7-2011

Effects of the Herbicide Atrazine on the Behavior of the Checkered Gartersnake (Thamnophis Marcianus)

Katie Chamberlain

EFFECTS OF THE HERBICIDE ATRAZINE ON THE BEHAVIOR OF THE CHECKERED GARTERSNAKE (*THAMNOPHIS MARCIANUS)*

By

KATIE CHAMBERLAIN

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science
Department of Biology

John S. Placyk, Jr., Ph.D., Committee Chair

College of Arts and Sciences

The University of Texas at Tyler
July 2011

The University of Texas at Tyler
Tyler, Texas

This is to certify that the Master's Thesis of

KATIE CHAMBERLAIN

has been approved for the thesis requirement on
July 19, 2011
for the Master of Science degree

Approvals:

Thesis Chair: John S. Placyk, Jr., Ph.D.

Member: Neil B. Ford, Ph.D.

Member: Ali Azghani, Ph.D.

Chair, Department of Biology

Dean, College of Arts and Sciences

Acknowledgments

This thesis could not have been completed without the support, encouragement and enthusiasm of many people. It was because of these people that I had the determination to push through the many hours of behavioral trials and complete the writing of this thesis.

First, I would like to thank the East Texas Herpetological Society and the University of Texas at Tyler Office of Sponsored Research for their help in funding this research project.

I would also like to thank my advisor, Dr. John S. Placyk Jr. for all of his support and mentoring these past two years. Firstly, for taking me under his wing and finding me a place at this university, and secondly, for guiding me towards a fulfilling research topic. It was a great comfort to know that I could rely on his help during every step of this process. I would also like to thank my committee members, Dr. John S. Placyk, Jr., Dr. Neil Ford, and Dr. Ali Azghani for all of their suggestions and feedback on my research proposal and drafts of my thesis.

Paula Hibbert also helped me in many ways throughout my time at the University of Texas at Tyler. I want to thank her for all of her support in everything from finding a job, to buying a car, to giving me an outlet for venting my frustrations. I would also like to thank the rest of the faculty and staff at UT Tyler. I believe that every one of them helped me in some way whether it was through excellent teaching, mentoring in my research, or simply providing me with friendly conversation.

I am especially grateful for the companionship of all my friends that I have gained in Tyler, TX. If I ever needed a break from my work or research, I always knew that I could depend on them to lend a hand. My family also played an important part in helping me reach my goals. If I ever needed someone to talk to or was in a bind financially, I knew that I could count on them. I am truly blessed to have so many people in my life that love and care for me.

Lastly, I would like to thank my husband, Jeremy Chamberlain. Without him, I truly wouldn't be where I am today. His motivation gave me the drive not only to pursue graduate school, but also the determination to continue towards my goals despite many roadblocks. I can't count the hours that he spent beside me while we performed behavior trials, graded biology student papers and exams, and read research paper after research paper. He has been a complete inspiration for my life and I am overwhelmingly happy to be his wife.

Table of Contents

Appendices

List of Tables

List of Figures

Abstract

EFFECT OF THE HERBICIDE ATRAZINE ON THE BEHAVIOR OF THE CHECKERED GARTERSNAKE (*THAMNOPHIS MARCIANUS*)

Katie Chamberlain

Thesis Chair: John S. Placyk, Jr., Ph.D.

The University of Texas at Tyler
July 2011

Atrazine is one of the most commonly used herbicides in the United States and one of the most popular weed-killers worldwide, being utilized in over 80 countries. Despite the effectiveness of atrazine, there has been growing concern over the potential harmful effects this chemical may have on non-target species exposed to this chemical. Few studies, however, have been performed on the effects of this chemical on reptiles, in particular snakes. This study examined the effects of environmentally relevant concentrations of atrazine on the foraging, antipredator, thermoregulatory, and courtship behaviors of the checkered gartersnake (*Thamnophis marcianus*). Effects of atrazine appeared negligible for all foraging and thermoregulatory trials. There were effects found on tail-wagging and body flattening antipredator behaviors and courtship behaviors of individuals receiving different concentrations of atrazine. These effects, however, were few and did not follow any noticeable trends.

Chapter 1

Overview of Atrazine and its Effects

Atrazine Characteristics

Atrazine (2-chloro-4-ethylamino-6-amino-s-triazine) is one of the most commonly used herbicides in the United States and one of the most popular weed-killers worldwide, being utilized in over 80 countries (Graymore et al., 2001; Hayes et al., 2002; Hayes et al., 2003; Kiely et al., 2004). This chemical is effective in killing a variety of broad leaf and grassy weeds in a wide range of crop systems including corn, sorghum, sugar cane and wheat (Graymore et al., 2001; Barr et al., 2007). Though it is used primarily on farms, atrazine is also used along highways and railroads (ATSDR, 2003). Atrazine was developed in 1952 by the Geigy Chemical Company in Basel, Switzerland (Knuesli, 1970) and was registered for commercial use in the United States in 1959 (USEPA, 1994). This chemical has been produced by Syngenta, a world-leading agri-business, for over 50 years, and used extensively in the United States since the 1960's (USDA, 1994). This chemical can be applied pre-plant (i.e., before planting seeds), pre-emergence (i.e., before the seedlings emerge), or post-emergence (after seedlings emerge from the ground), making it a very flexible herbicide. Atrazine's success can also be attributed to its low cost, high efficacy and versatility in most agricultural systems. The U.S Environmental Protection Agency (2003) estimates that farming without the use of

atrazine could cost corn, sorghum, and sugarcane growers in the United States over $2 billion in yield losses and additional weed control costs.

Atrazine is a chloro-triazine herbicide with a chemical structure based on a central carbon/nitrogen ring (Figure 1). The structure of this herbicide makes it highly water soluble. Atrazine also has the potential to adsorb to soil or become airborne, however, the physical properties of this chemical make it more prone to dissolving in water. Once atrazine is applied to soils, it can remain there for days to months, but is usually broken down within one growing season (ATSDR, 2003). If atrazine enters the air, it is usually broken down quickly by oxidation reactions. Atrazine can also bind to dust particles in the air and once bound, these particles are not likely to break down until they settle back on the Earth's surface.

The half-life of atrazine in the environment is variable depending on many factors, most importantly the type of soil, climate, and application history (Jablonowski et al., 2010; Kookana et al., 2010). The average half-life of atrazine ranges from 13 to 261 days (USEPA, 2003). Environmental conditions such as colder temperatures and anaerobic conditions may increase the half-life of this chemical dramatically. Atrazine also degrades at a much slower rate when it enters aquatic environments; in reservoirs, for example, the half-life can range anywhere from 1 to 2 years (Goolsby, 1993). As atrazine leaches through soil into underlying groundwater systems with less available oxygen, its degradation rate slowly decreases. This decrease in degradation rate allows the chemical to persist for long periods in the environment.

