EFFECT OF PH ON THE PERSISTENCE AND TOXICITY OF CYFLUTHRIN TO *CHIRONOMUS TENTANS*

Faith Ann Hambleton, B.A.

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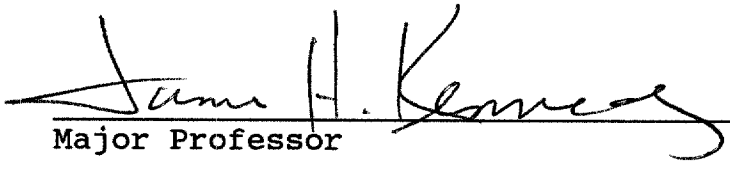

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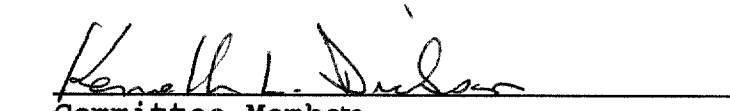

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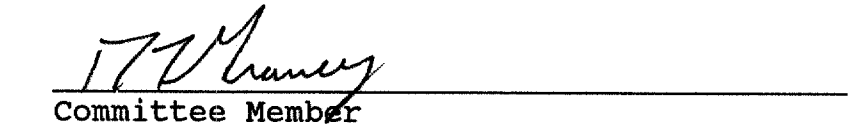

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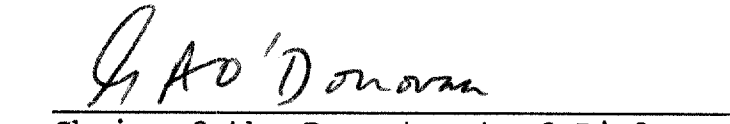

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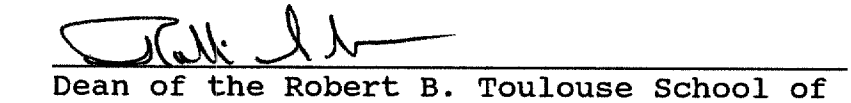

Dean of the Robert B. Toulouse School of Graduate Studies

EFFECT OF PH ON THE PERSISTENCE AND TOXICITY OF CYFLUTHRIN TO *CHIRONOMUS TENTANS*

THESIS

Presented to the Graduate Council of the
University of North Texas in Partial
Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Faith Ann Hambleton, B.A.
Denton, Texas
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Jan H. Kennedy

Hambleton, Faith Ann , Effect of pH on the Persistence and Toxicity of Cyfluthrin to *Chironomus tentans*. Master of Science (Biology), 1992, 119 pp.,11 Tables, 20 Figures, References, 82 Titles.

The effect of pH upon the aquatic toxicity of cyfluthrin was determined in 48 h static, acute toxicity tests using 2nd instar *Chironomus tentans* larvae. Tests were conducted in both dechlorinated tap water and lake water of pH 8.0, 7.2, and 6.6. After 48 h, immobilized and dead larvae were removed and replaced with 2nd instar larvae to assess the persistence of toxicity. Midges were cultured in water adjusted to the pH values used in testing.

Toxicity of cyfluthrin varied inversely with pH. An increase in the pH of tap water by 2 units resulted in a 2-fold decrease in toxicity. Toxicity of cyfluthrin also varied between tap and lake water of the same pH. EC50 values in lake water were approximately 2-3 times lower than those in tap water. Toxicity in lake water and tap water at every pH tested was also significantly different when regression line elevations were compared. Natural waters amended with cyfluthrin were consistently more toxic to the chironomids than tap water of the same pH. Persistence of cyfluthrin at low pH also influenced chironomid behavior. Recovery of normal behavior generally began within 24 h at

pH 8.0. At pH 6.0, recovery did not begin until one week after dosing. The persistence of cyfluthrin also varied with pH. Averaged across all concentrations, 30 % of the initial dose remained in tap water (pH 8.0) after 48 h, compared with 45 % (pH 7.2), and 75 % (pH 6.6).

Ten-day chronic sediment toxicity tests were conducted with overlying water of different pH's collected from the same source as the sediment. LC50 values in sediment/water of pH 8 were significantly different from water/sediment of pH 6.4. With the addition of sediment to the test system, toxicity of cyfluthrin to *C. tentans* was less than in the aqueous system under comparable pH. Results indicate that sediments with overlying lake water are able to detoxify cyfluthrin better than sediments with dechlorinated tap water.

ACKNOWLEDGEMENTS

I would like to thank all the members of my committee for their advice and comments on experimental design and statistical analysis. I would especially like to thank Dr. Kennedy for his friendship and unprecedented support throughout this endeavor.

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TABLE OF CONTENTS

LIST OF TABLES

Table

LIST OF ILLUSTRATIONS

Figure

CHAPTER 1

INTRODUCTION

Synthetic pyrethroid insecticides were first described as a new class of pesticides in 1973 (Hill, 1985). They are derivatives of naturally occurring pyrethrins which have been used since the mid 1930's. During the 1980's, several new synthetic pyrethroid insecticides were developed with greater insecticidal activities than those previous (Elliot, 1985; Bradbury and Coats, 1989). Synthetic pyrethroids combine high activity against insects with low mammalian toxicity (Elliot, 1976; Anderson, 1989). The present range of pyrethroids fulfill a need for lipophilic insecticides to replace environmentally persistent organochlorine compounds, which are no longer acceptable in the United States (Elliot 1985).

Cyfluthrin (Baythroid®) is a new generation synthetic pyrethroid insecticide. Baythroid® was issued conditional registration by the United States Environmental Protection Agency (USEPA) for use on cotton (December, 1987). Cyfluthrin is currently used in the southern United States for the control of insects on cotton. In 1987, 10 million

acres of cotton were harvested in the United States with a value of 4.5 billion dollars making protection of this crop paramount (US Dept. Agriculture 1988). Synthetic pyrethroids combine high activity against insects and low mammalian toxicity (Elliot 1985) with photostability, accounting for their increased use in agriculture (Mokry and Hoagland 1990). Laboratory toxicity studies have reported 96-hr LC50's in the low parts per billion (ppb) range for fish (McLeese et al. 1980) and in the parts per trillion range for invertebrates and zooplankton (Muir et al. 1985; Day 1989; Xiu et al. 1989; Mokry and Hoagland 1990).

In order to determine the ecological effects a pesticide may have on the environment, an extensive battery of aquatic tests are required by the United States Environmental Protection Agency (USEPA), under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) for registration of a compound (Touart 1988). In addition, computer models are used to predict exposures and are compared with laboratory measured effects levels to help determine the likelihood of an adverse impact caused by the application of a compound. During pesticide application in the field, a proportion of the chemical comes into contact with soil and/or water (Pimentel and Edwards 1982; Wauchope 1978). Run-off, which transports soil-bound insecticides and spray drift from aerial applications are the primary routes

of transport to aquatic systems (Zhang 1984). The risk of significant contamination of fresh waters is most likely to occur following aerial application of pesticides (Shires and Bennett 1985). Entry into the aquatic system can impact sensitive non target organisms such as cladocerans, amphipods, crayfish, and mayflies.

Pesticide Degradation in Water

A thorough knowledge of the physical and chemical properties of insecticides is needed to assess their fate and potential impacts when introduced into an aquatic system. Variables such as temperature, light, salinity, pH, water hardness, test organism size, nutritional state, and season of the year may significantly alter the outcome of a test (Buikema and Voshell 1990). Temperature and pH are two of the most influential environmental factors affecting pesticide fate and effects in aquatic systems (McEwen and Stephenson 1979).

Degradation can also occur by other routes such as hydrolysis. Hydrolysis occurs when a water molecule is split as the result of interaction with another ionic species. Pyrethroid, organophosphate, and carbamate insecticides are all esters of various acids. Hydrolysis plays an important role in their degradation. Hydrolytic rates in homogeneous systems such as water, are a function of chemical structure, kinetic parameters, and the polarity of the reaction medium.

Many pesticide studies on pH and hydrolysis rates have been done, but a variety of experimental conditions make it difficult to define the relative rates for any one class of insecticides (Chapman and Cole 1982).

The persistence of insecticides in water, along with their chemical and biological degradation, has been frequently studied for compounds such as parathion, diazinon, carbaryl, and chlorpyrifos (Lohner and Fisher 1990). Laboratory degradation studies in water have shown cyfluthrin stability at pH 5 (25°C) compared with a half-life of less than two days at pH 9. An extrapolated half-life of 193 days was determined at pH 7 for cyfluthrin (Mobay 1988). These patterns of degradation appear to hold true for pyrethroids as a class (Hill 1985a; Muir et al. 1985). The molecular stability of a compound can lead to prolonged environmental persistence and may cause adverse impacts on aquatic life. Carbamates are also reported to be stable under acidic conditions (Aly and El-Dib 1971; Sharom et al. 1980). For example, carbaryl, a carbamate insecticide, is more toxic to fourth instar midges under acidic conditions (Fisher and Lohner 1986).
Changes in the half-life for some carbamates and organophosphates between pH 8.0 and pH 4.5 have been as great as 1000x in sterile phosphate buffers at 25°C (Chapman and Cole 1982). Sterilization of any media however, negates the possibility of microbial degradation.

Dissociation is another way in which chemical compounds can break down. Dissociation results in the separation into ionic and non ionic species which in turn, can influence the toxicity of a compound. This relationship is not linear however, suggesting that other factors are involved in modifying toxicity (Doe et al. 1988). One explanation for this pattern states that undissociated organic particles pass across biological membranes more readily than do ionized forms of the same chemical (Jollow and Brodie 1972). Pesticides such as 2,4-D, are known to dissociate to a greater extent as the pH of the solution increases. Dissociation resulting in ionic and non-ionic species can influence the toxicity and the resultant bioavailability of a compound. Bioavailability of a compound to the organism is a determining factor in toxicity.

Since toxicity is a function of both exposure duration and concentration to a target organism, enhanced uptake and subsequent toxicity of a compound may result from pH changes which increase the proportion of the undissociated form. For example, Fenitrothion does not dissociate and should therefore cross biological membranes at a constant rate, regardless of the pH (Doe et al. 1988). The toxicity of aminocarb, a pesticide that dissociates to a greater extent as the pH decreases, has been shown to decline substantially under acidic conditions since it can no longer cross biological membranes (Doe et al. 1988).

Duration of a chemical compound due to environmental conditions can also modify toxicity. Studies clearly indicate that the hazard to aquatic life depends not only upon the intrinsic toxicity of the insecticide but also on exposure, i.e., the amount entering the environment, its distribution within the environment, and its persistence in the aqueous phase (Shires and Bennett 1985). Most agricultural chemicals have numerous applications prescribed throughout the growing season. Repeated exposure to a chemical can result in a pattern of impact and recovery of target and non-target organisms (Kingsbury 1982). In an aerial forest application (17.5 g active ingredient/ha) of a pyrethroid in Canada, depletion of benthic organisms was seen as the result of direct over spray. Recovery of the depleted organisms was being observed in 3 to 6 weeks. However, with a second application, recovery took several months longer (Kingsbury 1982). The extent of a pesticides' impact on the organisms in the environment is also dependent on season and environmental conditions.

Degradation of Pesticide in Sediment

There is great difficulty in extrapolating pH dependent disappearance behavior observed in homogeneous solution to heterogeneous systems such as soils. Degradation studies done on pesticide amended soils of varying pH suggest that neither the acidity or alkalinity of soils had the same

effect as when in solution (Chapman and Cole 1982). In aqueous solutions, toxicity tends to decrease as pH increases (Doe et al. 1988). Insecticide persistence on soils artificially amended to different pH values and natural soils of different pH have been examined (Chapman and Cole 1982). In these experiments, no correlation was observed between the measured pH of these solids and the rate of disappearance of selected insecticides applied to them.

The environmental fate and behavior of pyrethroids in aquatic systems are influenced mainly by adsorption to suspended organic matter, sediments, plants, by microbial degradation, and minimally by photodegradation (Hill 1985a; Hill 1985). The rate of degradation determines the half-life of insecticides. All highly active pyrethroids have octanol/water partition coefficients greater than 30,000. They are highly lipophilic with strong adsorption properties and have water solubilities of only a few ppm, falling to the low ppb range for deltamethrin and cyfluthrin (Table II) (Briggs 1985). These physical properties combined with high toxicity make accurate determinations of their toxicity to aquatic organisms difficult (Stephenson 1982). Special care must be taken in the generation of test solutions and it is desirable to make chemical determinations of exposure concentrations.

Figure 1. Technical Information for Cyfluthrin (Baythroid^{R})

Cyano(4-fluro-3-phenyoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropanecarboxylate

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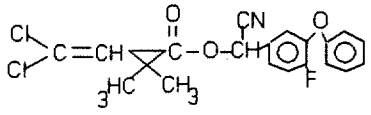
Chemical Structure:



Density: 1.25 g/ml @ 20^{0}C

Water Solubility: 2 ppb

Octanol/Water Partition Coefficient: 420,000

Aqueous Photolysis:

$T^{1/2}$ = 12.2 days @ 6700 $\mu W/cm^2$
$T^{1/2}$ = 1 day in natural light

Hydrolysis:

Stable at pH 5
$T^{1/2}$ = 193 days at pH 7
$T^{1/2}$ = < 2 days at pH 9

EEC: 8.3 - 26.8 ppt, median = 9.4 ppt (computer simulation)

Since pyrethroids are highly lipophilic and have low water solubilities, they rapidly adsorb to the soil. The rate of degradation in soil is generally fast, approximately 50 % of applied material is lost within a few weeks (Hill 1985). Microbial activity in soil is generally much greater than in water due to higher microbial counts in soil which often has been fertilized. Microbes play a major role in the degradation of many insecticides in soil (Williams and Brown 1979; Tu 1980; Chapman et. al. 1981). If degradation of parent compound can occur, the pesticide may be rendered inactive. Degradation also occurs in water-sediment mixtures. The degradative properties of pyrethroids in water-sediment systems are generally slower than in soil (Hill 1985). As a result, low residue concentrations can be recovered from bottom sediments after long periods of time (Hill 1985a).

In aquatic environments, the pattern of loss is more complex due to the varying nature of water bodies and the chemical properties of the compounds. The materials that pyrethroids encounter in the aqueous environment may act as a sink for adsorption and reduce the exposure of aquatic organisms by decreasing the dissolved concentrations of the chemical in water (Day 1989; Coats et al. 1989; Shires and Bennett 1985). For example, a synthetic pyrethroid permethrin, was applied at 5 day intervals to a cotton field in Alabama at the rate of 225 g/ha. Following heavy rains,

the concentration of permethrin in runoff from 12 ha was determined to be between 3.37 ppb and 15.3 ppb. Stream-water stations within 165 m of the runoff entry point showed residues of less than 0.42 ppb, and sediment residues no greater than 0.003 ppm (Kingsbury 1982). In another study, the toxicity of permethrin to first instar *Daphnia magna* was reduced 5-fold when a layer of soil was present in the bottom of the bioassay chamber (Kingsbury 1982). Other studies however, indicate that benthic organisms are at great risk from sediment-sorbed contaminants (Fry and Fisher 1990). In a study done with *Chironomus riparius*, the midges accumulated a significant amount of PCP, a chlorinated lipophilic compound, when they were in direct contact with the sediment (Fry and Fisher 1990). When midges were isolated from the sediment less uptake was observed. Uptake however, is not necessarily associated with toxicity since the compound must be bioavailable to the organism. Uptake studies done on a variety of chemicals from water and water-sediment systems have demonstrated less bio-availability from the adsorbed phase than from the aqueous phase (Luoma 1989; Shea 1988; Pritchard 1984). Such adsorption may decrease the potential for uptake, toxicity, and bioaccumulation of lipophilic contaminants.

Uptake of Contaminants by Organisms

The primary medium from which active uptake occurs is believed to be sediment interstitial water (Adams et al. 1985; Oliver, 1987; Landrum 1988). Freshwater macroinvertebrates are ideal organisms for sediment toxicity tests but few organisms are available for use. Midges are deposit feeders and are known to rework sediments by feeding and burrowing (Lee and Swartz 1980). Through such activity, midges may be exposed to contaminated particles in interstitial water and ingest these particles. Literature indicates however, that most bound residues are pesticide-humus adducts, and the majority of parent compound residues are unavailable for uptake (Huckins et al. 1986). The behavior and bioavailability of sediment-sorbed compounds are controversial due to the potential impact of these contaminants if they were to enter the food chain.

Environmental Factors and Compound Persistence

The Environmental Protection Agency has expressed concern over the persistence of cyfluthrin and the adverse effects it might have on non-target aquatic organisms. This concern is focused in regions where environmental factors such as pH, temperature, sunlight and nutrient input may differ greatly from the cotton growing regions where registration studies have been conducted.

Most registration studies on pyrethroids have been done in

waters with neutral to alkaline conditions under which pyrethroids rapidly degrade. Since environmental factors differ across geographical areas, it is difficult to extrapolate the results of one study to another region. The use of replicated ponds (mesocosms) for the assessment of the potential impact of xenobiotics in lentic and lotic ecosystems are accepted as tools in risk assessment and regulatory decision making (Touart 1988; Touart and Slimak 1989). Although the reproducibility of multi-trophic level tests is less than that of single species, site specific simulations are an improvement in the realism and accuracy of laboratory tests (Metcalf 1977).

Surface Water pH in the United States

Historical trends in pH profiles have been primarily examined in acidic lakes. In locations where lake water acidification is more than a transient phenomena, the surface layer of the lake sediment may also be acidified relative to the deeper layers (Morris and Kwain 1988). Sediment pH usually stabilizes about 10 centimeters below the sediment water interface, even in highly acidified lakes (Tolonen and Jaakkloa 1983, Stahl 1986). Thus, knowledge of pH is critical when determining the impact that a pesticide may have on organisms living in the surface sediments and at the water sediment interface of lakes and ponds that receive spray drift or surface run-off from agricultural areas.

Water Quality Data and Cotton Production

Water quality information for the year 1989 was obtained from the Environmental Protection Agency's Storage and Retrieval (STORET) computer data base. The pH values from various lakes and streams across the continental United States were preliminary examined. In order to understand the risk Cyfluthrin may pose, it is necessary to know if low pH water bodies are in the same vicinity as cotton crops receiving pesticide applications. Information on cotton production was obtained from the National Cotton Council in Memphis, TN. This information in conjunction with the laboratory toxicity test results, may help elucidate the potential risk of cyfluthrin use in areas of low pH in the future. A preliminary examination of water quality data in cotton growing regions is presented in this thesis.

Research Objectives

The objectives of this research were:

1) Develop test methods and extraction procedures that would adequately assess the toxicity of cyfluthrin to *Chironomus tentans* at various pH's.
2) Identify and collect acidic and basic sediments/water for use in the pH studies.
3) Compare toxicity in ambient and tap water of the same pH.
4) Determine if sediments of varying pH influence the persistence and toxicity of cyfluthrin to *Chironomus tentans*. Tests with sediments and lake water will be compared to sediments and tap water.
5) Obtain water quality data (pH) for various regions in the U.S. Obtain cotton production data. This information will be combined in a map to show potential risk of cyfluthrin in cotton production areas.

Hypotheses

1) Mortality of *C. tentans* is significantly influenced by pH.
2) Aquatic organism mortality differs using tap water or natural water of similar pH.
3) There is a difference in mortality when sediment is added as a substrate at any pH.

Scope of Research

Cyfluthrin is the active ingredient (26 %) in the compound Baythroid^{R}, a synthetic pyrethroid marketed for use on cotton in the United States. Pyrethroid compounds degrade quickly under alkaline conditions, and studies done on various pyrethroids have confirmed a short half-life in the field. The probability that cyfluthrin will exhibit persistence and adversely impact the environment under conditions of low pH has not been fully evaluated in the field or laboratory. Since the hydrolysis of cyfluthrin is base-catalyzed, it is likely that low pH will result in the increased persistence of the compound. Whether persistence of this compound will result in increased toxicity remains uninvestigated. There is little information in the literature addressing the fate of pyrethroids in natural sediments and water of varying pH's.

