Hydrolysis of Phorate Using Simulated Environmental Conditions: Rates, Mechanisms, and Product Analysis

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The hydrolysis of phorate, an organophosphorus pesticide, was studied under simulated environmental conditions. Several metal oxides (aluminum hydroxide, hematite, goethite, and ferrihydrite) were chosen to represent the solid phase in natural aquatic systems. Several degradation products were identified using GC-MS in combination with wet chemistry methods. Microtox was used to measure the toxicity of phorate and one of the degradation products, diethyl disulfide. Two potential reaction pathways were proposed for the alkaline hydrolysis of phorate that results in unusual products, such as formaldehyde and diethyl disulfide. No statistically significant catalytic effect was observed for the four metal (hydr)oxides used in the study. However, diethyl disulfide was found to be highly toxic (EC_{50} is 2.0 ppm). This study again confirmed the need for the study of the environmental impact of the degradation products of pesticides.

Keywords: *Phorate; organophosphorus pesticide; diethyl disulfide; formaldehyde; hydrogen sulfide; toxicity; Microtox*

INTRODUCTION

Organophosphorus pesticides, most of which are esters and thioesters of phosphoric acid and thiophosphoric acid, are widely used throughout the world due to their strong inhibitory activity to cholinesterase (an essential enzyme for the function of the neuronal system) (Khan, 1980). Wind and water transport the pesticides applied to agricultural crops, forest, and recreational lawns to various parts of natural aquatic systems. Aquatic degradation of organophosphorus pesticides has been attributed to both chemical and microbiological pathways (Cowart et al., 1971; Gomaa and Faust, 1972; Seiber and Markle, 1972; Beynon et al., 1973; Walker, 1976). Because the USA accounts for 45% of the world's pesticide use and the majority of those pesticides in use at present belong to the organophosphate family, the persistence and toxicity of these compounds and their degradation products have become a serious environmental concern as well as a public health priority. Meanwhile, some organophosphorus pesticides and their degradation products have already been shown to be deleterious to aquatic fauna (Juarez and Sanchez, 1989; Fernandez-Casalderrey et al., 1992, 1993).

Surprisingly, there are few data on the reactivity of organophosphorus compounds in aquatic systems despite their prevalence in the environment. Hydrolysis pathways, for which acid, base, or neutral species serve as the catalysts, have been proposed for phosphoric and thiophosphoric triesters (Schwarzenbach et al., 1993). Acid-catalyzed hydrolysis occurs only when the protonation of a certain functional group results in a better leaving group or a better internal nucleophile. The contribution of each of the three mechanisms toward the total hydrolysis depends on the pH. For nucleophilic attack at a saturated carbon atom, OH^- is found to be a better nucleophile than H_2O by a factor of $10^{4.2}$ (Hine, 1962); but for nucleophilic attack at a phosphorus atom in organophosphate, the relative nucleophilicity of OH^- compared to H_2O increases by several orders of magnitude (e.g., OH^- is 10^8 stronger than H_2O as a nucleophile for triphenyl phosphate (Barnard et al., 1961)). The initial hydrolysis product (phosphomonoesters and phosphodiesters) can undergo further hydrolysis, although at slower rates. It is important to realize that nucleophiles can attack both the phosphorus atom (with an alcohol (thiol) moiety being the leaving group) or the carbon atom bound to the oxygen (sulfur) of an alcohol (thiol) moiety (with the diester being the leaving group) (Figure 1).

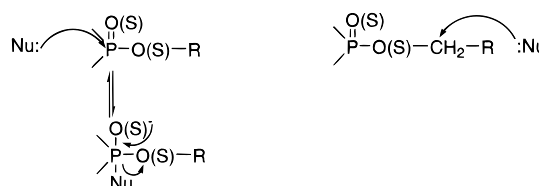

Figure 1. Nucleophilic attack on organophosphate.

In recent years, the hydrolysis of organophosphorus compounds in the presence of metal (hydr)oxide surfaces has received increased attention because these minerals are readily available in the aquatic environment (Ryan and Gschwend, 1990; Backhus et al., 1993) and because their catalytic activity on the hydrolysis of many organic compounds have been well-documented (Wilkins, 1991; Torrents and Stone, 1994; Stone and Torrents, 1995). (Hydr)oxides of iron and aluminum form an important group among these potential catalysts. In the aquatic environment, these metal (hydr)oxides often exist as colloids, whose activity is affected by the surface chemistry, surface speciation, and surface charge, which in turn are affected by the interaction of colloids with H^+, OH^-, metal ions (e.g., Cu^{2+}), and various ligands (e.g.,

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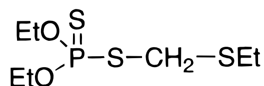

Figure 2. Structure of phorate.

oxalate, formate, acetate, etc.). Abundant experimental evidence supports the notion that complex formation equilibria for both dissolved and surface-bound metal species share common characteristics (Kummert and Stumm, 1980; Davis and Hayes, 1986). However, significant adsorption of the main reactants to the colloids is often a prerequisite for observable surface catalysis, yet little is known about the nature and the coordination environment of adsorbed species.

