ABSTRACT

Quantifying the Presence of Current-Use Insecticides and Toxicity of Sediments in Urban Residential Watersheds in Central Texas

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In the US, residential use of pyrethroid and other recently developed insecticides has increased substantially in recent years, yet the impact of these insecticides on benthic invertebrates in urban streams is largely unknown. The objective of this study was to determine the presence and concentration of current-use pesticides in the sediments of residential streams in central Texas. Additionally, the toxicity of these sediments to *Hyalella azteca* was evaluated. Sediment samples were collected from several sites in urban streams over the course of a pesticide application season. The sediments were extracted and analyzed using a Varian 2100 gas chromatograph with MS/MS for the presence of several pyrethroids and other common insecticides. Ten-day sediment toxicity tests using *H. azteca* were also conducted with the sediment samples. Results of this study suggest that pyrethroid insecticide contamination of urban sediments may indeed be a concern in central Texas.

Quantifying the Presence of Current-Use Insecticides and Toxicity of Sediments in Urban Residential Watersheds in Central Texas

by

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CHAPTER ONE

Introduction

Pesticides have been used by humans for thousands of years to kill or otherwise control weeds, insects, fungi, rodents and other pests. While they are indeed helpful in agriculture, horticulture, disease prevention, nuisance insect control and many other applications, pesticides also exact a toll on the environment, including the air, water, sediments and organisms (Baird and Cann 2005). The nature and extent of the unintended side effects of pesticides have been studied intensively, but much remains to be known, and results are often controversial. Since the early 1990's the United States Geological Survey (USGS) has been working on an in-depth survey of the nation's waters. The project, termed the National Water-Quality Assessment Program (NAWQA), examines natural variations in water quality as well as the impact of human activities, and includes both agricultural and urban waters (USGS 1999).

Urban Pesticides

One unintuitive finding of NAWQA is that insecticides were detected more frequently and often in greater concentrations in urban streams compared to agricultural streams (USGS 1999). Approximately 25% of overall pesticide use in the United States is non-agricultural. Urban pesticide applications include lawn and garden, structure protection, mosquito abatement, golf courses, roadsides, right-of-ways and medians, and pet products. Urban insecticide use may contribute more toxicity to surface waters than agricultural insecticide use (Hoffman et al. 2000). Researchers have just recently started investigating this issue.

Hoffman et al. (2000) conducted a large-scale investigation of pesticides in eight urban watersheds of the United States in conjunction with the NAWQA. Six of the eight urban sample cities were compared with a similar-sized nearby agricultural watershed. Water samples were analyzed for 75 pesticides (both current and historic) and seven transformation products. The authors concluded that agricultural herbicides contribute about 20 times more to surface waters than urban herbicides, but that urban insecticides contribute equally to surface waters as do agricultural insecticides.

Urban Insecticides

Diazinon, chlorpyrifos and malathion are organophosphate insecticides (OPs) that were frequently detected by Hoffman et al. (2000). The researchers detected diazinon in all eight urban watersheds with a 69.3 % frequency, and chlorpyrifos and malathion in seven urban watersheds with a 17.7% and 14.0% frequency, respectively. Chlorpyrifos may have had a low detection bias since it is capable of binding to sediment. All three of these insecticides were detected at maximum concentrations that exceeded U.S. or international water-quality criterion (Hoffman et al. 2000). According to EPA 2001 Pesticide Sales and Usage Reports, diazinon and malathion were the number one and number three insecticides used in the home and garden sector in the U.S. in 2001. Chlorpyrifos was the number one insecticide used in the industry, commercial and government sector in the U.S. in 2001. Overall, OPs made up 70% of insecticide use for all market sectors in the U.S. in 2001 with 73 million pounds of active ingredient (U.S. EPA 2004 a).

Recently, the use of diazinon and chlorpyrifos has been restricted by the EPA due to human health, wildlife and environmental concerns. Retail sales and product registrations for diazinon for residential indoor and outdoor use were terminated December 2002 and December 2004, respectively. Agricultural use is more restricted as well (U.S. EPA 2004 b). Retail sales of chlorpyrifos and non-agricultural applications where children may be present (schools, parks) were terminated December 2001. Other non-agricultural applications and agricultural use have been reduced as well (U.S. EPA 2002).

Banks et al. (2005 a & b) monitored the presence of the OPs chlorpyrifos and diazinon in the surface water in and around Denton, TX before and after the EPA's restrictions were implemented. The studies used a dense network of 70 water quality monitoring stations established using topological and hydrological considerations, and sampled monthly from March through August. The results of the studies indicated that the reduction of outdoor, non-agricultural use and retail ban on diazinon and chlorpyrifos resulted in a significant decrease of these OPs in surface waters.

As the urban and agricultural uses of OPs are decreasing, other insecticides such as pyrethroids are taking their place.

Pyrethroids

Pyrethroids are a class of insecticides that are synthetic versions of pyrethrum, a natural insecticide derived from the daisy *Chrysanthemum cinerariifolium* that has been in use for hundreds of years (Casida 1980). Pyrethroids affect the central and peripheral nervous systems of insects, causing hyper excitability, convulsions, paralysis and death (Cremlyn 1991, Nandi 2006). Similar to DDT, the primary mechanism of action of pyrethroids is binding to the sodium channels of neuronal membranes, causing the sodium channels to remain open for longer periods of time, which disrupts the normal action potential (Cremlyn 1991). This causes a prolonged channel depolarization which leads to repeated firing of the neurons, causing the convulsions and paralysis (Cremlyn 1991, Nandi 2006). This process is specifically caused by pyrethroids altering the gating kinetics of the sodium channels, which are large proteins embedded in the axon membranes. Normally the sodium channels are activated and inactivated by voltage changes in the membrane potential, which causes the proteins to undergo rapid conformational changes that open or close the gate. Pyrethroids slow both the activation and inactivation of the voltage-dependant sodium channels (Vais 2001). It is assumed that pyrethroids bind directly to the sodium channel protein; however, specific binding sites are unknown at this time (Vais 2001).

The mechanism of action is the same in insects and mammals, but insect sodium channels are much more susceptible to pyrethroids than those of mammals. Experiments have also shown that pyrethroids have a greater affinity for insect sodium channels (Vais 2001). In a study comparing the sodium channels of insects (Drosophila channels expressed by genetically modified Xenopus oocytes) to those in rat brains, it was found that the insect sodium channels had a 100-fold greater affinity for pyrethroid binding than did those of the mammals (Nandi 2006). Another study examined recombinant sodium channels from insects and mammals and found that the mammalian sodium channels were 1000-fold less sensitive to pyrethroid activity than those of regular insects (Vais 2001). Fish are also extremely susceptible to pyrethroids compared to mammals, and it is understood to be a function of toxicokinetics and toxicodynamics (Bradbury 1989).

In addition to altering sodium channels, there is also evidence that pyrethroids affect potassium channels, GABA-activated chloride channels, membrane-bound ATPases (Cremlyn 1991), nicotinic acetylcholine receptors, glutamate receptors and peripheral-type benzodiazepine receptors (Soderlund et al. 2002).

In recent years pyrethroids have been increasingly used in agriculture as a replacement for organophosphate pesticides (OPs), whose use has been decreasing in the U.S. due to human-toxicity concerns (Weston et al. 2004). Residential and commercial pest control usage of pyrethroids has increased substantially as well. There has been a shift from using less toxic first generation pyrethroids such as permethrin, to newer compounds that can be nearly 20 times more toxic (Amweg et al. 2005). Pyrethroid insecticides have been used to control insects in the U.S. for over 20 years, but researchers are still working to understand their impact on the environment (Maund et al. 2002).

Pyrethroids are a group of highly hydrophobic compounds with low water solubility (Liu et al. 2004) and log K_{oc} values in the 4.5 to 7 range (Laskowski 2002). These chemical properties indicate that pyrethroids will partition strongly to sediment, specifically the organic carbon (OC) portion, which has been demonstrated in numerous experiments. For example, a phase distribution study by Liu et al. (2004) found that the pyrethroids bifenthrin and permethrin will mostly adsorb to suspended solids, with lesser amounts adsorbing to dissolved organic matter (DOM). Pyrethroid concentrations in the freely dissolved phase, which is associated with bioavailability, were found to be 0.4-1.0% in natural surface waters, and 10-27% in runoff effluents. A study by Bennett et al. (2005) analyzing the effectiveness of vegetated agricultural ditches for the mitigation of the pyrethroids bifenthrin and lambda-cyhalothrin, found that they adsorbed strongly to aquatic vegetation, such as *Ludwiga* and *Lemna*, perhaps reducing their availability for sediment adsorption. A partitioning and bioavailability study of the pyrethroid cypermethrin by Maund et al. (2002) determined that the mean biota-sediment accumulation factors (BSAFs) in *Daphnia magna* and *Chironomus tetans* decreased as the organic carbon percentage of soils increased. The 10-day LC50s also increased with increasing organic carbon components of soil, and the authors concluded that toxicity is due to the pyrethroids that remain in the aqueous phase. Only one pyrethroid (*cis*-permethrin) was on the analyte list in the Hoffman et al. study (2000), and it was never detected, as would be expected given its strong tendency to adsorb to sediments.

Due to the affinity of pyrethroids for sediments, recent research has focused on sediment toxicity, rather than the water toxicity tests that have traditionally been used for pesticide testing and water quality monitoring. In 10-day exposure tests using three sediments with 1.1% to 6.5% organic carbon, Amweg et al. (2005) determined acute toxicity and growth impairment of six pyrethroids to *H. azteca,* and normalized the results for OC content. The study found that most pyrethroids (excluding permethrin) in sediment with 1% OC caused 50% mortality to *H. azteca* at concentrations of 2-10 ng/g, and reduced growth at half that concentration. Table 1 lists the sediment toxicity to *H. azteca* of insecticides analyzed for in this study, as well as some legacy organochlorine (OC) insecticides for comparison. Pyrethroids are highly toxic to benthic invertebrates compared to OCs.