In pure form, atrazine is a colorless crystalline powder that is not volatile, reactive, or flammable (ATSDR, 2003). Atrazine can be purchased as a pure substance or

as an ingredient in many commercial herbicides both for large-scale agriculture or individual consumer application. Because of its high water solubility, atrazine can easily be dissolved in water to get desired application concentrations. Atrazine is most commonly applied by spraying over crops with application rates depending on climate and soil texture (USDA, 1994). For example, lower application rates are recommended for sand, loamy sands, and sandy loams than other soil types. On average, it is estimated that the United States applies 13.2 to 16.5 million kg of this chemical annually (Kiely et al., 2004). The heaviest use of atrazine occurs in the Midwestern United States with high corn production, with three of the highest application rates being found in Illinois, Iowa, and Indiana) (USDA, 1994) (Figure 2).

Movement of Atrazine in the Environment

A pesticide's solubility is considered one of the most important properties influencing its transport in the environment (Gleason, 2006). When pesticides are applied, some of these chemicals adsorb or attach to soil particles and some will dissolve in the water either on the soil surface or between soil particles. As more water filters through the soil, some of the pesticide detaches from soil particles and dissolves into the water. Pesticides with high water solubility are therefore less likely to attach to soil particles. Atrazine is highly water soluble and is rated as having medium to high mobility in soils (Wauchope et al., 1992). Because of its high solubility, this chemical is most effective when applied to wet soils (Flynn and Spellman, 2009; Graymore et al., 1999; Graymore et al., 2001). Its high water solubility, therefore, allows for even distribution throughout a field. Because atrazine is an herbicide, its ability to readily

dissolve in water also allows for easy uptake of the chemical by plants. Application often occurs after winter rains when soils are at field capacity, holding their maximum amount of moisture, making atrazine highly prone to leaching and runoff (Graymore et al., 2001). Runoff transports pesticides and other pollutants along the surface of the ground, but does not penetrate into the soil. In the process of leaching, however, these pollutants are carried through the soil by rain and irrigation water percolating in response to gravity. The leaching potential of atrazine is rated as large, which is the highest rating available for pesticides (Wauchope et al., 1992). Consequently, atrazine is highly likely to infiltrate freshwater ecosystems.

After application, the ultimate fate of a pesticide depends on its persistence in the environment and its solubility in water (Gleason, 2006). The length of time a pesticide is able to persist in the environment is dependent on the chemical and microbial reactions occurring in the soil that break each chemical into its metabolites. The breakdown of atrazine is highly variable in different environmental conditions but is largely considered moderately persistent, meaning that compared to other chemicals, it is not quickly broken down (USEPA, 2003).

Because atrazine is so commonly used and because water is found almost everywhere on the planet, this herbicide is capable of entering a variety of different ecosystems. Organisms found in or near contaminated areas are therefore likely to come into contact with atrazine. All organisms need water for their daily metabolic activities. Plants uptake water for use in photosynthesis, amphibians need water to complete stages of their life cycle, and fish use water for habitat. Some organisms may be exposed directly by absorption through their skin, and others may be exposed through respiration.

Atrazine contamination can also occur by indirect exposure through ingestion. Organisms at higher trophic levels such as mammals and birds can be exposed to atrazine by ingesting organisms at lower trophic levels that have been directly exposed to the chemical.

Often times, pesticide contamination manifests itself in the form of bioaccumulation or biomagnification. These are two of the most common measurements of contamination used in toxicological research. Bioaccumulation is the process by which the concentration of a chemical in an organism increases as more contaminated food or water is ingested over time. The potential for bioaccumulation of atrazine is low, but has been documented in some organisms including bivalves (Jacomini et al., 2006) and oligochaete worms (*Lumbriculus variegates*) (Jantunen et al., 2008). Biomagnification is the process in which chemicals are transferred to an organism from the food they ingest, resulting in a higher concentration of that chemical (Gray, 2002). In biomagnification, the concentration of a chemical is magnified as it moves up through a food chain to higher trophic levels. Biomagnification of atrazine is considered negligible (Solomon et al., 1996). Even though the risk of bioaccumulation and biomagnification of atrazine is considered low, it is still possible that it may be detrimentally affecting organisms. These effects can easily be measured by examining the behavior, physiology, morphology, and life-history of organisms that have been exposed.

Effects on Plants and other Photosynthetic Organisms

Atrazine primarily enters plants through the uptake of water and nutrients by the roots. In unicellular photosynthetic organisms such as algae, atrazine is generally

transferred across cell surfaces (Hull, 1967). Photosynthesis, the process through which light energy is converted to usable chemical energy, is vital in the acquisition of nutrients for many plants. One component of this process is Photosystem II. Photosystem II is the first constituent of the light reactions that occur in photosynthesis, which allows for electrons to be energized by light and transferred to Photosystem I by the electron transport chain. The mechanism through which atrazine disrupts photosynthesis is by the inhibition of an electron carrier in this electron transport chain (Tischer and Srotmann, 1977; Forney and Davis, 1981; Solomon et al., 1996). Atrazine blocks the transport of electrons by competing with plastoquinone II, an enzyme, at its binding site. This binding site requires water as an electron donor, but with this site being blocked, the transfer of electrons ceases (Solomon et al., 1996). Inhibiting electron transport has many detrimental effects on the process of photosynthesis, such as chlorophyll destruction and buildup of carbon dioxide within the cell (Shabana, 1987; Solomon et al., 1996). Ultimately, this can lead to the death of the plant. Because of the effectiveness of this process in killing weed species, atrazine has become one of the most common herbicides used today.

While being effective for eradicating a variety of weeds and grasses, this function is somewhat indiscriminate, also causing interruption of photosynthetic processes in non-target species. Once in the ecosystem, this chemical can have indirect damage on native flora, especially algal species which are integral primary producers of aquatic food webs (Graymore et al., 2001). Stratton (1984) discovered that the green algae *Chlorella pyrenoidosa* is even more sensitive to atrazine metabolites than the parent compound itself. Growth inhibition in unicellular algae *Chlamydomonas reinhardii* and

Scenedesmus subspicatus was recorded in concentrations of atrazine as low as 2.0 to 5.0μg/L (Girling et al., 1999). Death of nonresistant species of algae begins occurring at levels of 10.0 to 20.0μg/L (Graymore et al., 2001). This allows more resistant phytoplankton species to progress and causes drastic changes in aquatic community structures. Algal groups most sensitive to atrazine exposure are Chlorophyta (green algae) and large filamentous algae (Hamilton et al., 1987). A recent study on the effect of atrazine on macrophyte (emergent water plant) communities showed that even short-term exposures of 8 weeks affected the community structure by inhibited growth of sensitive plant species (Coutris et al., 2011).