Strong pesticidal activity and presumably easy biodegradability were the two main reasons for the introduction of organophosphorus pesticides to replace organochlorine pesticides, which gained notoriety for their persistence in the environment, their cumulative deposition in fatty tissues of animals and their toxicity toward humans and the aquatic fauna. However, while the environmental toxicity of organophosphorus pesticides have later been acknowledged (Faust and Gomaa, 1972), there have been limited data on the toxicity of their degradation products. The high cost of animal tests has been a major deterrent for the lack of data in this critical area. Microtox, a commercial toxicity test system developed by Azur Environment Co. (Carlsbad, CA), is based on the metabolic response of *Photobacterium phosphoreum*, a pristine deep sea bacteria presumably exposed to few anthropogenic substances. Toxicity to the bacteria is reflected by the decline in the intensity of luminescence, which is emitted by the bacteria in the dark. The U.S. EPA has already recommended Microtox for testing of treated effluent samples. In the meantime, it has seen wide application in a variety of toxicity studies (Indorato et al., 1984; Calleja et al., 1986; Ribo and Kaiser, 1987; Symons and Sims, 1988; Kramer and Botterweg, 1991). This fast and economical assay is considered to be one of the best available options for rapid toxicity assessment.

The current study is intended for the evaluation of the environmental fate and environmental impact of phorate in an abiotic setting. Phorate, a dithiophosphate, is a restricted-use pesticide that is used to control chewing and sucking insects. Field crops on which phorate has seen significant application include corn, upland cotton and fall potatoes. For example, 0.65 million lbs of phorate was used on fall potatoes alone in 1995 (National Agricultural Statistics Service, 1996). Phorate (Figure 2) was chosen for the current study because it is a good representative of organophosphorus pesticides with its average water solubility and vapor pressure (Budavari et al., 1989).

MATERIALS AND METHODS

Hydrolysis Experiments. Boric acid, NaOH, $Fe(ClO_4)_3$, NaOAc, $Fe(NO_3)_3$, methanol, benzene, CCl_4, and HNO_3 were all obtained from Fisher Scientific (Pittsburgh, PA). Neat phorate was purchased from Supelco, Inc. (Bellefonte, PA). A methanol stock solution of phorate (1:60 v/v)) was kept at 4 °C. Amorphous $Al(OH)_3$ was purchased from Baker & Adamson Co. (New York, NY). Iron oxide suspensions (hematite, goethite, two-line ferrihydrite) were prepared as described in Schwertmann and Cornell (1991). All four metal (hydr)oxides were characterized with X-ray diffraction analysis using a Siemens D-500 X-ray Diffraction system (Siemens Inc., Munich, Germany). Pyrex glass flasks (260 mL) were used as reactors. Homogeneous and heterogeneous hydrolysis experiments were carried out at both pH 5.7 and pH 8.5. The background buffer of the reaction mixture was 0.01 M boric acid buffer for pH 8.5 reactions and 0.01 M sodium acetate buffer for pH 5.7 reactions. Homogeneous hydrolysis of phorate was also carried out at pH 9.4 and pH 10.25, where 0.01 M boric acid buffer was used. Carbon tetrachloride (0.1 mL) was added to each reaction mixture at the beginning to prevent microbial growth. For heterogeneous reactions, 1 g/L of metal (hydr)oxide was used and the reaction mixtures were sonicated for 10 min at the beginning to break up oxide agglomerates. The reaction mixtures were continuously stirred at ~600 rpm. The reactions were carried out at room temperature (i.e., 25−28 °C). The total volume of the reaction mixture was kept at 260 mL so that very little head space was left in the reactor. The starting concentration of phorate was 69 μM. The length of the hydrolysis experiments was 10−14 days during which six to seven samples were taken as 10 mL aliquots. A 10 mL aliquot of the corresponding buffer (i.e., 0.01 M boric acid buffer or 0.01 M sodium acetate buffer) was added back to the reaction mixture after sampling, and the pH of the system was adjusted back to the starting value (with NaOH or HNO_3). To measure the yield of diethyl disulfide (its identification is described in the results section) in pH 8.5 hydrolysis experiments, a 25 mL sample was taken at the end of a hydrolysis experiment and subsequently subjected to GC analysis.

Adsorption Experiments. Teflon bottles (250 mL, Fisher) were chosen as the vessels because the walls have minimal adsorption (i.e., 2−4%) for phorate. The experiments were carried out at room temperature and at both pH 5.7 and pH 8.5. Background buffers, oxide concentrations, and the starting phorate concentration of adsorption experiments were the same as those of heterogeneous hydrolysis experiments. For each experiment, a 100 mL mixture was stirred at ~600 rpm for 18 h before a 6 mL sample was collected into four 1.5 mL Eppendorf tubes. Two tubes were centrifuged at 10 000 rpm for 10 min in an Eppendorf Centrifuge 5415 (Brinkmann Instrument Inc., Westbury, NY), and the other two were placed in a beaker of water and stirred as control samples to account for the hydrolytic loss and the adsorption of the pesticide onto the walls of the Eppendorf tubes. For each adsorption experiment, 2 mL of supernatant from the centrifuged sample and 2 mL suspensions from the control sample were taken for GC analysis.