Table 1. Sediment toxicity of various insecticides to *H. azteca*

Insecticide	LC_{50} (μg/g OC)	Reference
DDT	260	Trimble et al. (in press)
α-Chlordane	516	Trimble et al. (in press)
γ-Chlordane	889	Trimble et al. (in press)
Diazinon	not available	
Chlorpyrifos	4.36	Trimble et al. (in press)
Bifenthrin	0.52	Amweg et al. (2005)
Lambda-cyhalothrin	0.45	Amweg et al. (2005)
Esfenvalerate	1.54	Amweg et al. (2005)
Cyfluthrin	1.08	Amweg et al. (2005)
Cypermethrin	0.38	Trimble et al. (in press)
Permethrin	10.83	Amweg et al. (2005)
Fipronil	7.70	Ma (unpublished)
Fipronil sulfide	4.06	Ma (unpublished)
Fipronil sulfone	9.70	Ma (unpublished)
Indoxacarb	not available	

Agricultural Pyrethroids

Pyrethroids are an important class of insecticide in agriculture, especially for fruits, vegetables and cotton. Much research has been conducted on the impact of pyrethroids used in agriculture. Research examining the effects of cypermethrin and esfenvalerate in mesocosm and agricultural field studies focused on water concentrations and concluded that most invertebrate populations affected by the treatment were able to fully recover by the end of the year, and some within weeks (Giddings et al. 2001). In a study by Weston et al. (2004), 10-day *H. azteca* and *C. tentans* sediment toxicity tests were conducted using sediments from California's agriculturally dominated Central Valley. The researchers collected 77 sediment samples from creeks, rivers and irrigation canals, and using a toxic units approach, found 32% of them to cause pyrethroid-related mortality to the test organisms.

Urban Pyrethroids

Several studies have begun to elucidate the possible effects of pyrethroids used in urban areas for structural pest control and home and garden uses. Weston et al. (2005) investigated residential pyrethroid usage and their associated aquatic toxicity in Roseville, California, a suburb of Sacramento. They sampled creeks that drain subdivisions of single-family homes, and found that 9 of 21 sites caused greater than 90% mortality in a 10-day *H. azteca* toxicity tests. Using a toxic units approach, toxicity was mostly attributed to bifenthrin, and a lesser extent to cyfluthrin and cypermethrin. They also sampled the distribution of native *H. azteca*, which are historically present in the creeks. Results showed that their abundance was inversely correlated with pyrethroid toxicity units, although environmental factors confounded the results. More recently, Amweg et al. (2006) analyzed sediments from urban streams in and around Sacramento, California and Nashville, Tennessee. In the California sediment, toxicity was observed in 12 out of 15 streams at least once and this generally correlated with the presence of pyrethroids, especially bifenthrin, using a toxic units approach. On the other hand, toxicity was not observed in the Tennessee sediments and pyrethroids were rarely detected (Amweg et al. 2006).

Other Urban Insecticides

Fipronil is a fairly new insecticide registered in the U.S. in 1996 that is widely used as a fire ant control. It is formulated for turf application and as ant and cockroach bait, and is a slow acting poison through contact or ingestion. Its mode of action is blocking GABA-gated chloride channels in the central nervous system, which causes neural excitation and death (Gunasekara 2007). Fipronil has four metabolites, some of which are more toxic than the parent compound, including fipronil sulfide, fipronil sulfone. Fipronil is somewhat hydrophobic, with a log K_{ow} of 3.9 - 4.1. Fipronil has an average K_{oc} of 803, while fipronil sulfide and fipronil sulfone K_{oc}'s are 2719 and 4209, respectively, suggesting that they all bind to sediment, but the metabolites would be less mobile (Gunasekara 2007). The USGS has monitored water for fipronil and its metabolites in both urban and agricultural settings, and has found that over half of the detections occur in urban streams, although most detections are at low concentrations (up to 0.158 μg/L in California) (Gunasekara 2007). To our knowledge, there has been no previous survey of fipronil or its metabolites in urban sediments.

Indoxacarb is another new insecticide that is widely used in urban settings for fire ant control. It was registered with the U.S. EPA in 2000 as a "reduced-risk" insecticide. It belongs to the oxidiazine chemical family, and its mode of action is to block sodium channels in insects, and acts via contact or ingestion routes (EPA October 2000) It is moderately hydrophobic, with a log K_{ow} value of 4.65 and a K_{oc} value between 2200 and 8200, so it can be expected to bind to sediments (Moncada 2003). Indoxacarb is considered moderately to very toxic to aquatic invertebrates, with EC50s ranging from 0.029 to 2.94 mg/l, however, it is considered safer than alternative such as the OPs it's expected to replace (EPA October 2000). To our knowledge, indoxacarb has not been monitored in urban sediments.

Insecticide Usage in Central Texas

Many residential neighborhoods in Central Texas are characterized by large, intensively maintained lawns. Additionally, imported fire ants are a serious problem affecting this region, causing an estimated $1.2 billion annual impact in Texas (Texas

A&M). Previous studies have reported OP insecticide contamination throughout streams in Denton, TX due to urban pest control. Federal restrictions on the retail sales and many urban uses of chlorpyrifos and diazinon appear to have resulted in their decline in the surface waters of Denton, TX (Banks et al. 2005 a & b). However, it is likely that urban customers will turn to widely available pyrethroids to replace OPs for home and garden use, and but there is no data to indicate whether these may be a problem in Central Texas.

Study Objectives

The objectives of the study were to 1) quantify the presence of pyrethroids and other hydrophobic insecticides in the sediments of the urban watersheds in central Texas, and 2) test the toxicity of the sediments to the amphipod *Hyalella azteca.* The goal was to determine if these insecticides are being transported into stream sediment, and if so, if they appear to be bioavailable and capable of causing toxicity to benthic stream organisms.

Since no data is currently available for pyrethroid contamination in this region, the sampling regime targeted sites that the authors considered to have high potential for contamination including low order streams with direct input from highly maintained residential neighborhoods. The presence or absence of pesticides within these streams has more than regional importance. Previous studies have indicated serious urban insecticide contamination in some areas around Sacramento, California and nearly no contamination in stream sediments collected from Nashville, Tennessee. The region sampled in this study may be representative of southern lawn care practice and fire ant control. Thus if contamination is found, concern may be warranted across a significant portion of the United States rather than just in the Central Valley of California.

CHAPTER TWO

Methods

Study Area and Sample Sites

This study used 16 sampling sites that were established by Banks et al. (2005 a & b) working for the City of Denton, Texas (Figure 1). Denton is a suburb of Dallas located in north central Texas, with a population of approximately 93,000 people and an area of approximately 207 km^2. There are three main watersheds that contain most of the city, with land uses including highly developed commercial and residential areas, mixed rangeland, cropland/pasture, agricultural, forest and the Interstate Highway 35 corridor (Banks et al. 2005 a & b). Sediment samples were also collected from two sites in urban creeks in Hewitt, Texas and one site in Temple, Texas, two smaller cities in central Texas.

Sites were selected to represent streams that drained residential single-family neighborhoods, and the land use classifications of the sub basins show that the areas are heavily residential for the most part. Low and/or high density residential land use averages 41.2% (16.0-94.4% range). Commercial and/or transportation averages 11.1% of land use (0.1-42.9% range), although for most sub basins it was mostly transportation and overall there was very little commercial land use. Grasslands, pasture and forest land use was clumped together as use that would typically not be treated with insecticides, and it averaged 44.1% of land use (0.0-76.7% range).

The individual sampling sites were selected based on several factors. Sites were either within 20 meters of residential yards or were in close proximity to, or by direct

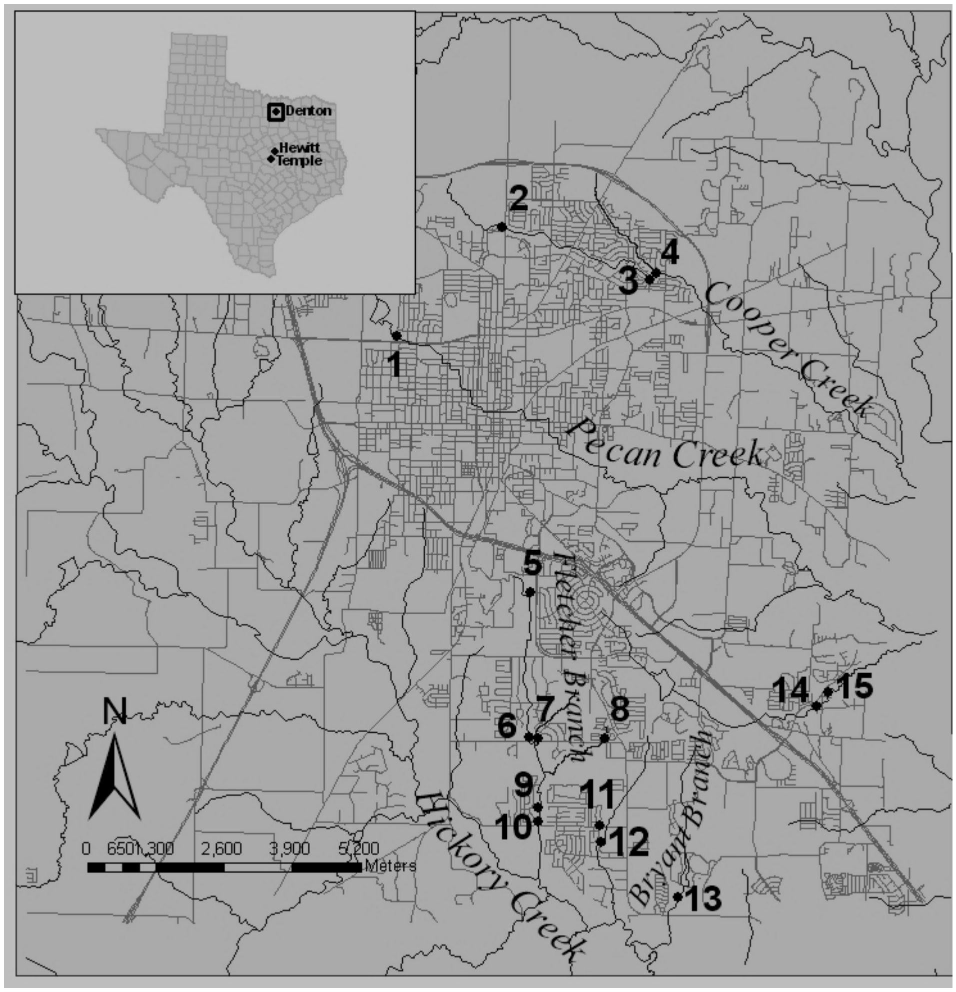

Figure 1. Map of streams and sample sites in Denton, TX. Sites are identified by numbers on Table 2. Stream flow is from West to East. Hewitt and Temple are shown in the inset.

