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Reaction of Chlorpyrifos-methyl in Aqueous Hydrogen Sulfide/Bisulfide Solutions

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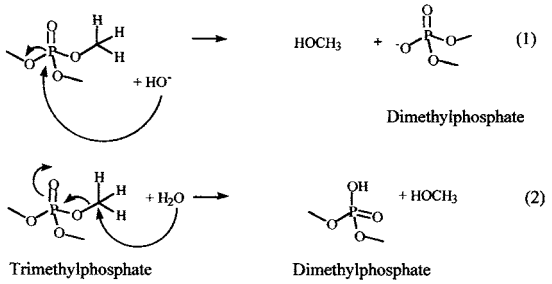
The kinetics of the reactions of chlorpyrifos-methyl, an organophosphorus insecticide, with hydrogen sulfide (H_2S) and bisulfide (HS^-) were determined in well-defined aqueous solutions. The resulting pseudo-first-order rate constant for chlorpyrifos-methyl with bisulfide yielded a second-order rate constant of $(2.1 \pm 0.3) \times 10^{-3}$ M^{-1} s^{-1}. The second-order rate constant for chlorpyrifos-methyl with hydrogen sulfide is significantly slower than the second-order rate constant with bisulfide. The contribution of H_2S to the observed degradation rate constant of chlorpyrifos-methyl at concentrations of up to 4 mM H_2S is not significant. The second-order rate constant of chlorpyrifos-methyl with H_2S was too low to be measured in this study. The results indicate that HS^- present at environmentally relevant concentrations may represent an important sink for phosphorothionate triesters in a coastal marine environment, while H_2S reacts too slowly to be environmentally relevant (pH 6−9). Trichloropyridinol, the major product of hydrolysis of chlorpyrifos-methyl, is only a minor product of the reaction of chlorpyrifos-methyl with bisulfide; however, trichloropyridinol was found to be stable under the experimental conditions.

KEYWORDS: Organophosphorus pesticide; degradation; reduced sulfur species; chlorpyrifos-methyl; trichloropyridinol

INTRODUCTION

Organophosphorus insecticides (OPs) are widely used throughout the world and have in many cases replaced organochlorine pesticides. In general, OPs are less persistent in the environment than organochlorine compounds. The major pathways of degradation for OPs are hydrolysis, oxidation, photolysis, and biodegradation (*1*); the half-lives for OPs in natural water are in the range of several days to several months (*2*−*4*). 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. In sediments, salt marshes, and the bottom layer of estuaries, anoxic conditions are typically prevalent. Transformation processes occurring under anoxic conditions may represent an important sink for OPs and have not yet been extensively studied.

Investigators have shown that abiotic processes can control the fate of organic contaminants under anaerobic conditions (*5*, *6*). It is therefore possible that OPs undergo significant transformation under anoxic conditions by chemical rather than microbial processes. The possible transformation processes are compound specific. They typically include reduction reactions and substitution reactions. While some organic pollutants are transformed through surface-catalyzed reactions on particles, others are degraded by homogeneous reactions. Anoxic conditions can give rise to high concentrations of reduced sulfur species. Reduced sulfur species are capable of reacting with a wide array of pollutants, including organic contaminants that undergo nucleophilic reactions (*7*, *8*). Reduced sulfur species can form whenever O_2 is consumed more rapidly than it can be replenished by mixing processes. Under such conditions, the microbial reduction of sulfate (abundant in seawater, but limited in freshwater) gives rise to reduced sulfur species such as HS^-. Concentrations of hydrogen sulfide species (HS^-/H_2S) as high as 5 mM have been reported in sediment pore water of estuaries and salt marshes (*9*).



