 probably can be largely, but not exclusively, associated with the hydrolysis of undissociated triclofos. Finally, evaluation of Eq. 5*b* implies that about 50% of *k′* at pH 7.80 is associated with monoanion hydrolysis and 50% is associated with dianion hydrolysis. By this analysis, the activation energy observed at pH 7.80 is apparently a mixed function of the hydrolysis associated with the two dominant forms of triclofos present.

Ionic Strength Dependency of Triclofos Hydrolysis—Figure 5 gives the results of the kinetic runs at pH 3.50 and 80.1° in which ionic strength was varied; the results of similar runs at pH 7.80 and 98.4° also are shown in Fig. 5. The data in Fig. 5 are plotted as log *k′ versus* $\mu^{1/2}/(1 + \mu^{1/2})$, where μ is the ionic strength, based on an extended and modified form of the Debye–Huckel equation (24). Plots based on this equation should yield a linear relationship whose slope is proportional to the product of the charges carried by the reactive species forming the activated complex. Although this modified equation holds, in theory, only up to about an ionic strength of 0.1, it has been shown (25) to predict kinetic salt effects reliably in some pharmaceutical systems to as high as unity ionic strength.

The slope of the linear regression line in Fig. 5 fitted to the data at pH 3.50 is 0.14; the slope of the line fitted to the data at pH 7.80 is −0.37. Neither of these slopes fits exactly any theoretical model predicted by the equation used, but the relatively low values of the slopes are probably more consistent with the absence of ionic charge on at least one reactant rather than charges on all reactants (26).

CONCLUSIONS

The kinetic data reported here provide a basis for comparison with other studies on monophosphate ester hydrolysis (2–13). These studies all show little or no hydrolysis at pH conditions where the dianion species is dominant and usually show a maximum hydrolysis rate where the monoanion predominates. At lower pH conditions, the observed rate may show evidence of neutral species hydrolysis; in some cases, at a pH of about 0.5 and less, the rate may increase consistent with formation of a conjugate acid form of the ester that experiences hydrolysis.

Table II gives the hydrolysis rate of the neutral and monoanion forms of several phosphate esters relative to methyl phosphate as well as the values of the Arrhenius preexponential factor, *A*, and activation energy, E_a, for k_{HA^-}. The activation energy for monoanion hydrolysis is very similar for all esters so that any variation in rate is more a function of the preexponential or entropy term. For both k_{H_2A} and k_{HA^-}, but especially the former, the rate constant increases as the acid strength of the alcohol, ROH, of the ester increases (or, alternatively, the stability of the RO^- group increases).

A fully satisfactory mechanistic explanation for monophosphate ester hydrolysis has not been established. The comparatively ready monoanion

Table II—Comparative Kinetic Parameters[a] for Selected Monophosphate Esters at 100°

Compound	Relative Rates[b] k_{H_2A}	Relative Rates[b] k_{HA^-}	E_a[c], kcal/mole	10^{-14} A[c], min^{-1}
Methyl phosphate	1.0	1.00	30.6	3.88
Glycerol 1-phosphate	—	1.66	29.9	2.57
Triclofos	136.0	7.94	28.1	1.47
p-Tolyl phosphate	240.0	28.6	29.0	10.80
Phenyl phosphate	305.0	32.0	29.0	12.6
p-Nitrophenyl phosphate	3000.0	66.8	30.0	216.0

[a] Values listed for triclofos are based on data obtained in this study; values for other esters are from Ref. 13. [b] Hydrolysis rates for each ester relative to methyl phosphate for which $k_{HA^-} = 4.94 \times 10^{-4}$ min^{-1} and $k_{H_2A} = 0.06 \times 10^{-4}$ min^{-1}. [c] Both E_a and A are Arrhenius equation parameters for k_{HA^-}.

hydrolysis is not easy to explain, but the presence of both an OH^- group and an O^- group is evidently necessary since diphosphate esters monoanions do not hydrolyze with the same facility. Most proposed mechanisms for monoanion hydrolysis involve formation of a hydrogen-bonded complex with water as the reactive entity. In the current study on triclofos, the modest dependency of k_{HA^-} on ionic strength at pH 3.50 is not inconsistent with a bimolecular reaction between the monoanion and water since the activity coefficient of either species might vary with an ionic strength change of the range studied.

The neutral species rate constants, k_{H_2A} (Table II), are rather sensitive functions of the stability of RO^- as a departing species. A heterolytic reaction involving the phosphorus–oxygen bond, yielding protonated metaphosphoric acid that then undergoes rapid hydration, is consistent with these results (11) (Scheme II).

$$ROPO(OH)_2 \rightarrow RO^- + PO^+(OH)_2$$

Scheme II

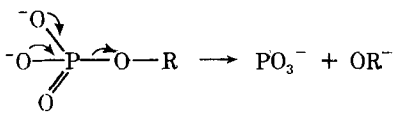
An alternative mechanism involving direct attack by water on the phosphorus atom can be hypothesized and is also consistent with the experimental observations (11) (Scheme III).

$$ROPO(OH)_2 + OH_2 \rightarrow RO^- + H_2O^+PO(OH)_2$$

Scheme III

The activation energy determined at pH 0.00 for triclofos, which can be largely associated with the hydrolysis of the unionized form, is less in magnitude than that found at pH 3.50 where monoanion hydrolysis dominates. Neither this observed difference in activation energies nor data obtained by other investigators in phosphate ester hydrolysis studies permit selection between these two possible mechanisms.

Dianion hydrolysis of monophosphate esters has been reported to be minimal or unobserved in other systems studied (11). Although a reaction between the monoanion and hydroxide ion is kinetically equivalent to a reaction between the dianion and water, previous evidence and/or arguments (5, 6, 11) make the former possibility unlikely. In addition to these mechanistic possibilities, it has been argued (11) that, for hydrolytically reactive phosphate ester dianions, the results can be interpreted most simply in terms of dianion heterolysis (Scheme IV).



Scheme IV

This type of reaction would be expected to be aided by the two negative charges on the oxygen atoms, providing considerable driving force for the heterolysis. Even so, the activation energy of such a heterolytic breakdown would be expected to be fairly high; for 2,4-dinitrophenyl phosphate (11), the Arrhenius activation energy associated with dianion hydrolysis was greater than that for monoanion hydrolysis. For triclofos hydrolysis, a higher activation energy was observed at pH 7.80, where the dianion species dominated, than at pH 3.50, where the monoanion was the dominant species. Also, the dependence of the reaction rate constant at pH 7.80 on ionic strength was negative rather than positive as at pH 3.50. These differences in magnitude or character of reaction parameters at pH 7.80 *versus* 3.50 support the dependency of the reaction rate on pH and also indicate that some triclofos dianion hydrolysis occurs.

In summary, although this study does not permit definitive judgments on the mechanism(s) of triclofos hydrolysis throughout the pH range studied, it does provide sufficient information, particularly when compared with other such hydrolyses, to assign a hydrolysis role to the unionized, monoanion, and dianion ester forms.

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