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Article

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Investigation of the Nucleophilic Attack of Dichlorvos by Reduced Sulfur Species using ^{1}H NMR

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The mechanism of the reaction of dichlorvos through hydrolysis reactions and through the reaction with polysulfide (S_n^{2-}) and thiophenolate (PhS^-) were investigated by proton nuclear magnetic resonance (^1H NMR). The study confirmed product identities of an organophosphorus insecticide reacting with reduced sulfur species using ^1H NMR in oxygen sensitive solutions. The experiments of dichlorvos with polysulfide led to the detection of a previously undetected product. The thiophenolate experiments were further advanced to investigate second-order rate kinetics using an internal standard. The experiments provide new evidence for a nucleophilic attack by the reduced sulfur species at the methoxy carbon of dichlorvos. In addition, the observation of in-situ reaction dynamics illustrates the applicability of ^1H NMR spectroscopy toward kinetic investigations in environmental science.

KEYWORDS: Dichlorvos, ^1H NMR, thiophenolate, methyl polysulfide, desmethyl dichlorvos

Introduction

Organophosphorus compounds (i.e., esters and thioesters of phosphoric acids) are among the most common insecticides encountered in surface waters in the U.S.[1] Dichlorvos (for structure see Fig. 2) is an organophosphorus pesticide that has been in widespread use for over 45 years. It is reported to be genotoxic in vitro.[2] It causes tumors in rats and mice in some studies but not others.[3,4] Apart from having actively been used as a pesticide, it has been found to be the breakdown product of the pesticide trichlorfon. It is also generated in many plants through metabolism of the pesticide naled – of which it is postulated as the chief "insecticidal" principle.[3,5-8] Application of dichlorvos for various pest control approaches have resulted in its release into sensitive environments such as salt marshes and estuaries where anoxic conditions are known to be prevalent.[9] Anaerobic conditions in these waters can result in the formation of reduced sulfur species such as bisulfide, polysulfide (S_n^{2-}), thiosulfate, and organosulfur species (e.g., glutathione and cysteine).[10-12] In order to determine dichlorvos' fate in these environments, hydrolysis at environmentally relevant pH and nucleophilic substitution by reduced sulfur species were considered to be the primary reaction pathways.

Phosphate triesters are known to hydrolyze via a nucleophilic substitution with the attack of OH^- on the phosphorus atom resulting in the cleavage of a P-O bond, while H_2O might also attack at the carbon of a methoxy or ethoxy group.[13,14] When OH^- attacks the phosphorus of dichlorvos, the vinyl phosphate bond is most susceptible to breakage due to electron withdrawing chlorines on the vinyl-group.[15] This has been supported by past investigations showing that dichlorvos hydrolysis resulted in the formation of dichloroacetaldehyde that is in equilibrium with dichlorovinyl alcohol

(dichloroethenol).[9,16] However, the formation of dimethyl phosphate, the other expected product of this reaction was not analyzed. In the current work, ^{1}H NMR was employed to further support the previously proposed reaction mechanism. Since ^{1}H NMR has the potential to provide insight into all hydrogen containing products formed, it can deliver valuable information on reaction pathways, as well as potential subsequent reactions of primary products.

Reduced sulfur species have been known to react with a wide array of organic pollutants that undergo nucleophilic substitution.[17-20] Concentrations of S_n^{2-} as high as 0.33 mM have been reported in porewaters of marine sediment.[21,22] While concentrations of thiophenol have not been reported, thiophenol-containing compounds are likely to form through reactions of dissolved natural organic matter with bisulfide. Previous work in our lab with S_n^{2-} and organophosphorus pesticide (e.g., chlorpyrifos-methyl) support a substitution reaction at the carbon of the methoxy group of the pesticide.[23] S_n^{2-} and thiophenolate are expected to react analogously with dichlorvos, resulting in the formation of *O*-desmethyl dichlorvos and methylated reduced sulfur species – methylated polysulfides for S_n^{2-} (Figure 2A) and thioanisol for thiophenolate. The attack of the reduced sulfur species at the phosphorus atom resulting in the formation of sulfur-substituted dimethyl phosphate and dichloroacetaldehyde (Figure 2C) was also considered, although less likely to occur due to unfavorable hard-soft interactions.

Previous investigation in our lab determined the second order reaction rate constant of dichlorvos with thiophenolate (pH≥9.0) by analyzing samples of aqueous reaction solutions periodically by gas chromatography.[9] In this work, reactions of

dichlorvos in D_2O were monitored in-situ and reaction rate constants were derived directly from NMR resonance changes over time.

^{31}P NMR has been used to study kinetics and to identify degradation products of reactions of organophosphorus insecticides.[24,25] Previously, ^{31}P NMR had been utilized to investigate the hydrolysis of dichlorvos at different pH values and the collected data supported expected product identities.[26] However, the reported kinetics conflicted with expectations, the measured reaction rate decreased with increase in basicity. It also reported the formation of dimethyl phosphate as the product of hydrolysis (at pH 5.6, 7, and 9).[26] Our work extended toward ^{1}H NMR utilization because of more structural elucidation possibilities than ^{31}P NMR. Minor chemical changes in structure that might remain undetected by ^{31}P NMR are more likely to be identified by ^{1}H NMR. Also, products lacking ^{31}P would effectively remain 'hidden' in ^{31}P NMR. However, the formation of new products can only be monitored in-situ, if the product protons are in a magnetic environment that is distinctly different from the environment of reactant protons. If this condition is satisfactorily met, following kinetic experiments by ^{1}H NMR has the advantage of revealing all products.

In this work, the hydrolysis of dichlorvos was monitored in D_2O at pH* 6.7, 7.7, and 9.1. In addition, reactions of dichlorvos with polysulfide and thiophenolate as nucleophiles were conducted in D_2O to elucidate the identity of the products formed and to test the suitability of ^{1}H NMR to measure reaction rate constants in oxygen sensitive solutions.