This research used the midge *Chironomus tentans* to assess the toxicity and persistence of cyfluthrin in natural sediments and overlying water pH's of 6, 8, and 9. This experiment was designed to simulate geographical regions with low and high pH water.

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CHAPTER 2

MATERIAL AND METHODS

General Organism Culture Methods

The test organism, *C. tentans* (Fabricius) (Diptera: Chironomidae), has a holartic distribution and is locally common in the mid-continental area of North America (Townes 1945). *C. tentans* is a large midge (second instar 5-10 mm) with a generation time of approximately 28-30 days at 25^{0}C. Although many chironomids are considered pollution tolerant (Hilsenhoff 1987; Buikema and Voshell 1990) they are considered ideal test organisms because of their importance to benthic communities, ease of culture (Anderson 1980b; Giesy et al. 1988), and the existence of a large data base documenting the effects of toxic chemicals on the midge (Lydy et al. 1990). Chironomids appears to be highly sensitive to some chemicals, but in general their toxicity responses are similar to other invertebrate test organisms (Buikema and Voshell 1990).

The test organism was maintained at the University of North Texas Aquatic Laboratory. Chironomids were cultured according to the method of Townsend et al. 1981, with the

at 25°C in 10 gallon class aquaria. Culture substrate consisted of boiled, shredded natural brown paper towels (Lind Paper Co., Carrollton, TX) to a depth of 4-5 cm. A suspension of Tetra Conditioning Fish Food was fed to the cultures 2X a day. Adults were removed from the tank daily and placed in a 500 ml Erlenmeyer flask for egg laying. Egg masses were collected the next day and placed into prepared rearing tanks. Culture methods are described in detail in Appendix A.

Culture Method in pH Adjusted Water

Paramount to this research was development of a method to maintain both the desired pH of the culture water and chironomid survival. Chironomids were cultured under the same pH conditions as used in the toxicity tests. Animals were cultured at three different pH levels (6.5, 7.2, 8.0). Various methods of maintaining a constant pH in the culture water were attempted and are briefly described below.

1) The addition of either a 1 N HCL or 0.05 N NaOH solution to the rearing tanks in order to gradually increase the pH by 0.2 units /day.

2) An apparatus to give a constant delivery of the HCL and NaOH solutions. This delivery apparatus consisted of a 500 ml separatory funnel with a 22 G needle attached to the end with tygon tubing (Fig 2). The funnel was refilled every 12 hours with dechlorinated tap water already adjusted to the desired pH with either the acidic or basic solution.
3) A solution change. The acidic media was changed to a 1 M potassium dihydrogen phosphate buffer, and the basic media to a mixture of 0.5 M sodium borate and 0.1 N sodium hydroxide 1:1. Each buffer solution was placed in a 500 ml separatory funnel as described previously, and refilled every 12 hours.
4) Addition of larvae to pre-adjusted water. One day old larvae were added into rearing tank water that was already adjusted and stabilized to the designated pH. Approximately 20 ml of the acidic buffer or 12 ml of the basic buffer were added to 1 liter of dechlorinated tap water to achieve the desired pH.

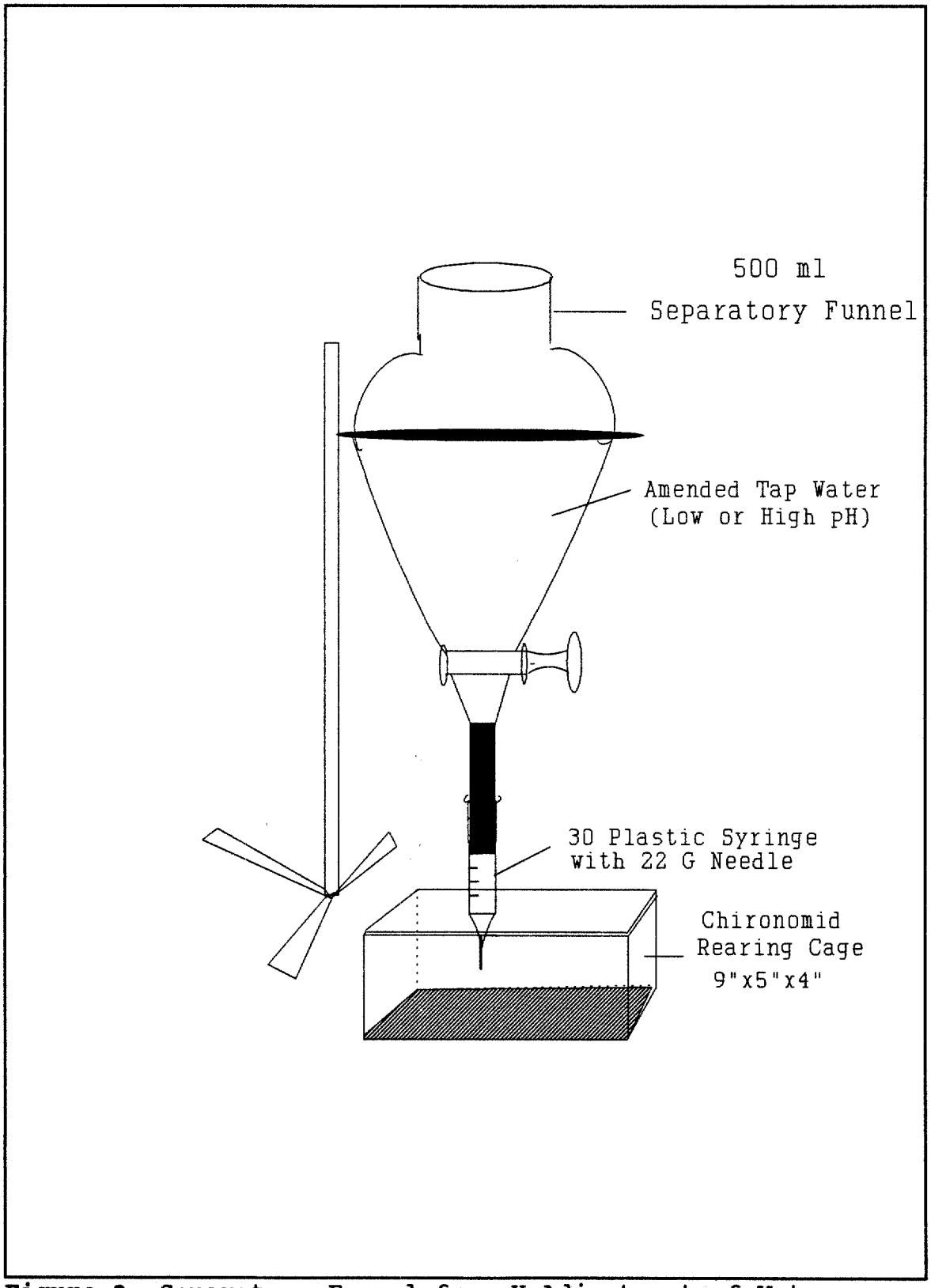


Figure 2. Separatory Funnel for pH Adjustment of Water.

Water and Sediment Collection

Since this research is concerned with the persistence and toxicity of cyfluthrin in water bodies of various pH, water and sediments were collected from areas with pH <7 and pH >8. The objective was to compare the toxicity of cyfluthrin in water collected from established water bodies of various pH with pH adjusted tap water.

A variety of area lakes and ponds were initially visited and checked for suitable pH values (Appendix B). Two sites were chosen as representative of low and high pH water bodies. A low pH site was selected in the piney woods of East Texas. Lake O' the Pines located in Marion and Morris Counties is a reservoir of approximately 19,000 acres (Figure 3). The lake water was tea colored due to the presence of organic acids such as tannic and humic, and salts of strong acids and weak bases. Water was collected from two sites at the extreme south end of the lake. The first collection site was located approximately 1.5 mi. east of highway 450, the other north of highway 726 near Watts Island. Both sites are fed by Big Cypress Creek.

The second water body chosen was a 1/12th acre man-made pond of high pH located in Denton County (Figure 3). This pond, fed by well water, is part of the Water Research Field Station facility and supports many types of aquatic fauna.

Water was collected from the littoral zone of all collection sites with a gas powered pump and transported back to the laboratory in sealed, black, 208 liter polyethylene containers. Water used for the toxicity experiments was filtered through filter floss to remove coarse suspended solids. A portion of the water, both filtered and non-filtered, was kept refrigerated at $4^0C \pm 4$ until water chemistry was performed.

Water quality parameters were chosen for their possible interactions with toxicity and overall importance to water quality. Detailed water quality methodologies are outlined in Appendix C.

Sediment Collection

The objective was to select sediments and retain some of the associated sediment microbes and characteristics that might be responsible for pesticide degradation. Sediments were taken from the same locations where water was collected, Lake O' the Pines, and the WRFS pond. Sediments were collected from the littoral region of each site in approximately 0.5-1 meters of water with a ponar grab. Sediments were refrigerated ($4^0C \pm 4$) immediately upon arrival at the University of North Texas. The procedure used for pH measurement is described in Appendix D.

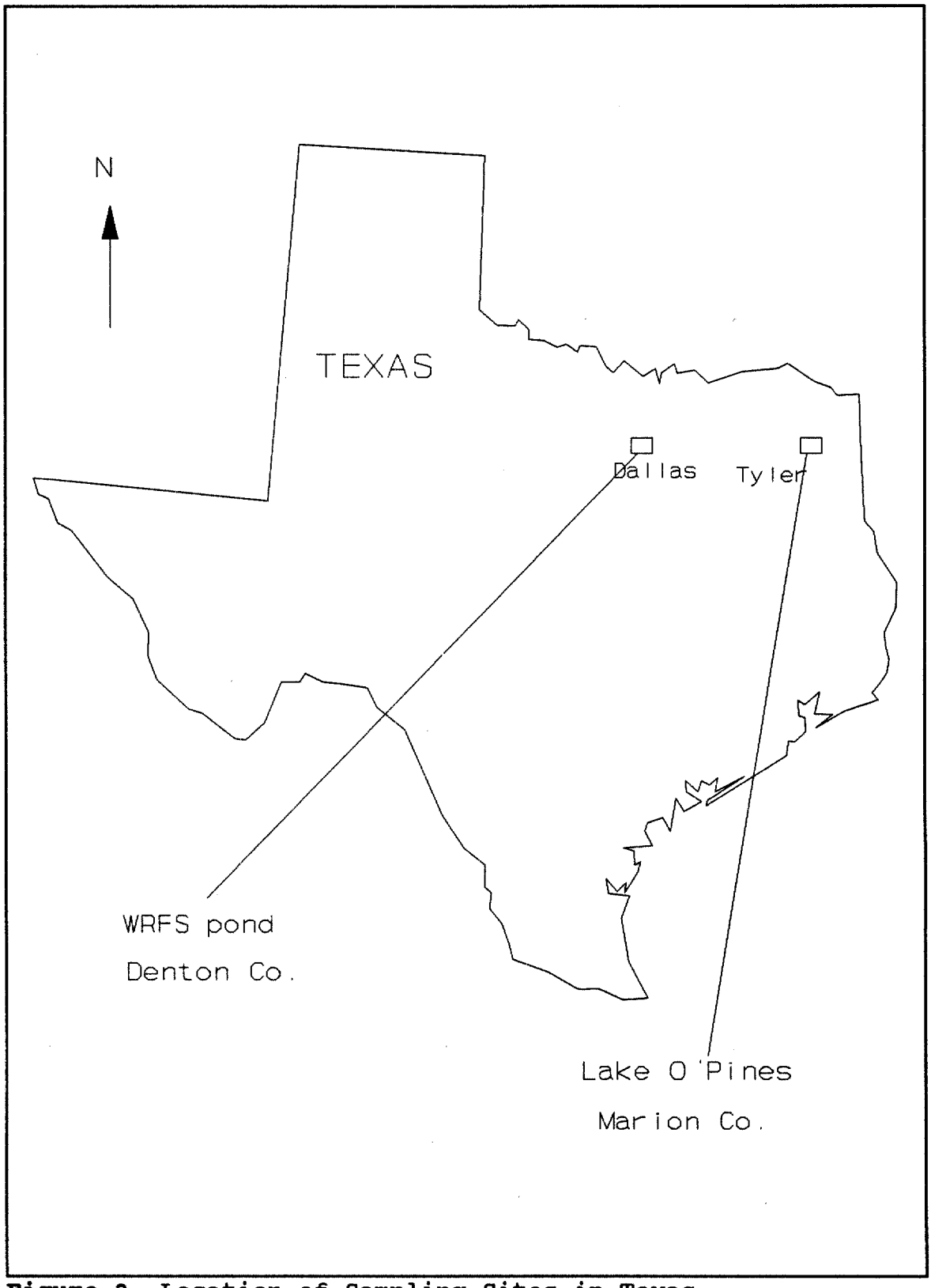


Figure 3. Location of Sampling Sites in Texas.

Evaluation of Test Vessels and Extraction Methods

Since cyfluthrin has a sorptive tendency due to its lipophilic nature, it was necessary to find a test vessel that maximized % recovery of pesticide while minimizing variability between replicate test chambers. Two test vessels were evaluated, a 250 ml beaker, and a 37.9 liter aquarium. Evaluation of both vessel designs was done with cyfluthrin treatment levels of 0.25, 0.75, and 1.00 ppb. Recovery of chironomids from the larger test vessel was also examined in this study. Chironomid containment inside the aquarium was evaluated in both a butyrate tube and glass petri dish.

The first test vessel was a 250 ml glass beakers filled to 200 ml. Glass beads (150-200 μm) were added to each beaker as a substrate to minimize stress to the chironomids. Five animals were added to each beaker. Six beakers were used per treatment; three replicates designated for residue analysis and three for toxicity. The water used in these tests was carbon filtered, dechlorinated tap water used in chironomid culture.

The second test vessel was a 37.9 liter aquarium (10 gallon) filled to a volume of 25 liters. The aquarium had a surface area of 6252 cm^2 which represents a surface to volume ratio of 250:1. During the aquarium experiments, two chironomid containment chambers were evaluated and are described below.

1) Butyrate Tube - Each aquarium contained 5 butyrate tubes (5" tall, 1 1/4 " diam.) with the sides partially removed and covered with Nitex® screen (3-500/49) (Fig. 4). Three chironomids were placed in a tube with glass beads (3 grams) used as a substrate.
2) Glass petri dish bottoms - Each aquarium contained 2 petri dishes (9.5 cm X 2 cm). Four chironomids were placed in each petri dish with glass beads (6 grams) as a substrate.

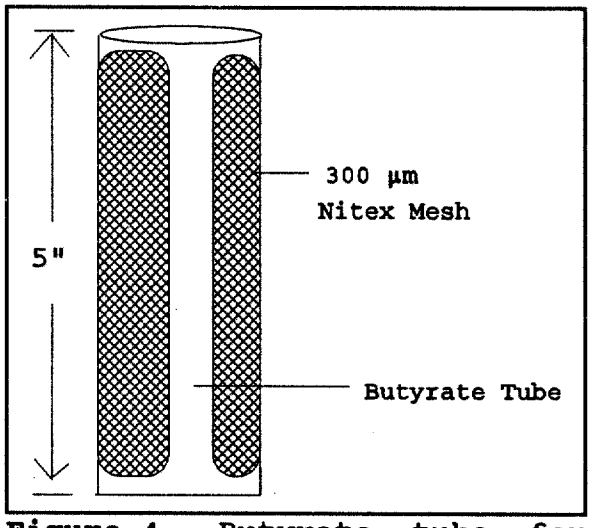


Figure 4. Butyrate tube for Chironomid Containment.

Aqueous Test Procedures

Dosing

Stock solutions of Baythroid^{R} 2 EC were prepared for each experiment in a carrier solvent (Optima acetone) due to the compounds' low water solubility (2 ppb). Stock solutions based on the active ingredient [25% cyfluthrin (w/v)] were made immediately prior to each experiment. Dosing was done by removing aliquots from the stock solution which was kept constantly stirring. Test aliquots were added under the surface of the water with a gas-tight Hamilton syringe to minimize volatilization. The solution was stirred into each test vessel (beaker or aquarium) for two minutes with a teflon spatula. Two drops of green food coloring were added with each aliquot to visually aid in making sure dispersal was complete. Solvent control animals were given food coloring and the same amount of acetone as the high dose, but without pesticide.

Range Finding Tests

Range finding tests were conducted to determine Baythroid^{R} concentrations needed to cause chironomid toxicity. Glass beads (150-200 μm) were added to each beaker or petri dish as a substrate to minimize chironomid stress. Five animals were added to each beaker. Six beakers were used per treatment; three replicates designated for residue analysis and three for toxicity. Range finding experiments

were simultaneously set up in the aquariums at the same concentrations. Two petri dishes with four chironomids per dish were added to each aquarium. Only 1 liter of water was withdrawn from each aquarium for residue analysis. The water used in these tests was carbon filtered, dechlorinated tap water. Water was removed from the test vessel one hour after dosing to determine pesticide recoveries as described in the analytical method section.

Definitive Tests

Definitive toxicity tests were performed to determine the effect of pH on cyfluthrin toxicity to chironomids and its persistence without the presence of a sorption matrix such as sediment. The aquarium test vessel with the petri dish containment chamber was utilized in these tests. For each set of experiments, petri dishes were filled with water from the test aquariums and 6 grams of glass beads. Four 2nd instar chironomids were placed into the water in each petri dish. Two dishes were lowered into each aquarium, randomly placed, and animals allowed to acclimate overnight. Every dish received 0.5 ml of the Tetra-Conditioning food suspension. Fluorescent lights were on a 16 hr. light - 8 hr. dark schedule in the test room, and ambient temperature ranged from 22-25 ^{0}C.

The next day, chironomids were observed for signs of stress, and any dead animals replaced. Analytical residue samples were taken 30 min., 24 hr and 48 hr post dosing. Visual observations of the animals were done 1-2X per day, and pH was monitored daily.

After 48 hours, animals were removed from each aquarium and prodded with a blunt probe. The criteria for mortality was failure to elicit a direct response to tactile stimulation (Stephenson, 1982). Since mortality was defined as immobility by the organisms, effective concentrations (EC50's) and not lethal concentrations (LC50's) were used as endpoints. After removal of immobilized animals at 48 hours, the persistence of toxicity was examined by placing new second instar *C. tentans* into each aquarium. Animals were monitored daily for signs of toxicity and replaced with new chironomids when found dead or immobilized. Animals were fed 0.25 ml food suspension every three days. This pattern of chironomid replacement continued until signs of recovery i.e, animals crawling and undulating normally, were seen.

Statistical Procedures

Differences in mortality within each water type were statistically compared using non - parametric analysis of variance (SAS, 1985). EC50's were calculated using the SAS probit procedure on log transformed data. Mortality response curves were judged to be significantly different if their 95 percent fiducial limits did not overlap.

Linear Regression was also performed to assess the relationship of concentration to mortality. Non linearity among concentrations was corrected by logarithmic transformation of dose. Raw percentages of chironomid response were normalized by arcsine transformation to meet assumptions of the regression analysis. Slopes of the lines between natural and tap water were compared to see if they approximated the same population (Zar 1984). Slope elevations were also compared if the slope lines were not determined to be significantly different.

Analytical Procedures

Residue samples for pesticide analysis during the range finding tests were taken 1 hr after dosing. In the beaker vessel, samples consisted of the whole amount of water (200 ml) which was poured into a 200 ml volumetric flask.
In the aquarium, 1 liter of water was removed for analysis. Water was removed from each aquarium using a 500 ml teflon bottle and poured into a 1 liter screw cap volumetric flask.

The extraction procedure was common to both test vessel samples. Optima grade hexane (5 ml) was added to each sample and the pesticide removed from the aqueous layer by vigorous stirring for 1 hour. After mixing, the organic layer was allowed to separate for 5 minutes. The organic layer containing the cyfluthrin was then removed from the narrow neck of the flask with a pasteur pipet and placed into a glass vial. Vials were sealed with an aluminum foil lined cap to prevent leaching of the cap glue into the sample. The extracted samples were stored in a -15 $\pm$ 10°C freezer until analysis.

