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Nucleophilic Substitution Reactions of Chlorpyrifos-methyl with Sulfur Species

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Chlorpyrifos-methyl is widely used in the control of insects on certain stored grain, including wheat, barley, oats, rice, and sorghum. The reactions of chlorpyrifos-methyl with hydrogensulfide/bisulfide (H_2S/HS^-), polysulfides (S_n^{2-}), thiophenolate (PhS^-), and thiosulfate ($S_2O_3^{2-}$) were examined in well-defined aqueous solutions over a pH range from 5 to 9. The rates are first-order in the concentration of the different reduced sulfur species. The resulting data indicate that chlorpyrifos-methyl undergoes a S_N2 reaction with the reduced sulfur species. The transformation products indicate that the nucleophilic substitution of reduced sulfur species occurs at the carbon atom of a methoxy group to form the desmethyl chlorpyrifos-methyl. The formation of trichloropyridinol, a minor degradation product, could be attributed entirely to hydrolysis. The reaction of chlorpyrifos-methyl with thiophenolate leads to the formation of the corresponding methylated sulfur compound. The resulting pseudo-first-order rate constant for chlorpyrifos-methyl with bisulfide yielded a second-order rate constant of 2.2 ($\pm$0.1) $\times$ 10^{-3} M^{-1} s^{-1}. The determined second-order rate constants show that the reaction of chlorpyrifos-methyl with HS^- is of the same order of magnitude as the reaction of chlorpyrifos-methyl with $S_2O_3^{2-}$ with a second-order rate constant of 1.0 ($\pm$0.1) $\times$ 10^{-3} M^{-1} s^{-1}. The second-order rate constant for chlorpyrifos-methyl with polysulfides (3.1 ($\pm$0.3) $\times$ 10^{-2} M^{-1} s^{-1}) is of the same order of magnitude as the one with thiophenolate (2.1 ($\pm$0.2) $\times$ 10^{-2} M^{-1} s^{-1}). The second-order rate constant for the reaction of polysulfides is approximately 1 order of magnitude greater than that for the reaction with HS^-. When the determined second-order rate constants are multiplied by the concentration of HS^-, polysulfides and thiosulfate reported in salt marshes and porewaters, predicted half-lives show that the inorganic reduced sulfur species present at environmentally relevant concentrations may represent an important sink for phosphorothionate triesters in coastal marine environments.

Introduction

Organophosphorus insecticides (OPs), most of which are esters and thioesters of phosphoric and thiophosphoric acid, are widely used throughout the world and have in many cases replaced organochlorine pesticides. The amount of organophosphate insecticides used has declined since 1980, from an estimated 131 million pounds in 1980 to 73 million pounds in 1999 in U.S. agriculture. However, organophosphate usage as a fraction of total insecticide usage has increased from 58% in 1980 to 70% in 2001 (*1*). OPs are also among the most prevalent pesticides encountered in ground and surface waters (*2*, *3*).

Organophosphorus compounds are used as insecticides for their inhibitory activity toward cholinesterase, a key enzyme involved in the metabolism of acetylcholine (one of the major neurotransmitters) (*4*). Because cholinesterase is a rather common enzyme among animals that have a neural system, these pesticides can actually cause harm to many nontarget species. In recent years, the U.S. EPA has begun to consider tougher regulations regarding the use of organophosphorus compounds (*5*).

In general, OPs are less persistent in the environment than organochlorine compounds. The half-lives for OPs in natural water are in the range of days to months (*6*−*9*). The major pathways of degradation for OPs are hydrolysis, oxidation, photolysis, and biodegradation (*8*). Oxidation of OPs to the corresponding oxons, sulfones, and sulfoxides has been reported widely (*10*). Oxidation can occur either biotically via specific enzymes or abiotically by radical mechanisms, ozone, dissolved oxygen, or aqueous chlorine. Photodegradation can occur either by a direct photolysis of OPs, which have an absorption spectrum overlap with the solar spectrum, or by indirect photodegradation, whereby dissolved humic and fulvic acids can act as sensitizer or when particles can lead to semiconductor-promoted photodegradation. Hydrolysis of OPs is perhaps the most thoroughly studied process. It can occur by a homogeneous pathway, where H_2O and OH^- (H^+ catalysis is less common) act as nucleophiles in an S_N2 mechanism. In addition, the presence of dissolved metal ions can enhance the rate of hydrolysis. For example, the presence of Hg^{2+} can increase the initial hydrolysis rate of malathion, fenitrothion, fenthion, and parathion-methyl in a pH 5.5 buffer by 2−3 orders of magnitude, and the hydrolysis was found to be first-order with respect to both the Hg^{2+} ion and the OPs (*11*). Cu^{2+} and Pb^{2+} show significant promotion at relatively low pH for zinophos, diazinon, chlorpyrifos-methyl, parathion-methyl, and fenchlorphos, but at high pH, a decrease in Cu^{2+} catalysis stems from decreased solubility of Cu^{2+} (*6*, *12*). Heterogeneous surfaces such as Fe and Al oxides and different clays can enhance the rate of hydrolysis by providing surface sites at which the nucleophiles and the OP can react (*13*, *14*). Biotransformation of OPs is also reported to be an important and rapid degradation pathway (*15*−*17*). However, the occurrence of the microorganisms and the enzymatic degradation mechanisms has not been thoroughly investigated. Therefore, it is difficult to predict the contribution of biotransformation to the overall fate of OPs in the environment.