input from impervious surfaces draining residential single-family neighborhoods. Additionally, preference was given to sites with potential quality habitat, and the presence of sediment was a requirement. Sites in Denton were selected due to the availability of historic OP data from established sampling sites, and sites in Hewitt and Temple were used to add breadth to the project.

Sites were qualitatively classified according to basic channel attributes and proximity of direct input from impervious surfaces in order to provide a more comprehensive description. “Natural” sites had stream beds that were relatively unimpacted, some amount of riparian zone and were not in immediate proximity to input from impervious surfaces, but still draining a residential neighborhood, while “natural sites with input” were in immediate proximity to a storm drain or pipe. Channelized sites had either earthen or concrete channels and input from impervious surfaces. Individual site information, including land use within the streams’ sub basin, is presented in Tables 2 and 3. Site selection criterion is intended to represent possible worst case scenarios of contamination by urban insecticides, but does not measure how far the insecticides may be transported in the streams.

GIS Analysis

GIS and watershed models were used to determine the land use in the sub basin for each sampling point. The Soil Water Assessment Tool (SWATX 2005, USGS) was used to delineate sub basins at each sample point using a 30 m resolution grid from the National Elevation Dataset (USGS). The National Land Cover Dataset (EPA 2001) was clipped to the sub basins to determine the percentages of different land uses, using the Anderson Land Use Classification (2001). ArcMap 9.2 (ESRI, Redlands, CA) was used to produce the map graphics.

Sample Collection

Sediment samples for most sites were collected on three occasions: April 2006 (spring), June 2006 (summer) and September 2006 (fall). The dates selected corresponded with probable application times of the insecticides and water being present in the streams after a significant rainfall. Samples of approximately the upper 2-cm of

Table 2. Sampling site descriptions and land use in Denton, TX

Sampling Site [a]	Water Body [b]	Season [c]	Latitude/ Longitude	Channel Type	Sub basin Land Use Percents [d]		
					Res	Com/T	Gr/Fr
1. University & Gay St	Pecan Creek	Sp, S, F	33.230552/-97.15376	Transition from underground storm sewer to natural	24.6	11.1	59.9
2. Locust N of Evers Park	Cooper Creek	Sp, S, F	33.249325/-97.132086	Concrete storm drain that empties into earthen channel	38.4	13.4	48.2
3. Burning Tree Rd (main channel)	Cooper Creek	Sp, S, F	33.240949/-97.10167	Natural, no direct input	56.4	13.2	26.8
4. Burning Tree Rd (side channel)	Trib to Cooper	Sp, S, F	33.240838/-97.10028	Natural, no direct input	49.1	0.9	50.0
5. Teasley	Storm drain to Fletcher Br	Sp, S, F	33.185812/-97.12595	Transition from underground storm sewer to concrete channel	49.5	42.9	7.6
6. Ryan Road West	Fletcher Branch	Sp, S, F	33.162121/-97.12573	Natural, no direct input	60.6	7.9	30.7
7. Ryan Road East	Trib to Fletcher Br	Sp, S, F	33.161871/-97.12351	Natural, no direct input	38.3	7.4	53.2
8. Ryan Road @ Fox Circle	Trib to Fletcher Br	Sp, S, F	33.162071/-97.11019	Natural, no direct input	57.2	7.1	35.7
9. Hickory Crk Rd @ park (up stream)	Fletcher Branch	S, F	33.147286/-97.13249	Natural, no direct input	35.8	11.3	51.9
10. Hickory Crk Rd @ park (down stream)	Fletcher Branch	Sp, S, F	33.147427/-97.12323	Natural, no direct input	35.8	11.3	51.9
11. Hickory Crk Rd @ Livingstone (concrete)	Unnamed Branch	Sp, S	33.146722/-97.10932	Concrete channel that drains a neighborhood	20.5	10.0	67.9
12. Hickory Crk Rd @ Livingstone (stream)	Unnamed Branch	Sp, S, F	33.145814/-97.10578	Natural, direct input site 12	20.5	10.0	67.9
13. Barrel Strap Lane	Bryant Branch	Sp, S, F		Natural, no direct input	47.9	19.8	31.5
14. Upstream from Pecan Crk Elem	Unnamed Creek	Sp, S, F	33.168104/-97.06663	Transition from underground storm sewer to natural channel	16.4	6.9	76.7
15. Pecan Crk Elementary	Unnamed Creek	Sp, S, F	33.17038/-97.06421	Natural, no direct input	41.5	23.8	34.2

[a] Where sample sites are on the same water body, they are listed from highest upstream to downstream; [b] All "branches" lead to Hickory Creek; [c] Sp = spring; S = summer; F=fall; [d] Res includes high and low residential, Com/T includes commercial and/or transportation, Gr/Fr includes pasture, grassland and/or forest (land use not usually treated with pesticides); agriculture and water are not included, as they are low percentages of total land use.

Table 3. Sampling site descriptions and land use in Hewitt and Temple, TX

Sampling Site [a]	Water Body	Season [b]	Latitude/ Longitude	Channel Type	Sub basin Land Use Percents [c]		
					Res	Com/T	Gr/For
Hewitt Park (Hewitt)	Castleman Creek	Sp, S, F	31.453484/-97.20660	Earthen channel through park with direct input	16.0	0.1	43.2
Spring Valley Rd (Hewitt)	Castleman Creek	Sp, S	31.445518/-97.19308	Natural, no direct input	44.5	2.2	47.9
Hickory Rd (Temple)	Trib to Bird Crk	Sp, F	31.068401/-97.39393	Natural, no direct input	94.9	0.5	0.9

[a] Where sample sites are on the same water body, they are listed from highest upstream to downstream; [b] Sp = spring; S = summer; F=fall; [c] Res includes high and low residential, Com/T includes commercial and/or transportation, Gr/For includes pasture, grassland and/or forest (land use not usually treated with pesticides); agriculture and water are not included, as they are low percentages, except Hewitt Park, which contains 39.9% row crops.

sediment were scooped from depositional areas using a small glass jar, placed in a Teflon sealed jar, and transported to the laboratory on ice. The samples were homogenized by hand mixing as soon as possible, and an aliquot for toxicity tests was stored in darkness at 4°C, and an aliquot for chemical analysis was frozen. Clear Creek, north of Denton in an agricultural area, served as a source of control sediment. Sediment from this site has been used in previous studies, is the regional reference stream for the City of Denton (Brooks et al. 2005), and was tested for the presence of our analytes and baseline toxicity to *H. azteca.*

Chemical Analysis

Extraction solvents were purchased from Burdick & Jackson (Honeywell International Inc., Morristown, NJ), except the diethyl ether was purchased from Alfa Aesar (Ward Hill, MA); all were pesticide grade or better. Most analytical standards were purchased from AccuStandard (New Haven, CT), except bifenthrin, permethrin decachlorobiphenyl (DCBP), 1-bromo-2-nitrobenzene and p-terphenyl were from Chem Service (West Chester, PA). All standards were at least 98% purity. Sample analysis techniques were derived from You et al. (2004). Sediments were centrifuged at 1500 rpm for 15 to 20 minutes to remove excess water, homogenized by hand mixing, and a precisely weighed aliquot around 30 grams was dried with magnesium sulfate powder and anhydrous 10-60 mesh sodium sulfate (both from EMD, Darmstadt, Germany). The sample was transferred to a cellulose extraction thimble (30 mm x 100 mm, Whatman International Ltd., Middlesex, UK), p-terphenyl was added to the samples as a surrogate, and they were extracted with methlyene chloride using a soxhlet apparatus. Extracts were transferred through a sodium sulfate-lined filter cone and reduced in volume to approximately 20 mL using nitrogen gas on a Turbovap apparatus (Zymark, Hopkinton, MA). They were solvent exchanged with 10-15 mL of hexane and further reduced to 7-10 mL. Columns made up of 6 g of activated Florisil (60-100 mesh, 6% water, JT Baker, Lopatcong Township, NJ) and 1 cm of anhydrous sodium sulfate, and preconditioned with 10 mL of hexane were used to clean up the extracts, which were then eluted with 50 mL of 70% hexane 30% diethyl ether (v/v). Extracts were reduced again on the Turbovap and further reduced using a heating block and a gentle nitrogen stream. Most samples were brought to 1 mL final volume with 1-bromo-2-nitrobenzene added to the final volume as an internal standard. A few samples were brought to 2 mL final volume due to waxy organics that fell out of solution at smaller volumes.