On the day of analysis, samples were brought to room temperature and an aliquot of the hexane fraction removed with a pasteur pipet and transferred to an amber 1.2 ml autosampler vial for analysis by gas chromatography. Analytical grade cyfluthrin (95%) was used for standards. The residue extracts and analytical standards were analyzed by electron capture (Ni^{63}) gas chromatography (Hewlett-Packard model 5890A) using cool on-column injection and an uncoated retention gap. A fused silica megabore column (5 meter x 0.53 mm ID) was used with hydrogen as the carrier gas and argon:methane (90:10) as the make up gas. Cyfluthrin values were determined from a regression line developed from standards made by diluting a stock solution of 48,300 pg/μl. The stock solution was prepared by diluting analytical grade

cyfluthrin in Optima grade hexane. Standards and stock solutions were stored at -15 ± 10°C. Data handling was done via a 286 computer and Nelson Analytical chromatography software v.3.0 (Bland and Eitelman 1983; Chapman and Harris 1987). A summary of dosing and analytical residue procedures are illustrated in a flow diagram (Figure 5).

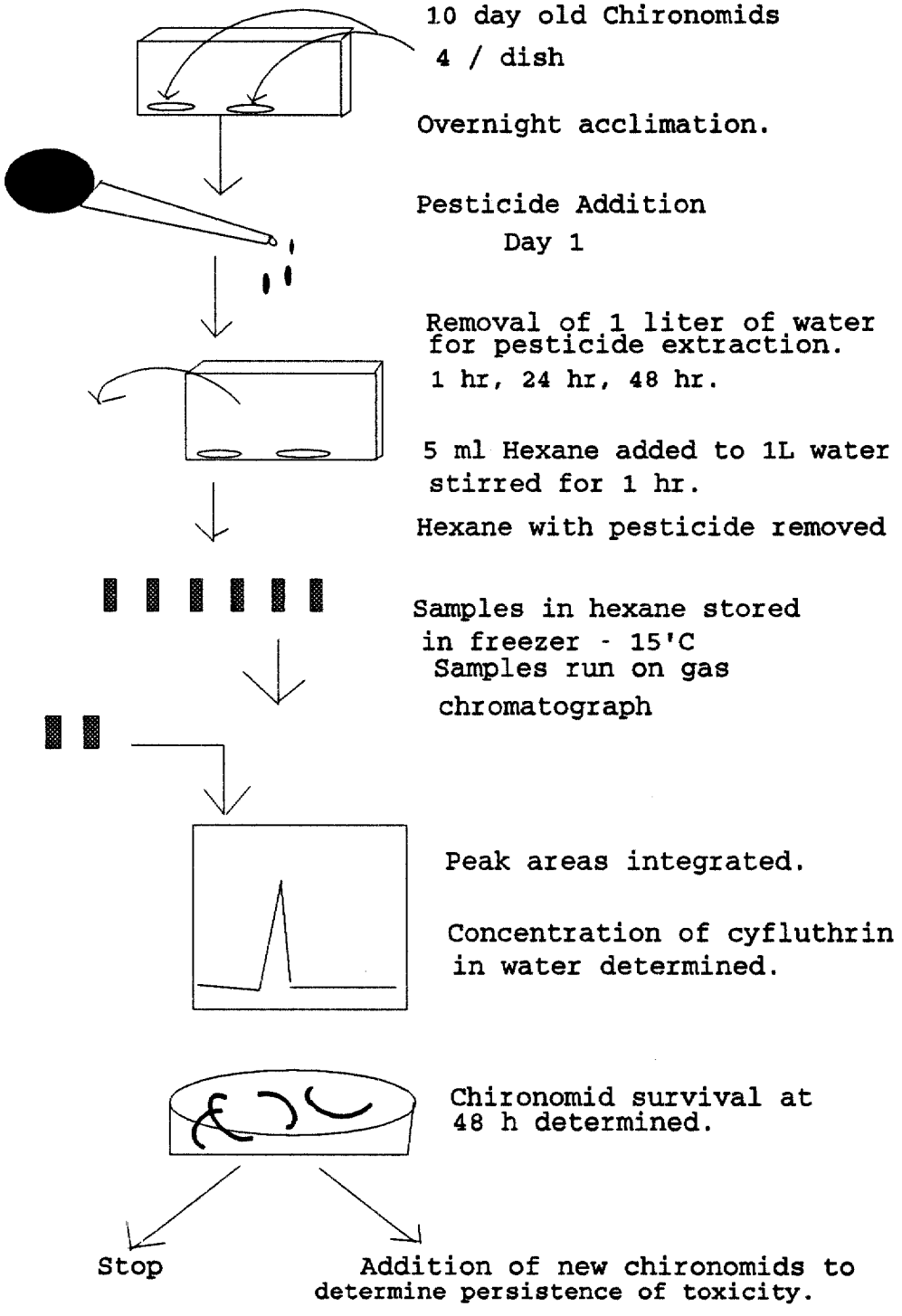


Figure 5. Experimental Flow Diagram of Procedures Used in the Aqueous Phase Toxicity Tests.

Sediment Test Procedures

Dosing

Due to previously determined interference of co-eluting isomers and a low detection limit (0.5 ppb) for cyfluthrin in sediment, ^{14}C labeled cyfluthrin was used in sediment toxicity tests. Radioactively labeled cyfluthrin (98 % pure) was made up in an acetone stock solution and a small aliquot counted using a liquid scintillation counter to determine activity. An appropriate aliquot was removed from the stock solution and added to a 250 ml amber glass jar containing 25 g of Kaolin potter's clay and 10 ml water. This solution was mixed on a stirplate for 1 h prior to dosing to ensure homogenous mixing. Cyfluthrin in the form of a sediment slurry was then added to each beaker. The control beakers received the same amount of acetone in a slurry as the high dose treatment, but without pesticide. The volume of acetone in the stock dosing solutions never exceeded 1 ml. Detailed dosing procedures and calculations are described in Appendix E.

Range Finding Tests

It was necessary was to establish a concentration of cyfluthrin that would result in chironomid mortality in sediments. All collected sediments were sieved to remove macroinvertebrates which could eat the chironomids. Sediments were tested for ambient toxicity by exposing *C. tentans* to each sediment for 10 days. Kaolin clay which was the dosing matrix, was also tested for ambient toxicity at various pH's.

Each beaker contained 10 ml of moist sediment and 40 ml of dechlorinated tap water (4:1 water to sediment ratio). The beakers were placed into an environmental chamber (22^{0}C; 16:8 light:dark) and suspended sediment allowed to settle over night. The following day two second instar (10 d old) chironomids were added to each beaker and allowed to burrow into the sediment. Dosing was done 8 hours later.

Definitive Tests

The purpose of the sediment tests was to add back one of the mitigating factors that would decrease toxicity under natural conditions. Being a sorptive compound with a high octanol/water partitioning coefficient, cyfluthrin will adsorb to macrophytes and particulate matter, removing a percentage of compound from the water column fairly quickly.

A Kaolinite slurry amended with cyfluthrin was added to

beakers to obtain concentrations that would span the exposure-response curve determined in the range finding tests. Test duration was 10 days and consisted of four treatment concentrations and an untreated control with 12-24 replicate beakers per concentration. Each beaker contained 10 ml of moist sediment and 40 ml of dechlorinated tap water amended to the desired pH with the buffers previously describe. Water was slowly trickled onto the sediment and suspended matter allowed to settle. When the water was clear, two early 2nd instar (10 d old) chironomids were added to each beaker and allowed to burrow into the sediment over night. Beakers were placed in an environmental chamber on a 16:8 L:D schedule at 22 ^{0}C. The next day, all the beakers were dosed with the cyfluthrin-Kaolin slurry. Controls received an identical amount of slurry prepared in the same manner, but without pesticide. Animals were fed 1 drop of Tetra food slurry every 3rd day to ensure adequate food supply.

Mortality was used as an endpoint so the lethal concentration to fifty percent of the chironomids (LC50) was calculated. After 10 days, the sediment in each beaker was screened through a 150 μm ASTM metal sieve and the number of dead animals recorded. Replicate beakers (without chironomids) were set up with each test for analytical analysis.

Statistical Procedures-Sediment

Differences in mortality within each collection site and between the two sites were statistically compared using one way analysis of variance (SAS 1985). LC50's were calculated using the probit procedure (SAS 1985) using log transformed concentration data (Finney 1971) or, if the data were insufficient for probit analysis, by the moving average angle method. Mortality response curves were judged to be significantly different if their 95 percent fiducial limits did not overlap.

Analytical Procedures-Sediment

From the replicate set of beakers for residue analysis, 2 ml of water was withdrawn from each of three replicates at each dose level and placed into a scintillation vial for counting. Fifteen ml of Aquasol cocktail was added to each vial and the mixture vigorously shaken to ensure a homogenous mixture. The remaining water in each beaker was carefully removed via vacuum suction so as not to disturb the sediment layer and discarded. The top 2 mm of sediment was then removed with a spatula and placed in a vial for counting. Removal of this surface layer was facilitated by the fact that the slurry previously added was white in color and easily seen. All sediment and water analyses were performed on a Beckman LS 100 liquid scintillation counter.

Samples were counted for a total of 5 minutes and a ^{14}C standard counted with each run.

Assessment of pH values in Natural Waters

Information obtained through STORET was downloaded to the mainframe computer system at the University of North Texas. The data was reformatted for the SAS system and a hardcopy generated. Within each cotton producing state, there were numerous sites at which pH had been taken throughout the water quality year 88-89. These values were taken by various state agencies throughout the country and no record of the methodologies or quality control used was provided.

Sites within a river system (or lake) were coded according to the majority of the pH values found there. The three pH criteria were: $pH \geq 8.00$, $pH \leq 6.99$, and $7.99 \geq pH \geq 7.00$. If there was no clear pattern of pH, site locations were further subdivided into pH categories. For example, a river system throughout 3 counties might have a different pH at the headwaters than at the river's end. Both sites in this case would be given their proper pH values.
Multiple sites on different water bodies were looked at within a county. Only five states that used pyrethroids on cotton were examined in this manner.

CHAPTER 3

RESULTS

Evaluation of Test Vessels

The two vessels evaluated for their ability to maximize pesticide recovery were a 250 ml beaker and 25 L aquarium. The experimental design with the beaker was repeated five times. Each resulted in varying toxicity to the chironomids using the same nominal concentration of cyfluthrin. In addition, there was poor reproducibility among replicate pesticide recoveries (Table I). Due to the pyrethroids chemical characteristics, sorption onto the glass walls was thought to be a problem (Sharom and Solomon 1982). The 250 ml beaker has a high surface area (190 cm^2) relative to the volume of water (0.250 L) which represents a surface to volume ratio of 750:1. To circumvent this problem, a larger test vessel with a greater volume to surface area was evaluated.

A 25 liter aquarium (10 gallon) was successfully used to conduct the aqueous phase tests. The aquarium has a surface area of 6252 cm^2. This represents a surface to volume ratio of 250:1. In a 25 L aquarium, there is much

less surface area relative to volume for sorption to occur when compared to the beaker.

Chironomid Containment Chambers

Within the aquarium, two chambers were evaluated for their ability to contain the test organism. The butyrate tube design was rejected due to poor recovery and survival of chironomids. Some chironomids became stuck in the nylon mesh seams and died. Also, the butyrate tube provided more surface area within the aquarium for sorption to occur.

Recovery of animals in the petri dish was excellent and control survival was 100 %. Petri dishes were therefore selected as the test chamber with the aquarium test vessel for the definitive aqueous toxicity experiments.

Aqueous Toxicity Tests

Basic pH

Chironomids at all dose levels were noticeably impacted by cyfluthrin within 8 hours of dosing. Animals which had been burrowed in the glass beads came up onto the substrate surface and ceased normal undulating behavior. Abnormal behavior such as coiling, twitching, and immobilization were seen. Abnormal behavior in the low doses (0.25 - 0.575 ppb) ceased after 24 hr. and normal undulating movements resumed, although the animals did not return to their burrows. In the higher doses (1.0 - 1.575 ppb)

however, larvae continued to exhibit abnormal behavior and remained immobilized. Some larvae at this point appeared to have lost their red coloring (hemoglobin) and to be dead. After 48 hr., EC50's and 95 % confidence intervals were calculated and are shown in Table II. All EC50's were calculated based on analytical concentrations of cyfluthrin in the aquariums. The EC50 values obtained with dechlorinated tap water were unexpectedly lower than those in pond water of the same pH. Therefore, a second experiment was run with tap water at pH 8.6 to verify the results previously obtained. The new EC50 in dechlorinated tap water was 0.62 ppb, a value almost identical to that obtained previously.

Results of water quality analyses (Table III) show the WRFS pond was considerably softer than the tap water and higher in conductivity. The dissolved oxygen in all experimental tanks was between 66 and 88 % saturation for the duration of the test. Cyfluthrin concentrations in tap water of pH 8.0 and pond water of pH 8.1 were measured (Table IV). Measured concentrations one hour after application were within 75.5 % of nominal in the tap water, and 89.2 % of nominal in pond water. The degradation of cyfluthrin over 48 hr for both tap and pond water at the high pH values was determined via gas chromatography (Figure 6). Averaged across all concentrations, only 30 % of the initial dose remained in tap water after 48 hr. compared

with 56 % in pond water (Figure 6B).

To determine if cyfluthrin concentration played a role in chironomid mortality, data were statistically evaluated. Mortality was significantly influenced by treatment level. In tap water of pH 8.0, ranked mortality was significantly different from control only at the highest dose (Kruskal-Wallis Analysis of Variance (AOV) by ranks, $p<0.002$, Dunn's MRT $\alpha=0.05$). In lake water (pH 8.1), mortality was significantly different from that of control only at the high dose (Kruskal-Wallis AOV, $p<0.001$, Dunn's MRT).

Linear regression was performed on each of the two data sets at high pH. Non linearity among concentrations was corrected by logarithmic (base 10) transformation of dose. Since the raw percentages of chironomid response were not distributed in a normal fashion, data were normalized by arsine square root transformation. R^2 values of 0.61 and 0.90 were obtained in tap water and pond water respectively (Figure 7). For pond water (pH 8) this means that 90 % of the variation in chironomid response is accounted for by the regression model. In both experiments, chironomid response and dose were highly significantly related ($p<0.001$, AOV). The slope of the regression lines in each experiment were also compared against each other to determine if they were significantly different. For any given slope, there exist an infinite number of possible regression lines, each with a

different Y intercept. The slopes relating response to dose were not significantly different between tap and lake water of pH 8 (t test for slopes, $p>0.20$).

These results indicate that two different populations (water types) had been sampled. Slope elevations between the two regression lines were also compared and found to be significantly different (t test for elevations, $0.02 > p > 0.01$). The lines describing each model are therefore parallel and for a given dose, the predicted response values will not be equal. Since the two population coefficients do not have different slopes but do have different elevations, the slopes computed from the two samples are both estimates of the common population regression coefficients and this common slope must be assigned to both equations.

Table I. Extraction Conditions and Analytical Recovery of Cyfluthrin. Concentrations are in μg/L. Volumetric Sizes 1000 ml and 500 ml Were Used for the Aquaria, and a 200 ml was Used for the 250 ml Beaker Extractions.

Sampling Time (Hr)	Nominal Dose	Volumetric Size (ml)	Extraction Time (min)	% Recovery mean ±SD
0.5	1.0	1000	60	125 ± 23.2
1	1.0	1000 500 200	15	73.0 ± 25.5 56.5 ± 17.7 9.50 ± 0.71
	3.0	1000 500 200		63.3 ± 0.35 52.3 ± 0.35 4.5 ± 2.1
	6.0	1000 500 200		101 ± 0.35 101 ± 0.35 11.0 ± 1.41
20	1.0	1000 500 200	15	29.0 ± 21.2 22.3 ± 7.50 7.50 ± 3.53
	3.0	1000 500 200		43.5 ± 0.71 26.5 ± 3.53 25.5 ± 26.2
	6.0	1000 500 200		95.0 ± 7.07 100 ± 0 7.50 ± 0.71
72	1.0	2000	45	48.5 ± 2.12
	3.0	1000 2000 1000	45 15 15	30.0 ± 11.3 41.0 ± 8.48 30.0 ± 4.24

Table II. Calculated EC50's of *C. tentans* Exposed to Cyfluthrin in Natural and Dechlorinated Water of Varying pH. Temperatures Ranged from 22 - 25 ^{0}C. Calculations Are Based on Measured Concentrations. (*n*= 9-12).
(α = calculation based on nominal concentration)

Time (hr)	EC50 (μg/L)	95% confidence limits	pH	Water Source
48	0.607	0.474 - 0.896	8.0	Denton tap
48	0.323 α	0.191 - 0.475 α	8.1	WRFS pond
48	0.434	0.212 - 0.627	7.2	Denton tap
48	0.221	0.017 - 0.386	7.2	Lake O' Pines
48	0.366	---------	6.6	Denton tap
48	0.214	0.183 - 0.247	6.0	Lake O' Pines

Table III. Water Quality Results for Aqueous Toxicity Experiments

Sample I.D.	Alkalinity mg L^{-1} as CaCO3	Hardness mg L^{-1} as CaCO3	pH	Conductivity μmHos/cm
TAP-1	94-120	98-120	7.20	660-880
LAKE	10-25	94-244	7.22	180-200
TAP-1	35-250	80-152	6.60	620-800
LAKE	5	20-26	5.97	-----
TAP-2	90-150	95-170	8.00	630-800
POND	190-235	40-60	8.10	710-750

* All water chemistry taken pre and post experiment.
Tap-1 = dechlorinated Denton City water + buffer + acetone.
Tap-2 = dechlorinated Denton City water + acetone.
Lake = water from Lake O'the Pines, near Tyler, TX.
Pond = Water Research Field Station pond #26 water, Denton, TX.

Table IV. Cyfluthrin (a.i) Loading Rates and Residue Results from Aqueous Phase Study. All concentrations are in µg/L. Percent Recoveries are Averaged Samples One Hour After Treatment ± S.D. (n=3).