OPs that are present in surface water may associate with particles and can eventually become part of the sediment phase. It is also likely that some organophosphorus insecticides are transported into salt marshes and into the anoxic bottom layers of estuaries, which may also contain high concentrations of hydrogen sulfide species (H_2S and HS^-), polysulfides (S_n^{2-}, where n = 2−5), thiosulfate, and organosulfur species. These reduced sulfur species are produced via different microbial sulfate reduction pathways under hypoxic conditions (*18*, *19*), while polysulfides are also produced under oxic conditions (*20*). Concentrations of hydrogen sulfide, polysulfides, and thiosulfate in coastal marine sediment porewaters have been reported to be as

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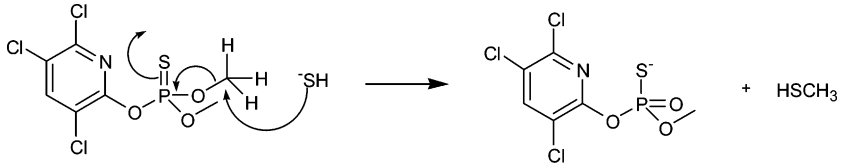
SCHEME 1



Chlorpyrifos-methyl Desmethyl chlorpyrifos-methyl

high as 5.6, 0.33, and 0.6 mM, respectively (*21*). Several organosulfur species were reported to be found in the same sediment; for example, glutathione and cysteine can reach concentrations as high as 2.4 mM and 12.4 μM, respectively (*22*).

Reduced sulfur species are capable of reacting with a wide array of pollutants, including organic contaminants that undergo nucleophilic substitution reactions (*23*−*26*). It has been reported that reduced inorganic and organic sulfur species are the most potent nucleophiles present in the environment (*22*, *27*, *28*). The potential therefore exists for such highly reactive reduced sulfur nucleophiles to serve as environmental "reagents" affecting the abiotic degradation of OPs. Because of its abundance in the subsurface environment, bisulfide (HS^-) has been shown to play an important role in the degradation of organochlorine compounds (*26*). It also has been reported that the lesser abundant polysulfides relative to HS^- can outweigh bisulfide due to their considerably greater reactivity toward relatively simple alkyl halides (*23*, *25*).

Chlorpyrifos-methyl (CPM) (Scheme 1) is a widely used organophosphorus insecticide. It is registered for use in the control of insects on certain stored grain, including wheat, barley, oats, rice, and sorghum as well as for empty grain bins. An average of about 80 000 pounds of active ingredient is used on 5−10% of all stored grain (*29*). Its ethyl analogue, chlorpyrifos, was reported to be used in amounts in excess of 10 million lb/year in U.S. agriculture in 2001 (*1*). Chlorpyrifos-methyl and chlorpyrifos are quite persistent under neutral and acidic conditions typical of surface waters, soils, and aquifer sediments. The use of these two compounds has been restricted due to their high adverse effects (*30*) and high-end residue value in food (*31*). Chlorpyrifos-methyl was chosen for this study because of its increased solubility in water.

In aqueous solution without any other nucleophiles present, phosphate triesters degrade via a S_N2 reactions with attack of OH^- occurring at the phosphorus atom resulting in cleavage of a P−O bond (*32*, *33*). It has also been reported that some phosphate triesters display cleavage of an O−C bond in water, indicating that organophosphate compounds can also undergo a nucleophilic substitution reaction at the carbon atom of an alkoxy group (*9*, *15*, *28*).

In a previous study (*34*), we proposed that in bisulfide solution chlorpyrifos-methyl is likely to undergo a nucleophilic substitution with the attack of bisulfide (HS^-) occurring at the carbon of the methoxy group (Scheme 1). The reaction rate constant of the reaction of chlorpyrifos-methyl with HS^- was reported; however, the major products of the reaction were not characterized.

Several studies indicate that polysulfides (S_n^{2-}), thiosulfate ($S_2O_3^{2-}$), and thiophenolate (PhS^-) are stronger nucleophiles than is bisulfide. Polysulfides and thiosulfate are abundant in anoxic and suboxic aqueous environments (*21*, *22*). Thiophenol was chosen as an organosulfur nucleophile; although the concentration of thiophenol in natural water is not reported, thiophenol-containing compounds are likely to result from the reaction of natural organic matter with bisulfide (*35*). Therefore, in this study, these four nucleophiles were chosen and the reactions with chlorpyrifos-methyl were monitored at varying pH and nucleophile concentration to obtain second-order rate constants.

The reaction of chlorpyrifos-methyl with the four reduced sulfur species is examined in detail; a reaction mechanism is proposed to account for the observed products, intermediates, kinetics, and stoichiometry. Detailed understanding of the pathways and rate constants whereby chlorpyrifos-methyl reacts with HS^-, S_n^{2-}, thiophenolate, and thiosulfate will improve our ability to predict the fate of chlorpyrifos-methyl and related compounds in diverse environmental settings.

Materials and Methods

Chemicals. All chemical reagents were used as received. *O*,*O*-Dimethyl *O*-(3,5,6-trichloro-2-pyridyl)phosphorothionate (chlorpyrifos-methyl) (99.7%, CAS registry no. 5598-13-0), desmethyl chlorpyrifos-methylsodium salt (DmCPM) (70−86%), and the hydrolysis product, 3,5,6-trichloropyridinol (98%, CAS registry no. 6515-38-4), were provided by Dow AgroSciences (Indianapolis, IN).