GC and GC-MS Analyses. HP 5890 Series II GC with an FID detector (Hewlett-Packard Co., Palo Alto, CA) was used for GC analysis. Diethyl disulfide was obtained from Aldrich Chemical Co. (Milwaukee, WI). When a sample was taken for GC analysis, one volume of benzene containing 50 mg/L 4-chloro-3-methylphenol (internal standard for GC) was added to five volumes of sample, shaken for 2 min, and then stored at 4 °C prior to analysis. GC conditions were as follows: 30 m × 0.53 mm i.d. fused silica capillary column with 1.5 μm film thickness (DB-5, J&W Scientific, Folsom, CA) and a carrier gas of nitrogen (10 psi) were used; initial temperature was 105 °C for 2 min; temperature increased at 13 °C/min up to 215 °C for 3 min; injector port temperature was 250 °C; detector temperature was 300 °C. GC standards for phorate were prepared by making aqueous solutions of phorate at 19.2 μM (5 ppm), 38.5 μM (10 ppm), 57.7 μM (15 ppm), and 76.9 μM (20 ppm); 10 mL of each solution was then extracted with 2 mL of benzene (with internal GC standard). The 25 mL sample for diethyl disulfide yield measurement was extracted with 1 mL of benzene (containing internal GC standard). GC standards for diethyl disulfide (aqueous concentrations were 34.5 17.25, 8.63, and 3.45 μM) were also made in 25 mL of H_2O and extracted with 1 mL of benzene (containing internal GC standard). Every other sample was injected twice during GC analysis to monitor the reproducibility.

HP 5890 Series II GC with an HP 5970 MS detector (Hewlett-Packard) was used for GC-MS analysis. GC-MS conditions were as follows: 30 m × 0.25 mm i.d. fused silica capillary column with 0.25 μm film thickness (DB-5ms, J&W Scientific) and a carrier gas of helium (5.5 psi) were used;

initial temperature was 40 °C; temperature increased at 8.5 °C/min up to 240 °C; injector port temperature was 225 °C; detector temperature was 250 °C. After the last samples were taken, the remaining phorate hydrolysis reaction mixtures were extracted with Empore SDB-RPS extraction disks (Fisher Scientific) which were then eluted with 20 mL of ethyl acetate (Fisher Scientific). The elutes served as GC-MS samples. Neat diethyl disulfide was also dissolved in ethyl acetate and subjected to GC-MS analysis as a standard.

Wet Chemistry Analyses. Ammonium acetate and formaldehyde were obtained from Fisher Scientific. Acetylacetone and diethyl disulfide were purchased from Aldrich. A Shimadzu UV-1201S spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD) with 1 and 5 cm quartz cells were used for absorbance measurement. [HCHO] was measured using the Nash method (Nash, 1953). Fresh Nash reagent (30% NH_4OAc (w/v) and 0.4% acetylacetone (v/v) in water) and formaldehyde standard solutions were prepared as described in Smith and Erhardt (1975). Formaldehyde, acetylacetone, and ammonia form 3,5-diacetyl-1,4-dihydrolutidine (the so-called Hantzsch reaction). Absorbance of 3,5-diacetyl-1,4-dihydrolutidine was determined at 415 nm. The blank was prepared by using doubly deionized H_2O instead of formaldehyde solution in sample preparation. To quantify the production of HCHO during phorate hydrolysis, phorate hydrolysis mixture (pH 8.5, starting [Phorate] was 69 μM) samples were prepared in the following two ways:

(1) The hydrolysis reaction was set up as described in the hydrolysis experiments section. One 5 mL sample was taken 10 days after the reaction was started.

(2) Four parallel hydrolysis reactions were set up as above except that the reaction mixtures were purged with N_2 for 20 min prior to the addition of phorate in order to reduce the dissolved oxygen to a negligible level. The reactions were stopped after 2, 5, 7, and 9 days, respectively, when 5 mL samples were taken and analyzed immediately afterward.

For a qualitative assessment of H_2S production, a phorate hydrolysis experiment at pH 8.5 was set up and purged with N_2 to remove dissolved oxygen. Samples of 20 mL each were taken by a syringe through a rubber septum, and the liquid volume was replaced by the N_2 stored in a balloon which was connected to the reactor via a needle through the septum. Samples were quickly mixed with an equal volume of a preservation buffer (2 M NaOH, 0.2 M Na_2EDTA, 0.2 M ascorbic acid in water) and stored at 4 °C. Sulfide concentrations of the samples was then analyzed by Model 94-16 silver/sulfide electrode from Orion Research Inc. (Boston, MA) following the manufacturer's protocol.

Toxicity Analysis. Microtox from Azur was used to measure the toxicity of phorate and one of its degradation products, diethyl disulfide. Twenty parts per million aqueous solutions of phorate (77 μM) or diethyl disulfide (164 μM) was prepared as the initial sample, while the rest of samples were prepared via a 2-fold serial dilution (the last sample was at 0.16 ppm). Phenol solution (100 μM) was used as a toxicity standard. A basic test procedure from the manufacturer's manual was used, and the assay was repeated three times.

