

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

**Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600**

UMI[®]

**Interactions and transformations of chlorpyrifos
in aqueous and colloidal systems**

by

Jigang Wu

**A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of**

DOCTOR OF PHILOSOPHY

Major: Soil Science (Soil Chemistry)

Major Professor: David A. Laird

Iowa State University

Ames, Iowa

2000

UMI Number: 9990501

UMI[®]

UMI Microform 9990501

Copyright 2001 by Bell & Howell Information and Learning Company.

**All rights reserved. This microform edition is protected against
unauthorized copying under Title 17, United States Code.**

**Bell & Howell Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346**

**Graduate College
Iowa State University**

**This is to certify that the Doctoral dissertation of
Jigang Wu
has met the dissertation requirements of Iowa State University**

Signature was redacted for privacy.

Major Professor

Signature was redacted for privacy.

For the Major Program

Signature was redacted for privacy.

For the Graduate College

TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION	1
Insecticides and Environment	1
Basic Terminology and Processes	2
Properties and Applications of Chlorpyrifos	5
Sorption and Desorption of Chlorpyrifos	7
Degradation and Transformation of Chlorpyrifos	10
Catalysis of Chlorpyrifos Transformation	13
Ecotoxicology of Chlorpyrifos	15
Hypothesis and Objectives	17
Organization of Dissertation	18
References	19
CHAPTER 2. INTERACTION OF CHLORPYRIFOS WITH COLLOIDAL MATERIALS IN AQUEOUS SYSTEMS	26
Abstract	26
Introduction	27
Materials and Methods	29
Results and Discussion	34
Conclusions	39

References	40
CHAPTER 3. HYDROLYSIS OF CHLORPYRIFOS IN AQUEOUS SYSTEMS	55
Abstract	55
Introduction	56
Materials and Methods	58
Results and Discussion	62
Conclusions	66
References	66
CHAPTER 4. FATE OF CHLORPYRIFOS SORBED ON COLLOIDAL MATERIALS IN AQUEOUS SYSTEMS	75
Abstract	75
Introduction	76
Materials and Methods	78
Results and Discussion	80
Conclusions	82
References	83
CHAPTER 5. CHEMICAL TRANSFORMATION OF CHLORPYRIFOS TO CHLORPYRIFOS OXON IN WATER	90
Abstract	90
Introduction	91

	Materials and Methods	93
	Results	95
	Discussion	98
	Implications	101
	Literature Cited	103
CHAPTER 6.	SUMMARY	114
APPENDIX	SPME-GC TECHNIQUE FOR DETERMINATION OF TRACE CHLORPYRIFOS IN AQUEOUS SOLUTIONS	117
	Principle of SPME	118
	Materials and Methods	119
	Results	120
	References	122
ACKNOWLEDGEMENTS		126

CHAPTER 1 GENERAL INTRODUCTION

Chlorpyrifos, one of the most important organophosphate insecticides, is widely used in agricultural, industrial and residential environments. This general introduction begins with a short review of insecticides and environmental concerns. The emphasis is placed on the fate of chlorpyrifos in soil and aquatic environments. The most important processes that govern the fate of chlorpyrifos in the environment are reviewed. Organization of the dissertation is given at the end of this chapter.

Insecticides and Environment

Insect control is an ancient art. The burning of plants to suppress locust invasions was recorded during the Shang Kingdom (ca. 1520-1030 BC) in China (Harpaz, 1973). Historically, inorganic chemicals, such as sulfur and arsenic, and botanicals, such as tansy and elder leaves and flowers, were used as repellants and insecticides (Menn and Hollingwoth, 1985). The age of insect control by synthetic organic chemicals began following World War II. The most notable of such chemicals is DDT, which was used extensively from the mid-1940s to the late 1950s. Other synthetics that followed are the chlorinated hydrocarbons, organophosphates and carbamates. Already in the 1950s there was a growing awareness among entomologists that more rational approaches to

pest control were needed to reduce the dependence on chemicals. They recognized the need to mobilize biological resources to work in concert with environmentally and toxicologically compatible crop protection chemicals. A well-known book, "Silent Spring" written by Carson (1962), initiated a reappraisal of pesticide technology and was partly responsible for the creation of the US Environmental Protection Agency (EPA) and amendment of the laws regulating pesticides. Today, the controversy over pesticide use continues despite tremendous change in pesticide chemistry.

Once a pesticide chemical has entered the environment, its fate is determined by physical and chemical properties of the chemical and is largely influenced by sorption, transformation and transport processes occurring in the environment. These processes mediate biological significance by determining the quantity of pesticide that will be present in any phases for a certain period of time. Some of the basic concepts and processes commonly used for describing the fate of a pesticide in soil and aquatic environments are therefore reviewed in the following section.

Basic Terminology and Processes

The process in which chemicals become associated with solid phase is generally referred to as sorption. Sorption is extremely important because it may dramatically affect the fate and impact of chemicals in the environment. When we are interested in assessing the equilibrium proportion of a particular

chemical's presence in association with solids for any particular volume of an aquatic environment, we begin by considering how the chemical concentration associated with sorbent, C_s ($\mu\text{g g}^{-1}$ or mol kg^{-1}), depends on the chemical concentration in the solution, C_{aq} ($\mu\text{g mL}^{-1}$ or mol L^{-1}), at a constant temperature. Such a relationship is commonly referred to as a sorption isotherm. The basic objective of sorption studies is to determine the change in solution concentration when a known volume of solution is equilibrated with a known amount of solids.

The sorption affinity for an organic chemical on solid material can be quantified by introduction of sorption distribution coefficient, K_d . The K_d value is defined by the following equation:

$$K_d = \frac{C_s}{C_{\text{aq}}} \quad (1)$$

where C_s is the amount of chemical on solid ($\mu\text{g g}^{-1}$) and C_{aq} is the amount of chemical in solution ($\mu\text{g mL}^{-1}$) at equilibrium. Generally, higher K_d values imply greater sorption affinity for the solid material in aqueous system.

The distribution of nonpolar organic compounds between water and natural solids (e.g., soils, sediments, and suspended particles) or organisms can, in many cases, be viewed as a partition process between the aqueous phase and the bulk organic matter in natural solids or in biota. As early as 1900, investigators studying the uptake of nonpolar drugs by organisms discovered that they could use water-immiscible organic solvents like *n*-octanol as a surrogate for organisms insofar as accumulation of these pharmaceutically important organic

molecules from the water was concerned (Schwarzenbach, 1993). Although the extent of uptake from water into these solvents was not identical to that into organisms, it was directly proportional; that is, within a series of compounds, higher accumulation into an organism corresponded to more favorable partitioning into the organic solvent. More recently, environmental chemists have found similar correlations with soil humic acid and other naturally occurring organic phases. These correlations exist because the same molecular factors controlling the distribution of compounds between water-immiscible organic solvents and water also determine environmental partitioning from water into natural organic phases. K_{ow} can be defined as:

$$K_{ow} = \frac{C_o}{C_w} \quad (2)$$

where C_o denotes concentration of organic chemical in octanol and C_w denotes concentration of organic chemical in water. K_{ow} express the relative affinity a compound has for organic vs. aqueous solution and are key parameters in the estimation of environmental partitioning (e.g., sediment/water). K_{oc} is defined as:

$$K_{oc} = \frac{C_{oc}}{C_w} \quad (3)$$

where C_{oc} denotes concentration of chemical on organic carbon and C_w denotes concentration of chemical in water. Similar relationship can be used to relate soil organic carbon with sorption behavior of a pesticide in soil environment.

Desorption from solid phase to aqueous phase is generally slower than adsorption, but its kinetic characteristics are less well known. Hysteresis is frequently observed and the use of desorption isotherms has been proposed (Savage and Wauchope, 1974; Hornsby and Davidson, 1973; Swanson and Dutt, 1973; Farmer and Aochi, 1974). Sorption-desorption hysteresis could be due to a relatively slow rate of desorption, modification of adsorbent occurring during the shaking period, or decomposition of the solute during the experiment. The true nature of desorption hysteresis needs to be further defined for each experimental system. This is important since transport phenomena and biological activity of pesticides may be closely controlled by desorption.

Adsorption-desorption processes largely influence chemical concentrations in the soil solution and, thus, are closely involved in transport and uptake by living organisms. The distribution of a pesticide between the liquid phase and the adsorbed phase affects its toxicity to organisms in aquatic environments. The physical and chemical properties of a specific pesticide also largely affect the behavior of the chemical in the environment.

Properties and Applications of Chlorpyrifos

Chlorpyrifos [o,o-diethyl o-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate] is a broad-spectrum organophosphorus or organophosphate insecticide. It was introduced by the Dow Chemical Company (now DowElanco, Midland, Michigan) in 1965 (Hayes and Laws, 1992). The trade names of chlorpyrifos products

include Dowco 179, Dursban, Lorsban, Empire, Paqant, Piridane, Scout, Stipend, etc. While originally used primarily to kill mosquitoes in the immature, larval stage of development, chlorpyrifos is no longer registered for this use. Chlorpyrifos is effective in controlling a variety of insects, including cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, and lice (USEPA, 1986). It is used as an insecticide on grain, cotton, fruit, nut and vegetable crops, as well as on lawns and ornamental plants (Berg, 1986). It is also registered for direct use on sheep and turkey, and for treatment of horsing sites, domestic dwellings, farm buildings, storage bins, and commercial establishments. For agricultural applications alone, more than 3 million kg of chlorpyrifos are applied to field crops in the USA annually (ERS, 1994). The standard application rate for corn ranges from 0.56 to 2.24 kg a.i. ha⁻¹. An extensive review of application rates and methods for various crops was presented by Racke (1993).

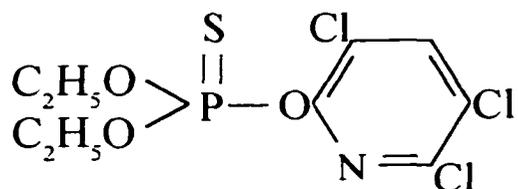


Fig. 1. Chemical structure of chlorpyrifos

The chemical structure of chlorpyrifos is depicted in Fig. 1. The available data on the physical and chemical properties of chlorpyrifos have been reviewed in detail by Racke (1993) and Giesy et al. (1999). Its water solubility is 1.4 mg/L at 25°C (Packard, 1987), and it exhibits a moderate level of hydrophobicity ($\log K_{ow}$ of 4.7-5.3). As a result of its hydrophobicity, chlorpyrifos partitions largely from the aqueous phase into the organic fractions of environmental matrices. Although chlorpyrifos has an intermediate vapor pressure (2×10^{-5} mm Hg at 25°C; Chakrabarti and Gennrich, 1987), volatilization has been shown to be a significant mechanism of dissipation from certain environmental surfaces (i.e., plant foliage, pond water), as summarized by Racke (1993). The sorption, desorption, degradation and transformation that govern the fate of chlorpyrifos in aquatic environments are further reviewed in the following sections.

Sorption and Desorption of Chlorpyrifos

It is generally believed that chlorpyrifos is strongly adsorbed by soil and sediment (Racke, 1993). Retention of chlorpyrifos by colloidal materials is one of the most important processes influencing its environmental fate. The sorptive behavior of chlorpyrifos in aqueous, soil and sediment systems has been investigated by a number of researchers (Felsot and Daham, 1979; Sharom, et al., 1980; MaCalady and Wolfe, 1985). Racke (1993) comprehensively reviewed sorption coefficients that have been determined in the laboratory using batch

equilibrium methods. For chlorpyrifos, adsorption K_d values ranged from as low as 13.4 to as high as 1862.0.

It is often assumed that the partitioning of pesticides proceeds rapidly (adsorption) and reversibly (desorption) to an equilibrium state in soil. Felsot and Dahm (1979) reported that adsorptive equilibrium for chlorpyrifos was reached within 2 hours in four different soils. Sharom et al. (1980) found that adsorptive equilibrium for chlorpyrifos was reached in a Beverly sandy loam and an organic soil in 4 and 6 hours, respectively. Macalady and Wolfe (1985) reported that chlorpyrifos adsorptive equilibrium was reached within 15 minutes in sediment/water systems. However, several experiments with chlorpyrifos have suggested that the sorption process may not be as simple as is often assumed. Sharom et al. (1980) reported that desorptive equilibrium was reached much more slowly than adsorptive equilibrium for a Beverly sandy loam soil. Approximately 70% of the adsorbed chlorpyrifos remained sorbed after four desorptive cycles, and the amount of chlorpyrifos desorbed as a relative percentage of total chlorpyrifos present declined with successive rinses. The sorption kinetics work by Cryer (1992), who recirculated chlorpyrifos-treated water (50-600 $\mu\text{g}/\text{mL}$) through a column containing Cecil sandy loam soil (0.34% organic carbon), indicated that it took from 11 to 50 hours for equilibrium to be approached and the time to reach equilibrium was concentration-dependent. Use of a sorption model that assumed Freundlich equilibrium provided a poor explanation of the observed kinetics. A model with better predictions was one in

which two types of sorbing sites were simulated: type I sites, which were assumed to be at equilibrium, and type II sites, which were assumed to be kinetically governed.

The empirically observed sorption of chlorpyrifos in soil may be due to more than one sorption mechanism. Mechanisms for sorption of organic chemicals in soils that have been demonstrated or postulated include hydrophobic bonding, electrostatic attraction, van der Waals affinity, hydrogen bonding, chelation/coordination, covalent bonding, and entrapment in soil micropores (Stevenson, 1972). However, the mechanisms involved in chlorpyrifos sorption by soil and sediment have not been extensively investigated (Racke, 1993). Partitioning into soil organic matter is commonly assumed to be a major mechanism of pesticide sorption in soils. However, the correlation between chlorpyrifos sorption coefficient and soil organic carbon is rather poor ($r^2 = 0.56$), and normalizing K_d values by organic carbon content does account for a significant component of the variability of chlorpyrifos sorption between soils. Felsot and Dahm (1979) calculated correlation coefficients for adsorption of chlorpyrifos and four other insecticides on four soils and found that K_d and organic matter ($r^2 = 0.469$, $\rho < 0.05$) and K_d and cation-exchange capacity ($r^2 = 0.439$, $\rho < 0.05$) were significantly correlated. Neither soil clay content nor pH was significantly correlated with chlorpyrifos sorption coefficients (K_d).

It is generally recognized that sorption is often the dominant reaction governing the fate and persistence of contaminants in aquatic systems (Lee and

Jones, 1987). The modeling of fate and effects of contaminants requires a reliable description of the sorption reactions that a contaminant may undergo. The inability to describe chlorpyrifos sorption is a major impediment to developing appropriate and predictive environmental chemistry-fate models. The mechanisms for sorption of chlorpyrifos should be more thoroughly investigated. The facts that sorption reactions do not follow the mass action law and that they show considerable hysteresis make them complex to investigate and model.

Degradation and Transformation of Chlorpyrifos

Chlorpyrifos will degrade by both abiotic and biotic transformation processes in terrestrial and aquatic environments. In soil, water, plants, and animals, the major pathway of abiotic and biotic degradation involves cleavage of the phosphorothioate ester bond to form 3, 5, 6-trichloro-2-pyridinol (TCP). TCP is further degraded in the environment via photolysis and microbial degradation (Bidlack, 1976; Dilling et al., 1984; Racke, 1993).

Hydrolysis and oxidation are the two most important degradation processes for chlorpyrifos (Fig. 2). The principal hydrolysis product, TCP, is the most important transformation product in water and soil environments. In addition, it is the metabolite formed *in vivo* by most organisms. The hydrolysis can be achieved either chemically or biologically, the latter mostly through the action of phosphatase enzymes. The primary oxidation product is chlorpyrifos oxon [o,o-diethyl o-(3,5,6-trichloro-2-pyridyl) phosphate], in which the thion sulfur has

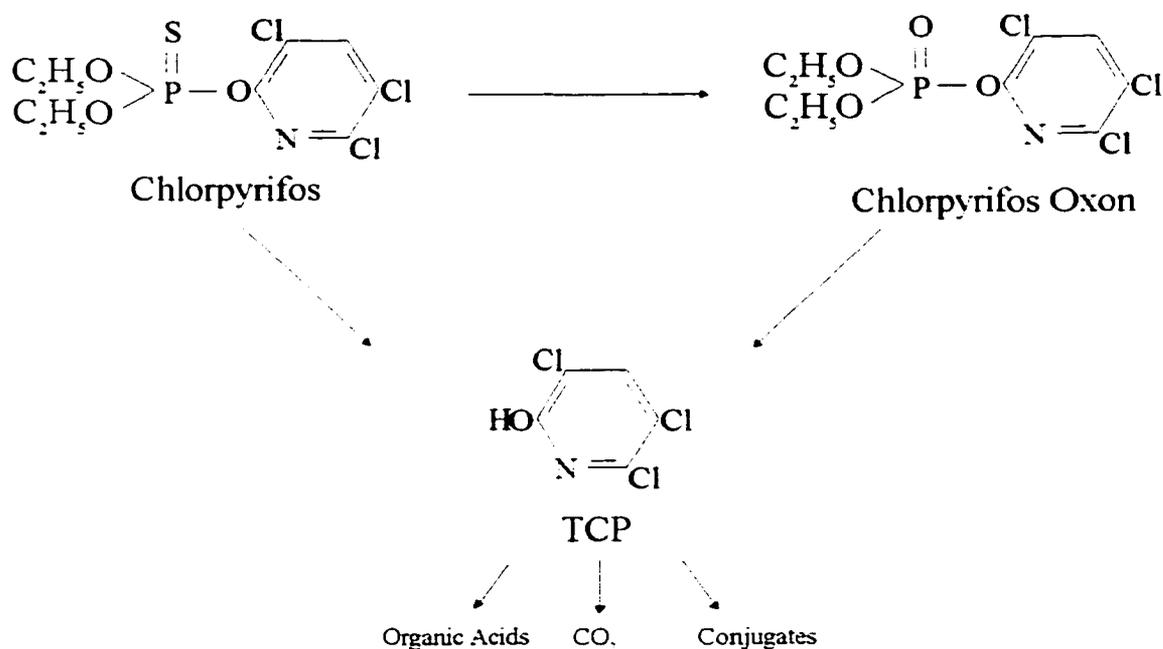


Fig. 2. Major pathway for transformation of chlorpyrifos in the environment

been oxidized and replaced by an oxygen atom. The oxon is very susceptible to hydrolysis and is hence quickly degraded further, principally to TCP. It is generally believed that transformation of chlorpyrifos to chlorpyrifos oxon occurs *in vivo*. Siegfried (1990) studied the biochemistry and genetics of chlorpyrifos resistance in the German cockroach and confirmed the mixed function oxidase was involved in activation of chlorpyrifos to chlorpyrifos oxon. Chlorpyrifos may also degrade to desethyl chlorpyrifos [o-ethyl o-(3,5,6-trichloro-2-pyridyl) phosphorothioate] in the environment. The more detailed pathways of environmental transformation of chlorpyrifos were described by Racke (1993).

Environmental factors (pH, temperature, etc.) significantly influence hydrolysis of chlorpyrifos in soil and aquatic environments (Cink and Coats, 1993). Most reports (Meikle and Youngson, 1978; Chapman and Cole, 1982) have indicated more rapid hydrolysis under alkaline conditions (pH > 7.5-8.0) than acidic or neutral conditions. At near neutral pH (~7) and ambient temperature (~25°C), chlorpyrifos is moderately stable, with reported hydrolysis half-lives between 29-35 days. Temperature has also been found to significantly influence chlorpyrifos hydrolysis. Meikle and Youngson (1978) reported that the rate of hydrolysis increased an average of 3.5-fold for each 10°C rise in temperature.

A number of field investigations and observations have focused on the fate of chlorpyrifos in aquatic ecosystems. Some of the earliest studies were initiated as a result of its former use as a mosquito larvicide. More recent research has been directed toward assessment of its fate following indirect or unintentional introduction of chlorpyrifos into aquatic environments due to surface runoff, spray drift and spills, etc. Several researchers have examined the fate of chlorpyrifos in freshwater ponds and lakes. Nelson and Evans (1973) found that after nominal application of 250, 500, or 1000 µg/L chlorpyrifos, residues in pond water remained relatively constant over a 22-week period, with average concentrations for the treatments of 0.37, 0.50, and 1.18 µg/L, respectively. Residue levels in sediments were higher, but also remained relatively stable over a 22-week period.

A few attempts have been made to predict the fate of chlorpyrifos in aquatic ecosystems. Neely and Blau (1977) utilized a three-compartment model (water, sediment/plants, and fish) to examine the ability of laboratory data to predict the fate of chlorpyrifos in a pond. It was reported that the modeling results based on laboratory-obtained kinetic and partitioning data compared favorably with those of a field-scale study. The authors conclude that the use of laboratory-derived fate data in modeling was a credible avenue for predicting the fate of chlorpyrifos in the real world.

Catalysis of Chlorpyrifos Transformation

Interactions of metals and organophosphorus pesticides have been the subject of several investigations (Blanchet and St-George, 1981). The hydrolysis of chlorpyrifos is catalyzed by dissolved copper ions in aqueous systems. Mortland and Raman (1967) first reported this phenomenon and observed nearly 100% hydrolysis of chlorpyrifos ($2.8 \mu\text{g L}^{-1}$) to TCP within 24 hours in aqueous methanol (50%) containing 0.1 mM Cu^{2+} . Limited hydrolysis (10% in 24 hours) occurred in the presence of 0.1 mM MgCl_2 , and negligible hydrolysis was observed in the presence of any other metal salt tested (CoCl_2 , ZnSO_4 , NiSO_4 , AlCl_3 , and CaCl_2). In aqueous systems containing different Cu^{2+} -saturated ion exchangers, 100% (montmorillonite), 20% (beidellite), 10% (nontronite), and 0% (vermiculite, organic soil) hydrolysis was observed within 24 hr. Mortland and Raman (1967) hypothesized a mechanism whereby Cu^{2+} coordinated with the S

and pyridyl N atoms and thus increased the electron deficiency of the P atom, rendering it more susceptible to nucleophilic attack. Blanchet and St-George (1981) also postulated the existence of a 6-member intermediate complex of Cu^{2+} and chlorpyrifos.

Aluminosilicate minerals have been observed to catalyze a number of transformation reactions (Theng, 1974). The mechanism for heterogeneous surface-catalyzed hydrolysis in soil is thought to be related to the activity within the film of hydration water (≥ 1 nm) that is associated with the surface of clay minerals and their surface counter-ions (e.g., Ca^{2+}) under both moist and air-dry conditions (Mingelgrin et al., 1977; Yaron, 1978). Camazano and Martin (1983) theorized that the clay-organophosphorus insecticide interaction that occurs in this zone may enhance the electrophilic nature of the P atom of organophosphates, thus facilitating nucleophilic attack by hydroxide ions.

Surface acidity of clay minerals is capable of catalyzing a number of pesticide reactions (Theng, 1974; McBride, 1994; Stevenson, 1994). The protonation reaction of atrazine is known to occur on montmorillonite (Russell, 1968). However, there are no reports in the literature of protonation of chlorpyrifos on the surface of clay minerals. It is not known whether the sorption of chlorpyrifos by colloids might catalyze the transformation process in aquatic systems.

Ecotoxicology of Chlorpyrifos

Chlorpyrifos is used in a variety of environments. Agricultural, industrial, and residential applications of chlorpyrifos have results in its intentional and accidental introduction into terrestrial and aquatic ecosystems throughout the world. Specific regional vulnerability of surface water to chlorpyrifos exposure resulting from natural runoff events was inferred by Giesy (1999) from the combined criteria of chlorpyrifos use volume and runoff potential. Because of agricultural uses, surface waters in the Midwestern corn belt and the Mississippi Delta region were predicted to have the greatest risk of chlorpyrifos exposure.

In agriculture, chlorpyrifos is often applied to the plant surface either aerially or through the use of ground spraying equipment. Studies have shown that as little as 0.5-2.0% of active ingredient applied is found on or in the target foliage (Wauchope et al., 1991). This same study found that approximately half of the active ingredient applied was found in the soil. The remaining 48-49% a.i. was probably lost through a combination of spray drift and volatilization from the plant or soil surface. Pesticide losses from treated cropland may cause off-site problems for aquatic and terrestrial environments and for human health.

Laboratory and field tests have shown that chlorpyrifos exhibits moderate persistence in natural systems (Marshall and Roberts, 1978; USEPA, 1986; Odenkirchen and Eisler, 1988). An extensive database on the toxicology of chlorpyrifos to many aquatic and terrestrial organisms has developed since the

initial production of this chemical in 1962 (Barron and Woodburn, 1995). It is well documented that chlorpyrifos is highly toxic to fish and aquatic invertebrates and typically has 96-hr LC_{50} values of 0.1-500 $\mu\text{g L}^{-1}$ (Odenkirchen and Eisler, 1988; Worthing and Hance, 1991; Wilcock et al., 1994).

There are a few documents on occurrence of chlorpyrifos in natural surface waters. One published study, limited to the Northern Chesapeake Bay region, included chlorpyrifos as a target analyte (Kroll and Murphy, 1993). The results indicated the occurrence of chlorpyrifos in virtually every sample collected at levels above limits of detection (McConnell et al., 1997). It has been reported that more than one million acres of corn soils are treated with chlorpyrifos in Iowa alone annually (Wintersteen and Hartzler, 1987). However, the occurrence of chlorpyrifos in Iowa streams and rivers has received little attention.

In residential applications, chlorpyrifos is the sixth most commonly used pesticide in home and garden environments. It is reported that chlorpyrifos is applied in more than 20 million American homes each year to protect families, children and pets from the harmful effects of insect pests (e.g. termites, ticks, cockroaches, fireants). According to the USEPA, 972 registered products contain chlorpyrifos, including widespread uses for termite and roach control (Davis and Ahmed, 1998).

In toxicological studies conducted according to EPA guidelines, chlorpyrifos has been shown not to be mutagenic, carcinogenic, or teratogenic, nor does it adversely affect reproduction. The only known mode of chlorpyrifos toxicity is

through cholinesterase inhibition. If human exposure is less than that causing significant cholinesterase depression, then no signs or symptoms related to chlorpyrifos exposure occur. Therefore, chlorpyrifos has been regarded as a safe pesticide for household use (Gibson, 1998).