All solvents and reagents that were used were of analytical grade or equivalent. They were used without further purification and were obtained from Fisher Scientific (Pittsburgh, PA) and EMD Chemicals (Gibbstown, NJ). All solutions were prepared inside a controlled-atmosphere glovebox (96% N_2, 4% H_2, Pd catalyst; Coy Laboratory Products, Grass Lake, MI) using argon-purged deionized water (Milli-Q water) with a resistivity of 18 MΩ cm (Millipore, Bedford, MA).

Reduced Sulfur Solution Preparation and Analysis. Na_2S stock solutions were prepared under argon from $Na_2S{\cdot}9H_2O$ (98%; EM Science) using deoxygenated Milli-Q water as reported by Jans (*34*). Polysulfide stock solutions were prepared under argon via dissolution of purified sodium tetrasulfide crystals (Na_2S_4, 90+% technical grade; Alfa Aesar). Na_2S_4 crystals were purified by grinding the crystal in a mortar, and rinsing with deoxygenated toluene in the glovebox. Thiophenol stock solutions were prepared via dissolution of thiophenol (99%, Lancaster Synthesis, Pelham, NH) in deoxygenated methanol. Thiophenol was placed inside the glovebox immediately at arrival in the lab. The concentration of thiophenol stock solution was monitored by iodometric titration and HPLC. To determine the extinction coefficient of thiophenol on the HPLC, the external standards were prepared in the glovebox and analyzed with HPLC as soon as possible. The results of the two methods were in good agreement (the difference of measured concentrations of thiophenol using these two methods was always smaller than 5%). Reaction solution containing thiosulfate was prepared by dissolving the sodium thiosulfate crystals directly. The concentration of thiosulfate was determined by iodometric titration.

All four nucleophile concentrations were also determined by iodometric titration, and the sodium thiosulfate solution for the titration was standardized against potassium iodate daily (*36*).

Experimental System. All glassware was soaked in 1 M HNO_3 overnight and was rinsed several times with Milli-Q water prior to use. Unless otherwise stated, reaction solutions were prepared in an anaerobic glovebox and equilibrated overnight. The reaction solutions were prepared in volumetric

flasks and then transferred to 20 mL glass syringes equipped with a polycarbonate stopcock and PTFE needle tubing. The syringes contained three PTFE rings to facilitate mixing. All reaction solutions contained 5% methanol and 50 mM buffer (sodium phosphate or sodium tetraborate). NaCl was added to all solutions to yield an ionic strength of 0.25 equiv/L except the reactions for ionic strength effects. The glassware for slow hydrolysis experiments was autoclaved to inhibit biological growth. In addition, the buffer solutions were filtered (0.2 μm, Anotop 25-sterile, Whatman Ltd., Maidstone, England). Filtering of the buffer solution and assembly of autoclaved glassware were carried out in a biological safety cabinet to prevent any microbial contamination. The polycarbonate stopcocks used in the hydrolysis experiments were rinsed with 80% 2-propanol and air-dried in the biological safety cabinet prior to their use in a hydrolysis experiment. The spike solutions of organophosphate were prepared by dissolving parent compounds in deoxygenated methanol. Experiments conducted at methanol concentrations from 0% to 20% indicated that these levels of methanol did not affect the reaction rates in either the presence or the absence of reduced sulfur species in the range of error. An Accumet pH meter (Fisher Scientific) with a Ross combination pH electrode (ThermoOrion, Beverly, MA) was used to measure the pH in the reaction solutions.

The total hydrogen sulfide content ($[H_2S]_T$) of bisulfide solutions, representing the sum of all hydrogen sulfide species ($[H_2S] + [HS^-] + [S^{2-}]$), was measured by iodometric titration. Bisulfide ion concentrations were computed from ($[H_2S]_T$) and pH values, using acidity constants (*37*) that were corrected for ionic strength through activity coefficients determined from the Davies approximation.

The total reduced sulfur content $[S(-II)]_T$ of polysulfide solutions represents the sum of $[H_2S]_T$ and $[H_2S_n]_T$, where the latter represents the total concentration of polysulfides, hydropolysulfides, and sulfanes numerically equal to $\sum([S_n^{2-}] + [HS_n^-] + [H_2S_n])$ for $n = 2-5$. For these solutions, $[S(-II)]_T$ was measured by iodometric titration. Methods appropriate for determining concentrations of individual polysulfide species in complex matrixes have not been developed. The polysulfide concentrations were calculated on the basis of the measurement of $S(-II)_T$ and the equilibrium constants reported by Giggenbach (*38*) with excess S(0) as described in detail by Lippa and Roberts (*24*).

Kinetic Experiments. Reaction kinetics were measured under pseudo-first-order conditions, with an initial chlorpyrifos-methyl concentration of 25 μM, which was typically less than 0.5−1% of the total reduced sulfur species concentration. The reaction solutions were spiked with aliquots (100 μL) of chlorpyrifos-methyl stock solution in deoxygenated methanol. Reactors were vigorously mixed for 30 s in the glovebox and were incubated in a water bath at 25.0 ± 0.1 °C. Aliquots (1 mL) were periodically taken; 2 drops of 6 M HCl were added, and the mixture was extracted with ethyl acetate, followed by analysis via HPLC. The acidification ensures the extraction of products (calculated pK_a value of desmethyl chlorpyrifos-methyl is reported to be 1.7 in 7% alcohol) (*39*). Pseudo-first-order rate constants were obtained by performing a linear regression of the natural logarithm of the parent compound concentration versus time. Reactions were monitored over sufficient time (two to three half-lives) to verify pseudo-first-order kinetics. For selected experiments, the pseudo-first-order rate constants for the degradation of parent compounds and for the formation of degradation products were concurrently determined via nonlinear regression techniques using Scientist for Windows, version 2.01 (MicroMath Scientific Software, Salt Lake City, UT).