Analysis of the extracts was performed using a Varian 2100 gas chromatograph (Varian Inc. Walnut Creek, CA) with MS/MS detection and an 8200 autosampler, with settings based on a study by Arrebola et al. (2003). Split/Splitless injection was used, with inlet in split mode for 0.70 minutes, followed by a 20:1 split for the remainder of the run. The injection volume was 1 µl, and the injector temperature was set at 270°C. The carrier gas used was ultra-high purity helium (Airgas Inc., Radnor, PA) with a flow rate of 1 ml/min. A Varian FactorFour capillary column (VF-5m, 30 m x 0.25 mm ID, DF = 0.25) was used to separate analytes. The column oven temperature program started at 100°C for 0.75 minutes, then it was ramped to 200°C at a rate of 8°C/min, then 212°C at 3 °C /min, then 250°C at 8°C/min, then 255°C at 1°C/min, finally it was ramped to 290°C at 3°C/min and held for a bake out period of 5 min. The total run time was 43.67 minutes. The mass spectrometer was set to non-resonant wave form and electron ionization for all analytes, with the emission current set at 80 µA. Other mass spectrometer settings were: ion trap temperature 220°C; manifold temperature 100°C; transfer line temperature 290°C, multiplier offset ±25 V, and automatic gain control (AGC) was turned on. The AGC pre-scan ionization time was 1500 µs, and the AGC target value was 5000 counts. The mass spectrometer was programmed to scan within a particular ion mass per charge (m/z) range, and hold an excitation storage level and excitation amplitude optimized for individual compounds. The GC MS/MS parameters, including retention time (RT), parent and quantification ions, ion scan range, excitation storage and excitation amplitude, and reporting limits for individual compounds are listed in Table 4. Figure 2 displays a chromatograph of all analytes, including surrogates.

Moisture analysis was performed on each sediment sample by drying in a 100 °C oven, and calculating percent loss. The organic carbon (OC) content of sediments was determined by measuring total carbon with a C/N elemental analyzer, and measuring inorganic carbon with a Shimadzu SSM-5000A TOC Analyzer (Kyoto, Japan). Chemical concentrations were normalized on an organic carbon basis to best reflect the bioavailability of the compounds, and match common reporting standards.

All sediment samples were analyzed for the presence of several contaminants. A survey of two retail home improvement centers revealed the most common pyrethroids available to purchase in the central Texas area: bifenthrin, permethrin and lambda-cyhalothrin. Other pyrethroids analyzed for were cypermethrin, cyfluthrin and esfenvalerate, as they were important in the California work (Amweg et al. 2006). Sediments were also tested for the presence of other insecticides commonly used in urban areas including fipronil, its metabolites fipronil sulfide and fipronil sulfone, and indoxacarb; and OP insecticides recently removed from the market, chlorpyrifos and diazinon.

Table 4. GC-MS/MS parameters and reporting limits for each compound

Compound	RT (min.)	Parent Ion (*m/z*)	Quant Ion (*m/z*)	Ion *m/z* Range	Ex. Storage (m/z)	Ex. Amplitude (V)	RL* (µg/ml)
1-Br-2-NB (IS)	6.988	575	203	40-650	89	40	na
TBP (SS)	11.278	99	99	40-650	43	40	na
Diazinon	13.313	304	179	100-320	110	68	0.023
Chlorpyrifos	16.267	314	258	80-325	170	100	0.020
Fipronil sulfide	17.291	643	351	40-650	155	70	0.014
Fipronil	17.539	533	367	40-650	162	70	0.016
Fipronil sulfone	19.547	384	383	40-650	169	20	0.019
p-terphenyl (SS)	19.924	562	244	40-650	108	40	na
Bifenthrin	23.427	181	165	100-350	50	40	0.019
λ-cyhalothrin	25.377	181	152	80-290	80	90	0.033
Permethrin	27.426	183	152	90-200	70	78	0.021
Cyfluthrin	28.926	206	150	90-215	86	96	0.072
Cypermethrin	29.827	181	127	90-215	70	53	0.043
DCBP (IS)	30.916	358	356	90-650	157	40	na
Esfenvalerate	33.026	225	119	90-360	70	51	0.066
Indoxacarb	34.339	546	203	40-650	89	70	0.192

*Reporting limit (RL) is the method detection limit multiplied by three; IS = internal standard, SS = surrogate standard, RT = retention time, Ex = excitation.

Toxicity Tests

Sediment samples were screened for toxicity using ten-day acute survival tests on the amphipod *Hyalella azteca* based on EPA methods. The *H. azteca* culture is maintained in a glass aquarium with a substrate of maple leaves that have been soaked in salt water for at least 30 days then rinsed, and a 16 hour light 8 hour dark cycle. They were fed Tetramin flake fish food three times per week. The water was dechlorinated and carbon filtered municipal water on a flow-through system, maintained around 23˚ C.

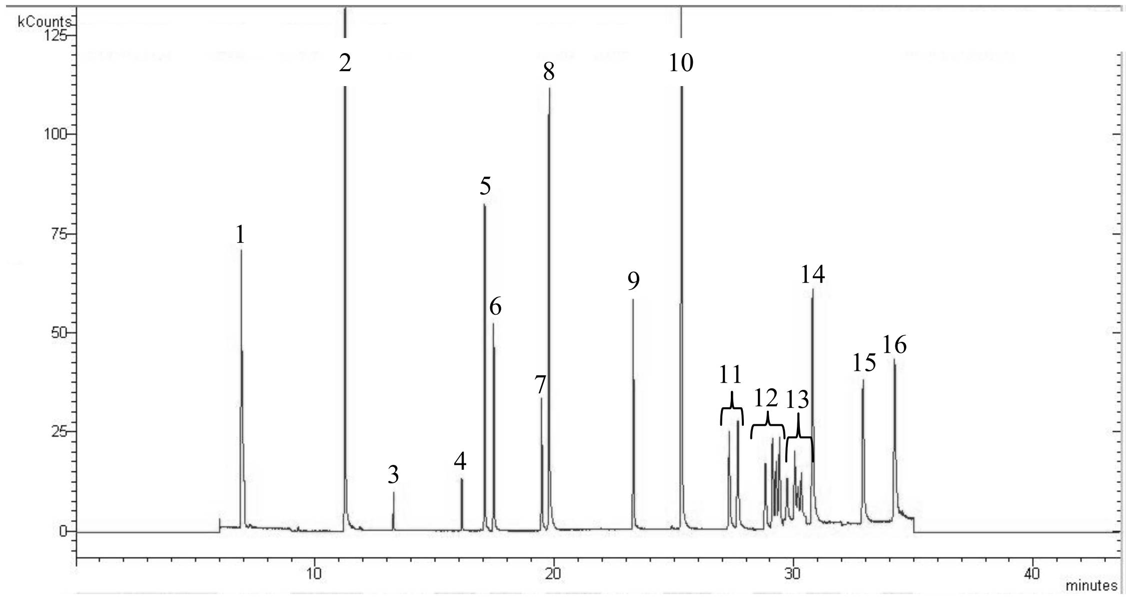

Figure 2. Gas chromatograph of all analytes. 1 = 1-bromo-2-nitrobenzene, 2 = TBP, 3 = diazinon, 4 = chlorpyrifos, 5 = fipronil sulfide, 6 = fipronil, 7 = fipronil sulfone, 8 = p-terphenyl, 9 = bifenthrin, 10 = lambda-cyhalothrin, 11 = permethrin (2 isomers), 12 = cyfluthrin (4 isomers), 13 = cypermethrin (4 isomers), 14 = DCBP, 15 = esfenvalerate, 16 = indoxacarb.

Toxicity tests of the spring and summer sediments were performed at four and eight weeks from collection, respectively. The toxicity test for the fall collection was delayed and the sediment was stored for 15 weeks prior to testing. Controls were the Clear Creek reference sediment and water only units. The toxicity tests require 7- to 14-day-old amphipods that are within a 1- to 2-day range in age. To obtain this standard we used amphipods that pass through a #40 sieve and are retained on a #45 sieve. They were held for three days to ensure they were healthy and the proper age (approximately 9 days old) before commencing the test (USEPA 2000).

Tables from the USEPA (2000) summarize the conditions, a work timeline, and acceptable standards for the 10-day *H. azteca* sediment toxicity test (Appendices A &B). Tests were conducted using an automatic renewal system set to renew approximately four volume additions, added over four renewals per day. Measures of temperature and DO were taken daily, and samples where the dissolved oxygen (DO) dropped to below 2.5 ppm were gently aerated. Temperature was held around 20˚ C and the organisms were fed 1 mL YCT mix per day, added between water renewals. At the completion of the test, live organisms were gently retrieved using a sieve and any individuals not found were considered dead.

Quality Control

A quad study using analyte-spiked control sediment was conducted to verify and optimize the sediment extraction method, and determine percent recoveries for individual analytes. A method detection limit (MDL, n = 7) study was performed to determine reporting limits for the analytes and was also used as a recovery study for low level insecticide concentrations. Results of these studies, including percent recoveries of each analyte at low and high concentrations are presented in Table 5. Each sediment sample to be analyzed was spiked with p-terphenyl as a surrogate chemical to measure percent recovery. The mean surrogate recovery on all sediment samples was 89.4% (SD = 9.9), with a range from 73.2 to 133.1%. Each concentrated sediment extract was spiked with 1-bromo-2-nitrobenzene and decachlorobiphenyl to use as internal standards on the GC/MS.

