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Pesticide Concentrations in Water from a Southern Louisiana Marsh Influenced by the Mississippi River

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PESTICIDE CONCENTRATIONS IN WATER FROM A SOUTHERN LOUISIANA MARSH INFLUENCED BY THE MISSISSIPPI RIVER

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ABSTRACT

The pesticides atrazine, chlorothalonil, and fipronil are common surface water contaminants due to their high rates of application and their chemical properties. This study investigates the introduction of contaminants into coastal wetlands via the Bayou Lamoque Mississippi River control structure in Plaquemines Parish, Louisiana. A protocol for environmental sampling using Continuous Low-level Aquatic Monitoring (C.L.A.M.) C-18 disks is described, along with effective solid phase extraction (SPE) and gas chromatography mass spectrometry (GC/MS) methodology for the determination of atrazine, chlorothalonil, and fipronil in surface water. Average concentrations of atrazine and chlorothalonil inside the Bayou Lamoque freshwater diversion structure for the months of June – August 2016, were 24.5 ng l^{-1} and 3.5 ng l^{-1}, respectively. Fipronil was not detected in concentrations exceeding its method detection limit of 12.6 ng l^{-1}. Though the detected concentrations are innocuous to non-target organisms based on current ecotoxicity data, more research is needed to identify additional contaminants entering the wetlands via freshwater diversions.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Coastal wetlands in Louisiana provide a variety of valuable ecosystem goods and services. They support numerous commercial interests, including the fishing, oyster, and shrimp industries. The wetlands also provide revenue through various recreational activities. Additionally, they deliver physical services, such as water purification, protection from storm surge, and carbon sequestration. Wetlands provide other supporting services through their intrinsic value: species diversity and cultural enrichment, for example. Many of Louisiana's wetlands are now influenced by the Mississippi River due to anthropogenic intervention such as sediment and freshwater diversions.

River control structures built into levees along the Mississippi River serve multiple purposes. Coastal restoration projects for the last 50 years have employed sediment and water diversions from the Mississippi River to compensate for southern Louisiana land loss. The river has also been deliberately redirected by the Army Corps of Engineers to prevent the flooding of cities that would otherwise fall in its future path. River water may be diverted in the case of pollution events such as oil spills to reduce damage to the marshes that fringe the northern Gulf of Mexico. These projects result in the introduction of water from the Mississippi River into wetland ecosystems, along with the contaminants it carries from rainwater drainage pumps at the parish level and runoff from the farms in the heart of America.

1.2. Louisiana Coastal Wetlands

1.2.1. Economic Value

Louisiana's coastal wetlands play a critical role in the state's economy. They are valued at $2,429—$6,400 to $8,977—$17,000 per acre due to the ecosystem services they provide. The

wide gap in these estimates is due to uncertainty introduced by the complexity of the system (Costanza, Farber, & Maxwell, 1989). One ecosystem service provided by estuaries and coastal wetlands is the shelter and nutritional source they provide to fish and aquatic and terrestrial invertebrate populations. Such resources are critical for the highly-sensitive larval stages of these organisms (Beck et al, 2001). Fin fishes, oysters, crabs, and shrimp are economically important to Louisiana, so disturbances to their habitat and juvenile development may have serious ramifications.

The Louisiana Department of Wildlife and Fisheries studied trends in the average commercial catch and dockside value of Louisiana seafood from 2000—2009. Commercial fishers sold an average of 872 million pounds of saltwater finfish per year between the years 2000—2008. Dockside value for fish during the years studied was highest in 2000, throughout which 24.4 million pounds of fish sold commercially for $32.3 million. These values decreased to a low of 10.7 million pounds sold for $16.8 million in 2009 (Bharadwaj, Lavergne, & Ogunyinka, 2012a). Crab sales were highest in 2009, during which 52.9 million pounds of crabs sold for $37.2 million (Bharadwaj, Lavergne, & Ogunyinka, 2012b). Commercial shrimp sales were the highest in 2000, with 147 million pounds sold for $260.1 million. In 2009, 110.9 million pounds of shrimp were sold for $117.9 million (Bharadwaj, Lavergne, & Ogunyinka, 2012c). Oysters also reached their peak value in 2009; 14.9 million pounds were sold for $50.7 million. Oysters are the most valuable game of the four listed, selling for $3.40 per pound in their peak year. (Bharadwaj, Lavergne, & Ogunyinka, 2012d).

Louisiana's economy also benefits from numerous recreation activities that take place in its coastal wetlands. Recreational fishing in the state is a popular pastime for locals and non-residents. In 2008, $807 million was spent within the state for non-commercial fishing per a

census survey of state residents; 66% went to trip-related expenditures such as food, housing, and transportation, while 30% went to equipment purchases (U.S. Department of the Interior et al, 2011). One in 100 Louisiana jobs can be attributed to fishing; the industry employs as many people as clothing and accessory stores (Southwick, 2008).

Wildlife watching is another popular activity that generates revenue for the state. These activities include feeding, watching, or photographing wild species in natural settings. Out of one million wildlife-watchers across the state, 371,000 people participated in wildlife watching activities greater than one mile away from their homes in 2011. Approximately 45% of these people were non-residents. In 2011, wildlife-watchers spent $543 million; 51% went to equipment and 41% was dedicated to trip-related expenses (USDOI, 2011).

A third recreational activity supported by Louisiana's coastal wetlands is boating. In 2008, 316,593 boats were registered in the state. Out of 1,306 vessels observed, the average number of trips for the year was 20 (Isaacs & Lavergn, 2010). One out of 85 jobs in Louisiana is attributable to boating activities, enough to employ all residents of West Baton Rouge Parish (Southwick 2008). This is due to the broad range of demands that arise from boating activities, including: sales, licensing, docking, launching, rental, maintenance, and other related services.

Commercial and recreational revenue is dependent on the health of Louisiana's coastal wetlands. Damage to the ecosystem could have a profound impact on the economic health of the state. Beyond finances, the wetlands serve critical ecosystem services, as well.

1.2.2. Physicochemical Services

Wetlands provide unique physical and chemical ecosystem services surpassed only by coral reefs. Wetland ecosystem services include water purification, protection from storm surge, and carbon sequestration. The yearly estimated values of these services range from $44,000 per

hectare for inland wetland systems to $79,000 for coastal systems and $215,000 for tidal marshes (Clarkson, Ausseil, & Gerbeaux; 2017).

The water purification abilities of wetlands are well-recognized. They receive water inputs from a variety of point and nonpoint sources. Point sources have a clear point of origin, such as a pipe discharging or a vessel containing a potential pollutant; therefore, monitoring and remediating issues related to point source pollution is relatively straightforward. According to the Clean Water Act, a nonpoint source is any origin of a contaminant that is not a point source. Nonpoint sources include agricultural runoff, precipitation, and atmospheric deposition. Pollution from nonpoint sources is challenging to address due to its vague point of origin (EPA, 2016). Wetlands are often referred to as "the kidneys of the landscape" due to their ability to remediate excess nutrients and contaminants generated from point and nonpoint sources (Haygarth & Jarvis, 2002).

A common nutrient in surface waters is nitrogen from agricultural runoff. Nitrogen concentrations in the lower Mississippi River increased 17% from 1980—2010 (Murphy, Hersch, & Sprague, 2013). Excess nitrogen in an aquatic system is problematic due to the potential for eutrophication. Photosynthetic organisms such as algae use the excess nutrients to produce more biomass. This shades the water column, making survival difficult for photosynthetic submerged aquatic vegetation. Additionally, when the algae die, their decomposition increases the oxygen demand of the water. This can eventually result in hypoxia, killing sessile organisms and driving mobile organisms away in search of a more suitable habitat (EPA, 2017).

Nitrogen is an example of a potentially harmful nutrient that can be tempered by wetlands. Delaune and Jugsujinda (2003) determined the denitrification potential of sediment-

water samples taken from Breton Sound, a southern Louisiana estuary that is influenced by the Mississippi River. The samples were spiked with nitrogen, and their denitrification rates were monitored over 11 days. An average denitrification rate of 21—32 g N m^{-2} yr^{-1} was calculated. The estimated total nitrogen input to Breton Sound from the Mississippi River in 1999 was 8.9—23.4 g N m^{-2} yr^{-1} (Lane, Day, & Thibodeaux; 1999). This suggests that the denitrification potential of this ecosystem is substantial enough to mediate nonpoint source nitrogen additions.

Heavy metal pollution from industrial effluents, fossil fuel burning, and domestic waste can also be mediated by wetlands. Some heavy metals have serious toxicological impacts on humans and wildlife. Cadmium, chromium, copper, arsenic, lead, and mercury have been detected in the lower Mississippi River at concentrations exceeding drinking water regulatory maximums (Newchurch & Kahwa, 1984). Membrane filtration and ion exchange systems can be used to remove metals from water, but these processes are expensive and impractical. However, wetlands are capable of metal removal via natural processes (Rai, 2008).

Heavy metals tend to adsorb to particulate matter due to their positive charges; when these particles settle out, the metals are removed from the water column, reducing the likelihood of exposure the bioavailability of the contaminants. Metals in wetlands may also precipitate as salts and settle into the sediments (Rai, 2008). Flora et al (1993) verified that suspended sediment concentrations increase with depth in a salt marsh near Terrebonne Bay, Louisiana. With rising tides, sediment concentrations peaked and then steadily decreased before maximum water level was reached, suggesting that sedimentation processes are occurring. The group also considered flow stresses on the sediments at the bottom of the marsh and determined that typical disturbances such as tides and wind are not conducive to resuspension of settled sediment (Flora

et al, 1993). These findings suggest that metals bound to sediments or precipitated as salts are likely to remain in their deposits at the bottom of the wetland.

Vegetation in wetlands has also demonstrated an affinity for metal removal from the water column via numerous mechanisms. Vegetation in wetlands slows the velocity of water flow, further encouraging sedimentation processes to take place. Additionally, plant roots introduce oxygen into anaerobic or hypo-aerobic soil and water, facilitating microbial decomposition of organic materials, which can aid in the immobilization of metals. Finally, some wetland plants can uptake the contaminants directly during their normal metabolism. This removes the contaminant from the water but may be problematic when the plant dies and degrades, releasing the contaminant back into the water column. Removal and proper disposal of contaminated plants can resolve the issue, but this is often not practical (Rai, 2008).

Beyond water purification, wetlands also protect coastal communities from flooding and damage caused by storm surge. In the United States, 17 of 20 of the fastest growing cities are in coastal areas, and more than half of the nation's population resides in these areas; therefore, the protective services produced by wetlands are of critical importance (Tibbetts, 2002). Costanza et al estimate that wetlands, acting as "horizontal levees," provide $23.2 billion annual storm surge protection (2008).

The shallow depths and dense vegetation in wetlands help prevent the formation of large waves that occur in open water and similarly dissipate the energy of strong waves before they reach mainland (Costanza et al, 2008). The degree of protection provided by wetlands is contingent on many interrelated factors, including the intensity and speed of the storm, the characteristics of the wetland, and the location of the community relative to the wetland; this complicates predictions of coastal protection potential (Saleh & Weinstein, 2016). Despite

uncertainty due to the complexity of the system, both measured and modeled values indicate that wetlands aid in storm surge attenuation.

Water level sensors deployed across western Louisiana measured 2005's Hurricane Rita storm surge. Four sensors were in the direct path of the storm and in suitable physical locations to provide data for the calculation of wetland storm surge attenuation. The attenuation rate of these wetlands falls between 1m per 25km and 1m per 4km of wetland (Wamsley et al, 2010).

Wamsley et al (2010) estimated similar storm surge attenuation rates for two southern Louisiana ecosystems, the Barataria and Caernarvon wetland areas. Their model considers four different storms each with varying combinations of central pressure and forward speed values. Central barometric pressure values, indicative of storm intensity, were 900 or 960 mb. Forward storm speeds were 5.7 or 3.1m s^{-1}. Estimated storm surge attenuation rates for the two wetland areas range between 1m per 6 km and 1m per 50 km (Wamsley et al, 2010). Regardless of the variations in attenuation rates, wetlands provide a valuable physical barrier capable of reducing storm impacts in coastal communities.

A third physicochemical service provided by wetlands is carbon sequestration. Wetlands can store atmospheric carbon dioxide for extended periods. Atmospheric CO_2 levels began increasing drastically during the Industrial Revolution due to anthropogenic activities such as deforestation and fossil fuel burning. This is demonstrated in Figure 1.1.

During January 2017, atmospheric carbon dioxide (CO_2) concentrations averaged 406.13 ppm (NOAA, 2017). Ice cores and historic monitoring data indicate that this level is unprecedented for the past 400,000 years (NASA, 2016). The only exceptions are April through June 2016, during which atmospheric CO_2 averaged 407.31 ppm (NOAA, 2017). The upward

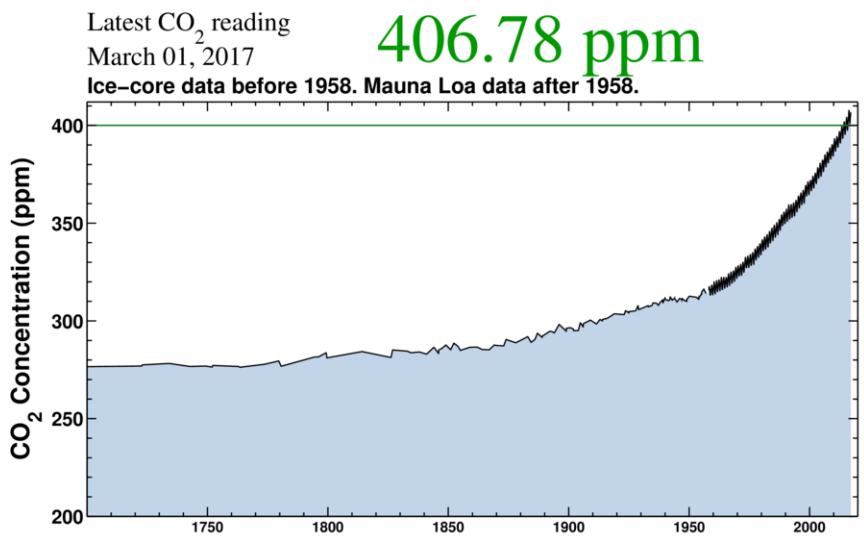


Figure 1.1. The Keeling Curve, 1700 – Present (Scripps, 2017).

trend in these concentrations is evident in Figure 1.2. High concentrations of atmospheric carbon dioxide are a key factor driving climate change, but wetlands can help mediate this.

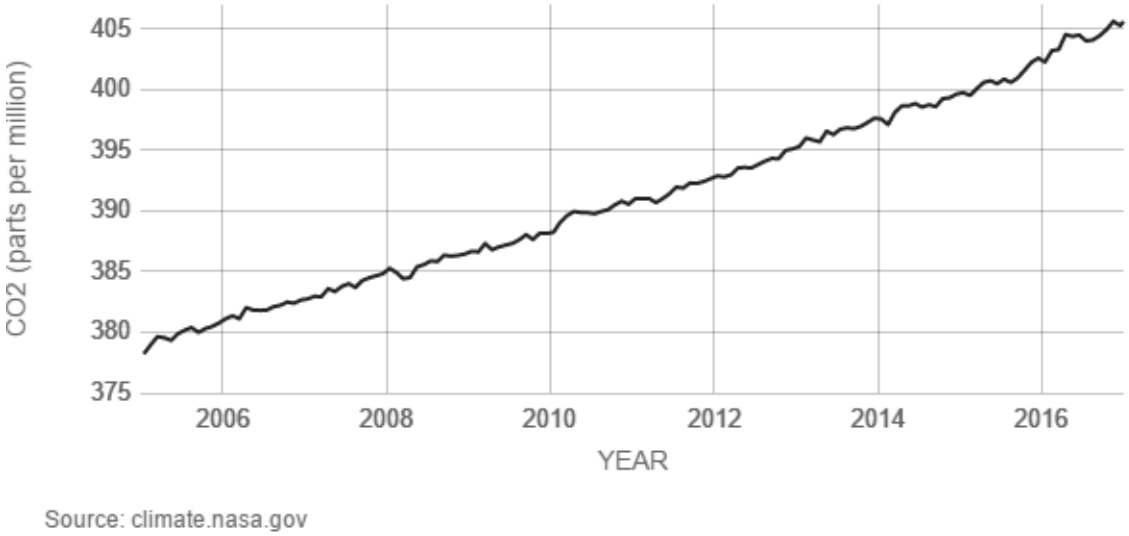


Figure 1.2. Atmospheric CO_2 Concentrations.