Treatment Parameters	Levels and	Tap Water pH 8.0	Pond Water pH 8.1
Dose 1	Nominal	0.250	0.075
	% Recovery	83.00	127
	Std. Dev.	± 0.030	± 0.010
Dose 2	Nominal	0.750	0.575
	% Recovery	68.6	93.0
	Std. Dev.	± 0.230	± 0.040
Dose 3	Nominal	1.00	1.08
	% Recovery	± 75.0	± 0.140
	% of Nominal	75.0	40.0
Dose 4	Nominal	-----	1.58
	% Recovery	-----	96.8
	Std. Dev.	-----	± 0.85

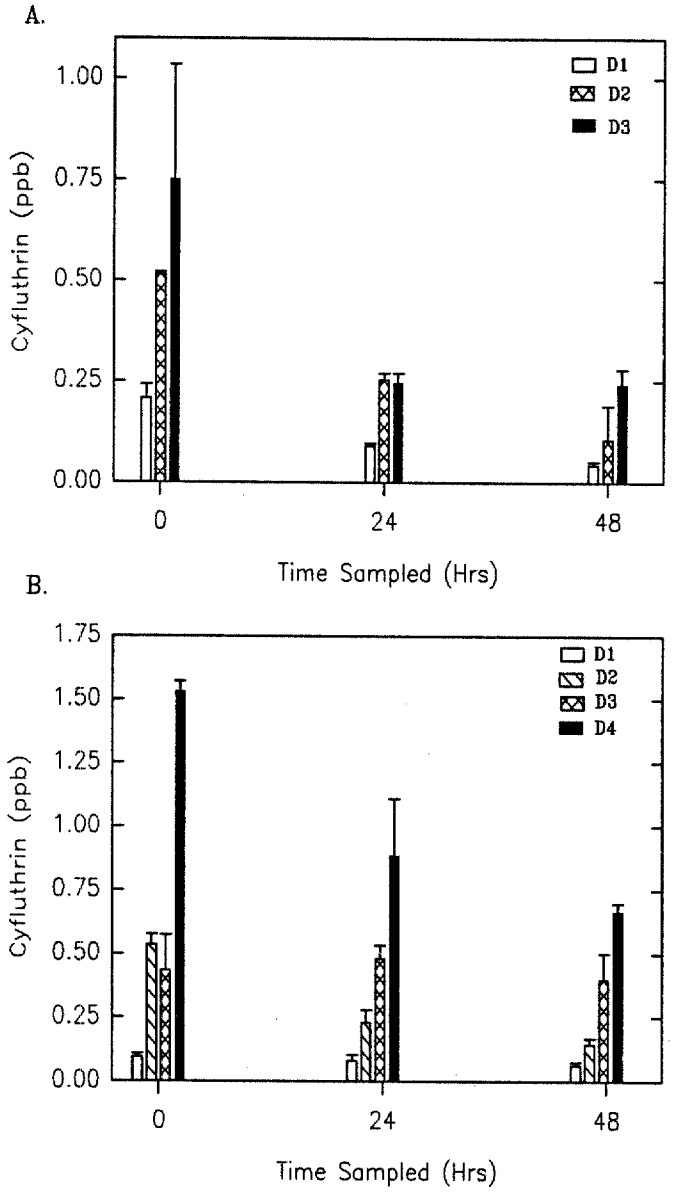


Figure 6. Degradation of Cyfluthrin in WRFS pond water (A) and tap water (B) of pH 8. Mean ± standard deviation, *n*=3.

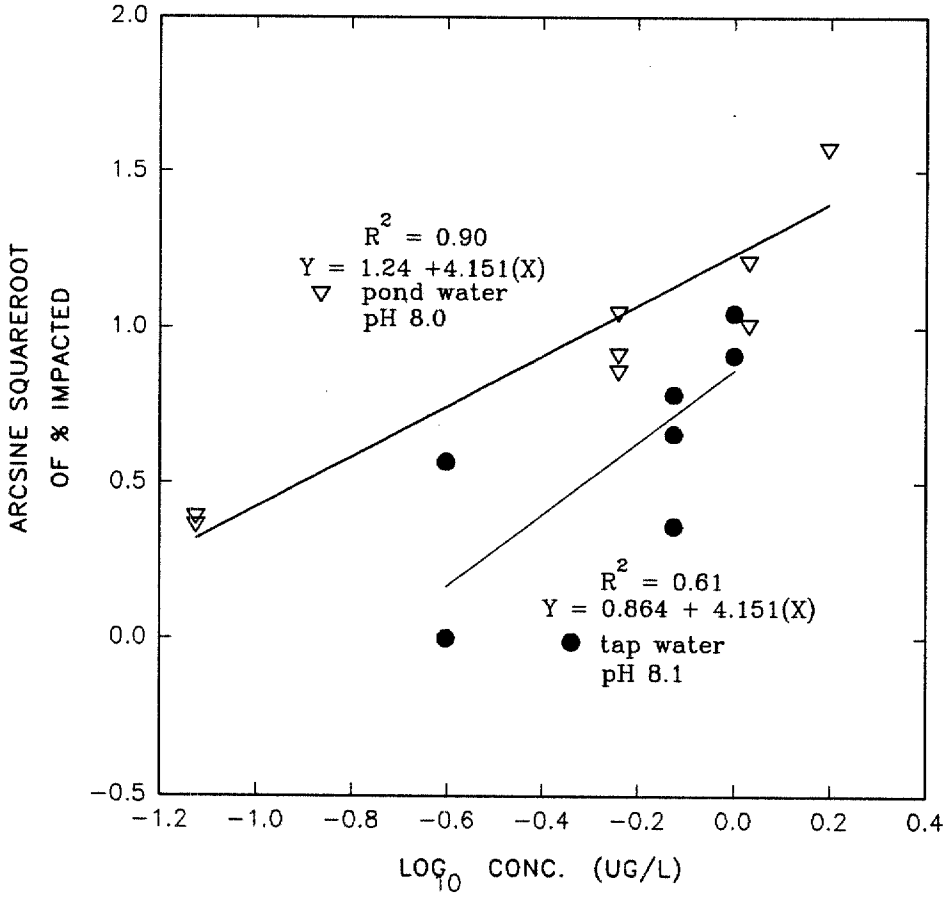


Figure 7. Comparison of Regression Analysis Between WRFS Pond and Tap Water Toxicity Results at pH 8. Response is Percent of Affected Chironomids.

Neutral pH

After overnight acclimation in the petri dishes, chironomids had formed burrows in the glass beads and were observed undulating normally. Addition of pesticide brought most larvae out of their burrows. This response was not seen in control tanks to which the carrier (acetone) alone had been added. Three hours after dosing, severe twitching and body spasms were seen in the medium and high dose animals (0.82 ppb and 1.42 ppb respectively). After approximately 8 hrs, chironomid larvae in treatment tanks were unburrowed and exhibiting aberrant behavior atypical of controls characterized by severe twitching and coiling of body, paralysis, and inability to crawl. All control and solvent control animals remained in their burrows. This behavior was characterized as normal. Acetone as a carrier introduced into the tanks never exceeded 0.1 ml/L (Stratton 1985) and dissolved oxygen levels were between 88 and 99 % saturation throughout the experiment.

Within 22 hours, little movement could be detected in treated animals. All the chironomids however, had retained their hemoglobin (red color) and from past experiments this meant they were probably immobilized, but not dead. At 24 hr., slight twitching was seen in animals that only two hours earlier had shown no movement. After 48 hr, animals were removed and the number of animals unable to exhibit a response to tactile stimulation recorded. Based on EC50

values, the toxicity of cyfluthrin to *C. tentans* appeared almost two times greater in lake water compared with tap water (Table II). In both tap and lake water of pH 7, ranked mortality was significantly influenced by only the highest dose when compared with control (Kruskal-Wallis AOV ranked, $p<0.001$, Dunn's MRT).

Water quality results for the neutral pH experiments are presented in Table III. Alkalinity values increased slightly (10 to 25 mg/L as $CaCO_3$) and hardness decreased (244 to 94 mg/L as $CaCO_3$) when pesticide was added. An increase in only 0.23 pH units was observed throughout the test duration.

Measured concentrations of cyfluthrin throughout the neutral pH tests are shown in Table V. Average recovery was 110 % of nominal in the tap water, and 106 % of nominal in lake water one hour after application. Degradation of cyfluthrin over 48 hr for both tap and lake water at neutral pH values was determined by gas chromatography (Figure 8). Averaged across all doses, 45 % of the initial measured dose was left in the tap water after 48 hr (Figure 8 A) compared with only 25 % in lake water (Figure 8 B).

Linear regression was performed on each of the two data sets at neutral pH. Regression coefficients (R^2) of 0.61 and 0.59 were obtained in tap water and pond water respectively (Figure 9). For both experiments, only about three fifths of the variation in response was accounted for by the variation in dose level. However, response and dose were highly significantly related ($p<0.001$, AOV) indicating a dependence of chironomid response on dose. The slope of the regression lines were compared against each other to see if there was a statistical difference. No significant difference between the slopes of the two lines at pH 7.2 was determined (t test for slopes, $p>0.50$). The elevations between the two regression lines were also compared and found to be significantly different (t test for elevations $p<0.001$). Since the two population coefficients do not have different slopes but do have different elevations, the slopes computed from the two samples are both estimates of the common population regression coefficients. Since the elevations but not the slopes are different, the lines are parallel and for a given dose, the predicted response will not be equal.

This set of experiments suggest that different water types of neutral pH may influence the toxicity of cyfluthrin to *C. tentans*. As in the previous experiment with basic water, cyfluthrin in lake water (pH 7) appeared more toxic to chironomids than tap water of the same pH.

Table V. Cyfluthrin (a.i) Loading Rates and Residue Results from Aqueous Phase Study. All Concentrations are in µg/L. Percent Recoveries are Averaged Samples One Hour after Treatment ± S.D. (*n*=3).

Treatment Parameters	Levels and	Tap Water pH 7.2	Lake Water pH 7.2
Dose 1	Nominal	0.220	0.250
	% Recovery	130	100
	Std. Dev.	± 0.06	± 0.05
Dose 2	Nominal	0.820	0.750
	% Recovery	102	100
	Std. Dev.	± 0.180	± 0.140
Dose 3	Nominal	1.42	1.25
	% Recovery	98.0	117
	Std. Dev.	± 0.250	± 0.190

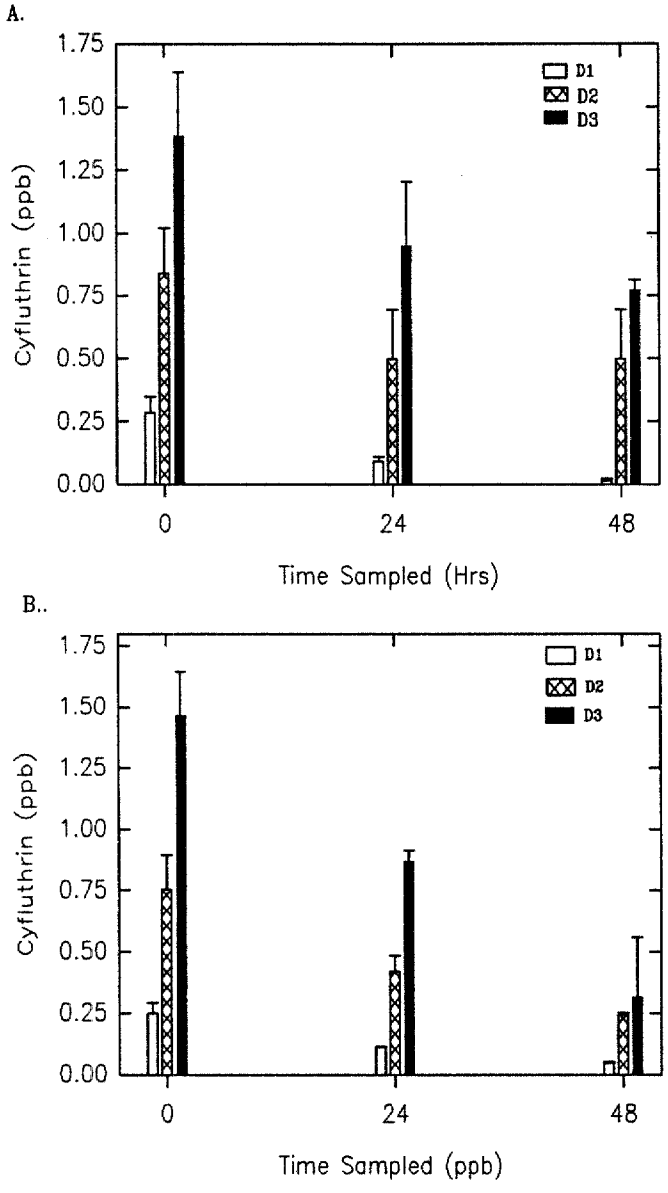


Figure 8. Degradation of Cyfluthrin in Lake O' the Pines water (A) and tap water (B) of pH 7. Mean ± standard deviation.

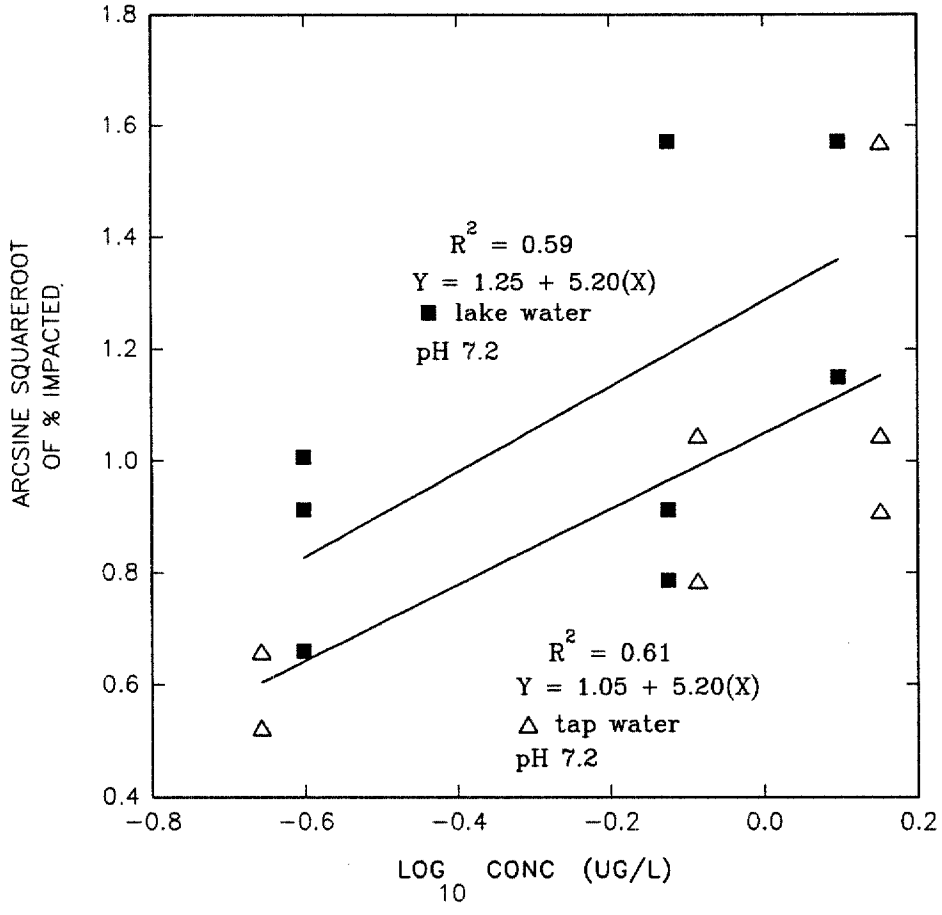


Figure 9. Comparison of Regression Analysis Between Lake O' the Pines and Tap Water Toxicity Results at pH 7. Response is Percent of Chironomids Affected.

Acidic pH Tests

From preliminary range finding experiments, it was necessary to drastically lower the treatment levels at low pH in order to obtain a dose response curve. Similar results in terms of abnormal behavior were observed at this pH as were seen in the experiments at higher pH levels with one exception. The recovery of normal undulating behavior that was seen at 24 hr in the higher pH experiments was not observed. Animals in all doses continued to remain immobilized after 48 hr. and only some sporadic twitching was seen at the low dose (0.15-0.035 ppb). Animals did not start to recover after 24 hr, or even after 48 hr when toxicity results were recorded. EC50 results for both tap and lake water were obtained by probit analysis (Table II). Based on the EC50, lake water dosed with cyfluthrin appeared to be more toxic to the chironomids than tap water of the same pH. These findings are consistent with those in both basic and neutral water.

After 48 h, new 10 day old chironomids were added to replace immobilized chironomids at each dose level. This was done in an effort to determine the persistence of cyfluthrin as measured by toxicity. New 10 day old chironomids were added 5, 8 and 14 days after initial dosing to tap water of pH 6.6. On day eight (192 h), many chironomids added on day 5 were beginning to crawl and undulate normally in the low dose (0.15 ppb). No survival was seen in the other doses.

Animals added on day 8 were surviving by day 11 in the low dose, but neither the medium (0.75 ppb) nor high (1.35 ppb) doses supported life. After fourteen days (336 h) however, chironomids added on day 8 were starting to survive in all doses and exhibit normal behavior (Figure 11). No original chironomids were alive after 48 h in the high dose. There was 90 % survival of all control animals throughout the test duration.

Water quality in Lake O' the Pines differed in both alkalinity and hardness from acidified tap water (Table III). Lake water was softer and lower in alkalinity than the tap water.

Analytical residue recoveries in both tap water and lake water of pH 6 remained close to or above nominal values throughout the 48 hr. test duration (Table VI). The degradation of cyfluthrin throughout time was slow and a half-life was not obtained within 48 hours in either tap or lake water of pH 6 (Figure 10). Averaged across all doses, 75 % of the initial measured dose remained in tap water at 48 hours (Figure 10 A), and 133 % remained in lake water (Figure 10 B). The persistence of cyfluthrin in the water column lead to analytical recoveries over 100 %, especially in the lowest doses near the detection limit.

In order to determine if there was any difference in mortality, data were evaluated by analysis of variance. Ranked mortality was significantly influenced by the high

level dose in each experiment (Kruskal-Wallis AOV ranked, $p<0.001$, Dunn's MRT).

Regression analysis was performed on each of the two data sets at low pH. R^2 values of 0.80 and 0.81 were obtained in tap water and pond water respectively (Figure 12). In both experiments, chironomid response and dose were significantly related ($p<0.001$, AOV) indicating a dependence of chironomid response on dose concentration. The slope of the lines in each experiment were also compared against each other to see if they were equal. There was no significant difference in the slopes of the lines between tap or lake water of pH 6 ($p>0.50$). Elevations between the two lines were then compared and found to be highly significantly different (t test for elevations, $p<0.001$).

Since the two population coefficient do not have different slopes but do have different elevations, the slopes computed from the two samples are both estimates of the common population regression coefficients.

These results indicate that cyfluthrin persistence at pH 6.0 - 6.6 is accompanied by persistent toxicity to *C. tentans* in both tap water and Lake O'the Pines water. The combination of lake water and cyfluthrin was again more toxic to the chironomids than tap water of the same pH.

Table VI. Cyfluthrin (a.i) Loading Rates and Residue Results from Aqueous Phase Study. All concentrations are in µg/L. Percent Recoveries are Averaged Samples One Hour after Treatment ± S.D. (n=3).

Treatment Parameters	Levels and	Tap Water pH 6.6	Lake Water pH 6.0
Dose 1	Nominal	0.150	0.035
	% Recovery	96.7	470
	Std. Dev.	± 0.09	± 0.03
Dose 2	Nominal	0.750	0.150
	% Recovery	89.6	151.7
	Std. Dev.	± 0.46	± 0.06
Dose 3	Nominal	1.35	0.315
	% Recovery	25.0	63.7
	Std. Dev.	± 0.38	± 0.22
Dose 4	Nominal	-----	0.945
	% Recovery	-----	100
	Std. Dev.	-----	± 0.05

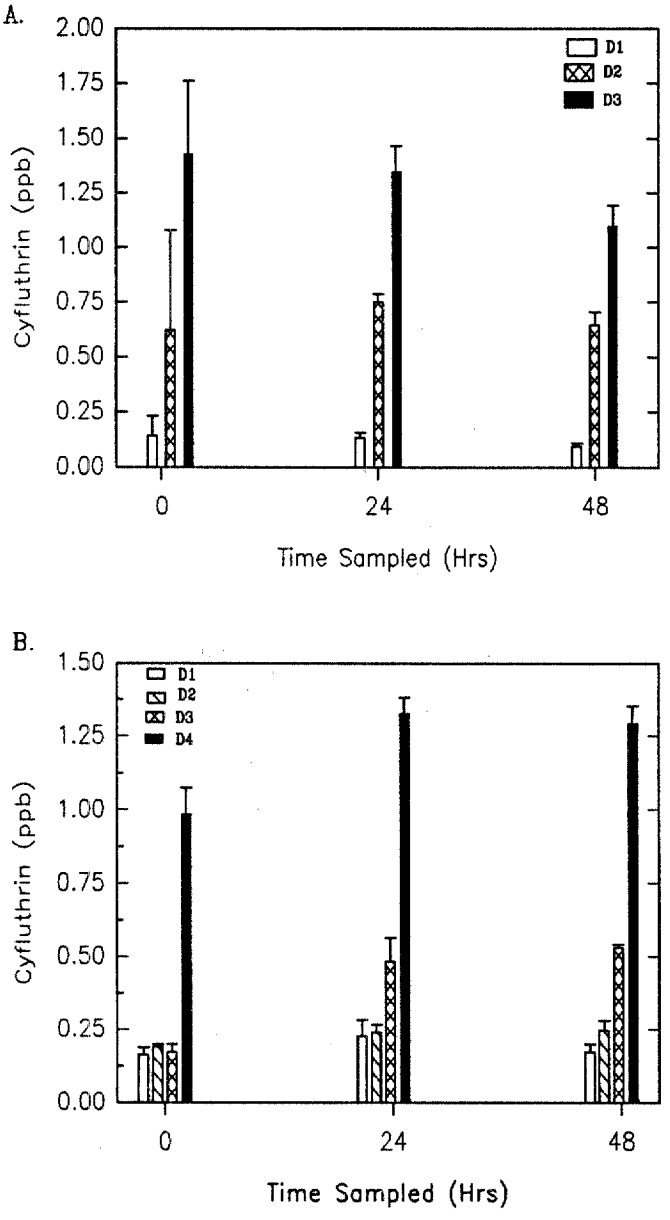


Figure 10. Degradation of Cyfluthrin in Lake O' the Pines water (A) and tap water (B) of pH 6. Mean ± standard deviation.