Product Derivatization. The chlorpyrifos-methyl transformation products were alkylated using CH_3I (Alfa Aesar, 99%). A 2-mL aliquot of reaction mixture (containing in most cases 25 μM organophosphate product and up to 10 mM sulfur nucleophile) was added to 1 mL of a 0.1 M CH_3I/0.02 M $Na_2B_4O_7$ buffer solution (pH 9) and then heated at 60 °C in a water bath for 1 h. After being cooled to room temperature, the solution was extracted with ethyl acetate, and the extract was analyzed via GC/MS. Additional analyses of the reaction mixtures were conducted without derivatization, after acidifying samples to below pH 1 using hydrochloric acid, followed by extraction into ethyl acetate. This allowed the determination of the chlorpyrifos-methyl fraction that had converted to desmethyl chlorpyrifos-methyl. However, the derivatization condition led to some hydrolysis of the formed product, and therefore the quantification was not possible (compare Figure S-1 in Supporting Information).

GC and GC/MS Analysis. Ethyl acetate extracts were analyzed using a Series 8000 GC (Fisons Instruments) equipped with a split/splitless injector, a FID (EL980, Fisons Instruments), and an EC-5 fused-silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness; Alltech, Deerfield, IL). Chlorpyrifos-methyl transformation products were analyzed using a Trio1000 quadrupole GC/MS (Fisons Instruments) equipped with a split/splitless injector and an AT-1 fused-silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness; Alltech). Ionization mode for MS analyses of products was electron impact (EI). The EI mass spectra were generated using an electron energy of 70 eV and were monitored for ions m/z 50−400 in full scan mode.

HPLC Analysis. The reactions of chlorpyrifos-methyl were followed by Waters 2690 liquid chromatograph equipped with a Waters 996 photodiode array detector. To determine the concentration of chlorpyrifos-methyl and its products, a wavelength of 289 nm was chosen, and a wavelength of 254 nm was chosen to analyze thiophenol and thioanisole. Detailed HPLC methods are provided in the Supporting Information.

Product Quantitation. Trichloropyridinol could be quantified using reference material that was commercially available. Because of a lack of pure desmethyl chlorpyrifos-methyl (DmCPM), the quantification was based on the following assumptions. An impure standard of DmCPM (70−86%) from Dow AgroSciences allowed us to develop analytical methods and determine the retention time of DmCPM. The extraction efficiency of DmCPM into ethyl acetate (after acidifying the aliquots of the reaction mixture to below pH 1) was obtained by analyzing both the organic and the aqueous phase immediately after extraction. Because no DmCPM could be detected in the acidified aqueous phase after extraction, the extraction efficiency was assumed to be 100%. The UV spectrum of DmCPM obtained with the PDA detector was identical to chlorpyrifos-methyl (Supporting Information, Figure S-2), so we assumed that the extinction coefficients (wavelength equal to 289 nm) of DmCPM and chlorpyrifos-methyl are the same. These assumptions were supported by the results of the reaction of chlorpyrifos-methyl with thiophenol (see Results and Discussion).

Results and Discussion

Kinetics of Reactions with Sulfur Nucleophiles at 25 °C. The reaction of chlorpyrifos-methyl with hydrogen sulfide was assessed at different pH values. The plots of ln[parent compound] versus time are linear, indicating that the reactions of chlorpyrifos-methyl in these bisulfide solutions are first-order with respect to chlorpyrifos-methyl. The slope in such a semilogarithmic plot yields a pseudo-first-order rate constant, k_{obs}. Figure S-3 shows representative time-course profiles for reactions of chlorpyrifos-methyl with HS^-. Linear regression analysis of log k_{obs} versus log$[H_2S]_T$ yielded a slope equal to 0.98 ± 0.05 (where the stated uncertainties reflect the 95% confidence limits) at pH 8.6. This result indicates that the reaction of chlorpyrifos-methyl with HS^-

TABLE 1. Second-Order Rate Constants Determined for the S_N2 Reactions of Chlorpyrifos-methyl at 25.0 °C

nucleophile	k'' (M^{-1} s^{-1})[a]	log k''	R^2(adj)
H_2S	1.0 (±2.1) × 10^{-4}		
PhSH	0.3 (±9.4) × 10^{-4}		
$S_2O_3^{2-}$	1.0 (±0.1) × 10^{-3}	−3.0	0.964
HS^-	2.2 (±0.2) × 10^{-3}	−2.7	0.978
PhS^-	2.1 (±0.2) × 10^{-2}	−1.7	0.985
S_n^{2-}	3.1 (±0.3) × 10^{-2}	−1.5	0.989

[a] Second-order rate constant of chlorpyrifos-methyl at 0.050 M sodium phosphate and sodium tetraborate buffer, ionic strength adjusted with NaCl to 0.25 equiv/L, 5% or 10% MeOH at 25 °C.

is an overall second-order process, first-order both in HS^- and in chlorpyrifos-methyl concentrations.