Wetlands store carbon through microbial processes. Carbon dioxide is released when organic material is decomposed by microorganisms in soil. This process occurs more rapidly under aerobic conditions, because some microbes metabolize organic matter much more

efficiently when oxygen is present. Microbe species *Pseudomonas* and *Brevibacillus* are nitrate-reducing, facultative anaerobes; they preferentially use oxygen for their metabolic processes but can switch to using nitrate under anaerobic conditions. Microbes were exposed to petroleum-contaminated soil to assess their metabolic efficiency under aerobic and anaerobic conditions. After 10 days of aerobic conditions, the microbes degraded 90-95% of the alkanes in the soil; in contrast, 50 days of anaerobic exposure only resulted in 20-25% degradation (Grishchenkov et al, 2000). This study is evidence that microbial carbon degradation and the resulting carbon dioxide release is more rapid under aerobic conditions.

In flooded wetland soils, anaerobic conditions predominate, therefore the decomposition rate of organic material and the resulting release of carbon dioxide is greatly reduced. Organic material inputs into the system may be autochthonous, such as detritus, or allochthonous, such as sediments deposited by wind or outside water inputs. When anaerobic soil conditions persist, this carbon will accumulate rather than being released into the atmosphere as carbon dioxide (Reddy & DeLaune, 2008).

Wetland biomass also aids in carbon sequestration. Primary producers such as plants and algae use carbon dioxide to build biomass through the process of photosynthesis. Although this does not permanently sequester the carbon, it immobilizes it. As the plants shed leaves, or when the organisms die, they become part of the detrital layer. If conditions are anaerobic, less of the original carbon will be returned to the atmosphere, and the emission will occur at a reduced rate (Reddy & DeLaune, 2008).

It is estimated that wetlands sequester 210 ± 20g CO_2 m^{-2}, per year (Chmura et al, 2003). The average vehicle emits 255g CO_2 km^{-1} (411g mi^{-1}) driven (EPA, 2014). This means that every two square meters of wetland can negate the carbon dioxide emissions from a 1.61 km car trip.

Effects of climate change such as sea-level rise and ocean acidification can be mediated by this carbon sequestration, a valuable ecosystem service for coastal communities. Wetlands provide additional important ecosystem services that are more difficult to quantify.

1.2.3. Instrumental and Intrinsic Values

Whereas coastal wetlands provide an abundance of lucrative and helpful ecosystem services, they also have instrumental and intrinsic worth that is sometimes overlooked. These ecosystems become an integral part of the culture of those who live near them. They provide habitat and breeding areas to broad range of organisms; these species are critical to the health of the wetlands, and some of them are in danger of extinction. Wetlands also serve as an education source for young and old alike. Louisiana's coastal wetlands provide perfect examples of these services; though the benefits cannot be easily enumerated in dollars or grams, they add to the importance of maintaining healthy wetland ecosystems.

New Orleans, Louisiana, is the eighth most popular travel destination in the United States due to its rich, unparalleled culture (Polland, 2015). The city is surrounded by productive wetlands that have shaped and melded the traditions of Native American, French, Spanish, and African residents over the past 300 years (History, 2010). South Louisiana's famous Cajun and creole cooking is the result of this blending of culture; the dishes frequently incorporate local seafood provided by the wetlands. Another cultural curiosity birthed from the wetlands is Cajun French. This dialect developed among self-sustaining communities of settlers in wetland habitats and is still spoken by some in South Louisiana parishes (Hebert, 2009).

Productive wetlands also gave rise to one of Louisiana's favorite social pastimes: crawfish boils. These events may be hosted at private homes, public venues, or restaurants, bringing together friends, family, and coworkers. Events such as the Crawfish Festival held

annually in Breaux Bridge, Louisiana, attract local artisans, musicians, and vendors, and provide a sense of community wellbeing among the participants (Breaux, 2017). Louisiana's wetlands support far more biodiversity beyond these appetizing invertebrates.

Louisiana's coastal wetlands fringe the Gulf of Mexico and are thereby subject to tidal influences. Tide cycles allow water and nutrient exchange between the marsh and the gulf; a salinity gradient exists where the salty gulf water intrudes into the freshwater marshes creating intermediate and brackish marshes. Certain plants and animals are adapted to living in exclusively high or low-salinity environments, and some thrive under intermediate concentrations. The saltmarsh ecosystem can accommodate all three types due to the gradient created by the saltwater intrusion, providing ideal habitats for a wide variety of organisms (Bobbink, 2008).

The health and productivity of a wetland is critical to its provision of ecosystem goods and services, and productivity is contingent on biodiversity. Every organism serves one or more functions in an ecosystem. High species diversity allows provides the system with resilience to disturbances, because species' functions can shift in response to change. However, due to the interrelatedness of species functions, the local extinction of an organism can negatively impact system productivity (Bobbink, 2008). It is important to maintain the health of wetlands to prevent biodiversity loss, including the loss of threatened and endangered species.

Plaquemines Parish in south Louisiana contains vast estuarine and freshwater wetlands that shelter ten threatened or endangered species: the West Indian manatee (*Trichechus manatus*); the piping plover (*Charadrius melodus*); the red knot (*Calidris canutus*); the Atlantic sturgeon (*Acipenser sturio*); the pallid sturgeon (*Scaphirhynchus albus*); the green sea turtle (*Chelonia mydas*); the hawksbill sea turtle (*Eretmochelys imbricate*); the Kemp's ridley sea turtle

(*Lepidochelys kempii*); the leatherback sea turtle (*Dermochelys coriacea*); and the loggerhead sea turtle (*Caretta caretta*). The characterization of these wetlands is available in Figure 1.3.

The area is critical habitat for the piping plover (USFWS et al, 2017). These species depend on the health of the marsh for their survival, and their extinction could disturb the function of the ecosystem. Loss of a species also removes the potential for future instrumental value, such as health remedies and food sources (Endangered, 2011).

The complexity of wetlands and array of services they provide culminate in a final benefit: public education. The United States Geological Survey (USGS) is actively working with scientists across the nation to gather information about our wetlands and disseminate it to the public via Wetland Aquatic Research Centers in Lafayette, Louisiana, and Gainesville, Florida (WARC, 2017). The United States Environmental Protection Agency (EPA) provides wetland education resources on their website. Teaching tools, such as curriculum and activities, as well as education programs seeking community involvement are publicly available (EPA, 2016b). The accessibility of this information is important so that people who cannot visit wetlands may still gain appreciation for them; however, the most effective wetland education is experience-based.

The Nature Conservancy offers a wetlands education program called Wings & Water that is mandatory fourth-grade curriculum in Utah. The program includes a field trip to the marsh as one of its education strategies. Students who participated in the field trip more proficiently identified species that belong in a Utah marsh than students who received the classroom training only. Discussions with the students who visited the wetlands indicated that they were concerned about conservation of the marsh and motivated to visit again for further educational activities. The students who received only class instruction did not reflect these sentiments, but were rather more negative or apathetic (Cachelin, Paisley, & Blanchard; 2009).

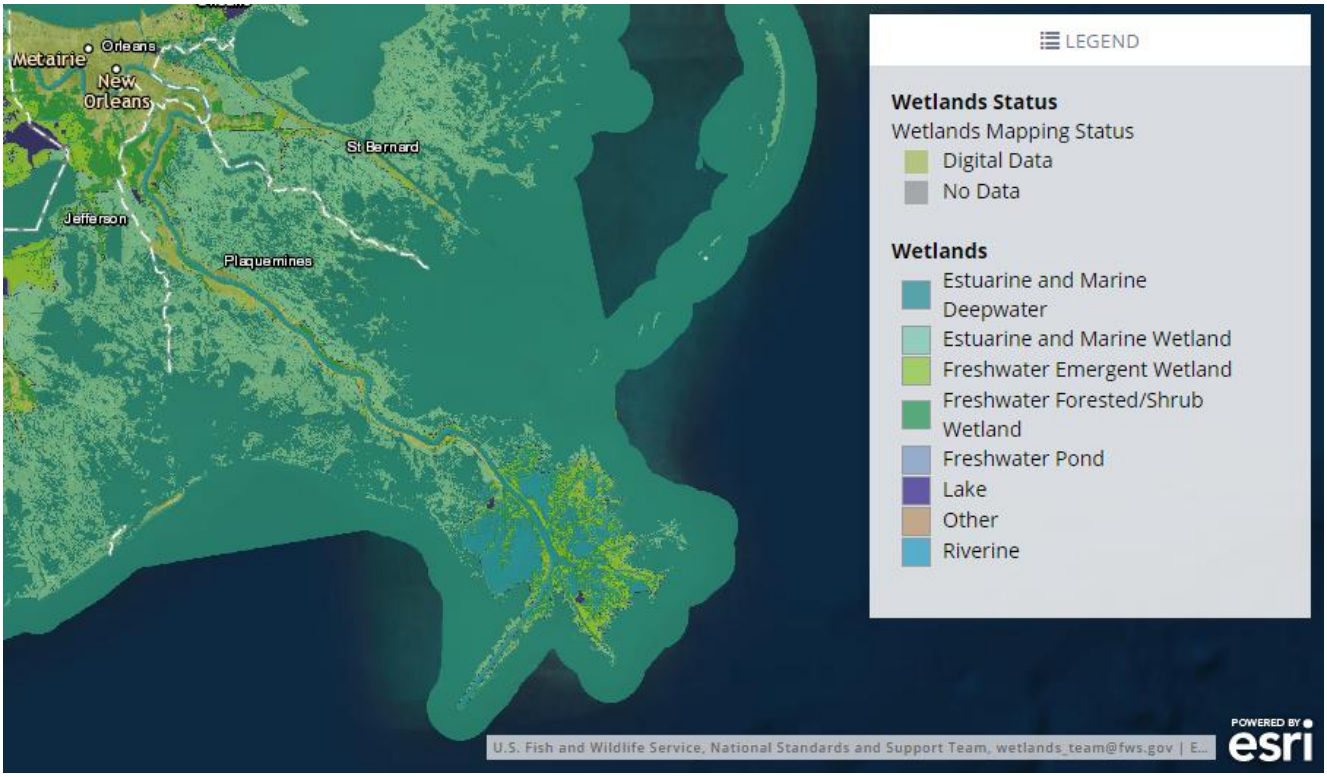


Figure 1.3. Plaquemines Parish Wetlands (LAFWS, 2017).

Louisiana's coastal wetlands provide a wide range of provisioning and supporting services. They help support the state's economy, protect its residents, and enrich its culture. The ecosystems are complex and highly productive, but human activities are disrupting their natural processes. A particularly profound anthropogenic modification to Louisiana's wetlands was initiated by one of the worst natural disasters in United States history.

1.2.4. Mississippi River Control Structures

Heavy rain during spring of 1927 filled the Mississippi River to its highest recorded level at that point in history. On April 16, the first levee broke in Illinois, beginning the "Great Flood of 1927." Over the following weeks, the entire levee system collapsed, submerging 60,000 km^2 of land, displacing hundreds of thousands of people, and killing 250. Over 9 meters of water

submerged neighborhoods in some areas, and the floodwater took over two months to subside (Editors, 2013).

This catastrophe inspired the drafting of the Flood Control Act of 1928, which authorized the Mississippi Rivers and Tributaries (MR&T) project. The United States Army Corps of Engineers are responsible for this project, the largest flood control effort in the world. The MR&T project incorporates four components to prevent a reoccurrence of the Great Flood: the construction of levees to contain flood flows; the construction of floodways to help dissipate excess flow; stabilization of the channel to stabilize the levees and facilitate navigation along the river; and improvements to major drainage basins such as the addition of reservoirs and pumping stations (USAC, 2017).

Following the MR&T project, the Army Corps rebuilt larger, sturdier levees along the Mississippi River down into the reaches of Southern Louisiana. Despite good intentions, this action had unanticipated consequences. The healthy, brackish estuarine wetlands were adapted to freshwater and nutrient inputs from periodic flooding and natural crevices in the riverbanks. When the levees were built, this supply was cut off, and an estimated 3,885 km^2 of wetland loss resulted. In 1956, a modification to the Mississippi River levee in South Plaquemines Parish, LA laid the groundwork for many more to come (Caffey & Schexnayder, 2002).

The Bayou Lamoque river control structure was the first controlled freshwater diversion constructed to supplement fishery productivity, particularly oyster production (Boshart & MacInnes, 2000). The original structure allowed a freshwater input of about 113 $m^3 s^{-1}$. It was expanded in 1978 to double the throughput. This diversion was the first of many to be built into the Mississippi River levee to aid in fishery productivity and coastal restoration (Caffey &

Schexnayder, 2002). However, along with the beneficial nutrients and freshwater flowing into the wetlands, the river also carries a host of environmental contaminants.

The Mississippi River collects and transports runoff from two Canadian provinces and 31 states in the United States; its 3,107,986 km^2 (1.2 million square mile) watershed covers 40% of the contiguous US, as can be seen in Figure 1.4. The river allows for urban population growth by facilitating commerce and supports agriculture development by supplying water for crops; it also provides a convenient solution for waste water disposal. Unfortunately, the growth of cities and agriculture results in the environmental issue of pesticide contamination.

Property owners apply pesticides around their homes and lawns to control nuisance insects. They are used heavily around schools, daycare centers, and parks to kill potentially dangerous insects, namely fire ants. Commodities such as nursery-grown plants and turf grass, which require protection from damaging insects, are in high demand for use in housing and industrial developments. Increasing population numbers also necessitate increased agricultural productivity to feed people and livestock; Syngenta sold over $1.7 billion in pesticides for crop protection in 2015 (Syngenta, 2016). The global pesticide industry is expected to exceed $81 billion by 2021, and North America is considered the region of greatest potential growth (Lucintel, 2016).

Improper residential application practices combined with heavy agricultural and commercial use result in large quantities of pesticide introduction into the Mississippi River via rainfall and irrigation runoff. Because these chemicals are specifically formulated to induce a toxicological response in organisms, they can accumulate to dangerously high concentrations and impact nontarget organisms in receiving wetland ecosystems at the bottom of the river. Water bodies containing sediments with high organic content would further facilitate the transport of

Figure 1.4. Mississippi River Watershed (DOI, 2016).

these chemicals after their introduction via runoff (Bobé, Coste, & Cooper; 1997). An estimated 9.3E8 kg of particulate organic carbon is released from the MS River into the Gulf of Mexico every year, making this watershed an ideal site for pesticide transport (Bianchi et al, 2007).

Atrazine, chlorothalonil, and fipronil are three common-use pesticides that have adequately high application rates and environmental persistence to cause toxicity to wetland species. Each is described in the following sections.

1.3. Atrazine

1.3.1. Background and Current Use

Atrazine is a triazine herbicide that is widely used to control broadleaf and grassy weeds for agricultural, commercial, and residential applications (Farruggia, 2016). It is effective in pre- and post-emergence applications (Hertfordshire, 2017). Atrazine, along with 34 similar triazine herbicides, were field-tested in December 1955 on oat, mustard, cucumber, and onion plants; promising results led to testing on more varieties of crops. Atrazine's efficacy made it the most

popular triazine herbicide in history, and it was introduced to the US market in 1960 (McFarland Burnside, 1975).

From 2006 – 2010, atrazine's highest agricultural use was on corn, sorghum, and sugarcane, respectively; corn applications accounted for 88% of the total. Non-agricultural uses include applications to golf courses, institutional turf, and turf farms, as well as use by lawn care companies, nurseries, and greenhouses. It is estimated that the amount of atrazine applied for these uses more than tripled between 2002 and 2006, rising from 360,660 to 1,155,281 pounds of active ingredient applied (Farruggia, 2016).

1.3.2. Mode of Action

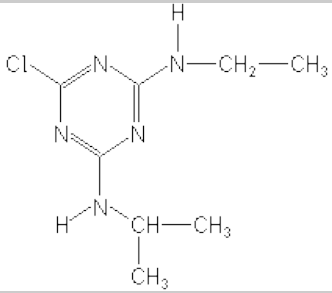
Triazine herbicides like atrazine function primarily by inhibiting photosynthetic processes in target organisms. The first step of photosynthesis occurs in Photosystem II (PSII) protein complex, in which electrons are obtained from light energy for the generation of organic material. The molecule plastoquinone transports electrons through the protein complex to aid in this process. Atrazine works by blocking plastoquinone's electron binding sites, halting plant metabolism. Additionally, the resulting excess energy converts typically single chlorophyll molecules into triplet molecules. The triplet molecules bind with oxygen, causing peroxidation processes that break down the plant's cellular membranes (Fuerst & Norman; 1991). The mode of action of this chemical makes it a threat to nontarget organisms when it is introduced to unintended environments.