In order to verify the sensitivity of the *H. azteca* culture as compared to literature LC_{50} values, a sediment toxicity test with bifenthrin-spiked reference sediment was performed using our culture. Bifenthrin in acetone was added to small portions of reference sediment (0.4% OC) in order to bring the nominal concentrations to 0.04, 0.18, 0.44, 0.88, and 1.94 μg/g OC, and an equal amount of acetone was mixed, and aged at

Table 5. Percent recoveries of analytes at high and low concentrations

Compound	High Level Spike/Quad Study (n=4)			Low Level Spike/MDL Study (n=7)		
	Expected Conc (μg/g OC)	Mean % Recovery	SD	Expected Conc (μg/g OC)	Mean % Recovery	SD
Diazinon	3.75	89.3	4.8	0.12	115.1	7.3
Chlorpyrifos	3.75	115.6	6.9	0.12	93.3	6.4
Fipronil sulfide	3.75	110.3	6.2	0.12	92.5	4.5
Fipronil	3.75	144.2	25.0	0.12	146.4	5.0
Fipronil sulfone	3.75	100.5	8.1	0.12	125.8	5.9
p-terphenyl (SS)	0.720	103.3	4.4	0.720	93.2	2.6
Bifenthrin	3.75	125.0	8.4	0.12	131.3	5.9
λ-cyhalothrin	16.25	58.7	7.4	0.257	152.1	4.9
Permethrin	3.84	125.0	12.0	0.12	131.0	6.7
Cyfluthrin	6.25	137.4	25.2	0.197	123.2	14.1
Cypermethrin	6.25	114.7	17.0	0.197	180.1	8.3
Esfenvalerate	16.25	110.7	9.9	0.510	127.8	5.0
Indoxacarb	25.0	70.6	3.8	0.783	117.6	9.4

Expected concentrations assume a spike in 30g of 1% OC sediment; MDL = method detection limit; SD = standard deviation; SS = surrogate standard.

4°C for two weeks before use. Three replicates of each level, plus four solvent controls, and eight of each reference sediment and water-only controls were used for the toxicity test.

Data Analysis

Toxicity data was analyzed using analysis of variance (ANOVA) and Fischer's PLSD to evaluate whether survival rates between sediment samples were significantly different from reference sediment and water-only controls ($\alpha = 0.05$). All statistics were performed using JMP (Version 6.0.0, SAS Institute, Cary, NC).

Chemical concentration data was normalized on a toxicant concentration scale using toxic units (TU), calculated as follows:

$$sumTU = \sum_{i=1}^{n} \frac{E_i}{LC_{50i}} \qquad (1)$$

where E_i is the exposure concentration of the *i*th chemical in the mixture and LC_{50i} is the LC50 of the *i*th chemical. The LC50s used are the *H. azteca* sediment values listed in Table 1. Sum TUs (henceforth referred to as TUs) were plotted against the toxicity test data (percent mortality) to determine if there was a correlation between observed toxicity and insecticide contamination.

To determine if there were significant differences in TUs between the seasons, we used a two factor ANOVA using season (spring, summer or fall) and site as treatments. Sites that were not sampled in all three seasons were eliminated from this analysis.

CHAPTER THREE

Results

Chemical Analysis

In total, 51 sediment samples, not including the reference sediment, were analyzed for the twelve insecticides and metabolites. All insecticides were detected in at least one sediment sample, and all sediment samples contained at least one insecticide. Table 6 displays the frequency of detection (percent of samples containing the analyte) and mean and maximum detected concentrations for each analyte. Appendix C contains all analytical chemistry results and percent OC for each sediment sample.

Table 6. Reporting limit (RL) and frequency, mean and maximum detections

Insecticide	RL (µg/ml)	Detection Freq	Mean (µg/g OC)	Max (µg/g OC)
Diazinon	0.023	21.6%	0.103	0.360
Chlorpyrifos	0.020	56.9%	0.215	2.981
Fipronil sulfide	0.014	58.8%	0.067	0.371
Fipronil	0.016	51.0%	0.129	0.632
Fipronil sulfone	0.019	64.7%	0.178	0.567
Bifenthrin	0.019	94.1%	0.740	2.877
λ-cyhalothrin	0.033	56.9%	0.497	1.718
Permethrin	0.021	41.2%	0.769	3.672
Cyfluthrin	0.072	9.8%	0.271	0.404
Cypermethrin	0.043	5.9%	0.516	0.938
Esfenvalerate	0.066	2.0%	0.886	0.886
Indoxacarb	0.192	37.3%	8.073	68.800

Bifenthrin was the most ubiquitous with detections in 94.1% of sediment samples, and nearly 50% of the concentrations were above the published LC50 value for *H. azteca* sediment tests. Lambda-cyhalothrin was detected in 56.9% of the samples, with 34.5%

of concentrations above its published LC50 value. Bifenthrin and lambda-cyhalothrin were often detected together, and in all but one instance, lambda-cyhalothrin was accompanied by bifenthrin. Fipronil and its metabolites, chlorpyrifos, permethrin, indoxacarb and diazinon were frequently detected compounds, but had concentrations below the LC50 values for *H. azteca*. In most cases their TUs were 0.14 or below, except on three occasions when there were TU values up to 0.68. Cyfluthrin, cypermethrin and esfenvalerate were infrequently detected but in some cases had high enough concentrations to possibly contribute to toxicity, with TUs between 0.11 and 2.47.

The reference sediment was collected in both the spring and the fall, and both collections were analyzed for the twelve insecticides. The spring collection had 0.78 μg/g OC of fipronil sulfone and the fall collection contained no detectable insecticides. Overall, the reference sediment was considered clean and appropriate for use as control sediment in toxicity tests and laboratory analytical spikes.

Toxicity Tests

Control survival for all toxicity tests was high, with an overall mean mortality of 4.3% and 6.7% in the reference sediment and water-only controls, respectively. The reference sediment control was used for comparison in the ANOVA. Out of the 51 samples, 45% were significantly different from the reference sediment control (15 had a p-value of <0.0001, and eight had p-values from 0.0011-0.0479). Mean mortality for each sediment sample and whether the mortality is significantly different from the reference sediment, is shown along with TUs in Table 7.

Table 7. Toxic units and mortality by site and season

Sampling Sites	Spring			Summer			Fall		
	TU	Mean Mortality	p-value*	TU	Mean Mortality	p-value*	TU	Mean Mortality	p-value*
University & Gay St	0.94	40%	0.0011*	2.79	33%	0.0068*	2.38	40%	0.0022*
Locust N of Evers Park	1.08	13%	.4445	1.33	30%	0.0124*	1.26	25%	.0798
Burning Tree Rd (main channel)	1.59	3%	.8107	0.98	18%	.1505	0.12	15%	.4085
Burning Tree Rd (side channel)	1.11	8%	.8432	1.37	18%	.1505	0.35	13%	.5445
Teasley	1.01	17%	.2732	1.48	10%	.4361	1.07	23%	.1276
Ryan Rd West	0.55	10%	.7107	0.36	5%	.7381	1.36	20%	.2415
Ryan Rd East	3.94	70%	<.0001*	2.60	10%	.4361	3.16	63%	<.0001*
Ryan Rd @ Fox Circle	2.19	55%	<.0001*	1.88	23%	.0615	2.03	28%	0.0479*
Hickory Crk Rd @ park (up stream)	…	…	…	1.51	18%	.1505	1.56	63%	<.0001*
Hickory Crk Rd @ park (down stream)	1.32	8%	.8432	5.12	3%	.9112	0.84	3%	.8027
Hickory Crk Rd @ Livingstone (concrete)	7.99	90%	<.0001*	4.54	80%	<.0001*	…	…	…
Hickory Crk Rd @ Livingstone (stream)	1.63	63%	<.0001*	1.75	43%	0.0005*	0.85	15%	.4085
Barrel Strap Lane	0.32	0%	.5659	3.57	10%	.4361	0.38	0%	.5927
Upstream from Pecan Crk Elem	5.58	100%	<.0001*	7.39	93%	<.0001*	6.60	95%	<.0001*
Pecan Crk Elementary	7.01	83%	<.0001*	0.57	65%	<.0001*	0.34	10%	.7225
Hewitt Park (Hewitt)	6.92	97%	<.0001*	0.97	35%	0.0036*	6.71	100%	<.0001*
Spring Valley Rd (Hewitt)	0.03	17%	.2732	0.11	0%	.9312	…	…	…
Hickory Rd (Temple)	0.18	30%	0.0093*	…	…	…	0.15	58%	<.0001*

* indicates that sample mortality is significantly different from control sediment, α level 0.05.

Toxic Units

The concentrations of insecticides present in sediments were normalized for toxicity using a toxic unit (TU) approach for the compounds that had LC50 values available. These values ranged from 0.03 – 7.99 TUs, with a mean of 2.18 TU (SD = 2.21) and a median of 1.36 TU. Out of the 51 samples, 35.3% contained less than one TU, 41.2% contained 1-3 TU, and 23.5% contained more than 3 TUs. Figure 3 shows the TU plotted against observed mortality from the toxicity tests, and Table 6 shows TU data for each sample.