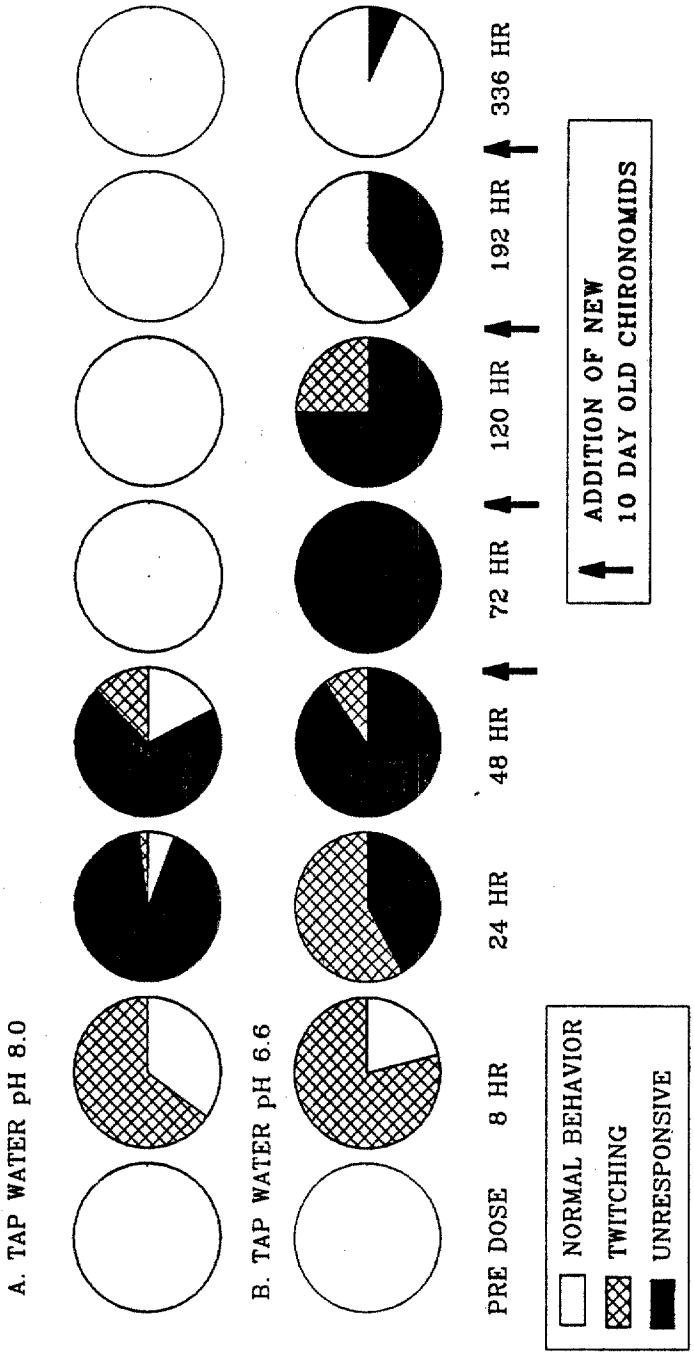


Figure 11. Behavioral Characteristics of Chironomids Exposed to Cyfluthrin at High and Low pH. (1.00 ppb at pH 8.0, 0.625 ppb at pH 6.6). Normal = responsive and/or burrowed. Unresponsive Animals were Replaced Until Normal Behavior Occurred.

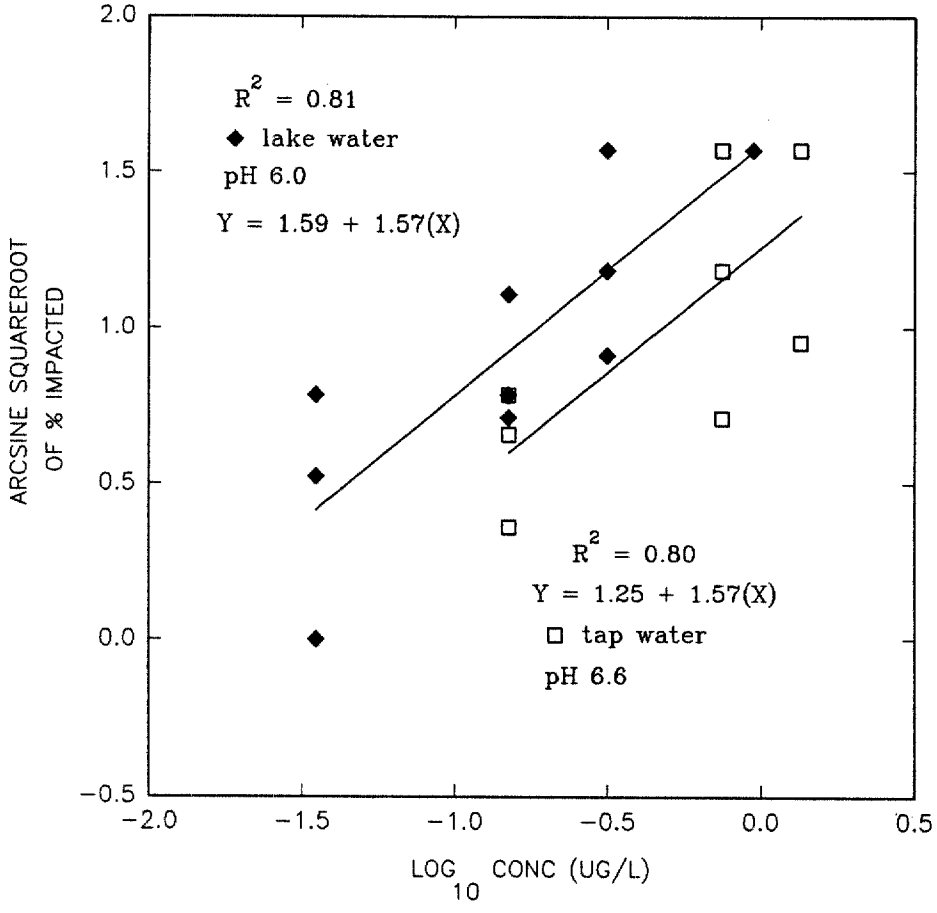


Figure 12. Comparison of Regression Analysis Between Lake O' Pines and Tap Water Toxicity Results at pH 6. Response is Percent of Affected Chironomids.

Toxicity of Kaolinite Clay

Sediment toxicity tests were conducted with overlying water collected from the same source as the sediment. The matrix of the dosing slurry however was a clay. Kaolinite is a mineral clay which was chosen for its negligible organic carbon content (<0.01 %) and its homogenous nature. The clay's make up is given in Table VII. When added to water, layers of aluminosilicate alternate between water layers and swell when exposed to water or hydrocarbons. Bilayers of hydrocarbons can easily be accommodated between the aluminosilicate sheets via adsorption.

Initial test results indicated that the clay itself would not cause toxicity at various pH's. No significant difference in ranked mortality at any pH was found when clay was used as a substrate (Kruskal-Wallis AOV ranked, $p>0.1$). The dosing matrix also did not clump when added to each beaker. The matrix spread evenly across the water surface and quickly settled out onto the bottom. Using pesticide amended clay, an LC50 of 1.518 ppb was determined via probit analysis. Due to these results the potter's clay (Kaolinite) was used as the dosing matrix in the definitve sediment toxicity experiments. Sediment dosing calculations are fully outlined in Appendix E.

Table VII. Analysis of EPK (Kaolinite) Clay Used as the Dosing Matrix

COMPOUND	% COMPOSITION
SiO_2	46.62
Al_2O_3	38.31
Fe_2O_3	0.38
TiO_2	0.07
CaO	0.33
MgO	0.25
K_2O	0.68
Na_2O	0.30
Other ingredients	13.2

Sediment Toxicity Tests

Alkaline Sediments and Natural Water

Sediments and water were collected from the littoral region of the WRFS pond and water chemistry conducted (Table VIII). The pond water was soft with a pH of 8.55. The sediments collected from the pond were a sandy loam of pH 8.6. Chironomids burrowed readily into the sediments and their burrows were often visible. The dosing matrix settled out evenly over the surface of the sediment and after a few hours, water clarity returned. No abnormal behavior was seen since the chironomids remained burrowed. Dosing did not bring the animals out of their burrows. The control animals were dosed with an identical slurry of Kaolinite minus

pesticide. Since there was a carrier solvent involved, controls were dosed with the same amount of acetone added to the high dose beakers. No adverse effects were seen in control animals.

After 10 days, the contents of each beaker were sieved and an LC50 of 2.64 ppb was determined by the probit method (Table IX). Mortality in treatment levels 4 and 5 were determined to be different from control and treatments 1 and 2 (AOV w/Bonferroni t-test, p=0.05).

In order to determine cyfluthrin loadings, water and sediment were removed from each beaker and analyzed for cyfluthrin over a period of 9 days. Sediment levels of cyfluthrin decreased after 24 hr. while the amount present in water increased slightly in each dose over time (Figure 13).

In pond sediment and water of high pH, a transition occurred after day 2 of the test. Cyfluthrin residue leached out of the sediment and into the water column. This trend however was only discernable in the higher doses. Sediment and water counts reached an equilibrium by the end of the test, with degradation occurring.

Table VIII. Characterization of Water and Sediments from WRFS Pond.

Parameter	Quantity (n=3)
WATER	
Alkalinity mg $CaCO_3$/L	310
Hardness mg $CaCO_3$/L	21
TSS (mg/L)	30.67
TOC (mg/L)	5.809
DOC (mg/L)	3.531
POC (mg/L)	2.279
Salinity (mg/L)	240
SEDIMENT	
% sand	59.68
% clay	20.88
% silt	19.44
pH	8.6
% organic matter	0.2

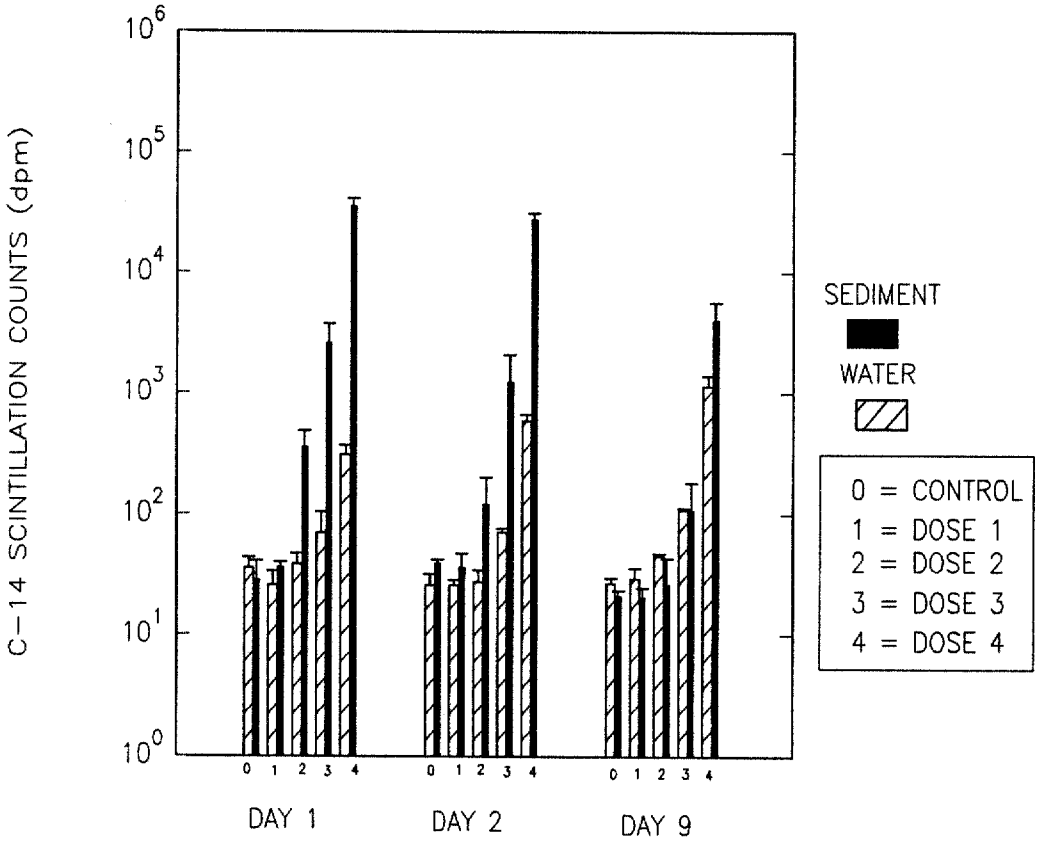


Figure 13. ^{14}C Cyfluthrin in WRFS Pond Water and Sediments, pH 8. Samples Taken on Days 1, 2, 9. Mean ± standard deviation, *n*=4.

Table IX. Probit Analysis of Chironomid Mortality in Sediments of Different pH values.

Water and Sediment Identification	LC50 (ppb)	95 % C.I. (lower/upper)
WRFS pond, Denton, TX (water and sediment)	2.64	0.980/6.24
Lake O' Pines, Tyler TX (water and sediment)	0.232	0.103/0.425
Lake O' Pines sediment (tap water)	0.095	0.055/0.246

Table X. Water Quality Characteristics Lake O' the Pines (Fall 1990).

WATER QUALITY	
Parameter	Result
Alkalinity as mg/L $CaCO_3$	25
Hardness as mg/L $CaCO_3$	26
pH	6.40
Temperature ^0C	30
Conductivity (μmHos/cm)	230

Acidic Sediments and Natural Water

Sediment (pH 7.0) and water (pH 6.4) were collected from Lake O' the Pines during Fall 1990. Results of the water quality analyses are shown in Table VIII. Both alkalinity and hardness tests indicate soft water.

During the acclimation period, the chironomids burrowed effortlessly into the soft sediments and tube openings could be seen the next day. No abnormal behavior was observed in the pre dose period. Dosing did not bring the chironomids out of their burrows. However, some of the animals in the high dose beakers were unburrowed by the fourth day and were observed crawling on top of the sediment. Three days later those same chironomids were no longer moving, appearing white and dead. After 10 days, the content of the beakers were sieved and mortality recorded. Using the probit method an LC50 of 0.232 ppb was established (Table IX). Ranked mortality in treatment groups D2 D3 and D4 were significantly different from control (D0) and D1 (Kruskal-Wallis AOV ranked, $p>0.05$, Dunn's MRT $\alpha=0.05$).

Sediments and water were removed from each residue beaker at three time points for analysis of labeled cyfluthrin. Two days after dosing, scintillation counts in sediment at all dose levels (except dose 4) were no different than control counts. After 2 days, counts in the water were higher than control in all doses except dose 1

(Figure 14). Levels of cyfluthrin in the sediment remained fairly constant with those of day 2 throughout the test duration. Counts in dose 3 and 4 increased slightly by day 8 when compared to control. Most concentrations however do not differ in recovery from control values. Accuracy of this method is brought up in the discussion. Water samples were not taken after the second day, so no data is available for water on day 8 of the test.

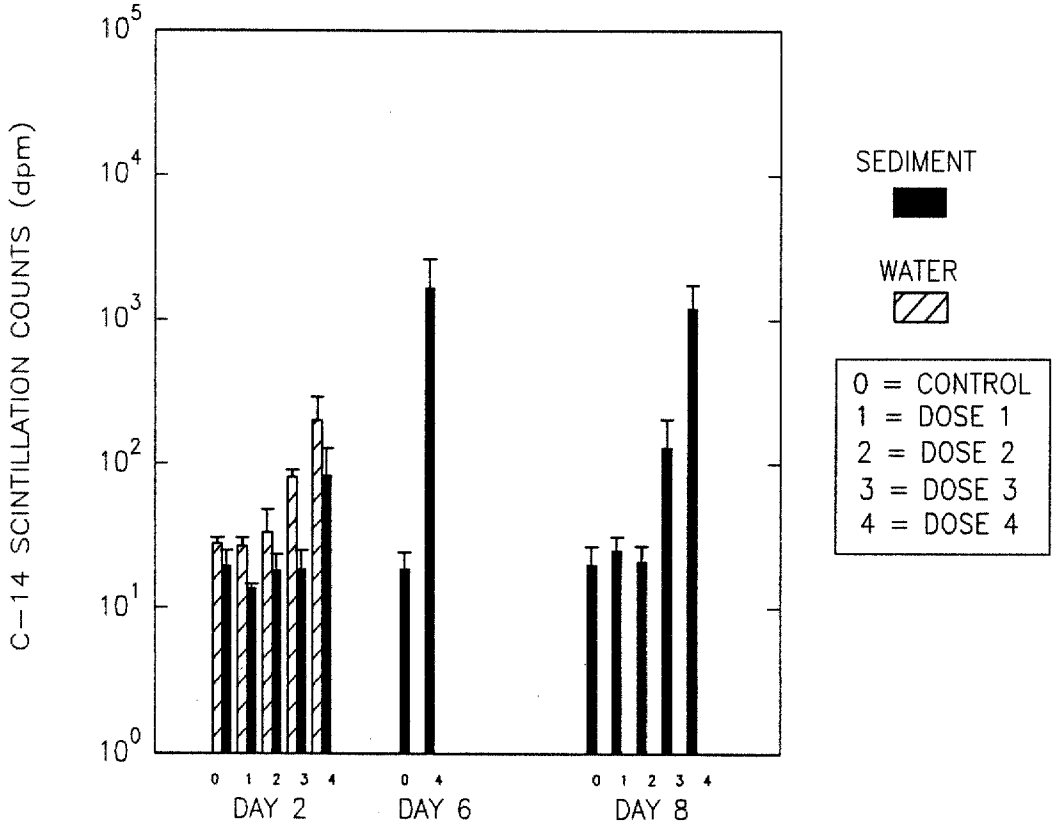


Figure 14. ^{14}C Cyfluthrin in Lake O' the Pines Sediment and Water (pH 6.5). Samples Taken on Days 2, 6, 8. Mean ± standard deviation, *n*=6.

Acidic Sediments and Tap Water

To determine if lake water was aiding in degradation of the pesticide, sediment was used from Lake O' the Pines but acidified tap water (pH 6.3) was used as the overlying test water. It was hypothesized that microbial degradation taking place in the natural water would not occur in the tap water to any substantial degree due to its previous chlorination.

After 10 days, the contents of the beakers were sieved and the number of dead recorded. Probit analysis determined an LC50 of 0.095 ppb (Table IX). This LC50 indicated a higher toxicity than in sediments with overlying lake water. Only ranked mortality associated with the high dose treatment (0.0316 ppb) was found to be statistically different from that of control (Wilcoxon Rank Sum w/Bonferroni Adjustment, $p< 0.05, \alpha=0.05$). These results indicated that the toxicity of cyfluthrin to *C. tentans* in sediments with overlying tap water was greater than when lake water was used.