1.3.3. Environmental Fate

Table 1.1 lists the chemical properties of the herbicide, atrazine, including the structure and IUPAC name. Atrazine is a contaminant of environmental concern due to its chemical properties and high application rates. It has a soil adsorption coefficient of 100 and an aerobic

soil metabolism half-life of 66 days. These values suggest that atrazine is moderately mobile and moderately persistent in soils. Atrazine's relatively low water solubility further encourages binding to soil. Its hydrolysis half-life is 86 days, which suggests that it is moderately persistent in aquatic environments. However, the compound is sensitive to light; the aqueous photolysis of

Table 1.1. Atrazine Chemical Properties.

	Atrazine (MW: 215.7 g mol^{-1})	Interpretation	Ref
IUPAC Name	6-chloro-4-N-ethyl-2-N-isopropyl-1,3,5-triazine-2,4-diamine		1.1.1
Structure		$C_8H_{14}ClN_5$	1.1.2
Chemical Class and Type	selective triazine herbicide		1.1.4
CAS No.	1912-24-9		1.1.4
Water Solubility	35 ppm	Low	1.1.3
pKa	1.7	Very Weak Base	1.1.3
Vapor Pressure	0.039 mPa	Low Volatility	1.1.3
Octanol-Water Partition Coefficient (log K_{ow})	2.7	Moderate	1.1.3
Soil Adsorption Coefficient (K_d or K_{oc})	100	Moderately Mobile	1.1.3
Hydrolysis DT_{50}, 20°C	86 days	Moderately Persistent (Aqueous)	1.1.3
Aerobic Soil Metabolism DT_{50}, 20°C	66 days	Moderately Persistent	1.1.3
Aquatic Photolysis DT_{50}	2.6 days	Moderately Fast	1.1.3
Water-Sediment DT_{50}	80 days	Moderately Fast	1.1.3

Ref 1.1.1: BRPO, 2006. Ref 1.1.2: Wood, 2017. Ref 1.1.3: Hertfordshire, 2017a. Ref 1.1.4: NCBI, 2017c.

atrazine occurs moderately quickly, with half of the compound degrading (DT_{50}) in 2.6 days. The DT_{50} increases to 80 days in water-sediment system, also a relatively fast rate (Hertfordshire, 2017). The presence of humic acids further increase the longevity of atrazine in aquatic systems,

possibly due to shielding effects. However, high concentrations of humic acids are thought to catalyze hydrolysis of the compound, increasing degradation rates (Prosen, 2005).

Atrazine has a relatively low log octanol/water partition coefficient value of 2.7, indicating that it has a low proclivity to bioaccumulate in living organisms (Hertfordshire, 2017). Tang et al (1998) investigated the uptake and bioaccumulation of 40 ppb atrazine in eight freshwater algal and diatom species. All species approached 90% of their total uptake values following 60 minutes of exposure. Green algae exhibited a greater potential to uptake atrazine than diatoms, and maximum uptake occurred within six hours of exposure. The algal species also demonstrated higher bioconcentration factors (BCFs) than diatoms, though all BCFs were relatively low; algal BCFs ranged from 130.5 to 324.1 based on dry mass and from 94.4 to 200.9 based on cell biovolume. Different algal species exhibited different capacities for the uptake and bioconcentration of atrazine, possibly due to structural outer membrane differences (Tang, Hoagland, & Siegfried; 1998).

Spray drift and direct soil applications allow soil-bound atrazine to move into surface waters due to erosion after rainfall and watering events (Sherman, 2006). The USGS compiled a report on the occurrence of pesticides in surface water using data from 463 sites in the Mid-Atlantic region. Atrazine was the most frequently detected compound, appearing in over half of the samples analyzed; 3% of samples contained levels of atrazine exceeding the Federal Maximum Contaminant Level (MCL) of 3 ppb (Ferrari et al, 2017).

Similarly, Ryberg and Gilliom (2015) studied trends in estimated pesticide applications and surface water concentrations in 38 US rivers from 1992 – 2010. They split the study period into three overlapping segments: 1992 – 2001, 1997 – 2006, and 2001 – 2010. For the first period, during which the EPA increased its stringency on atrazine regulations, four out of nine

sites exhibited downtrends in atrazine concentrations, whereas the remaining five were nonsignificant with one uptrend. The second segment exhibited 14 sites with downtrends, 16 with nonsignificant trends, and six with uptrends. The final period showed a smaller proportion of downtrends despite a decline in agricultural applications. Magnitudes of the trend deviations only exceeded 10% in one case for the study areas in the Mississippi River and Great Lake regions. This suggests that agricultural use is a reasonable predictor for surface water concentrations of atrazine in this watershed (Ryberg & Gilliom, 2015).

Between 1992 and 2014, agricultural application quantities ranged between approximately 58 and 70 million pounds per year, most of which was applied to corn, as is demonstrated in Figure 1.5. Application rates surrounding the Mississippi River and its watershed often exceeded 64 pounds per square mile in 2012, a conservative estimate formulated primarily using survey data. This data is demonstrated in Figure 1.6. The magnitude of these applications results in a large amount of agricultural atrazine runoff into the Mississippi River, which is compounded by unreported and nonagricultural use.

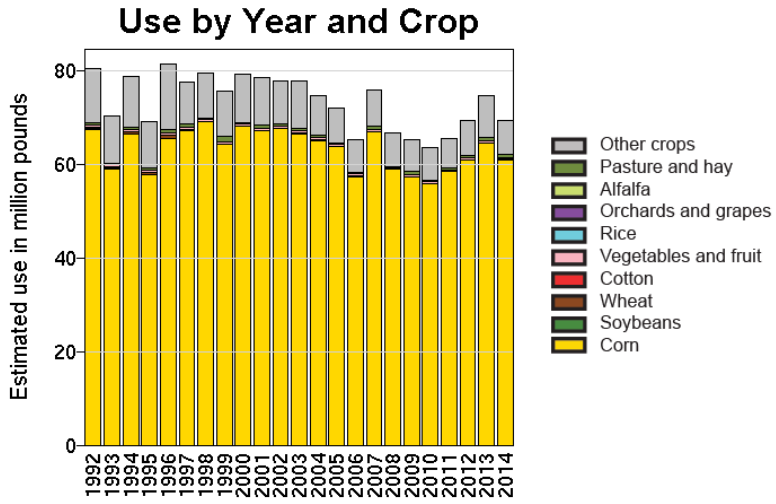


Figure 1.5. Atrazine Use by Year and Crop (USGS, 2017a).

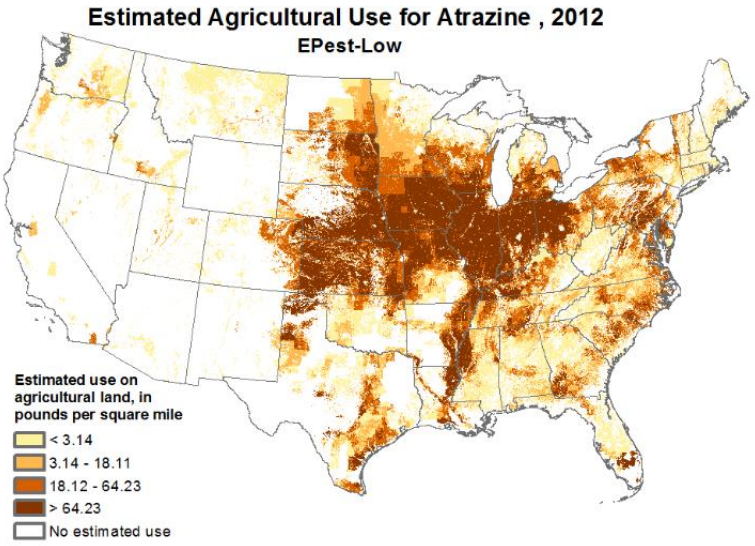


Figure 1.6. Estimated Agricultural Use for Atrazine, 2012 (USGS, 2017a).

1.3.4. Toxicity

Table 1.2 includes the toxicity information on atrazine found in literature, including aquatic and terrestrial organisms. The high application rates and environmental mobility of

Table 1.2. Atrazine Toxicity.

	Atrazine Concentration	Interpretation	Ref
Bobwhite Quail Acute Oral LD_{50}	940 ppm	Slightly Toxic, *Colinus virginianus*	1.2.1
Albino Rat Acute Oral LD_{50}	1869 mg kg^{-1}	Practically Non-Toxic, *Rattus norvegicus*	1.2.1
Brook Trout LOAEC	120 ppb	Moderately Toxic, *Salvelinus fontinalis*	1.2.1
Freshwater Midge 96-hr LC_{50}; LOAEC	720; 230 ppb	Moderately Toxic, *Chironomus tentans*	1.2.1
Duckweed EC_{50} (10-day; >10-Day)	170; 37 ppb	Highly Toxic, *Lemna gibba*	1.2.1
Algal Short Term 50% Growth Reduction	49 ppb	Highly Toxic, *Kirchneria subcapitata*	1.2.1
Ref 1.2.1: (Sherman, 2006).			

atrazine raise toxicity concerns for nontarget organisms. The chemical is only slightly toxic to bobwhite quail, *Colinus virginianus*, with an LD_{50} of greater than 950 ppm. Atrazine is practically non-toxic to rats, with a minimum acute oral LD_{50} of 1,859 mg kg^{-1}. Brook trout

(*Salvelinus fontinalis*) exhibited reduced body weight and length at concentrations as low as 120 ppb (Sherman, 2006). These values exceed typical concentration ranges detected in the environment. However, aquatic invertebrates, amphibians, and aquatic plants are more sensitive to the chemical.

African clawed frogs (*Xenopus laevis*) have demonstrated hormonal abnormalities resulting from exposure to environmentally relevant levels of atrazine. Mature males exposed to 25 ppb atrazine solutions exhibited a ten-fold reduction in plasma testosterone levels. When exposed to solutions of 1 ppb or greater, males suffered demasculinized larynges. Most remarkably, concentrations as low as 0.1 ppb resulted in the hermaphroditism of male frogs (Hayes et al, 2002). These results are unprecedented and could have profound implications for amphibian populations exposed to environmentally relevant levels of atrazine. However, a lack of experimental data, particularly information on control parameters, makes the validity of this study questionable.

Atrazine is highly toxic to the freshwater midge (*Chironomus tentans*) with an acute LC_{50} of 720 ppb. In the same species, a reduction in pupation and adult emergence was observed at concentrations as low as 230 ppb (Sherman, 2006). Another study on *C. tentans* demonstrated that concentrations as low as 1 ppb atrazine increase oxygen consumption by 47%. The increased respiration indicates metabolic stress in the midges, probably caused by decreased hemoglobin gene expression (Anderson et al, 2008).

Aquatic plants are nontarget organisms of concern due to atrazine's ability to inhibit photosynthetic processes. Gibbous duckweed (*Lemna gibba*) exhibited a 50% reduction in growth after ten days of exposure at 170 ppb. For longer exposure periods, the same growth reduction was seen at levels as low as 37 ppb. Freshwater algal species *Kirchneria subcapitata* is

also extremely susceptible to atrazine. Cell growth reduction of 50% was observed after a short-term exposure to 49 ppb atrazine (Sherman, 2006). These are environmentally relevant concentrations, and a reduction in primary producers can impact the entire food web.

1.4. Chlorothalonil

1.4.1. Background and Current Use

Chlorothalonil is a broad-spectrum, non-systemic organochlorine fungicide used to prevent and kill mildew, mold, bacteria, algae, and some insects. The chemical was first registered in the United States for use on turf grass in 1966 and for use on potatoes four years after (Vincelli, 2003). It is commonly sold under the following trade names: Bravo Ultrex®, Bravo Weather Stik®, Chloronil 720®, Chlorostar DF®, Concord DF®, Countdown L + G®, Daconil Ultrex®, Daconil Weather Stik®, Daconil Zn®, and Ensign 720® (Douglas, 2007). In addition to turf and crop protection, chlorothalonil is incorporated into paints, caulk, and grout and used to treat wood. Chlorothalonil is most commonly used to treat peanuts, potatoes, tomatoes, and golf courses (RED, 1998). However, it is not approved for use on home lawns due to residential exposure to applicators and young children (Vincelli, 2003).

In a market survey, chlorothalonil was ranked the most important fungicide for use on golf courses, ornamentals, and turf. The most commonly treated ornamentals are Christmas trees, conifers nurseries and orchards, and roses. Turf treatments include sod farms, professional athletic fields, and commercial landscaping. The amount of active ingredient used between the three is distributed as follows: golf courses (86%), ornamentals (6%), and turf treatments (8%) (Kelly, 2012). Golf courses, sod farms, and general turf treatments may be treated with up to 1.12 kg ha^{-1} (11.3 lb ac^{-1}) active ingredient every two weeks; golf course green treatments cannot exceed 81.8 kg ha^{-1} (73 lb ac^{-1}) active ingredient per year, while sod farms and turf use are

restricted to 13.21 kg kg ha^{-1} (26 lb ac^{-1}) per year. Maximum application rates aim to reduce exposure to aquatic species via surface runoff and erosion (Vincelli, 2003).

Its agricultural use in the United States has ranged from about 3,175,147 – 5,443,108 kg (7 - 12 million lb) per year since 1992 (USGS, 2017b). Its agricultural applications are broken down by crop type in Figure 1.7, and its estimated agricultural use for 2012 is shown in Figure 1.8.

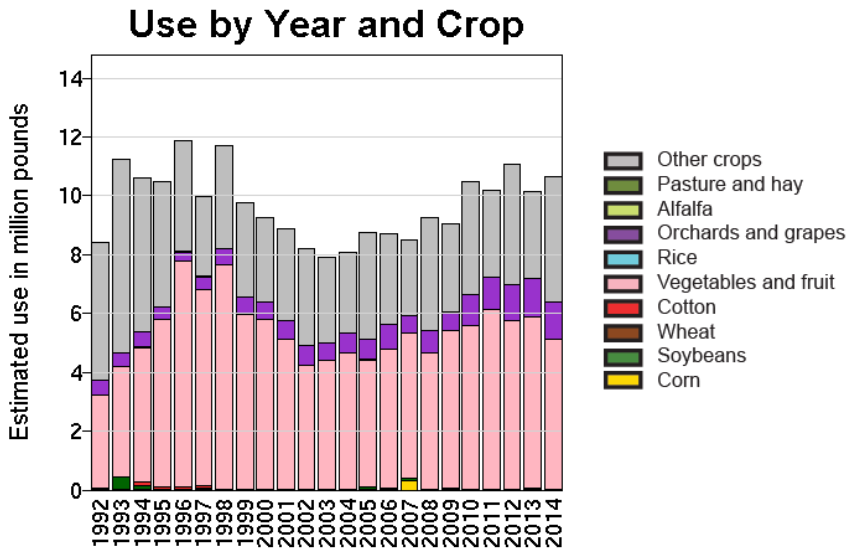


Figure 1.7. Chlorothalonil Use by Year and Crop (USGS, 2017b).

Across 121,406 hectares (300,000 acres) of crop use in California, nearly 43% of chlorothalonil was applied to tomatoes. Approximately 28% was used on potatoes and 14% on almonds. Chlorothalonil is only effective where it is directly applied; therefore, all parts of the plant must be sprayed with the chemical to be protected, as is evident in Figure 1.9 and in Figure 1.10. Despite this inconvenience, the lack of resistance development after over 40 years of use makes this chemical a popular choice (Kelly, 2012).

1.4.2. Mode of Action

Chlorothalonil's mechanism of action was not well understood upon its EPA reregistration in 1999, but research has since been conducted that illuminates the matter (RED, 1998).

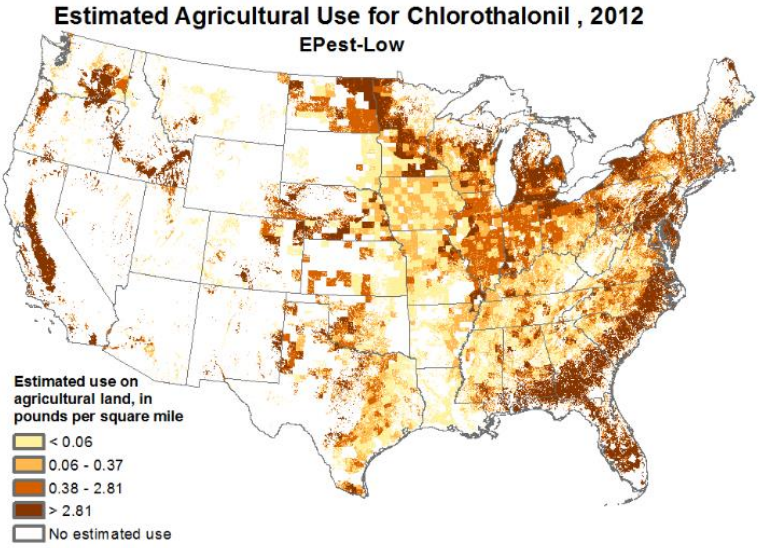


Figure 1.8. Estimated Agricultural Use for Chlorothalonil, 2012 (USGS, 2017b).