In recent years, however, EPA formally reviewed a number of legal claims related to chlorpyrifos products for home use. Among the symptoms reported to be linked with chlorpyrifos applications are headache, dizziness, abdominal cramps, nausea, vomiting, diarrhea, blurred vision, increased secretions (tearing, sweating, salivation), mental confusion, and muscular weakness. Because exposure from spray applications was assumed to reach peak levels within a few hours after use and to fall off rapidly, researchers have generally been unable to understand or have been slow to accept how these disease symptoms could be associated with chlorpyrifos exposure. Davis (1998) suggested that exposure from indoor spraying of chlorpyrifos poses greater health risks than currently estimated.

Hypothesis and Objectives

A high mortality of larval walleye fish (*Stizostedion vitreum*) was observed in the Upper Cedar River, Iowa (Menzel, 1981; Paragamian, 1990). It is hypothesized that pollution by agricultural chemicals is the cause of the fish mortality. Most of the commonly used agricultural chemicals in the watershed of the Upper Cedar River are only slightly to moderately toxic to fish. However,

widely used chlorpyrifos is highly toxic to freshwater fish. Because chlorpyrifos is believed to be strongly bound to soil particles it is most likely to be transported to the river with suspended sediment in surface runoff events.

This research project, supported by grants from USDA-NRICGP (9700882) and the Iowa State University Leopard Center for Sustainable Agriculture (98-08), was initiated to test the hypothesis that chlorpyrifos bound to suspended sediment may contribute to the mortality of larval walleye fish in the Upper Cedar River, Iowa. The toxicity of aqueous chlorpyrifos and chlorpyrifos-colloid complexes to fish were determined in a cooperative study by Dr. R. C. Summerfelt and Dr. T. A. Phillips. Their results indicate chlorpyrifos remains toxic to larval walleye when bound to humic colloids (Phillips, 2000). The specific objectives of the present study are: 1) to quantify sorption and desorption of chlorpyrifos on colloidal materials in aqueous systems; 2) to study effects of suspended colloids on hydrolysis of chlorpyrifos in aqueous systems; 3) to determine whether colloidal materials may catalyze the abiotic transformation of sorbed chlorpyrifos in aqueous systems; and 4) to investigate mechanism for chemical transformation of chlorpyrifos in water. Each of above objectives is further addressed under a separate study.

Organization of Dissertation

This dissertation includes six chapters and an appendix. Chapter 1 is a general introduction. Chapters 2-5 were prepared as independent articles for

submission to referred journals. Chapter 2 describes the interaction of chlorpyrifos with colloidal materials in aqueous systems. The effects of water chemistry and suspended colloidal materials on hydrolysis of chlorpyrifos in aqueous systems are discussed in Chapter 3. Chapter 4 addresses the fate of the chlorpyrifos sorbed on colloidal materials in aqueous systems. Chemical transformation of chlorpyrifos to chlorpyrifos oxon in water, which was first found in this study, is reported in chapter 5. Chapter 6 summarizes the results obtained in this study. A SPME-GC technique developed in our laboratory for determination of trace chlorpyrifos in aqueous samples is described in the Appendix.

References

- Barron, M.G., and K.B. Woodburn. 1995. Ecotoxicology of chlorpyrifos. *Rev. Environ. Contam. Toxicol.* 144:1-93.
- Berg, G.L. 1986. *Farm chemical handbook*. Meister Publishing Company. Willoughby, OH.
- Bidlack, H.D. 1976. Degradation of ¹⁴C-labeled 3, 5, 6-trichloro-2-pyridinol in 15 select agricultural soils. Report GH-C 953. DowElanco, Indianapolis, IN.
- Blanchet, P.F., and A. St-George. 1981. Kinetics of chemical degradation of organophosphorus pesticides; hydrolysis of chlorpyrifos and chlorpyrifos-methyl in the presence of copper (II). *Pestic. Sci.* 13:85-91.

- Camazano, M.S., and M.J.S. Martin. 1983. Montmorillonite-catalyzed hydrolysis of phosmet. *Soil Sci.* 136:89-93.
- Carson, R. 1962. *Silent Spring*. Houghton Mifflin Co., Boston.
- Chakrabarti, A., and S.M. Gennrich. 1987. Vapor pressure of chlorpyrifos. Report ML-AL-87-40045. DowElanco, Indiannapolis, IN.
- Chapman, R.A., and C.M. Cole, 1982. Observations on the influence of water and soil pH on the persistence of insecticides. *J. Environ. Sci. Health B.* 15:39-46.
- Cink, J.H., and J.R. Coats. 1993. The effect of concentration, temperature, and soil moisture on the degradation of chlorpyrifos (Dursban TC) in an urban Iowa soil. In K.D. Racke and A.R. Leslie (eds). *Pesticides in urban environments: Fate and significance*. ACS. Washington DC. ACS Symp. Series 522:62-69.
- Cryer, S.A. 1992. A method for evaluating kinetic and non-equilibrium sorption models: preliminary results using chlorpyrifos pesticide formulations in Cecil soil. Report of DowElanco, DowElanco, Indiannapolis, IN.
- Davis D.L., and A.K. Ahmed. 1998. Exposures from indoor spraying of chlorpyrifos pose greater health risks to children than currently estimated. *Environ. Health Perspect.* 106:299-301.
- Dilling, W.L., L.C. Lickly, T.D. Lickly, P.G. Murphy, and R.L. McKellar. 1984. *Organic photochemistry*. 19. Quantum yields for o,o-diethyl o-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate (chlorpyrifos) and 3, 5, 6-trichloro-2-pyridinol in dilute aqueous solutions and their environmental

- phototransformation rates. *Environ. Sci. Technol.* 18:540-543.
- Economic Research Service (ERS). 1994. *Agricultural resources and environmental indicators*. U.S. Department of Agriculture, Natural Resources and Environment Division. *Agricultural Handbook 705*, 205.
- Farmer, W.J., and Y. Aochi. 1974. Picloram sorption by soils. *Soil Sci. Soc. Am. Proc.* 38:418-423.
- Felsot A., and P.A. Dahm. 1979. Sorption of organophosphorus and carbamate insecticides by soil. *J. Agric. Food Chem.* 27:557-563.
- Gibson J.E., R.K.D. Peterson, and B.A. Shurdut. 1998. Human exposure and risk from indoor use of chlorpyrifos. *Environ. Health Prospect.* 106:303-306.
- Giesy, J.P., K.R. Solomon, J.R. Coats, K.R. Dixon, J.M. Giddings, and E.E. Kenaga. 1999. Chlorpyrifos: ecological risk assessment in North American aquatic environments. *Rev. Environ. Contam. Toxicol.* 160:1-129.
- Harpaz, I. 1973. Early entomology in the middle East. In R.F. Smith, T.E. Mittler, and C.N. Smith (eds). *History of Entomology*, *Ann. Rev. Entomol. Annual Reviews*. Palo Alto, CA. 21-26.
- Hayes, W.J., and E.R. Laws. 1992. *Handbook of pesticide toxicology*, Vol. 3, classes of pesticides. Academic Press, Inc., NY.
- Hornsby, A.G., and J.M. Davidson. 1973. Solution and adsorbed fluometuron concentration distribution in a water saturated soil: experimental and predicted evaluation. *Soil Sci. Soc. Am. Proc.* 37:823-828.

- Kroll, R.B., and D.L. Murphy. 1993. Pilot monitoring project for 14 pesticides in Maryland surface water. Maryland Dept. of Environ. Baltimore, MD.
- Lee, G.F., and R.A.Jones. 1987. Water quality significance of contaminants associated with sediments: an overview. In K.L.Dickson et. al. (ed). Fate and Effects of Sediment-bound Chemicals in Aquatic Systems. Pergamon Press. 3-34.
- MaCalady, D.L., and N.L. Wolfe. 1985. Effects of sediment sorption and abiotic hydrolysis. 1. organophosphorothioate esters. J. Agric. Food Chem. 33:167-173.
- Marshall, W.K., and J.R. Roberts. 1978. Ecotoxicology of chlorpyrifos. National Research Council of Canada Publication 16079. NRCC. Ottawa.
- McBride, M.B. 1994. Environmental chemistry of soils. Oxford University Press, New York, Oxford.
- McConnell, L.L., E. Nelson, C.P. Rice, J.E. Baker, W.E. Johnson, J.A. Harman, and K. Bialek. 1997. Chlorpyrifos in the air and surface water of Chesapeake Bay: predictions of atmospheric deposition fluxes. Environ. Sci. Technol. 31:1390-1398.
- Meikle, R.W., and C.R. Youngson. 1978. The hydrolysis rate of chlorpyrifos, o-o-diethyl-o-(3,5,6-trichloro-2-pyridyl) phosphorothioate, and its dimethyl analog, chlorpyrifos-methyl, in dilute aqueous solution. Arch. Environ. Contam. Toxicol. 7:13-22.

- Menn, J.J., and R.M. Hollingwoth. 1985. Introduction. In G.A. Kerkut and L.I. Gilbert (eds). *Comprehensive Insect Physiology Biochemistry and Pharmacology*. Pergamon Press, Oxford. 12:1-8.
- Menzel, B.W. 1981. Iowa's waters and fishes: A century and a half of change. *Proceedings of the Iowa Academy of Science*. 88:17-23.
- Mingelgrin, U., S. Saltzman, and B. Yaron. 1977. A possible model for the surface-induced hydrolysis of organophosphorus pesticides on kaolinite clays. *Soil Sci. Soc. Am. J.* 41:519-523.
- Mortland, M.M., and K.V. Raman. 1967. Catalytic hydrolysis of some organic phosphate pesticides by copper (II). *J. Agric Food Chem.* 15:163-167.
- Neely, W.B., and G.E. Blau. 1977. The use of laboratory data to predict the distribution of chlorpyrifos in a fish pond. In S.U. Khan (ed). *Pesticides in aquatic environments*. Plenum Press, NY. 145-163.
- Nelson, J.H., and E.S. Evans. 1973. Field evaluation of the larvicidal effectiveness: effects on nontarget species and environmental residues of a slow-release polymer formulation of chlorpyrifos. March-October. Army Environ Hygiene Agency. Aberdeen, MD.
- Odenkirchen, E.W., and R. Eisler. 1988. Chlorpyrifos hazards to fish, wildlife, and invertebrates: a synoptic review. *US Fish Wildl. Serv. Biol. Rep.* 85, 1.13, 34.
- Packard, S.R. 1987. Determination of the water solubility of chlorpyrifos. Report ML-AL 87-71102. DowElanco, Midland, MI.

- Paragamian, V.L. 1990. Stream walleye fisheries investigation. Federal Aid to Fish Restoration Annual Performance Report, Stream Fisheries Investigation. Iowa Department of Natural Resources, Des Moines, IA.
- Phillips, T.A. 2000. Toxicity of chlorpyrifos adsorbed on clay and humic acid to larval walleyes. PhD Dissertation, Iowa State University, Ames, IA.
- Racke, K.D. 1993. Environmental fate of chlorpyrifos. *Rev. Environ. Contam. Toxicol.* 131:1-154.
- Russell, J.D., M.Cruz, and J.L. White. 1968. Mode of chemical degradation of s-triazines by montmorillonite. *Science* 160:1340-1342.
- Savage, K.E., and R.D. Wauchope. 1974. Fluometuron adsorption-desorption equilibria in soil. *Weed Sci.*, 22:106-110.
- Schwarzenbach, R.P., P.M. Gschwend, and D.M. Imboden. 1993. *Environmental organic chemistry*. John Wiley & Sons, Inc. New York.
- Sharom M.S., J.R.W. Miles, C.R. Harris, and F.L. McEwen. 1980. Persistence of 12 insecticides in water. *Water Res.* 14:1089-1093.
- Siegfried, B.D., J.G. Scott, R.T. Roush, and B.C. Zeichner. Biochemistry and genetics of chlorpyrifos resistance in the German cockroach, *Blattella germanica* (L). 1990. *Pesticide Biochemistry and Physiology*. 38:110-121.
- Stevenson, F.J. 1972. Organic matter reactions involving herbicides in soil. *J. Environ Qual.* 1:333-343.
- Stevenson, F.J. 1994. *Humus chemistry: Genesis, composition, reaction*. John Wiley & Sons, New York.

- Swanson, R.A., and G.R. Dutt. 1973. Chemistry and physical processes that affect atrazine movement and distribution in soil systems. *Soil Sci. Soc. Am. Proc.* 37:872-876.
- Theng, B.K.G. 1974. *The chemistry of clay-organic reactions*. John Wiley & Sons, New York.
- US Environmental Protection Agency (EPA). 1986. *Ambient water quality criteria for chlorpyrifos*. Office of Water Regulations and Standards, Criteria and Standards Division, Washington, DC.
- Wauchope, R.D., J.R. Young, R.B. Chalfant, L.R. Marti, and H.R. Summer. 1991. Deposition, mobility and persistence of sprinkler-irrigation-applied chlorpyrifos on corn foliage and in soil. *Pestic. Sci.* 32:235-243.
- Wilcock, R.J., G.L. Northcott, and J.W. Nagels. 1994. Mass losses and change in concentration of chlorpyrifos and cis- and trans-permethrin applied to the surface of a stream. *Bull. Environ. Contam. Toxicol.* 53:337-343.
- Wintersteen, W., and R. Hartzler. 1987. *Pesticides used in Iowa crop production in 1985*. Iowa Coop. Ext. Serv. Pamph. 1288.
- Worthing, C.R., and R.J. Hance. 1991. *The pesticide manual*, 9th ed. Unwin Brothers, Surrey, UK.
- Yaron, B. 1978. Some aspects of surface interactions of clays with organophosphorus pesticides. *Soil Sci.* 125:210-216.

CHAPTER 2

INTERACTION OF CHLORPYRIFOS WITH COLLOIDAL MATERIALS IN AQUEOUS SYSTEMS

A paper to be submitted to Soil Science Society of America Journal

Jigang Wu and David A. Laird

Abstract

An understanding of sorptive processes is key to describing the fate of chlorpyrifos in aquatic systems. The objectives of this study were to evaluate isotherms for sorption and desorption of chlorpyrifos on colloidal materials and elucidate the mechanism for their interactions. Six Ca-saturated reference smectites, one humic acid (Ca-humate), and one suspended sediment sample collected from the Upper Cedar River, Iowa were studied. A batch equilibrium technique was employed to quantify sorption and desorption isotherms for chlorpyrifos over the 0 to 100 $\mu\text{g L}^{-1}$ concentration range in 0.01 M CaCl_2 background. A large difference in sorption affinity and variation in desorption hysteresis was found among smectites. Neither chlorpyrifos sorption nor its desorption was correlated with cation-exchange capacity, surface area or surface charge density of smectites. The fabric of smectite quasicrystals may be related to sorption affinity for chlorpyrifos. It was postulated that physical interaction between chlorpyrifos and smectite was the dominant mechanism for sorption of

chlorpyrifos in aqueous systems. Chlorpyrifos was very strongly sorbed on humic acid and was not desorbed from humic acid in aqueous solution. Chlorpyrifos was moderately sorbed on river sediment and a large sorption-desorption hysteresis was also found. The study implies that humic acid and suspended sediment can be important vectors for transport and exposure of chlorpyrifos to aquatic organisms in rivers.

Introduction

Chlorpyrifos [o,o-diethyl o-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate], an active ingredient in Dursban® and Lursban®, has been used for many years in agriculture for the control of various crop insect pests (Racke, 1993). It is estimated that more than 3 million kg of chlorpyrifos are applied to field crops in the USA annually (ERS, 1994). In Iowa alone, over 1,000,000 acres of corn soils are treated with chlorpyrifos annually (Wintersteen and Hartzler, 1987). Chlorpyrifos bound to soil constituents may be introduced into rivers by surface runoff from agricultural lands, which is a major concern for aquatic ecosystems in many Midwestern streams and rivers.

Kratzer (1998) reported a considerable increase in the concentrations of suspended solids, organic compounds, pesticides, metals and other contaminants that may be toxic to biota in surface waters as a result of surface runoff from storm events. While larger particles may represent a significant fraction of

suspended materials, the majority of the available surface area on the particles in suspension is certain to reside within the smaller size fractions. Colloidal materials, which are made up predominantly of clay minerals and organic matter, are generally responsible for retention of pesticides in soils (Koskinen and Harper, 1990). Understanding the interaction between chlorpyrifos and colloidal materials in aqueous systems is fundamental for assessing fate and potential toxicity of chlorpyrifos bound to suspended sediment in aquatic environments.

The sorptive behavior of chlorpyrifos in aqueous soil or sediment systems has been investigated by a number of researchers (Felsot and Daham, 1979; Sharom, et al., 1980; MaCalady and Wolfe, 1985). The moderately high partition coefficients that have been determined for chlorpyrifos result from its nonpolar nature and indicate its tendency to associate strongly with organic materials in the environment. Racke (1993) comprehensively reviewed sorption coefficients that have been determined in the laboratory using the batch equilibrium method. For chlorpyrifos, sorption K_d values ranged from as low as 13.4 to as high as 1862.0.

Laird et al. (1992) investigated the effects of surface charge density on adsorption of atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) by Ca-smectites. They found that anywhere from 0 to 100% of added atrazine was adsorbed on the Ca-smectites from 0.01M CaCl_2 aqueous systems, and the affinity of smectites for atrazine increased with decreasing surface charge

density of reference smectites. Atrazine has both polar and nonpolar moieties while chlorpyrifos is dominantly a nonpolar compound. Therefore, it is not known whether chlorpyrifos sorption on smectites will exhibit a similar relationship.

The objectives of this study were: 1) to evaluate isotherms for sorption and desorption of chlorpyrifos on colloidal materials in aqueous systems; 2) to find the relationship between sorptive behavior and surface and physical properties of smectites and to elucidate the mechanism for their interactions in aqueous systems. The significance of this study lies in the prediction of the fate and toxicity of chlorpyrifos associated with suspended sediment in aquatic environments.

Materials and Methods

Chemicals

Reagent grade chlorpyrifos with purity of 99.2% was obtained from Chem Service (West Chester, PA). Ethanol (100%) was used to help dissolve chlorpyrifos for preparation of stock solutions. All solutions and suspensions were prepared using Milli-Q water with a resistivity of 18.2 M Ω -cm (Milli-Q Plus system, Millipore, Bedford, MA).

Sample Preparation

Six reference smectites: Amory montmorillonite (Amory), Polkville montmorillonite (Polkville), Panther Creek beidellite (Panther), Otay white

montmorillonite (Otay), IMV saponite (Saponite), Floridin hectorite (Hectorite) were evaluated in this study. Amory and Polkville were obtained from Ward's Natural Science Establishment, Inc., Rochester, NY. Panther was acquired from the A.D. Scott collection, Iowa State University, Ames, IA. Otay was collected at an exposure near San Diego, CA. Saponite and Hectorite were obtained from IMV cooperation, division of Floridin Company (Amargosa Valley, Nevada). Carbonates were removed by treatment with pH 5.0 1.0 M $\text{NaC}_2\text{H}_3\text{O}_2$ (Kunze and Dixon, 1986). The clay fraction ($< 2 \mu\text{m}$) was separated by sedimentation from the bulk ore. The clays were Ca-saturated by treatment with 1 M CaCl_2 . The Ca-saturated clays were dialyzed using Spectra/Por[®] 3500 MWCO molecularporous membrane tubing against Milli-Q water until the conductivity of dialysate was $< 0.2 \text{ mS/m}$. The Ca-saturated samples were then freeze-dried.

The humic acid (HA) was obtained from Aldrich Chemical Company (Milwaukee, WI). The HA was washed with 1.0 M CaCl_2 four times to prepare Ca-saturated humic acid (Ca-humate), dialyzed using Spectra/Por 3500 MWCO molecularporous membrane tubing against Milli-Q water until no Cl^- was detected by AgNO_3 and then freeze-dried.

A large volume of river water (~950 L) containing suspended sediment was collected from the Upper Cedar River near Janesville, Iowa on October 16, 1997. The suspended sediment was separated from the river water by freezing-induced flocculation, thawing and sedimentation. The concentrated suspended sediment

sample was then freeze-dried. No chemical treatments were used to prepare the dried suspended sediment sample.

Characterization of Samples

The clay mineralogy of the smectites and the sediment sample was analyzed by X-ray diffraction (XRD) using oriented specimens on the glass slide and CuK α radiation with a Siemens D5000 X-ray diffractometer (Moore and Reynolds, 1989).

Total chemical analysis (Si, Al, Fe, Mg, Ca, K, Li, Zn, Mn, Ti) of the clay samples was performed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Thermo Jarrell Ash ICAP 61E, Franklin, MA) using suspension nebulization (Laird et al., 1991). The elemental compositions were used to calculate smectite structural formulae, cation-exchange capacities (CEC), surface areas, and surface charge densities (Gast, 1977). Total C and N of the smectites and the sediment sample were determined by dry combustion using a Carlo Erba NA 1500 NSC elemental analyzer (Haake Buchler Instruments, Paterson, NJ). Organic C of the smectites was determined after treatment with 1:3 HCl to remove trace carbonate, and inorganic C was estimated after treatment with 30% H₂O₂ to oxidize trace organic C in the samples. Inorganic C of the sediment was determined by a titrimetric method (Bundy and Bremner, 1972). Organic C of the sediment was determined by the difference between total C and inorganic C. The particle-size analysis of the sediment sample was performed using the pipet method (Gee and Bauder, 1986).

For scanning electron microscopy (SEM), 0.2-g samples were shaken with 20 mL Milli-Q water for 24 hours. The suspensions were then diluted to $1.0 \mu\text{g mL}^{-1}$ with Milli-Q water and one drop of the diluted suspensions was deposited using Pasteur pipet on Ag foil covered SEM-stubs, and dried in a desiccator above Drierite (97% CaSO_4 and 3% CoCl_2). The samples were lightly sputter coated (30 seconds) with palladium/gold alloy (60/40) using a Denton Vacuum LLC Desk II Cold Sputter/Etch Unit (Denton Vacuum Inc., Moorestown, NJ). Images were taken using a JEOL 5800LV Scanning Electron Microscopy (Japan Electron Optics Laboratory, Japan) at 10kV.

Sorption and Desorption of Chlorpyrifos

Chlorpyrifos sorption and desorption isotherms were obtained using the batch equilibration procedure. Triplicate 0.2-g samples of clay, sediment or Ca-humate were equilibrated with 20 mL of 0.01 M CaCl_2 solution containing 0 to $100 \mu\text{g L}^{-1}$ of chlorpyrifos by shaking for 24 hours at room temperature ($23 \pm 2^\circ\text{C}$) in 25-mL Corex glass centrifuge tubes. Aqueous and solid phases were separated by centrifugation ($\times 17,210 \text{ g}$) for 30 minutes. Supernatant (10-mL) was withdrawn to quantify chlorpyrifos in the aqueous solution. The amount of chlorpyrifos sorbed on the samples was calculated from the difference between the initial and equilibrium solution concentrations.

Desorption of chlorpyrifos was performed by resuspending samples initially equilibrated with $100 \mu\text{g L}^{-1}$ of chlorpyrifos in 10 mL of fresh 0.01 M CaCl_2 . The

suspensions were shaken for 24 hr at $23\pm 2^{\circ}\text{C}$. Aqueous and solid phases were separated by centrifugation as described above. The desorption procedure was repeated five times for each sample.

SPME-GC Analysis

Chlorpyrifos in aqueous samples was quantified using solid phase microextraction-gas chromatography technique (SPME-GC) developed in our laboratory. The SPME device was obtained from Supelco (Bellefonte, PA). The SPME fiber coated with 85- μm polyacrylate was used for the determination of chlorpyrifos after evaluation of a variety of available fibers.

A 10-mL aqueous sample was pipetted into an amber glass vial. The SPME fiber was completely immersed into the solution, which was stirred at a constant rate during a 30-min equilibration. The SPME fiber was then inserted directly into the gas chromatograph injector port for desorption and analysis of chlorpyrifos.

All analyses were performed using a Hewlett Packard 5890 series II Gas Chromatograph equipped with a flame ionization detector (Wilmington, DE). A split/splitless GC injection port, maintained at 220°C , and a $30\text{ m} \times 0.25\text{ mm ID}$ DB-1701 fused silica capillary column with a $0.25\text{ }\mu\text{m}$ stationary film (J & W Scientific; Folsom, CA) were utilized. The GC oven temperature programming was: initial temperature of 50°C ramped to a final temperature of 260°C at $5^{\circ}\text{C min}^{-1}$. The final temperature was held for 10 min. Analyte desorption from the fiber and purge off time were 5 min. The carrier gas was helium with the head

pressure set to 10 psi. The detector temperature was maintained at 260 °C. The retention time of chlorpyrifos was found to be 43.2 min based on analysis of calibration standards. A linear calibration with a correlation coefficient (r^2) of greater than 0.9995 was achieved for chlorpyrifos over the concentration range of the study (0~1000 $\mu\text{g L}^{-1}$).

Results and Discussion

Characterization of Smectites and Suspended Sediment Sample

X-ray diffraction analysis indicates that smectite was the dominant mineral phase in all of the reference clay samples used in the study (Fig. 1). The saponite sample was dominated by smectite, but contained lesser amounts of illite and quartz. Table 1 presents the oxide composition of the reference smectites determined using ICP-AES. The difference in elemental composition among smectites is substantial, especially for Fe, Mg, K, Li, and Al. Structural formulae of the smectites can be derived from the chemical analysis (Table 2). This calculation was based on a unit cell consisting 44 negative charge [i.e., $\text{O}_{20}(\text{OH})_4$] which has eight tetrahedral and six octahedral sites (Gast, 1977). Among those reference smectites Hectorite and Saponite are trioctahedral smectites and the others are dioctahedral smectites. These structural data are further used to calculate surface charge density, cation-exchange capacity and surface area using the method described by Gast (Table 3). The data in Table 3

demonstrate that the selected smectites have a large range in surface charge properties.