Chlorpyrifos-methyl is a typical OP. It contains a P═S moiety and three ester bonds, which makes it a phosphorothionate triester. Chlorpyrifos and parathion-methyl are two phosphorothionate triesters that are among the most widely used insecticides in the United States (*10*). Chlorpyrifos-methyl was

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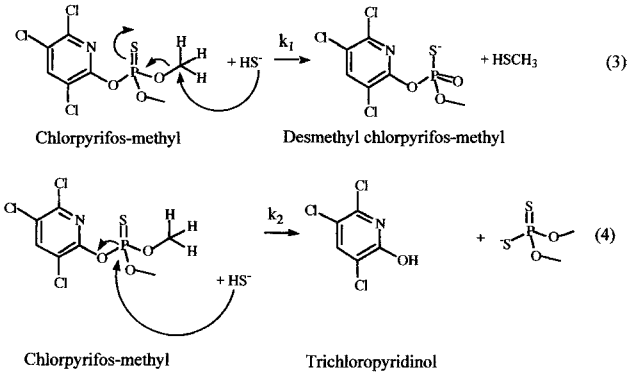
chosen for this study because it has a higher water solubility than chlorpyrifos. It has been shown that HO^- reacts with phosphate triesters in a S_N2 reactions with attack occurring at the phosphorus atom, resulting in cleavage of a P−O bond (eq 1) (*11*, *12*). It has also been reported that some phosphate triesters display cleavage of an O−C bond in water, indicating that H_2O undergoes a nucleophilic substitution reaction at the carbon atom of a methoxy group (eq 2) (*13*). On the basis of these findings, it is hypothesized that phosphorothionate triesters are likely to undergo displacement reactions with a sulfur nucleophile (e.g., HS^-) either with attack occurring at the carbon of a methoxy group (eq 3) or with attack occurring at the phosphorus atom (eq 4) (*14*). Actual experiments that test this hypothesis have not yet been reported to the best of our knowledge.



The primary purpose of this research was to explore the potential impact of hydrogen sulfide/bisulfide solutions on the abiotic transformation of chlorpyrifos-methyl. The second-order rate constant was determined in well-defined systems. In addition, the formation rates of trichloropyridinol (a degradation product of this reaction) was measured and compared with the formation rate of trichloropyridinol in hydrolysis experiments.

MATERIALS AND METHODS

Chemicals. *O*,*O*-Dimethyl *O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate (chlorpyrifos-methyl) (99.7%, CAS registry no. 5598-13-0) and the hydrolysis product, 3,5,6-trichloropyridinol (98%, CAS registry no. 6515-38-4), were obtained from Chem Service (West Chester, PA). All solvents and reagents that were used were analytical grade or equivalent. They were used without further purification, and were obtained from Fisher Scientific (Pittsburgh, PA). Ethyl acetate and methanol were HPLC grade.

Experimental Setup. All aqueous solutions were prepared from deionized water (DW) (Milli-Q gradient system, Millipore, Bedford, MA). All glassware was soaked in 1 M HNO_3, rinsed several times with DW, and dried at 200 °C. Glassware used with bisulfide solutions was washed with a methanol/NaOH mixture to remove traces of sulfur impurities prior to acid washing.

Sodium sulfide stock solutions were prepared under argon from $Na_2S{\cdot}9H_2O$ crystals using deoxygenated DW. Crystals were rinsed with Ar-purged water to remove surface oxidation products, blotted with a cellulose wipe, and then transferred to an Ar-purged three-necked flask. The flask was then connected to an Ar-purged closed glassware system consisting of a reservoir bottle (containing DW), glass tubing, stopcocks, and an argon tank. Before rerouting the argon flow and forcing the reservoir content into the flask containing the washed sodium sulfide crystals, we purged the DW with argon for 1 h. Then the sodium sulfide stock solutions were transferred in the anaerobic chamber.

Unless otherwise stated, reaction solutions where prepared in an anaerobic chamber (5% H_2 and 95% N_2). The reaction solutions were prepared in volumetric flasks and then transferred to 20 mL glass syringes equipped with a polycarbonate stopcock and a Teflon needle. The syringes contained five glass rings to facilitate mixing. All reaction solutions contained 5% methanol, 100 mM NaCl, and 50 mM buffer (sodium phosphate). 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 solution of chlorpyrifos-methyl was prepared by dissolving chlorpyrifos-methyl in deoxygenated methanol. Pilot experiments conducted at varying methanol concentrations (0−15%) indicated that these levels of methanol did not alter the reaction rates.