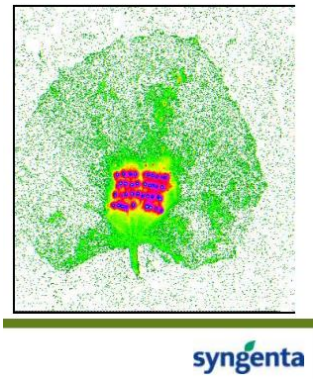


Figure 1.9. Radiolabeled Chlorothalonil Demonstrates Its Non-Systemic Nature (Kelly, 2012).

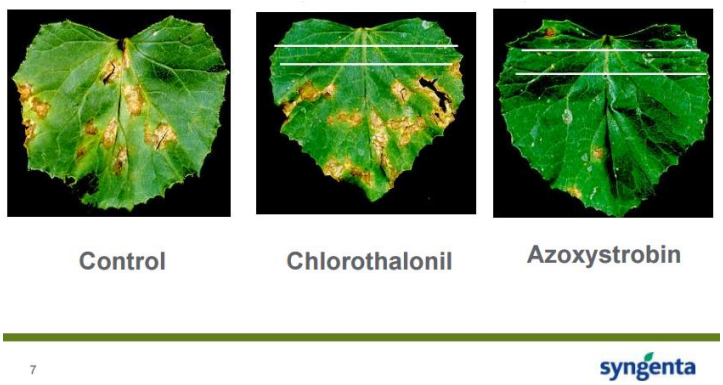


Figure 1.10. Chlorothalonil Only Effective Where Applied (Kelly, 2012).

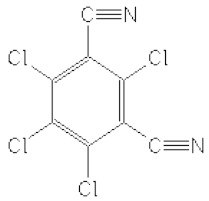
Fungi have not developed resistance to chlorothalonil because it affects multiple enzymes simultaneously, making adaptation difficult for the organism.

Chlorothalonil's effectiveness is largely due to its interference with the sulfhydryl group on a thiol called glutathione (GSH) (Vincelli, 2003). Glutathione is critical in maintaining fungus cell membrane and mitochondrial structures and in facilitating numerous cellular stress-response processes. For example, the GSH serves as an antioxidant by reacting with potentially-harmful oxygen species in the cell. Similarly, GSH sequesters heavy metals through a binding process called chelation, protecting the organism from toxicity. GSH also plays a role in cell development, differentiation, and destruction (Pocsi, 2004). The broad-spectrum nature of this fungicide makes it a contaminant of environmental concern.

1.4.3. Environmental Fate

Table 1.3 lists the chemical properties of the fungicide, chlorothalonil. Chlorothalonil is introduced to non-target environments by spray drift, runoff, and erosion. It is not likely to volatilize due to its low vapor pressure of 0.076 mPa. Its low solubility in water (0.81 mg l^{-1}) and its high soil adsorption coefficient (K_{oc}: 2632) make sedimentary transport the most likely route for chlorothalonil into the environment. Additionally, its aerobic soil metabolism half-life of 10 – 40 days means that chlorothalonil could persist in sediments long enough to undergo transport.

Table 1.3. Chlorothalonil Chemical Properties.

	Chlorothalonil (MW: 265.91 g/mol)	Interpretation	Ref
IUPAC Name	Tetrachloro-isophthalonitrile		1.3.1
Structure		C_8Cl_4N	1.3.2

(Table 1.3 continued)

	Chlorothalonil (MW: 265.91 g/mol)	Interpretation	Ref
Chemical Class and Type	chloronitrile fungicide		1.3.1
CAS No.	1897-45-6		1.3.1
Water Solubility	0.81 ppm	Low	1.3.1
pKa	n/a	No Dissociation	1.3.1
Vapor Pressure	0.076 mPa	Low Volatility	1.3.1
Octanol-Water Partition Coefficient (log K_{ow})	2.94	Moderate	1.3.1
Hydrolysis DT_{50}	Stable at pH 4 and 7, 29.6 days at 50°C; 38.1 days at pH 9	Moderately Persistent	1.3.1; 1.3.3
Aerobic Soil Metabolism DT_{50}, 20°C	10 - 40 days	Non-Persistent to Moderately Persistent; four different soil types	1.3.4
Aquatic Photolysis DT_{50}, pH 7.0	0.72 days; 65 days	Fast; Slow	1.3.1; 1.3.4
Aerobic Aquatic DT_{50}	2 hours to 6 - 8 days	Fast	1.3.4

Ref 1.3.1: Hertfordshire, 2017b. Ref 1.3.2: Wood, 2017. Ref 1.3.3: NCBI, 2017a. Ref 1.3.4: RED, 2017.

With an octanol-water partition coefficient of 2.94, the compound has a moderate potential to bioaccumulate; thus, application rates, and their resulting runoff concentrations, should be minimized whenever possible (Hertfordshire, 2017b).

Chlorothalonil has the potential to be moderately persistent in the water column. The chemical is stable at pH 2 and 7; a hydrolysis DT_{50} of about 30 days occurs at pH 9 or 50°C, but these are not typically environmentally relevant values (Hertfordshire, 2017b; NCBI, 2017). Aerobic aquatic metabolism half-life values ranging from 2 hours to eight days have been reported for chlorothalonil; the disparity in values suggests that the analysis is sensitive to experimental conditions and that this half-life may be concentration-dependent (RED, 1998).

1.4.4. Toxicity

Table 1.4 includes the toxicity data on chlorothalonil on both aquatic and terrestrial organisms. The potential of this pesticide to persist in the environment raises concern for the safety of non-target organisms. Chlorothalonil is practically non-toxic to birds and mammals; the Japanese quail (*Coturnix japonica*) and albino lab rat (*Rattus norvegicus*) acute oral LD_{50} values were greater than 2,000 mg kg^{-1} and greater than 5,000 mg kg^{-1}, respectively. However, the compound is moderately toxic to aquatic vegetation. Duckweed (*Lemna gibba*) growth was reduced by half over one week at a concentration of 0.29 mg l^{-1} chlorothalonil. In 96 hours, chlorothalonil reduced green algae (*Raphidocelis subcapitata*) growth by half in a 0.21 mg l^{-1} solution (Hertfordshire, 2017b).

Table 1.4. Chlorothalonil Toxicity.

	Chlorothalonil Concentration	Interpretation	Ref
Japanese Quail Acute Oral LD_{50}	>2,000 mg kg^{-1}	Practically Non-Toxic, *Coturnix japonica*	1.4.1
Albino Rat Acute Oral LD_{50}	>5,000 mg kg^{-1}	Practically Non-Toxic, *Rattus norvegicus*	1.4.1

	Chlorothalonil Concentration	Interpretation	Ref
Rainbow Trout Acute LC_{50} (96-hour)	0.017 mg l^{-1}	Highly Toxic, *Oncorhynchus mykiss*	1.4.1
Freshwater Invertebrate LC_{50} (96-hour)	0.054 mg l^{-1}	Highly Toxic, *Daphnia magna*	1.4.1
Duckweed EC50 (7-day)	0.29 mg l^{-1}	Moderately Toxic, *Lemna gibba*	1.4.1
Algal Short Term 50% Growth Reduction (72-hour)	0.21 mg l^{-1}	Moderately Toxic, *Raphidocelis subcapitata*	1.4.1
Ref 1.4.1: Hertfordshire, 2017b.			

Fish and aquatic invertebrates are more sensitive to chlorothalonil. Rainbow trout (*Oncorhynchus mykiss*) exhibited an acute LC_{50} of 0.017 mg l^{-1}, and water flea (*Daphnia magna*) had a 96-hour LC_{50} of 0.054 mg l^{-1}. These values suggest that both organisms are highly sensitive to the fungicide (Hertfordshire, 2017b). Copepod (*Tigriopus japonicas*) is also susceptible to low concentrations, with an acute LC_{50} of 16 ug l^{-1} (Reilly et al, 2012). Fortunately, these toxicity levels exceed typical environmental levels.

1.5. Fipronil

1.5.1. Background and Current Use

Fipronil was registered on the US market in 1985 and is used to control insects in home and agricultural applications, including: ants, beetles, cockroaches, fleas, ticks, termites, mole crickets, rootworms, and weevils (Jackson et al, 2009). For domestic use, fipronil may be purchased in concentrations exceeding 9% without a license (Taurus, 2017). Pesticides containing Fipronil as an active ingredient include: Blitz®, Icon®, Termidor®, Chipco Choice®, Frontline®, CeaseFire®, Over and Out®, Regent®, and Top Choice® (Schowalter, 2005).

Agricultural fipronil use peaked in 2002 for corn protection from root worms and dropped thereafter, as can be seen in Figure 1.11. Estimated agricultural use of fipronil in 2012 is shown in Figure 1.12. In 2010, the EPA removed fipronil from the list of chemicals approved for corn treatment, claiming that other alternatives were available (Whalen, 2010). Fipronil is currently permitted for agricultural use on potatoes and for the treatment of common domestic pests such as ants, spiders, and termites.

1.5.2. Mode of Action

Fipronil is a phenylpyrazole insecticide that causes neurotoxicity in target organisms via contact or ingestion. The pesticide works by blocking receptors in the central nervous system that control muscle contractions (Jackson et al, 2009). Ordinarily, γ-aminobutyric acid, a

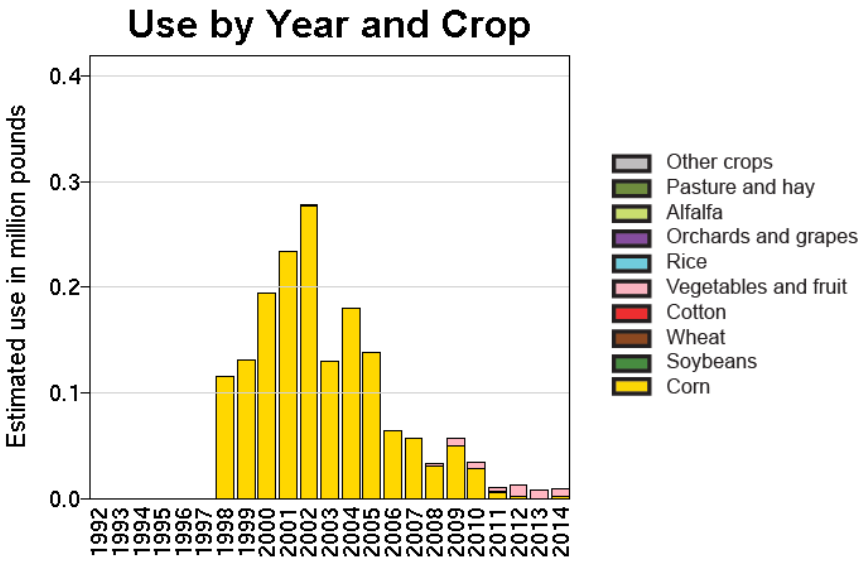


Figure 1.11. Fipronil Use by Year and Crop (USGS, 2017c).

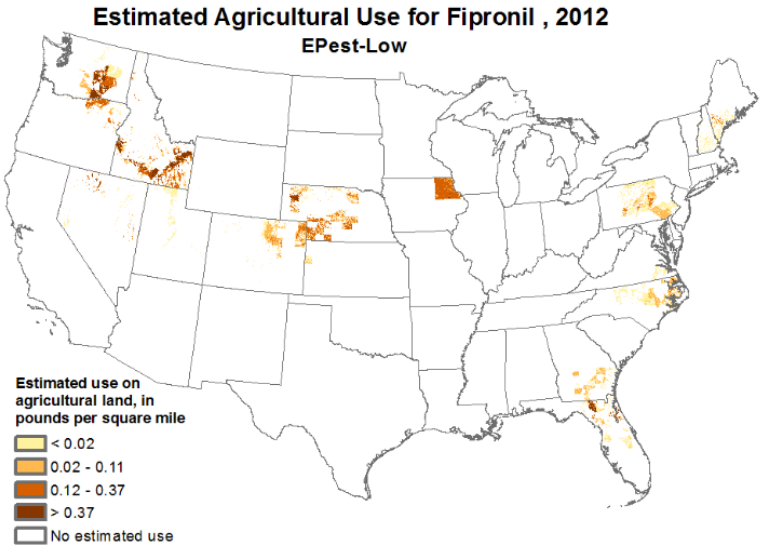


Figure 1.12. Estimated Agricultural Use for Fipronil, 2012 (USGS, 2017c).

neurotransmitter, binds to these "GABA" receptors, releasing chloride ions and allowing a muscle contraction; when the neurotransmitter unbinds, the chloride ions are reabsorbed and the contraction ceases (Gilbert & Gill, 2010). Fipronil mimics this neurotransmitter, binding to the GABA receptor and triggering chloride ion outflow from the complex. However, it does not detach from the site, which causes sustained contraction and eventual death. Though mammal neurotransmission functions similarly, fipronil has a much higher binding affinity for insect GABA receptors than mammals', reducing the risk of exposure to the chemical (Jackson et al, 2009).

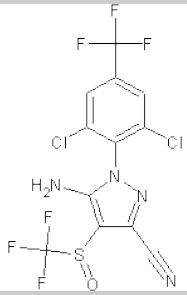
1.5.3. Environmental Fate

Table 1.5 lists the chemical properties of the insecticide fipronil. Fipronil has a soil sorption coefficient (K_{OC}) of 825 ± 214, meaning that it has low to no mobility once it is bound to soil (NCBI, 2017b). The solubility of fipronil in water is low: 3.78 ppm (Hertfordshire, 2016). These characteristics make soil/sediment an ideal vector for fipronil in natural waters. Additionally, fipronil bonds more strongly to media with high organic content; therefore, water

bodies containing sediments with high organic content, such as the Mississippi River, would further facilitate the transport of these chemicals after their introduction via runoff (Bobé et al, 1997).

Fipronil has a low vapor pressure of 0.002 mPa, therefore it is not volatile under environmentally relevant conditions. It has an octanol-water partition coefficient of 3.75, making bioaccumulation a high concern (Hertfordshire, 2016). The compound is stable at moderate to slightly acidic pH values, but it hydrolyzes in 28 days at pH 9.0 (NCBI, 2017b). It is also persistent in soils, with an aerobic soil metabolism of 142 days. Fipronil degrades in soil-water systems after approximately 68 days. Despite its persistence under various conditions, fipronil is susceptible to light, hydrolyzing in water in about eight hours (Hertfordshire, 2016).

Table 1.5. Fipronil Chemical Properties.

	Fipronil (MW: 437.141 g mol^{-1})	Interpretation	Ref
IUPAC Name	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)pyrazole-3-carbonitrile		1.5.1
Structure		$C_{12}H_4Cl_2F_6N_4OS$	1.5.2
Chemical Class and Type	phenylpyrazole insecticide		1.5.3
CAS No.	120068-37-3		1.5.4
Water Solubility	3.78 ppm	Low	1.5.4
pKa	n/a	No Dissociation	1.5.4
Vapor Pressure	0.002 mPa	Low Volatility	1.5.4
Octanol-Water Partition Coefficient (log K_{ow})	3.75	High	1.5.4

(Table 1.5 continued)

	Fipronil (MW: 437.141 g mol^{-1})	Interpretation	Ref
Soil Adsorption Coefficient (K_{oc})	825 (average)	Low Mobility	1.5.1
Hydrolysis DT_{50}, 20°C	Stable at pH 5.5 and 7; 28 days at pH 9	Stable	1.5.1
Aerobic Soil Metabolism DT_{50}, 20°C	142 days	Persistent	1.5.4
Aquatic Photolysis DT_{50}	0.33 days	Fast	1.5.4
Water-Sediment DT_{50}	68 days	Moderately Fast	1.5.4

Ref 1.5.1: NCBI, 2017b. Ref 1.5.2: Wood, 2017. Ref 1.5.3: Jackson et al, 2009. Ref 1.5.4: Hertfordshire, 2016.

Four main degradation products of fipronil result from reduction, oxidation, hydrolysis, and photolysis. The degradation products are: fipronil-sulfide (MB45950), formed through reduction in soil; fipronil-amide (RPA200766) formed by biotically in soil or via hydrolysis in water; fipronil-desulfinyl (MB46513) formed by photolysis on soil or foliage; and fipronil-sulfone (MB46136) formed through oxidation or biotic processes in soil or photolytically on foliage (Gunasekara, 2007).