The suspended sediment sample contained 105.7 g kg⁻¹ total C (among total C are 18.4 g kg⁻¹ organic C, 87.3 g kg⁻¹ inorganic C) and 2.8 g kg⁻¹ total N.

Substantial amount of calcite in the whole sediment was identified by XRD (Fig. 2A). The particle size analysis indicated that the suspended sediment contained 0.9 % sand (>0.05 mm), 72.9% silt (0.002-0.05 mm) and 26.2% clay (<0.002 mm).

The clay fraction was dominated by smectite with lesser amounts of illite, kaolinite, quartz, and calcite (Fig. 2B).

Sorption and Desorption Isotherms

The isotherms for sorption of chlorpyrifos on smectites are shown in Fig. 3. The isotherms indicate a large variability in sorption affinity for chlorpyrifos among the smectites. More chlorpyrifos tended to be adsorbed on Hectorite, Saponite, and Panther while Otay and Polkville had relatively less affinity for chlorpyrifos. The results indicate that sorption affinities differ substantially though the selected samples are all Ca-saturated smectitic clays. Chlorpyrifos was not readily desorbed in the aqueous solutions. A large sorption-desorption hysteresis was found among smectites (Fig. 4).

Chlorpyrifos was virtually 100 percent sorbed on the Ca-humate and none of the sorbed chlorpyrifos was desorbed back into the aqueous solution (Fig. 5), indicating that chlorpyrifos has a strong tendency to be sorbed from water by organic materials probably due to its nonpolar nature. This strong tendency is

consistent with its high octanol/water partition coefficient ($K_{ow}=50,000$) reported in the literature (Racke, 1993). The composition of the Cedar River suspended sediment was much more complicated as it contained various mineral and organic materials. Fig. 5 shows that the affinity of chlorpyrifos for the suspended sediment is between that of reference smectites and the Ca-humate, implying that both organic and inorganic components of sediment contribute to the sorption of chlorpyrifos in aqueous systems. Some of chlorpyrifos sorbed on sediment was desorbed in the aqueous solution, most likely from the clay mineral phases in the sediment.

Sorption Affinity and Mechanism

The sorption affinity for chlorpyrifos on smectites can be quantified by introduction of the sorption distribution coefficient, K_d . The K_d value is defined by the following equation:

$$K_d = \frac{C_s}{C_{aq}} \quad (1)$$

where C_s is the amount of chlorpyrifos on smectites ($\mu\text{g g}^{-1}$) and C_{aq} is the amount of chlorpyrifos in solution ($\mu\text{g mL}^{-1}$). The K_d values for different smectites (Table 4) indicate a large variation in sorption and desorption for chlorpyrifos among smectites. The K_d values ranged from 45 for Otay to 1315 for Hectorite. After 5-cycles of desorption, the amount of chlorpyrifos desorbed from smectites varied from as low as 5.2% of the initially adsorbed chlorpyrifos on the solid phase for Hectorite up to 78.0% for Polkville. The K_d values from desorption isotherms

(Kd_{de}) were generally greater than Kd_{ad} from sorption isotherms. The Kd_{de} values increased with each desorption cycle. The difference between Kd_{de} and Kd_{ad} indicates the magnitude of the sorption-desorption hysteresis.

Relationships between the Kd_{ad} values and properties of the smectites are plotted in Fig. 6. The Kd_{ad} values apparently were not correlated with CEC, surface area, or surface charge density, suggesting that sorption affinity for chlorpyrifos is not related to surface properties of smectites. This is contrary to the findings under the similar experiment by Laird et al. (1992), who found that the affinity of smectites for atrazine linearly decreased with increasing surface charge density ($r^2=0.83$). However, this study shows that surface chemistry of smectites did not control the sorption behavior of chlorpyrifos on the Ca-saturated reference smectites. Smectite surfaces are likely polar and expose a combination of hydroxy- and oxy-moieties to their exterior. Naturally, these polar surfaces strongly favor interactions which allow them to form H-bonds with water. As a result, replacing the water molecules at a mineral surface with nonpolar chlorpyrifos is unfavorable from an energetic point of view. On the other hand, penetration of neutral chlorpyrifos into humic acid does not require displacement of tightly bound water molecules. Such materials contain 40-45% carbon, but they also have oxygens in their structure (Stevenson, 1994). Thus, they offer a relatively nonpolar environment into which a hydrophobic compound may partition without undue competition with water.

Trace amounts of total carbon were detected in the smectite samples (Table 5). The results indicate trace C in Otay, Polkville, Amory and Saponite, and a slightly higher C in Hectorite and Panther samples. About half of the residual C appeared to be carbonate due to incomplete removal of carbonate by treatment with 1.0 M $\text{NaC}_2\text{H}_3\text{O}_2$ buffer (pH 5.0) during the preparation of clay samples. Regression analysis demonstrates a weak relationship ($r^2=0.63$) between $K_{d,d}$ and organic C content of the smectite samples, suggesting that organic C may account for some but not all of the variation in sorption affinity for the smectites.

The SEM images of reference smectites are illustrated in Fig. 7. The images generally reveal a lot of thin flakes and fine particles in the samples. The general appearance or fabric of the fine particles was different in the various smectite samples. Many fine debris and fine needle-like structures exist in the Hectorite while fewer fine particles are seen in the SEM image of the Otay. The amount of fines in the other smectites is somewhere between that of the Hectorite and the Otay. The effects of sample preparation, quasicrystal structure, chemical composition and the mechanism of formation of different smectite morphologies are largely not understood (Borchardt, 1989). The fine needle-like particles observed in the Hectorite SEM could be sepiolite, which displays lath-like or fibrous forms (Singer, 1989). XRD evidence for sepiolite includes the broad shoulder around 1.2 nm, and a weak reflection at 0.43 nm. The presence of sepiolite in the sample may affect the sorption behavior of Hectorite.

The mechanism for the difference in sorption affinity among smectites is complicated. The morphology of smectite quasicrystals, the distribution of fine and coarse particles and the presence of both organic and inorganic contaminants all may influence sorption affinity. The fabric of smectite particles will influence the distribution of internal and external surfaces, and the size and distribution of micro- and nano-pores within the quasicrystals. The large variation in sorption affinity and desorption characteristics together with the lack of correlation between Kd_{ad} and smectite surface properties suggests that chlorpyrifos is retained by physical interactions rather than chemical sorption.

SEM images of suspended sediment (Fig. 8) illustrate that sediment particles are composed of inorganic and organic materials and biota debris such as diatoms. Obviously, chlorpyrifos sorption by sediment is governed by multiple mechanisms as both clay and organic materials would contribute to sorption-desorption processes.

Conclusions

Chlorpyrifos is a highly toxic insecticide widely used in both agricultural and urban environments. Understanding interaction of chlorpyrifos with colloidal materials in aqueous systems is fundamental for assessing the fate and toxicity of chlorpyrifos in soil and aquatic environments. The present study reveals a large difference in sorption affinity and variation in desorption hysteresis among

smectites. Neither chlorpyrifos sorption nor desorption was correlated with cation-exchange capacity, surface area or surface charge density of the smectites. The fabric of smectite quasicrystals may be related to sorption affinity for chlorpyrifos, suggesting that physical properties of the smectite rather than surface chemistry of smectite controls chlorpyrifos sorption in aqueous systems. Chlorpyrifos was very strongly sorbed on humic acid and was not desorbed into aqueous solution. A large sorption-desorption hysteresis was also found for river sediment. The results suggest that little chlorpyrifos sorbed on humic materials in suspended sediment will be released to aquatic environments while some chlorpyrifos adsorbed on suspended clay minerals may be released to the aqueous systems depending on properties of the clays.

References

- Borchardt, G. 1989. Smectites. In J.B. Dixon and S.B. Weed (eds). Minerals in Soil Environments. 2nd edition. Soil Sci. Soc. Am. Book Series 1.
- Bundy, L.G., and J.M. Bremner. 1972. A simple titrimetric method for determination of inorganic carbon in soils. Soil Sci. Soc. Am. Proc. 36:273-275.
- Economic Research Service (ERS). 1994. Agricultural resources and environmental indicators. U.S. Department of Agriculture, Natural Resources and Environment Division. Agricultural Handbook. 705, 205.

- Felsot, A., and P.A. Dahm. 1979. Sorption of organophosphorus and carbamate insecticides by soil. *J. Agric. Food Chem.* 27:557-563.
- Gast, R.G. 1977. Surface and colloid chemistry. In J.B. Dixon. et al. (eds). Minerals in soil environments. *Soil Sci. Soc. Am. Madison, WI.* 27-73.
- Gee, G.W., and J.W. Bauder. 1986. Particle-size analysis. In A. Klute (ed.) *Methods of Soil Analysis: Part 1, physical and mineralogical methods.* 2nd edition. ASA, Inc. Madison, WI, USA. 383-409.
- Koskinen, W.C., and S.S. Harper. 1990. The retention process: mechanisms. In *Pesticides in the Environment: Processes, Impacts, and Modeling.* SSSA Book Series 2. *Soil Sci. Soc. Am, Inc. Madison, WI, USA.* 51-73.
- Kratzer, C.R. 1998. Pesticide in storm runoff from agricultural and urban areas in the Tuolumne River Basin in the vicinity of Modesto, California. *Water-Resources Investigation Report 98-4017, U.S. Geological Survey.*
- Kunze, G.W., and J.B. Dixon. 1986. Pretreatment for mineralogical analysis. In A. Klute (ed.). *Methods of Soil Analysis: Part 1, physical and mineralogical methods.* 2nd edition. ASA, Inc. Madison, WI, USA. 91-99.
- Laird, D.A., R.H. Dowdy, and R.C. Munter. 1991. Suspension nebulization analysis of clays by inductively coupled plasma-atomic emission spectroscopy. *Soil Sci. Soc. Am. J.* 55:274-278.
- Laird, D.A., E. Barriuso, R.H. Dowdy, and W.C. Koskinen. 1992. Adsorption of atrazine on smectites. *Soil Sci. Soc. Am. J.* 56:62-67.

- MaCalady, D.L., and N.L. Wolfe. 1985. Effects of sediment sorption and abiotic hydrolysis. 1. organophosphorothioate esters. *J. Agric Food Chem.* 33:167-173.
- Moore, D.M., and R.C. Reynolds. 1989. X-ray diffraction and the identification and analysis of clay minerals. Oxford University Press, Oxford, New York. 179-200.
- Racke, K.D. 1993. Environmental fate of chlorpyrifos. *Rev. Environ. Contam. Toxicol.* 131:1-154.
- Sharom, M.S., J.R.W Miles, C.R. Harris, and F.L. McEwen. 1980. Persistence of 12 insecticides in water. *Water Res.* 14:1089-1093.
- Singer, A. 1989. Palygorskite and sepiolite group minerals. In J.B. Dixon and S.B. Weed (eds). *Minerals in Soil Environments*. 2nd edition. Soil Sci. Soc. Am. Book Series 1. Madison, WI, USA.
- Stevenson, F.J. 1994. *Humus chemistry: Genesis, composition, reaction*. John Wiley & Sons. New York.
- Wintersteen, W., and R. Hartzler. 1987. Pesticides used in Iowa crop production in 1985. Iowa Coop. Ext. Serv. Pamph. 1288.

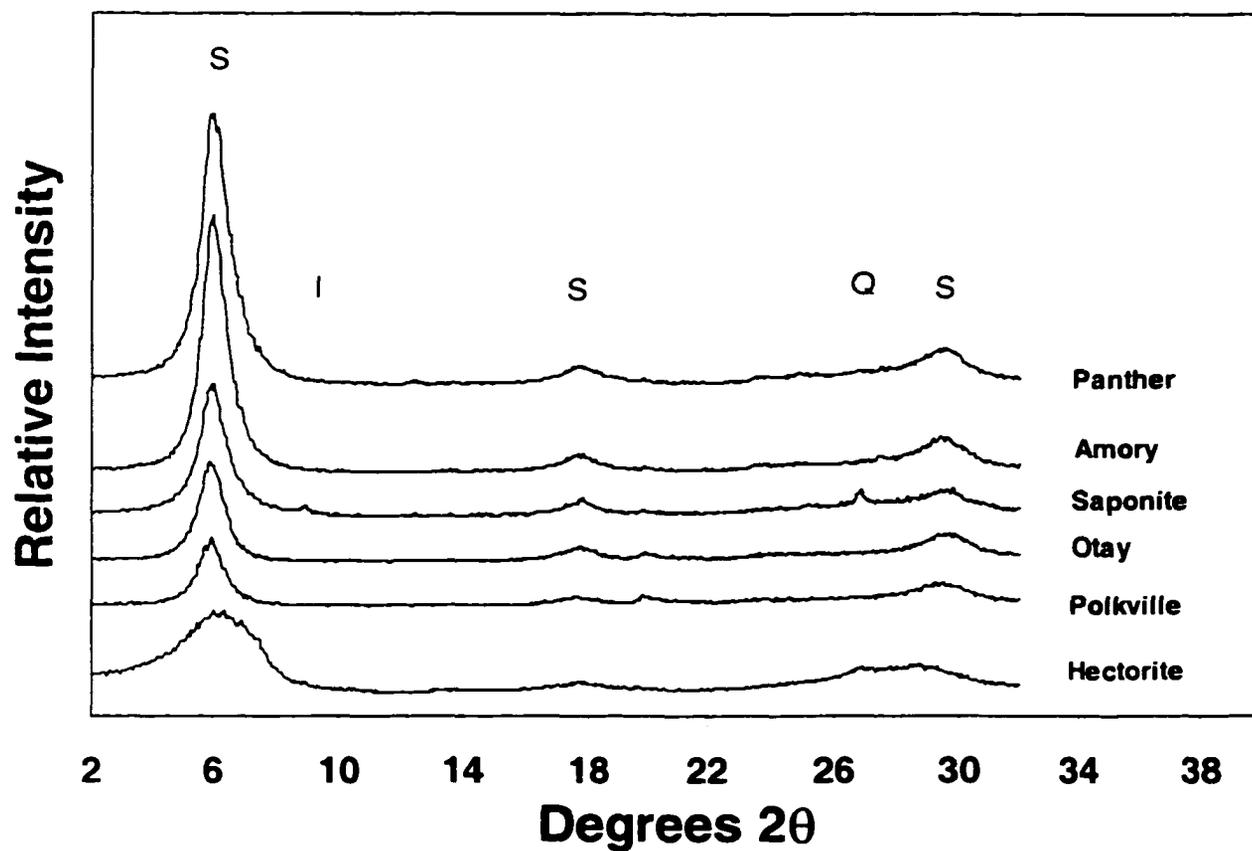


Fig. 1. X-ray diffraction patterns of Ca-saturated reference smectites equilibrated at 54% relative humidity (S: smectite; I: illite; Q: quartz)

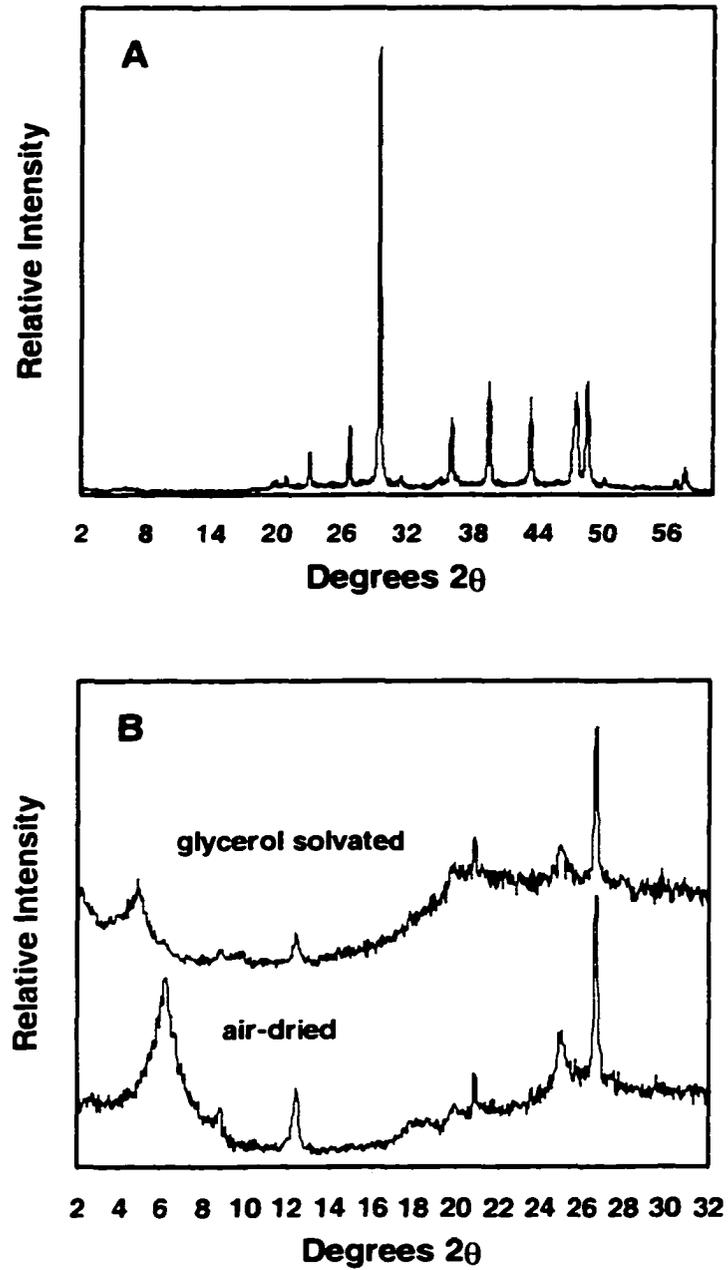


Fig. 2. XRD patterns of suspended sediment

A: whole sediment;

B: clay fraction ($< 2 \mu\text{m}$) of sediment.

Table 1. Total oxide composition of selected smectites

Smectites	SiO ₂	Al ₂ O ₃	CaO	Fe ₂ O ₃	K ₂ O	Li ₂ O	MgO	MnO	TiO ₂	ZnO
g kg ⁻¹										
Hectorite	652.9	21.9	23.4	6.9	5.1	6.7	282.1	0.1	0.8	0
Panther	653.1	210.7	24.9	76.3	5.4	0	21.1	0.1	8.2	0.2
Saponite	650.5	153.6	31.1	19.1	12.0	1.1	130.0	0.6	1.9	0.1
Amory	665.0	213.0	27.3	60.1	3.1	0	23.3	0	2.4	0.2
Polkville	673.2	202.8	33.3	48.1	0.1	0.2	39.6	0	2.1	0.1
Otay	676.7	197.4	35.9	14.3	0	0.5	72.9	0.3	1.9	0.1

Table 2. Structural formulae of reference smectites

Hectorite	[Si _{7.916} Al _{0.084}]	[Al _{0.229} Fe _{0.063} Mg _{5.099} Li _{0.327}]	O ₂₀ (OH) ₄ K _{0.079} Ca _{0.303}
Panther	[Si _{7.867} Al _{0.133}]	[Al _{2.858} Fe _{0.691} Mg _{0.378}]	O ₂₀ (OH) ₄ K _{0.082} Ca _{0.322}
Saponite [*]	[Si _{7.816} Al _{0.184}]	[Al _{1.991} Fe _{0.173} Mg _{2.329} Li _{0.052}]	O ₂₀ (OH) ₄ K _{0.184} Ca _{0.400}
Amory	[Si _{7.949} Al _{0.051}]	[Al _{2.950} Fe _{0.541} Mg _{0.415}]	O ₂₀ (OH) ₄ K _{0.048} Ca _{0.350}
Polkville	[Si _{7.987} Al _{0.013}]	[Al _{2.823} Fe _{0.430} Mg _{0.700}]	O ₂₀ (OH) ₄ K _{0.001} Ca _{0.424}
Otay	[Si _{7.976} Al _{0.024}]	[Al _{2.719} Fe _{0.126} Mg _{1.280}]	O ₂₀ (OH) ₄ Ca _{0.453}

^{*} Saponite contained small amount of quartz, therefore, 2% of quartz was assumed for the calculation of its structural formula.

Table 3. Surface properties of selected reference smectites

Smectites	CEC [†]	SA [‡]	SCD [§]
	cmol kg ⁻¹	m ² g ⁻¹	μmol _c m ⁻²
Hectorite	91.2	766	11.9
Panther	99.0	757	13.1
Saponite	134.0	771	17.4
Amory	102.1	762	13.4
Polkville	117.0	764	15.3
Otay	163.5	773	21.1

[†] CEC: Cation-Exchange Capacity

[‡] SA: Surface Area

[§] SCD: Surface Charge Density

Table 4. Distribution coefficient for sorption and desorption of chlorpyrifos on selected smectites

Smectites	Adsorbed $\mu\text{g g}^{-1}$	$\text{Kd}_{\text{ad}}^{\dagger}$	Desorbed [‡] $\mu\text{g g}^{-1}$	$\text{Kd}_{\text{de}}^{\S}$	Total desorbed %
Hectorite	9.67	1315	0.50	1224-6846	5.2
Panther	9.07	1069	1.15	1046-1796	12.7
Saponite	9.13	1132	0.92	1099-2857	10.1
Amory	8.07	473	1.46	476-1245	18.1
Polkville	4.96	89	3.87	80-105	78.0
Otay	3.07	45	1.98	45-162	64.5

[†] Average Kd_{ad} for the sorption isotherm.

[‡] Total amount desorbed after five repeated equilibrations.

[§] Range in Kd_{de} values for the desorption isotherm

Table 5. Carbon content of reference smectite samples

Smectites	Total C	Organic C	Inorganic C
		g kg⁻¹	
Hectorite	1.5	0.9	0.8
Panther	1.1	0.8	0.3
Saponite	0.7	0.4	0.4
Amory	0.6	0.5	0.3
Polkville	0.4	0.4	0.3
Otay	0.4	0.3	0.2