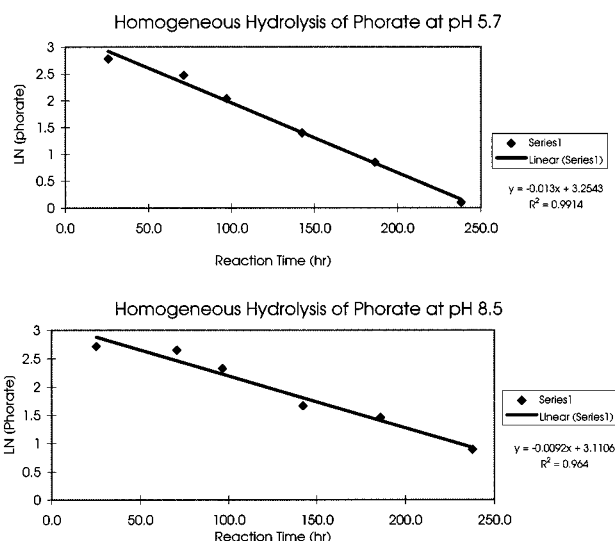

Figure 3. Pseudo-first-order kinetics of phorate hydrolysis.

RESULTS AND DISCUSSION

Hydrolysis Experiments. The three iron oxides are proved to be of high purity with x-ray diffraction analysis. Values of *d* spacing of goethite and hematite are successfully matched to those of previous determinations (Brindley et al., 1958). However, the amorphous $Al(OH)_3$ contains a small amount of γ-Al_2O_3, ι-Al_2O_3, and χ-Al_2O_3.

The hydrolysis reaction is treated as a pseudo-first-order reaction and the following equation is applied: $dC/dt = -k_{obs}C$. The observed rate constant for the hydrolysis reaction, k_{obs}, actually represents the sum of three terms: $k_{obs} = k_a[H^+] + k_N + k_b[OH^-]$, where k_a, k_b, and k_N represent the rate constants for acid-catalyzed, base-catalyzed, and neutral hydrolysis pathways, respectively. The k_{obs} is measured as the slope of the plot of ln[pesticide] vs the reaction time (Figure 3). The kinetics data of most of the experiments display good fit with pseudo-first-order reaction profile, which in turn strengthens the validity of the treatment. Observed homogeneous hydrolysis rate constants of phorate at four different pH values (5.7, 8.5, 9.4, and 10.25) are shown in Table 1 (each rate constant represents more than one experiment). The values of k_a, k_b, and k_N for homogeneous hydrolysis are calculated via nonlinear regression to be 2.67×10^{-2} M^{-1} s^{-1}, 1.45×10^{-2} M^{-1} s^{-1}, and 3.19×10^{-6} s^{-1}. It turns out that the acid-catalyzed pathway is quite significant for phorate, despite that the hydrolysis of most phosphoric and thiophosphoric acid esters is quite insensitive to

Table 1. Hydrolysis Rate Constants of Phorate

reaction conditions	$k_{obs} \pm SD^a$ (s^{-1})	$t_{1/2}$ (h)	no. of exp
homogeneous, pH 5.7	$3.71 \times 10^{-6} \pm 3.69 \times 10^{-7}$	52	4
homogeneous, pH 8.5	$3.15 \times 10^{-6} \pm 5.03 \times 10^{-7}$	61	4
homogeneous, pH 9.4	$3.11 \times 10^{-6} \pm 5.89 \times 10^{-8}$	62	2
homogeneous, pH 10.25	$5.83 \times 10^{-6} \pm 5.89 \times 10^{-7}$	33	2
heterogeneous with hematite, pH 5.7	$3.19 \times 10^{-6} \pm 7.73 \times 10^{-7}$	60	3
heterogeneous with hematite, pH 8.5	$3.64 \times 10^{-6} \pm 5.46 \times 10^{-7}$	53	3
heterogeneous with goethite, pH 5.7	$2.78 \times 10^{-6} \pm 4.82 \times 10^{-7}$	69	3
heterogeneous with goethite, pH 8.5	$3.97 \times 10^{-6} \pm 5.30 \times 10^{-7}$	48	3
heterogeneous with ferrihydrite, pH 5.7	2.86×10^{-6}	67	2
heterogeneous with ferrihydrite, pH 8.5	$3.67 \times 10^{-6} \pm 3.74 \times 10^{-7}$	52	2
heterogeneous with $Al(OH)_3$, pH 5.7	$3.49 \times 10^{-6} \pm 8.84 \times 10^{-7}$	55	2
heterogeneous with $Al(OH)_3$, pH 8.5	$3.33 \times 10^{-6} \pm 5.11 \times 10^{-7}$	58	2

[a] SD = standard deviation.