1.5.4. Toxicity

Table 1.6 includes the toxicity data on fipronil, including aquatic and terrestrial organisms. Fipronil's mobility, persistence, and bioaccumulation potential make toxicological effects to nontarget organisms a concern. The compound poses the least threat to birds, mammals, and macrophytes. The bobwhite quail (*Colinus virginianus*) acute oral LD_{50} is 11.3 mg kg-1, a level of exposure that is unlikely to occur under natural circumstances. Similarly, albino rats (*Rattus norvegicus*) must consume 92 mg kg-1 body weight in a short period to reach their LD_{50} (Hertfordshire, 2016). However, some organisms are more affected by the chemical. Smaller organisms with more direct routes of exposure are increasingly sensitive to fipronil; the chemical is moderately toxic to some aquatic plants and fish species. Concentrations of 160 ppb

caused a 50% reduction in duckweed (*Lemna gibba*) growth over a seven-day period. Green algae (*Scenedemus subspicatus*) is much more susceptible to fipronil, reaching its EC_{50} at 68 ppb. The 96-hour LC_{50} of bluegill sunfish (*Lepomis macrochirus*) was reached with an 83 ppb fipronil solution; fipronil sulfone, one of fipronil's common soil metabolites, is three times more toxic to *L. macrochirus* (Jackson et al, 2009). Rainbow trout (*Oncorhynchus mykiss*) are more tolerant of the compound, with an acute LC_{50} of 248 ppb (Hertfordshire, 2016).

Invertebrates are the most vulnerable nontarget organisms affected by fipronil. Mysid shrimp (*Americamysis bahia*) demonstrated an acute LC_{50} at 0.14 ppb, and their metabolism and reproduction were affected at concentrations as low as 0.005 ppb (Jackson et al, 2009). Amphipod crustaceans (*Hyalella azteca*) have an acute LC_{50} of 0.54 ppb fipronil (Lizotte et al,

Table 1.6. Fipronil Toxicity.

	Fipronil Concentration	Interpretation	Ref
Bluegill Sunfish Acute LC_{50} (96-hour)	83 ppb	Moderately Toxic, *Lepomis macrochirus*	1.6.1
Mysid Shrimp Acute LC_{50} (96-hour)	0.14 ppb	Highly Toxic, *Americamysis bahia*	1.6.1
Freshwater Trout Acute LC_{50} (96-hour)	248 ppb	Moderately Toxic, *Oncorhynchus mykiss*	1.6.2
Amphipod Acute LC_{50} (96-hour)	0.54 ppb	Highly Toxic, *Hyalella azteca*	1.6.3
Honeybee Acute LD_{50} Contact; Oral (48-hour)	5.9 ng bee^{-1}; 4.17 ng bee^{-1}	Highly Toxic, *Apis mellifera*	1.6.2
Bobwhite Quail Acute Oral LD_{50}	11.3 mg kg^{-1}	Moderately Toxic, *Colinus virginianus*	1.6.2
Albino Rat Acute Oral LD_{50}	92 mg kg^{-1}	Moderately Toxic, *Rattus norvegicus*	1.6.2
Duckweed EC_{50} (7-day)	160 ppb	Moderately Toxic, *Lemna gibba*	1.6.2
Green Algae EC_{50} (72-hour)	68 ppb	Moderately Toxic, *Kirchneria subcapitata*	1.6.2

Ref 1.6.1: Jackson et al, 2009. Ref 1.6.2: Hertfordshire, 2016. Ref 1.6.3: Lizotte et al, 2009.

2009*).* Honeybees *(Apis mellifera*) have 48-hour LD_{50} values of 5.9ng and 4.17ng per bee for contact and oral exposure, respectively (Hertfordshire, 2016).Fipronil sulfone is over six times more toxic to freshwater invertebrates than the parent compound, so fipronil's presence in a freshwater system is cause for concern (Demcheck & Skrobialowski, 2003). The threshold fipronil concentrations that impact invertebrates are easily surpassed in the environment when nearby agricultural activity is high.

The Mermentau River Basin in southwestern Louisiana is a "wet prairie" underlain by an impermeable layer of clay that makes it ideal for the flooded conditions necessary in rice farming. Fipronil is used on rice crops for protection against damaging water weevils. Throughout the growing season, contaminated rice field "tailwater" is periodically discharged into nearby surface water. Fipronil concentrations ranging from 0.829 – 5.29 ug l^{-1} (ppb) were detected at 17 surface water sites surrounding rice paddies, harmful levels for vulnerable nontarget invertebrates (Demcheck & Skrobialowski, 2003).

Another study summarized results for more than 3200 samples taken from urban areas across California. The water and sediment samples were analyzed for fipronil and its primary degradates. Fipronil was detected in 39% of the water samples and 19% of the sediment samples. The average concentration detected was 89.7 ppt; the maximum was 10,004 ppt. These concentrations do not exceed LC_{50} values for sensitive invertebrate populations (Madsen, Sandstrom, & Zaugg; 1995). However, they exceed the United States EPA freshwater invertebrate chronic benchmark value of 11 ppt, indicating that harmful effects could occur over extended periods of time (EPA, 2017b).

1.6. Environmental Levels

The toxicological features of each of these compounds, atrazine, chlorothalonil, and fipronil, have raised concerns regarding ecological and public health in both scientific and non-scientific communities. The United States Geological Survey, Environmental Protection Agency, and United States Department of Agriculture have collaborated to establish a database of water quality monitoring results from across the United States (NWQMC, 2017). Additionally, scientific studies are plentiful and ongoing regarding the concentrations of anthropogenically-introduced chemicals in the environment.

A 2012 – 2013 USGS study in California tested surface water in the Sacramento River and San Joaquin River basins for common pesticides. The Sacramento River receives runoff from a 22,300 mi^2 watershed, encompassing urban, forest, and agricultural areas; the predominant crops grown are rice, alfalfa, and orchard crops like almonds. The San Joaquin River watershed is roughly one-third the size of the Sacramento and receives runoff primarily from cotton, orchard, and vegetable crop production; the watershed also includes some forests, grasslands, and urban populations (Orlando et al, 2014). The primary agricultural products of both watersheds are all treatable with chlorothalonil, and cotton can be treated with atrazine (Gray, Fernandez, & Horton; 1987; RED, 1998; Sherman, 2006). Fipronil could originate from agricultural or urban sources.

Out of 35 grab samples taken from the Sacramento River basin, chlorothalonil was detected in 6% of samples, and fipronil was detected in 15% of samples; atrazine was not detected. The maximum concentration measured for fipronil was 3.0 ng l^{-1}, and the highest detected level of chlorothalonil was 20.4 ng l^{-1}. Atrazine was detected in 4% of the 24 San

Joaquin River basin samples with a maximum concentration of 39.1 ng l^{-1}; chlorothalonil and fipronil were not detected (Orlando et al, 2014).

Reilly et al (2012) examined local-level fungicide concentrations due to agriculture; they complemented the study with herbicide and insecticide analyses, as well. Farms reporting high potato yields and associated fungicide use were chosen as sampling sites; the farms were in Idaho, Maine, and Wisconsin. Surface water sample aliquots were taken from locations near the farms, with watershed sizes ranging from 23 hectares to 20,589 hectares (Reilly et al, 2012).

In Idaho, land use within three of the four watersheds sampled exceeded 83% agricultural use, with alfalfa, beans, corn, potatoes, and wheat being the primary crops grown; samples were drawn from agricultural drainage ditches. The Maine sites consisted of three springs and one groundwater-fed pond; the watershed use consisted of forest and less than 55% agricultural for the growth of barley, oats, potatoes, and rye. Finally, Wisconsin samples were drawn from four agricultural drainage ditches with watersheds containing 63 – 89% agricultural land for the growth of beans, corn, potatoes, and soybeans. Sampling began after the first fungicide application and continued every three weeks throughout the growing season (Reilly et al, 2012).

Despite the variation in site characteristics, the results were not significantly different across sites. Fipronil was not detected at any of the sites. Atrazine was detected at a median concentration of 14.7 ng l^{-1}, with a maximum concentration of 132 ng l^{-1}. The median concentration detected of chlorothalonil was only 1.1 ng l^{-1}, but its maximum value was 228 ng l^{-1} (Reilly et al, 2012). This value would be alarming if detected during typical surface water monitoring circumstances, but the value is believable given the deliberate location of the sampling sites near areas of high use.

Environmental detections of atrazine, chlorothalonil, and fipronil are typically at low parts per billion (ppb) to parts per trillion (ppt) concentrations. Though these concentrations may be harmless in an individual body of water, runoff from multiple nonpoint sources can compound in streams to elevate concentrations to toxic levels. The enormous watershed of the Mississippi River, the heavy agricultural activities that it supports, and the high volume of organic carbon that it carries make the river an ideal environment for this scenario. This study proposes a methodology for completing solid phase extraction (SPE) of atrazine, chlorothalonil, and fipronil into a composite sample in a remote field location and investigates the introduction of these contaminants into the Ballandock Canal via the Mississippi River.

CHAPTER 2: METHODS AND MATERIALS

2.1. Overview

Environmental surface water samples were collected in a Plaquemine Parish marsh that is heavily influenced by the Mississippi River. Peristaltic pumps were used to concentrate samples onto C-18 disks for various dates and durations. Disks were sealed in a zippered bag, refrigerated upon collection and then stored in an -80°C freezer until extraction and analysis by GCMS.

2.2. Site Selection

The Ballandock Structure (N29° 26.401'; W89° 35.651'), as shown in Figure 2.1., is a Mississippi River control structure located south of Port Sulphur, LA; it was irreversibly opened in late 2010, allowing unrestricted flow of fresh MS River water and sediments into the formerly brackish marsh.

Figure 2.1. Ballandock Structure (Google, 2017).

The water flow from the Ballandock structure leads primarily to California Bay but may also influence nearby Allen and Gasper Bays; the pinned location of this site is shown in Figure 2.2.

Figure 2.2. Ballandock Pinned Image (Google, 2017).

This site was chosen due to concerns that contaminants introduced by the Mississippi River, due to the control structure opening, may negatively impact the marsh ecosystem.

2.3. Sample Collection and Storage

Samples were collected onto CI-Agent Solid Phase C-18 Extraction Disks connected to lofted glass pre-filtration disks to remove suspended sediments. The C-18 media and glass fiber filters are incased in a cartridge made of high-density polypropylene, which is resistant to solvent exposure and leaching of chemicals; they have threaded Luer Lock ends and screw together to prevent sample loss when connected correctly.

2.3.1. Cleaning and Conditioning Disks (June)

In the field, prior to deployment, the connected C-18 disks and pre-filtration disks were primed with 100 mL bottled distilled water at maximum flow rate using the pump system. The sample Mississippi River/marsh water was introduced immediately behind the distilled water with minimal introduction of air bubbles.

2.3.2. Cleaning and Conditioning Disks (August and October)

In the lab, disks were cleaned and conditioned using an adaptation of CI-Agent Stormwater Solution's recommended method (Hepner, 2013). A 50-mL Luer Lock syringe was used to inject the following through the C-18 cartridges: 30 mL dichloromethane (DCM; Sigma-Aldrich; St. Louis, MO; CAS 75-09-2; Lot SHBH2577V) with a one minute residence time, followed by two syringe volumes of air; 30 mL methanol (Fisher Scientific; Waltham, MA; CAS 67-56-1; Lot 153779) with a two-minute residence time, followed by one syringe volume of air; and then 30 mL deionized (DI) water, followed by one syringe volume of air. The cartridges were capped with Luer Lock plugs in re-sealable metal foil pouches at room temperature until after sample collection.

2.3.3. Pump Setup

Three contact pump drives (L/S COMPACT DRV 12V DC 'CE'; Model No. 77200-12; Cole-Parmer; Vernon Hills, IL) and peristaltic easy-load pump heads (EZ LOAD PUMP CRS SHRT SHFT PPS; Model No. 07516-00; Cole-Parmer; Vernon Hills, IL) were manually wired to Commander Series boat batteries to facilitate long-term operation in the field.

The pump/battery setups were housed inside large Rubbermaid containers with locking lids to prevent environmental exposure during sample collection; additionally, the containers were wrapped in aluminum foil during sampling to shield from heat and sunlight. Two small holes were drilled in the front of these containers for insertion of the tubing that runs from the sampling location to the C-18/pf setup. (Tubing: 0.8 mm with 3.1 mm adapter for connection to disk; Masterflex Platinum-Cured Silicone #13, 25'; Cole-Parmer; Vernon Hills, IL)

The outflow was collected in 37.85 liter buckets with locking lids for the calculation of final volume and flow rate. A small, drilled hole allowed for the suspension of the collection

cartridges inside the bucket to reduce environmental exposure and guarantee that all outflow was collected.

2.3.4. Deploying samplers

Triplicate pumps were positioned next to each other on a concrete ledge on the marsh side of the Ballandock Structure. Approximately 2 m of the tubing was suspended with a fishing float in the water for sample uptake. Pumps were run on the maximum flow rate setting to push water to the output end of the tubing, and then filters and cartridges were connected; this step minimizes air introduction into the cartridge.

Sample collection was conducted at a rate of approximately 4 mL per minute, corresponding to ¼ turn of the rate knob on the pump drive. Pumps were checked periodically to verify proper functionality and to measure water volumes. Water volumes were measured with a 950 mL glass beaker and a 100-mL plastic graduated cylinder for smaller volumes.

Upon completion of sample collection, the C-18 cartridges and pre-filtration disks were closed with Luer Lock plugs and stored on ice in re-sealable foil pouches until they could be stored in a -80°C freezer.

Environmental data was taken during each visit to the sampling site, including: air temperature (Milwaukee Laser Temp-Gun 2265-20; Brookfield, WI), water temperature (YSI Pro20 DO-T; Yellow Springs, Ohio), salinity (YSI Pro30 COND-T; Yellow Springs, Ohio), and dissolved oxygen concentration (YSI Pro20 DO-T; Yellow Springs, Ohio). This information, as well as final flow rates, final volumes, and other relevant field notes recorded during acquisition can be found in the Appendix.

2.4. Sample Processing

2.4.1. Extraction Preparation

All glassware was triple rinsed with DI water two times, triple rinsed with methanol and then triple rinsed with DCM to prevent sample contamination. Anhydrous sodium sulfate (VWR International, LLC; Solon, Ohio; CAS No. 7757-82-6; Lot No. 1076C063) was stored in a 104°C oven overnight and allowed to cool in a desiccator to remove moisture. 100 mL DI water per sample was reduced to pH 2 with sulfuric acid (EMD Chemicals Inc.; Gibbstown, NJ; CAS 7664-93-9; Lot 51246) on a magnetic stir plate (Fisher Scientific; Waltham, MA), monitored by a pH meter (Mettler Toledo InLab Expert Pro-ISM; Columbus, OH).

2.4.2. Extraction

Samples, method detection limits (MDL), method blanks (MB), and method spikes (MS) were extracted by the same procedure, an adaptation of CI-Agent Stormwater Solution's recommended method (Hepner, 2013).

On the day of extraction, samples were removed from the -80°C freezer and allowed to thaw for 30 minutes. Using a 50 mL glass Lauer lock syringe, 50 mL methanol was eluted slowly through the disk into a 500 mL separatory funnel. Then, using the same syringe, a total of 75mL DCM was eluted slowly through the disk into the separatory funnel. Slow elution is critical for recovery of compounds adsorbed to the disk. The disk was capped with Lauer lock plugs and set aside to prevent analyte loss during the following steps.

100 mL of DI water, adjusted to pH 2 with sulfuric acid (CAS 7664-93-9; EMD Chemicals, Billerica Massachusetts, USA) was added directly to the separatory funnel using a 100mL graduated cylinder. The separatory funnel was sealed with a glass stopper and shaken gently for three minutes; pressure was vented periodically by carefully opening the release valve.

This step aids in removing the methanol from the DCM by partitioning it into the water phase. After settling for 10 minutes, the bottom DCM layer was drawn into a 250 mL flask through a long-stemmed glass funnel containing filter paper (150 mm; Whatman; Buckinghamshire, UK; Cat No 1004 150) filled with prepared anhydrous sodium sulfate (CAS 7757-82-6; VWR International, Solon Ohio USA).

The steps were repeated twice more by eluting an additional 50 mL DCM slowly through the disk, shaking the sample, and drawing off the DCM layer into the concentration vessel through the sodium sulfate.

2.4.3. Reduction and Storage (Parent Samples)

Extracted solvent was then reduced on an R-114 Rotavapor over a B-480 water bath (BÜCHI; New Castle, DE) not exceeding 30°C. Water bath temperatures greater than 30°C resulted in lower recoveries during method development. A BÜCHI PLASTIC+GLAS cooling column attached to a DI cooling system (Lauda-Brinkmann; Delran, New Jersey) was also connected to the Rotavapor.