Materials and Methods

Chemicals. All chemicals were used as received. Dichlorvos (2,2-dichlorovinyl dimethyl phosphate, 99%) was obtained from Chem Service (West Chester, PA). D_2O (99.9 atom % D) and methanol-D4 (D, 99.8%) were received from Cambridge Isotope Laboratories, Inc (Andover, MA). Iodomethane (CH_3I, 99%), sodium sulfide (Na_2S_4), anhydrous sodium tetraborate ($Na_2B_4O_7$, 99.998 %) and anhydrous potassium phosphate (K_3PO_4, 97%) were received from Alfa Aesar (Ward Hill, MA). D_3PO_4 (D-enrichment 99%, nitrogen flushed) was received from ACROS Organics (New Jersey, USA). Thiophenol was received from Lancaster Synthesis Inc (99%, Pelham, NH). Thioanisole was obtained from TCI (Portland, OR). 4,4-Dimethyl-4-silapentane-1-sulfonic acid (DSS) was obtained from Sigma-Aldrich (St. Louis, MO). All solvents and reagents that were used were analytical grade or equivalent.

Instrument. 500 MHz Varian Inova NMR Spectrometer was utilized. The spectra were acquired in D_2O and at 25 ˚C. In general spectra were obtained as 32 scans with a relaxation delay (d1) of 5 seconds. This allowed the acquisition of spectra in just under 5 minutes. It is of primary importance to verify beforehand the nuclear relaxation times of all compounds of interest. For DDS as an internal standard we noticed a lack of quantitative correlation between various DSS peaks applying the previously mentioned parameters. Therefore, the relaxation delay (d1) was increased to 30 seconds while decreasing the number of scans to 8 per spectrum in experiments with DDS. This still produced very acceptable spectra with each spectrum still acquired in less than 5 minutes.

Preparation of Solutions: *Buffer Stock Solution*: All solutions were prepared in an anoxic glovebox (5% H_2, 95% N_2) with argon-purged solutions. Borate buffer stock solutions were prepared in argon-purged D_2O. 5 mM deuterated phosphoric acid/potassium phosphate buffer was prepared for hydrolysis experiments conducted at pHs 6.7 and 7.7. For reduced sulfur experiments and hydrolysis experiments at pH 9, borate buffer was utilized. 100 mg of anhydrous sodium tetraborate was dissolved in 5 mL deoxygenated D_2O resulting in a borate stock solution of 400 mM. The buffer solution was stored in the anoxic glove box.

Dichlorvos Stock Solution: Various dichlorvos stock solutions ranging in concentrations from 40 mM to 70 mM were prepared in deoxygenated D_2O or deoxygenated CD_3OD in a 1.0 mL volumetric flask and stored in the glove box.

^1H NMR Internal Standard Preparation: DSS stock solution was prepared in 10 mL deoxygenated D_2O and stored in the anoxic glovebox. Stock solution concentrations ranged from 15 mM to 30 mM for all solutions prepared throughout the study.

Polysulfide Stock Solution Preparation: Na_2S_4 powder was used to prepare 5 mL deoxygenated D_2O solutions in 100 mM borate buffer and 100 mM NaCl. The final polysulfide solution concentration was determined through iodometric titration using a starch endpoint.

Thiophenolate Stock Solution: Thiophenolate stock solutions, ranging in concentrations from 30 mM to 60 mM, were prepared by diluting pure thiophenol in 100 mM tetraborate buffer in the anoxic glovebox. The final thiophenol stock solution concentrations were determined through iodometric titration using a starch endpoint.

126 *Polysulfide and Thiophenolate Reaction Solution Preparation.* Reduced sulfur species
127 reaction solutions were prepared by adding an appropriate amount of the respective
128 reduced sulfur species stock solution to a 5-mL volumetric flask and filled up with D_2O.
129 0.5 mL of this reaction solution was used for the 5 mm NMR glass tube to which an
130 appropriate amount of dichlorvos with/without DSS was added prior to it. For selected
131 experiments, dichlorvos and DSS were added to the thiophenolate reaction solution (in 5
132 mL volumetric flask) before transferring approximately 0.5 mL to the NMR glass tube.
133 Three time one milliliter of the remaining reaction solution were used for iodometric
134 titration with a starch endpoint to determine the concentrations of total reduced sulfur
135 concentration $[S(-II)]_T$. $[S(-II)]_T$ of polysulfide solutions represents the sum of $[H_2S]_T$ and
136 $[H_2S_n]_T$, where the latter represents the total concentration of polysulfides, hydrogen-
137 polysulfides, and sulfanes numerically equal to $\sum([S_n^{2-}]+[HS_n^-]+[H_2S_n])$ for n = 2-5. The
138 polysulfide concentrations were calculated on the basis of the measurement of $S(-II)_T$ and
139 the equilibrium constants reported by Giggenbach[27] with excess S(0) as described in
140 detail by Lippa and Roberts.[18] In case of thiophenolate reaction solutions, the
141 concentrations determined by iodometric titration were adjusted for speciation using the
142 pK_a of thiophenol, as reported to be 6.50.[28]
143 *^{1}H NMR Reaction Solution Preparation:* All steps to prepare the reaction solutions were
144 performed in the anoxic glovebox with thoroughly washed glassware that was dried
145 overnight at 200 ºC. The NMR tube was deoxygenated prior to its transfer to the
146 glovebox by flushing it with argon to ensure that all air was removed. Appropriate
147 amount of dichlorvos that would maintain pseudo-first order kinetics (dichlorvos reaction
148 concentrations ranging from 10 μM to 50 μM) was withdrawn from the dichlorvos stock

149 solution and transferred to a 5 mm NMR glass tube. Appropriate amount of DSS
150 (concentrations maintained at values in par with the reduced sulfur concentrations) was
151 added to the NMR tube for selected experiments where DSS was used as an internal
152 standard and internal reference. All transfers were carried out using either microsyringes
153 (excluding reduced sulfur solutions) or micropipettes. Finally, 0.5 mL of the reduced
154 sulfur reaction solution was transferred to the NMR tube. The tube was closed with a
155 pressure cap and sealed with parafilm. The contents were mixed by shaking the NMR
156 tube vigorously prior to probing with a 500 MHz NMR for at least three half-lives of
157 dichlorvos degradation. The amount of time from mixing the reaction contents to
158 obtaining the initial NMR spectrum was about 10 minutes. All experiments were
159 conducted at 25 °C. Pseudo-first-order rate constants were determined by regression of
160 the natural logarithm of applicable dichlorvos NMR chemical signal versus time.
161 **pH measurement**. The remaining reaction solution that was not transferred to the NMR
162 tube was used for pH* determination using an Accumet pH meter (Fisher Scientific,
163 Pittsburg, PA) with a Ross combination pH electrode (ThermoOrion, Beverly, MA). The
164 measured values are reported as pH*, which is an uncorrected reading in a D_2O solution
165 of the H_2O calibrated pH-meter, where pD = pH* + 0.4.[29]
166
167 **Results and Discussion**
168 **Degradation of dichlorvos through hydrolysis**
169 ***A) Qualitative determination*.** Hydrolysis experiments were conducted at pH* 6.7, 7.7
170 and 9.1. ^{1}H NMR peaks that were changing in intensity with reaction time were
171 scrutinized. Figure 1 shows the ^{1}H NMR spectra of dichlorvos hydrolysis at pH* 9.1