Both sediment and tap water samples were analyzed for pesticide content throughout the test. Counts of water in all 4 doses did not differ from control after 4 hr. (Figure 15). Sediment counts in dose 4 decreased over time while those in the overlying water increased. Sediment levels in dose 3 declined to control levels by day 4. Due to the inability to detect cyfluthrin in the sediments, the accuracy of any analytical results are questionable.

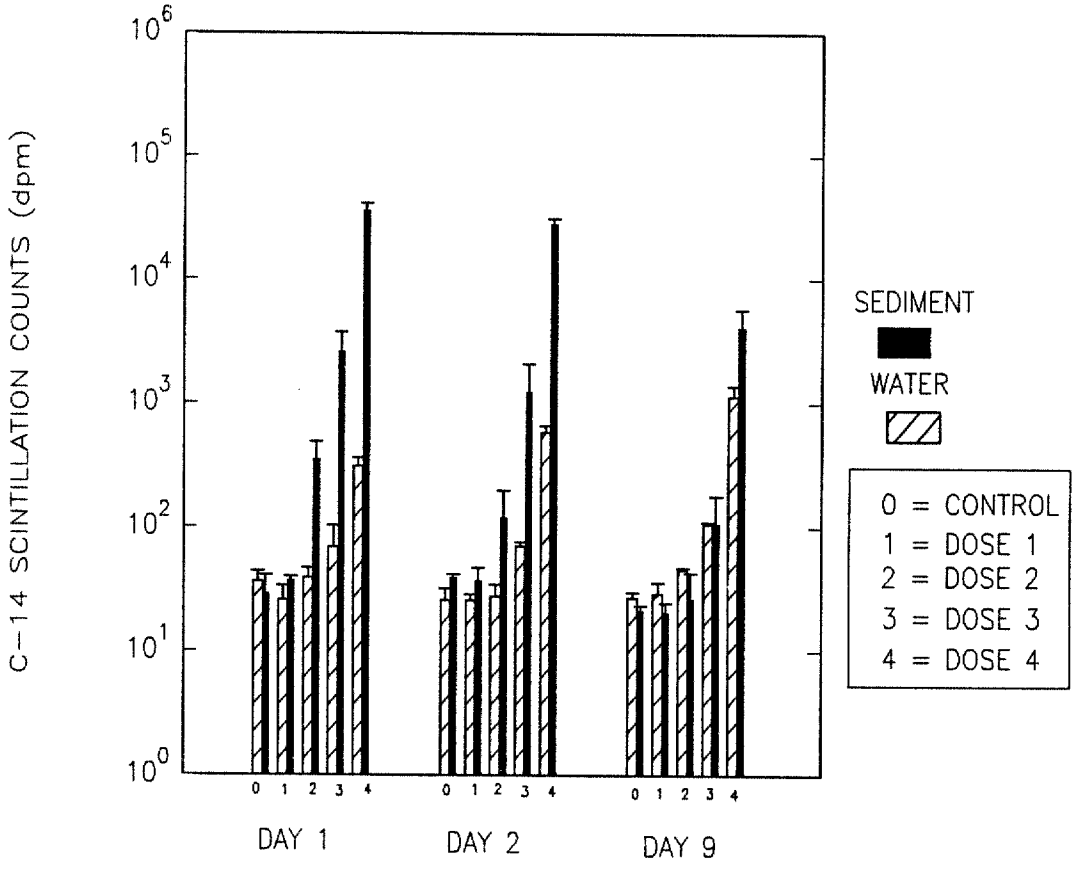


Figure 15. ^{14}C Cyfluthrin in Lake O' the Pines Sediments (pH 6.4) with Overlying Tap Water (pH 6.5). Samples Taken on Days 1, 2, 9. Mean counts ± standard deviation, *n*=4.

Water Quality in Cotton Production Regions

Water quality data (pH) obtained from the Environmental Protection Agency's data base (STORET) for the water quality year 88-89 was examined. Due to the format the data was received in and the extensive amount of time to reformat for the SAS system, only preliminary analyses were able to be performed. Approximately 90 % of the information was for riverine systems, both small and large. The rest of the data encompassed lakes, ponds, swamps, and bayous.

In conjunction with cotton production data obtained from the National Cotton Council, a listing of cotton growing counties was merged with pH data for water bodies (streams, swamps, bayous and lakes) in that county. Due to time constraints, only 5 states using pyrethroids on cotton were examined. These were Alabama, Georgia, Louisiana, Mississippi, and Texas.

For simplicity reasons, pH results were divided out into three groups. 1) water with pH $\geq$ 8.00

2) water with pH $\leq$ 6.99

3) water pH $\geq$ 7.00 and $\leq$ 7.99

Regions of different pH within cotton producing counties were delineated by polygons on each state map. Major Rivers and waterways for each state were also mapped (Appendix G). Alabama was mainly characterized by neutral pH water (Figure 16). Bayous in the SW part of the state were more acidic.

Two main areas were found to be basic, the Tennessee River which feeds Lakes Wilson and Wheeler in the NW corner, and the head waters of the Alabama river coming out of the Dannelly Reservoir in the middle of the state. In Georgia, the majority of lakes and rivers were neutral in pH (Figure 17). The areas found to be acidic were parts of the Ogeechee River, Satilla River, and the Etowah River which feeds Lake Allatoona in the NW portion of the state. Little pH information was available for Louisiana. Both the Red River and Lake Wallace in the NW corner of the state were basic (Figure 18). The confluence of the Red and Black River in the middle of the state was also basic. The Boeuf and Ouachita Rivers in the NE portion of the state were acidic. The water quality information available on Mississippi indicates extensive acidic areas along the Sunflower River near the Louisiana border (Figure 19). The confluence of the Tallahatchie and Yalobusha Rivers was also acidic. The Yallobusha River feeds Lake Grenada (also acidic) further upstream. The final state examined was Texas (Figure 20). All water bodies for which information was obtained indicate water of neutral to basic pH in cotton growing counties. Due to the size of Texas, no river information is provided in Appendix G.

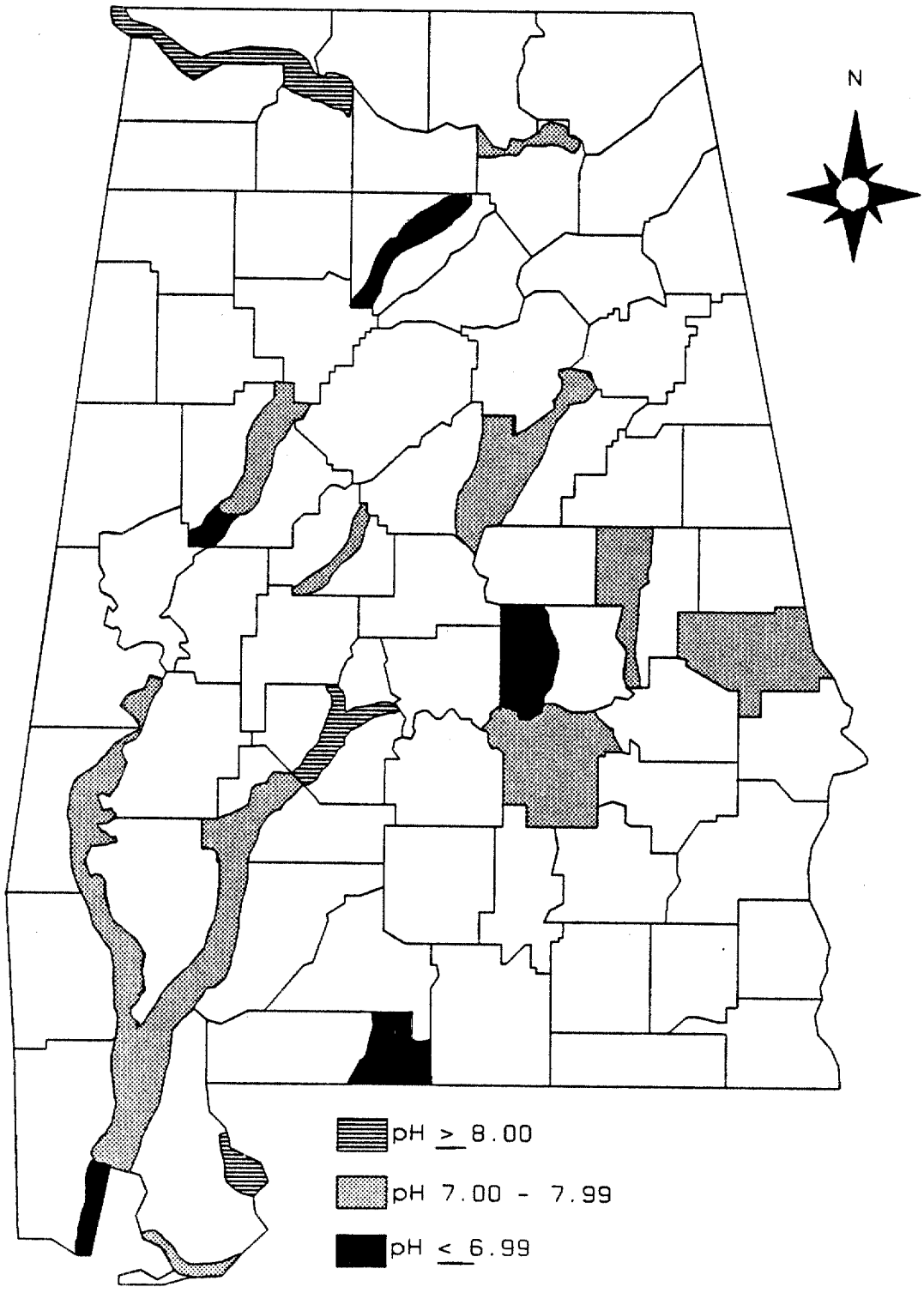


Figure 16. Alabama - pH in Cotton Producing Counties.

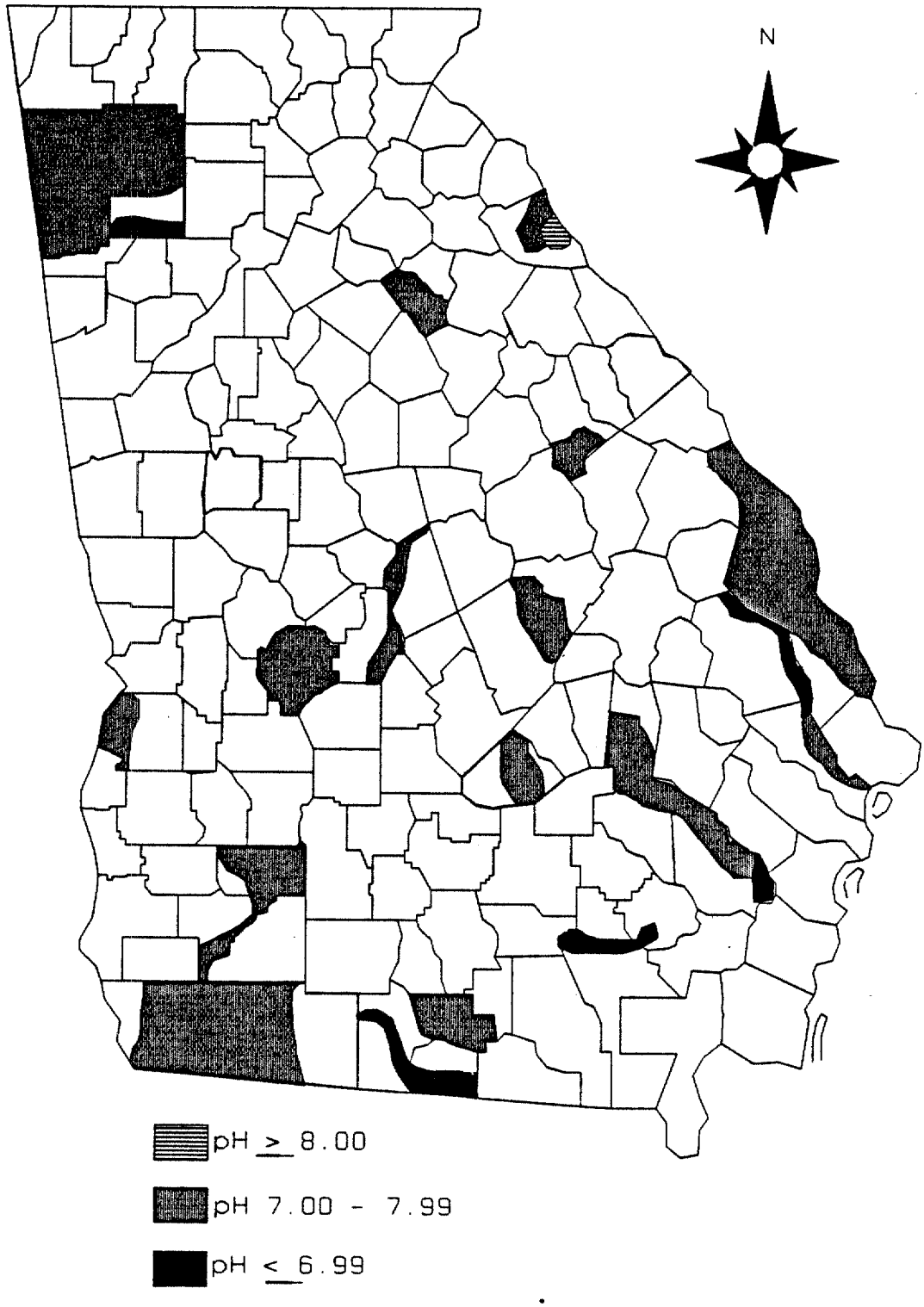


Figure 17. Georgia - pH in Cotton Producing Counties.

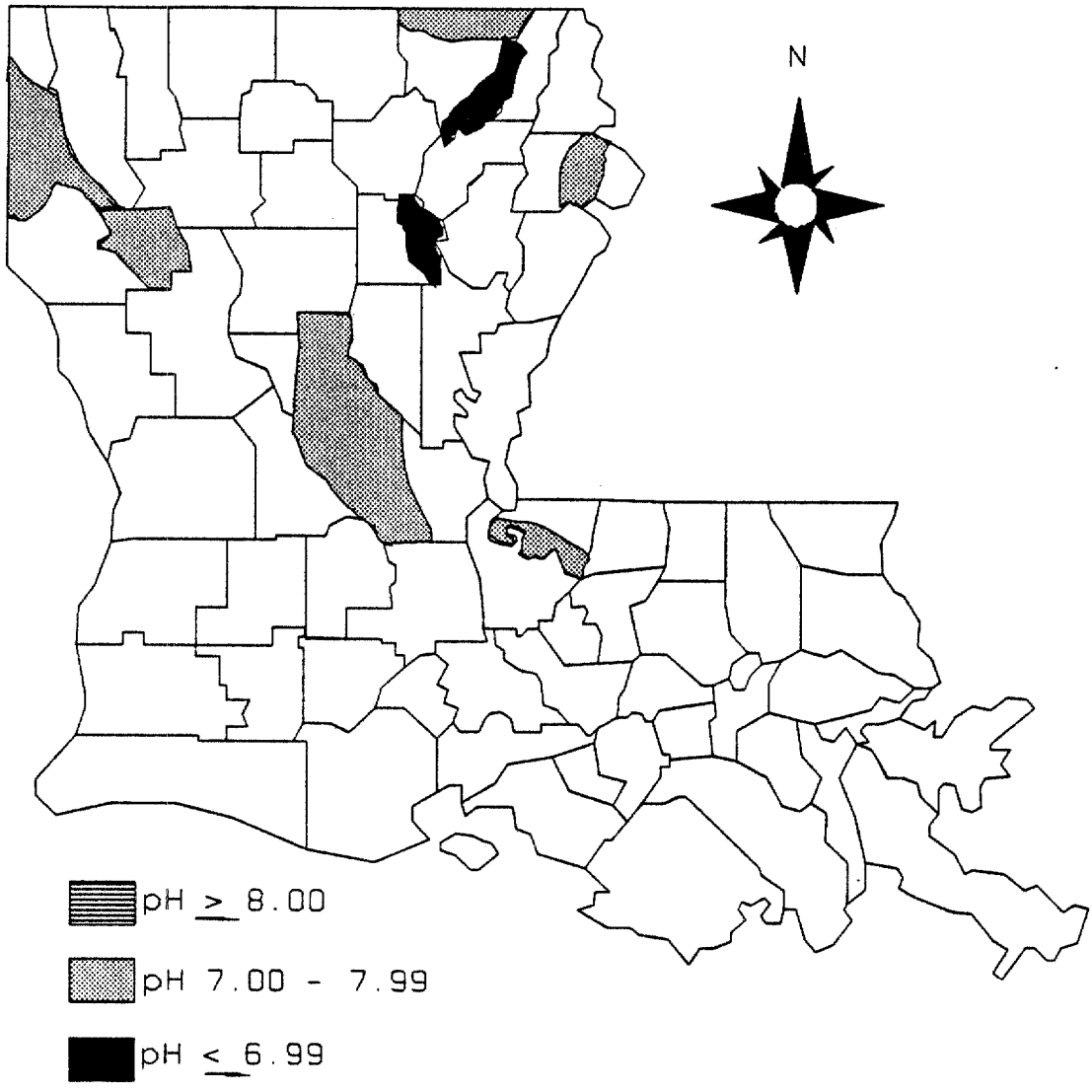


Figure 18. Louisiana - pH in Cotton Producing Counties.

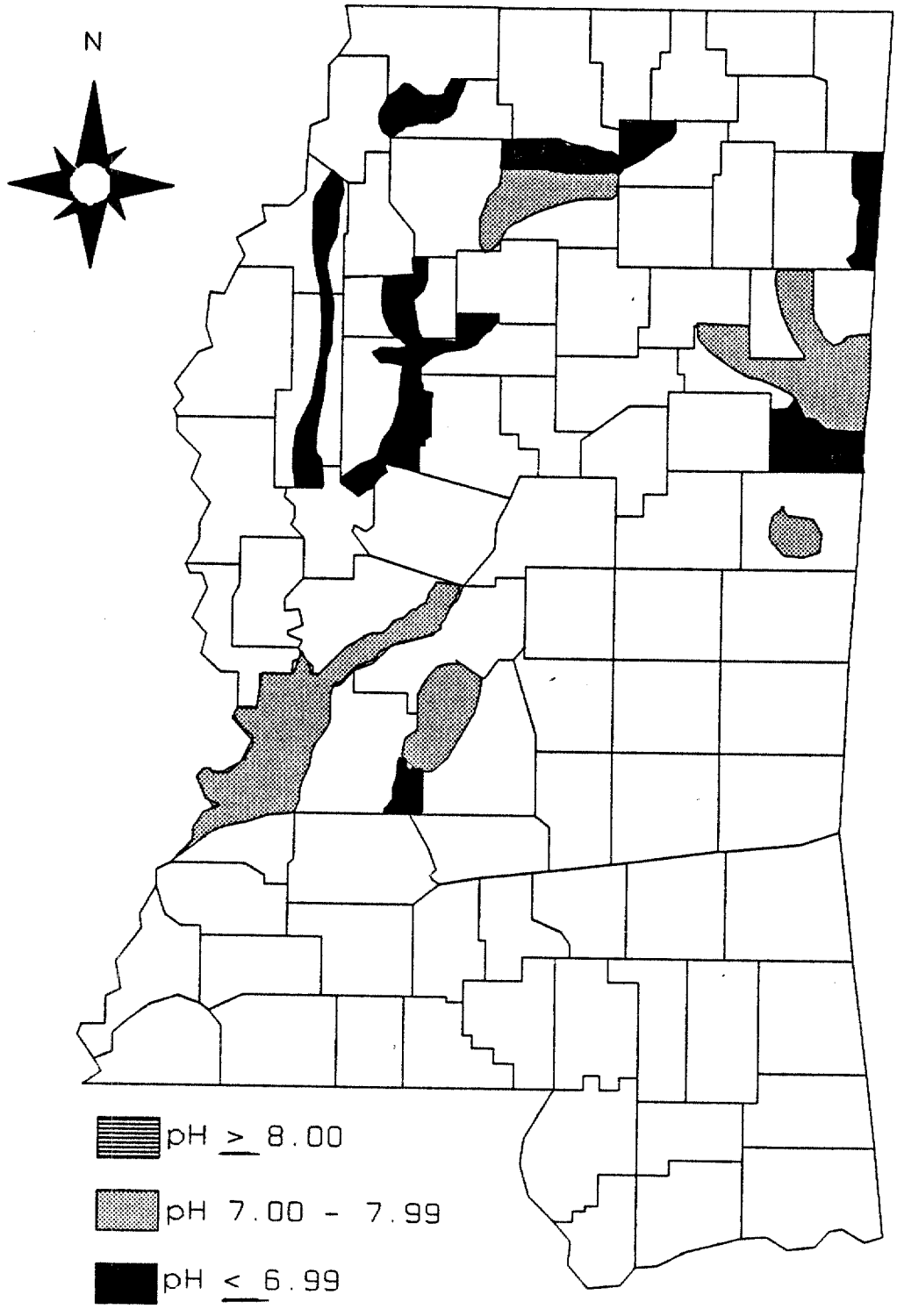


Figure 19. Mississippi - pH in Cotton Producing Counties.