Table 2. Data from Phorate Adsorption Experiments

oxide and pH	$[\text{phorate}]_{\text{control}}$ (ppm)	$[\text{phorate}]_{\text{supernatant}}$ (ppm)	adsorption[a] (mg/g)
$Al(OH)_3$ pH 5.7	15	15	0
$Al(OH)_3$ pH 5.7	12	11	1
$Al(OH)_3$ pH 8.5	21	21	0
$Al(OH)_3$ pH 8.5	13	13	0
goethite pH 5.7	13	8	5
goethite pH 5.7	11	7	4
goethite pH 8.5	15	12	3
goethite pH 8.5	10	9	1
hematite pH 5.7	11	11	0
hematite pH 8.5	16	18	0
hematite pH 8.5	8	8	0
ferrihydrite pH 5.7	9	9	0
ferrihydrite pH 5.7	9	8	1
ferrihydrite pH 8.5	10	10	0
ferrihydrite pH 8.5	10	9	1

[a] Milligrams of pesticide adsorbed per gram of oxide.

acid catalysis (Schwarzenbach et al., 1993). Although at pH 5.7 heterogeneous hydrolysis experiments all exhibit smaller k_{obs} values than that of the homogeneous experiments, while the opposite trend is observed for hydrolysis experiments at pH 8.5, the difference is not statistically significant enough to indicate any catalytic or inhibitory effect of metal oxides on the hydrolysis of phorate. The lack of significant adsorption of phorate onto the (hydr)oxide surface is one possible reason for the lack of catalytic effect. The presence of methanol and carbon tetrachloride makes the cosolvent effect a viable candidate for the disruption of the phorate–surface interaction (Torrents and Stone, 1994). However, the concentrations of methanol (0.1% (v/v)) and carbon tetrachloride (0.04% (v/v)) were so low that they were unlikely to have any large impact. On the other hand, the pH values of the hydrolysis experiments were not very low; hence, there was abundant supply of nucleophiles such as OH^- in the solution and the nucleophilic attack by the surface-coordinated hydroxide ions became less important.

While the acid-catalyzed hydrolysis of phorate is comparable to that of the base-catalyzed hydrolysis, most phosphoric and thiophosphoric acid esters are quite insensitive to acid-catalyzed hydrolysis (Schwarzenbach et al., 1993). A good example is malathion, which is also a dithiophosphate. The k_b:k_a ratio of malathion at room temperature (27 °C) is more than 10^5 (Wolfe et al., 1977) compared to the k_b:k_a ratio of phorate, 0.54, found in this study.

Adsorption Experiments. Data for adsorption experiments of phorate with the four metal oxides are shown in Table 2. $[\text{Phorate}]_{\text{control}}$ represents the total concentration of phorate in the adsorption mixture, while $[\text{phorate}]_{\text{supernatant}}$ represents the unadsorbed concentration of phorate. Because these adsorption experiments were conducted over a time period of several months, there was a certain degree of pipeting error and variations among different batches of phorate stock solutions. Overall, no significant adsorption is observed with the exception that goethite sorbs about a third of the phorate at pH 5.7 and about a fifth of the phorate at pH 8.5. The lack of adsorption of phorate by the four metal oxides can be attributed to several possible factors. First, phorate has a P=S bond; while the S atom is more polarizable, it carries a much smaller negative charge compared to the O atom in the P=O bond and, hence, is a less favorable target for complexation to a hard metal ion (e.g., Fe^{3+} and Al^{3+}). Second, phorate does not have any lone-pair-electron-donating O or N atom in their side chains (Hay, 1987). Third, the S atom is a much weaker lone-pair electron donor, and it is also a soft base, while Fe^{3+} and Al^{3+} are both hard acids. Finally, the concentration of metal (hydr)oxides may not be high enough to result in significant adsorption. In fact, the lack of adsorption is likely to be one of the reasons for the absence of catalytic activity by these metal oxides on phorate hydrolysis (Stone and Torrents, 1995). A more conclusive study could employ the ATR–FTIR (attenuated total reflection–Fourier transform infrared spectroscopy) (Tejedor-Tejedor and Anderson, 1986) to pinpoint the exact interaction between phorate and the four metal (hydr)oxides in the aqueous environment.

Product Analysis. Figure 4 shows the GC spectrum of the ethyl acetate extract of the final reaction mixture from a pH 8.5 hydrolysis experiment of phorate. The MS spectrum of the GC peak at 5.786 min (retention time) is in the middle of the same figure (the three other peaks were found to be impurities in the solvent). A search of the on-line MS spectra library selected diethyl disulfide as a good match (87%) for the compound whose retention time on GC was 5.786 min. The MS spectrum of diethyl disulfide in the library is shown in the bottom of the figure. Neat diethyl disulfide, purchased from Aldrich and dissolved in ethyl acetate, yielded the same GC retention time (~5.8 min) and MS spectrum as that of the suspected diethyl disulfide found in the extract of the phorate hydrolysis experiment at pH 8.5. When a custom library was built with the MS spectrum of neat diethyl disulfide obtained in our laboratory, the match improved to 95%. Diethyl disulfide was still identified in the phorate hydrolysis mixture at pH 8.5 even when the starting phorate concentration was reduced to 13.8 μM (one-fifth of that used in most experiments). This result suggests that diethyl disulfide results from a major degradation pathway of phorate because it is observed even when the starting phorate concentration is low. However, the mass balance experiment showed that the yield of diethyl disulfide was only about 10% in pH 8.5 hydrolysis experiments. Oxidation of ethanethiol to diethyl disulfide by oxygen is a rapid reaction, and it is reported that prolonged reaction time results in oxidation of thiols beyond the disulfide level in aqueous reactions (Capozzi and Modena, 1974). The more oxidized products, RSO_2^- and RSO_3^-, are more hydrophilic than diethyl disulfide and not detectable by extraction and GC-MS.