showing all relevant peaks. The signal centered at 3.47 ppm was identified as dimethyl phosphate (the expected products when hydroxide attacks at the phosphorus atom). The signal further downfield at 3.54 ppm was identified to be desmethyl dichlorvos, that resulted from water attacking dichlorvos at one of the methoxy carbons. The two equivalent dichlorvos methyl groups produces a phosphorus-split doublet at 3.81 ppm. Two more signals are observed at 3.71 ppm and 3.22 ppm. But their lack of change in intensity over the duration of the experiment led to the conclusion that these peaks correspond to compounds that are either impurities or breakdown products that formed in the dichlorvos stock solution prior to the experiment. A similar lack in intensity change was observed for the desmethyl dichlorvos signal at 3.54 ppm. Overall, these three peaks had no significant contribution to the dichlorvos degradation under the given conditions. These observations indicate that the hydrolysis reaction at pH* 9.1 proceeded with an attack of the nucleophile (e.g., hydroxide ion) at the phosphorus atom as published in previous work. The hydrolysis experiment at pH* 7.7 also shows dimethyl phosphate as the major product, however it also showed a minor increase in the integral for the desmethyl dichlorvos and methanol peak (signal at 3.20 ppm). This observation seems to indicate that at pH* 7.7 the nucleophilic attack at the phosphorus in no longer the only reaction pathway. There also seems to be nucleophilic attack of water at the methoxy carbon leading to the formation of desmethyl dichlorvos and methanol. In our previous work investigating the hydrolysis of dichlorvos, the formation of dichloroacetaldehyde was characterized by GC-MS after derivation with PFBHA-HCl.[9] In general, an aldehyde is expected to be the more stable than the corresponding enol form (tautomer). However, the expected aldehyde peak centered around 10 ppm was completely absent in this study.

195 This lack of an aldehyde proton can be explained by the enol – aldehyde and the aldehyde
196 – diol equilibria. The SPARC online calculator (developed at the University of Georgia
197 using the computational approach outlined by Carreira et al.[30]) was used to calculate the
198 equilibrium constants of these two equilibria. Between the enol (dichloroethenol) and the
199 respective aldehyde (dichloroacetaldehyde), the enol is expected to be the major tautomer
200 by approximately 95%. In addition, the aldehyde can also react with water and form a
201 diol (dichloroethanediol). According to SPARC, the diol of dichloroacetaldehyde
202 (dichloroethanediol) occurs at more than 99% when water is the solvent at pH 7.
203 Therefore, if we consider all species at pH 9.0, the dichloroethanediol occurs at about
204 90%, while the deprotonated dichloroethanediol and the deprotonated enolate occur at
205 1% and 9%, respectively. Thus, the most likely structure responsible for the resonating
206 proton at approximately 6.8 ppm in Figure 1 is dichloroethanediol. While ChemDraw
207 (version 7.0.1) NMR calculations assign an estimated shift of 5.58 ppm to the
208 dichloroethanediol, it assigns an almost identical shift (5.60 ppm) to the dichloroethenol.
209 Thus, it is possible that the vinylic proton of the 9% of the dichloroethenol present may
210 be resonating at the same frequency as the proton of the dichloroethanediol.
211 ***B) Kinetic treatment.*** No internal standard was used for hydrolysis experiments. The
212 NMR reaction tube was removed from the instrument between acquisitions of
213 consecutive spectra since slow hydrolysis rate even at pH 9.1 required a considerable
214 wait time before significant changes in dichlorvos concentration could occur. As a result,
215 spectral shift assignments could not be compared directly as NMR parameters were reset
216 every time the reaction tube was reintroduced into the instrumental probe. This
217 shortcoming was overcome by normalization of all relevant peak integrals (shown

below), which in this case were the methyoxy protons in dichlorvos and their eventual translation to methoxy protons in dimethyl phosphate.

$$relative\ \%\ integral\ of\ dichlorvos = 100 \cdot \left(\frac{Integral\ of\ dichlorvos}{Integral\ of\ dichlorvos + Integral\ of\ Products}\right) \quad (1)$$

However, this step requires that all products of all competing reaction pathways are included. To ensure that all products were included a carefully comparison of ratios of all observed signals with one another over the entire spectrum was undertaken. This resulted in identification of those signals that were increasing or decreasing with time as opposed to those staying constant. The signals that remained constant with time indicates non-reactive compounds. Pseudo first-order rate constants were obtained from the slope of the semi-logarithmic plots of the normalized peak integral of dichlorvos versus time. The peak integral was normalized by dividing a peak integral by the sum of all integrals of peaks that change in area over time, Eq. 1. Rate of degradation increased with pH confirming our previous observation[8] that OD^- anion is a stronger nucleophile than D_2O, which is the dominant nucleophile in acidic solutions. The so determined reaction rate constants for the hydrolysis in D_2O at pH* 6.7, 7.7, and 9.1 were found to be 0.055 d^{-1}, 0.086 d^{-1}, and 0.19 d^{-1}, respectively. Hydrolysis experiment in water and analyzed with gas chromatography performed in our lab at pH 7.12 and 25 °C showed a reaction rate constant of 0.32 d^{-1} which is considerably faster than the rate observed here in D_2O.[9] Solvent effects (H_2O vs D_2O) are reported for the nucleophilic attack at phosphate esters[31,32] and might explain the slower reaction rates in D_2O versus H_2O. The results of the hydrolysis experiments allowed us to confirm the products identities from our

previous work and locate the associated peaks in the ^{1}H NMR spectrum. The knowledge of the spectral peaks of dimethyl phosphate and dichloroaldehyde/dichloroethenol made the interpretation of the NMR spectra of the experiments with polysulfide and thiophenolate significantly easier.