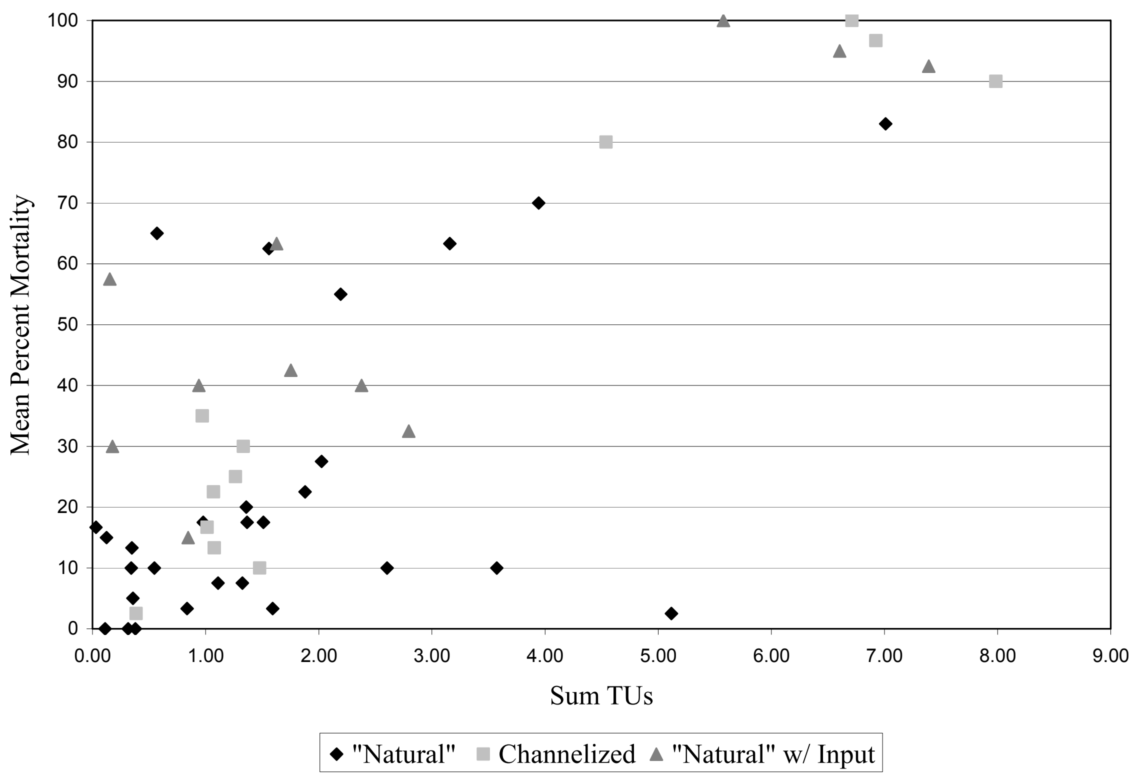

Figure 3. Sum toxic units versus mean percent mortality by site characteristics

Bifenthrin was the most widely detected pyrethroid in all samples, and it was by far the largest contributor to TUs, with a mean of 65.3% of TUs associated with bifenthrin (SD = 29.5, median = 73.2%). Lambda-cyhalothrin was the second largest contributor to TUs, but was much less than bifenthrin, with a mean of 23.0% of TUs associated with it (SD = 25.5, median = 12.1%). In contrast, the next largest contributors to TUs, permethrin and cypermethrin, were associated with means of 4.0% and 1.4%, respectively.

Influence of Season

Despite some seasonal variation in TUs within sites and overall, there were no significant differences related to season (p-value 0.6916). The mean TUs for spring, summer and fall, respectively, are 2.29, 2.50 and 1.95, with a pooled standard error of 0.4523.

CHAPTER FOUR

Discussion

Pyrethroid Chemical Analysis and Toxic Units

Overall, 65% of sediment samples contained at least 1 TU of the insecticides analyzed for, suggesting widespread sediment toxicity. The maximum TU in this study was about eight in the concrete drainage ditch at Hickory Creek Road and Livingstone. This is very similar to previous research conducted in urban streams in California, where in Sacramento 64% of their sampling sites had at least 1 TU of pyrethroids (Amweg et al. 2006), and in the heavily residential Sacramento suburb of Roseville, 73% of samples had at least 1 TU (Weston et al. 2005). However, in California the range of TUs was much greater, with several samples over 10 TUs and a maximum of about 40 TUs at one site.

Bifenthrin was by far the largest contributor to TUs in the sediment samples, comprising an average of 65% of the TUs. It was detected in all but three of the individual samples and was detected at least once at each site. In fact, graphing just bifenthrin TUs against mortality results in a better correlation than the Sum TUs, with r^2 values of 0.66 (Figure 4) and 0.59, respectively. Lambda-cyhalothrin was the next largest contributor to TUs, comprising an average of 23% of the TUs. Bifenthrin was also the largest contributor in California, where bifenthrin contributed an average of 58% and 70% of the TUs in the Sacramento area (Amweg et al. 2006) and Roseville (Weston et al. 2005), respectively. However, lambda-cyhalothrin was not as important in the

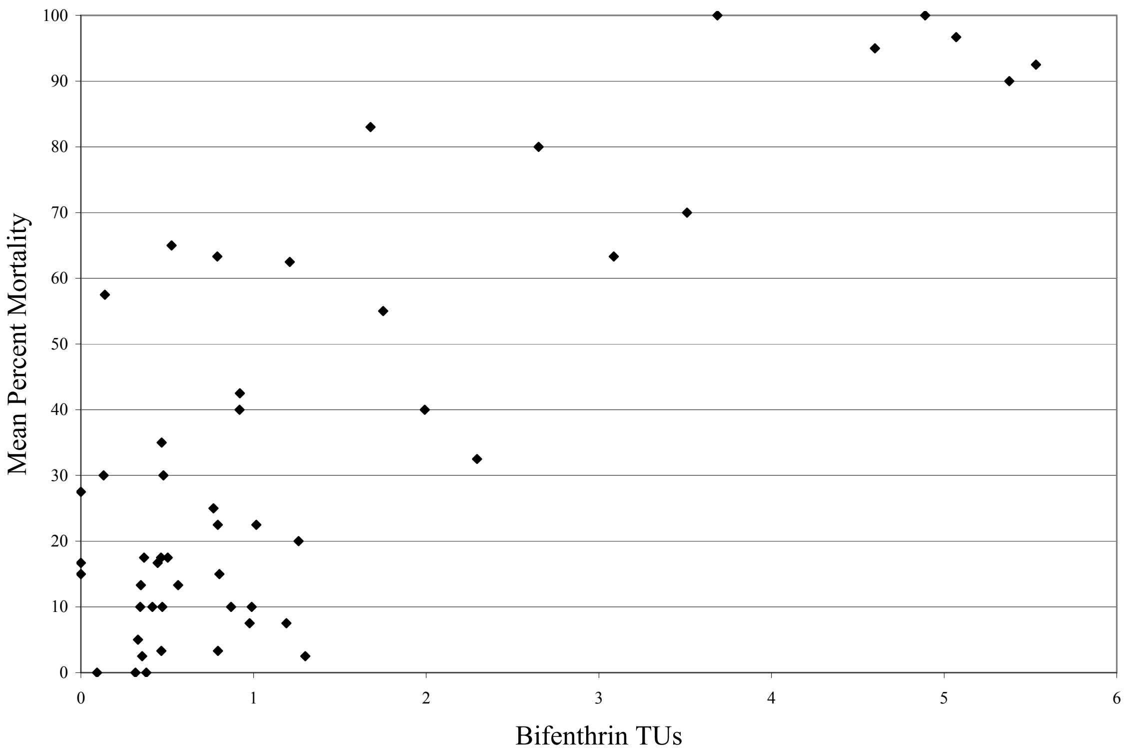

Figure 4. Bifenthrin toxic units versus mean percent mortality

California studies with an average of 10% of the TUs in Sacramento (Amweg et al. 2006), and a low frequency of detections and low concentrations in Roseville (Weston et al. 2005).

The prevalence of bifenthrin and lambda-cyhalothrin in the sediments is most likely explained by their wide availability in central Texas for control of imported fire ants and grub worms. Bifenthrin is heavily used in both applications. Additionally, these pyrethroids have a longer half-life than some of the others, so they may remain in the sediments for longer periods of time. Bifenthrin has an aerobic soil half-life of 96 days, and lambda-cyhalothrin has an aerobic half life of 43 days. In comparison, the other pyrethroids have half-lives that range from 12 days for cyfluthrin to 40 days for permethrin. Bifenthrin is particularly stable in anaerobic environments, with a half-life of over 400 days (Laskowski 2002).

To a lesser extent permethrin, cyfluthrin and cypermethrin also contributed to TUs. Permethrin was detected in 41% of samples, but it is substantially less toxic than the other pyrethroids (10.83 μg/g OC) and at most contributed 0.3 TU to an individual sample, and just 4% overall. Cypermethrin is the most toxic pyrethroid we tested for (0.38 μg/g OC) but it was rarely detected, and therefore did not contribute significantly to the overall TUs (1.4%). However, on the three occasions that it was detected, it contributed between 0.5 and 2.5 TUs. Cyfluthrin and esfenvalerate contributed even less to the overall TUs. In California, permethrin was similarly widely detected, but due to its lower toxicity did not contribute much to the overall TUs. In contrast to central Texas, cypermethrin and cyfluthrin and were much more important in California, where they contributed an average of 16% and 9%, respectively, to TUs in Sacramento (Amweg et al. 2006) and were detected much more frequently in both studies.

Non-Pyrethroid Chemical Analysis and Toxic Units

Overall, the non-pyrethroids on the analyte list were somewhat widespread, but apparently rarely contributed to toxicity. Fipronil and its metabolites, while widely detected, did not significantly contribute to sediment toxicity, with the largest contribution to TUs being one instance of fipronil sulfone contributing 0.14 TUs. This is similar to the findings of the USGS where they found widespread occurrence of fipronil and its metabolites in urban surface water, but always at very low concentrations (USGS 2006). The difference in K_{oc} values between fipronil and its metabolites may explain why the metabolites were detected slightly more frequently than the parent compound. This study suggests that fipronil is not causing a sediment contamination problem.

Chlorpyrifos was detected in 57% of the sediment samples, but did not appear to contribute significantly to sediment toxicity, except in one instance at the Upstream from Pecan Creek Elementary site where it contributed 0.68 TUs. Diazinon does not have an *H. azteca* sediment LC50 value available, but was only detected in 22% of samples and always in low concentrations. While these OP insecticides do not appear to contribute significantly to TUs, they have been banned for retail sale by the EPA, but are apparently still being used. This is likely due to homeowners that still have a personal supply from before the ban, as the City of Denton does not use these chemicals for treatment of public areas (personal communication, David Hunter). It is impossible to compare levels of these insecticides to the studies conducted by the City of Denton because they analyzed water samples but did not investigate sediments. However, their study did find a significant decrease in concentrations of chlorpyrifos and diazinon after the EPA ban (Banks et al. 2005 a. & b.), so presumably their use will continue to decrease as homeowners use up their supplies.