Diethyl disulfide is an interesting hydrolysis product because it most likely forms from the dimerization of ethanethiol (a molecular segment of phorate's side chain). Diethyl disulfide was later identified in all subsequent GC-MS analyses of the phorate hydrolysis experiment at pH 8.5 (also in pH 9.4 experiments and in some pH 5.7 experiments). Two control experiments were also conducted. One was a phorate hydrolysis experiment at pH 8.5 where deoxygenated condition (dissolved oxygen was removed by purging the reaction mixture with N_2 for 15 min prior to the onset of the experiment) was used; the phorate hydrolysis rate remained the same, while the diethyl disulfide peak disappeared from the GC spectrum. In the other control experiment, 70 μM ethanethiol was dissolved in 0.01 M boric acid buffer (pH 8.5) under oxygenated and deoxygenated conditions. After overnight mixing, the mixtures were extracted with Empore extraction disks and

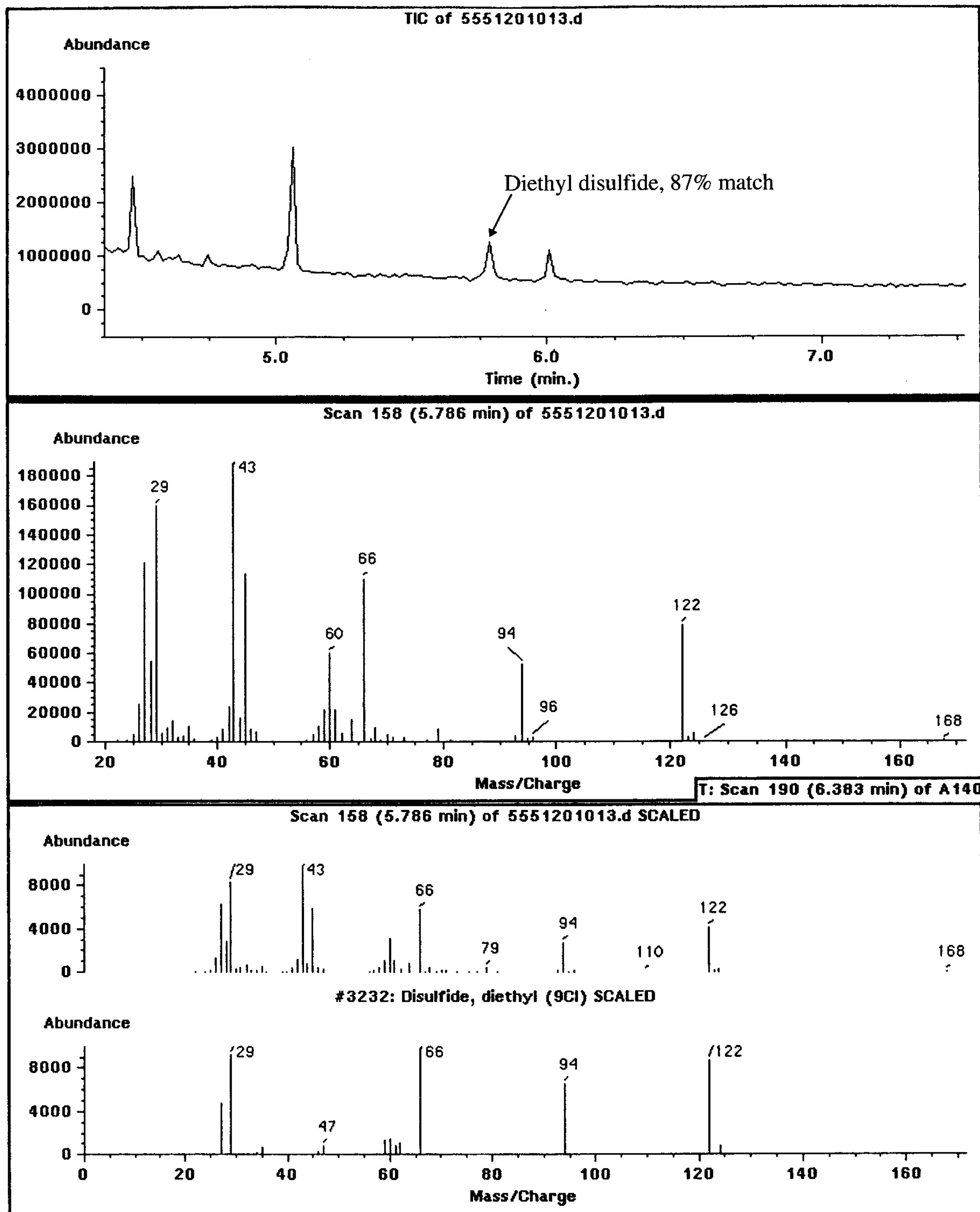

Figure 4. GC-MS spectra leading to the identification of diethyl disulfide.