Reaction of dichlorvos with polysulfide. It has been proposed that polysulfides react with structurally related organophosphorus insecticides by attacking a methoxy carbon.[33] This potential attack of polysulfide at a methoxy carbon of dichlorvos would result in the formation of *O*-desmethyl dichlorvos and methylated polysulfides (Figure 2A). It might also be possible that polysulfide attacks the phosphorus atom or the carbon in the vinyl group (Figure 2B). Attack by polysulfide at the phosphorous atom (Figure 2C) would result in the formation of dimethyl phosphate and dichloroacetaldehyde/dichloroethanediol. ^{1}H NMR was used again to monitor the reaction with the intend to identify the forming products. Based on the analysis of the product peaks increasing in intensity over time, we conclude that polysulfide attacks at a methoxy carbon of dichlorvos. Figure 3 shows three consecutive ^{1}H NMR spectra of the reaction of dichlorvos in 2.0 mM polysulfide at pH* 8.3. (For this experiment the NMR tube was not removed from the instrument thus no resetting the measurement parameters was necessary). While there is a dimethyl phosphate peak centered at 3.43 ppm, a peak centered at 6.88 ppm could signify the presence of dichloroethandiol/dichloroethenol. This could result in erroneous conclusion that the alternative mechanism (Figure 2C) is occurring. However, the dimethyl phosphate peak maintained a constant intensity throughout the course of the experiment, therefore the formation of dichloroethandiol/dichloroethanol is unlikely. The peak at 6.88 ppm is much more likely

attributed to the lone proton on the dichloroethenoxy moiety of desmethyl dichlorvos, which would resonate at an almost identical frequency as the dichloroethandiol. Additional support for the polysulfide attack at the methoxy carbon is obtained by the observation of increase in area of the signal for multiple singlets between 2.0 ppm and 2.7 ppm. These singlets agree with the expected signals resulting from various methylated polysulfide molecules that would form as products along with desmethyl dichlorvos. Four such peaks are observed at 2.16 ppm, 2.36 ppm, 2.48 ppm and 2.61 ppm. The sum of those peak intensities agreed with the signal intensity resulting from desmethyl dichlorvos at 3.54 ppm. Although these two products were not individually probed for their respective relaxation times, a near agreement between the integral of the two supports the mechanism postulated in Figure 2A wherein the three methoxy protons in desmethyl dichlorvos and the three methyl protons of methylated polysulfide should produce similar intensities due to the same number of resonating protons. To verify the identity of the signals between 2.16 ppm and 2.61 ppm, a spectrum was obtained of 0.5 mL of 50 mM polysulfide in excess iodomethane. Figure 3 shows this spectrum; the highest peak is assigned to the unreacted excess of iodomethane. The inset provides further verification when a secondary spectrum is obtained after adding more polysulfide to the NMR tube. It should be noted that any given polysulfide molecule is methylated twice resulting in six protons per molecule when the electrophile is in excess (as in the case for iodomethane here). But even in the experimental case where dichlorvos is the limiting electrophile, for any dimethylated polysulfide, two molecules of desmethyl dichlorvos would be produced resulting in six methoxy protons resonating at 3.54 ppm. This would preserve linearity between the two signal intensities, which is what is

observed. The reaction rate for dichlorvos in 2.0 mM S_n^{2-} at pH* 8.3 was determined to be 5.2 d^{-1} while the reaction rate of dichlorvos at pH* 9.1 through hydrolysis was 0.19 d^{-1}. This indicates that the contribution of hydrolysis in this experiment can be neglected.

Reaction of dichlorvos with thiophenolate. Reaction of dichlorvos with thiophenol was previously examined showing that nucleophilic attack by thiophenol/thiophenolate at the methoxy carbon resulted in the formation of thioanisol.[9] The observed degradation rate of dichlorvos in a thiophenol solution is the sum of three individual reaction rates resulting from hydrolysis, reaction with thiophenol and reaction with thiophenolate:

$$k_{obs} = k_h + k_{PhSH}[PhSH] + k_{PhS-}[PhS^-] \qquad (2)$$

If the thiophenol/thiophenolate concentration is large enough, the contribution of hydrolysis (k_h) can be neglected. The mole fraction of thiophenolate (α_{PhS}-) at a given pH is given by the equation below.

$$\alpha_{PhS-} = \frac{[PhS^-]}{[PhSH]+[PhS^-]} = \left(1 + \frac{[H^+]}{K_a}\right)^{-1} \qquad (3)$$

All thiophenol experiments were carried out at pH* 9.0 where the pK_a of thiophenol (pK_a 6.50 at 25 °) favors the deprotonated form (thiophenolate) by more than 99%.[28] Hence the observed degradation of dichlorvos in these experiments can solely be attributed to thiophenolate.

^{1}H NMR spectra for the reaction the dichlorvos with thiophenolate indicate a nucleophilic substitution pathway through an attack of thiophenolate at a methoxy carbon resulting in the formation of *O*-desmethyl dichlorvos and thioanisol. Spectral results showed three separate aromatic peaks centered at 6.8 ppm, 7.0 ppm, and 7.3 ppm that were assigned to *para*-, *meta*-, and *ortho*-protons on thiophenolate, respectively.