The sample volume was reduced to approximately 6 mL and then transferred to a 15 mL graduated glass vial using a small glass funnel; after sample transfer, the flask was rinsed three times with approximately 2 mL DCM to recover any residual compounds; this was added to the sample. The sample in the graduated vial was reduced to dryness on an N-EVAP 111 nitrogen evaporator (Organomation Associates, Inc; Berlin, MA) with a water bath not exceeding 30°C and then reconstituted in 1mL acetonitrile (ACN; Fisher Scientific; Waltham, MA; CAS 75-05-8; Lot 161620). Reduced samples were refrigerated in solvent-rinsed amber glass vials with septa-less polyethylene screw cap lids (Waters; Milford, MA) until analysis.

Reconstitution in ACN was added during method development, because leaving the solvent in DCM resulted in technical difficulties and lower chlorothalonil recoveries. In a method development analysis, samples in DCM were reduced to 0.100 mL rather than dryness, and then 5 mL ACN was added; the reduction by nitrogen blowdown was continued to a final volume of 1 mL. Analyte recoveries were lower using this technique than when samples were reduced to dryness, so it was not used.

2.4.4. Reduction (Final Analysis)

Once all samples were analyzed following the initial reduction procedure (final volume of 1 mL), the remaining volume of each sample was reduced to 0.100 mL by nitrogen blowdown to analyze for compounds present in lower concentrations. The sample storage vials were triple rinsed with ACN, which was added into the sample volume prior to reduction for optimal compound recovery.

2.4.5 Sample Preparation for Analysis

On the day of analysis, samples and standards were allowed to warm to room temperature. Then, a 20-200 uL micropipette was used to dispense 100 uL sample into a 150 uL vial insert (Waters; Milford, MA). The sample was then spiked with 5 uL internal standard and capped with a septum for analysis (Agilent; Santa Clara, California).

2.5. Sample Analysis

2.5.1. GC/MS and Quantitative Analysis

The instrument parameters used were an adaptation of methods outlined by Cunha and Fernandez (2011). Chemical analyses were performed using an Agilent 6890 gas chromatograph (GC) equipped with an Agilent 5973 mass selective detector (MSD) and fitted with a Zebron-5MS high-resolution capillary column (30 m long x 250 mm diameter x 0.25-micron thick film).

The carrier gas was ultrahigh purity helium (Air Liquide; Houston, TX) at a constant flow rate of 1 ml min^{-1}. An Agilent 7693 autosampler was used for making splitless injections. The injector port was set at 280°C and was fitted with an Agilent deactivated borosilicate liner. The oven temperature program was as follows: the initial temperature was set to 80°C and was held for 2min. The temperature was then increased incrementally, as follows: from 80°C to 180°C at a rate of 20°C per minute; from 180°C to 230°C at a rate of 5°C per minute; from 230°C to 280°C at a rate of 20°C per minute; and, finally, from 280°C to 300°C at a rate of 40°C per minute. The temperature of the MSD interface to MS was set at 280°C. The mass spectrometer had an ion source temperature of 230°C, quadrupole temperature of 150°C, and ionization energy of 70eV. The MSD was operated in the selected ion monitoring (SIM) mode for quantifying atrazine, chlorothalonil, fipronil, and phenanthrene d10.

The MS was tuned to PFTBA (perfluorotributylamine) before each set of analyses. No samples were analyzed if any of the tune parameters were outside of acceptable limits. Targeted analytes detected in SIM mode were subsequently run in Scan mode set to molecular weight range 50 – 550. Atrazine detections were confirmed in Scan for every sampling date. Chlorothalonil and fipronil concentrations were too low for a definitive confirmation in Scan.

2.6. Method Detection Limit Determination

Method detection limits (MDLs) were performed to quantify the lowest concentrations that the extraction and analytical procedures can produce with 99% confidence that the detection is not due to contamination or noise. Confidence of 95% in a detection is represented by the lower confidence limit (LCL), which can be calculated from the MDL value (USGPO, 2012). Two sets of MDLs were utilized in this study. The first set, MDL_1, corresponds to a final sample volume of 1 mL. The second set, MDL_2, corresponds to the further reduction of original samples

to 0.100 mL. The MDL solution was prepared in 1 L DI water using the following volumes and concentrations listed in Table 2.1.

Table 2.1. Method Detection Limit (MDL) Solution Preparation Calculations.

	Initial Conc.	Initial Vol.[1]	+	Solvent	=	Final Vol.[1]	Final Conc.
MDL stock solution	2.0 ug/mL	0.375 mL ACN	+	1.125 mL ACN	=	1.5 mL	0.5 ug/mL
MDL sample solution	0.5 ug/mL	0.100 mL DI H_2O	+	1900 mL DI H_2O	=	1000 mL DI H_2O	0.00005 ug/mL
Unit Conversion				0.00005 ug/mL (ppb)	=	50 ng/L (ppt)	

[1] Volumes were measured with a 20-200 uL micropipette, calibration-verified each day of use. Stock solution contains equal parts of atrazine, chlorothalonil, and fipronil.

MDL standard solutions were prepared in 1 L volumetric flasks with glass stoppers and inverted ten times to mix. One pump was assembled in the laboratory, and tubing was fitted and flushed with 50 mL methanol followed by 50 mL DI water prior to analysis. The uptake tube was suspended in the MDL solution, and the solution was drawn to the tip of the outflow end before clean, prepared disks were attached. Figure 2.3 illustrates the laboratory pump setup.

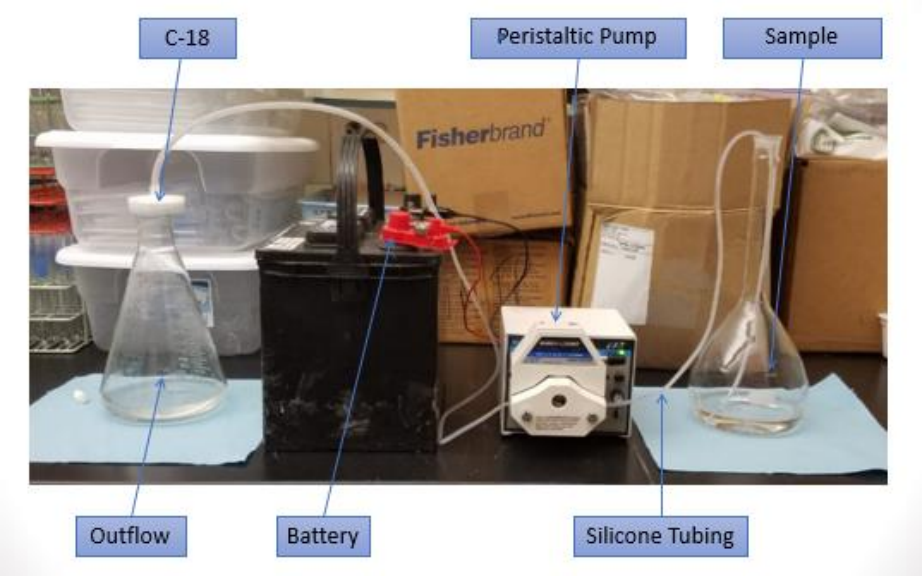


Figure 2.3. Example Pump Setup for MDL Preparation.

The solution was drawn through at approximately 4 mL min^{-1}, and then the disks were sealed with Luer Lock plugs and stored in the refrigerator overnight to simulate sample hold-times in the field. After 24 hours, the disks were transferred to the -80°C freezer, where they were stored until extraction and analysis. The equations used to calculate the MDLs are shown below (Source: USGPO, 2012). MDLs are displayed in Table 2.2.

$$MDL = 3.143\ x\ STD$$

$$LCL = 0.62\ x\ MDL$$

STD = Standard Deviation of recovered concentrations

3.14 is the 99% confidence t value for (n – 1) replicates.

Table 2.2. Method Detection Limit (MDL_1) Results.

	MDL expected (ng/L)	Mean observed (ng/L)	Standard deviation (ng/L)	Relative standard deviation (%)	Mean recovery (%)	MDL_1 (ng/L)	LCL_1 (ng/L)
A	50	27.45	4.002	15.23	54.9	12.58	8.0
C	50	33.78	9.002	32.32	55.7	28.29	18.1
F	50	22.24	3.999	17.58	45.5	12.57	8.0

A: Atrazine. C: Chlorothalonil. F: Fipronil.

Following analysis of the 1 mL sample aliquots, sample volumes were further reduced to 100 uL to allow for low-level detection of chlorothalonil and fipronil. The MDLs were thereby adjusted (Corl, Owens, & Pollack; 2002). MDLs for samples with a final volume of 100 uL (MDL_2) are listed in Table 2.3: Method Detection Limit (MDL_2) Results.

Table 2.3. Method Detection Limit (MDL_2) Results.

	MDL_2 (ng/L)	LCL_2 (ng/L)
A	1.26	0.80
C	2.83	1.81
F	1.26	0.80

A: Atrazine. C: Chlorothalonil. F: Fipronil.

2.7. Quality Assurance and Calculations

Reported concentrations validity was insured using multiple quality assurance and quality control measures. Samples, method detection limits (MDL), method blanks (MB), and method spikes (MS) were all extracted by the same procedure, an adaptation of CI-Agent Stormwater Solution's recommended method (Hepner, 2013). A five-point (minimum) calibration curve was analyzed at the beginning of each analytical batch; average R^2 values for reported samples throughout the study are listed in Table 2.4.

Table 2.4 Average Calibration Curve R^2 Values.

Analyte	Average R^2
Atrazine	0.992
Chlorothalonil	0.973
Fipronil	0.992

In GC/MS analyses, an internal standard (ISTD) must be added to every calibration standard, quality control standard, and sample. This is performed for the calculation of a response factor (RF) to quantify sample concentrations. The RF is calculated by determining the ratio of a known ISTD response to a known standard concentration response, such as a point on the calibration curve. The ratio represents the mass of analyte that is present per mass of ISTD, i.e. a comparison of how each chemical responds to the analytical parameters specified in the GC/MS instrument method. This ratio can then be used to calculate the mass of analyte in a sample of unknown concentration. The application of an RF is necessary to account for confounding variables such as sample matrix and instrument variation.

Phenanthrene d10 (Sigma-Aldrich; St. Louis, MO; CAS 1517-22-2) was used as an ISTD in this study; the California Department of Agriculture uses this compound for the same purpose in surface water analysis for fipronil by GC/MS (CDFA, 2008). During method development, captan d6 (CDN Isotopes; Pointe-Claire, Quebec, Canada; CAS 1330190-00-5; Lot C-424) was

used as an internal standard due to its structural similarity and affordability in comparison to deuterated standards of the target analytes. The compound response was unstable across multiple analyses in acetonitrile. Because the phenanthrene d10 was used successfully in a prior fipronil experiment within the academic department, this compound was used instead.

The phenanthrene d10 area response and the area response of target analytes from the daily calibration curve, a response factor was calculated using the following formula (ADHS, 2017):

$$RF = \frac{(Ax)(Cis)}{(Ais)(Cx)}$$

Where:

Ax = Area of the compound response

Cx = Concentration of the compound

Ais = Area of the internal standard response

Cis = Concentration of the internal standard

The response factors for each calibration standard were then averaged and applied to the sample results calculation (CDFA, 2008):

$$ppt = \frac{(\mathrm{Ax})(\mathrm{Mis})(\mathrm{Vf})(\mathrm{DF})(1000\ \mathrm{ng/mL})}{(Ais)(RFavg)(IV)(Vi)}$$

Where:

Ax = Area of the compound response

Mis = Mass of the internal standard (ng)

Vf = Final volume (mL)

Ais = Area of the internal standard response

RFavg = Average Response Factor

IV = Injection Volume (uL)

Vi = Initial sample volume (mL)

Method blanks (MB) were analyzed to monitor for contamination introduced during sample processing. They were prepared in the same manner as the MDLs minus the analytical standard addition. 1 L DI water was pumped through the C-18 disks at a flow rate of approximately 4 mL min^{-1}. After the entire volume was passed through the disk, the samples were stored in the -80°C freezer and treated identically to the field samples. None of the target analytes were detected in the method blanks during the study.

Method spikes (MS) were performed to monitor target analytes' recovery throughout the analytical process. These samples were prepared in the same manner as the MDLs. After the standard spiking process was complete, the matrix spike C-18 disks were stored with field samples in the -80°C freezer. They were treated identically to the field samples thereafter. The method spike standard solution preparation concentrations and volumes are listed in Table 2.4.

Table 2.4. Method Spike (MS) Solution Preparation Calculations.

	Initial Conc.	Initial Vol.[1]	+	Solvent	=	Final Vol.[1]	Final Conc.
MDL stock solution	2.0 ug/mL	0.375 mL ACN	+	1.125 mL ACN	=	1.5 mL	0.5 ug/mL
MDL sample solution	0.5 ug/mL	0.100 mL DI H_2O	+	1900 mL DI H_2O	=	1000 mL DI H_2O	0.00005 ug/mL
Unit Conversion				0.00005 ug/mL (ppb)	=	50 ng/L (ppt)	

[1] Volumes were measured with a 20-200 uL micropipette, calibration-verified each day of use. Stock solution contains equal parts of atrazine, chlorothalonil, and fipronil.

A continuing calibration variable (CCV) was analyzed with every analytical batch, between four and six samples. Average percent recoveries for batches containing reportable atrazine and chlorothalonil results, as well as MS recoveries, are listed in Table 2.5. Fipronil

CCV recoveries are not applicable, because all detections fell below the method detection limit. Atrazine CCV results fell within the acceptable range of 80-120%. Only one analytical batch contained reportable chlorothalonil values. The CCV percent recovery of 129.3% is within the acceptable range (68.5-130.4%) defined by the California Department of Food and Agriculture for chlorothalonil determination in surface water (CDFA, 2010).

Table 2.5. Continuing Calibration Verification (CCV) and Matrix Spike (MS) Percent Recoveries (%Rec).

	CCV %Rec Range	CCV % Rec Average	MS %Rec Range	MS %Rec Average
Atrazine	81.4 - 119.8	99.1	67.1 - 120.7	90.0
Chlorothalonil	129.3	129.3	111.7 - 129.8	120.4
Fipronil	n/a	n/a	60.7 - 81.3	70.3

Instrument blanks (IB) were analyzed at least every five samples during the study to monitor for sample carry-over and solvent contamination. None of the target analytes were detected in IBs during the study.

2.8. Statistical Analyses of Data

Field-measured data taken during sampling trips, climate data from the National Oceanic and Atmospheric Administration (NOAA), and additional data from USGS-07374525 were compiled for the dates of (or nearest to) the study period using the national water quality monitoring database (NOAA, 2017b, NWQMC, 2017). These data were then organized chronologically and paired with the atrazine mean concentrations from corresponding sample start dates.

Chemical concentrations were compared with measured environmental data and other published water level and water quality data using multiple regression. Regressions and T-tests were performed GraphPad Prism version 7.00 for Mac (GraphPad Software, La Jolla California USA, www.graphpad.com (2017)). Excel for Windows (Excel 2016, Microsoft, Redmond

Washington USA) was used for descriptive statistics and for correlations of final volumes and atrazine concentrations.

CHAPTER 3: RESULTS AND DISCUSSION

3.1. Occurrence of Pesticides

The MDL_2 and detection frequency for each compound are listed in Table 3.1.

Table 3.1. Bayou Lamoque Pesticide Detection Frequency, June – October 2016.

	Type	MDL_2 (ng/L)	Detection Frequency (%) (n = 15)	Detections > MDL_2
Atrazine	H	1.26	100	14
Chlorothalonil	F	2.83	13.3	2
Fipronil	I	1.26	53.3	0

H: herbicide. F: fungicide. I: insecticide. S:N, 1:4.

Atrazine, chlorothalonil, and fipronil were all detected over the course of the study. Samples with concentrations lower than the target analyte's respective method detection limit (MDL_1, MDL_2) cannot be distinguished from a non-detect with 99% confidence (USGPO, 2012). Atrazine was the most frequently detected compound with a 100% detection frequency. This is directly compatible with 2016 USGS water quality monitoring data from their station on the Mississippi River in Belle Chasse, Louisiana (NWQMC, 2017). One atrazine sample concentration fell below the method detection limit (MDL_1). This result was reported and included in statistical analysis of data due to confirmation of the detection in the 0.100 mL volume analytical batch at concentrations above MDL_2. Chromatograph screenshots and further explanation may be found in the Appendix.

Fipronil was identified in 53% of the samples at concentrations below the method detection limit; all fipronil results were reported as less than the MDL_2 value, 1.26 ng/L. This detection frequency is higher than those of similar studies, suggesting that nearby upstream runoff is contributing to the study-area fipronil load. Chlorothalonil was the least frequently detected compound, appearing in 13% of samples, which aligns with other environmental

studies. Both detection concentrations met the MDL_2 criteria and were reported (Orlando et al, 2014; Reilly et al, 2012).