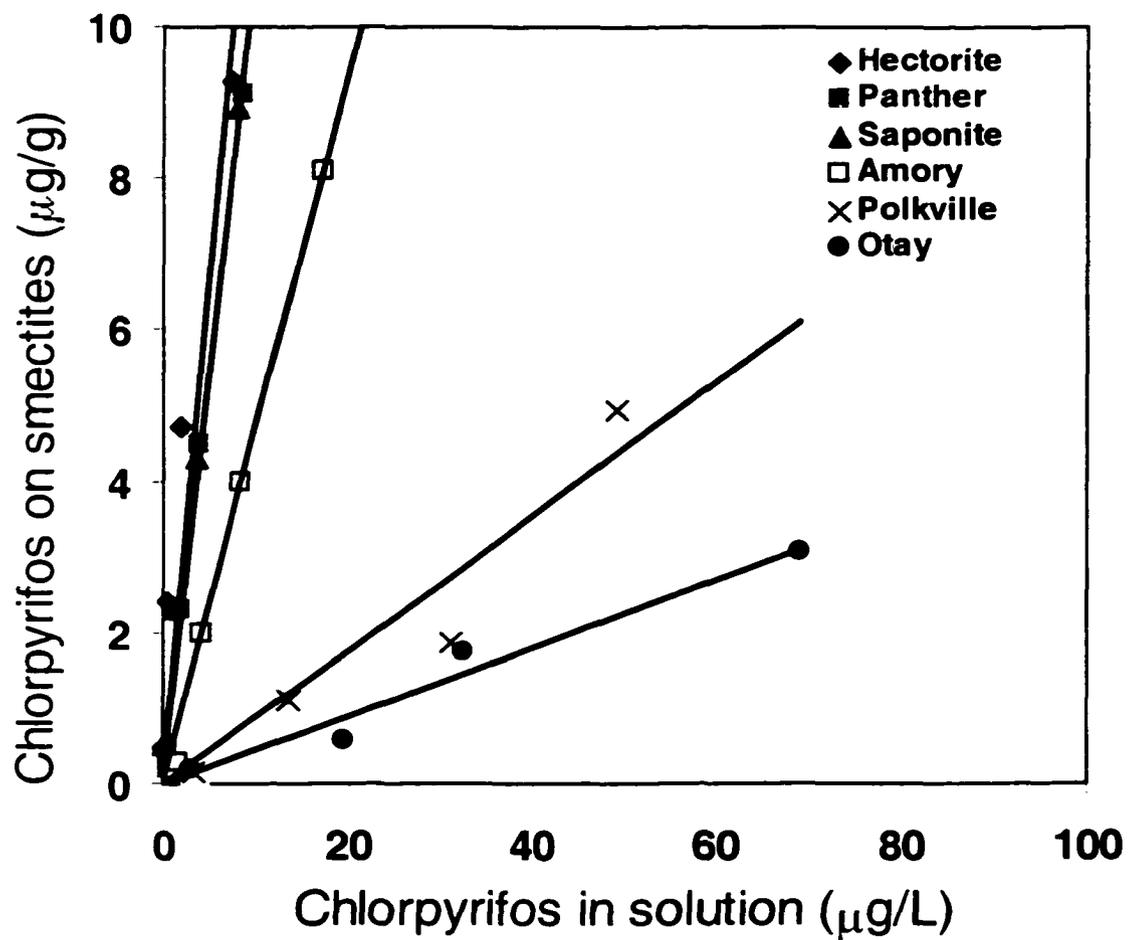


Fig. 3. Isotherms for sorption of chlorpyrifos on smectites

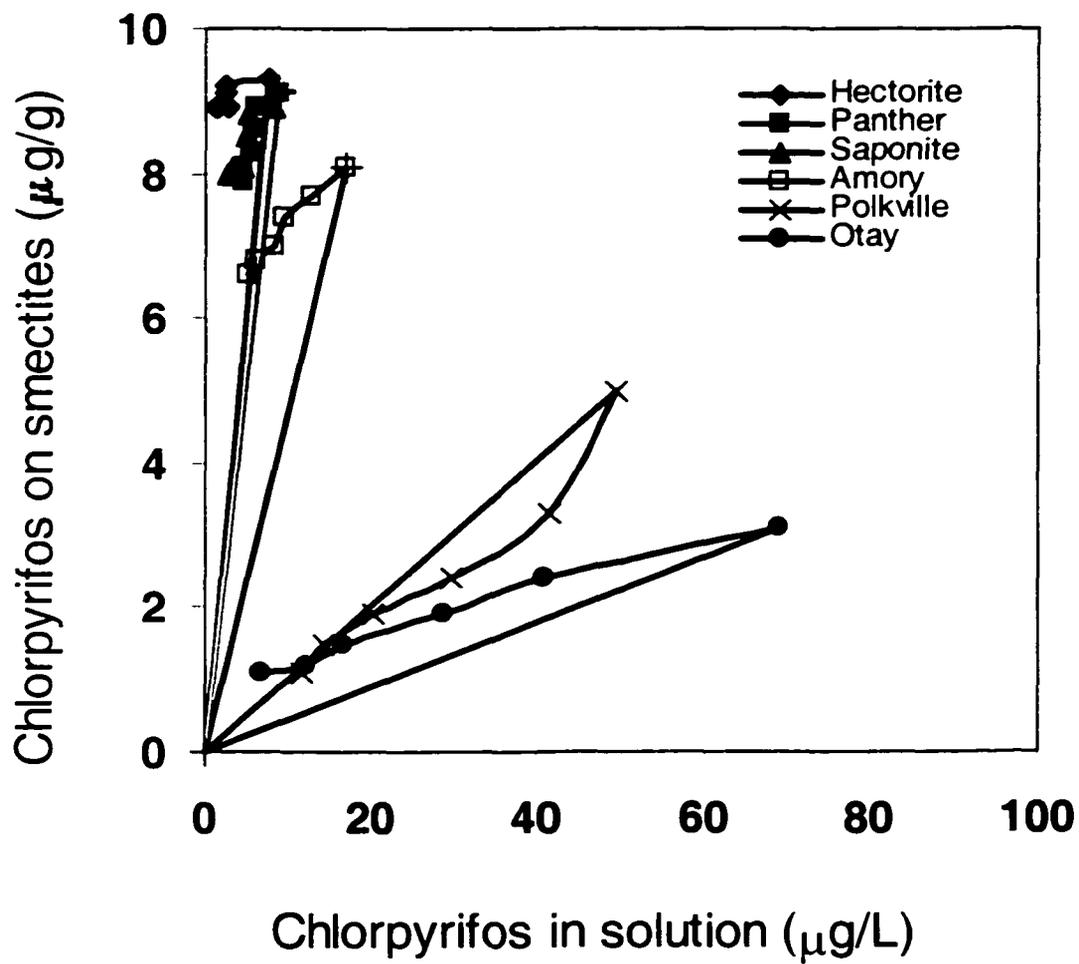


Fig. 4. Isotherms for desorption of chlorpyrifos from smectites

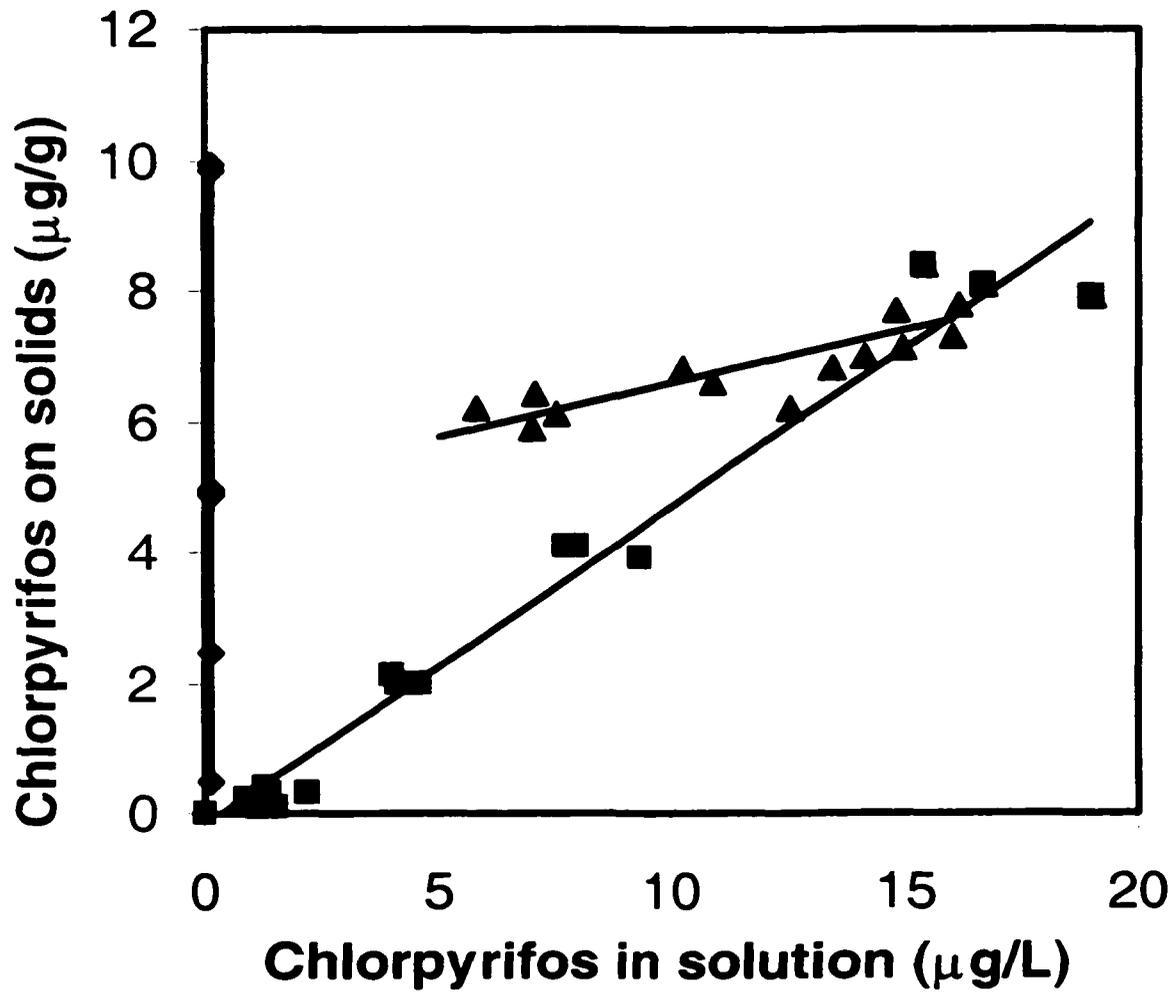


Fig. 5. Isotherms for sorption and desorption of chlorpyrifos on HA and sediment (◆ sorption on humic acid; ■ sorption on sediment; ▲ desorption from sediment).

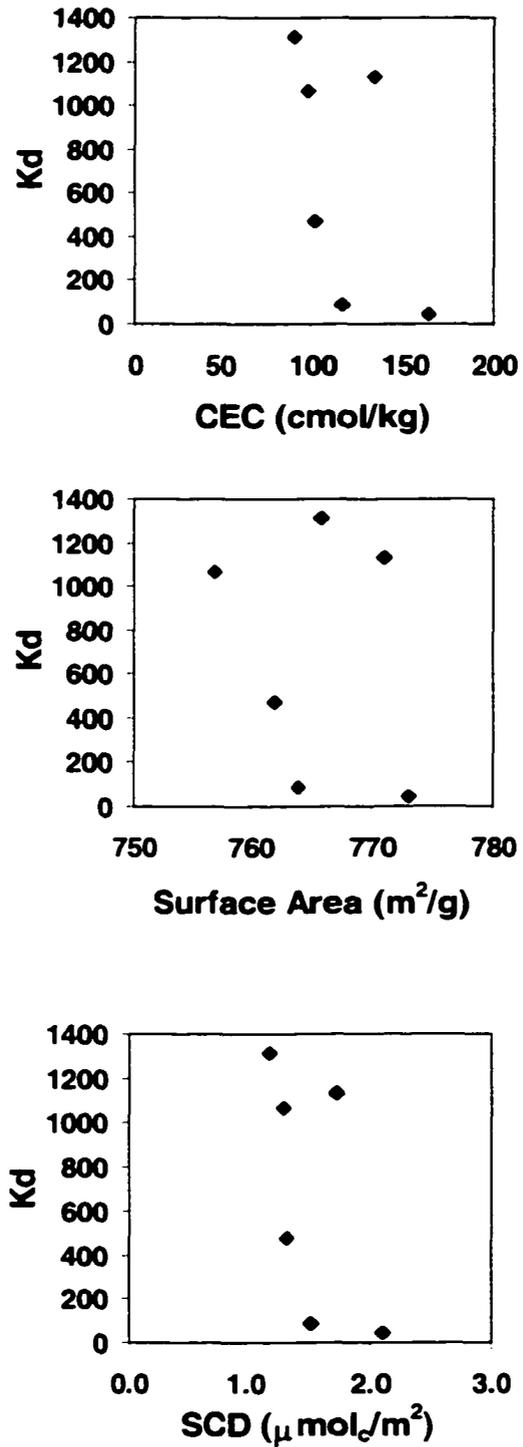


Fig. 6. Relationship between $K_{d_{ad}}$ value and CEC, surface area, surface charge density of smectite



Otay



Amory



Polkville



Saponite



Panther



Hectorite

Fig. 7. SEM images of selected smectites

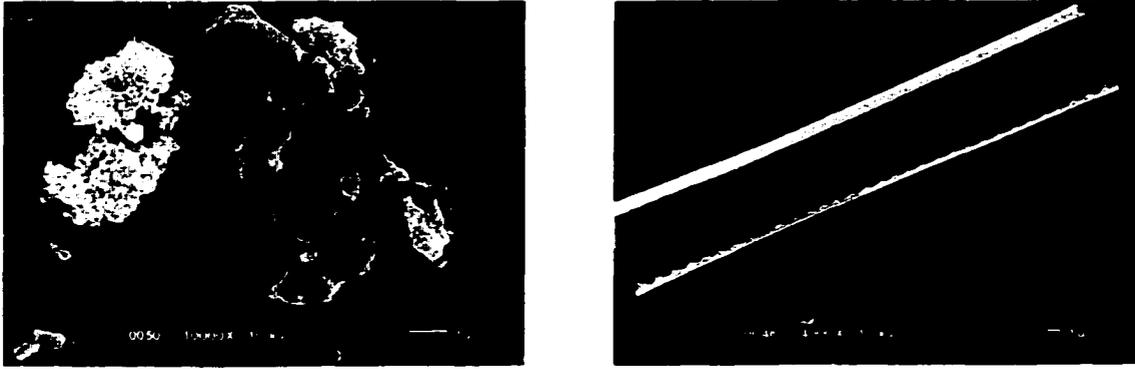


Fig. 8. SEM images of the whole suspended sediment

CHAPTER 3

HYDROLYSIS OF CHLORPYRIFOS IN AQUEOUS SYSTEMS

A paper to be submitted to Journal of Environmental Quality

Jigang Wu and David A. Laird

Abstract

Degradation is one of the most important processes that govern the fate of chlorpyrifos in aquatic environments. The effects of water chemistry, suspended smectites and suspended sediment collected from the Upper Cedar River in North Central Iowa on the rate of chlorpyrifos hydrolysis were investigated. HPLC was employed to determine concentrations of chlorpyrifos and its degradation product, TCP (3,5,6-trichloro-2-pyridinol), in various chlorpyrifos solutions incubated in amber glass bottles at $23 \pm 2^\circ\text{C}$. The results indicate that chlorpyrifos degraded in aqueous solutions with half-lives ranging from 27 to 158 days, depending on the initial concentration of chlorpyrifos and the chemistry of the aqueous solutions. The rate of degradation was slower in the presence of suspended smectites than in the absence of smectites for 0.01 M CaCl_2 solutions, suggesting that sorption of chlorpyrifos on clays inhibited the degradation process. This effect implies that chlorpyrifos sorption by suspended sediment may prolong the potential exposure of aquatic organisms to chlorpyrifos.

Introduction

Chlorpyrifos [o,o-diethyl o-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate] is a widely used organophosphorus insecticide effective against a broad-spectrum of insect pests of important crops (Giesy et al., 1999). Both biological and chemical degradation are important processes that govern the fate of chlorpyrifos in soil and aquatic environments.

Chlorpyrifos is characterized by the same P-O-C linkage as in diazinon (diethyl 2-isopropyl-4-methyl-6-pyrimidyl thionophosphate), parathion (diethyl 4-nitrophenyl thionophosphate) and methyl parathion (o,o-dimethyl o,p-nitrophenyl phosphorothioate). But unlike diazinon (Sethunathan and Pathak, 1972), parathion (Sethunathan and Yoshida, 1973) and methyl parathion (Misra et al., 1992), chlorpyrifos is not affected by accelerated biodegradation in retreated soils. Therefore, efforts to isolate chlorpyrifos-degrading microorganisms from chlorpyrifos-acclimatized soils have not been successful (Racke et al., 1990). Racke and Coats (1987) found that chlorpyrifos was neither metabolized nor cometabolized by an *Arthrobacter* sp., which could hydrolyze a variety of other organophosphorus compounds such as methyl parathion, parathion, fenitrothion (o,o-dimethyl o-4-nitro-m-tolyl phosphorothioate), etc. Few microorganisms isolated from environmental matrices have been unequivocally demonstrated to be capable of degrading chlorpyrifos. Microbial enzymes, however, have been shown to hydrolyze chlorpyrifos under controlled

conditions (Munnecke and Hsieh, 1975). Nevertheless, Racke (1993) stated that soil microorganisms are likely very important in the complete mineralization of the major chlorpyrifos metabolites such as TCP and TMP (trichloro-2-methoxy pyridine).

Susceptibility to hydrolytic degradation is one of the distinguishing features of organophosphate insecticides and is related to their mode of action in cholinesterase enzyme inhibition via phosphorylation (Racke, 1993). Most organophosphorus pesticides will generally undergo rapid chemical hydrolysis. It is well documented that pH has a profound effect on the degradation of chlorpyrifos in water. Several reports (Meikle and Youngson, 1978; Chapman and Cole, 1982) have indicated more rapid hydrolysis under alkaline conditions (pH > 7.5-8.0). At near neutral pH (~7) and ambient temperature (~25°C), chlorpyrifos is moderately stable, with reported hydrolysis half-lives from 29 to 35 days (Meikle and Youngson, 1978). Temperature has also been found to significantly influence chlorpyrifos hydrolysis (Cink and Coats, 1993). Meikle and Youngson (1978) reported that the rate of hydrolysis increased an average of 3.5-fold for each 10°C rise in temperature. Some cations present in the aqueous solutions also substantially catalyzed cleavage of the P-O bond. Mortland and Raman (1967) reported that the aqueous hydrolysis of chlorpyrifos is catalyzed by dissolved Cu ions. Clays have been found to catalyze degradation of a number of pesticides in the environment (Theng, 1974). However, clays are

reported to both promote and inhibit the degradation of pesticides in aqueous systems (Mortland and Raman, 1967; Konrad et al., 1967, 1969).

The objective of this study was to investigate chemical hydrolysis of chlorpyrifos in aqueous systems and to examine the effects of water chemistry and presences of suspended smectites and suspended sediment on the degradation of chlorpyrifos in aqueous systems.

Materials and Methods

Chemicals

Reagent grade chlorpyrifos and TCP were obtained from Chem Service (West Chester, PA) with purities of 99.6% and 98.7%, respectively. Ethanol (100%) was used to help dissolve chlorpyrifos for preparation of chlorpyrifos stock solutions. Milli-Q water (Milli-Q plus system, Millipore, Bedford, MA) with a resistivity of 18.2 M Ω -cm was used for preparation of all solutions and suspensions.

Sample Preparation

Otay white montmorillonite (Otay), Panther Creek beidellite (Panther) and suspended sediment collected from the Upper Cedar River in North Central Iowa were used for this study. Otay was collected at an exposure near San Diego, CA. Panther was acquired from the A.D. Scott collection, Iowa State University, Ames, IA. The clay fraction (< 2 μ m) of the two smectites (Otay and Panther)

was prepared by sedimentation from bulk ore. The clays were Ca-saturated by washing 4 times with 1.0 M CaCl₂. The Ca-saturated samples were dialyzed using Spectra/Por 3500 MWCO molecularporous membrane tubing against Milli-Q water until the conductivity of dialysate was < 0.2 mS/m. The Ca-saturated samples were then freeze-dried.

A large volume of river water (~950 L) containing suspended sediment was collected from the Upper Cedar River near Janesville, Iowa on October 16, 1997. The suspended sediment was separated from the river water by freezing-induced flocculation, thawing and sedimentation. The concentrated suspended sediment sample was then freeze-dried. No chemical treatments were used to prepare the dried suspended sediment sample. Natural river water from the Upper Cedar River near Janesville, Iowa was collected on December 14, 1999. The river water used for an incubation experiment was filtered using Whatman 0.2 μm membrane filter (Whatman, Maidstone, England) to remove suspended particles.

Incubation of Chlorpyrifos

Milli-Q water, 0.01M CaCl₂ solution and natural river water were used to investigate degradation of chlorpyrifos in those water systems. An aliquot of chlorpyrifos stock solution was added to Milli-Q water, 0.01M CaCl₂ solution and river water, respectively, to make a large volume (8L) of various chlorpyrifos aqueous solutions. The low and high initial concentrations of chlorpyrifos were 0.257 and 1.283 μmol L⁻¹, respectively. The aqueous solutions contained 0.25%

ethanol which was necessary to help dissolve chlorpyrifos in water. The pH of aqueous solution was measured using Orion Research Analog pH meter (model 301). Amber glass bottles (500 mL) were used for incubations of chlorpyrifos at $23\pm 2^{\circ}\text{C}$. Each amber glass bottle contained 450 mL of chlorpyrifos aqueous solution. The whole solution in each bottle was sacrificed at different time intervals for analysis of chlorpyrifos and its degradation products. Analytes were extracted from the aqueous solutions into tetrahydrofuran (THF) using solid phase extraction technique with a Varian (Harbor City, CA) Bond Elut LRC[®] C-18 cartridge. Prior to extraction, the aqueous solutions were adjusted to pH 3.0 using HPLC grade H_3PO_4 .

Panther, Otay and the suspended sediment samples were used to investigate the effects of suspended colloids on the degradation of chlorpyrifos in aqueous systems. Duplicate 0.1 g samples were dispersed in 100 mL 0.01M CaCl_2 containing $0.442 \mu\text{mol L}^{-1}$ chlorpyrifos and incubated in glass amber bottles at $23\pm 2^{\circ}\text{C}$. The pH of Panther, Otay, and sediment suspensions was measured using Orion Research Analog pH meter (model 301). The suspensions were periodically shaken using a laboratory shaker. At various time intervals during the incubations whole bottles were sacrificed and the aqueous phase was analyzed for chlorpyrifos and its degradation products. For each sample, the suspension was filtered using Anodisc 0.02 μm filter membrane (Whatman, Maidstone, England) to separate solid from aqueous phases. The analytes in the

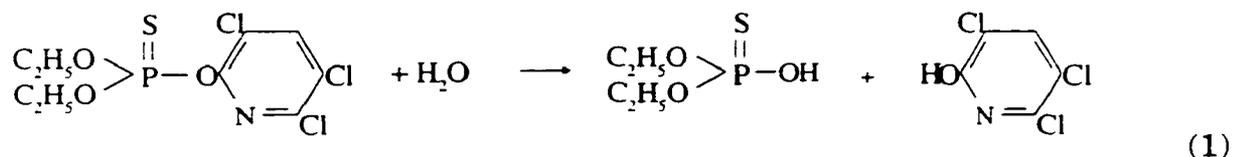
aqueous phase were then extracted into THF using solid phase extraction cartridge as described above and analyzed by HPLC.

HPLC Analysis

Chlorpyrifos and its degradation products were determined using HPLC. The analytes were separated using a reversed-phase SUPELCOSIL™ LC-18 column (15.0 cm × 4.6 mm ID) from Supelco (Bellefonte, PA). The mobile phase was HPLC grade acetonitrile and acidified water (pH was adjusted to 3.0 using HPLC grade H₃PO₄). A gradient program started at 10% acetonitrile + 90% water and increased linearly to 90% acetonitrile + 10% water over 20 min, held for 5 min and returned to the initial gradient state in 5 min. A 50 µL sample injection loop was used and the flow rate was set to 1.0 mL/min. The analytes were detected with a Hewlett Packard (series 1100) Diode array UV-Vis detector. Under the above conditions, retention times were 19.8 min for chlorpyrifos and 12.1 min for TCP. Absorbance was measured continuously in the range of 190 ~ 400 nm. Chlorpyrifos and TCP were quantified at 230 and 302 nm, respectively. Linear calibration curves with correlation coefficients (r^2) of 0.9999 were achieved for chlorpyrifos and TCP over the concentration ranges of 0~0.5 mmol L⁻¹ and 0~0.25 mmol L⁻¹, respectively.

Results and Discussion

A general hydrolytic reaction of chlorpyrifos in water involves cleavage of the phosphorothioate ester bond to form TCP as shown in equation (1).



By the law of mass action and assuming first-order kinetics, the reaction rate would be described as equation (2):

$$-\frac{dC_t}{dt} = K C_t \quad (2)$$

where C_t is the concentration of chlorpyrifos at time t .

Integrating equation (2) yields:

$$\log C_t = \log C^0 - \frac{K}{2.303} t \quad (3)$$

where C_0 is the concentration of chlorpyrifos at time zero. Fig. 1 shows the relationship between $\log C_t$ and time (t) for Milli-Q water, 0.01M CaCl_2 and natural river water. The linear relationships imply that the degradation of chlorpyrifos fits fairly well in first-order kinetics. Thus, first-order rate constants are listed in Table 1. It appears that CaCl_2 had little effect on hydrolysis when the initial concentration of chlorpyrifos was low. However, at high initial concentrations, the half-life ($t_{1/2}$) for chlorpyrifos in Milli-Q water was 2.6 times longer compared with chlorpyrifos in 0.01M CaCl_2 . Mortland and

Raman (1967) reported that the hydrolysis of chlorpyrifos may be catalyzed by dissolved Cu ions in aqueous systems. They observed nearly 100% hydrolysis of chlorpyrifos (2.8 mg L^{-1}) within 24 hr in aqueous methanol (50%) containing 0.1 mM Cu^{2+} . Limited hydrolysis (10% in 24 hours) occurred in the presence of 0.1 mM MgCl_2 , and negligible hydrolysis was observed in the presence of CaCl_2 . The present results indicate that such influence is also related to the concentration of chlorpyrifos present in aqueous system.

The natural river water contained Ca and several other metals including Al, Mg, Fe, Mn, Na, as detected by a quick scan using ICP-AES (inductively coupled plasma-atomic emission spectroscopy). The effects of various metals in the river water on the rate of chlorpyrifos hydrolysis were certainly different. The pH values of Milli-Q water, 0.01 M CaCl_2 and natural river water were 6.50, 6.30, 8.05 respectively. The pH of river water was obviously higher than Milli-Q water and 0.01 M CaCl_2 solution. The overall effect of the river water (pH and metals) was apparently to promote hydrolysis. The half-lives of chlorpyrifos decreased by 17.6 days for low initial concentration of chlorpyrifos and 130.4 days for high initial concentrations of chlorpyrifos in river water compared to Milli-Q water.

The disappearance of chlorpyrifos from the aqueous phase in the clay-water systems is illustrated in Fig. 2. Chlorpyrifos disappeared relatively slower from the aqueous phase in the clay-water systems than in 0.01 M CaCl_2 . In the sediment-water system, chlorpyrifos disappeared from the aqueous phase

rapidly during 0 to 50 days of incubation, and approached a stable concentration after 50 days. The measured $t_{1/2}$ for chlorpyrifos in the Otay, Panther, and 0.01M CaCl_2 systems over 100 days of incubation are 115.5, 126.0, and 57.8 days, respectively. The $t_{1/2}$ for the suspended sediment system over 50 days of incubation is 27.6 days. It seems that sorption of chlorpyrifos by the two smectites inhibited hydrolysis of chlorpyrifos in clay-water systems as compared with 0.01M CaCl_2 aqueous system. Microbial activity would be minimized for the two smectite systems due to a lack of a carbon source and other nutrients under the experimental conditions, and any hydrolysis observed would be abiotic in nature. The pH values in Panther, Otay, and sediment suspensions were measured to be 5.46, 5.75, and 8.02 respectively. The higher pH in the sediment suspension due to substantial amount of carbonates present may facilitate alkaline hydrolysis of chlorpyrifos. However, in addition to the high pH effect, microorganisms that existed in the suspended sediment may have contributed to the fast degradation of chlorpyrifos in sediment-water system.

The same amount of chlorpyrifos was added into the clay-water system initially. The Panther Creek beidellite has a greater sorption affinity for chlorpyrifos than the Otay white montmorillonite (Chapter 2). The $t_{1/2}$ of the Panther-water system is slightly longer than the $t_{1/2}$ for the Otay-water system. The slower rate of hydrolysis in clay-water systems suggests that the clay may act as a buffer slowly releasing chlorpyrifos to the aqueous phase.

Chlorpyrifos possesses three ester bonds that are candidates for hydrolytic cleavage: two tertiary alkyl ester bonds and one phosphate ester (pyridyl) bond. Four hydrolysis products of chlorpyrifos have been reported in the literature, and their presence indicates that all three ester bonds in the molecule may be hydrolyzed (Racke, 1993). TCP, a major chlorpyrifos degradation product in aqueous solutions was measured at different time intervals of incubation (Fig. 3 and Fig. 4). TCP clearly appeared in the aqueous systems but could not account for all of disappearance of chlorpyrifos in the aqueous solutions over the time. The loss of chlorpyrifos due to volatilization from the aqueous solution was minimized in the cap-covered amber glass bottle. An unidentified peak was noted in the HPLC chromatograms, however no attempt was made for this study to identify and quantify other compounds that may have been in the solutions as a result of hydrolysis.