The major factor that determines whether a plant is tolerant to atrazine is the plant's ability to metabolize and detoxify the parent molecule (Shimabukuro, 1967). There are two major pathways through which higher plants are able to detoxify atrazine (Figure 3). The first results in complete detoxification through a hydroxylation reaction in which a hydroxyl group is added to atrazine (Shimabukuro, 1967). Species such as corn and sorghum are resistant to atrazine because of their ability to quickly metabolize atrazine into a non-toxic metabolite using this pathway. The second pathway to detoxify atrazine consists of the de-alkylation of either of its two alkyl groups to form a partially detoxified intermediate. These intermediates also have the possibility of being further metabolized into a completely detoxified metabolite (Shimabukuro, 1967).

Effects on Animals

In addition to inhibiting photosynthesis in plants, it is speculated that atrazine disturbs the cellular functions in animal cells as well, although the exact mechanisms

remain unclear (Goldman, 1999). At the organismal level, however, the effects of atrazine are better understood. Researchers have examined the effects of atrazine encompassing everything from endocrinology and immunology to reproductive biology and behavior incorporating a broad range of taxa including invertebrates, amphibians, reptiles, mammals, and birds (Goldman, 1999; Benotti et al., 2009; Forney and Davis, 1981; Victor-Costa et al., 2010; Hayes et al., 2002; De Solla et al., 2006; Dewey, 1986; Flynn and Spellman, 2009; Ottinger et al., 2008; Biradar and Rayburn, 1995). A large majority of research, in particular, has focused on life-history and behavioral effects of atrazine.

The major modes of entry into animal systems can occur through direct or indirect exposure to the chemical. Direct uptake of atrazine can occur by means of absorption through the skin, respiration, or ingestion of contaminated water. Uptake can also occur indirectly through ingestion of plants or animals from lower trophic levels that have come into contact with atrazine. As was seen in certain species of plants, some taxa such as fish (Fisher-Schrel et al., 1991) also have the ability to metabolize atrazine and excrete the products as waste. Higher trophic levels, in general, tend to show a decrease in sensitivity to this chemical (Solomon et al., 1996).

Invertebrates

Invertebrates perform many roles in ecosystem processes and have a diverse array of feeding habits (Coleman, 2000). Because of their high diversity in habitat and prey preferences, these organisms are at risk of contamination through many different routes. Often occupying roles as lower level consumers in food webs, invertebrates tend to be

highly susceptible to effects of atrazine, even at very low concentrations. In particular, aquatic invertebrates are at risk due to the large portion of their life cycles being spent in water, putting them at risk of both direct exposure through absorption and respiration and indirect exposure through the food web. Numerous studies have been performed on the effects of atrazine on aquatic invertebrates encompassing many taxa.

Changes in zooplankton community structures have been noted following applications of atrazine (deNoyelles et al., 1982). This noticeable change in structure has been attributed more to changes in available food sources, such as algae, rather than direct physiological effects on zooplankton (Graymore et al., 2001). For example, aquatic gastropods can be influenced negatively due to decreased algal food sources and loss of macrophytes for habitat (Graymore et al., 2001). In the aquatic pulmonate gastropod, *Ancyclus fluviatilis*, decreased hatch rates were seen when animals were exposed to 1000 µg/L atrazine (Streit and Peter, 1978). Decreases of mussel aggregations in highly contaminated streams have been documented (Flynn and Spellman, 2009). Survival, growth, and development of aquatic insects have been negatively influenced at concentrations of atrazine between 20 and 100 µg/L (Dewey, 1986). The aquatic midge *Chironomus tentans* exhibited reduced hatchling success, abnormal larval development, and reduced numbers of pupating larvae at concentrations greater than 230µg/L (Dewey, 1986; Macek et al., 1976; Graymore et al., 2001). Anderson et al. (2007) found that this midge species exposed to ecologically relevant concentrations of atrazine, could significantly increase its oxygen consumption, most likely in response to physiological impairment of the respiratory system.

Fish

Because fish are aquatic creatures, one of the most significant exposure pathways is through absorption and respiration of contaminated water. In fish, atrazine is metabolized by the kidneys and excreted through the gills. It is not surprising that because of this, many of the reported physiological changes occur in these organs. Fisher-Scherl et al. (1991) noticed changes in kidney tissues and increased protein levels in urine when fish were exposed to concentrations of atrazine and gill damage has been recorded in many fish species exposed, including elephantnose fish (*Gnathonemus pertersii*) and carp (*Cyprinus carpio*) (Alazemi et al., 1996). Because gills are such an integral part of many physiological processes in fish, damage to these structures is inherently detrimental.

Even very short-term exposure to atrazine in goldfish can cause numerous behavioral changes, including increased burst behavior and decreased grouping (Saglio and Trijasse, 1998). Zebrafish exhibited changes in behavior including habitat preferences and swimming behavior (Steinberg et al., 1995). Behavioral changes are very important because they often reflect changes in the nervous system (Steinberg et al., 1995). Rainbow trout (*Oncorhynchus mykiss)* exhibited changes in behavior including reduced motility and balance when exposed to atrazine (Steinberg et al., 1995). These changes can negatively influence many aspects of an organism's life history and natural history including predator avoidance, timing of reproduction, and use of micro-habitats (Graymore et al., 2001).

Reptiles and Amphibians

One of the most studied taxonomic groups in response to atrazine contamination is amphibians. Amphibians are often used as indicators of ecosystem health because they are highly susceptible to environmental changes. This group is also important to study in conjunction with atrazine because early developmental stages of many amphibians are entirely aquatic and often, organisms are most susceptible to effects of environmental contaminants during early development.

Storrs and Semlitsch (2008) demonstrated that amphibians with shorter larval periods and more rapid somatic development rates are more susceptible to somatic effects of atrazine contamination. Amphibians with rapid ovarian development, however, are more susceptible to ovarian effects of this chemical (Storrs and Semlitsch, 2008). In addition, exposure to atrazine can cause hermaphrodism, demasculinization, and impairment of gonadal development in leopard frogs (*Rana pipiens*) and African clawed frogs (*Xenopus laevis*) (Hayes et al., 2003; Hayes et al., 2002). Hayes et al. (2002) also noted a 10-fold decrease in testosterone in the African clawed frogs exposed to this chemical. Because of these effects, it is hypothesized that atrazine may induce activity of aromatase, an enzyme responsible for converting male sex hormones into female sex hormones (Hayes et al., 2002). Atrazine may also modify life history traits of leopard frogs by making them more susceptible to parasitism, most likely due to suppressed immune systems (Gendron et al., 2003; Goldman et al., 1999). Similar effects were found on immune in tiger salamanders (*Ambystoma tigrinum*) exposed to atrazine. Increasing levels of atrazine were found to correlate with increases in pathogen susceptibility

(Forson and Storfer, 2006). Salamanders exposed to atrazine pre-metamorphosis had increased risk of desiccation as eight months post-exposure (Rohr and Palmer, 2005).