the ethyl acetate elutes were analyzed on GC-MS. Diethyl disulfide forms under the oxygenated condition but not under the deoxygenated condition. These provide further support to the notion that diethyl disulfide is an oxidation product of ethanethiol. The fact that diethyl disulfide has been observed more frequently in pH 8.5 experiments than in pH 5.7 experiments (where the yield was also lower) is likely due to the following: EtS^- is the key intermediate in the oxidation of ethanethiol to disulfide (Capozzi and Modena, 1974), and the ratio $[EtS^-]$:[EtSH] increases with pH. On the basis of the identification of diethyl disulfide and the general scheme of organophosphorus pesticide hydrolysis proposed by Schwarzenbach et al. (1993), two hydrolysis pathways are proposed (Figures 5 and 6). Because diethyl dithiophosphate and diethyl phosphorothioate are more acidic than ethanethiol, their conjugate bases are better leaving groups than ethanethiol anion during nucleophilic attack. Therefore, we suggest the formation of ethanethiol at a later step in mechanism 2 (Figure 6). The structure of the phosphorus-containing products (Figure 7) in the two proposed mechanisms is similar to those reported for other dithiophosphates (Wolfe et al., 1977).

Wet Chemistry Analyses. A series of wet chemistry tests were designed in an attempt to detect the formation of other possible products (i.e., HCHO and H_2S) and to evaluate the soundness of the two possible pathways

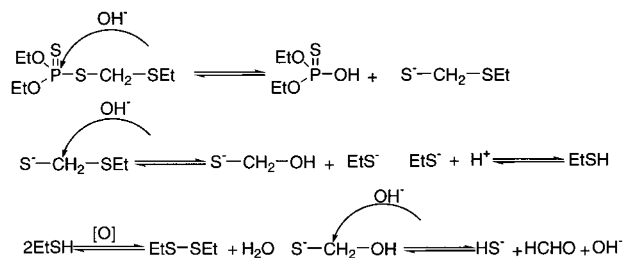

Figure 5. Mechanism 1 for the hydrolysis of phorate at pH 8.5.

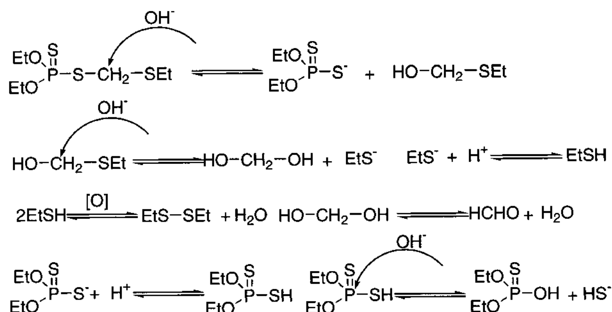

Figure 6. Mechanism 2 for the hydrolysis of phorate at pH 8.5.

Figure 7. Two potential P-containing products of phorate hydrolysis.

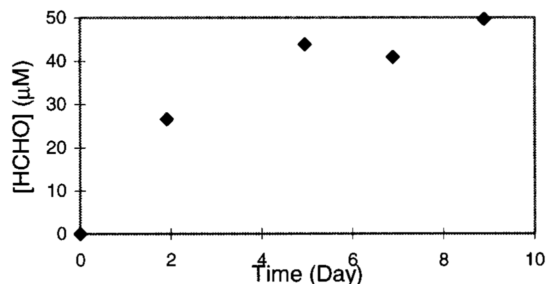

Figure 8. Time profile of HCHO formation during phorate hydrolysis at pH 8.5.

Table 3. Measurement of HCHO Concentration

	absorpn at 415 nm	HCHO concn (μM)
standards	0.092	15
	0.185	31
	0.353	62
	0.688	123
	1.286	246
sample	0.112	21.2
sample[a]	0.263	49.6

[a] Taken from the deoxygenated experiment with N_2 purging.

for phorate hydrolysis. Formation of HCHO was confirmed by the Nash method, and HCHO concentrations at the end of phorate hydrolysis experiments at pH 8.5 are shown in Table 3. Figure 8 shows the time course of HCHO production in a deoxygenated (with N_2 purging) phorate hydrolysis experiment at pH 8.5. The starting concentration of phorate was 69 μM in the hydrolysis experiments; therefore, $[HCHO]_{MAX}$ at the end of the hydrolysis reaction should also be 69 μM. Evaporation to the gaseous phase, polymerization to paraformaldehyde, and oxidation (formaldehyde is a strong reducing agent under alkaline conditions) most likely account for the difference between the observed $[HCHO]_{MAX}$ of 49.6 μM and the projected $[HCHO]_{MAX}$ of 69 μM. Interestingly, Chen and Morris (1972) reported that the oxidation of sulfide induced the oxidation of HCHO at alkaline pH. The significant difference between the HCHO yield of the oxygenated and deoxygenated experiments (21.2 μM and 49.6 μM) suggests that oxidation by dissolved oxygen is a major sink for HCHO. Considering the mass balance (49.6 μM vs 69 μM), it indicates that HCHO-producing pathways are responsible for the majority of phorate hydrolysis. However, it is still possible that other degradation pathways exist where HCHO is not a product.