An Accumet pH meter (Fisher Scientific) with a Ross combination pH electrode (ThermoOrion, Beverly, MA) was used to measure the pH in the bisulfide solutions. The concentration of the hydrogen sulfide/bisulfide solutions was determined by iodometric titration. The sodium thiosulfate solution for the titration was standardized against potassium iodate daily (*15*).

Kinetic Experiments. Reaction kinetics were measured under pseudo-first-order conditions, with an initial chlorpyrifos-methyl concentration that was typically 0.5−1% of that of the total hydrogen sulfide concentration. The reaction solutions were spiked with aliquots (80 μL) of deoxygenated methanol containing the chlorpyrifos-methyl, yielding an initial concentration of ~20 μM. 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 was added, and the mixture was extracted into ethyl acetate, followed by analysis via HPLC. The acidification ensures the extraction of trichloropyridinol. Pseudo-first-order rate constants were obtained by performing a linear regression of the natural logarithm of the chlorpyrifos-methyl 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 chlorpyrifos-methyl and for the formation of trichloropyridinol were concurrently determined via nonlinear regression techniques using Scientist for Windows, version 2.01 (MicroMath Scientific Software, Salt Lake City, UT).

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, k'_{obs}, is expected. This rate constant can be corrected for hydrolysis and divided by the total concentration of hydrogen sulfide species, which results in the apparent second-order rate constant, k''_{app}. This apparent second-order rate constant, k''_{app}, can be given by the expression

$$k''_{app} = k''_{H_2S}\frac{[H_2S]}{[H_2S]_T} + k''_{HS^-}\frac{[HS^-]}{[H_2S]_T} \quad (5)$$

where $[H_2S]_T = [HS^-] + [H_2S]$.

Substituting expressions for $[H_2S]$ and $[HS^-]$ concentrations as a function of $[H_2S]_T$ yields

$$\alpha_{H_2S} = \frac{[H_2S]}{[H_2S]_T} = \left(1 + \frac{K'_a}{[H^+]}\right)^{-1} \quad \alpha_{HS^-} = \frac{[HS^-]}{[H_2S]_T} = \left(1 + \frac{[H^+]}{K'_a}\right)^{-1} \quad (6)$$

where K'_a, the acid dissociation constant of H_2S, equals $10^{-6.98}$ (*16*). After rearrangement, the apparent second-order rate constant k''_{app} can be given in terms of α_{HS^-}:

$$k''_{app} = k''_{H_2S} + \alpha_{HS^-}{\cdot}(k''_{HS^-} - k''_{H_2S}) \quad (7)$$

Therefore, a plot of k''_{app} versus α_{HS^-} shows a linear correlation, and it allows extraction of second-order rate constants k''_{H_2S} and k''_{HS^-}.

Liquid Chromatographic Analysis. Ethyl acetate extracts containing chlorpyrifos-methyl and trichloropyridinol were analyzed using a Waters 2690 separations module (Waters, Milford, MA) equipped with

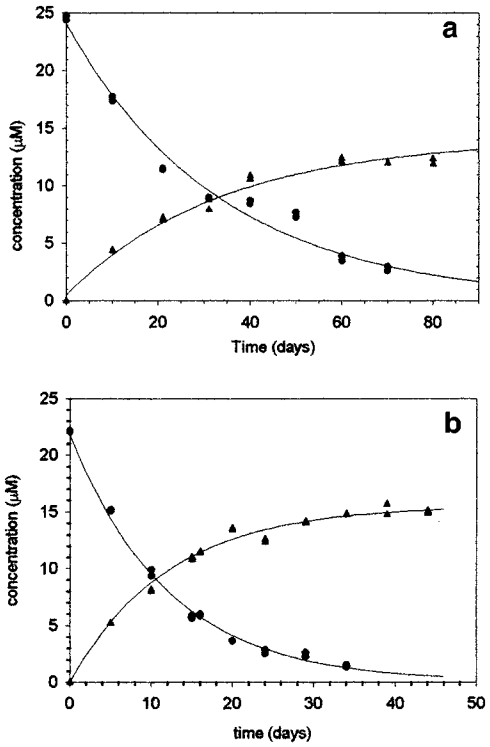


Figure 1. Hydrolysis of chlorpyrifos-methyl (●) and formation of trichloropyridinol (▲) in 0.050 M phosphate, 0.10 M NaCl, and 5% methanol at 25 °C and (a) pH 5.9 and (b) pH 7.6.