Aromatic peaks from the forming thioanisol are overlapping with the peaks of thiophenolate. The mechanistic pathway whereby dichlorvos undergoes nucleophilic attack by thiophenolate at the methoxy carbon was supported by observation of a singlet at 2.4 ppm, which was assigned to the resonating methyl group on thioanisol. This peak was confirmed by observing a singlet at 2.4 ppm in a spectrum obtained from commercial thioanisol. The peak at 3.2 ppm remained constant throughout all experiments. It was attributed to resonance of protons of the solvent methanol. No dimethyl phosphate peak growth was observed. Unlike spectra of dichlorvos in polysulfide where 1:1 signal intensities were observed between desmethyl dichlorvos and methylated polysulfides, signal intensities between desmethyl dichlorvos and thioanisol showed a slightly slower growth for the methyl group of thioansiol compared to the growth of the methyl group in desmethyl dichlorvos. Differential relaxation time between the two products is not expected to affect the relative signal intensities of these two products. At the same time, thioanisol solubility in water is 7.0 mM which was well above the expected concentration of thiosanisol formed in the experiment. However, the cause of the slower peak growth of thioanisol versus desmethyl dichlorvos was hypothesized to be the adsorption of thioanisol to the surface of thiophenolate dimers (diphenyl disulfide) particles which can form by (photo)-oxidation of thiophenolate (see Figure 4). Diphenyl disulfide has a very low solubility in water and therefore precipitates and forms colloids. It is possible that thioanisol may adsorb to those diphenyl disulfide colloids. (It was observed that the thiophenol reaction solution turned slowly turbid over the course of several hours). In order to address this issue an internal standard (DSS) was added to the reaction solutions. Two consecutive spectra of commercial thioanisol in borate buffer (pH* 9.0) with

internal standard DSS were obtained within an interval of three days. It was observed that all thioanisol resonance signals (aromatic and methyl) had decreased significantly indicating a decrease in concentration of the compound. Except for a weak signal from methanol, the 'missing' protons from thioanisol were not observed elsewhere in the spectra, which indicated some removal of thioanisol from solution. This observation supports the hypothesis that thioanisol might be removed from solution by adsorption. In particular, since thioanisol is slowly hydrolyzed at elevated pH to thiophenolate and then can oxidize to diphenyl disulfide.

Internal Standard and Kinetic Measurements. The hydrolysis experiments were conducted over serval days and the NMR reaction tube was removed frequently. Therefore, the signal integral had to be normalized. The experiments with polysulfide had no such requirement since they were much faster. In those cases, three half-lives of dichlorvos degradation were observed with a dedicated NMR instrument. In either case, decreasing dichlorvos signal strength was plotted against time to obtain first-order exponential decay plots. Semi-logarithmic translation of these plots provided pseudo-first order rate constants for the respective nucleophile concentrations. However, at low thiophenolate concentration, due to a slower reaction rate, the NMR tube was removed from the NMR magnet in between measurements. In such a case, the ^{1}H NMR signals could not directly be assigned a concentration value. Normalization of the ^{1}H NMR signals was not possible, since we noticed that the signal loss due to dichlorvos reacting did not correspond to the signal increase due to thioanisol and desmethyl dichlorvos formation. Therefore, 2,2-dimethyl-2-silapentane-5-sulfonic acid (DDS) was used as an internal standard for experiments where thiophenolate was the nucleophile. DSS was

chosen as the internal standard after assessing its non-interference in terms of ^{1}H NMR resonance at frequencies of interest and its lack of reactivity towards the electrophile or the nucleophile. The singlet arising from its nine tri-methyl protons was chosen to determine concentrations of all reaction participants. The internal standard was also used to determine the thiophenolate concentration. Since thiophenolate might undergo photooxidation, it is stored in a dark vial to minimize this process. However, during transfer to the NMR tube and the transport to the NMR some exposure to light is difficult to avoid completely. The possibility of in-situ oxidation could effectively reduce the reaction concentration of thiophenolate in the reaction (in the NMR tube). Since the concentrations determined by iodometric titration were measured with an amount of the reaction solution that was not transferred to an NMR tube, the concentration determined by iodometric titration could potentially be higher than the concentration in the NMR tube. The proton in *para*-position of thiophenolate which resonates at a relatively 'uncrowded' distance from the other aromatic protons was used to obtain in-situ thiophenolate concentrations. These concentrations of thiophenolate obtained for experiments where DSS was utilized were compared to the concentration values obtained through iodometric titration. For the lower thiophenolate concentrations, the values obtained through the internal standard tend to be lower than those obtained through iodometric titration. However, values for higher concentrations of thiophenolate showed that spectral values were slightly larger than iodometric determinations. This can be explained by significant resonance overlap that was observed between various aromatic peaks (including the lone *para*-proton) when experiments were designed with very high thiophenolate concentrations. Figure 5 shows a plot of pseudo-first order rate constants

379 against thiophenolate concentrations determined through iodometric titrations. The
380 second order rate constant for the reaction of thiophenolate with dichlorvos in D_2O was
381 derived from this plot. Its value of 0.80 ± 0.16 min^{-1} M^{-1}, when compared to the reported
382 value determined in H_2O and with GC-FID of 1.30 ± 0.09 min^{-1} M^{-1} might be indicative
383 of a deuterium effect.

384 The use of an internal standard allowed the determination of reaction rate
385 constants even when some of the products were not stable over time and therefore the
386 total area of protons considered was decreasing with time.

387

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390 willingness to engage in numerous discussions involving this project.

391

392 **Supporting Information**

393 Supporting Information is available free of charge on the ACD Publication website:
394 Figure S1: calculated equilibrium constants determining the speciation of
395 dichloroacetaldeyde, dichloroethandiol, and dichloroethenol. Figure S2: plot of natural
396 logarithm of relative signal integral at 3.8 ppm versus time for the hydrolysis of
397 dichlorvos at pH* 7.7. Figure S3: ^{1}H NMR spectra (500 MHz) of mixtures of polysulfide
398 and iodomethane in D_2O at 25 ˚C. Figure S4: ^{1}H NMR spectrum (500 MHz) of
399 dichlorvos reacting with thiophenolate in D_2O, pH* 9.0 at 25 ˚C. Table S1: normalized
400 signal integral of dichlorvos and its products for the hydrolysis in D_2O at pH* 7.7 at 25
401 ˚C. Table S2: signal integrals for the reaction of 2.0 mM polysulfides with dichlorvos in

402 D_2O, pH* 8.25. Table S3: signal integrals of the various methoxy groups for the reaction
403 of dichlorvos with 39 mM thiophenolate at pH* 9.0. Table S4: concentrations for
404 thiophenolate determined through iodometric titration and spectral assignment with
405 internal standard.
406

References

(1) Stone, W. W.; Gilliom, R. J.; Ryberg, K. R., Pesticides in U.S. streams and rivers: occurrence and trends during 1992-2011. *Environ. Sci. Technol.* **2014,** *48*, 11025-11030.