Table 4. Data of Microtox Test

	phenol	phorate	diethyl disulfide
EC_{50} (ppm)	13−26	11.50	2.0
EC_{50} (mM)	0.14−0.28	0.044	0.016

Formation of H_2S was also qualitatively confirmed by the sulfide electrode measurement. However, sulfide exists mainly in HS^- form at pH 8.5, and the oxidation of HS^- is sensitive to base catalysis. At pH 8.5, the half-life of sulfide in seawater saturated with oxygen was found to be 1−2 h, depending on the initial concentration of sulfide (Almgren and Hagström, 1974); at pH 7.94, the half-life of sulfide was about 50 h in freshwater (Chen and Morris, 1972). Interestingly, HCHO was found to accelerate the oxidation of sulfide by nearly 10-fold when the pH is about 8.5 (Chen and Morris, 1972). Because the hydrolysis experiment was not conducted under stringent anoxic conditions and the duration of the experiment was about 10 days, the yield of sulfide was low and it declined with time (data not shown). Wet chemistry experiments have strengthened the likelihood of the proposed pathways by confirming the production of HCHO and H_2S. However, both pathways can potentially produce these compounds. Hence no conclusion can be drawn to deny the existence of either at this point. A very practical scenario is that both pathways are valid while contributing differently to the hydrolysis of phorate. Identification of the P-containing hydrolysis products and the ensuing kinetics study will be necessary to gain further insight into the complicated system.

Toxicity Analysis. Microtox, when compared with acute lethality tests, such as *Daphnia* or rainbow trout bioassay, has been found to be just as sensitive to most pure organic compounds (Munkittrick and Power, 1991). EC_{50} is defined as the concentration of a compound that reduces the luciferase activity of *Photobacterium phosphoreum* by 50%. EC_{50} values for phenol standard, phorate, and diethyl disulfide are shown in Table 4. Diethyl disulfide is found to be about two and a half times as toxic as phorate on a molar basis (10 times as toxic as phenol). Organophosphorus pesticides were considered to be toxic toward humans (Faust and Gomaa, 1972). However, the toxicities of their degradation products have not received enough attention. In the case of phorate, diethyl disulfide is a potential toxic agent. In cells, diethyl disulfide can be easily reduced back to ethanethiol, which can potentially disrupt the function of many enzymes by forming a S−S bond with cysteine, an amino acid commonly found in active sites of enzymes (Mathews and van Holde, 1990). Formaldehyde is a suspected carcinogen (Perara and Petito, 1982), while hydrogen sulfide is an insidious poison

(Gosselin and Gleason, 1976). Considering that the thiol group is a common molecular segment of many organophosphorus pesticides, it seems reasonable to say that the potential of seemingly environmentally friendly organophosphorus pesticides to be the precursors to toxic compounds has been underestimated.

CONCLUSIONS

At room temperature, the hydrolysis rates of phorate at pH 5.7 and at pH 8.5 were found to be comparable. The basic and neutral hydrolysis rate constants of phorate are similar in magnitude to those of other phosphoric and thiophosphoric triesters (Faust and Gomaa, 1972; Mabey and Mill, 1978; Wanner et al., 1989). While most of the organophosphorus triesters are relatively insensitive to acidic hydrolysis, the acidic hydrolysis rate constant of phorate was found to be quite significant. Interestingly the acidic hydrolysis of disulfoton, which is only one $-CH_2-$ different from phorate, is negligible (Faust and Gomaa, 1972). Some of the major metal (hydr)oxides found in the natural environment do not seem to have significant influence on the half-life of phorate under the experimental conditions employed here.

Abiotic hydrolysis of phorate under alkaline conditions produces diethyl disulfide, hydrogen sulfide, and formaldehyde, all of which are toxic compounds. What is also noteworthy is the uniqueness of degradation products identified here. Neither diethyl disulfide nor formaldehyde has ever been reported as the degradation product of organophosphorus pesticides. Even H_2S is rarely mentioned in the literature as a product of organophosphorus pesticide hydrolysis. While the structure of the phosphorus-containing bulky product is important, it is also more predictable on the basis of previous work. The more variable side chain gives each organophosphorus pesticide its distinct physical, chemical, and pesticidal activity, and the hydrolysis products derived from the side chain deserve more attention. For example, compared to phorate, both malathion and disulfoton are structurally similar dithiophosphates, yet both produce very different hydrolysis products (Wolfe et al, 1977; Dannenberg and Pehkonen, 1998). The current study underscores the importance of research on the hydrolysis pathways, product identification, and product toxicity analysis prior to the introduction of new organophosphorus pesticides into the environment.

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