TCP does not cause cholinesterase inhibition and is of low to moderate toxicity to aquatic and terrestrial biota (Barron and Woodburn, 1995). Fig. 3 shows that TCP was relatively stable in the amber glass bottles in our experimental conditions, which is consistent with the findings that TCP is degraded in the environment via photolysis (Dilling et al., 1984) and microbial degradation (Bidlack, 1976). Natural river water may contain microorganisms in the aqueous systems. As evident in Fig. 3, TCP was not detectable until on day 45 at low initial concentration of chlorpyrifos and day 20 at high initial concentration in the river water system. At the same time of incubation, TCP

already appeared in both Milli-Q water and 0.01M CaCl₂ systems. It is possible that TCP transformed from chlorpyrifos was further degraded by microbial degradation in the river water system. However, it is unlikely that microorganism would exist in Milli-Q water and 0.01 M CaCl₂ solution. Therefore, TCP was detected earlier in those aqueous systems. Both photolysis and microbial degradation were minimized in the aqueous solutions used in the present study, however, TCP will most likely undergo further biotic and abiotic degradation in aquatic environments.

Conclusions

Chlorpyrifos undergoes a rapid chemical hydrolysis in aqueous systems. The rate of hydrolysis is affected by the water chemistry and colloids present in the systems. The presence of suspended smectites reduced the rate of chlorpyrifos hydrolysis in aqueous systems, suggesting sorption of chlorpyrifos by colloidal materials in aquatic environments may inhibit hydrolysis and prolong potential exposure time to aquatic organisms such as fish to chlorpyrifos.

References

- Barron, M.G., and K.B. Woodburn. 1995. Ecotoxicology of chlorpyrifos. *Rev. Environ. Contam. Toxicol.* 144:1-93.

- Bidlack, H.D. 1976. Degradation of ^{14}C -labeled 3, 5, 6-trichloro-2-pyridinol in 15 select agricultural soils. Report GH-C 953, DowElanco. Indianapolis, IN.
- Chapman, R.A., and C.M. Cole. 1982. Observations on the influence of water and soil pH on the persistence of insecticides. *J. Environ Sci Health B.* 15:39-46.
- Cink, J.H., and J.R. Coats. 1993. The effect of concentration, temperature, and soil moisture on the degradation of chlorpyrifos (Dursban TC) in an urban Iowa soil. In K.D. Racke and A.R. Leslie (eds). *Pesticides in urban environments: Fate and significance.* ACS. Washington DC. ACS Symp. Series 522:62-69.
- Dilling, W.L., L.C. Lickly, T.D. Lickly, P.G. Murphy, and R.L. McKellar. 1984. Organic photochemistry: Quantum yields for o,o-diethyl o-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate (chlorpyrifos) and 3, 5, 6-trichloro-2-pyridinol in dilute aqueous solutions and their environmental phototransformation rates. *Environ. Sci. Technol.* 18:540-543.
- Giesy, J.P., K.R. Solomon, J.R. Coats, K.R. Dixon, J.M. Giddings, and E.E. Kenaga. 1999. Chlorpyrifos: ecological risk assessment in North American aquatic environments. *Rev. Environ. Contam. Toxicol.* 160:1-129.
- Konrad, J.G., D.E. Armstrong, and G. Chesters. 1967. Soil degradation of diazinon. *Agron. J.* 59:591-594.
- Konrad, J.G., G. Chesters, and D.C. Armstrong. 1969. Soil degradation of malathion. *Soil Sci. Soc. Am. Proc.* 33:259-262.

- Meikle, R.W., and C.R. Youngson. 1978. The hydrolysis rate of chlorpyrifos, o-o-diethyl-o-(3,5,6-trichloro-2-pyridyl) phosphorothioate, and its dimethyl analog, chlorpyrifos-methyl, in dilute aqueous solution. *Arch. Environ. Contam. Toxicol.* 7:13-22.
- Misra, D. S., T.K. Adhya, and N. Sethunathan. 1992. Accelerated degradation of methyl parathion, parathion and fenitrothion by suspensions from methyl parathion- and p-nitrophenol-treated soils. *Soil Biol. Biochem.* 24:1035-1042.
- Mortland, M.M., and K.V. Raman. 1967. Catalytic hydrolysis of some organic phosphate pesticides by copper (II). *J. Agric. Food Chem.* 15:163-167.
- Munnecke, D.M., and D.P.H. Hsieh. 1975. Development of microbial systems for the disposal of concentrated pesticide suspensions. *Med Facul Landbouww Rijksuniv Gent.* 40:1237-1247.
- Racke, K.D. 1993. Environmental fate of chlorpyrifos. *Rev. Environ. Contam. Toxicol.* 131:1-154.
- Racke, K.D., and J.R. Coats. 1987. Comparative degradation of organophosphorus insecticides in soil: specialty of enhanced microbial degradation. *J. Agric. Food Chem.* 36:193-199.
- Racke, K.D., D.A. Laskowski, and M.R. Schultz. 1990. Resistance of chlorpyrifos to enhanced biodegradation in soil. *J. Agric. Food Chem.* 38:1430-1436.
- Sethunathan, N., and M.D. Pathak. 1972. Increased biological hydrolysis of diazinon after repeated application in rice paddies. *J. Agric. Food Chem.*

20:586-589.

Sethunathan N, and T. Yoshida. 1973. A *Flavobacterium* sp. that degrades diazinon and parathion. *Can. J. Microbiol.* 19:873-875.

Theng, B.K.G. 1974. *The chemistry of clay-organic reactions.* John Wiley & Sons, New York.

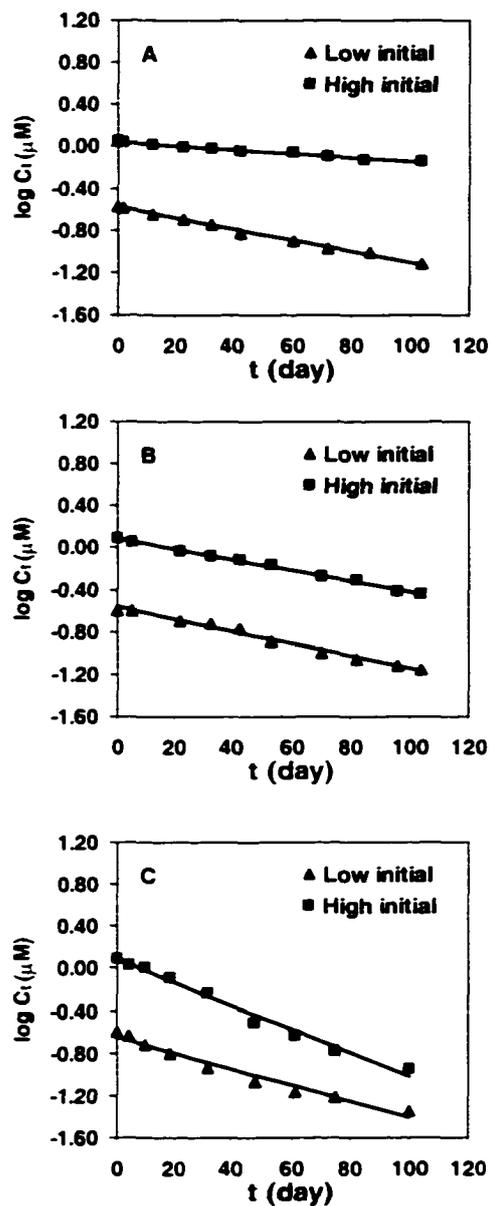


Fig. 1. First-order plots of hydrolysis of chlorpyrifos in aqueous systems (A: Milli-Q H_2O ; B: 0.01M CaCl_2 ; C: river water. Low and high initial concentrations of chlorpyrifos are 0.257 and $1.283 \mu\text{mol L}^{-1}$, respectively.)

Table 1. Kinetic parameters of chlorpyrifos hydrolysis in aqueous systems

Waters	Low initial		High initial	
	K (day ⁻¹)	t _½ (days)	K (day ⁻¹)	t _½ (days)
Milli-Q water	0.0122	56.8	0.0044	157.5
0.01M CaCl ₂	0.0131	52.9	0.0115	60.3
River water	0.0177	39.2	0.0256	27.1

Table 2. Kinetic parameters of chlorpyrifos hydrolysis in colloidal systems

Treatments	K	t _½
	day ⁻¹	days
Otay-system	0.0060	115.5
Panther-system	0.0055	126.0
Sediment-system	0.0251	27.6
0.01M CaCl ₂	0.0120	57.8

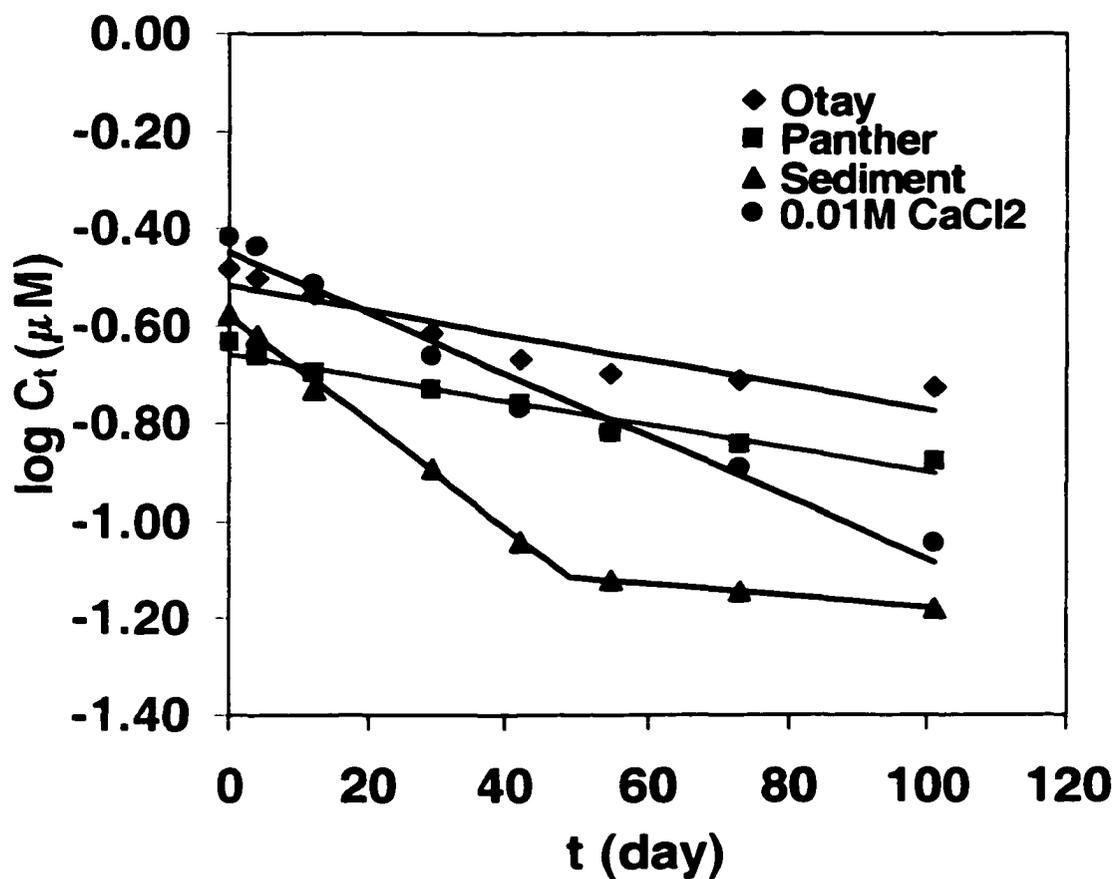


Fig. 2. First-order plots of degradation of chlorpyrifos in aqueous and colloidal systems. The same amount of chlorpyrifos was present in all systems at time zero. Difference relative to the control (0.01 M CaCl₂) in aqueous concentrations of chlorpyrifos at time zero is due to sorption by Panther, Otay and sediment.

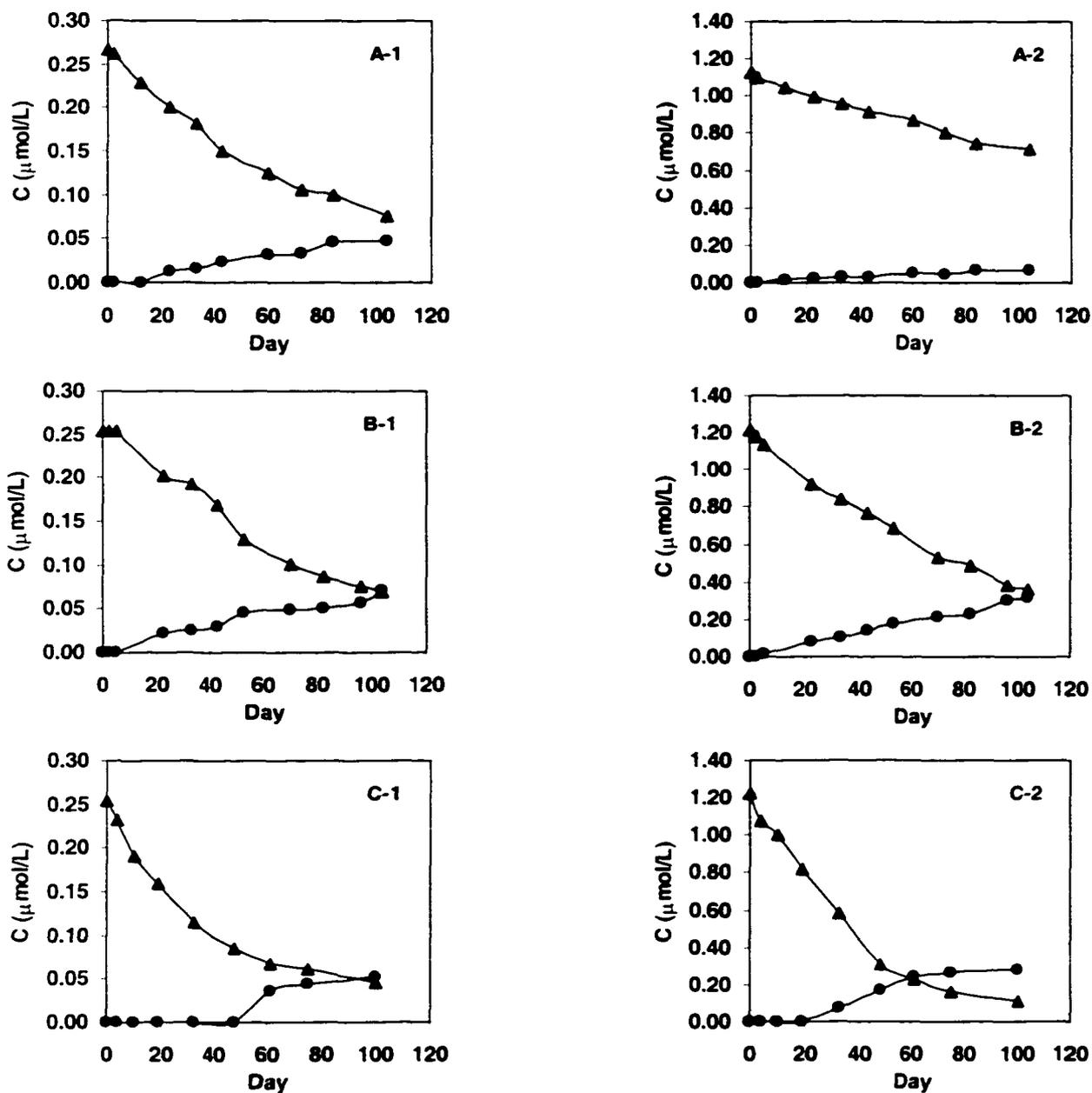


Fig. 3. Disappearance of chlorpyrifos (▲) and appearance of TCP (●) in aqueous solutions as a function of time (A-1: low initial conc. in Milli-Q H_2O ; A-2: high initial conc. in Milli-Q H_2O ; B-1: low initial conc. in 0.01M CaCl_2 ; B-2: high initial conc. in 0.01M CaCl_2 ; C-1: low initial conc. in river water; C-2: high initial conc. in river water)

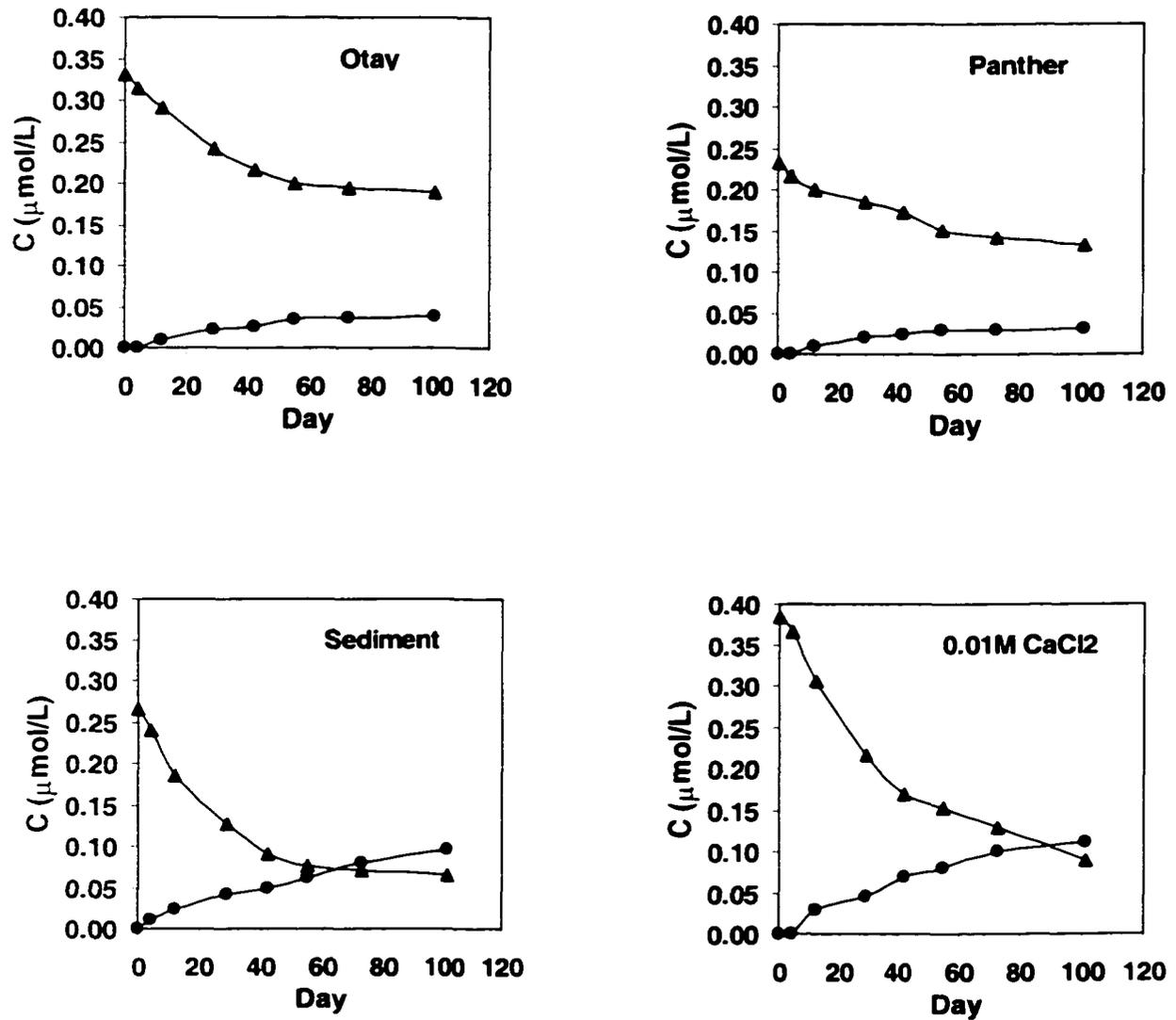


Fig. 4. Disappearance of chlorpyrifos (▲) and appearance of TCP (●) in aqueous and colloidal systems as a function of time. Difference relative to the control (0.01M CaCl₂) in initial concentrations of chlorpyrifos at time zero is due to sorption by Panther, Otay and sediment.

CHAPTER 4

FATE OF CHLORPYRIFOS SORBED ON COLLOIDAL MATERIALS IN AQUEOUS SYSTEMS

A paper to be submitted to Journal of Environmental Quality

Jigang Wu and David A. Laird

Abstract

The fate of chlorpyrifos sorbed on colloidal materials may influence its potential toxicity to organisms in aquatic environments. The objective of this study was to determine whether the degradation of chlorpyrifos is catalyzed by sorption on colloidal materials in aqueous systems. Chlorpyrifos was incubated with smectite, humic acid and Cedar River suspended sediment samples in 0.01M CaCl₂ at 23±2°C for 30 days. Chlorpyrifos and its primary degradate, TCP (3,5,6-trichloro-2-pyridinol) in both the aqueous phase and solid phase were quantified after 1, 5, 10, 15, 20, 25, and 30 days of incubation using high performance liquid chromatography (HPLC). The results indicate negligible degradation of chlorpyrifos to TCP in colloid-water systems. Total recoveries of sorbed chlorpyrifos ranged from 78% to 97% with a tendency to decrease with incubation time. The study indicates that colloidal materials are not effective catalysts for the degradation of chlorpyrifos in aqueous systems. On the contrary, the formation of chlorpyrifos-colloid complexes may inhibit the hydrolysis process and thereby preserve chlorpyrifos in the aqueous systems.

The results suggest sorption of chlorpyrifos on suspended sediment may prolong the exposure time of organisms to chlorpyrifos in aquatic environments.

Introduction

Chlorpyrifos [o,o-diethyl o-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate] has been widely used in agricultural and urban environments for many years (Giesy et al., 1999). It is estimated that more than 3 million kg of chlorpyrifos are applied to field crops in the USA annually (ERS, 1994). Most of the chlorpyrifos applied in agricultural fields will degrade and disappear in the environment, however, some chlorpyrifos residues may be retained by soil constituents for an extended period of time (Racke, 1993).

A variety of organic molecules including pesticides react with clay minerals and organic matter in soils (Theng, 1974; Stevenson, 1994). Surface acidity of soil minerals catalyzes protonation, hydrolysis and other transformation reactions (McBride, 1994). The protonation of atrazine (with a pKa of 1.68) is known to occur on montmorillonite (Russell, 1968). Aluminosilicate minerals have also been observed to catalyze a number of hydrolysis reactions. Surface-catalyzed parathion (o,o-diethyl-o-p-nitrophenyl phosphorothioate) hydrolysis, for example, has been observed following addition of kaolinite and soil samples (Saltzman et al., 1974, 1976; Yaron, 1975; Mingelgrin and Saltzman, 1979). Sanchez-Camazano and Sanchez-Martin (1991) reported that adsorption of

azinphosmethyl on clay surface, especially adsorption into the interlayer space, may be involved in the hydrolysis process.

Chlorpyrifos degradation and transformation in aqueous systems have been extensively studied (Racke, 1993; Barron and Woodburn 1995; Giesy et al., 1999). It is well documented that chemical hydrolysis is a major pathway for chlorpyrifos degradation. Surface heterogeneous catalysis of chlorpyrifos was reported in soil. The mechanism for heterogeneous surface-catalyzed hydrolysis in soil is thought to be related to the activity within the film of hydration water (≥ 1 nm) that is associated with the surface of clay minerals and their surface counter-ions (e.g. Ca^{2+}) under both moist and air-dry conditions (Mingelgrin et al., 1977; Yaron, 1975). Camazano and Martin (1983) theorized that the clay-organophosphorus insecticide interaction that occurs in this zone may enhance the electrophilic nature of the P atom of organophosphates, thus facilitating nucleophilic attack by hydroxide ions. On the contrary, it has also been reported that chlorpyrifos sorption on soil inhibits hydrolytic degradation (Macalady and Wolfe, 1985). Racke et al. (1992) examined the hydrolysis of chlorpyrifos to TCP in moist, sterilized soil samples, and reported that under acidic to neutral pH conditions the presence of soil appears to retard hydrolysis compared to that which occurs in water of a similar pH. To date, however, relatively little is known about the fate of the chlorpyrifos sorbed by colloidal materials in aquatic environments.

A study of the fate of chlorpyrifos sorbed on colloidal materials in aqueous systems is essential to better understand the potential toxicity to aquatic organisms of chlorpyrifos sorbed on suspended sediment. The objective of this study was to determine whether the degradation of chlorpyrifos is catalyzed by sorption on colloidal materials in aqueous systems.

Materials and Methods

Otay white montmorillonite (Otay), Panther Creek beidellite (Panther), humic acid (HA), and suspended sediment (Sediment) collected from the Upper Cedar River, Iowa were used for this study. Otay was collected at an exposure near San Diego, CA. Panther was acquired from the A.D. Scott collection, Iowa State University, Ames, IA. The clay fraction ($< 2 \mu\text{m}$) of the two smectites (Otay and Panther) was prepared by sedimentation from bulk ore. HA was obtained from Aldrich Chemical Company (Milwaukee, WI). The clays and HA were Ca-saturated by washing 4 times with 1.0 M CaCl_2 . The Ca-saturated samples were dialyzed using Spectra/Por 3500 MWCO molecularporous membrane tubing against Milli-Q water until the conductivity of the dialysate was $< 0.2 \text{ mS/m}$. The Ca-saturated samples were then freeze-dried. A large volume of river water ($\sim 950 \text{ L}$) containing suspended sediment was collected from the Upper Cedar River near Janesville, Iowa on October 16, 1997. Suspended sediment was separated from the river water by freezing-induced

flocculation, thawing and sedimentation. The concentrated suspended sediment sample was then freeze-dried. No chemical treatments were used to prepare the dried suspended sediment sample.