All measured first-order rate constants, k_{obs}, were corrected by subtracting $k_{control}$ values obtained in buffer control experiments conducted at the same temperature and pH to account for competing reactions by other nucleophiles (e.g., HO^-, HPO_4^{2-}, Cl^-). Because of differences in reactivity of the different hydrogen sulfide species (e.g., H_2S vs HS^-), a pH-dependent pseudo-first-order rate constant is expected. This corrected rate constant can be divided by the total concentration of hydrogen sulfide species, which results in the apparent second-order rate constant, k''_{app}. The apparent second-order rate constant can be given as the sum of the second-order rate constant of hydrogensulfide times the mole fraction of hydrogensulfide plus the second-order rate constant of bisulfide times the mole fraction of bisulfide (eq 1). From a plot of k''_{app} versus ($[HS^-]/[H_2S]_T$) (Supporting Information, Figure S-4), k''_{H_2S} and k''_{HS^-} can be determined. The second-order rate constant for the reaction of bisulfide with chlorpyrifos-methyl is 2.2 (±0.1) × 10^{-3} M^{-1} h^{-1}, and the second-order rate constant for the reaction of hydrogen sulfide with chlorpyrifos-methyl is 1.0 (±2.1) × 10^{-4} M^{-1} h^{-1}, which is small and not significantly different from zero at the 95% confidence level (Table 1).

$$k''_{app} = \frac{k_{obs} - k_{control}}{[H_2S]_T} = k''_{H_2S} \times \frac{[H_2S]}{[H_2S]_T} + k''_{HS^-} \times \frac{[HS^-]}{[H_2S]_T}$$

$$= k''_{H_2S} + \frac{[HS^-]}{[H_2S]_T}(k''_{HS^-} - k''_{H_2S}) \quad (1)$$

Similar results to the reaction with HS^- were obtained for reactions of chlorpyrifos-methyl with all other nucleophiles investigated. Linear regression analysis of $\log(k_{obs} - k_{control})$ versus $\log [S_n^{2-}]$ yielded a slope equal to 1.08 ± 0.13. Linear regression analysis of $\log(k_{obs} - k_{control})$ versus $\log[S_2O_3^{2-}]$ yielded a slope equal to 0.98 ± 0.11, and $\log k_{obs}$ versus $\log [PhS^-]$ yielded a slope equal to 1.10 ± 0.17. Therefore, the reactions of chlorpyrifos-methyl with these three nucleophiles also exhibited first-order dependence, and the overall second-order rate constants are listed in Table 1.

The dependence of the pseudo-first-order rate constant ($k_{obs} - k_{control}$) on polysulfide concentration was determined by conducting experiments at pH 8.0−9.5. Experimental solutions contained substantial concentrations of HS^- in addition to S_n^{2-} species. Second-order rate constants ($k''_{S_n^{2-}}$) were estimated by dividing pseudo-first-order rate constants by $\sum[S_n^{2-}]$, after first correcting k_{obs} to account for the contribution from hydrolysis and reaction with HS^-. Confidence limits on the second-order rate constants were calculated by propagating the errors associated with analysis of the reduced sulfur nucleophile and the pseudo-first-order rate constants. The resulting $k''_{S_n^{2-}}$ value is 3.1 (±0.3) × 10^{-2} M^{-1} s^{-1}.

The dependence of the corrected pseudo-first-order rate constant on PhS^- and $S_2O_3^{2-}$ concentration was determined by conducting experiments at constant pH and temperature. The pseudo-first-order rate constants were corrected for the contribution from hydrolysis, and the resulting second-order rate constants for the reaction of chlorpyrifos-methyl with PhS^- and $S_2O_3^{2-}$ are 2.1 (±0.2) × 10^{-2} and 1.0 (±0.1) × 10^{-3} $M^{-1}s^{-1}$, respectively. The dependence of the pseudo-first-order rate constant ($k_{obs} - k_{control}$) on thiophenol concentration was determined by conducting experiments at pH 6.5−9.3, and the resulting second-order rate constant for the reaction of chlorpyrifos-methyl with PhSH is 0.3 (±9.4) × 10^{-4} M^{-1} h^{-1}, which is close to zero.

Products of Chlorpyrifos-methyl Reactions with Sulfur Nucleophiles at 25 °C. Prior to discussing the products of chlorpyrifos-methyl reactions with reduced sulfur species, the hydrolysis should be briefly discussed. Chlorpyrifos-methyl, like most of the other organophosphorus insecticides, can undergo hydrolysis. Hydrolysis for most of the organophosphorus triesters includes neutral reactions and base-catalyzed reactions. In the hydrolysis of chlorpyrifos-methyl, the cleavage of the P−O bond will lead to the formation of trichloropyridinol (TCP), and the cleavage of the C−O bond will lead to the formation of desmethyl chlorpyrifos-methyl (DmCPM). In hydrolysis of organophophorus triesters, the OH^- (a "hard" nucleophile) will attack the relatively "harder" electrophilic center, the phosphorus atom, to form trichloropyridinol; the "soft" nucleophile H_2O will attack both the "soft" carbon atom and the "harder" center phosphorus atom to form DmCPM and trichloropyridinol. By calculating the formation rate of TCP and DmCPM, we can obtain the reaction rates for attack to both the carbon and the phosphorus atom. Hydrolysis rate constants from pH 5.4 to 9.7 are shown in the Supporting Information (Figure S-5). The observed hydrolysis rate is independent of pH over a wide pH range (5.4−8.6), indicating that H_2O is the dominant nucleophile under those conditions.