3.2. Analytical Results and Validity

The MDL_1, MDL_2, and results for each sample are listed in Table 3.2. Only MDL_2 values are given for chlorothalonil and fipronil, because all sample results were obtained from the final (lowest volume) analysis. For atrazine, significant figures indicate whether MDL_1 or MDL_2 was used.

Table 3.2. Bayou Lamoque Pesticide Concentrations, June – October 2016.

Sample ID	Atrazine (ng/L)(ppt)	Chlorothalonil (ng/L)(ppt)	Fipronil (ng/L)(ppt)
MDL_1	12.58	n/a	n/a
MDL_2	1.26	2.83	1.26
6/23/2016a	33.73	3.37	nd
6/23/2016b	51.20	nd	<1.26
6/23/2016c	32.67	nd	<1.26
8/2/2016a	18.92	nd	<1.26
8/2/2016b	52.23	nd	nd
8/2/2016c	30.00	nd	nd
8/4/2016a	3.30	nd	nd
8/4/2016b	20.01	nd	nd
8/4/2016c	104.1	nd	nd
10/6/2016a	24.32	nd	<1.26
10/6/2016b	14.07	nd	<1.26
10/6/2016c	41.11	nd	<1.26
10/7/2016a	38.03	3.71	<1.26
10/7/2016b	3.54	nd	nd
10/7/2016c	32.42	nd	<1.26

n/a: Not applicable. nd: not detected. S:N, 1:4.

The low number of reportable values for chlorothalonil and fipronil did not allow for a descriptive statistical analysis. The concentrations of chlorothalonil detected were compatible with surface-water levels reported in other environmental studies (Orlando et al, 2014; Reilly et al, 2012). Though the fipronil detections were not reportable, the analyte's MDL_2 insures with

99% certainty that the environmental concentrations were not greater than 1.26 ng l^{-1}. Descriptive statistics for atrazine are listed in Table 3.3 and mean atrazine concentrations are listed in Figure 3.1.

Table 3.3. Atrazine Concentration Descriptive Statistics.

	6/23/2016	8/2/2016	8/4/2016	10/6/2016	10/7/2016
Mean (ng/L)(ppt)	39.20	33.72	42.5	26.50	24.7
Standard Error	6.01	9.79	31.18	7.88	10.68
Median	33.73	29.99	20.01	24.32	32.42
Standard Deviation	10.40	16.96	54.00	13.65	18.50
Sample Variance	108.26	287.76	2916.40	186.39	342.41
Range	18.52	33.31	100.77	27.04	34.48
Minimum	32.67	18.92	3.30	14.07	3.54
Maximum	51.20	52.23	104.07	41.11	38.03
Count (n)	3.00	3.00	3.00	3.00	3.00

A t-test indicated there are not significant differences in the amounts of atrazine between any of the dates. A correlation of 88% (R = 0.88) was determined between average final water volume per sample set and sample variance. High sample variance is inherent to the environmental sampling process and matrix. A grab sample is an aliquot that is taken at a single point in time, whereas composite samples represent an average of the measured parameters over time. The continuous nature of the sampling process in this study, drawing directly from the Ballandock Canal for over 24 hours, means that the extracts should be classified as composite rather than grab samples. Additionally, Mississippi River water is heterogenous in nature due to the variety of inputs it receives. The sample variance values are acceptable due to these considerations. Sample set means were used to investigate further correlations.

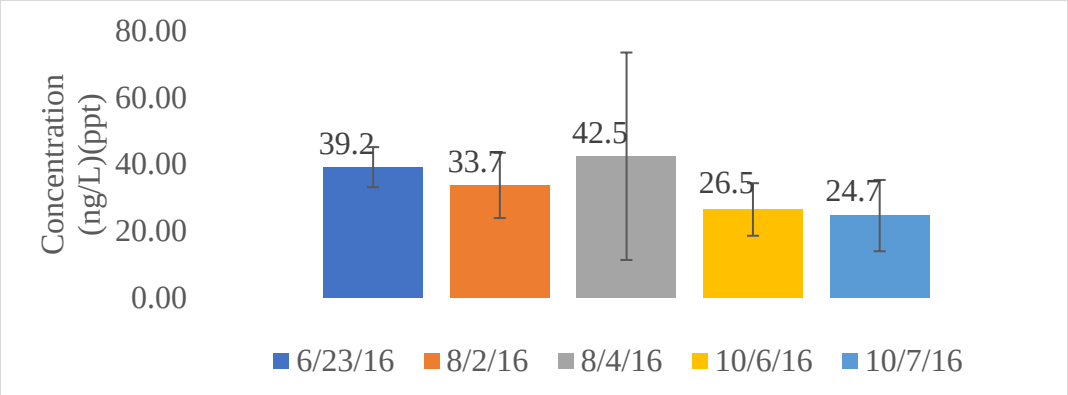


Figure 3.1. Atrazine Mean Concentrations.

3.3. Atrazine Results

Numerous significant correlations were identified by best linear fit statistical regression analyses. Rainfall in New Orleans from June – October 2016 was positively correlated to the measured atrazine concentrations ($R^2 = 0.831$, $p = 0.0312$). The regression is shown in Figure 3.2. This is a logical correlation, as rainfall would increase the amounts of pesticide runoff draining into the Mississippi River. Due to its hydrolysis DT_{50} of 86 days, atrazine could be carried by the river for large spans of time and distance under favorable conditions (Hertfordshire, 2017a).

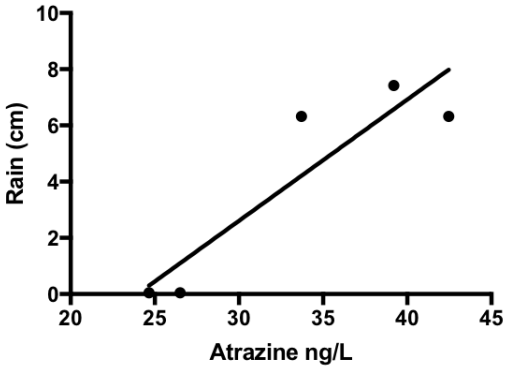


Figure 3.2. Regression Analysis of Mean Atrazine Concentrations and Rainfall.

A positive association was also identified between atrazine and turbidity ($R^2 = 0.83$, $p = 0.0314$), as is demonstrated in Figure 3.3. Turbidity is a water quality parameter that evaluates

how much light can pass through water, i.e. how cloudy it is. High turbidity is caused by dissolved or extremely small bits of matter suspended in the water column; among other substances, this matter may include algae, silt, soluble organic compounds, and organic matter (Perlman, 2016). Elevated turbidity levels may help shield atrazine from photodegradation, increasing its environmental persistence. Turbidity may be related to rainfall.

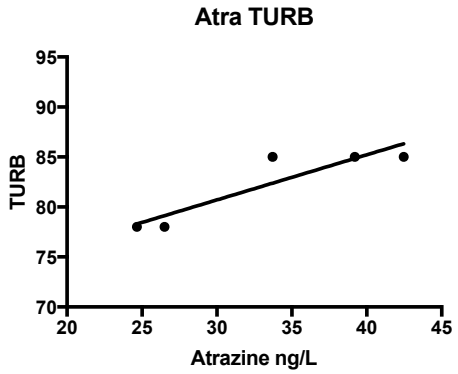


Figure 3.3. Regression Analysis of Mean Atrazine Concentrations and Turbidity.

Mudhoo and Garg (2010) discussed atrazine's high affinity to bind to organic matter and humic acids. If high turbidity is a result of dissolved or particulate organics, atrazine will bind to them preferentially, facilitating its transport. This may explain the positive correlation observed between suspended organic carbon and atrazine, as well ($R^2 = 0.83$, $p = 0.0314$). Suspended organic carbon concentrations are also affected by rainfall. Figure 3.4 demonstrates a regression analysis of mean atrazine concentrations and suspended organic carbon.

Positive correlations were identified between atrazine concentrations and six additional water quality parameters. Though the validity of each correlation may be argued individually, the most logical explanation is that the additional correlations result from rainfall. These correlations and their resulting R^2 and P values are listed in Table 3.4.

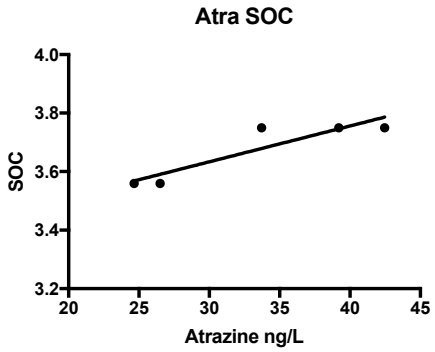


Figure 3.4. Regression Analysis of Mean Atrazine Concentrations and Suspended Organic Carbon (SOC).

Table 3.4. Factors Related to Measured Atrazine Concentration.

	R^2	P value
Alkalinity	0.813	0.0363
Dissolved Oxygen	0.83	0.0314
pH	0.83	0.0314
Specific Conductivity	0.83	0.0314
Total Dissolved Solids	0.83	0.0314
Total Kjeldahl Nitrogen	0.83	0.0314

3.4 Possible Sources

Identifying the source of these pesticides is challenging due to their ubiquitous application practices. Agricultural practices near the study site include the maintenance of citrus groves and the production of row crops, such as tomatoes; this is demonstrated in aerial imagery in Figures 3.5 and 3.6. In addition to local agricultural practices, farming, residential, and commercial areas along the Mississippi River north of the site are likely contributors to pesticide-containing runoff. Additional research including point source runoff concentrations and transport processes in the river would be necessary to hypothesize specific sources of the pesticides with any confidence.

Figure 3.5. Circled Sample Site (Right) and Agricultural Land (Left) (Google, 2017).

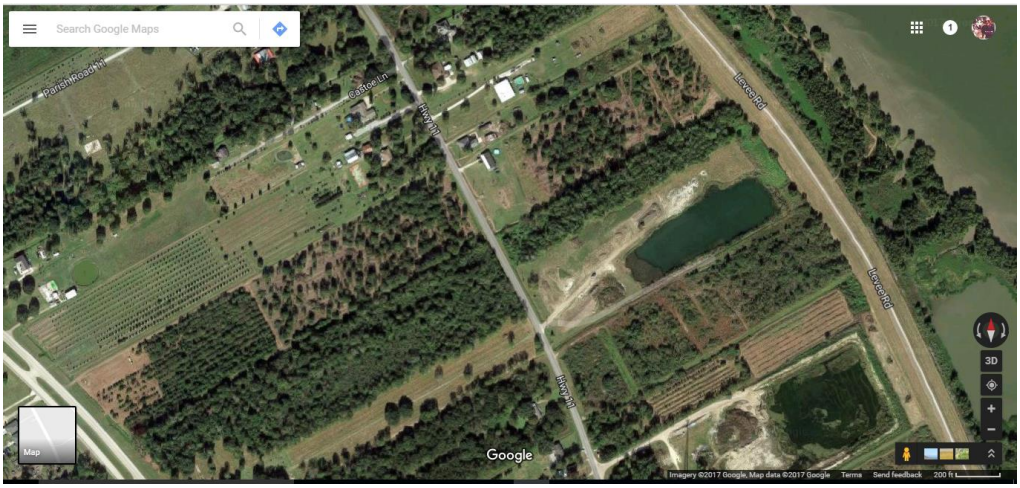


Figure 3.6. Nearby Agricultural Land, Including Row Crops and Citrus Groves (Google, 2017).

CHAPTER 4: FUTURE WORK AND CONCLUSION

4.1 Future Work

Future work includes the addition of site locations, sampling dates, and flow volume monitoring to the study design, and further development of the sampling and extraction methodology. Sampling at multiple site locations simultaneously could provide insight into route of introduction and transport processes for these pesticides in the Mississippi River. Additional sampling dates would provide helpful information regarding the seasonality of certain detections. Also, determining the flow volume and rate of water entering the Bayou Lamoque control structure could facilitate the calculation of estimated contaminant loads entering the canal due to the diversion.

The extraction process could be tested on other compounds to expand the scope of the method. Due to their structural similarity to the target analytes in this study, additional triazine herbicides, chloronitrile fungicides, and phenylpyrazole insecticides would be logical starting points. Scan results suggest that the extraction procedure may be effective for diethyltoluamide (DEET), dibutyl phthalate (DBP), metolachlor, and terbucarb.

On the day of sampling, grab samples could be taken in the field, stored on ice, and extracted as soon as possible to compare results to the time-composited samples. This step will pose practical challenges, as a minimum of 2 L must be sampled to detect concentrations of atrazine relevant to this study. At least 20 L would be necessary to approach detectable chlorothalonil or fipronil concentrations.

The addition of a surrogate standard to aid in determining extraction efficiency would also supplement the method. A standard that is structurally similar to the target compounds should be used. Additionally, the standard must not occur naturally in the sampling environment, a challenging fulfillment when sampling Mississippi River water.

Finally, a method could be added to facilitate analysis of the sediment trapped in the pre-filtration disks. An initial dry weight should be recorded for each pre-filter disk so that sediment mass can be calculated after collection. Methodological development and method detection limit determination comparable to those completed in this study would be necessary to generate usable data.

4.2 Conclusion

Louisiana's coastal wetlands provide a variety of ecosystem services that are critical to the livelihood and well-being of state residents. These services could be impacted by freshwater diversion projects rerouting Mississippi River water into the ecosystems due to the unintentional introduction of contaminants from its large watershed. The Bayou Lamoque freshwater diversion in Plaquemines Parish, Louisiana, is an appropriate location for assessing these processes.

This study outlines a protocol for environmental sampling in a remote field location using Continuous Low-level Aquatic Monitoring (C.L.A.M.) C-18 disks, along with effective solid phase extraction (SPE) and gas chromatography mass spectrometry (GC/MS) methodology for the determination of atrazine, chlorothalonil, and fipronil in surface water. Average concentrations of atrazine and chlorothalonil inside the Bayou Lamoque freshwater diversion structure for the months of June – August, 2016, were 24.5 ng l^{-1} and 3.5 ng l^{-1}, respectively. Fipronil was not detected in concentrations exceeding its volume-adjusted lower confidence limit (LCL_2) of 0.80 ng l^{-1}. These concentrations do not pose a threat to sensitive non-target organisms based on current, published ecological toxicity data. More research is needed to identify additional contaminants of concern entering the coastal wetlands because of freshwater diversion projects.

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APPENDIX A: FIELD NOTES

FIELD NOTES

BLM CONTROL STATION

http://www.timeanddate.com/date/timeduration.html

22 June 2016

09:15—Pumps set up and containers wrapped in foil. Cartridge details recorded on packaging. Cartridges were primed with 100 mL Great Value Distilled Water.

09:51—Pumps turned on.

Table A1. Environmental Data 6/22/16, 09:51.

COORDINATES	29° 26.401'; 89° 35.651'
AIR TEMPERATURE (°C)	29.2
WATER TEMPERATURE (°C)	28.0
SALINITY (ppt)	0.2
DISSOVED OXYGEN (ppm)	7.8-8.1; jumped to 8.27

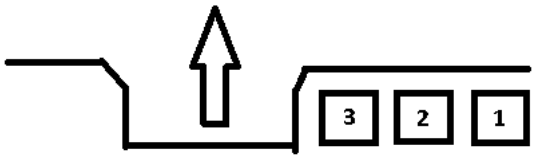

Figure A1. Pump Setup, 6/22/16.

23 June 2016

08:37—Checked pumps. Pump 1 and Pump 2 stalled; battery still charged. Visible sediment accumulation in Pre-filter 06221603.

Table A2. Environmental Data 6/23/16, 08:37.

COORDINATES	29° 26.401'; 89° 35.651'
AIR TEMPERATURE (°C)	32.4
WATER TEMPERATURE (°C)	28.2
SALINITY (ppt)	0.2
DISSOVED OXYGEN (ppm)	8.11

Table A3. Flow Rates 3 of 3 (06/22/16 09:51 - 06/23/16 08:37).

SAMPLE ID	VOLUME (mL)	TIME ELAPSED (min.)	FLOW RATE (mL/min.)
06221601*	216	1366	0.16
06221602*	140	1366	0.10

06221603	1284	1366	0.94

*Pumps stalled overnight.

Sample cartridges were removed and stored on ice until transfer to -80°C freezer on campus. Collected water was dumped.

23 June 2016

New sample cartridges were installed, and pumps were rearranged:

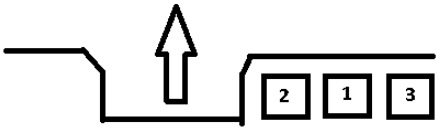

Figure A2. Pump Setup, 6/23/16.