(2) Booth, E. D.; Jones, E.; Elliott, B. M., Review of the in vitro and in vivo genotoxicity of dichlorvos. *Regul. Toxicol. Pharmacol.* **2007,** *49*, 316-326.

(3) Dichlorvos: Initiation of special review, Notice OPP-30000/56. EPA, Ed. Fed. Regist.: 1988; Vol. 53, pp 5542-5549.

(4) Dichlorvos (DDVP, Vapone) Revocation of food additive tolerance: Final rule, Notice OPP-300237A, FRL-4188-7. EPA, Ed. Fed. Regist.: 1993; Vol. 58, p 59663.

(5) Menzie, C. M., Fate of pesticides in the environment. *Annu. Rev. Entomol.* **1972,** *17*, 199-222.

(6) *PIP-Naled. ECOTOXNET Profile for Naled*; Extension Toxicology Network, Pesticide Information Network, Pesticide Information Network, Cornell Univiersty Network, Ithaca, NY: 1994.

(7) *PIP-Trichlorfon, EXOTOXNET Profile for Trichlorfon*; Extension Toxicology Network, Pesticide Information Network, Pesticide Information Network, Cornell Univiersty Network, Ithaca, NY: 1993.

(8) Gan, Q.; Singh, R. M.; Jans, U., Degradation of naled and dichlorvos promoted by reduced sulfur species in well-defined anoxic aqueous solutions. *Environ. Sci. Technol.* **2006,** *40*, 778-783.

(9) Gan, Q.; Singh, R. M.; Wu, T.; Jans, U., Kinetics and mechanism of degradation of dichlorvos in aqueous solution containing reduced sulfur species. *Environ. Sci. Technol.* **2006,** *40*, 5717-5723.

(10) Barbash, J. E.; Reinhard, M., Reactivity of sulfur nucleophiles toward halogenated organic compounds in natural waters. In *Biogenic Sulfur in the Environment*, Satzman, E. S.; Cooper, W. J., Eds. ACS: Washington, DC, 1989; pp 101-138.

(11) Howarth, R. W.; Teal, J. M., Sulfate reduction in a New England salt marsh. *Limnol. Oceanogr.* **1979,** *24*, 999-1013.

(12) Gun, J.; Goifman, A.; Shkrob, I.; Kamyshny, A.; Ginzburg, B.; Hadas, O.; Dor, I.; Modestov, A. D.; Lev, O., Formation of polysulfides in an oxygen rich freshwater lake and their role in the production of volatile sulfur compounds in aquatic systems. *Environ. Sci. Technol.* **2000,** *34*, 4741-4746.

(13) Cox, J. R.; Ramsay, O. B., Mechanisms of nucleophilic substitution in phosphate esters. *Chem. Rev.* **1964,** *64*, 317-352.

(14) Faust, S. D.; Gomaa, H. M., Chemical hydrolysis of some organic phosphorus and carbamate pesticides in aquatic environments. *Environ. Lett.* **1972,** *3*, 171-201.

(15) Lamoreaux, R. J.; Newland, L. W., The fate of dichlorvos in soil. *Chemsophere* **1978,** *7*, 807-814.

(16) Mühlmann, R.; Schrader, G., Hydrolyse der insektiziden Phosphorsäureester. *Zeitschrift fur Naturforschung B* **1957,** *12*, 196-208.

(17) Lippa, K. A.; Demel, S.; Lau, I. H.; Roberts, A. L., Kinetics and mechanism of the nucleophilic displacement reactions of chloroacetanilide herbicides: Investigation and α-substituent effects. *J. Agric. Food Chem.* **2004,** *52*, 3010-3021.

(18) Lippa, K. A.; Roberts, A. L., Nucleophilic aromatic substitution reactions of chloroazines with bisulfides (HS^-) and polysulfides (S_n^{2-}). *Environ. Sci. Technol.* **2002,** *36*, 2008-2018.
(19) Loch, A. R.; Lippa, K. A.; Carlson, D. L.; Chin, Y. P.; Traina, S. J.; Roberts, A. L., Nucleophilic aliphatic substitution reaction of propachlor, alachlor, and metolachlor with bisulfide (HS^-) and polysulfide (S_n^{2-}). *Environ. Sci. Technol.* **2002,** *36*, 4065-4073.
(20) Roberts, A. L.; Sanborn, P. L.; Gschwend, P. M., Nucleophilic substitution reactions of dihalomethanes with hydrogen sulfide species. *Environ. Sci. Technol.* **1992,** *26*, 2263-2274.
(21) Luther, G. W., Polarographic analysis of sulfur species in marine porewaters. *Limnol. Oceanogr.* **1985,** *30*, 727-736.
(22) MacCrehan, W.; Shea, D., Temporal relationship of thiols to inorganic sulfur compounds in anoxic Chesapeake Bay sediment porewater. In *Geochemical Transformations of Sedimentary Sulfur*, Vairavamurthy, M. A.; Schoonen, M. A. A., Eds. ACS: Washington, DC, 1995; pp 294-310.
(23) Wu, T.; Jans, U., Nucleophilic substitution reactions of chlorpyrifos-methyl with sulfur species. *Environ. Sci. Technol.* **2006,** *40*, 784-790.
(24) Pehkonen, S. O.; Judeh, Z. M. A., Mechanism of interactions between Hg(II) and demeton S: an NMR study. *Environ. Sci. Technol.* **2005,** *39*, 2586-2591.
(25) Choi, H.; Yang, K.; Koo, I. S., ^{31}P NMR Kinetic studies on fenithrothion hydrolysis in the presence of Ag^+ and Hg^{2+} ions. *Bull. Korean Chem. Soc.* **2015,** *36*, 707-710.
(26) Benoit-Marquie, F.; De Montety, C.; Gilard, V.; Martino, R.; Maurette, M. T.; Malet-Martino, M., Dichlorvos degradation studied by ^{31}P NMR. *Environ. Chem. Letters* **2004,** *2*, 93-97.
(27) Giggenbach, W., Optical spectra and equilibrium distribution of polysulfide ions in aqueous solution at 20°. *Inorg. Chem.* **1972,** *11*, 1201-1207.
(28) Dean, J. A., *Lange's Handbook of Chemistry*. McGraw-Hill: New York, 1985.
(29) Krezel, A.; Bal, W., A formula for correlating pK_a values determined in D_2O and H_2O. *J. Inorg. Biochem.* **2004,** *98*, 161-166.
(30) Carreira, L. A.; Hilal, S.; Karickhoff, S. W., Estimation of chemical reactivity parameters and physical properties of organic molecules using SPARC. In *Theoretical and Computational Chemistry, Quantitative Treatments of Solute/Solvent Interactions*, Politzer, P.; Murray, J. S., Eds. Elsevier Publishers: 1994.
(31) Kovach, I. M.; Bennett, A. J.; Bibbs, J. A.; Zhao, Q., Nucleophilic and protolytic catalysis of phosphonate hydrolysis: isotope effects and activation parameters. *J. Am. Chem. Soc.* **1993,** *115*, 5138-5144.
(32) Khan, S. A.; Kirby, A. J., The reactivity of phosphate esters. Multiple structure-reactivity correlations for the reactions of triesters with nucleophiles. *J. Chem. Soc. (B)* **1970,** (6), 1172-1182.
(33) Wu, T.; Gan, Q.; Jans, U., Nucleophilic substitution of phosphorothionate ester pesticides with bisulfide (HS^-) and polysulfide (S_n^{2-}). *Environ. Sci. Technol.* **2006,** *40*, 5428-5434.