Figure 1 shows the degradation of chlorpyrifos-methyl and formation of the products for a hydrolysis experiment, for the reaction with HS^-, for the reaction with polysulfides, and for the reaction with thiophenolate. In Figure 1, trichloropyridinol is one of the products. In the hydrolysis experiment at pH 9.0, trichloropyridinol accounts for 76% of the chlorpyrifos-methyl that reacted. In the solution with 3.53 mM HS^-, more than 80% of chlorpyrifos-methyl reacted to form desmethyl chlorpyrifos-methyl, and in the reaction with polysulfides, the formation of desmethyl chlorpyrifos-methyl in percentage of chlorpyrifos-methyl consumed was even higher. The reactions of chlorpyrifos-methyl with thiophenolate and thiosulfate, which are shown in Figure 1d and the Supporting Information (Figure S-6), respectively, show tendencies similar to those of the reactions with bisulfide and polysulfides in terms of products distribution. From the data above, we also conclude that the deprotonated form of desmethyl chlorpyrifos-methyl, which is expected to be the dominant species at pH > 3, is stable under the experimental conditions over the time period of the experiment. Only the nonionic form of desmethyl chlorpyrifos-methyl, which is present at low pH values, might undergo further hydrolysis or attack by negatively charged nucleophiles. The reactivity of trichloropyridinol toward HS^- was tested in separate experiments, showing that there is no reaction between trichloropyridinol and HS^- over the time interval relevant for the experiments presented here. Based on these observations, the following reaction scheme was used to model the experiments.

Because in our experiments the reaction conditions did not lead to a significant degradation of trichloropyridinol or desmethyl chlorpyrifos-methyl, only two major products were considered. The data analysis of these experiments was

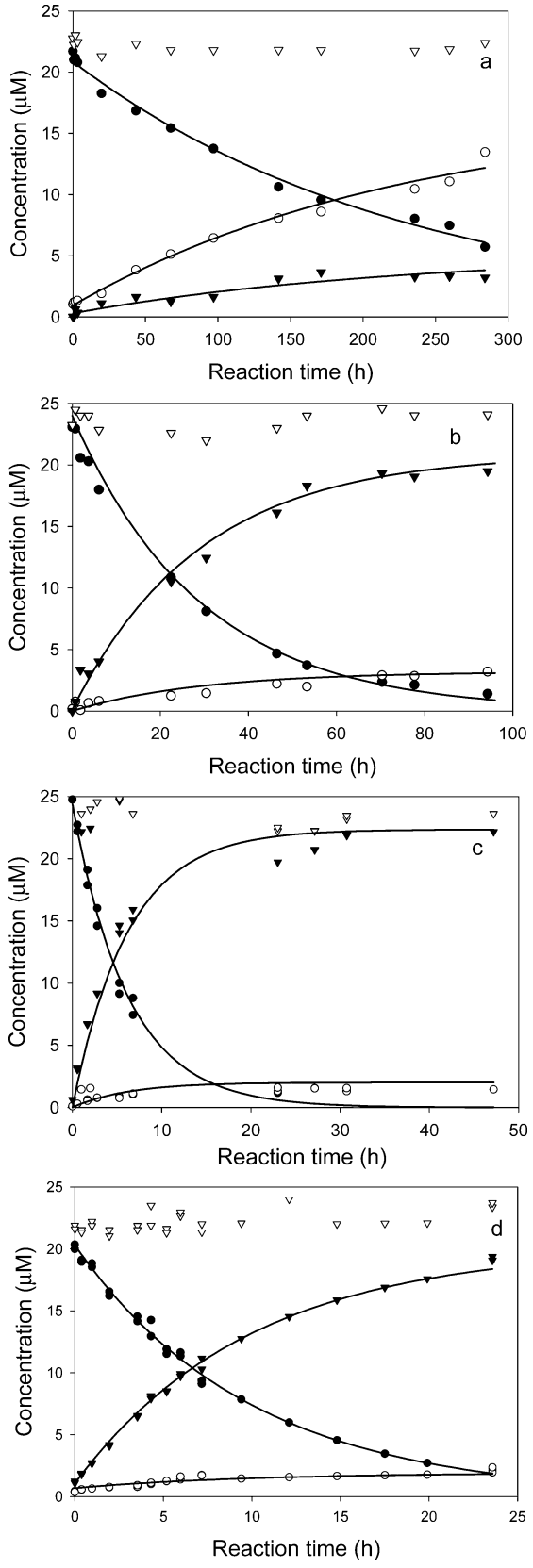


FIGURE 1. Reaction of chlorpyrifos-methyl (a) at hydrolysis at 9.0, (b) with 3.53 mM HS^- at pH 8.6, (c) with 2.16 mM $\sum[S_n^{2-}]$ at pH 9.2, (d) with 1.2 mM PhS^- at pH 8.0, showing the degradation of chlorpyrifos-methyl (●), the formation of 3,5,6-trichloropyridinol (○), the assumed concentration of desmethyl chlorpyrifos-methyl (▼), and the mass balance (▽), 0.050 M sodium phosphate and sodium tetraborate buffer, and ionic strength is 0.25 equiv/L (established with NaCl) and at 25.0 °C.

performed using nonlinear regression allowing the determination of k_{DmCPM} and k_{TCP} by fitting the degrading chlorpyrifos-methyl concentration and the forming trichloropyridinol and desmethyl chlorpyrifos-methyl concentration simultaneously (Table 2).