Samples of 0.2 g (actual weight measured to 4 decimal points) of colloidal materials (Otay, Panther, HA and Sediment) were equilibrated with solutions containing 9 mL of 0.01M CaCl₂ and 1 mL of 107 µg mL⁻¹ chlorpyrifos (in ethanol) in Corex glass centrifuge tubes. The suspensions were periodically shaken and stored at 23±2°C. Samples were sacrificed at certain time intervals during 30 days of incubation for analysis of chlorpyrifos and its major degradate, TCP, in both the aqueous and solid phases. For each sample, the aqueous and solid phases were separated by centrifugation (×17210 g) for 30 minutes using a Du Pont Sorvall® RC 28S centrifuge (Wilmington, DE). The analytes in the aqueous solutions were extracted into tetrahydrofuran (THF) using a solid phase extraction technique with a Varian (Harbor City, CA) Bond Elut LRC® C-18 cartridge. For the solid phase remaining in the centrifuge tube, 10-mL of THF was added to the tube and shaken for 2 hours to extract the sorbed chlorpyrifos and degradates. The THF extract was separated again from the solid using centrifugation (×17,210 g) for 30 minutes. Then 1 mL of the extractant was filtered through a 0.02 µm Anotop membrane filter (Whatman International Ltd, Maidstone, England) to remove ultra-fine particles.

Chlorpyrifos and degradates extracted into THF were determined using HPLC. A 50 μL aliquot of extractant was injected into a Hewlett Packard LC system with 1100 series Diode array UV-Vis detector (Wilmington, DE). The analytes were determined using a reversed-phase SUPELCOSIL™ LC-18 column (15.0 cm x 4.6 mm ID) from Supelco (Bellefonte, PA). The mobile phase was HPLC grade acetonitrile and acidified water (pH was adjusted to 3.0 using HPLC grade H_3PO_4). The solvent gradient program started at 10% acetonitrile + 90% water, increased linearly to 90% acetonitrile + 10% water over 20 min, held for 5 min and then returned to the initial gradient state in 5 min. The flow rate was 1.0 mL min^{-1} . Absorbance was measured continuously in the range of 190 ~ 400 nm. Chlorpyrifos and TCP were quantified at 230 nm and 302 nm, respectively.

Results and Discussion

Tables 1-4 present the distribution of chlorpyrifos in the aqueous and solid phases in different colloid-water systems as a function of incubation time. In these systems, chlorpyrifos was dominantly sorbed on the colloids, however, some chlorpyrifos remained in the aqueous phase, depending on the affinities of the various colloids for chlorpyrifos in aqueous systems. The concentrations of chlorpyrifos in the aqueous phases varied from 0.5 $\mu\text{g mL}^{-1}$ for the HA system to 2.22 $\mu\text{g mL}^{-1}$ for the Otay system. It is known that chlorpyrifos had a strong

tendency to be associated with organic material due to its nonpolar nature (Racke, 1993). Meikle and Youngson (1978) reported that at near neutral pH (~7) and ambient temperature (~25°C), chlorpyrifos is moderately stable, with reported hydrolysis half-lives from 29 to 35 days in water. However, in this study the concentration of chlorpyrifos in the aqueous phase of the colloid-water systems did not decrease as would be expected for the degradation of chlorpyrifos in aqueous systems for the 30 days of incubation.

During the 30 days of incubation, TCP did not appear in either the aqueous or solid phase of the Otay-, Sediment- and HA-systems while only a trace amount of TCP ($0.034 \mu\text{g mL}^{-1}$) was detected on day 30 in the aqueous phase for the Panther-system. In comparison with our findings in Chapter 3, TCP clearly appeared before 30 days of incubation in an aqueous system of 0.01M CaCl_2 background. These findings indicate that Ca-saturated colloidal materials may inhibit hydrolysis of chlorpyrifos in aqueous systems.

The total recoveries for chlorpyrifos extracted by THF from the solid phase and chlorpyrifos measured in the aqueous phase during the 30 days of incubation are 82-97%, 87-93%, 78-85%, 79-97% for suspended Otay-, Panther-, Sediment- and HA-systems, respectively. The amount of extracted chlorpyrifos from colloidal materials gradually decreased with incubation time, suggesting that chlorpyrifos either transformed to a degradation product other than TCP, or that chlorpyrifos formed a tightly-bound residue with colloidal materials that could not be extracted with THF.

The chemical environment of the solution and solid phases differ greatly. As is well known, the thin layer of water surrounding silicate surface is typically more acidic than bulk water (McBride, 1994), and thus reactions involving protons or hydroxide ions proceed at different rates for sorbed molecules which are otherwise structurally identical to dissolved molecules. Hydrolysis of chlorpyrifos is known to be rapid in aqueous systems, especially under basic condition (Racke, 1993). It is possible that chlorpyrifos sorbed on the particles are substantially less exposed to hydroxide ions which hydrolyze the chlorpyrifos molecules in the aqueous systems. Therefore, chlorpyrifos may actually be preserved when sorbed on smectites or tightly associated with macromolecules of humic acid. It is clearly evident that concentrations of chlorpyrifos in the aqueous phase remained relative stable in the studied colloid systems. The results indicate that chlorpyrifos-colloid complexes may act as a buffer to slowly release chlorpyrifos back to the aqueous solutions. These findings suggest that the chlorpyrifos sorbed on suspended sediment may buffer aqueous concentrations of chlorpyrifos and prolong the exposure time of organisms to chlorpyrifos in aquatic environments.

Conclusions

The fate of chlorpyrifos sorbed on colloidal materials may influence its potential toxicity to organisms in aquatic environments. The results from the

present study indicate that none of the studied colloidal materials catalyzed the hydrolysis of chlorpyrifos in 0.01M CaCl₂ aqueous systems. The concentration of chlorpyrifos in aqueous phases remained relative stable during the 30 days of incubation. A trace amount of TCP was detected in the aqueous phase for the day 30 sample of the Panther-system while no TCP was found in any other systems. Thus the results indicate that the formation of chlorpyrifos-colloid complexes may preserve chlorpyrifos and inhibit the hydrolysis process in the aqueous systems. The total recovery of aqueous and sorbed chlorpyrifos gradually decreased during the 30 days of incubation, suggesting that chlorpyrifos either forms tightly-bound residue with colloidal materials or undergoes a transformation process to a product other than TCP over an extended time in aqueous systems. The implication of this study is that the suspended sediment in contaminated aquatic environments may prolong the exposure time of aquatic organisms to chlorpyrifos.

References

- Barron, M.G., and K.B. Woodburn. 1995. Ecotoxicology of chlorpyrifos. Rev. Environ. Contam. Toxicol. 144:1-93.
- Camazano, M.S., and M.J.S. Martin. 1983. Montmorillonite-catalyzed hydrolysis of phosmet. Soil Sci. 136:89-93.

- Economic Research Service (ERS). 1994. Agricultural resources and environmental indicators. U.S. Department of Agriculture, Natural Resources and Environment Division. Agricultural Handbook 705, 205.
- Giesy, J.P., K.R. Solomon, J.R. Coats, K.R. Dixon, J.M. Giddings, and E.E. Kenaga. 1999. Chlorpyrifos: ecological risk assessment in North American aquatic environments. *Rev Environ Contam Toxicol.* 160:1-129.
- Macalady, D.L., and N.L. Wolfe. 1985. Effects of sediment sorption and abiotic hydrolysis. 1. Organophosphorothioate insecticide chlorpyrifos. *J. Agric. Food Chem.* 31:1139-1147.
- McBride, M.B. 1994. Environmental chemistry of soils. Oxford University Press, New York, Oxford.
- Meikle, R.W., and C.R. Youngson. 1978. The hydrolysis rate of chlorpyrifos, o-o-diethyl-o-(3,5,6-trichloro-2-pyridyl) phosphorothioate, and its dimethyl analog, chlorpyrifos-methyl, in dilute aqueous solution. *Arch. Environ. Contam. Toxicol.* 7:13-22.
- Mingelgrin, U., and S. Saltzman. 1979. Surface reactions of parathion on clays. *Clays Clay Miner.* 27:72-78.
- Mingelgrin, U., S. Saltzman, and B. Yaron. 1977. A possible model for the surface-induced hydrolysis of organophosphorus pesticides on kaolinite clays. *Soil Sci. Soc. Am. J.* 41:519-523.
- Racke, K.D., R.N. Lubinski, D.D. Fontaine, J.R. Miller, P.J. MaCall, and G.R. Oliver. 1992. Comparative fate of chlorpyrifos insecticide in urban and

- agricultural environments. In K.D. Racke and A.R. Leslie (eds). Fate and significance of pesticides in urban environment. ACS, Washington D.C. ACS Symp. Series.
- Racke, K.D. 1993. Environmental fate of chlorpyrifos. *Rev. Environ. Contam. Toxicol.* 131:1-154.
- Russell, J.D., M. Cruz, and J.L. White. 1968. Mode of chemical degradation of s-triazines by montmorillonite. *Science* 160:1340-1342.
- Saltzman, S., U. Mingegrin, and B. Yaron. 1976. Role of water in the hydrolysis of parathion and methylparathion on kaolinite. *J. Agric. Food Chem.* 24:739-743.
- Saltzman, S., B. Yaron, and U. Mingelgrin. 1974. The surface catalyzed hydrolysis of parathion on kaolinite. *Soil Sci. Soc. Am. Proc.* 38:231-234.
- Sanchez-Camazano, M, and M.J. Sanchez-Martin. 1991. Hydrolysis of azinphosmethyl induced by the surface of smectites. *Clays Clay Miner.* 39:609-613.
- Stevenson, F.J. 1994. *Humus chemistry: Genesis, composition, reaction.* John Wiley & Sons. New York.
- Theng, B.K.G. 1974. *The chemistry of clay-organic reactions.* John Wiley & Sons, New York.
- Yaron, B. 1975. Chemical conversion of parathion on soil surfaces. *Soil Sci. Soc. Am. Proc.* 39:639-643.

Table 1. Distribution of chlorpyrifos in the aqueous and solid phases in Otay-water system

Time (day)	Chlorpyrifos Distribution		Extracted Chlorpyrifos		TCP ($\mu\text{g mL}^{-1}$)
	Aqueous ($\mu\text{g mL}^{-1}$)	Kd [‡]	Solid ($\mu\text{g g}^{-1}$)	Recovery [‡] (%)	
1	2.22	186	409	97	nd [§]
5	1.57	283	426	97	nd
10	1.49	300	391	89	nd
15	1.63	270	379	88	nd
20	1.72	252	384	90	nd
25	1.82	236	361	87	nd
30	1.91	222	332	82	nd

[‡] Kd value refers to the distribution coefficient of chlorpyrifos as a ratio of the amount of chlorpyrifos on the solid to that in the solution. The amount of chlorpyrifos on the solid was calculated from the difference between the total amount of chlorpyrifos added initially in the systems and the amount of chlorpyrifos measured in the aqueous solution.

[‡] Recovery (%) denotes a percentage of the sum of chlorpyrifos extracted by THF from the solid phase and chlorpyrifos measured in the aqueous phase to the total amount of chlorpyrifos added initially in the system.

[§] not detectable in either aqueous or solid phase.

Table 2. Distribution of chlorpyrifos in the aqueous and solid phases in Panther-water system

Time (day)	Chlorpyrifos Distribution		Extracted Chlorpyrifos		TCP ($\mu\text{g mL}^{-1}$)
	Aqueous ($\mu\text{g mL}^{-1}$)	Kd [†]	Solid ($\mu\text{g g}^{-1}$)	Recovery [‡] (%)	
1	0.638	763	443	92	nd [§]
5	0.642	757	443	92	nd
10	0.659	739	449	93	nd
15	0.649	749	437	91	nd
20	0.662	733	435	90	nd
25	0.767	628	426	89	nd
30	0.654	745	421	87	0.034

[†] Kd value refers to the distribution coefficient of chlorpyrifos as a ratio of the amount of chlorpyrifos on the solid to that in the solution. The amount of chlorpyrifos on the solid was calculated from the difference between the total amount of chlorpyrifos added initially in the systems and the amount of chlorpyrifos measured in the aqueous solution.

[‡] Recovery (%) denotes a percentage of the sum of chlorpyrifos extracted by THF from the solid phase and chlorpyrifos measured in the aqueous phase to the total amount of chlorpyrifos added initially in the system.

[§] not detectable. On day 30, trace TCP was detected in the aqueous phase.

Table 3. Distribution of chlorpyrifos in the aqueous and solid phases in Sediment-water system

Time (day)	Chlorpyrifos Distribution		Extracted Chlorpyrifos		TCP ($\mu\text{g mL}^{-1}$)
	Aqueous ($\mu\text{g mL}^{-1}$)	K_d^{\dagger}	Solid ($\mu\text{g g}^{-1}$)	Recovery [‡] (%)	
1	1.42	319	366	84	nd [§]
5	1.37	332	374	85	nd
10	1.37	329	372	84	nd
15	1.46	306	365	84	nd
20	1.67	262	339	81	nd
25	1.57	282	335	80	nd
30	1.31	349	344	78	nd

[†] K_d value refers to the distribution coefficient of chlorpyrifos as a ratio of the amount of chlorpyrifos on the solid to that in the solution. The amount of chlorpyrifos on the solid was calculated from the difference between the total amount of chlorpyrifos added initially in the systems and the amount of chlorpyrifos measured in the aqueous solution.

[‡] Recovery (%) denotes a percentage of the sum of chlorpyrifos extracted by THF from the solid phase and chlorpyrifos measured in the aqueous phase to the total amount of chlorpyrifos added initially in the system.

[§] not detectable in either aqueous or solid phase.

Table 4. Distribution of chlorpyrifos in the aqueous and solid phases in HA-water system

Time (day)	Chlorpyrifos Distribution		Extracted Chlorpyrifos		TCP ($\mu\text{g mL}^{-1}$)
	Aqueous ($\mu\text{g mL}^{-1}$)	Kd [†]	Solid ($\mu\text{g g}^{-1}$)	Recovery [‡] (%)	
1	0.10	5444	503	97	nd [§]
5	0.06	8452	440	86	nd
10	0.05	10962	446	87	nd
15	0.07	7304	432	83	nd
20	0.07	7975	411	79	nd
25	0.06	8086	405	79	nd
30	0.06	8194	406	79	nd

[†] Kd value refers to the distribution coefficient of chlorpyrifos as a ratio of the amount of chlorpyrifos on the solid to that in the solution. The amount of chlorpyrifos on the solid was calculated from the difference between the total amount of chlorpyrifos added initially in the systems and the amount of chlorpyrifos measured in the aqueous solution.

[‡] Recovery (%) denotes a percentage of the sum of chlorpyrifos extracted by THF from the solid phase and chlorpyrifos measured in the aqueous phase to the total amount of chlorpyrifos added initially in the system.

[§] not detectable in either aqueous or solid phase.

CHAPTER 5

CHEMICAL TRANSFORMATION OF CHLORPYRIFOS TO CHLORPYRIFOS OXON IN WATER

A paper to be submitted to Environmental Science & Technology

Jigang Wu and David A. Laird

Abstract

In vivo transformation of chlorpyrifos to chlorpyrifos oxon is believed to be a prerequisite for this compound to display appreciable toxicity to organisms. An abiotic transformation of chlorpyrifos to chlorpyrifos oxon in tap water is first reported in this study. Active chlorine (HOCl and OCl⁻) dispersed in tap water for drinking water treatment was found to be responsible and effective for the chemical transformation. The pH of the aqueous solution controls the speciation of HOCl and OCl⁻ in water and thereby influences the transformation process. The proposed mechanism for the transformation is an electrophilic attack by HOCl on the thion (P=S) double bond of chlorpyrifos followed by desulfuration of chlorpyrifos to its oxon analog. Chlorpyrifos oxon is a potent anticholinesterase that is about 1000 times more toxic than chlorpyrifos. Because water chlorination is commonly used for treatment of domestic water supplies, the findings raise a new concern about the safety of domestic use of chlorpyrifos products.

Introduction

Chlorpyrifos [*o,o*-diethyl *o*-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate] is a broad-spectrum organophosphorus insecticide widely used in agricultural, industrial, and residential environments. The direct toxicity of chlorpyrifos is assumed to result from initial metabolic transformation of chlorpyrifos to chlorpyrifos oxon, and the subsequent inactivation of acetylcholinesterase (AChE) by chlorpyrifos oxon at neural junctions. Inactivation of AChE occurs by phosphorylation of the enzyme active site. AChE inactivation is dose- and exposure- dependent, and results in overstimulation of the peripheral nervous system and subsequent toxicity (1). It is well documented that chlorpyrifos is highly toxic to freshwater fish, aquatic invertebrates, and many estuarine and marine organisms (2).

A generalized pathway for chlorpyrifos transformations in the environment was comprehensively reviewed by Racke (2). Chlorpyrifos degrades by both abiotic and biotic transformation processes in terrestrial and aquatic environments. In soil, water, plants, and animals, the major pathway of abiotic and biotic degradation involves cleavage of the phosphorothioate ester bond to form 3, 5, 6-trichloro-2-pyridinol (TCP) (Figure 1). TCP does not cause cholinesterase inhibition and is less toxic to aquatic and terrestrial biota such as fish, birds and mammals than is chlorpyrifos.

Most organophosphorus insecticides are activated *in vivo* to potent inhibitors of AChE (3). Generally, the phosphorothionates can be bioactivated by the cytochrome P-450-dependent monooxygenases to their highly reactive oxon metabolites. The oxons are about 3 orders of magnitude more potent as an inhibitor of AChE than the corresponding phosphorothionates (4, 5). Because of the great difference in anticholinesterase potency between chlorpyrifos and chlorpyrifos oxon, the bioactivation reaction is critical for chlorpyrifos to display appreciable toxicity. The phosphorothioates (P=S) can therefore be regarded as proinsecticides because they need *in vivo* activation before they are insecticidal (6).

Siegfried (7) studied biochemistry and genetics of chlorpyrifos resistance in the German cockroach and confirmed the mixed function oxidase was involved in activation of chlorpyrifos to chlorpyrifos oxon. Commercial chlorpyrifos oxon can be prepared in the laboratory by oxidation from chlorpyrifos in organic solvent (7). However, no study has reported the abiotic transformation of chlorpyrifos to chlorpyrifos oxon in the environment. Walia (8) studied superoxide induced oxidative transformation of chlorpyrifos and found no evidence for oxidative desulfuration of thion (P=S) to oxon (P=O).

During recent investigations of the toxicity and fate of chlorpyrifos in aqueous systems we observed the rapid transformation of chlorpyrifos to chlorpyrifos oxon in chlorinated tap water. In this paper we report our evidence for the abiotic transformation of chlorpyrifos to chlorpyrifos oxon in aqueous solution

and postulate a chemical mechanism for the transformation. The significance and implications of the findings to human health are also discussed in this manuscript.

Materials and Methods

Chemicals

Reagent grade chlorpyrifos, chlorpyrifos oxon and TCP standards were obtained from Chem Service (West Chester, PA) with purities of 99.6%, 98% and 98.7%, respectively. Commercial Ortho Dursban[®] specially formulated for residential use (EPA Reg. No. 239-2633) was purchased from a local market. Dursban[®] has 4.38% active ingredient (chlorpyrifos) and 95.62% inert ingredients. Ethanol (100%) was used to help dissolve chlorpyrifos for preparation of chlorpyrifos stock solution. Milli-Q water (Milli-Q plus system, Millipore, Bedford, MA) used in our laboratory has a resistivity of 18.2 M Ω -cm. Tap water used in the study was from Ames, Iowa. The Ames tap water has a pH of 9.0, chlorine residual 2.55 mg L⁻¹, total hardness 172 mg L⁻¹, Fe 0.01 mg L⁻¹, F 1.05 mg L⁻¹ (Data were from the Ames Water Treatment Plant, Ames, Iowa).

Transformation of Chlorpyrifos in Aqueous Solutions

An aliquot of chlorpyrifos stock solution was added to Milli-Q water and Ames regular tap water to make a large volume (8L) of 500 μ g L⁻¹ chlorpyrifos aqueous solutions. Amber glass bottles (500 mL) were used for incubations at 23 \pm 2 °C.

Each bottle contained 450 mL of chlorpyrifos aqueous solution. The whole solution in each bottle was sacrificed over different time intervals for analysis of chlorpyrifos and its degradation products.

The commercial chlorpyrifos product, Dursban[®] was mixed with both Milli-Q water and Ames regular tap water to make 1,100 $\mu\text{g L}^{-1}$ chlorpyrifos solutions according to the label instructions. Both the Milli-Q water and tap water systems were incubated in amber glass bottles at 23 ± 2 °C for 24-hours.

Transformation of chlorpyrifos in the presence of OCl⁻ in aqueous solutions was investigated. Reagent grade NaOCl was used to prepare a range of OCl⁻ concentrations from 0 to 1000 mg L^{-1} in Milli-Q water. HCl and NaOH were used to adjust pH of the aqueous solutions for evaluation of the pH effect. An aliquot of the stock chlorpyrifos solution was then added to the aqueous systems to make 500 $\mu\text{g L}^{-1}$ chlorpyrifos in each amber glass bottle and incubated at 23 ± 2 °C for 24-hours.

HPLC Analysis

Chlorpyrifos and its degradation products were determined using high performance liquid chromatography (HPLC). Analytes were extracted from aqueous solutions into tetrahydrofuran using a solid phase extraction technique with a Varian (Harbor City, CA) Bond Elut LRC[®] C-18 cartridge. Prior to extraction, aqueous solutions were adjusted to pH 3.0 using HPLC grade H_3PO_4 .

A 50 μL aliquot of sample was injected into a Hewlett Packard LC system with a Series 1100 Diode array UV-Vis detector (Wilmington, DE). Chlorpyrifos

and its degradates were determined using a reversed-phase SUPELCOSIL™ LC-18 column (15.0 cm × 4.6 mm ID) from Supelco (Bellefonte, PA). The mobile phase was HPLC grade acetonitrile and acidified water (pH was adjusted to 3.0 using HPLC grade H₃PO₄). The HPLC gradient program started at 10% acetonitrile + 90% water and increased linearly to 90% acetonitrile + 10% water over 20 min, held for 5 min and then returned to the initial gradient state in 5 min. Flow rate was set to 1.0 mL min⁻¹. This separation method allowed the simultaneous measurement of chlorpyrifos, chlorpyrifos oxon, and TCP. Under above conditions, the retention times were 19.8 min for chlorpyrifos, 15.4 min for chlorpyrifos oxon, and 12.1 min for TCP. Absorbance was measured continuously in the range of 190 ~ 400 nm. Chlorpyrifos, chlorpyrifos oxon, and TCP were quantified at 230, 290, and 302 nm, respectively. Linear calibration curves with correlation coefficients (r^2) of 0.9999 were achieved for all analytes over the concentration ranges of 0~150, 0~100, 0~50 µg mL⁻¹ for chlorpyrifos, chlorpyrifos oxon, and TCP, respectively.

Results

Chlorpyrifos Transformation in Aqueous Solutions

Chlorpyrifos underwent a slow hydrolysis to TCP in Milli-Q water (Figure 2). Although TCP was clearly detected as a degradation product, total amount of TCP measured in water could not account for the disappearance of chlorpyrifos

in the Milli-Q water system. An unidentified peak was noted in the chromatographs from near the end of incubation period. For the present study, however, no attempt was made to isolate and identify other products which may also form as a result of hydrolysis of chlorpyrifos. Chlorpyrifos incubated in tap water was rapidly transformed to chlorpyrifos oxon (Figure 3). It was also found that chlorpyrifos oxon rapidly degraded and underwent hydrolysis to TCP in tap water. The half-life for chlorpyrifos oxon in Ames tap water was approximately 10 days in this study. The TCP was relatively stable in water, which is consistent with previous studies of chlorpyrifos hydrolysis in aqueous systems (1, 2).

Chlorpyrifos Transformation in the Presence of OCl^- in Water

We have found no other reports of the rapid transformation of chlorpyrifos to chlorpyrifos oxon in aqueous systems. That the rapid transformation occurred in tap water and not in Milli-Q water suggests that the chemistry of the tap water was responsible for the reaction. The phenomenon was reduced after tap water was boiled for 2 hours. This would likely eliminate the possibility that metals or other cations in tap water catalyzed the chemical transformation. We also tested whether active oxygen in water would contribute to the reaction by adding H_2O_2 solution into Milli-Q water (1% H_2O_2) and found no chlorpyrifos oxon after 24 hours (data not shown). Because the Ames Water Treatment Plant uses chlorination for treatment of domestic water supplies, we hypothesize that Cl residue in the tap water is responsible for the observed transformation.

To test our hypothesis, reagent grade NaOCl was added to Milli-Q water to prepare a range of OCl⁻¹ concentrations. No chlorpyrifos oxon was detected in the control (no NaOCl added) while chlorpyrifos oxon was clearly detected in aqueous solutions with 0.01 to 1000 mg L⁻¹ of OCl⁻¹ (Figure 4). At around 1.00 mg L⁻¹ Cl, all of the added chlorpyrifos was transformed to chlorpyrifos oxon. This is consistent with our findings in tap water, which contained about 2 mg L⁻¹ Cl residues. When Cl concentration was greater than 100 mg L⁻¹, the amount of chlorpyrifos, chlorpyrifos oxon and TCP in aqueous solution was reduced substantially. At 1000 mg L⁻¹ of Cl, none of the analytes were detected, indicating complete decomposition of the added chlorpyrifos in water.

Dursban[®] Transformation in Tap Water

To assess whether chlorpyrifos in a commercial insecticide product would undergo the same transformation in tap water, or whether inert ingredients in the formulation would stabilize the chlorpyrifos, Dursban[®] was tested in both Milli-Q water and tap water systems. The result (Figure 5) indicates that 99.8% of chlorpyrifos added to Milli-Q water remained as chlorpyrifos and only 0.2% of chlorpyrifos was hydrolyzed to TCP in a 24-hour period of incubation at 23±2°C. In tap water, however, 65.5 % of chlorpyrifos was transformed to chlorpyrifos oxon, 31.1% remained as chlorpyrifos, and 3.4% was hydrolyzed to TCP. The results clearly indicate that the Dursban[®] formulation did not inhibit or protect the transformation of chlorpyrifos to chlorpyrifos oxon when Dursban[®] was mixed with chlorinated tap water following label directions for residential use.