Very few studies, however, have been performed on the effects of atrazine on reptiles. Many ecotoxicological studies on reptiles utilize crocodiles, alligators, and turtles, in particular due to their unique feature of temperature dependent sex determination (TDSD) (Sparling et al., 200). This is advantageous because many toxicological studies involve the effects of chemicals on sex hormones. With TDSD, the sexes of individuals can easily be manipulated through temperature alteration and therefore changes in sex ratios are clearly visible. One of these studies examined changes in snapping turtle gonadal development in response to different concentrations of this herbicide (De Solla et al., 2006). A recent study performed by Neuman-Lee and Janzen (2005) revealed that atrazine exposure during embryonic development of map turtles (*Graptemys ouachitensis* and *Graptemys pseudogeographica*) inhibited nest escape behavior and reduced post-hatching survival.

Birds

To date, very few studies examined the direct effects of atrazine on avian species. Recently this number has been increasing but is still minimal, most of them utilizing quail as a model species. In birds, one primary route of chemical exposure is through maternal transfer into the developing egg (Ottinger et al., 2008). Another route is through bioaccumulation in the food chain. Because atrazine has been known to bioaccumulate in some species, there is potential that even small amounts of chemicals released in the

environment can result in high concentrations in the food of a predatory bird species (Sibley, 2001).

Japanese quail (*Coturnix* japonica) exposed orally to varying atrazine concentrations exhibited numerous physiological effects. Individuals exposed to higher concentrations showed significant decreases in food consumption, body weight, testes size, sperm count, and noticeable liver degeneration (Hussain et al., 2011). Japanese quail exposed to concentrations of atrazine in the egg before incubation had decreased hatchling weight and adult females exposed exhibited decreased ovarian weights (Wilhelms et al., 2006). Ottinger et al. (2008) also documented changes in behavior, including impaired male sexual behavior of quail exposed to atrazine.

Mammals

Studies on the effects of atrazine on mammals are particularly important for the extrapolation of data to potential effects this chemical may have on human populations. Ovary cells of hamsters have exhibited chromosomal damage after exposure to atrazine (Newman, 1995). Similar abnormalities have been documented in bone marrow chromosomes of mice and lymphocyte cultures of farm workers exposed to atrazine (Biradar and Rayburn, 1995). This chromosomal damage may potentially lead to cancer or birth defects in future generations (Newman, 1995; Biradar and Rayburn, 1995). Atrazine also causes tumors in the mammary tissues of rats (Eldridge et al., 1994). Fakhouri et al. (2010) also found that treatment of rat pituitary cells resulted in a reduction in growth hormone gene expression.

Another study utilizing rats resulted in decreased body weight, short-term increases in testis weight followed by testis atrophy, reductions in testosterone, and increases in estradiol levels (Victor-Costa et al., 2010). These results also support the idea that atrazine may be increasing aromatase activity. A recent study by Higley et al. (2010) utilizing human adrenocarcinoma (H295R) cells demonstrated that atrazine did not directly induce aromatase activity but significantly induced indirect aromatase activity.The direct increase in aromatase activity was attributed to cyclic adenosine monophosphate (cAMP) which was a second messenger that up-regulated gene expression in response to atrazine (Higley et al., 2010).

Reasons for Concern

In the 1963 classic, Silent Spring, Rachel Carson noted "when you put something in the water anywhere, it ends up in the water everywhere" (Lannoo, 2008; Carson and Darling, 1962). This holds true for the fate of atrazine. Once atrazine is applied to fields and roadways, it will eventually be transferred to rivers, streams, ponds, and all other sources of water. Therefore, it is important to understand what effects this chemical can cause after it enters these environments.

Although there are many case studies in which organisms are impaired due to atrazine exposure, there are also many studies that show little effect (Lutz et al., 2009; Storrs and Semlitsch, 2008; LaFiandra et al., 2008; Jaeger, 1999). In some cases, atrazine was capable of causing negative ecological effects, but only at concentrations considerably higher than those considered ecologically relevant (Ceh and Majdic, 2010). This is most likely due to the difference in sensitivity of different species and taxonomic

groups to this chemical. Further studies are needed to determine how atrazine is potentially having negative effects, which organisms are at risk, and how these negative effects may affect ecosystem and community structures.

Concentrations of atrazine in drinking waters have been found exceeding the Maximum Contaminant Level (MCL) of 3µg/L set by the U. S. EPA (Koplin et al., 1998; Champman and Stranger, 1992). This is the highest level of a contaminant that is allowed in drinking water. However, even at these restricted levels, there has been growing concern over the potential health risks that atrazine may have on humans, in particular birth defects, making further studies on the effects of this chemical vitally important (Winchester et al., 2009; Jaeger, 1999; Benotti, 2008). Currently, the leading cause of infant mortality in the United States is birth defects (NVSS, 2002/2003). Due to the growing concern that atrazine may potentially be causing birth defects, application of this chemical became a growing target of apprehension. Elevated concentrations of agrichemicals in surface water in common application month significantly correlated with times of increased risk of birth defects (Winchester et al., 2009).In 1999, the U.S. Environmental Protection Agency classified atrazine as a possible human carcinogen (U.S. EPA, 1999). Long term- exposure to at non-toxic doses can cause inhibition of human intestinal cell maturation and decreased epithelial barrier integrity (Olejnik et al., 2010). This suggests that chronic ingestion of low levels of atrazine may potentially inhibit intestinal transport, absorption, and barrier functions.

If atrazine is causing detrimental effects to humans or wildlife, the usage of this chemical may need to be modified. Application methods may be altered, or restrictions made on allowable concentrations and volumes of the chemical that can be applied.

Alterations could be made to the structure of atrazine to make it more easily broken down in the environment or make it more selective toward target weed species.

Reptiles and Ecotoxicology

Recently, there has been growing concern on the decline of reptile populations. However, due to the lack of long term studies, the low densities of many reptile populations, and the reclusive nature of some reptiles, our ability to define reptile declines has been considered "inadequate" (Gibbons et al., 2000). There are six major factors thought to be contributing to reptile declines: habitat loss and degradation, introduced species, global climate change, disease and parasitism, unsuitable harvest, and environmental contaminants (Gibbons et al., 2000; Hopkins, 2000)

Though many studies have been performed on the effects of atrazine on animals, there are very few studies utilizing reptiles to examine these effects. Few toxicological studies in general have utilized reptiles, in particular snakes, compared to other taxa (Sparling et al., 2000; Campbell and Campbell, 2009; Hopkins, 2000). The studies conducted on atrazine contamination are exclusive to alligators and turtles. This disproportion is surprising given that snakes hold pivotal roles in ecosystems ranging from secondary consumers to top predators, occupy a wide range of niches and habitats, and have numerous features making them ideal ecological indicators of environmental contamination (Sparling et al., 2000). In food webs, reptiles play a pivotal role in the transfer of energy. Unlike other taxa, reptiles invest a very small portion of the energy they ingest into metabolism; instead, the majority of their energy intake is converted to their biomass (Pough, 1980). In many ecological systems, reptiles consume invertebrates

that are unavailable to other organisms that cannot survive on these low-energy food sources (Sparling et al., 2000). Because of their high production efficiency, they also are responsible for increasing accumulation rates. Reptiles also serve as a trophic link between small invertebrates and larger carnivores, which also links possible transfer of chemicals between these two groups (Sparling et al., 2000).