Therefore, the experiments were modeled by assuming parallel reactions given by the expression:

$$\frac{-\mathrm{d[CPM]}}{\mathrm{d}t} = (k'_{TCP} + k'_{DmCPM}) \times [\mathrm{CPM}] = k'_{CPM} \times [\mathrm{CPM}] \quad (2)$$

$$\frac{\mathrm{d[TCP]}}{\mathrm{d}t} = k'_{TCP} \times [\mathrm{CPM}] \quad (3)$$

$$\frac{\mathrm{d[DmCPM]}}{\mathrm{d}t} = k'_{DmCPM} \times [\mathrm{CPM}] \quad (4)$$

The fitted rate constants in Table 2 show that all of the observed trichloropyridinol formation in experiments with reduced sulfur species can be explained by the trichloropyridinol formation due to hydrolysis (see k_{TCP}' values in Table 2). On the basis of these findings, it can be concluded that the observed results can be explained by the reduced sulfur species reacting with chlorpyrifos-methyl via an attack at the carbon of a methoxy group (Scheme 3).

This reaction of chlorpyrifos-methyl with thiophenolate leads to the formation of methylated sulfur species (thioanisole) that can also be used to determine the formation rate constant of desmethyl chlorpyrifos-methyl (Figure 1d and Figure S-7). The concentrations of the formed thioanisole are not significantly different from the concentrations of desmethyl chlorpyrifos-methyl at any point in the time-course (the slope of [DmCPM] vs [thioanisole] equal to 1). This observation supports the proposed mechanism that chlorpyrifos-methyl reacts with sulfur nucleophiles by a nucleophilic substitution at the carbon of a methoxy group via the leaving of DmCPM.

Our data indicate that the reduced sulfur species react with chlorpyrifos-methyl likely via a S_N2 mechanism. This will also allow us to evaluate the nucleophilicities of these environmentally relevant sulfur nucleophiles. Table 1 lists the measured second-order rate constants for the S_N2 reactions of chlorpyrifos-methyl with the nucleophiles. The Swain−Scott equation was used to evaluate the relative nucleophilicity of the measured reduced sulfur nucleophiles (*40*). The Swain−Scott equation is given as $\log k/k_o = sn$, where s is a constant characteristic of the substrate ($s = 1$ for MeBr), k_o is the rate constant for the reaction of water, and n is the nucleophilicity of the nucleophile reacting with MeBr. The relative order of k_{Nuc} values for the reactions of chlorpyrifos-methyl with three sulfur nucleophiles (HS^-, PhS^-, S_n^{2-}) tends to parallel that previously reported for S_N2 reactions of methyl bromide (Figure 2). Thiosulfate reacts 10 times faster than bisulfide with CH_3Br, whereas chlorpyrifos-methyl reacts about 2 times faster with bisulfide than with thiosulfate. It is quite likely that steric hindrance is responsible for the lower reactivity of the larger thiosulfate nucleophile toward chlorpyrifos-methyl. Similar observations of a relatively reduced nucleophilicity of thiosulfate are reported by Lippa and Roberts for the nucleophilic substitution reaction of chloroacetanilides with reduced sulfur species (*23*).

Environmental Significance

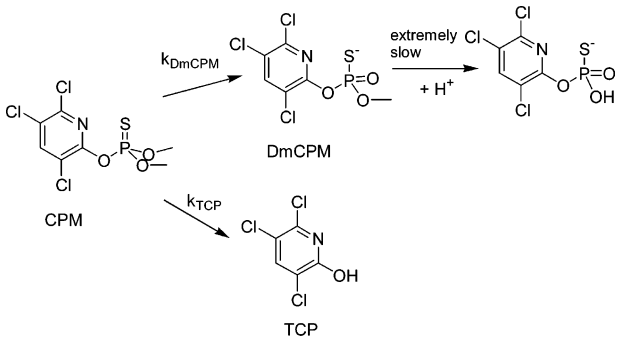
The environmental fate of chlorpyrifos-methyl is controlled by a number of abiotic and biotic processes. Our results suggest that HS^-, S_n^{2-}, PhS^-, and $S_2O_3^{2-}$ are sufficiently reactive as to control the fate of chlorpyrifos-methyl in anoxic and suboxic environments where the reduced sulfur species are abundant. Under these conditions, the organophosphorus

TABLE 2. Reaction Rate Constants of Chlorpyrifos-methyl[a]