Effect of pH on the Transformation of Chlorpyrifos in Cl Aqueous Solution

It is well known that the rate of chlorpyrifos hydrolysis is largely influenced by solution pH (1, 2). Generally, alkaline conditions facilitate hydrolysis of chlorpyrifos to TCP in water. As shown in Figure 6, the pH of the solution also affected the transformation process of chlorpyrifos in OCl^- aqueous solution. The amount of chlorpyrifos oxon in water increased with decreasing pH of aqueous solution. As pH increased, however, more chlorpyrifos was hydrolyzed to TCP while the amount of chlorpyrifos oxon decreased. The results demonstrate that pH had an apparent influence on the transformation process occurring in OCl^- aqueous solution.

Discussion

Chemistry of Water Chlorination

When chlorine is dispersed in water as is commonly done for treatment of municipal water, a variety of molecular and ionic species are produced, including H_2OCl^+ , HOCl , OCl^- , and Cl_2 . The reactivity of those species differs significantly. Active chlorine (including HOCl and OCl^-) is generally responsible for most chemical reactions and disinfections occurring in tap water (9, 10).

When NaOCl is added to water, the following reaction occurs:



As shown above, the active ingredient of NaOCl is the hypochlorite (OCl^-), which hydrolyzes to form hypochlorous acid (HOCl). HOCl tends to undergo partial dissociation to produce a H^+ and a OCl^- as follows:



In water between pH 6.0 and 9.0 the reaction is incomplete and both species are present to some degree according to the following equation:

$$K_a = \frac{(\text{H}^+)(\text{OCl}^-)}{(\text{HOCl})} \quad (3)$$

where K_a varies with temperature. Morris developed a best-fit formula for the relationship between K_a and temperature (9):

$$\text{p}K_a = \frac{3000}{T} - 10.0686 + 0.0253 T \quad (4)$$

where T is absolute temperature, $\text{p}K_a$ is acid dissociation constant.

As shown in equation (3), the pH of the aqueous solution largely influences equilibrium and distribution of undissociated HOCl species. The measured water temperature in our experiment was 22°C , thus, we can calculate the percent distribution of the OCl^- and HOCl for various pH based on the following equation:

$$\frac{(\text{HOCl})}{(\text{HOCl}) + (\text{OCl}^-)} = \frac{1}{1 + \frac{K_a}{\text{H}^+}} \quad (5)$$

The calculation result for speciation of HOCl and OCl^- is plotted in Figure 7. It indicates that virtually no HOCl would exist at $\text{pH} > 10$ and no OCl^- would be

present at $\text{pH} < 5$ in Cl aqueous solutions. This feature is noteworthy for elucidating the mechanism for chemical transformation of chlorpyrifos to chlorpyrifos oxon in Cl aqueous solutions.

Mechanism for Chemical Transformation of Chlorpyrifos to Chlorpyrifos Oxon

It is generally known that HOCl is a strong oxidizing and electrophilic agent. Therefore we postulate a mechanism for abiotic transformation of chlorpyrifos to chlorpyrifos oxon in chlorinated water (Figure 8). It is proposed that HOCl first attacks the thion (P=S) double bond of chlorpyrifos. We hypothesize two possibilities for this electrophilic reaction in that OCl^- may attach to either P or S atom. As the Cl^- and H^+ are eliminated simultaneously by dehydrohalogenation both of these forms will react to form a cyclic phosphorus-sulfur-oxygen intermediate analogous to the oxathiiran that has been proposed by numerous investigators to be an intermediate in the reaction pathways of various S-oxides (11). The resultant phosphooxathiiran then undergoes a cyclic electron shift with the loss of S, forming chlorpyrifos oxon. The S atom that is released will be rapidly oxidized to SO_4^{2-} with a strong oxidizing agent present in the aqueous solution.

At high concentrations of active Cl in aqueous solutions, it is likely that successive electrophilic chlorination of pyridinol would occur, which would explain the complete decomposition of chlorpyrifos in aqueous solution with 1000 mg L^{-1} Cl (Figure 4).

This mechanism could also explain the trend that the amount of chlorpyrifos oxon transformed in active Cl aqueous solution decreased with increasing pH from 3 to 11 in water (Figure 6). At pH 11.0, HOCl was almost completely dissociated and OCl⁻ became the dominant species in water (Figure 7). It seems that OCl⁻ species did not undergo nucleophilic attack on the P atom of chlorpyrifos. As the solution pH decreased, more HOCl species were present in the water and more chlorpyrifos was transformed to chlorpyrifos oxon by electrophilic reaction with HOCl.

Implications

Chlorpyrifos is one of the most widely used pesticides in the United States. Sold under commercial brand names such as Dursban[®], Lorsban[®], etc., it is the sixth most commonly used pesticide for home and garden applications. Chlorpyrifos is applied in more than 20 million American homes each year to protect families, children and pets from the harmful effects of insect pests (e.g., termites, ticks, cockroaches, fireants). According to the USEPA, 972 registered products contain chlorpyrifos, including widespread uses for termite and roach control (12).

Chlorine was first introduced in drinking water as a disinfectant in 1903. Since that time the use of Cl in potable water treatment, in addition to disinfectant, has expanded to include several other important functions such as

taste, odor and color removal, and prevention of water quality degradation in distribution systems (13, 14). As a result, Cl is used in at least 99 percent of the municipal water supplies that are being chemically disinfected in the USA (10).

The combination of widespread domestic use of chlorpyrifos products and predominant chlorination of domestic water supplies has significant implications for human health. In studies conducted according to EPA guidelines, chlorpyrifos has been shown not to be mutagenic, carcinogenic, or teratogenic, nor does it adversely affect reproduction. The only known mode of chlorpyrifos toxicity is through cholinesterase inhibition. If exposures are less than those that cause significant cholinesterase depression, then no signs or symptoms related to chlorpyrifos exposure occur. Therefore, chlorpyrifos has been regarded as a safe pesticide for household use (15).

In recent years, however, EPA formally reviewed a number of legal claims related to chlorpyrifos products for home use. Among the symptoms reported to be linked with chlorpyrifos applications are headache, dizziness, abdominal cramps, nausea, vomiting, diarrhea, blurred vision, increased secretions (tearing, sweating, salivation), mental confusion, and muscular weakness. Because exposure from spray applications was assumed to reach peak levels within a few hours after use and to fall off rapidly, researchers have generally been unable to understand or have been slow to accept how these disease symptoms could be associated with chlorpyrifos exposure (12). Davis suggested

that exposure from indoor spraying of chlorpyrifos poses greater health risks than currently estimated.

It is beyond scope of this paper to say whether the above symptoms are actually associated with chlorpyrifos oxon or chlorpyrifos. However, the present findings raise a new concern for human exposure to chlorpyrifos products for in-home use. As mentioned before, chlorpyrifos oxon is a potent cholinesterase inhibitor, which can cause acute toxicity to all organisms with nervous systems. Whether humans are exposed to chlorpyrifos or chlorpyrifos oxon makes three orders of magnitude difference in the acute toxicological effect. Iwata (16) reported that residue level of chlorpyrifos oxon on foliage after application to California Citrus was detected although no indication was given whether chlorpyrifos oxon was from biotic transformation or occurred when the product was mixed with tap water before use. Further investigation on the linkage between chlorpyrifos oxon and human exposure is indeed needed.

Literature Cited

1. Barron, M.G.; Woodburn, K.B. *Rev. Environ. Contam. Toxicol.* **1995**, *144*, 1-93.
2. Racke, K.D. *Rev. Environ. Contamin. Toxicol.* **1993**, *131*, 43-66.

3. Coats, J.R. In **Pesticide Transformation Products: Fate and Significance in the Environment**. ACS Symposium Series 459. Somasundaram, L., Coats, J.R. Eds. **1991**, 10-30.
4. Chambers, J.E.; Chambers, H.W. In **Pesticide Transformation Products: Fate and Significance in the Environment**. ACS Symposium Series 459. Somasundaram, L.; Coats, J.R. Eds. **1991**, 32-42.
5. Forsyth, C.S.; Chambers, J.E. *BioChem. Pharmacol.* **1989**, 10. 1597-1603.
6. Cremlyn, R.J.W. **Agrochemicals: preparation and mode of action**. West Sussex, England, New York, Wiley. **1991**, 106-141.
7. Siegfried, B.D. et al. *Pestic. Biochem. and Physiol.* **1990**, 38, 110-121.
8. Walia, S.; Dureja, P.; Mukerjee, S.K. *Toxicol. Environ. Chem.* **1988**, 18, 21-29.
9. Morris, J.C. In **The Environmental Impact of Water Chlorination**. Jolley, R.L. Ed. National Technical Information Service, US Dept. of Commerce. **1976**, 27-41.
10. White, G.C. **Handbook of chlorination and alternative disinfectants**. 3rd. ed. van Nonstrand Reinhold, New York. **1992**, 184-191.
11. Snyder, J.P. *J. Amer. Chem. Soc.* **1974**, 96, 5005.
12. Davis D.L.; Ahmed, A.K. *Environ Health Perspect.* **1998**, 106, 299-301.

13. Minear, R. A.; Amy, G.L. In **Water Disinfection and Natural Organic Matter**. Minear, R.A.; Amy, G.L. Eds. ACS Symposium Series 649. **1996**, p1.
14. White, G.C. In **The Environmental Impact of Water Chlorination**. Jolley, R.L. Ed. National Technical Information Service, US Dept. of Commerce. **1976**, p7.
15. Gibson, J. E. et al. **Environ Health Perspect.** **1998**, 106, 303-306.
16. Iwata, Y. et al. **J. Agric. Food Chem.** **1983**, 31, 603-610.

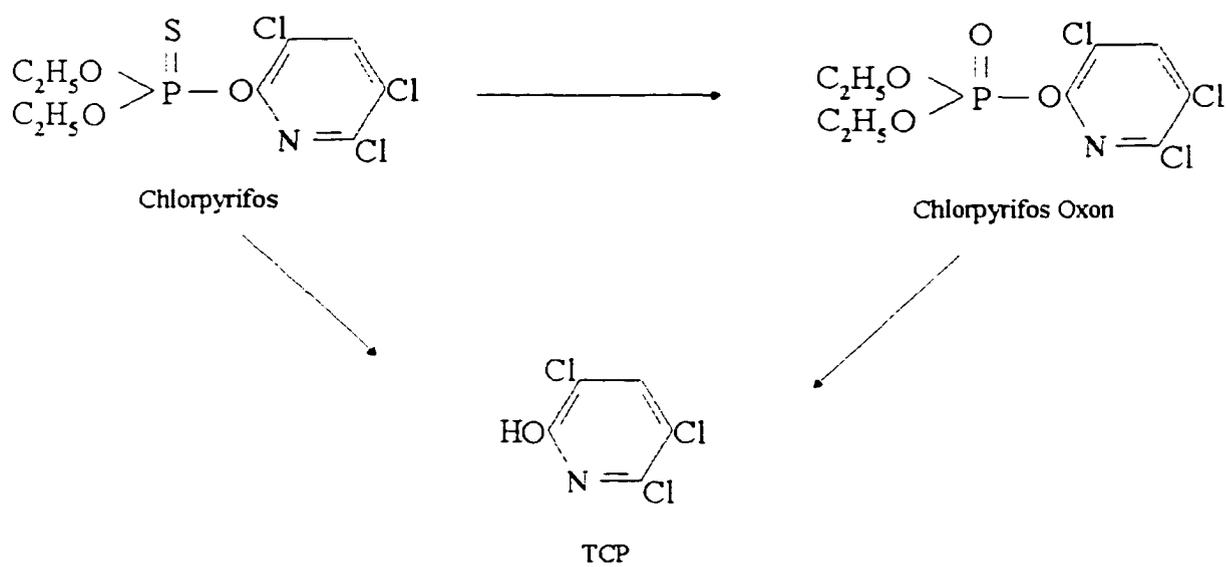


Figure 1. Major pathways of chlorpyrifos transformation in the environment

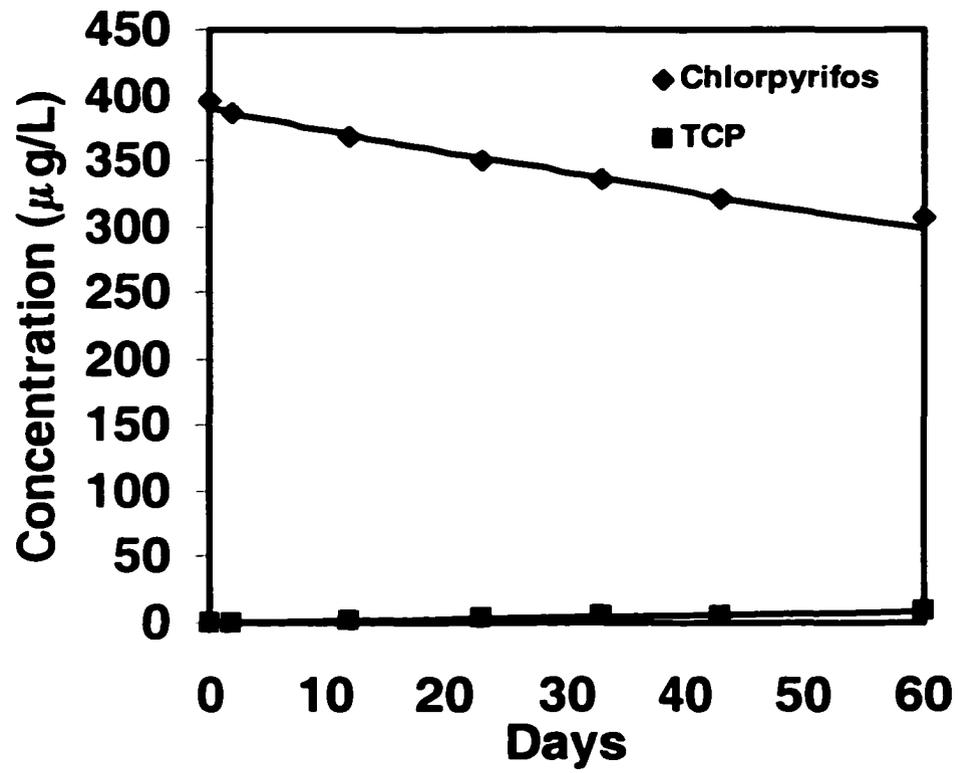


Figure 2. Transformation of chlorpyrifos in Milli-Q water

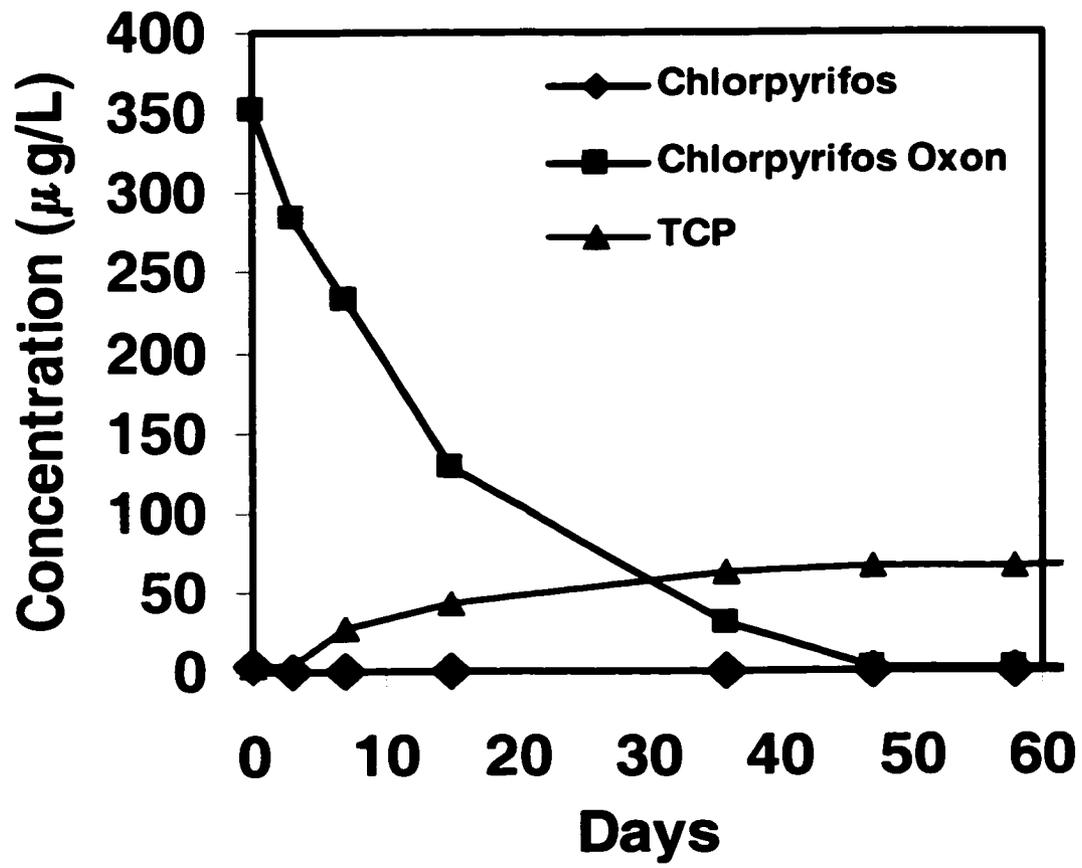


Figure 3. Transformation of chlorpyrifos in tap water

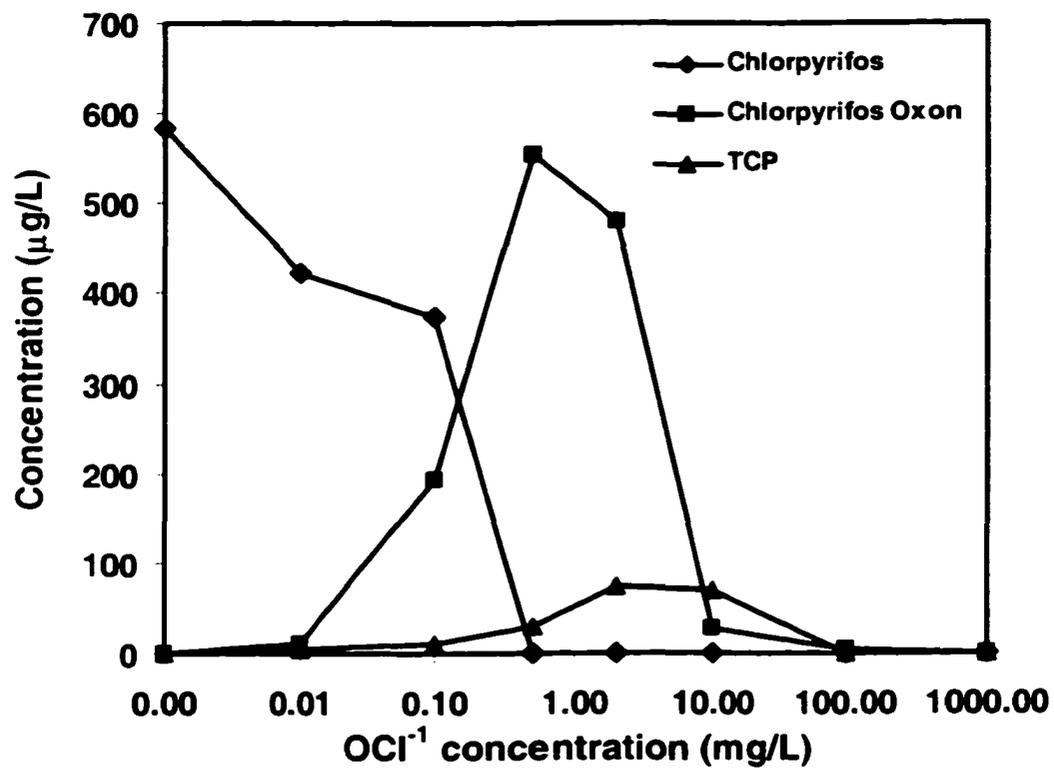


Figure 4. Transformation of chlorpyrifos as a function of OCl^- concentrations in water (24-hr incubation)

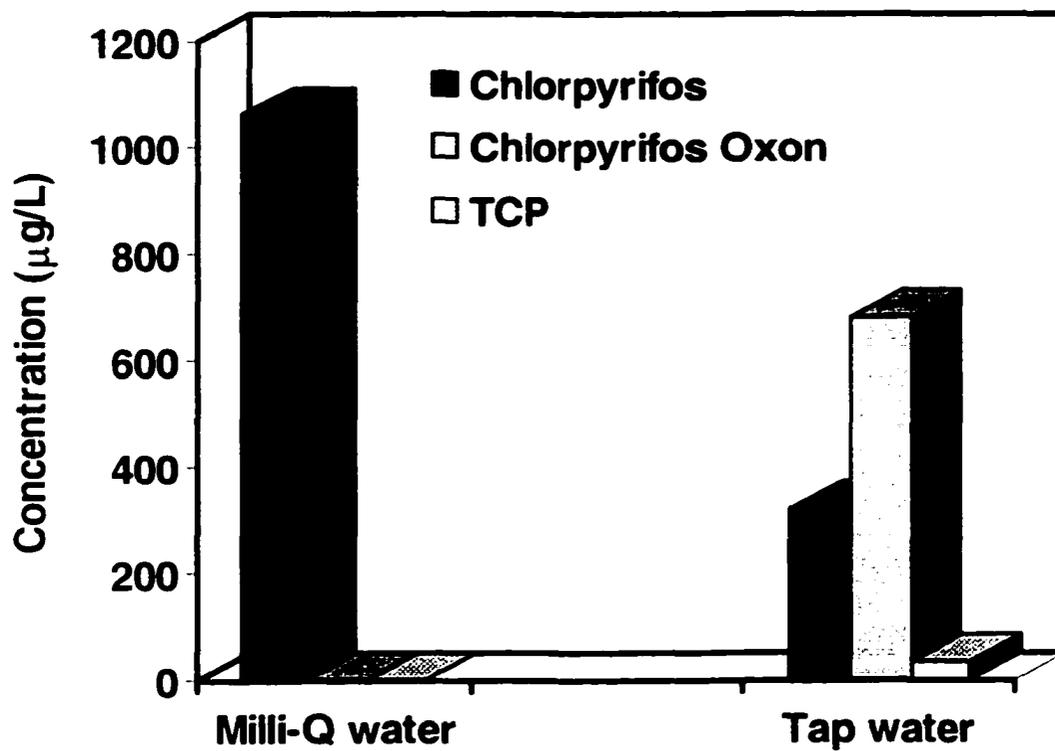


Figure 5. Dursban[®] transformation in Milli-Q and tap waters (24-hr incubation)

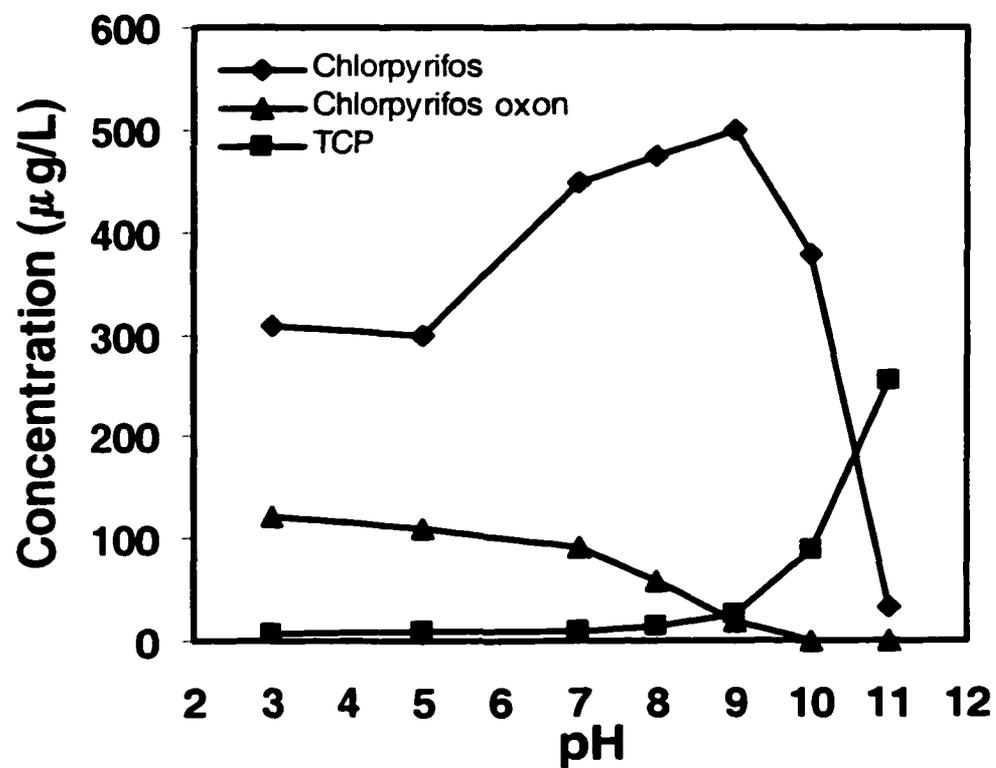


Figure 6. The effect of pH on transformation of chlorpyrifos in 0.1 mg L^{-1} NaOCl aqueous solutions (24-hr incubation.) The initial amount of chlorpyrifos in each pH treatment was $550 \text{ } \mu\text{g L}^{-1}$.