a photodiode array detector (996, Waters). The stationary phase was an Xterra MS C_{18}, 3.9 mm × 150 mm, 5 μM column (Waters) with a guard column (3.9 mm × 20 mm) of the same material. The detector wavelength was 289 nm for chlorpyrifos-methyl and trichloropyridinol. The mobile phase was isocratic at 75% methanol and 25% 1 mM H_3PO_4 in DW.

RESULTS AND DISCUSSION

Hydrolysis Experiments. The hydrolysis of chlorpyrifos-methyl can be treated as a pseudo-first-order reaction in buffered solutions with a constant pH; hence, the following equation applies: $dC/dt = -k'_{obs}C$. The observed rate constant for hydrolysis, k'_{obs}, is equal to the slope in a plot of ln[chlorpyrifos-methyl] versus reaction time. The hydrolysis of chlorpyrifos-methyl followed pseudo-first-order kinetics, and chlorpyrifos-methyl decomposed to yield trichloropyridinol as a major degradation product (**Figure 1**). Hydrolysis rates of 0.029 ± 0.006 day^{-1} at pH 5.9 and 0.081 ± 0.009 day^{-1} at pH 7.6 were observed. The results are in approximate agreement with that found in previous studies, 0.040 day^{-1} at pH 6.7 and 0.055 day^{-1} at pH 7.8 in 5 mM phosphate buffer at 25 °C (*17*). This agreement supports the assumption that the increased buffer concentration chosen in the present experiments does not have a significant effect on the hydrolysis rate of chlorpyrifos-methyl.

The data analysis of the hydrolysis experiment was performed using nonlinear regression allowing the determination of k'_{obs} and k_2 (eq 4) by fitting the degrading chlorpyrifos-methyl concentration and the forming trichloropyridinol concentration simultaneously (**Table 1**). At pH 7.6 (**Figure 1b**), a pyridinol formation rate of 6.7×10^{-7} s^{-1} was determined; this is equal to 69% of the hydrolysis rate constant of chlorpyrifos-methyl. At pH 5.9, the determined trichloropyridinol formation rate was 59% of the hydrolysis rate of chlorpyrifos-methyl (**Figure 1a**). Meikle et al. (*17*) report that at pH 6.7 and 35 °C, 46% of the product that was formed was pyridinol. These results can be explained by the hypothesis that two mechanisms are controlling the degradation of chlorpyrifos-methyl. While mechanism 2 (eq 4) leads directly to the formation of trichloropyridinol, mechanism 1 (eq 3) leads to the formation of desmethyl chlorpyrifos-methyl. The lack of authentic standards prevented the identification and quantification of desmethyl chlorpyrifos-methyl. The deprotonated form of desmethyl chlorpyrifos-methyl, which is expected to be the dominant species at pH >3 (*18*), might not be very susceptible to further hydrolysis, and might be considered to be stable under the experimental conditions. Only the nonionic form of desmethyl chlorpyrifos-methyl, which is present at low pH values, might undergo further hydrolysis. This hypothesis is based on the observation that at pH >0 the hydrolysis of dimethyl phosphate is a function of the fraction of the nonionic form present, indicating that only the nonionic form is undergoing hydrolysis (*18*).