Ecotoxicological studies on reptiles are also important to understand how contaminants such as atrazine are affecting all aspects of an ecosystem. Reptiles have many physiological and behavioral features that make them unique from other taxonomic groups. One feature in particular is behavioral thermoregulation. Temperature is an influential part of many physiological processes, and therefore, individuals will move to temperatures of optimal physiological performance (Sparling et al., 2000; Lillywhite, 1987; Pough, 1998). Since certain pesticides alter sensitivity to temperature extremes (Gordon and Rowsey, 1998; Heath et al., 1997), thermoregulation in reptiles would be an important process to examine in response to contamination.

Another feature that makes reptiles unique from other taxonomic groups is their thick integument layer, which may impact absorption of different contaminants (Gans and Liner, 1969; Pough, 1998). Reptiles are also one of the few taxonomic groups that lay amniotic eggs (Gans and Liner, 1969). Maternal transfer of contaminants into the amniotic egg is another potential pathway of contaminant exposure.

Ecotoxicological studies on snakes, in particular, are an integral component in understanding the effects of contaminants on ecosystems. Snakes are the most diverse of all the reptiles (Sparling et al., 2000). They are found all over the world, occupy habitats from arid to aquatic, and hold roles from primary consumers to top predators. Behavioral

and physiological properties of snakes can easily be used as indicators of ecosystem function due to the rapid response of this group to environmental change (Beaupre and Douglas, 2009). Also, being predators that lead generally sedentary lifestyles, snakes in particular are susceptible to bioaccumulation of these contaminants (Beaupre and Douglas, 2009; Campbell and Campbell, 2009).Currently, there are no published studies examining the direct effects of atrazine on snakes (Sparling et al., 2000). Given that snakes are biologically and physically distinct from other taxonomic groups and that they are highly susceptible to coming into contact with environmental contaminants, this group is inherently important to understanding the full effects of atrazine contamination.

The objective of this work is to determine whether ingestion of environmentally relevant doses of atrazine affects foraging, antipredator, thermoregulatory, and/or reproductive behavior of checkered gartersnakes (*Thamnophis marcianus*). These behaviors are vital components of the natural history of snakes and therefore, alterations to any of these traits could have detrimental effects on wild populations exposed to this chemical. Furthermore, this study will be one of the few experiments examining the influence of atrazine on reptiles and one of the first to examine its effects on snakes.

References

Agency for Toxic Substances and Disease Registry (ATSDR). 2003. Toxicological Profile for Atrazine. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Alazemi, B. M., J. W. Lewis, E. B. Andrews. 1996. Gill damage in the freshwater fish, *Gnathonemus petersii* (family: Mormyridae) exposed to selected pollutants: an ultrastructural study. Environmental Technology17: 225-235.

Anderson, T. D., Y. Jin-Clark, K. Begum, S. R. Starkley, and K. Y. Shu. 2007. Gene expression profiling reveals decreased expression of two hemoglobin genes associated with increased consumption of oxygen in *Chironomus tetans* exposed to atrazine: A possible mechanism for adapting to oxygen deficiency. Aquatic Toxicology 86: 148-156.

Barr, D. B., P. Panuwet, J. V. Nguyen, S. Udunka, and L. L. Needham. 2007. Assessing exposure to atrazine and its metabolites using biomonitoring. Environmental Health Perspectives 115:1474-1477.

Beaupre, S. J. and L. Douglas. 2009. Snakes as Indicators and Monitors of Ecosystem Properties. In S. J. Mullin and R. A. Siegel (Eds.), *Snakes: Ecology and Conservation.* (pp 244-261). Ithaca, NY: Comstock Publishing Associates.

Benotti, M. J., R. A. Trenholm, B. J. Vanderford, and J. C. Holady. 2009. Pharmaceuticals and endocrine disrupting compounds in US drinking water. Environmental Science and Technology 43: 597-603.

Biradar, D. P. and A. L. Rayburn. 1995. Chromosomal damage induced by herbicide contamination at concentrations observed in public water supplies. Journal of Environmental Quality 24: 1222-1225.

Campbell, K. R. and T. S. Campbell. 2009. A logical starting point for developing priorities for lizard and snake ecotoxicology: A review of available data. Environmental Toxicology and Chemistry 21: 894-898.

Carson, R. and L. Darling. *Silent Spring*. Boston: Houghton Mifflin, 1962. Print.

Ceh, K. and G. Majdic. 2010. Pesticides as endocrine disruptors. Slovenian Veterinary Research 47: 163-166.

Chapman, R. N. and J. W. Stranger. 1992. Horticultural pesticide residues in water: a review of potential for water contamination by pesticides used in the vegetable industry in Victoria. Department of Food and Agriculture, Melbourne, Australia.

Coleman, David C., and Paul F. Hendrix, eds. *Invertebrates as Webmasters in Ecosystems*. Wallingford, Oxon, UK: CABI Pub., 2000. Print.

Coutris, C., G. Merlina, J. Silvestre, E. Pinelli, and A. Elger. 2011. Can we predict community wide effects of herbicides from toxicity tests on macrophyte species? Aquatic Toxicology 101: 49-56.

Davies P. E., L. S. J. Cook, and J. L. Barton. 1994. Triazine herbicide contamination of Tasmanian streams: sources, concentrations and effects on biota. Australian Journal of Marine and Freshwater Research 45:209-226.

De Solla, S., P. Martin, K. Fernie, B. Park, and G. Mayne. 2006. Effects of environmentally relevant concentrations of atrazine on gonadal development in snapping turtles (*Chelydra serpentina*). Environmental Toxicology and Chemistry 25: 520-526.

deNoyelles F., W. D. Kettle, D. E. Sinn. The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. Ecology 63:12851293.

Dewey, S. L. 1986. Effects of the herbicide atrazine on aquatic insect community structure and emergence. Ecology 67: 148-162.

Eldridge, C., M. Tennant, L. Wetzel, C. Breckenridge, and J. Stevens. 1992. Factors affecting mammary tumor incidence in Chlorotriazine-treated female rats: Hormonal properties, dosage, and animal strain. Environmental Health Perspectives 102: 29-36.

Ernst, C. and E. Ernst. 2003. Snakes of the United States and Canada. Washington and London: Smithsonian Institution.

Fakhouri, W. D. , J. Nunez, and F. Trail. 2010. Atrazine binds to the growth hormone releasing hormone receptor and affects growth hormone gene expression. Environmental Health Perspectives 118:1400-1406.

Flynn, K. and T. Spellman. 2009. Environmental levels of atrazine decrease spatial aggregation in the freshwater mussel, Elliptio complanata. Ecotoxicology and Environmental Safety72:1228-1233.