Figure Captions:

Figure 1. ^{1}H NMR of dichlorvos and its degradation product in D_2O buffer and 25 ˚C at 500 MHz. The residual water signal was suppressed before signal acquisition.

Figure 2. Three potential reaction pathways of dichlorvos with polysulfides that were considered

Figure 3. Three ^{1}H NMR spectra of the reaction of dichlorvos with 7.6 mM polysulfide in D_2O at pH* 8.3 and 25 ˚C acquired after 10 min (bottom), 26 min (middle), and 175 min (top).

Figure 4. Oxidation of thiophenolate to diphenyl disulfide.

Figure 5. Plot of thiophenolate concentration (iodometric titration) versus observed reaction rate constants of dichlorvos with thiophenolate in D_2O, 100 mM borate buffer at pH* 9.0 and 25 ˚C.

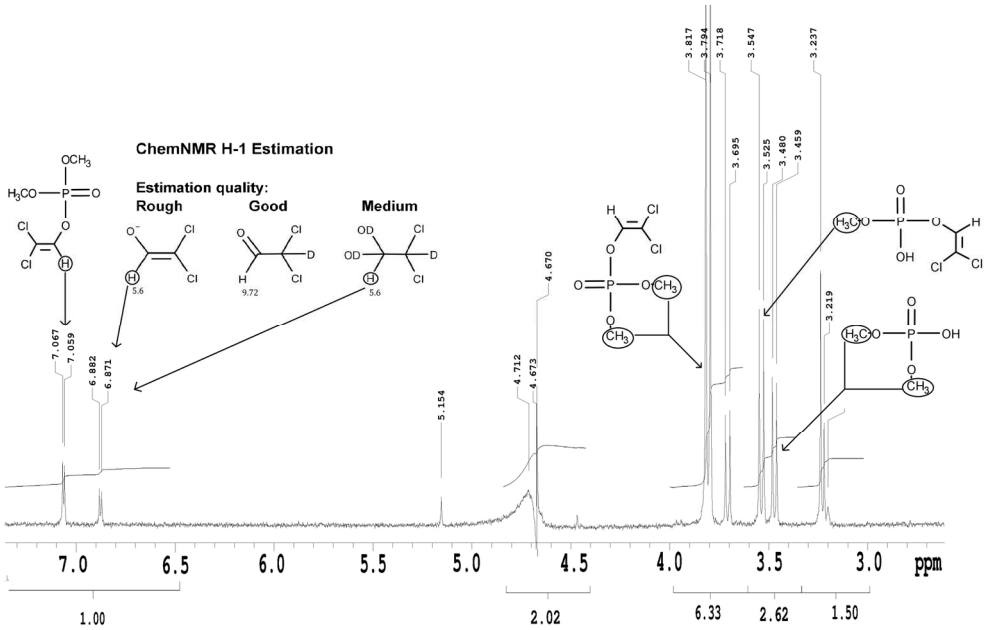

Figure 1. ^{1}H NMR of dichlorvos and its degradation product in D_2O buffer and 25 °C at 500 MHz. The residual water signal was suppressed before signal acquisition.

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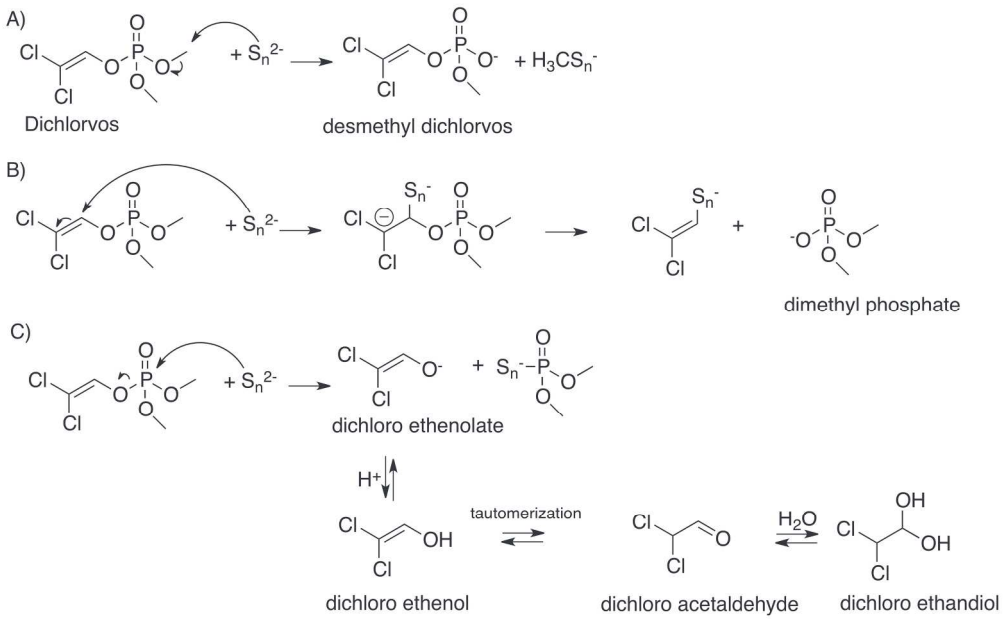