Table 1. Reaction Rate Constants for Chlorpyrifos-methyl[a]

reaction conditions	$k'_{obs} \pm SD^b$ (s^{-1})	$k_2 \pm SD^b$ (s^{-1})
pH 5.9, hydrolysis	$3.4 \times 10^{-7} \pm 1.3 \times 10^{-8}$	$1.9 \times 10^{-7} \pm 1.2 \times 10^{-8}$
pH 7.6, hydrolysis	$9.7 \times 10^{-7} \pm 2.0 \times 10^{-8}$	$6.7 \times 10^{-7} \pm 2.0 \times 10^{-8}$
pH 6.0, 3.14 mM H_2S_{tot}	$1.5 \times 10^{-6} \pm 3.3 \times 10^{-8}$	$2.1 \times 10^{-7} \pm 1.3 \times 10^{-8}$
pH 7.1, 3.44 mM H_2S_{tot}	$4.1 \times 10^{-6} \pm 1.4 \times 10^{-7}$	$6.1 \times 10^{-7} \pm 5.2 \times 10^{-8}$
pH 7.9, 3.33 mM H_2S_{tot}	$6.2 \times 10^{-6} \pm 1.4 \times 10^{-7}$	$6.6 \times 10^{-7} \pm 5.1 \times 10^{-8}$

[a] Determined via simultaneous nonlinear regression techniques using Scientist for Windows. [b] Standard deviation resulting from the nonlinear regression.

In connection with the experiments presented here, the influence of divalent metal cations on the hydrolysis of chlorpyrifos-methyl and the product distribution should be mentioned. It is reported that at pH 4.5, 5 mM acetate, and 10 mM NaCl and in the presence of 1 mM Cu(II) the hydrolysis of chlorpyrifos-methyl is significantly faster and that the trichloropyridinol formation is almost 100% of the chlorpyrifos-methyl loss (*19*). This result is explained by formation of Cu(II) complexes that are more susceptible to hydrolysis than the uncomplexed compound. A similar effect might be expected for the hydrolysis rate of the desmethyl chlorpyrifos-methyl in the presence of Cu(II).

Reaction with Hydrogen Sulfide. The reaction of chlorpyrifos-methyl with hydrogen sulfide was assessed at different pH values. The experiments can be treated as pseudo-first-order reactions in buffered solutions at a constant pH. The observed pseudo-first-order rate constant, k'_{obs}, is obtained from the slope of a plot of ln[chlorpyrifos-methyl] versus time. The apparent second-order rate constant, k''_{app}, was determined from k'_{obs}. **Figure 2** shows the plot of k''_{app} versus α_{HS^-} [for the calculation of α_{HS^-}, $K'_a(H_2S)$ was corrected for ionic strength]. The linear correlation supports our assumption that HS^- has a reactivity toward chlorpyrifos-methyl different from that of H_2S (eq 7). The second-order reaction rate constant for the reaction of bisulfide with chlorpyrifos-methyl that can be derived from the slope of **Figure 2** is 7.5 ± 1.0 M^{-1} h^{-1} [=(2.1 ± 0.3) × 10^{-3} M^{-1} s^{-1}]. The intercept in **Figure 2** (−0.01 ± 0.65 M^{-1} h^{-1}), which is equal to k''_{H_2S}, is small and not significantly different from zero. This indicates that the reaction of chlorpyrifos-methyl with H_2S (at the experimental H_2S concentrations) is too slow compared to hydrolysis and cannot be quantified from the experiments presented here.

Figure 3 shows time courses of the reaction of chlorpyrifos-methyl with hydrogen sulfide at pH 6.0, 7.1, and 7.9. Trichloropyridinol is a product of the reaction and accounts for 11−15% of the chlorpyrifos-methyl that reacted. However, almost all of the observed trichloropyridinol formation at this pH can

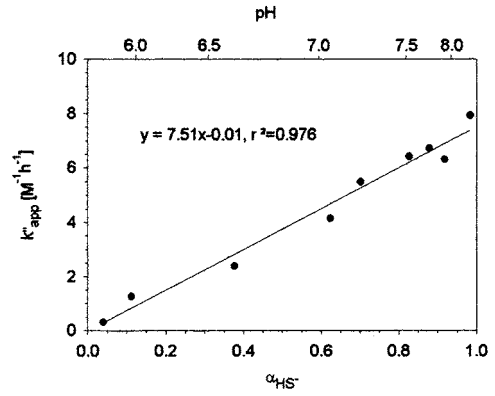


Figure 2. Plot of apparent second-order reaction rate constants, k''_{app} (M^{-1} h^{-1}), versus α_{HS^-} for reactions of chlorpyrifos-methyl with hydrogen sulfide/bisulfide solutions for different pH values in 0.050 M phosphate buffer, 0.10 M NaCl, and 5% methanol at 25 °C.