Indoxacarb also does not have an *H. azteca* sediment LC50 value available, but evidence indicates that it did not contribute to toxicity. Indoxacarb was detected in high concentrations in some sediments (up to 68.8 μg/g OC at the summer Ryan Road @ Fox site), but is not associated with observed toxicity. Indoxacarb was registered with the EPA as a “low-risk” insecticide, and presumably poses less risk to aquatic invertebrates than do the pyrethroids. More research should be done with indoxacarb to determine sediment toxicity and help determine if it poses a threat to aquatic invertebrates.

Sediment Toxicity

Overall, 45% of the 51 sediment samples had mortality to *H. azteca* that was significantly different than the control sediment, and 16% of sediments caused 80% or greater mortality. This is somewhat less mortality than what was observed in the California work where 73% of sediments in Roseville had greater than 20% mortality, and 43% had greater than 90% mortality (Weston et al. 2005). In Sacramento 67% of the samples in (Amweg et al. 2006) were acutely toxic to *H. azteca*. This is probably due to the fact that the California sediments had higher ranges of TUs than central Texas

Toxic Units and Toxicity

In general TUs were a good predictor of toxicity, although there were some outliers in the data (Figure 4). Sites with less than 1 TU were generally non-toxic, although some sites were more toxic than the TUs predicted. This is probably due to the presence of other chemicals that were not analyzed for, such as PAHs or metals. There was some variability between predicted and observed mortality in sediment with 1-3 TUs. Most samples with up to 3 TUs had 30% or less mortality, whereas the TU assumption would predict that sediment with greater than 1 TU should have at least 50% mortality. This discrepancy has been noted in previous work, and is probably due to differing bioavailability between sediment types, even when normalized for organic carbon content (Weston et al. 2005). There appears to be a threshold between 2 – 3 TUs before toxicity is observed, and may be related to the bioavailability of the hydrophobic pyrethroids. Except for two outliers, sediments with greater than three TUs had mortality of 60% or greater, as would be expected.

The two outliers in the data that have high TUs (5.12 and 3.57) but mortality of 10% or less may be explained by several possible factors. Both outliers have significant TUs from lambda-cyhalothrin (the sample with 5.12 TUs has 3.82 of those from lambda, and the other one has 1.11 TU from it). Removing lambda-cyhalothrin from the TU analysis improves the relationship between TUs and mortality, and removes both of the outliers (Figure 4). This could be a relic of the sediment storage time before they were tested for toxicity, as the sediment for chemical analysis was frozen soon after collection but the portion for toxicity testing was stored at 4°C until the toxicity test was conducted. Both samples were from the summer sampling, which was tested for toxicity eight weeks after collection. Since bifenthrin has a longer half-life than lambda-cyhalothrin (96 versus 43 day aerobic soil half-life (Laskowski 2002)), it is possible that the lambda in the refrigerated sample degraded and did not cause as much mortality, while the lambda in the frozen sample was preserved for the chemical analysis. Both of the sediments had low OC content, in particular, the sample with 5.12 TUs had the lowest OC of all samples with 0.25%. The sample with 3.57 TUs was 0.83% OC, and while low, was not among the lowest and there were also other samples with low OC that had similarly high TUs, but where the mortality was more on par with the TUs.

Seasonality

There were no significant differences in TUs between the different collection times, although there was some variation within the sites. In the neighborhoods we sampled in central Texas the lawns are maintained year round except for a brief dormancy in winter, thus it is assumed that pest control is applied throughout most of the year, except perhaps winter. Another factor to consider is the central Texas climate, which averages 34.75 inches of rain per year that is fairly evenly distributed, with May being the wettest month with an average of 5.15 inches of rain and January being the driest and averaging 1.90 inches (DFW Annual Summary of Normal, Means and Extremes). 2006 was drier than usual, with an annual total rain fall of 29.75 inches . The sampling months of April, June and September had monthly rain totals of 1.86, 0.34 and 2.60 inches of rain, respectively (DFW Monthly and Annual Precipitation). The sampling was conducted after a significant rainfall in an attempt to coincide with a flush of insecticides from lawns. A lack of seasonal differences in our data is likely due to a combination of nearly year-round application, and year-round rain that flushes new sediment and insecticides into the streams.

Site Characteristics

The stream classifications were included as a description for each site and the study was not designed to test differences in stream classification. However, there did appear to be patterns in the mortality and TUs associated with the different site characterizations. Twenty-eight of the samples were collected from "natural" sites, and of those, 75% did not have mortality that was significantly different than the reference sediment. The 12 sediment samples collected from channelized streams (earthen or concrete), were evenly divided between significant and non-significant mortality. The 11 samples collected from "natural" sites with direct input tended to be the most toxic, with only one (9%) being non-toxic. Further, there were several samples in the channelized and input categories that had mortality that did not appear to be related to pyrethroids, suggesting that the storm drain input carried other chemicals that we didn't analyze for. When TUs were examined with regard to stream classification, there did not appear to be much difference between the sites, with 71%, 55% and 67% of “natural”, “natural” with input and channelized sites, respectively, having two or fewer TUs. It appears that all types of sites were receiving pyrethroid input from residential neighborhoods, as that was what the study was designed to detect, however site type might help explain the data outliers that caused mortality, but did not contain enough TUs to explain it.

Twelve of the sampling sites had water concentrations of diazinon monitored by the City of Denton from April of 2001 to June of 2005 in conjunction with a study that monitored this OP before and after its EPA ban. Historic data indicates that insecticides have been used at all of our current sampling sites that overlap with the historic monitoring. In the current study, 83% of sites with historic diazinon detections had a mean of <1 TU, and 17% of those had a mean of <3 TUs.

Uncertainties

The sampling regime for this study was intended to be a snapshot of worst case scenario insecticide contamination. Sample sites were selected to be within close proximity of, or direct input from impervious surfaces or storm drains from residential neighborhoods. Although there was some connectivity between the sites, the study design did not test how widespread the contamination was, or how far the insecticides might be flushed downstream. Studies of vegetated agricultural ditches suggest that pyrethroids do not move very far. Bifenthrin and lambda-cyhalothrin were released into a 650-m agricultural ditch, and were detected at the inlet and 200 m downstream, but were never detected at the 400 m point, even after seven days (Bennett et al. 2005). An interesting set of our sampling sites seems to follow this trend. The Pecan Elementary sites were approximately 250 m apart in a stream that drained a new subdivision, but that had a park and riparian zone immediately adjacent. The upstream sample was taken directly from where the stream exited an underground culvert that had direct input from the storm sewers of the neighborhood, while the downstream sample was taken at a point where the steam flowed through about 250 m of "natural" area, although there was another, less direct input. The upstream site had very high mortality (93-100%) and high TUs (5.6 – 7.4) in every season. The downstream site was more varied, with high TUs in the spring, and less than 1 TU in the summer and fall. However, high mortality was observed in both spring and summer but not the fall. During the summer collection there was a concrete trail being constructed near the stream, which caused the upstream site to be silty. The downstream site did not appear to be affected by the construction, but it is possible another chemical or factor from the construction caused high mortality not related to insecticides. While it's difficult to know exactly what caused the variability in mortality and TUs at the downstream site, this does show that pyrethroid contamination may be localized, and not travel far downstream.

Conclusions

The overall findings of this study suggest that pyrethroids are indeed a problem at the sites we sampled. Moreover, it is likely that these sampling sites are representative of all southern states that have imported fire ant problems and practice intensive lawn maintenance, and these findings are likely a regional issue. As in the California work, the main culprit is bifenthrin, although some other pyrethroids do contribute as well. Since pyrethroids are causing sediment contamination in both California and central Texas, more effort should be spent to monitor sediment quality in other regions of the country.

APPENDICES

APPENDIX A

EPA Toxicity Test Conditions

Table 11.1 Test Conditions for Conducting a 10-d Sediment Toxicity Test with *Hyalella azteca*

	Parameter	Conditions
1.	Test type:	Whole-sediment toxicity test with renewal of overlying water
2.	Temperature:	23 ± 1°C
3.	Light quality:	Wide-spectrum fluorescent lights
4.	Illuminance:	About 100 to 1000 lux
5.	Photoperiod:	16L:8D
6.	Test chamber:	300-mL high-form lipless beaker
7.	Sediment volume:	100 mL
8.	Overlying water volume:	175 mL
9.	Renewal of overlying water:	2 volume additions/d (Appendix A); continuous or intermittent (e.g., 1 volume addition every 12 h)
10.	Age of organisms:	7- to 14-d old at the start of the test (1- to 2-d range in age)
11.	Number of organisms/chamber:	10
12.	Number of replicate chambers/treatment:	Depends on the objective of the test. Eight replicates are recommended for routine testing (see Section 16).
13.	Feeding:	YCT food, fed 1.0 mL daily (1800 mg/L stock) to each test chamber. The first edition of the manual (USEPA, 1994a) recommended a feeding level of 1.5 mL of YCT daily; however, this feeding level was revised to 1.0 mL to be consistent with the feeding level in the long-term tests with *H. azteca* (Section 14).
14.	Aeration:	None, unless dissolved oxygen in overlying water drops below 2.5 mg/L.
15.	Overlying water:	Culture water, well water, surface water, site water, or reconstituted water
16.	Test chamber cleaning:	If screens become clogged during a test, gently brush the *outside* of the screen (Appendix A).
17.	Overlying water quality:	Hardness, alkalinity, conductivity, pH, and ammonia at the beginning and end of a test. Temperature and dissolved oxygen daily.
18.	Test duration:	10 d
19.	Endpoints:	Survival and growth
20.	Test acceptability:	Minimum mean control survival of 80% and measurable growth of test organisms in the control sediment. Additional performance-based criteria specifications are outlined in Table 11.3.