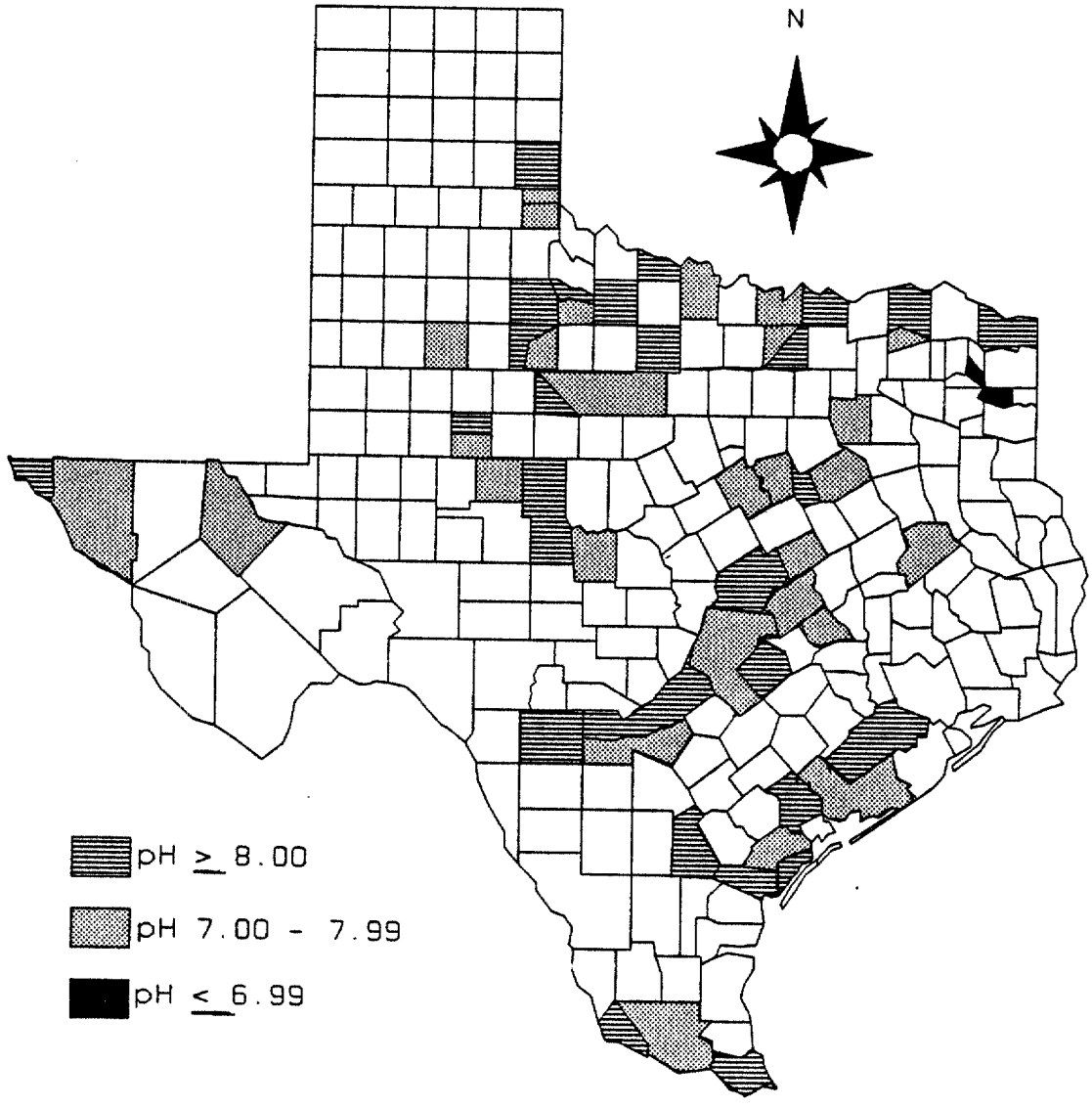


Figure 20. Texas - pH in Cotton Producing Counties.

CHAPTER 4

DISCUSSION

Aqueous Phase

The acute toxicity results obtained in the aqueous phase compare favorably with published LC50 and EC50 values for synthetic pyrethroids using similar insects (Table XI) (Lohner and Fisher, 1990; Mokry and Hoagland, 1990; Hill 1985; Anderson 1989; Mulla et. al. 1980, Bradbury and Coats 1989).

The toxicity of cyfluthrin to *C. tentans* was found to vary inversely with changing pH. A 2-fold increase in toxicity was measured in tap water adjusted to pH 6.6 compared to that at pH 8.0. This was seen as a decrease in EC50 values (607 ppt to 366 ppt). Toxicity was significantly higher in natural waters compared with tap water of the same pH in only one case. This was due to confidence limit overlap which were probably a result of using analytical concentrations in the probit analysis instead of nominal values which have no variance. Natural waters were characteristically softer than the tap water and this difference in water quality parameters probably influenced the toxicity results.

Hardness, alkalinity and conductivity values in all experiments with tap water were similar. Lake water however, was less alkaline than tap water. Pond water on the other hand was more alkaline than tap water. Since natural waters were found to have a greater impact on toxicity compared to tap water of the same pH, alkalinity and hardness by themselves do not appear as single contributing factors to toxicity. It seems that Ca^{++} and Mg^{++} ions by themselves do not affect toxicity of the pesticide unless accompanied by both pH and alkalinity changes. These aqueous phase experiments point to both pH and water source as important factors influencing the response of *C. tentans* to cyfluthrin. Further experiments should be done in which tap water is adjusted to have the exact same water quality as natural waters.

Table XI.
Acute Toxicities for Select Aquatic Organisms Exposed to Pyrethroids. Concentrations are in μg/l.

CP = Cypermethrin, P = Permethrin, F = Fenvalerate

ORGANISM	ENDPOINT	CP	P	F
Water Flea *Daphnia magna*	24H EC50	1-5	2.06	----
D. magna	48H LC50	2	0.6	----
Mosquito *Culex tarsalis*	24H LC50	----	2.0	4.0
Cx. quinquefasciatus	24H LC50	0.07	1.4	4.7
Midge *Chironomus decorus*	24H LC50	----	----	≥ 4
C. thummi	24H EC50	0.2	----	----
C. thummi	24H LC50	> 5	----	----

Reported toxicity of relevant compounds.
(Bradbury and Coats 1989):
cyfluthrin> cypermethrin> fenvalerate> permethrin

There is a paucity of information in the literature indicating that water hardness (Mauk et al. 1976, Dyer et al. 1990) alters the toxicity of pyrethroids to aquatic species. Many aquatic organisms however, are known to be more sensitive to chemical compounds and metals in soft waters of low pH (Rand and Petrocelli 1985). Bayluscide, a molluscicide used in South African streams, caused fish

death within minutes in soft water at 0.1 ppm. When used in hard water at twice the concentration, death did not occur until 2 hr after spraying (Marking and Hogan 1967). TFM, a compound targeting larval lamprey, was most effective in soft acidic waters (Howell et al., 1964).

The results obtained in the aqueous phase tests suggest that different water types may influence the toxicity of cyfluthrin to *C. tentans*. The results also show that WRFS pond water elicited a more severe response than tap water of the same pH from *C. tentans*. Both cyfluthrin concentration and water quality influenced toxicity results. Due to the extremely toxic nature of this compound, differences in mortality between doses in each experiment could not be statistically confirmed as different. Only mortality associated with the high dose in each experiment could be statistically separated from the low dose. A broader range of treatment concentrations which usually corrects for this problem, was not possible with cyfluthrin.

The agreement between measured and nominal concentration of a pesticide is an important consideration when conducting toxicity tests. Good agreement is an essential requirement of a valid test protocol. Analytically, the recoveries obtained in the aqueous experiments correlate well with nominal concentrations. Cyfluthrin is known for its high sorptive qualities making precise residue recoveries difficult. These recoveries were also closely examined in relation to the toxicity results obtained. In the low pH experiments, little mortality was seen at low treatments despite residue results indicating cyfluthrin was present in concentrations that would annihilate anything in the tank. The detection limit of cyfluthrin on the gas chromatograph was 10 ppt and contamination might also have played a role at low concentrations and must be considered when evaluating results.

The effects observed in such research must be related to the Environmental Effective Concentration (EEC) if one is to make a judgement about the risk posed by a material in the environment and thus estimate its ecological significance. Many concentrations of cyfluthrin causing impacts in the toxicity tests were above the estimated EEC (8 - 26 ppt) for this compound (Mobay, 1988). Since this is a computer model derived value, a range of concentrations

can be expected based on different input variables. *C. tentans* was behaviorally impacted at concentrations as low as 0.025 ppb. In addition, secondary defense mechanisms such as evasive action were not possible for most of the chironomids for approximately 20 hours after cyfluthrin entered the water column. Even though the laboratory test scenario was unnatural, the results should probably be considered an estimate of the potential effects of the test material in the field. In the test set up, there was an absence of mitigating factors (sunlight, sediment, microbes) that would tend to decrease toxicity under natural conditions. However, these test results indicate that in static water bodies of low pH, certain organisms with short life-cycles such as zooplankton, may be impacted due to the persistence of cyfluthrin especially in a realistic multi dose field application.

Sediment Phase

With the addition of sediments to the test system, it was hypothesized that toxicity of cyfluthrin to *C. tentans* would be less than in the previous aqueous phase study under comparable pH. It was also hypothesized that the pattern of persistence seen at low pH in the aqueous phase would be apparent in the sediment study.

In general, pesticide residues in the sediments started off high relative to the water. This was probably due to the

quick settling (< 24 hr) of the dosing matrix through the water column. Sediment residue concentrations were indistinguishable from controls the first day after treatment. From these limited results, degradation of cyfluthrin appeared to be no different in sediment with overlying tap water than sediment with lake water of the same pH. However, the LC50's between these two sediment systems were extrememly different. An LC50 of 0.095 ppb was determined with overlying tap water of pH 6.5. This value is in the same range as the EC50's determined in the aqueous phase study under similar pH. When lake water (pH 6.5) was added to the sediment system, an LC50 of 0.232 ppb occurred, indicating less toxicity. A higher LC50 of 2.64 ppb was determined with sediments and lake water (pH 8.5) indicating the least amount of toxicity. All of these LC50's are above the estimated EEC for this compound. Persistence of cyfluthrin in sediments/water of low pH was not reflected in the toxicity results and residue recoveries were inconclusive. These preliminary toxicity results however, appear to indicate that sediments with lake water are able to detoxify cyfluthrin better than sediments with tap water. This may be due to microbes associated with the natural water. More testing is necessary to confirm these results.

The analytical method using ^{14}C labeled cyfluthrin was not successful at providing quantitative concentrations. When the results were calculated, they were in extreme disagreement with the toxicity observed. Most residue samples indicated little difference in from control samples. Quenching of radioactivity in the sediments was one possible problem. After consultation with outside sources, it was determined that oxidation of the sediments was the best method for such a test system. The necessary instruments for doing this were not available at the University of North Texas at the time of this research.

Risk Assessment

In order for a decision to be made regarding the use of an insecticide in the environment, a risk assessment should be undertaken. Information needed includes, 1) types of water bodies and aquatic fauna in the vicinity of agricultural fields 2) the pH of those water bodies during the use season 3) toxicity tests, both laboratory and field. No information to date has been gathered on the number or type of water bodies in the vicinity of cotton fields using pyrethroids or other pesticides. Water quality data was obtained from the E.P.A.'s water quality database (STORET) in order to shed light on this issue. Approximately 90 % of the information was for riverine systems, both small and

large. The rest of the data encompassed lakes, ponds, swamps, and bayous.

Additional data on cotton growing counties was obtained from the National Cotton Council of America (Memphis, TN). Cyfluthrin (Baythroid ^{R}), introduced as a cotton insecticide in 1981, is on the market and extensively used in the southern cottonbelt which is plagued by the cotton Boll Weevil (*Anthonomus grandis* Boheman). This region is generally characterized by waters of neutral to basic pH. However, this insecticide due to its efficacy and low mammalian toxicity, has potential for use on other crops. These crops may lie in regions where the waters are not well buffered. The impact of cyfluthrin under different physio-chemical scenarios as described in this research appear quite different. In order to protect the aquatic fauna in ponds and streams and abate deleterious effects from xenobiotics, toxicity information should be looked at in conjunction with water quality data. Integrating STORET pH information with geographical location of cotton fields (and other potential use crops) and the pH of water bodies at risk should be one of the next steps in making an informed risk assessment about this chemical.

Conclusion

The physical and chemical properties of aquatic ecosystems can have a profound impact on the biological activity of chemical (Rand and Petrocelli 1985). This was demonstrated with these experiment at various pH. The vulnerability of the environment to chemical insult depends on many factors. These include the (1) physical properties of the chemical and its degradative by-products; (2) chemical concentration entering the environment; (3) persistence and type of input (intermittent or continuous); (4) properties of the ecosystem that enable it to resist impacts; and (5) location of sensitive environments in relation to the chemical discharge. It is further recognized that similar ecosystems are not always equally affected by addition of the same chemical. Minor differences in the chemical and physical environment and in species composition can result in heterogenous impacts on the system. Therefore, specific environmental conditions must be considered when evaluating potential chemical hazards.

The main objective of this study was to determine the effect that pH had on toxicity and environmental persistence of the pyrethroid cyfluthrin. This study was conducted under static conditions and the results should not be extrapolated to flowing systems. The results indicate that in geographical regions characterized by small static water bodies of low pH and alkalinity, cyfluthrin has the

potential to remain in the environment for up to at least one week. With a persistent compound there is potential for greater adverse impact to aquatic fauna. How long the compound remains in the aqueous environment however, depends upon the size and surface mixing of the water body as well as the pH. These factors as well as those enhancing degradation (microbes, photolysis etc.) should not be overlooked.

If it is the goal of the U.S. Environmental Protection Agency to protect the aquatic fauna in ponds and streams and abate deleterious effects from xenobiotics, toxicity information should be looked at in conjunction with site specific water quality data. Integrating STORET pH information with geographical location of cotton fields (and other potential use crops) and the pH of water bodies at risk should be one of the next steps in making an informed risk assessment about this chemical.

APPENDIX A

GENERAL CULTURE METHOD FOR *C. TENTANS*

Maintenance of Culture:
1. Add 5 g Tetra-Min B to aqyarium on the third or fourth day after the egg mass was added.
   Add 5 g per weeksubsequently.
2. Add dechlorinated water as need to replace evaportaion losses.

Isolation of Egg Masses:
1. Using an aspirator, withdraw adults from tanks in the morning just after emergance.
   Under most situations an equal number of males and females will be removed and adequate fertilization will take place.
2. Transfer adults to laying flask.
3. After the adults' wings have dried, add dechlorinated water to the flask for egg laying.
4. Egg masses will appear 24-48 h after fertilization.
5. Decant water and egg masses from flask into a petri dish.
6. Observe the egg masses in 2-3 days to make sure the eggs have hatched.

Production:
This culture procedure will provide a regular supply of adults in low numbers with a minimum of management. The first genertaion will emerge synchronously but subsequently, all life stages will be present. If a large number of adults are required, more food added more frequently, must be provided.
1. Hatching (egg to 1st instar), ±S.D.: 97±1 % in 55-57 h.
2. Emergence Pattern:
   a) males - start at day 21, peakat day 24.
   b) females - start at day 23, peak at day 26.
3. Standard production for 1 egg mass (n=18, ±SD):
   a) % emergence: 49 ± 16.
   b) sex ratio (M:F): 1:4.
   c) peak emergence: day 25.
   d) Average number emerging adults from 1 egg mass:
      1) male: 589 ± 152
      2) female: 417 ± 162

Physical/Chemical Conditions:
1) temperature: 20 C
2) conductivity: 400-800 µS/cm.
3) Photoperiod: 18L: 6D
4) pH: 7.2 - 7.4
* at pH 5.5-6.5, Normal development in system described by Lillie and Klaverkamp (1977).

APPENDIX B

FIELD PH OF VARIOUS LAKES - PRELIMINARY SURVEY

Field pH of Various Lakes - Preliminary Survey

DATE	LAKE	SITE LOCATION	pH	TEMP ^{0}C
3/14/91	Lewisville	The Colony, at Stewart Creek Park	8.17 8.44	12.2 13.3
3/14/91	Grapevine	Twin Cove Murrell Park Murrell Park	8.01 7.80 8.13	14.4 13.8 13.6
3/14/91	Benbrook	Main Entrance, dam to the right	8.38	15.0
3/14/91	Granbury	Granbury, behind Walmart	8.34	16.3
3/14/91	Whitney	Whitney, at Lofters Bend	8.45 8.50	13.7 13.7
3/12/91	Lake O Pines	Bridge, Rt. 760	7.00	13.6

APPENDIX C

PROCEDURES FOR WATER ANALYSIS

Dissolved Oxygen and Temperature

Dissolved oxygen and temperature were measured using a calibrated YSI Dissolved Oxygen meter and a YSI field probe. Dissolved oxygen and temperature are reported in mg/ L and degree celcius (°C) respectively.

Conductivity

Conductivity was measured using a YSI salinity-conductivity temperature meter with a YSI field probe. Conductivity is reported in μmhos/cm as specific conductance.

pH

The pH was determined using a Orion Series 200 portable meter. Before each use, the instrument was calibrated according to the instruction manual using buffers of 2 different pH's.

Alkalinity

For each replicate, 100 ml of sample water was titrated with 0.1 H_2SO_4 while the pH was being monitored. The volume of titrant (ml) used to reduce the pH of the sample water to 4.3 ± 0.2 was recorded. Alkalinity was determined by the following equation:

$$\text{Alkalinity (mg/ L as } CaCO_3) = (50)(\text{ml titrant})$$

Alkalinity is expressed in mg/ L as $CaCO_3$.

Hardness

Twenty-five ml of each replicate sample was diluted with 25 ml of deionized tap water in a porcelain casserole dish. The pH was raised to 10 by adding 2 drops of ammonia solution. Three drops of Eriochrome Black-T dye (0.5 g dye + 4.5 g hydroxyl amine hypochloride dissolved in 100 ml 95 % ethanol) were added. The burgandy colored sample was the totrated with 0.02N Ethylenediamine tetracetic acid (EDTA) until a clear blue color appeared. The volume of titant used was recorded and hardness calculated as follows:

Hardness (mg/ L as $CaCO_3$) = (40) (ml titrant)

Hardness is reported in mg/ L as $CaCO_3$.