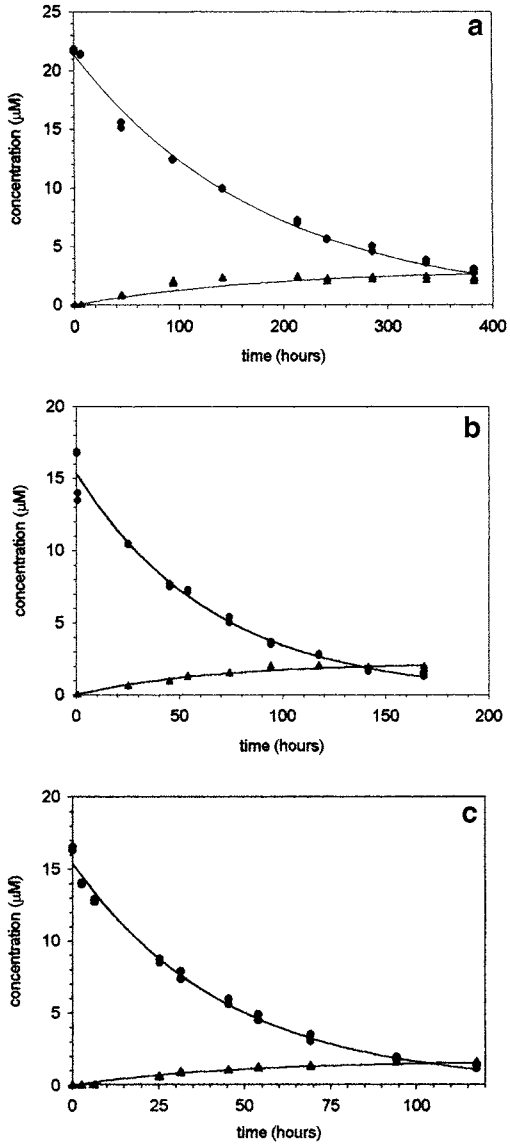


Figure 3. Reaction of chlorpyrifos-methyl (●) with hydrogen sulfide and formation of trichloropyridinol (▲) in 0.050 M phosphate, 0.10 M NaCl, and 5% methanol at 25 °C: (a) $[H_2S]_T = 3.14$ mM and pH 6.0, (b) $[H_2S]_T = 3.44$ mM and pH 7.1, and (c) $[H_2S]_T = 3.33$ mM and pH 7.9.

be explained by the trichloropyridinol formation due to hydrolysis (see k_2 values in **Table 1**). This observation indicates that the reaction of HS^- with chlorpyrifos-methyl does not lead to the formation of trichloropyridinol. 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. Therefore, it can be concluded that the observed results can be explained by HS^- reacting with chlorpyrifos-methyl predominantly via an attack at the carbon of a methoxy group (eq 3) and not via a nucleophilic attack at the phosphorus atom. Although the results support the postulated hypothesis, further studies are necessary to elucidate the mechanisms. The analysis and quantification of desmethyl chlorpyrifos-methyl will be an important step in this endeavor.

The determined reaction rate constant between HS^- and chlorpyrifos-methyl is environmentally relevant. At a total hydrogen sulfide concentration of 5 mM, at pH 7, the reaction of chlorpyrifos-methyl with HS^- would result in a half-life of 37 h for chlorpyrifos-methyl; at pH 8, the expected half-life of chlorpyrifos-methyl would be 21 h. In comparison, the half-lives due to hydrolysis at these pH values are expected to be 13 and 17 days, respectively. On the basis of these calculations, it can be expected that in anoxic coastal marine environments (e.g., sediment pore water of estuaries), the reaction of HS^- with chlorpyrifos-methyl can be an important sink.

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