Forney, D. R. and D. E. Davis. 1981. Effects of low concentrations of herbicides on submersed aquatic plants. Weed Science 29:677-685.

Forson, D. and A. Storfer. 2006. Atrazine increases ranavirus susceptibility in the tiger salamander, *Ambystoma tigrinum*. Ecological Applications 16:2325-2332.

Gans, C. and E. A. Liner. *Biology of the Reptilia*. London: Academic, 1969. Print.

Gendron, A., D. Marcogliese, S. Barbeau, A. Christin, P. Brousseau, S. Ruby, D. Cyr, and M. Fournier. 2002. Exposure of leopard frogs to a pesticide mixture affects life history characteristics of the lungworm *Rhabdias ranae*. Oecologia 135: 469 476.

Gibbons, J. W., D. E. Scott, T. J. Ryan, K. A. Buhlmann, T. D. Tuberville, B. S. Metts, J. L. Greene, T. Mills, Y. Leiden, S. Poppy, and C. T. Winne. 2000. The global decline of reptiles. BioScience 50: 653-666.

Girling, A. E., D. Pascoe, C. R. Janssen, A. Peither, A. Wenzel, H. Schafer, B. Neumeier, G. C. Mitchell, E. J. Taylor, S. J. Maund, J. P. Lay, I. Juttner, N. O. Crossland, R. R. Stephenson, and G. Persoone. 1999. Development of methods for evaluating toxicity to freshwater ecosystems. Ecotoxicology and Environmental Safety 45:148-175.

Gleason, John. 2006. *Mobility of Herbicide Active Ingredients: Atrazine and 2, 4 D*. City of Austin. Web. 17 Apr 2011. <http://www.ci.austin.tx.us/growgreen/downloads/atrazine_mobility.pdf>.

Goldman, J., T. Stoker, and R. Cooper. 1999. Neuroendocrine and reproductive effects of contemporary-use pesticides. Toxicology and Industrial Health 15: 26-36.

Goolsby, D.A., W.A. Battaglin, J.D. Fallon, D.S. Aga, D.W. Kolpin, and E.M. Thurman. Persistence of Herbicides in Selected Reservoirs in the Midwestern United States: Some Preliminary Results. U.S. Geological Survey, Open-File Report 93-418. Denver, CO, 1993.

Gordon, C. J. and P. J. Rowsey. 1998. Reduced fever in the vagotomized rat following exposure to the organophosphate (OP) insecticide chlorpyrifos (CHP). Journal of the Federation of American Societies for Experimental Biology 12: 5560.

Gray, J. S. 2002. Biomagnification in marine systems: the perspective of an ecologist. Marine Pollution Bulletin 45: 1-12.

Graymore M., G. Allinson, M. Allinson, F. Stagnitti, Y. Shibata, and M. Morita. 1999. Environmental fate of pesticides used in Australian viticulture: V. Behavior of atrazine in the soils of the South Australian Riverland. Toxicology and Environmental Chemistry 70:427-439.

Graymore, M., F. Stagnitti, and G. Allinson. 2001. Impacts of atrazine in aquatic ecosystems. Environment International 26:483-495.

Hall, J. C., T. D. van Deynze, J. Struger, and C. H. Chan. 1993. Enzyme immunoassay based survey of precipitation and surface water for the presence of atrazine, metolachlor and 2,4-D. Journal of Environmental Science and Health, Part B 28:577-598.

Hamilton, P. B., G. Jackson, N. K. Kaushik, and K. R. Solomon. The impact of atrazine on lake periphyton communities including carbon uptake dynamics using track autoradiography. Environmental Pollution 46:83-103.

Hayes, T. B., A. Collins, M. Lee, M. Mendoza, N. Noriega, A. Stuart, and A. Vonk. 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Ecology 99:5476-5480.

Hayes, T. B., K. Haston, M. Tsui, A. Hoang, C. Haeffele, and A. Vonk. 2003. Atrazine induced hermaphroditism at 0.1 ppb in American Leopard Frogs (*Rana pipiens*): Laboratory and field evidence. Environmental Health Perspectives 111:568-575.

Heath, A. G., J. J. Cech, Jr., L. Brink, P. Moberg, and J. G. Zinkl. 1997. Physiological responses of fathead minnow larvae to rice pesticides. Ecotoxicology and Environmental Safety 37: 280-288.

Higley, E. B., J. l. Newstead, X. Zhang, J. P. Giesy, and M. Hecker. 2010. Assessment of chemical effects on aromatase activity using the H295R cell line. Environmental Science and Pollution Research 17: 1137-1148.

Hopkins, W. A. 2000. Reptile toxicology: Challenges and opportunities on the last frontier in vertebrate ecotoxicology. Environmental toxicology and Chemistry 19: 2391-2393.

Hull, H. M. 1967.*Herbicide Handbook of the Weed Society of America*. W. F. Humphrey Press, Geneva, NY, USA.

Hussain, R., F. Mahmood, M. Z. Khan, A. Khan, and F. Muhammad. 2011. Pathological and genotoxic effects of atrazine in male Japanese quail (*Coturnix japonica*). Ecotoxicology 20:1-8.

Jablonowski, N. D., G. Hamacher, R. Martinazzo, U. Langen, S. Koppchen, D. Hofmann, and P. Burauel. 2010. Metabolism and persistence of atrazine in several field soils with different atrazine application histories. Journal of Agricultural and Food Chemistry 58:12869-12877.

Jacomini, A. E., W. E. P. Avelar, A. S. Martinez, and P. S. Bonato. 2006. Bioaccumulation of atrazine in freshwater bivalves *Anodontites trapesialis* (Lamarck, 1819) and *Corbicula fluminea* (Muller, 1774). Environmental Contamination and Toxicology 51:387-391.

Jaeger, J., I. Carlson, and W. Porter. 1999. Endocrine, immune, and behavioral effects of aldicarb (carbamate), atrazine (triazine) and nitrate (fertilizer) mixtures at groundwater concentrations. Toxicology and Industrial Health 15:133-151.

Jantunen, A. P. K., A. Tuikka, J. Akkanen, and J. V. K. Kukkonen. 2008 Bioaccumulation of atrazine and chlorpyrifos to *Lumbriculus variegates* from lake sediments. Ecotoxicology and Environmental Safety 71: 860-868.

Kiely, T., D. Donaldson, and A. Grube. 2004. Pesticide industry sales and usage: 2000 and 2001 market estimates. EPA-733-R-04-001. U.S. Environmental Protection Agency, Washington, D.C., USA.

Knuesli, E. 1970. History of the development of traizine herbicide. Residue Review 32: 1-9.

Kookana, R., G. Holz, C. Barnes, K. Bubb, R. Fremlin, and B. Boardman. 2010 Impact of climatic and soil conditions on environmental fate of atrazine used under plantation forestry in Australia. Journal of Environmental Management 91:2649 2656.