Organic Carbon

Analysis of total, dissolved, and particulate organic carbon in water was determined using a Dohrmann Carbon Analyzer (model CD-80). TOC, POC, and DOC are measured by converting C to CO2 and then measuring CO2 with an infra-red detector. Samples were collected in acid cleaned amber glass jars with teflon lined caps. Samples were analyzed within 24 h of collection. Calibration of the machine was done according to the Dohrmann manual. Twenty mls of sample was poured into a acid washed 18 X 150 mm test tube. Standards were run at 100 ppm and incuded spikes and checks. TOC samples were analyzed by filtering triplicate samples through separate GFF glass fiber filters using a

polyethylene 50 ml syringe. DOC samples were analyzed by filtering the samples and then acidifing to pH <2 with 1-2 ml of 1N H_3PO_4. Filtered Milli-Q water was used for blanks. Data were automatically printed out. The organic carbon values were obtained as follows: The blank values obtained were averaged and then subtracted from both the mean TOC and DOC values. Particulate organic carbon was determined by subtracting DOC from TOC (TOC = POC + DOC).

APPENDIX D

PROCEDURE FOR SEDIMENT PH MEASUREMENT

The pH meter is standardized as described for water samples.

1) Transfer an aliquot of blended, moist sedimentt sample to a 250 ml beaker. Do not use dried or frozen sediment as the dehydration process is not known to be reversible.

2) Insert the electrode into the sample while it is spinning gently on a stirplate. Record the pH. Check the standardization of the pH meter at regular intervals using known buffer solutions.
(Plumb, 198 p.3-51)

APPENDIX E

SEDIMENT DOSING AND CALCULATIONS

Radioactively labeled cyfluthrin (98 % pure) was made up in an acetone stock solution and a small aliquot counted using a liquid scintillation counter to determine activity. An appropriate aliqout was removed and added to a amber glass jar containing 10 g of Kaoloin potter's clay and 20 ml water. This solution was mixed on a stirplate for 1 h prior to dosing to ensure homogenous mixing. Aliquots were removed for analysis both before and after dosing to ensure homogeniety of the 0.5 ml aliquot delivered to each beaker. Cyfluthrin in the form of a sediment slurry was then added to each beaker. The control beakers received the same amount of acetone as the high dose treatment, but without pesticide. The volume of acetone in the stock dosing solutions never exceeded 1 ml. Two complete replicate experiments were set up, one for residue analysis over time, the other for toxicity results after 10 days. Animals were placed in an environmental chamber on a 16:8 L:D schedule. All beakers were covered with saran wrap to prevent evaporation of water over the 10 day period of testing. Animals were fed 1 drop of tetramin slurry every 3 rd day to ensure adequate food supply.

Calculations

Estimate the desired concentration of cyfluthrin

$$\text{cyf. } (\mu g)/\text{amt. soil (kg)} = \text{ppb}$$

Calculate amount of Baythroid dose (μg)= (μl)

$$\frac{\text{cyf.(g)} \quad (1 \text{ ml Baythroid}) \quad (1000\ \mu l)}{\%\ \text{A.I.} \quad (0.9599) \quad 1 \text{ ml}}$$

Calculate amount of active ingredient this will be.

$$\frac{\text{amt. } (\mu l) \text{ Baythroid} \quad (0.9599) \quad 1 \quad (0.262)}{(1 \text{ ml}) \quad (1000\ \mu l)}$$

Dosing slurry is Kaolinite clay (10 grams). Add this to 20 ml water. This equals a volume of 25 mls, consistently.

Stock Preparation in acetone :

% purity = 99.2

1.04 g X 0.992/100 ml = 9.95 μg/ml = 995 ng/ml.

Sample Dose Preparation:

To deliver approximately a 200 ng/0.5 ml concentration (400 ng/ml = 400 μg/kg, (μg/kg = ppb):

1. Remove 5.95 μg of stock (995 ng/ml). This = 600 μl.
2. Add 5.95 μg (600 μl) to the spinning slurry (25 ml) = 25.6 ml total.
3. The slurry now contains 0.232 μg/ml = 232 ng/ml.
4. The volume that the concentration is to be delivered however is 0.5ml, which = 116 ng, close enough to the desired concentration.
5. The 116 ng dose is delivered to the 50 ml beaker filled with 40 ml dechlorinated water and 10 ml sediment.

APPENDIX F

QUANTITATIVE ANALYSIS OF DOSING SLURRIES

Counts of Dosing Solutions at Different Time Intervals

	D1	D2	D3	D4
1a	63	91	557	4406
1b	65	87	517	4396
2a	58	70	509	6320
2b	43	72	490	5709

1 = Dosing solution (0.5ml) before adding to beakers.
2 = Dosing solution (0.5ml) after all beakers had been dosed. (a and b are replicates)

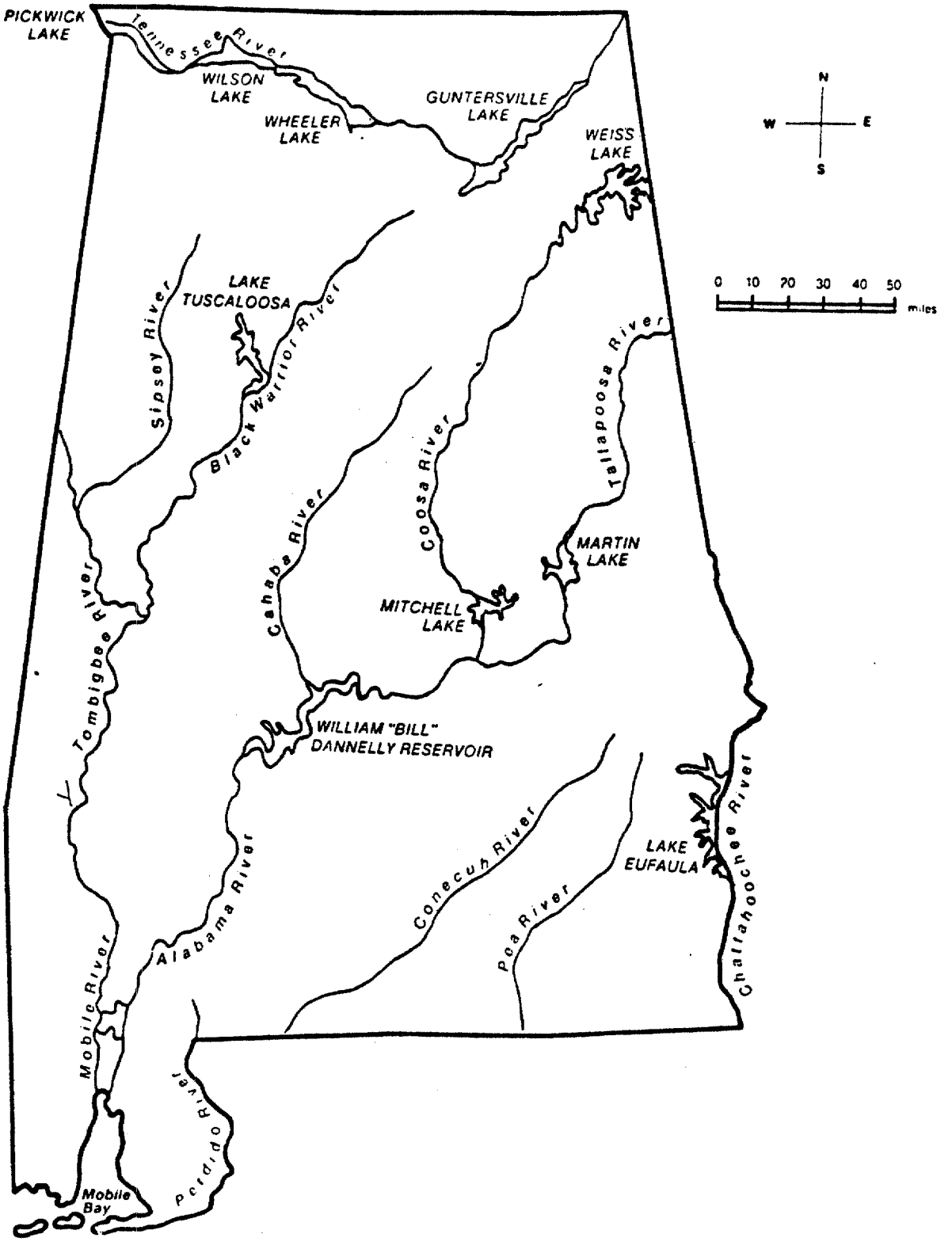
NOMINAL DOSES:

D1 = 0.0316 ng/0.5ml
D2 = 0.063 ng/0.5ml
D3 = 0.090 ng/0.5ml
D4 = 1.19 ng/0.5ml

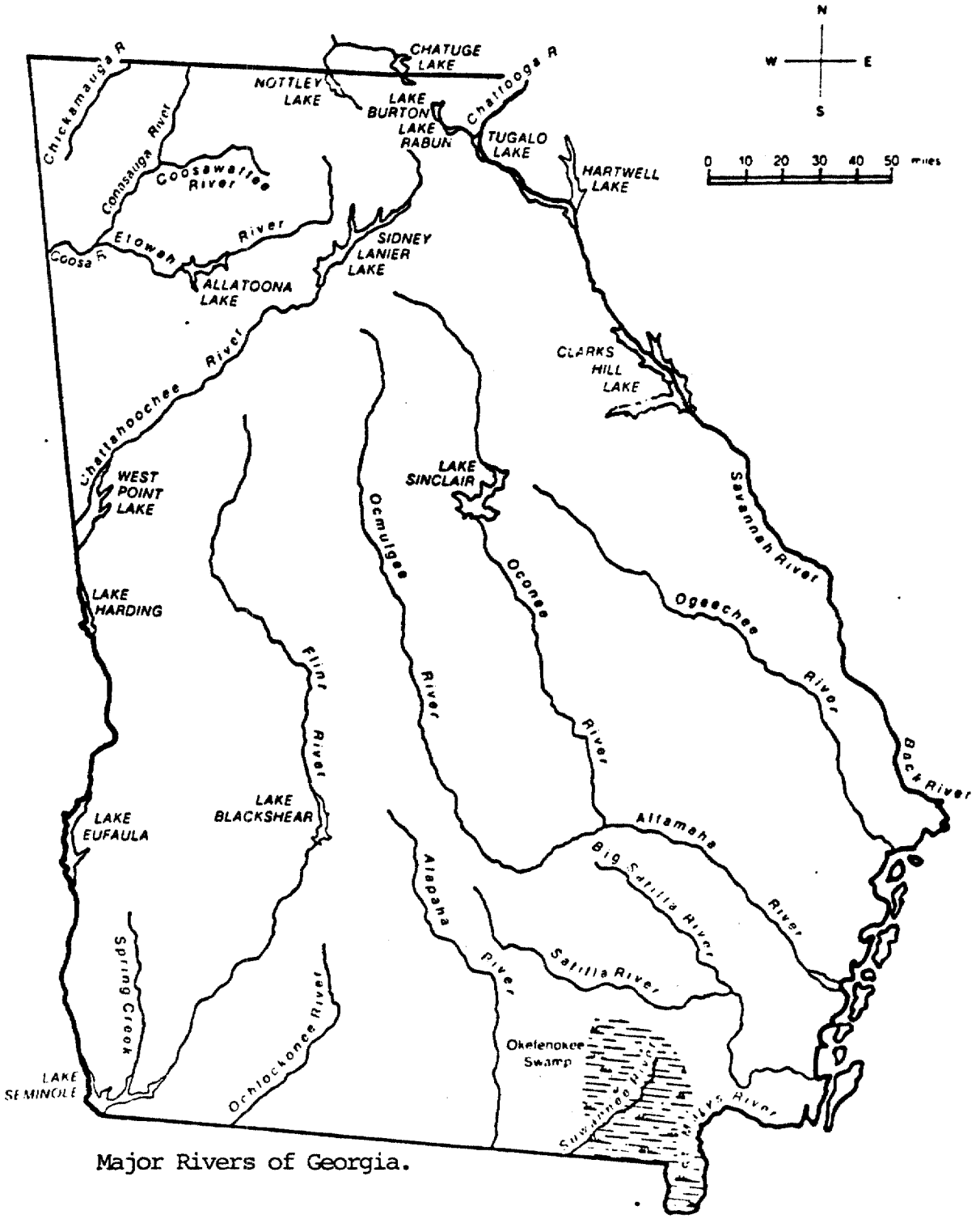
APPENDIX G

MAJOR RIVERS OF SELECT COTTON PRODUCING STATES

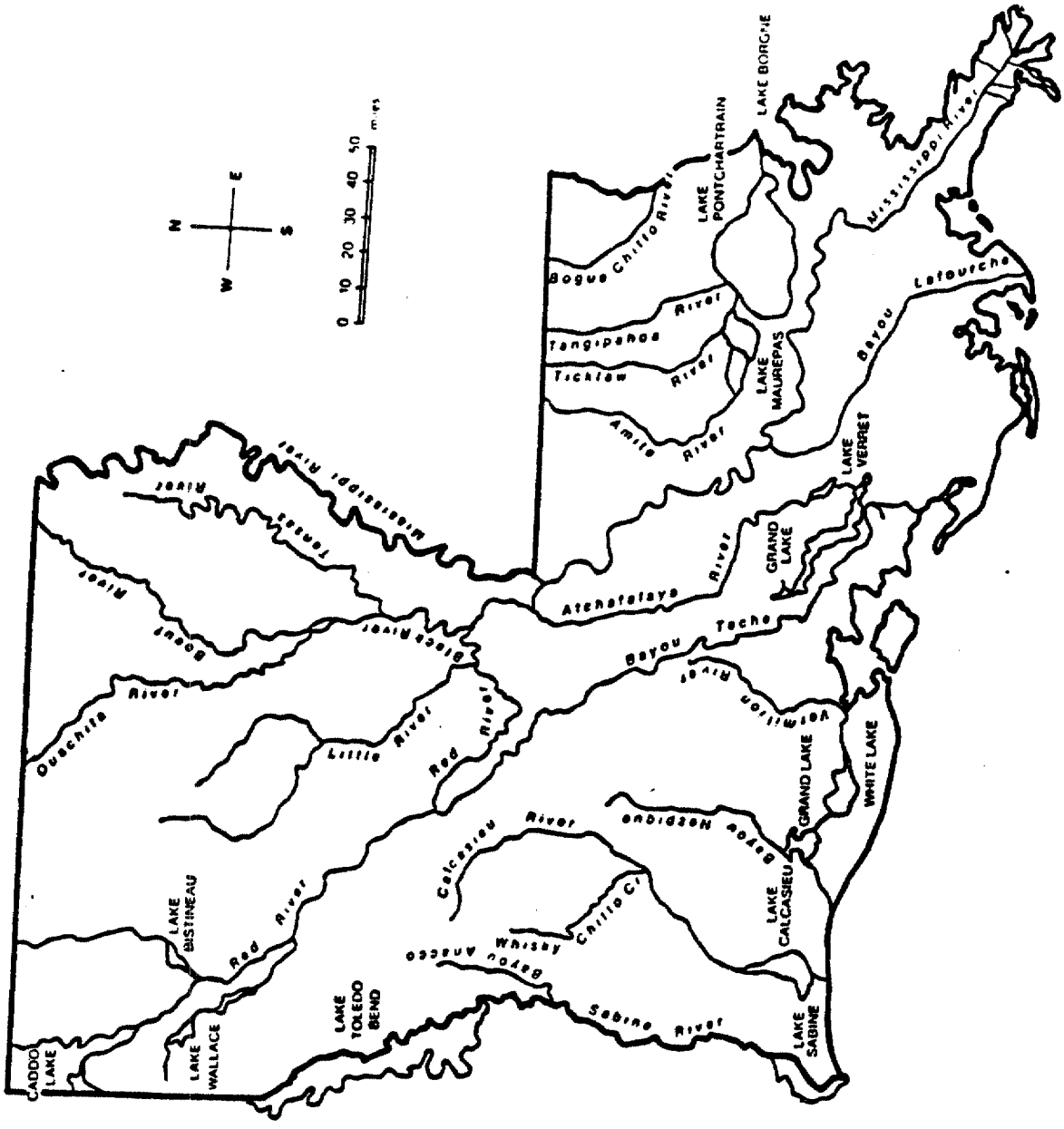
APPENDIX G



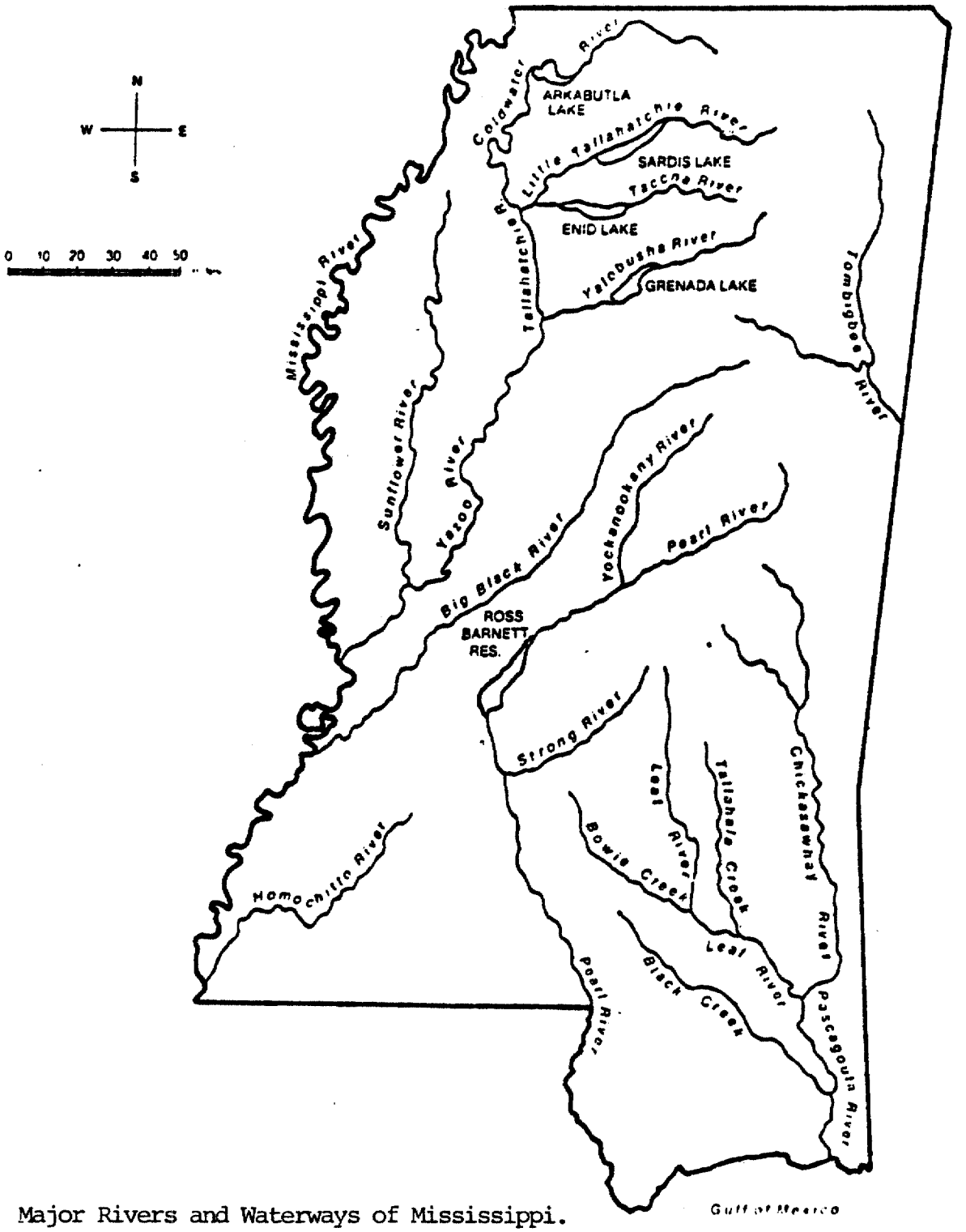
Major Rivers and Waterways of Alabama.



Major Rivers of Georgia.



Major Rivers and Waterways of Louisiana.



Major Rivers and Waterways of Mississippi.

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