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APPENDIX B

EPA Timeline for Toxicity Test

Table 11.2 General Activity Schedule for Conducting a 10-d Sediment Toxicity Test with *Hyalella azteca* [1]

Day	Activity
-7	Separate known-age amphipods from the cultures and place in holding chambers. Begin preparing food for the test. There should be a 1- to 2-d range in age of amphipods used to start the test.
-6 to -2	Feed and observe isolated amphipods (Section 10.3), monitor water quality (e.g., temperature and dissolved oxygen).
-1	Feed and observe isolated amphipods (Section 10.3), monitor water quality. Add sediment into each test chamber, place chambers into exposure system, and start renewing overlying water.
0	Measure total water quality (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity, ammonia). Transfer 10 7- to 14-day-old amphipods into each test chamber. Release organisms under the surface of the water. Add 1.0 mL of YCT into each test chamber. Archive 20 test organisms for length determination or archive 80 test organisms for dry weight determination. Observe behavior of test organisms.
1 to 8	Add 1.0 mL of YCT food to each test chamber. Measure temperature and dissolved oxygen. Observe behavior of test organisms.
9	Measure total water quality.
10	Measure temperature and dissolved oxygen. End the test by collecting the amphipods with a sieve (Section 11.3.7.1). Count survivors and prepare organisms for weight or length measurements.

[1] Modified from Call et al., 1994

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APPENDIX C

Table C.1—Complete analytical chemistry results

	Season	%OC [a]	Detected Insecticides (μg/g OC): [b] Diaz	Chl	Bif	λ-Cy	Perm	Cyf	Cyp	Esfen	Fip	Sulfide	Sulfone	Ind
University & Gay St	Sp	1.75	<RL	0.062	0.478	<RL	<RL	<RL	<RL	<RL	<RL	0.062	<RL	<RL
	S	2.85	0.042	0.216	1.193	0.179	0.462	<RL	<RL	<RL	0.053	0.026	<RL	<RL
	F	3.40	0.024	0.050	1.036	0.122	0.885	<RL	<RL	<RL	0.041	0.019	0.058	0.785
Locust N of Evers Park	Sp	1.95	<RL	<RL	0.293	0.217	<RL	<RL	<RL	<RL	0.086	<RL	0.081	<RL
	S	1.45	<RL	0.085	0.4786	0.329	0.424	<RL	<RL	<RL	0.085	0.064	0.102	16.866
	F	0.76	<RL	0.038	0.417	<RL	<RL	<RL	<RL	<RL	<RL	<RL	0.143	<RL
Burning Tree Rd (main channel)	Sp	1.20	<RL	0.044	0.242	0.328	<RL	0.382	<RL	<RL	0.181	0.102	<RL	<RL
	S	1.90	<RL	<RL	0.190	0.252	0.185	<RL	<RL	<RL	0.067	0.054	0.089	7.407
	F	0.95	<RL	0.021	<RL	<RL	0.951	<RL	<RL	<RL	<RL	0.021	0.115	0.781
Burning Tree Rd (side channel)	Sp	0.40	<RL	<RL	0.508	<RL	<RL	<RL	<RL	<RL	<RL	<RL	0.537	<RL
	S	0.95	<RL	<RL	0.242	0.377	0.233	<RL	<RL	<RL	<RL	0.076	0.144	6.441
	F	0.70	<RL	<RL	0.181	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL
Teasley	Sp	1.85	0.041	0.075	0.231	0.219	0.333	<RL	<RL	<RL	0.085	0.032	0.075	0.983
	S	1.70	<RL	0.098	0.452	0.246	<RL	<RL	<RL	<RL	0.061	0.066	0.103	<RL
	F	3.08	<RL	0.084	0.528	<RL	<RL	<RL	<RL	<RL	<RL	0.031	0.126	<RL
Ryan Rd West	Sp	1.04	<RL	0.330	0.245	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL
	S	1.57	0.054	0.051	0.172	<RL	<RL	<RL	<RL	<RL	0.070	0.051	<RL	6.065
	F	0.98	<RL	0.047	0.655	<RL	0.664	<RL	<RL	<RL	<RL	<RL	0.111	1.821

Table C.1—Continued

	Season	%OC	Detected Insecticides (µg/g OC): Diaz	Chl	Bif	λ-Cy	Perm	Cyf	Cyp	Esfen	Fip	Sulfide	Sulfone	Ind
Ryan Rd East	Sp	2.32	0.062	0.102	1.825	0.170	<RL	<RL	<RL	<RL	0.083	0.021	0.073	0.919
	S	0.55	<RL	0.097	0.514	0.686	<RL	<RL	<RL	<RL	<RL	0.142	0.216	<RL
	F	1.21	0.058	0.119	1.605	<RL	<RL	<RL	<RL	<RL	0.101	0.036	0.112	<RL
Ryan Rd @ Fox Circle	Sp	1.90	<RL	<RL	0.910	0.200	<RL	<RL	<RL	<RL	<RL	<RL	<RL	0.813
	S	0.81	<RL	0.040	0.412	0.473	<RL	<RL	<RL	<RL	0.126	0.101	<RL	68.752
	F	0.47	<RL	<RL	<RL	0.876	<RL	<RL	<RL	<RL	<RL	0.061	0.298	1.637
Hickory Crk Rd @ park (up stream)	S	0.88	<RL	<RL	0.262	0.428	<RL	<RL	<RL	<RL	0.119	0.086	0.128	6.528
	F	0.54	<RL	<RL	0.629	<RL	0.604	0.319	<RL	<RL	<RL	<RL	<RL	1.090
Hickory Crk Rd @ park (down stream)	Sp	0.41	<RL	<RL	0.619	<RL	<RL	<RL	<RL	<RL	<RL	<RL	0.545	<RL
	S	0.25	<RL	<RL	0.676	1.718	<RL	<RL	<RL	<RL	<RL	<RL	<RL	12.626
	F	1.21	<RL	<RL	0.413	<RL	0.210	<RL	<RL	<RL	<RL	0.017	0.088	0.822
Hickory Crk Rd @ Livingstone (concrete)	Sp	0.34	<RL	0.562	2.796	1.028	0.576	<RL	<RL	<RL	0.384	0.069	0.343	<RL
	S	0.42	<RL	0.075	1.379	0.759	1.176	<RL	<RL	<RL	<RL	<RL	0.310	<RL
Hickory Crk Rd @ Livingstone (stream)	Sp	1.11	<RL	<RL	0.4109	0.355	<RL	<RL	<RL	<RL	<RL	<RL	0.194	<RL
	S	1.45	<RL	0.085	0.4786	0.329	0.424	<RL	<RL	<RL	0.085	0.064	0.102	16.866
	F	0.76	<RL	0.038	0.417	<RL	<RL	<RL	<RL	<RL	<RL	<RL	0.143	<RL
Barrel Strap Lane	Sp	1.06	<RL	<RL	0.164	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL
	S	0.83	<RL	0.042	0.215	0.498	<RL	0.404	0.404	0.886	0.147	0.115	<RL	15.912
	F	0.46	<RL	<RL	0.197	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL

Table C.1—Continued

	Season	%OC	Detected Insecticides (μg/g OC):											
			Diaz	Chl	Bif	λ-Cy	Perm	Cyf	Cyp	Esfen	Fip	Sulfide	Sulfone	Ind
Upstream from Pecan Creek Elem	Sp	0.67	<RL	0.043	1.917	0.794	0.391	<RL	<RL	<RL	0.177	0.073	0.195	<RL
	S	1.59	<RL	0.021	2.877	0.791	0.379	<RL	<RL	<RL	0.112	0.100	0.146	1.885
	F	3.01	<RL	2.981	2.392	0.360	3.641	0.138	<RL	<RL	0.111	0.036	0.158	<RL
Pecan Crk Elementary	Sp	0.45	0.294	0.164	0.872	1.156	<RL	<RL	0.938	<RL	0.632	0.371	0.567	<RL
	S	0.60	<RL	<RL	0.273	<RL	<RL	<RL	<RL	<RL	<RL	<RL	0.184	<RL
	F	0.62	<RL	<RL	0.178	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL
Hewitt Park (Hewitt)	Sp	1.00	0.095	0.560	2.636	0.705	0.605	<RL	<RL	<RL	0.270	0.055	0.250	<RL
	S	2.05	<RL	0.027	0.243	0.218	<RL	<RL	<RL	<RL	0.059	0.047	<RL	<RL
	F	5.60	0.360	0.022	2.543	0.356	3.672	0.114	0.206	<RL	0.070	0.031	0.121	1.258
Spring Valley Rd (Hewitt)	Sp	2.45	0.049	0.020	<RL	<RL	0.294	<RL	<RL	<RL	<RL	<RL	<RL	<RL
	S	2.85	<RL	<RL	0.049	<RL	<RL	<RL	<RL	<RL	0.054	<RL	0.045	<RL
Hickory Rd (Temple)	S	3.60	0.057	0.167	0.068	<RL	<RL	<RL	<RL	<RL	0.049	<RL	<RL	<RL
	F	4.00	<RL	<RL	0.072	<RL	0.083	<RL	<RL	<RL	<RL	<RL	0.029	<RL

[a] %OC = percent organic carbon; [b] Diaz = diazinon, Chl = chlorpyrifos, Bif = bifenthrin, λ-Cy = lambda-cyhalothrin, Perm = permethrin, Cyf = cyfluthrin, Cyp = cypermethrin, Esfen = esfenvalerate, Fip = fipronil, Sulfide = fipronil sulfide, Sulfone = fipronil sulfone, Ind = indoxacarb; RL = reporting limits.

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