Koplin, D., J. Barbash, and R. Gillom. 1998. Occurrence of pesticides in shallow groundwater of the United States: Initial results from the National Water Quality Assessment Program. Environmental Science and Technology 32:558-566.

LaFiandra, E. M., K. J. Babbitt, and S. A. Sower. 2008. Effects of atrazine on anuran development are altered by the presence of a nonlethal predator. Journal of Toxicology and Environmental Health 71:505-511.

Lannoo, M. J. *Malformed Frogs: the Collapse of Aquatic Ecosystems*. Berkeley: University of California, 2008. Print.

Lillywhite, H. B. "Temperature, Energetics, and Physiological Ecology." *Snakes: Ecology and Evolutionary Biology*. Ed. Richard A. Seigel, Joseph T. Collins, and Susan S. Novak. New York: McGraw-Hill, 1987. Print.

Lutz, K. W., T. Springer, H. Krueger, L. Holden, and A. Hosmer. 2009. Does atrazine influence larval development and sexual differentiation in *Xenopus laevis*? Toxicological Sciences 107:376-384.

Macek, K. J., R. S. Buxton, S. Sauter, S. Ginilka, J. W. Dean. Chronic toxicity of atrazine to selected aquatic invertebrates and fishes Washington DC: Environmental Protection Agency, 1976 (Ecological Research Series 600/3-76-047).

Neuman-Lee, L. A. and F. Janzen. 2011. Atrazine exposure impacts behavior and survivorship of neonatal turtles. Herpetologica 67: 23-31.

National Vital Statistics System 2002/2003. Infant, neonatal, and postneonatal deaths, percent of total deaths, and mortality rates for the 15 leading causes of infant death by race and sex: United States, 1999-2005. Web. May 2011. <http://www.cdc.gov/nchs/datawh/statab/unpubd/mortabs/lcwk7_10.htm>

Newman A. 1995. Atrazine found to cause chromosomal breaks. Environmental Science and Technology 29: 450A.

Olejnik, A. M., R. Marecik, W. Bialas, P. Cyplik, and W. Grajek. 2010. In vitro studies on atrazine effects on human intestinal cells. Water, Air, and Soil Pollution 213: 401-411.

Ottinger M. A., E. Lavoie, N. Thompson, A. Barton, K. Whitehouse, M. Barton, M. Abdelnabi, M. Quinn Jr., G. Panzica, and C. Viglietti-Panzica. 2008. Neuroendocrine and behavioral effects of embryonic exposure to endocrine disrupting chemicals in birds. Brain Research Review 57: 376-385.

Pough, F. H. 1980. The advantages of ectothermy for tetrapods. American Naturalist 115:92-112.

Pough, F. H. *Herpetology*. Upper Saddle River, NJ: Prentice Hall, 1998. Print.

Rohr, J. R. and B. D. Palmer. 2005. Aquatic herbicide exposure increases salamander desiccation risk eight months later in a terrestrial environment. Environmental Toxicology and Chemistry 24:1253-1258.

Ruthven, A. G.. 1908. Variations and Genetic Relationships of the Garter-snakes. Washington, U.S.A.: Government Printing Office.

Salgio P. and S. Trijasse. 1998. Behavioral responses to atrazine and diuron in goldfish. Environmental Contamination and Toxicology 35:484-491.

Shabana, E. F. 1987. Use of batch assays to assess the toxicity of atrazine to some selected cyanobacteria. I. Influence of atrazine on the growth, pigmentation and carbohydrate contents of *Aulosira fertilissima, Anabaena oryzaw, Nostoc muscorum* and *Tolypothyrix tenius*. Journal of Basic Microbiology 2:113-119.

Shimabukuro, R. H. 1967. Atrazine metabolism and herbicidal selectivity. Plant Physiology 42: 1269-1276.

Sibley, D., C. Elphick, and J. B. Dunning, eds. *The Sibley Guide to Bird Life & Behavior*. New York: Alfred A. Knopf, 2001. Print.

Solomon, K. R., D. Baker, R. P. Richards, K. R. Dixon, S. J. Klaine, T. W. La Point, R. J. Kendall, C. P. Weisskopf, J. M. Giddings, J. P. Giesy, L. W. Hall, Jr., and W. M. Williams. 1996. Ecological risk assessment of atrazine in north American surface waters. Environmental Toxicology and Chemistry 15:31-76.

Sparling, D. W., G. Linder, and C. A. Bishop (Eds.). 2000. Ecotoxicology of amphibians and reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Stratton, G. W. 1984. Effects of the herbicide atrazine and its degradation products, alone and in combination, on phototrophic microorganisms. Archives of Environmental Contamination and Toxicology 13:35-42.

Steinberg, C.E. W., R. Lorenz, O. H. Spieser.1995. Effects of atrazine on swimming behavior of zebrafish, *Brachydanio rerio*. Water Research 29:981-985.

Storrs, S. and R. Semlitsch. 2008. Variation in somative and ovarian development: Predicting susceptibility of amphibians to estrogenic contaminants. General and Comparative Endocrinology 156: 524-530.

Syngenta. "Atrazine." *Syngenta US*. Syngenta, 2 Feb. 2010. Web. 13 Mar. 2011. <http://www.atrazine.com/AtraMain.aspx>.

Streit, B. and H. M. Peter. 1978. Long-term effects of atrazine to selected freshwater invertebrates. Archives of Hydrobiology Supplement 55:62-77.

Tischer, W. and H. Strotmann. Relationship between inhibitor binding by chloroplasts and inhibition of photosynthetic electron transport. Biochemical Biophysical Act 460:113-125.

United States Department of Agriculture (USDA). 1994. Atrazine: Environmental Characteristics and Economics of Management. Washingtong, D. C., USDA Economic Research Service.

United States Environmental Protection Agency. 1994. Atrazine, simazine and cyanazine; notice of initiation of special review. Federal Reg. 59: 60412-60443.

United States Environmental Protection Agency. Office of Pesticide Programs. 1999. *Guidelines for carcinogen risk assessment*. Web. May. 2011. <http://www.epa.gov/scipoly/sap/meetings/2000/june27/finalatrazine.pdf>

United States Environmental Protection Agency. Office of Pesticide Programs. *Interim Reregistration Eligibility Decision for Atrazine*. Jan. 2003. Web. 14 Mar.2011. <http://www.epa.gov/opp00001/reregistration/atrazine>

Victor-Costa, A. B., S. M. C. Bandeira, A. G. Oliveira, F. A. B. Mahecha, and C. A. Oliveira. 2010. Changes in testicular morphology and steroidogenesis in adult rats exposed to Atrazine. Reproductive Toxicology 29: 323-331.

Wauchope, R. D., T. M. Buttler, A. G. Hornsby, P. W. Augustijn-Beckers, and J. P. Burt. 1992. The SCS/ARS/CES pesticide properties database for environmental decision-making. Rev Environ Contam Toxicol. 123:1-155.