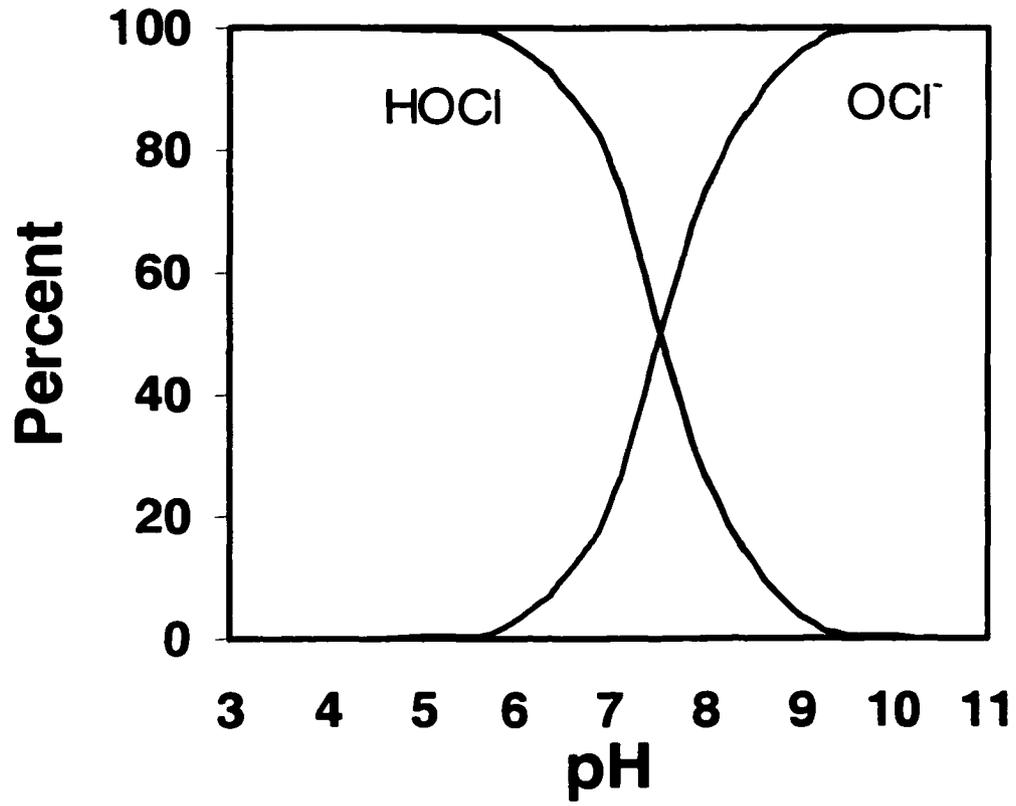


Figure 7. Speciation of HOCl in aqueous solution (22°C)

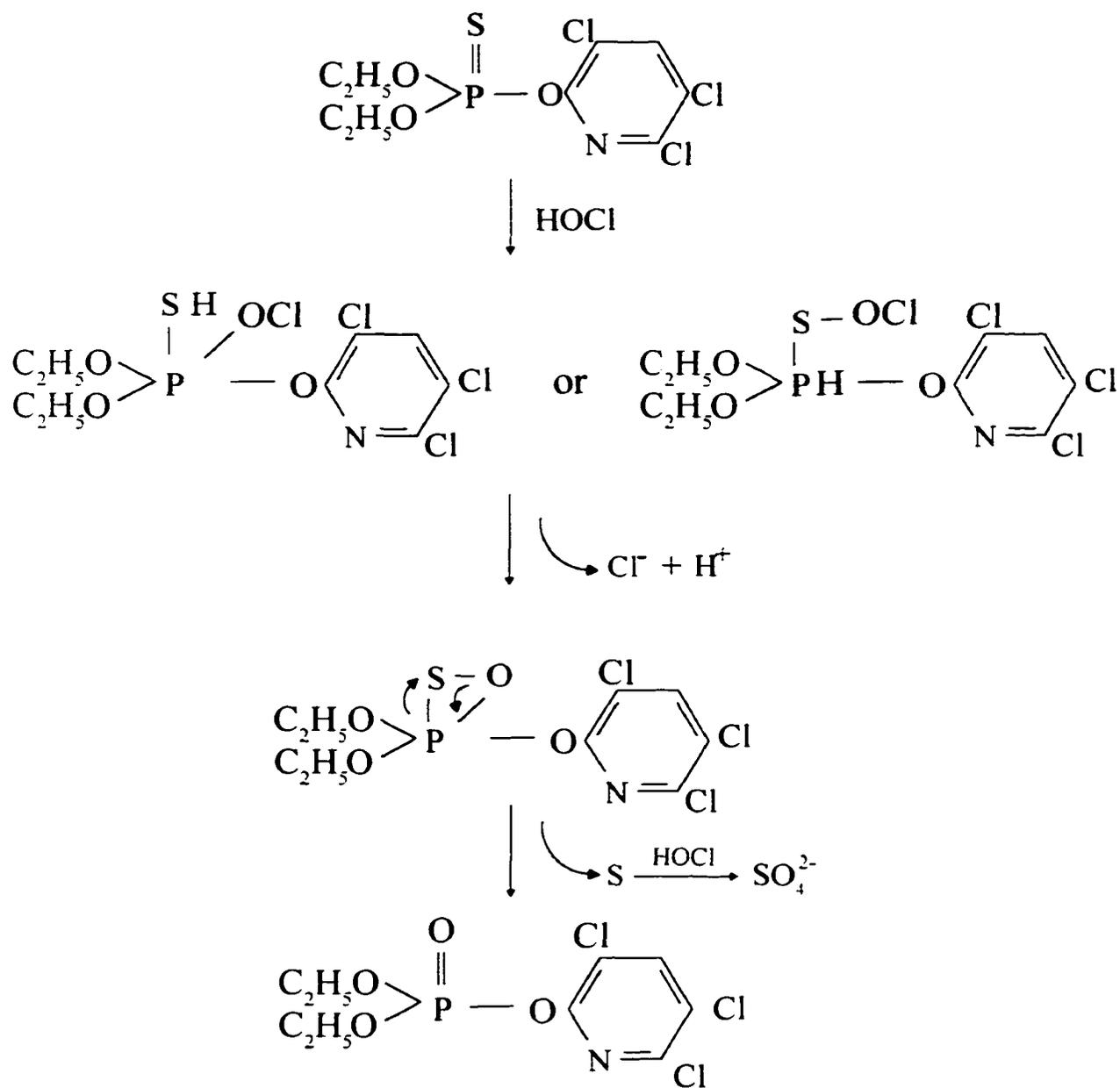


Figure 8. Proposed mechanism for transformation of chlorpyrifos to chlorpyrifos oxon in the presence of HOCl in aqueous system.

CHAPTER 6 SUMMARY

Chlorpyrifos is a broad-spectrum organophosphorus insecticide widely used in agricultural, industrial and residential environments. This compound is highly toxic to many aquatic organisms. Introduction of colloid-bound chlorpyrifos into rivers by surface runoff is a major concern for aquatic ecosystems in many Midwestern streams and rivers. The specific objectives of the present study are: 1) to quantify sorption and desorption of chlorpyrifos on colloidal materials in aqueous systems; 2) to study effects of water chemistry and suspended colloids on hydrolysis of chlorpyrifos in aqueous systems; 3) to determine whether colloidal materials may catalyze the abiotic transformation of sorbed chlorpyrifos in aqueous systems; and 4) to investigate mechanism for chemical transformation of chlorpyrifos in water. The implication of this study is to predict the fate and toxicity of chlorpyrifos in aquatic systems.

Understanding interactions of chlorpyrifos with colloidal materials in aqueous systems is fundamental for assessing fate and toxicity of chlorpyrifos in aquatic environments. The present study reveals a large difference in sorption affinity and variation in desorption hysteresis among smectites. Neither chlorpyrifos sorption nor desorption was correlated with cation-exchange capacity, surface area or surface charge density of smectites. Trace levels of organic matter and the fabric of smectite quasicrystals may be related to

sorption affinity for chlorpyrifos. Physical properties rather than surface chemistry of smectites may control the sorption behavior of chlorpyrifos on smectites. Chlorpyrifos was very strongly sorbed on humic acid and was not desorbed in aqueous solution. A large hysteresis was found for the sorption-desorption of chlorpyrifos on river suspended sediment. The results imply that suspended sediment can be important vector for transport and exposure of chlorpyrifos to aquatic organisms in rivers and streams.

Chlorpyrifos undergoes chemical hydrolysis in aqueous systems. The rate of hydrolysis was apparently affected by the water chemistry, clays and suspended sediment present in the systems. The rate of chlorpyrifos hydrolysis was substantially reduced in the presence of suspended smectites compared to the aqueous systems without smectites.

The fate of sorbed chlorpyrifos on colloidal materials in aqueous systems was investigated in this study. It was found that colloidal materials inhibited the hydrolytic degradation of chlorpyrifos in aqueous systems. The formation of chlorpyrifos-colloid complexes may actually preserve chlorpyrifos in aqueous systems. The implication of this finding is that the chlorpyrifos associated with suspended sediment and introduced into rivers due to surface runoff events from agricultural lands may prolong potential exposure time of organisms such as fish to chlorpyrifos in aquatic environments.

In vivo transformation of chlorpyrifos to chlorpyrifos oxon is believed to be a prerequisite for this compound to display appreciable toxicity to organisms. An

abiotic transformation of chlorpyrifos to chlorpyrifos oxon in tap water is first reported in this study. Active chlorine (HOCl and OCl⁻) dispersed in tap water for drinking water treatment was found to be responsible and effective for the chemical transformation. The pH of an aqueous solution controls the speciation of HOCl and OCl⁻ in water and thereby influences the transformation process. The proposed mechanism for the transformation is an electrophilic attack by HOCl on the thion (P=S) double bond of chlorpyrifos followed by desulfuration of chlorpyrifos to its oxon analog. Chlorpyrifos oxon is a potent anticholinesterase that is about 1000 times more toxic than chlorpyrifos. Because chlorination is commonly used for treatment of domestic water supplies, the findings raise a new concern about the safety of domestic use of chlorpyrifos products.

APPENDIX SPME-GC TECHNIQUE FOR DETERMINATION OF TRACE CHLORPYRIFOS IN AQUEOUS SOLUTIONS

Analyses of pesticides and many other organic pollutants in environmental samples usually begin with concentrating the analytes of interest through solid phase extraction, liquid-liquid extraction, purge-and-trap, headspace, or various other techniques. These procedures usually require excessive time, complicated equipment, and costly use of organic solvents. Solid phase extraction is commonly used to concentrate trace levels of pesticides from large volume aqueous samples, however, for limited volume samples, a more sensitive technique may be required.

The solid phase microextraction (SPME) technique, invented by Pawliszyn and commercially introduced by Supelco in 1993, has been widely applied to analyze organic pollutants in environmental samples such as soil, water, air, food, natural products and clinical samples (Supelco, 2000). Numerous SPME applications and techniques for analysis of pesticides in water have been documented (Choudhury et al., 1996; Magdic et al., 1996; Sng et al., 1997; Massat and Laurent, 1999). However, we have not found a report documenting the measurement of chlorpyrifos in aqueous solutions by SPME. Therefore, a solid phase microextraction-gas chromatograph (SPME-GC) technique for

determination of trace chlorpyrifos in aqueous solutions was developed and validated in our laboratory.

Principle of SPME

The theory of SPME is described in detail by Pawliszyn (1997). The SPME process is depicted in Fig. 1. In SPME, equilibria are established among the concentrations of an analyte in the sample, in the headspace above the sample and in the polymer coating on the fused silica fiber. The amount of analyte adsorbed by the fiber depends on the thickness of the polymer coating and on the distribution constant for the analyte.

For liquid polymeric SPME, the amount of analyte adsorbed by the coating at equilibrium is directly related to the concentration of the analyte in the sample (Pawliszn, 1997; Zhang et al., 1994):

$$n = \frac{K_{fs} V_f C_0 V_s}{K_{fs} V_f + V_s} \quad (1)$$

where n = mass of analyte adsorbed by the coating,

C_0 = initial concentration of analyte in sample,

K_{fs} = partition coefficient for the analyte between the coating and the sample matrix,

V_f = volume of the coating,

V_s = volume of the sample.

Equation (1) demonstrates that the relationship between the initial concentration of analyte in the sample and the amount of analyte adsorbed by the coating is linear. This is the basis for analyte quantitation (Pawliszyn, 1997). Because the coatings used in SPME are selected to have strong affinities for the organic compounds they are intended to extract substantial amounts of analytes from the aqueous phase. Consequently, SPME has a very effective concentrating effect and leads to good sensitivity. K_{fs} values usually are not sufficiently large to exhaustively extract the analyte from the matrix, hence, SPME is an equilibrium sampling method. Through proper calibration, SPME can be used to accurately determine the concentrations of analytes of interest in a sample.

Materials and Methods

Reagent grade chlorpyrifos was obtained from Chem Service (West Chester, PA) with purities of 99.6%. Ethanol (100%) was used to help dissolve chlorpyrifos for preparation of chlorpyrifos stock solution. Milli-Q water (Milli-Q plus system, Millipore, Bedford, MA) with a resistivity of 18.2 M Ω -cm was used for preparation of chlorpyrifos aqueous solutions.

SPME devices were obtained from Supelco (Bellefonte, PA). After preliminary evaluation of a variety of available fibers (7 μ m polydimethylsiloxane coating, 100 μ m polydimethylsiloxane coating, and 85 μ m polyacrylate coating), the SPME fiber coated with 85- μ m polyacrylate was used for the determination of

chlorpyrifos based on the best response for chlorpyrifos. A 10-mL aqueous sample containing chlorpyrifos was pipetted into an amber glass vial for extraction. As depicted in Fig. 1, the SPME fiber was completely immersed into the solution, which was stirred using a magnetic stirring bar at a constant rate during equilibration. The SPME fiber was then inserted directly into the GC injector port for desorption and analysis of chlorpyrifos.

All GC analyses were performed using a Hewlett Packard 5890 series II Gas Chromatograph equipped with a flame ionization detector (Wilmington, DE). A split/splitless GC injection port, maintained at 220°C, and a 30 m × 0.25 mm ID DB-1701 fused silica capillary column with a 0.25 µm stationary film (J & W Scientific; Folsom, CA) were utilized. The GC oven temperature was ramped from 50°C to 260°C at rate of 5°C min⁻¹. The final temperature was held for 10 min. Chlorpyrifos desorption from the fiber and purge off time were 5.00 min. The carrier gas was helium with the head pressure set to 10 psi. The detector temperature was maintained at 260°C. The retention time of chlorpyrifos was found to be 43.2 min based on analysis of calibration standards.

Results

During solid phase microextraction an equilibrium of chlorpyrifos between the aqueous phase and the polymer phase was established. Fig. 2. displays GC peak area for chlorpyrifos as a function of equilibration time using the 85 µm

polyacrylate SPME fiber. This pattern of extraction versus time for chlorpyrifos is similar to some of those reported by Pawliszyn (1997), who extensively examined a variety of analytes extracted from water. While the time required to reach true equilibrium is long, an equilibration time of 30 minutes is a reasonable compromise and practical for extraction of chlorpyrifos from water. For routine analysis, it is not necessary to reach a complete equilibrium as long as the exposure time of the fiber is constant (Pawliszyn, 1997).

At constant 30 min of equilibration time the linear dynamic range and precision of the method were determined. A typical calibration curve for chlorpyrifos determined by the SPME-GC technique is shown in Fig. 3. The r^2 values are generally greater than 0.9990 over the concentration range from 0 to 1000 $\mu\text{g L}^{-1}$. The precision of the measurement, reflected by relative standard deviation (RSD%), were from 2% to 10% over the same concentration range, which is generally good for GC measurements. The limit of detection, which was determined by successive dilution of chlorpyrifos solutions until no distinct response peak in GC, was as low as less than 0.1 $\mu\text{g L}^{-1}$ in water for the SPME-GC technique.

The results are generally characterized by low limits of detection, good linearity and precision, which proves that SPME is a valid method for analysis of chlorpyrifos at parts per billion levels in water. In conclusion, the SPME-GC technique is effective for determination of trace chlorpyrifos in aqueous solutions.

References

- Choudhury, T., K. Gerhardt, and T. Mawhinney. 1996. Solid phase microextraction of nitrogen-and phosphorus-containing pesticides from water and GC analysis. *Environ. Sci. Technol.* 30:3259-3265.
- Magdic, S., A. Boyd-Boland, K. Jinno, and J. Pawliszyn. 1996. Analysis of organophosphorus insecticides from environmental samples using SPME. *J. Chromatogr. A* 736:219-228.
- Massat, F., and A. Laurent. 1999. Quantitative analysis of pesticides in water by SPME coupled with Mass Spectrometry. *Spectra. Anal.* 28:23-29.
- Pawliszyn, J. 1997. *Solid phase microextraction: Theory and practice.* Wiley-VCH, New York.
- Sng, M. , F. Lee, and H. Lakso. 1997. Solid phase microextraction of organophosphorus pesticides from water. *J. Chromatogr. A* 759:225-230.
- Supelco. 2000. *SPME application guide. Bulletin 925A.* Supelco, Bellefonte, PA.
- Zhang, Z., M.J. Yang, and J. Pawliszyn. Solid phase microextraction: A solvent-free alternative for sample preparation. *Anal. Chem.* 66:844A-853A.

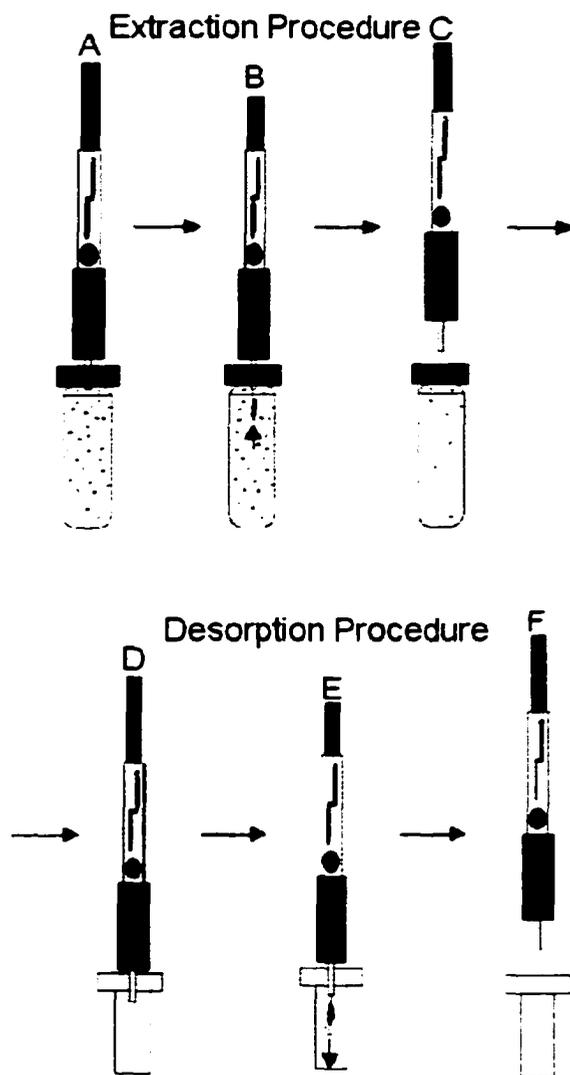


Fig. 1. Steps in SPME: A) with the fiber retracted, the outer metal sheath punctures the sample vial; B) the stationary phase fiber is then inserted into the sample and analytes are adsorbed onto the fiber; C) the unit is withdrawn from the vial; D) the sheath is inserted through the GC septum; E) the fiber is extended and heat of the injector desorbs the analytes; and F) the unit is then withdrawn from the injector. Modified from Pawliszyn (1997).

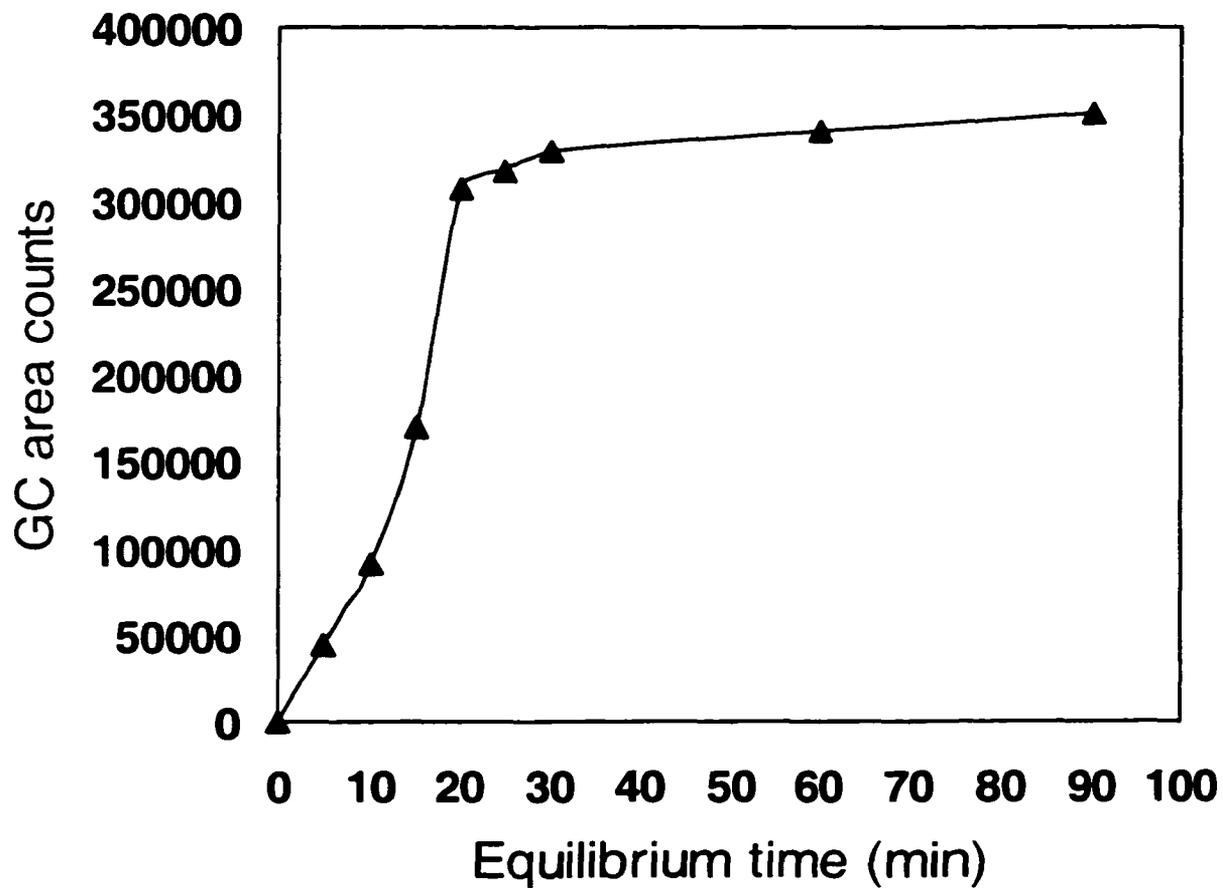


Fig. 2. Response of GC peak area for chlorpyrifos to equilibrium time for the SPME fiber in 10-mL aqueous samples containing $15 \mu\text{g L}^{-1}$ chlorpyrifos.

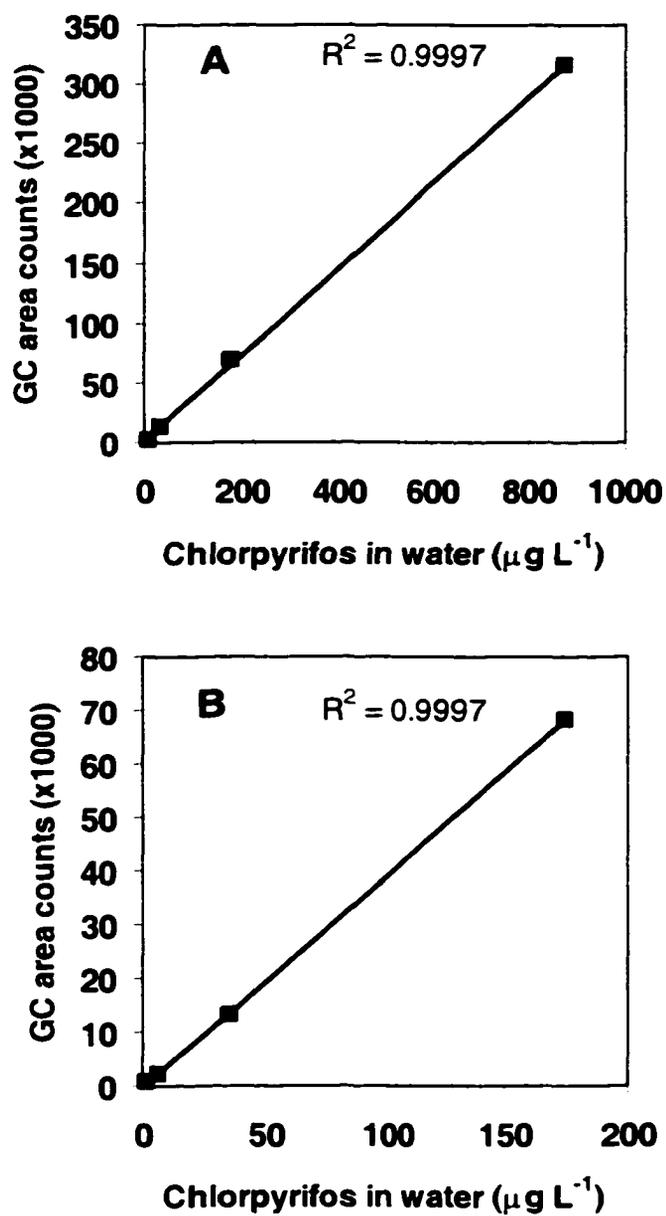


Fig. 3. A calibration curve for chlorpyrifos determined by the SPME-GC technique (A and B are the same data points plotted at two different scales showing a large linear dynamic range)

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and gratitude to my major professor, Dr. David A. Laird, for his continuing support, encouragement, and direction throughout my research program. Special thanks are due to my committee member, Dr. R. C. Summerfelt, for his insightful discussions for the research project. I would also like to extend my thanks to Dr. M. L. Thompson, Dr. R. Horton, and Dr. M. A. Tabatabai for their valuable suggestions and comments on the dissertation and time serving on my graduate committee.

I am especially thankful to Mr. Pierce Fleming for his technical support and friendship. It is my pleasure working with him in the National Soil Tilth Laboratory, USDA-ARS where most of my research work was conducted. I would also like to extend my special thanks to Mrs. Linda Schultz and Ms. Tracey Pepper for their technical assistances.

I would like to acknowledge my parents, Wanming Wu and Xueying Zhu, whose love, care and expectation are always treasured in my life. Finally, I wish to express my heartfelt thanks to my wife Yulian, my daughter Lucia, and my newborn son James who has given me new inspiration and hope. Completion of this dissertation is just a new beginning!