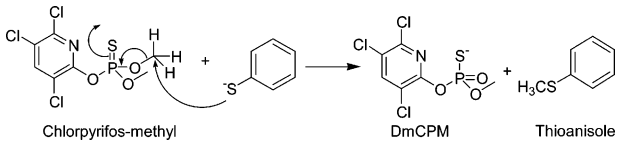
reaction condition	k_{obs} (s^{-1})[b]	k_{TCP} (s^{-1})[b]	k_{DmCPM} (s^{-1})[b]
pH 7.2, control buffer	8.3 (±0.9) × 10^{-7}	5.7 (±0.7) × 10^{-7}	NA[c]
pH 9.0, control buffer	2.4 (±0.1) × 10^{-6}	1.8 (±0.1) × 10^{-6}	0.56 (±0.02) × 10^{-6}
pH 7.4, 3.8 mM HS^-	7.1 (±0.4) × 10^{-6}	0.55 (±0.03) × 10^{-6}	6.5 (±0.3) × 10^{-6}
pH 8.3, 3.0 mM HS^-	9.6 (±0.4) × 10^{-6}	1.31 (±0.01) × 10^{-6}	8.3 (±0.03) × 10^{-6}
pH 9.2, 1.8 mM $\sum S_n^{2-}$	4.5 (±0.1) × 10^{-5}	0.17 (±0.01) × 10^{-6}	4.3 (±0.2) × 10^{-5}
pH 9.2, 3.6 mM $\sum S_n^{2-}$	1.2 (±0.01) × 10^{-4}	1.5 (±0.03) × 10^{-6}	1.2 (±0.01) × 10^{-4}
pH 8.1, 2.1 mM PhS^-	3.2 (±0.05) × 10^{-5}	9.4 (±0.95) × 10^{-7}	3.2 (±0.04) × 10^{-5}
pH 8.0, 1.2 mM PhS^-	2.6 (±0.2) × 10^{-5}	0.11 (±0.01) × 10^{-6}	2.6 (±0.2) × 10^{-5}
pH 7.0, 7.9 mM $S_2O_3^{2-}$	6.6 (±0.4) × 10^{-6}	0.44 (±0.07) × 10^{-6}	6.1 (±0.4) × 10^{-6}
pH 7.0, 5.2 mM $S_2O_3^{2-}$	4.9 (±0.2) × 10^{-6}	0.42 (±0.03) × 10^{-6}	4.5 (±0.1) × 10^{-6}

[a] Determined via simultaneous nonlinear regression techniques using Scientist for Windows based on the model shown in Scheme 2. [b] Standard deviation resulting from the nonlinear regression. [c] Not measured. All reactions were conducted at 0.050 M sodium phosphate and sodium tetraborate buffer with ionic strength adjusted with NaCl to 0.25 equiv/L, 5% methanol and 25.0 °C.

SCHEME 2



SCHEME 3



diester is the major degradation product resulting from a nucleophilic attack by reduced sulfur species (e.g., HS^-, S_n^{2-}, PhS^-, $S_2O_3^{2-}$) at the carbon atom of the methoxy groups of the phosphorothionate ester. The calculated half-life of chlorpyrifos-methyl with just hydrolysis at pH 8 is 206 h; this is 10 times greater than the calculated half-life in the presence of 5 mM of bisulfide that is 19 h. The calculated half-life of chlorpyrifos-methyl with 0.33 mM polysulfides is 7 h, and the calculated half-life with 0.5 mM thiosulfate is 170 h. These values of reduced sulfur species represent the maximum concentrations reported for Great Marsh, DE, sediment porewater (*41*). Therefore, reduced sulfur species present at environmentally relevant concentrations represent an important sink for chlorpyrifos-methyl in anoxic coastal marine environments.

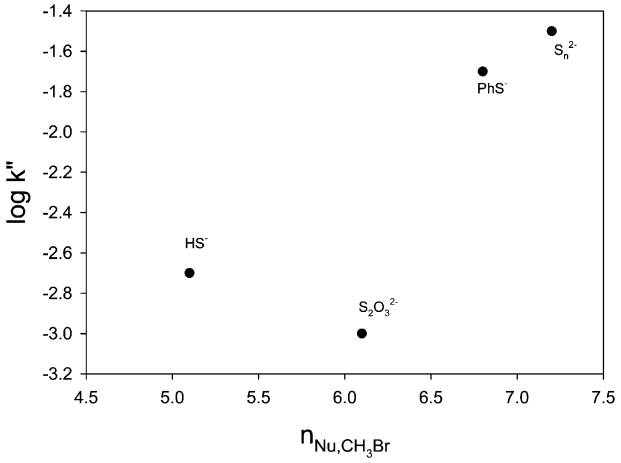


FIGURE 2. Logarithm of rate constants for reactions of chlorpyrifos-methyl with various reduced sulfur nucleophiles versus n_{Nu,CH_3Br}.

Our results also show that the reduced sulfur species have to be deprotonated to be good nucleophiles. Hydrogen sulfide and thiophenol in their protonated form are not environmentally relevant for chlorpyrifos-methyl degradation. For the same reason, some of the organosulfur species present in sediments, such as cysteine and glutathione, are quite likely not relevant as nucleophiles due to their high pK_a values.

It is also worth mentioning that polysulfides are actually employed as a 30% aqueous solution in commercial preparations used for agricultural soil conditioning to take advantage of fungicidal qualities as well its use as an essential nutrient (*42*). Therefore, a possible application of polysulfides and phosphorothionate pesticides to the same agriculture soils may result in significant sulfur species involved in reactions and significantly reduce the lifetime of the pesticide in the soil.

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Supporting Information Available

Figures showing gas chromatogram and EI mass spectra of methylated DmCPM, a figure showing the UV−vis spectrum of DmCPM and chlorpyrifos-methyl, a figure showing the time-courses for reactions of chlorpyrifos-methyl with HS^- at 25 °C and pH 8.6, a figure showing the dependence of second-order reaction rate constants with [HS^-], a figure showing the k_{obs} in controlled buffer solution, a figure showing the reaction time-course of chlorpyrifos-methyl with 5.2 mM thiosulfate at pH 7.1, a figure showing the correlation of the formation of DmCPM and thioanisole in reaction, and the detailed HPLC methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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