Figure 2. Three potential reaction pathways of dichlorvos with polysulfides that were considered

106x65mm (600 x 600 DPI)

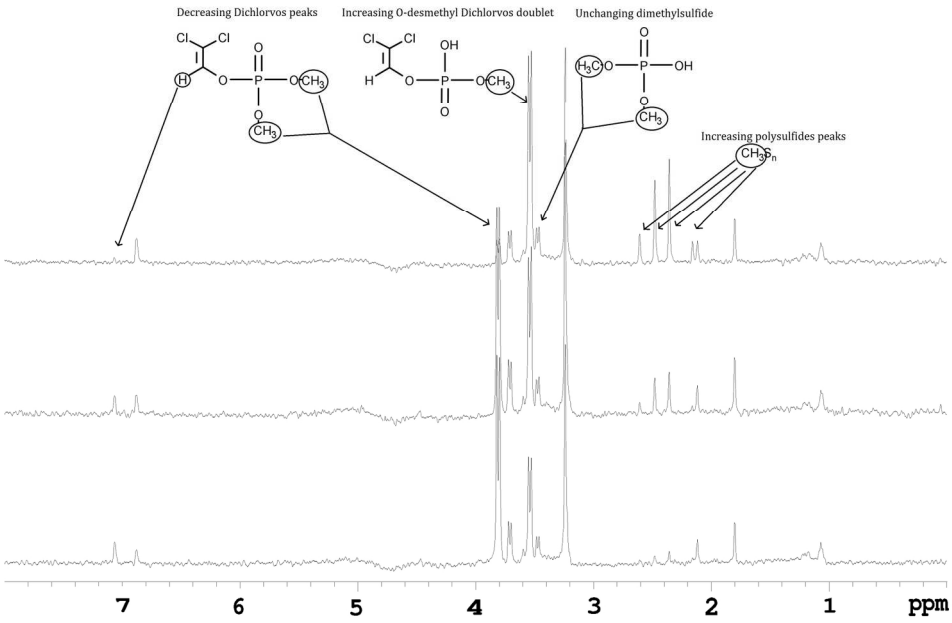

Figure 3.Three ^{1}H NMR spectra of the reaction of dichlorvos with 7.6 mM polysulfide in D_2O at pH* 8.3 and 25 °C acquired after 10 min (bottom), 26 min (middle), and 175 min (top).

170x109mm (300 x 300 DPI)

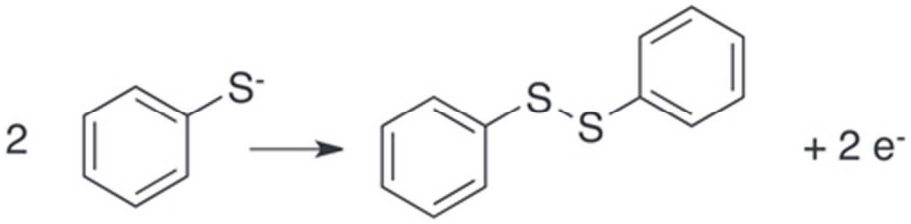

Figure 4. Oxidation of thiophenolate to diphenyl disulfide.

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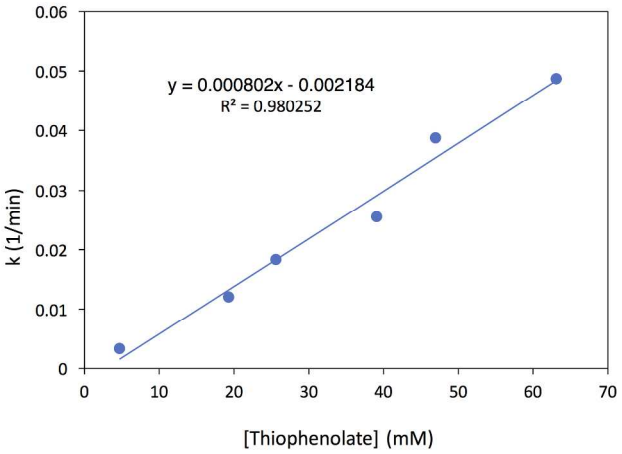
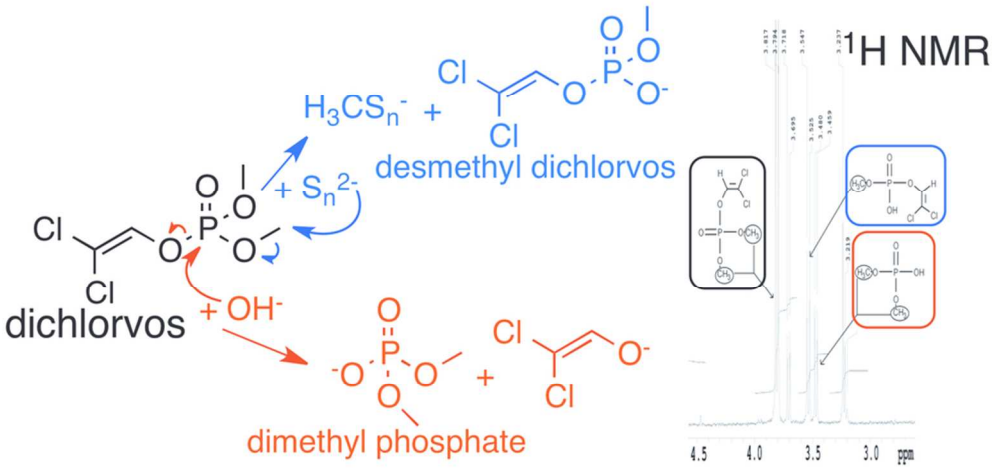


Figure 5. Plot of thiophenolate concentration (iodometric titration) versus observed reaction rate constants of dichlorvos with thiophenolate in D_2O, 100 mM borate buffer at pH* 9.0 and 25 °C.

215x279mm (300 x 300 DPI)



TOC Graphic

41x20mm (600 x 600 DPI)