09:18— Cartridges were primed with 100 mL Great Value Distilled Water. Pumps were started; turned knob ¼ turn clockwise from lowest setting.

Table A4. Environmental Data 6/23/16, 08:37.

COORDINATES	29° 26.401'; 89° 35.651'
AIR TEMPERATURE (°C)	32.4
WATER TEMPERATURE (°C)	28.2
SALINITY (ppt)	0.2
DISSOVED OXYGEN (ppm)	8.11

14:50—Pre-filters changed, water saved.

24 June 2016

11:47—No pump stalls overnight. A screw came loose on Pump Head 3; it was clunking with rotations. All three pumps pulled tubing into the head, but visible flow was still going through. Sample cartridges were removed and stored on ice until transfer to -80°C freezer on campus. Collected water was measured and dumped.

Table A5. Environmental Data 6/24/16, 11:47.

COORDINATES	29° 26.401'; 89° 35.651'
AIR TEMPERATURE (°C)	31.5
WATER TEMPERATURE (°C)	28.9
SALINITY (ppt)	0.2
DISSOVED OXYGEN (ppm)	8.20

Table A6. Flow Rates 2 of 2 (06/23/16 09:18 - 06/24/16 11:47).

SAMPLE ID	TOTAL VOLUME (mL)	TIME ELAPSED (min.)	FLOW RATE (mL/min.)
06231601	4941	1589	3.11
06231602	2569	1589	1.62
06231603	3700	1589	2.33

2 August 2016

Table A7. Environmental Data 8/2/16, 08:02.

COORDINATES	29° 26.401'; 89° 35.651'
AIR TEMPERATURE (°C)	29.9
WATER TEMPERATURE (°C)	30.9
SALINITY (ppt)	0.2
DISSOVED OXYGEN (ppm)	8.17

8:40—Pumps started. Cartridges were primed as detailed in Methods. Set-up below:

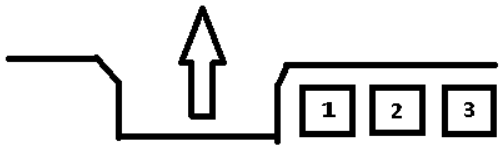

Figure A3. Pump Setup, 08/02/16.

Table A8. Environmental Data 8/2/16, 13:13.

COORDINATES	29° 26.401'; 89° 35.651'
AIR TEMPERATURE (°C)	33.4
WATER TEMPERATURE (°C)	29.6
SALINITY (ppt)	0.4
DISSOVED OXYGEN (ppm)	6.5

3 August 2016

Table A9. Environmental Data 8/3/16, 10:47.

COORDINATES	29° 26.401'; 89° 35.651'
AIR TEMPERATURE (°C)	30.7
WATER TEMPERATURE (°C)	30.9
SALINITY (ppt)	0.2
DISSOVED OXYGEN (ppm)	8.07

4 August 2016

Table A10. Environmental Data 8/4/16, 11:02.

COORDINATES	29° 26.401'; 89° 35.651'
AIR TEMPERATURE (°C)	37.3
WATER TEMPERATURE (°C)	31.0
SALINITY (ppt)	0.2
DISSOVED OXYGEN (ppm)	8.29

Table A11. Flow Rates 3 of 3 (08/02/16 08:40 – 08/04/16 11:02).

SAMPLE ID	VOLUME (mL)	TIME ELAPSED (min.)	FLOW RATE (mL/min.)
08021601	7190 + 6350 = 13540	3022	4.48
08021602	7423 + 6876 = 14299	3022	4.73
08021603	6341 + 5400 = 11741	3022	3.89

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4 August 2016

11:32—Pumps started at ¼ turn. Tubing rinsed with distilled water prior to redeployment. Cartridges primed as noted in Methods.

9 August 2016

Table A12. Environmental Data 8/9/16, 08:05.

COORDINATES	29° 26.401'; 89° 35.651'
AIR TEMPERATURE (°C)	29.4
WATER TEMPERATURE (°C)	31.0
SALINITY (ppt)	0.2
DISSOVED OXYGEN (ppm)	8.46

Table A13. Final Flow Rates (08/04/16 11:32 – 08/09/16 08:05).

SAMPLE ID	TOTAL VOLUME (mL)	TIME ELAPSED (min.)	FLOW RATE (mL/min.)
08041601*	26000	6993	3.72
08041602	30700	6993	4.39
08041603*	7800	6993	1.12

*08041601: Pump head 1 came loose, but still pulling water through.

*08041603: Pump head 3 pulled tubing in, not pulling water through, low final volume.

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6 October 2016

Table A14. Environmental Data 10/6/16, 08:06.

COORDINATES	29° 26.401'; 89° 35.651'
AIR TEMPERATURE (°C)	27.3
WATER TEMPERATURE (°C)	26.5
SALINITY (ppt)	0.1
DISSOVED OXYGEN (ppm)	10.47

Pump 1 has formerly used tubing. Pump 2 and 3 have brand new tubing. Drive #2 would not power on due to a corroded control panel. The power supply was hardwired directly to the motor, so the pump would only run on maximum in one direction. Arranged 1, 2, 3.

Table A15. Environmental Data 10/7/16, 07:29.

COORDINATES	29° 26.401'; 89° 35.651'
AIR TEMPERATURE (°C)	25.6
WATER TEMPERATURE (°C)	26.3
SALINITY (ppt)	0.1
DISSOVED OXYGEN (ppm)	10.66

Table A16. Flow Rates 3 of 3 (10/06/16 08:49 – 10/07/16 07:59).

SAMPLE ID	VOLUME (mL)	TIME ELAPSED (min.)	FLOW RATE (mL/min.)
10061601	987 + 6234 = 7221	1390	5.19
10061602* (on max)	2910 + 11400 = 14310	1390 – 21 - 19 = 1350	10.60
10061603	1166 + 6770 = 7936	1390	5.71

*Outflow from Pump 2 was cloudy. Pump 2 tubing split. Cartridge was stored at 07:40. Spit portion was removed and good tubing pulled into setup.

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07 October 2016

New cartridges deployed at 08:32. Pump 2 still running on max due to control panel corrosion bypass.

Table A17. Environmental Data 10/7/16, 07:29.

COORDINATES	29° 26.401'; 89° 35.651'
AIR TEMPERATURE (°C)	25.6
WATER TEMPERATURE (°C)	26.3
SALINITY (ppt)	0.1
DISSOVED OXYGEN (ppm)	10.66

*10071602pf was leaking and appeared to be impacted by sediment. We modified the setup to determine water volume through the C18 only.

8 October 2016

Table A18. Environmental Data 10/7/16, 07:29.

COORDINATES	29° 26.401'; 89° 35.651'
AIR TEMPERATURE (°C)	22.8
WATER TEMPERATURE (°C)	29.5
SALINITY (ppt)	0.2
DISSOVED OXYGEN (ppm)	10.64

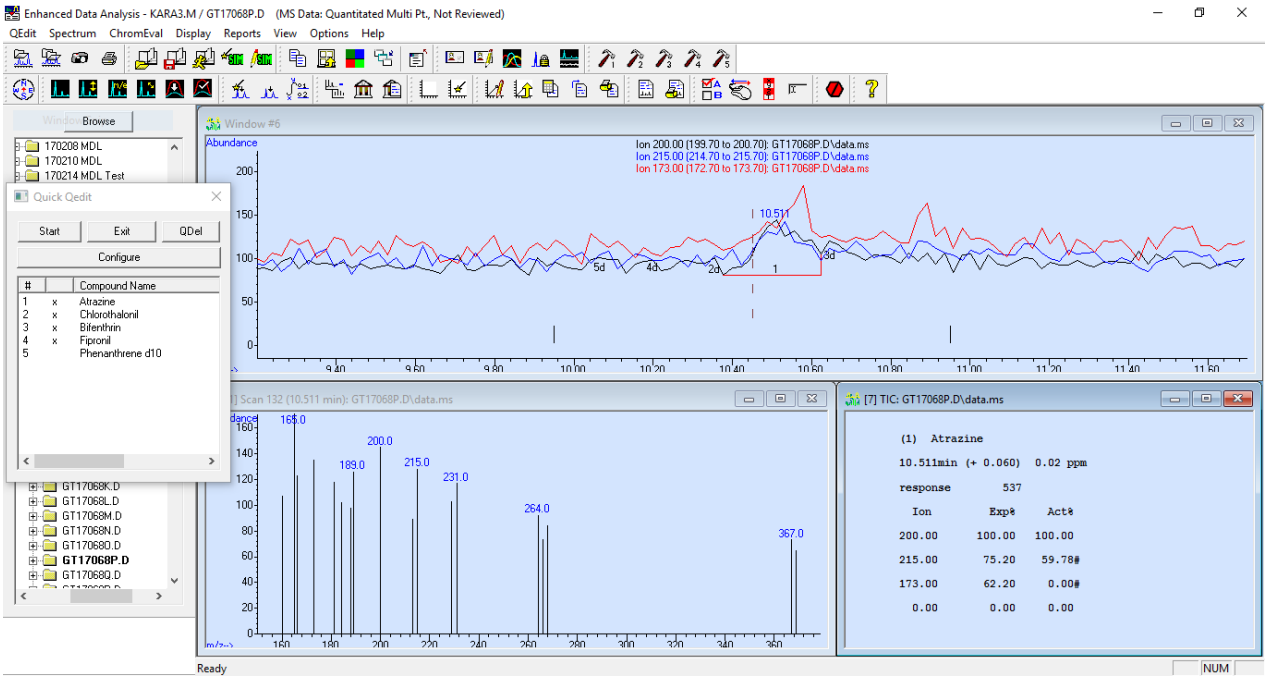
Table A19. Flow Rates 2 of 2 (10/07/16 08:32 – 10/08/16 07:23.

SAMPLE ID	VOLUME (mL)	TIME ELAPSED (min.)	FLOW RATE (mL/min.)
10071601	1923 + 6903 = 8826	1371	6.44
10071602* (on max)	6795 + 2471 = 9266	1371	6.76*
10071603	1797 + 6417 = 8214	1371	5.99

*10071602pf was impacted and spraying water from the cracks. We modified the setup to determine water volume through the C18 only.

APPENDIX B: SAMPLE INFORMATION

Explanation for inclusion of atrazine sample below MDL_1:



FigureB1: GT17068P(1). Atrazine peak falls within calibration curve. Analyte MDL/LCL is reduced due to sample concentration to 0.100 mL by Nitrogen Blowdown.

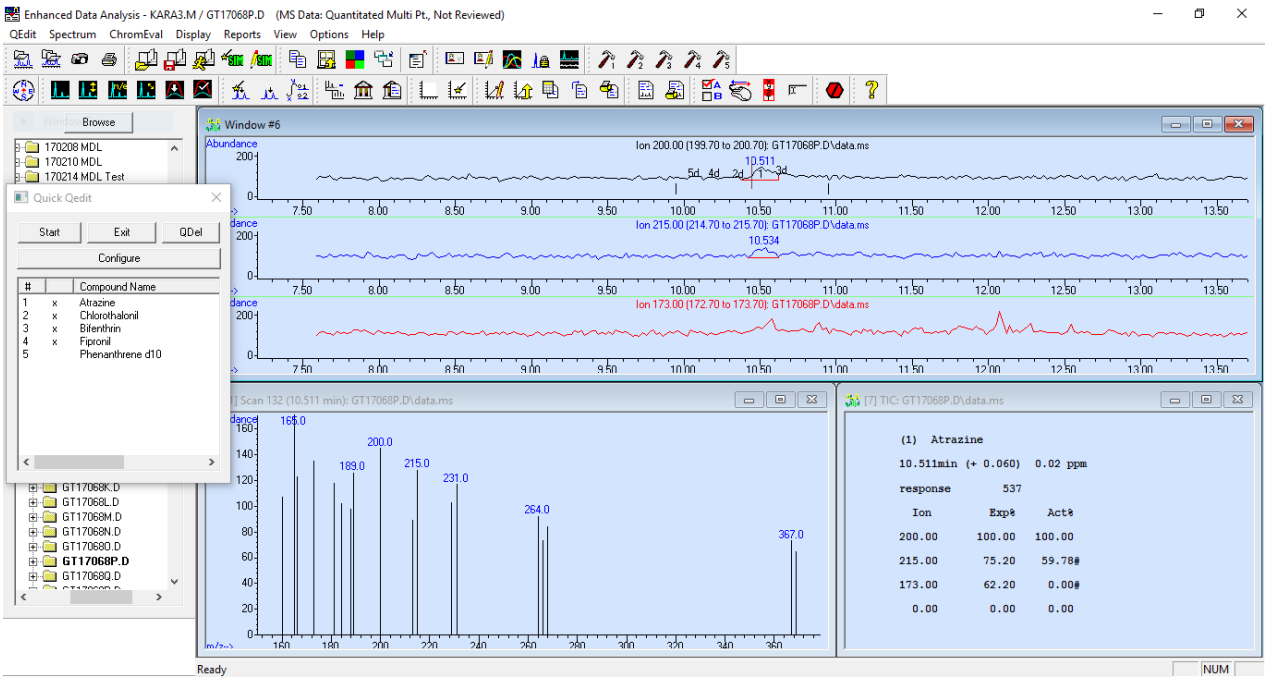


Figure B2: GT17068P(2). Qualifying ions confirm the detection within acceptable signal to noise ratio (1:4).

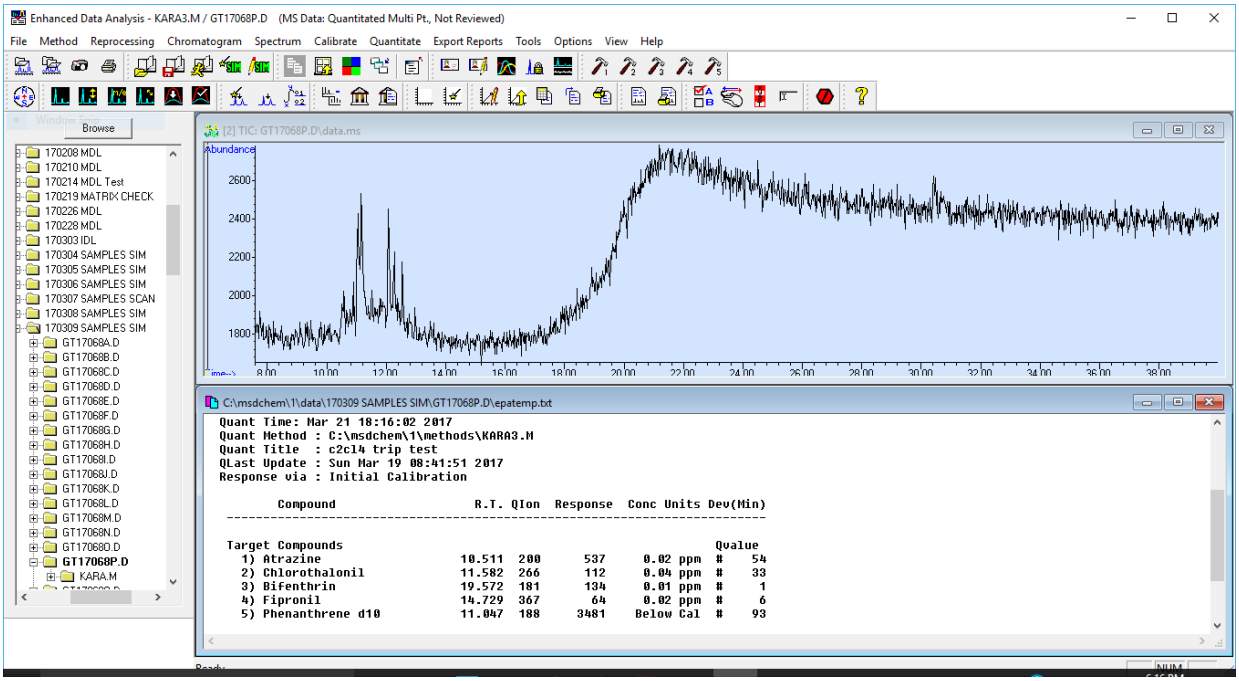


Figure B3: GT17068P(3). Chromatogram and low ISTD recovery suggest solvent interference due to analyst error (ACN addition) subsequent to sample reduction and addition of ISTD.

VITA

Kara Leigh Callicott, of Tupelo, Mississippi, received her bachelor's degree from the University of Mississippi in Biology in Spring 2013. She gained experience in the environmental field working at Gulf Coast Analytical Laboratory in Baton Rouge, Louisiana. She began her master's studies at Louisiana State University in the Department of Environmental Sciences during Fall 2015. Kara anticipates graduation in May 2017, after which she plans to continue her professional pursuits in the environmental field.