Wilhelms, K. W., K. F. Fitzpatrick, C. G. Scanes, and L. L. Anderson. 2006. In ovo exposure to a triazine herbicide: Effects of atrazine on circulating reproductive hormones in gonadal histology in young Japanese quail. Archives of Environmental Contamination and Toxicology 51: 117-122.

Winchester, P., J. Huskins, and J. Ying. 2009. Agrichemicals in surface water and birth defects in the United States. Acta Paediatrica 98:664-669.

Chapter 2

Effect of the Herbicide Atrazine on the Behavior of the Checkered Gartersnake (*Thamnophis marcianus)*

Abstract

Atrazine is one of the most commonly used herbicides in the United States and one of the most popular weed-killers worldwide, being utilized in over 80 countries. Despite the effectiveness of atrazine, there has been growing concern over the potential harmful effects this chemical may have on non-target species exposed to this chemical. Few studies, however, have been performed on the effects of this chemical on reptiles, in particular snakes. This study examined the effects of environmentally relevant concentrations of atrazine on the foraging, antipredator, thermoregulatory, and courtship behaviors of the checkered gartersnake (*Thamnophis marcianus*). Effects of atrazine appeared negligible for all foraging and thermoregulatory trials. There were effects found on tail-wagging and body flattening antipredator behaviors and courtship behaviors of individuals receiving different concentrations of atrazine. These effects, however, were few and did not follow any noticeable trends.

Introduction

Atrazine (2-chloro-4-ethylamino-6-amino-s-triazine) is one of the most commonly used herbicides in the United States and one of the most popular weed-killers

worldwide (Graymore et al., 2001; Hayes et al., 2002; Hayes et al., 2003; Kiely et al., 2004). This herbicide is utilized by over 80 countries for the treatment of a wide range on broadleaf and grassy weed species including corn, sorghum, sugar cane, and wheat (Graymore et al., 2001; Barr et al., 2007). Atrazine's success can be attributed to many factors including its low cost, high efficacy, and versatility in many agricultural systems.

Atrazine is highly water soluble which makes it most effective when applied to saturated soils (Flynn and Spellman, 2009; Graymore et al., 1999; Graymore et al., 2001). Although this allows atrazine to easily dissolve and disperse evenly throughout the field, it also makes this chemical highly prone to leaching, runoff, and infiltration of freshwater ecosystems (Gleason, 2006; Graymore et al., 2001; Wauchope et al., 1992). Consequently, organisms living in or near these contaminated ecosystems are susceptible to contact with this chemical, thus putting them at risk of atrazine contamination. There are many potential routes through which organisms can uptake atrazine. Some organisms may be exposed directly through ingestion of contaminated water, absorption through their skin, or less commonly, respiration. Atrazine contamination can also occur indirectly through ingestion of organisms at lower trophic levels that have been directly exposed to the chemical.

Recently, there has been growing concern over the potential harmful effects this chemical may have on non-target species (Goldman, 1999; Winchester et al., 2009). Although the exact metabolic pathway of atrazine in non-photosynthetic organisms is poorly understood, there have been many studies demonstrating the negative effects of this chemical on a variety of taxa (Goldman, 1999; Benotti et al., 2009; Forney and Davis, 1981; Victor-Costa et al., 2010; Hayes et al., 2002; De Solla et al., 2006; Dewey,

1986; Flynn and Spellman, 2009; Ottinger et al., 2008; Biradar and Rayburn, 1995). In particular, a large proportion of this research has focused on two major aspects of these organisms' natural histories, behavior and reproduction.

Atrazine causes decreases in mussel aggregation behavior (Flynn and Spellman, 2009), increased burst and decreased grouping behavior in goldfish (Salgio and Trijasse, 1998), impaired male sexual behavior in Japanese quail (*Coturnix japonica*) (Ottinger et al., 2008), and inhibited nest escape behavior in map turtles, *Graptemys ouachitensis* and *Graptemys pseudogeographica* (Neuman-Lee and Janzen, 2005). Changes in behavior such as these often indicate that the nervous system has been impinged (Steinberg et al., 1995). Effects on reproduction include induction of hermaphrodism, demasculinization, and impairment of gonadal development in leopard frogs (*Rana pipiens*) (Hayes, 2003) and African clawed frogs (*Xenopus laevis*) (Hayes, 2002), decreased hatch rate and hatchling success in an aquatic pulmonate gastropod, *Ancyclus fluviatilis* (Streit and Peter, 1978), an aquatic midge (*Chironomus tentans*) (Dewey, 1986 and Macek et al., 1976), and map turtles (*Graptemys ouachitensis* and *Graptemys pseudogeographica*) (Neuman-Lee and Janzen, 2005), and decreases in testosterone in African clawed frogs (Hayes, 2002) and Wistar rats (*Rattus norvegicus*) (Victor-Costa et al., 2010). It is hypothesized that atrazine may be inducing aromatase activity, an enzyme responsible for the conversion of androgens (i.e., male sex hormones) into estrogens (i.e., female sex hormones), which may be responsible for these negative effects on male reproductive systems (Hayes et al., 2002).

An aspect of natural history that remains unexamined in response to atrazine is thermoregulatory behavior. Thermoregulation is an integral component of the physiology

of ectothermic organisms (Sparling et al., 2000; Lillywhite, 1987; Pough, 1998). It is necessary for maintenance of homeostasis and also plays major roles in many other elements of their life history including behavior and reproduction (Johnston and Bennett, 1996; Rogers et al., 2004). Thus, altering thermoregulation may have a cascading effect on other aspects of that organism's life. Because little research has been performed on the effects atrazine may have on thermoregulation, it remains an important subject of investigation.

Reptiles are model organisms for the study of behavior, reproduction, and thermoregulation, as each of these can easily be measured in some members of this taxa (e.g. Ford and Karges, 1987; Krohmer, 2004; Mori and Burghhart, 2004; Burghardt, 1969; Mori, 1991). Interestingly, there are very few toxicological studies utilizing reptiles, in particular snakes, compared to other groups (Sparling et al., 2000; Campbell and Campbell, 2009). This disproportion is surprising given that snakes hold pivotal roles in ecosystems as predators, occupy a wide range of niches and habitats, and have numerous features making them ideal ecological indicators of environmental contamination (Sparling et al., 2000; Rossman et al., 1996; Ernst and Ernst; 2003). For example, behavioral and physiological properties of snakes can easily be used as indicators of ecosystem function due to the rapid response of this group to environmental change (Beaupre and Douglas, 2009). Also, being predators that lead relatively sedentary lifestyles, snakes in particular are susceptible to bioaccumulation of contaminants (Beaupre and Douglas, 2009).

This study will utilize one such species, the checkered gartersnake (*Thamnophis marcianus*) to examine potential behavioral effects of atrazine. Gartersnakes are

considered fairly representative of snake physiology and behavior (Rossman et al., 1996) and are therefore employed in many studies of behavior (e.g. Mori, 1991; Burghardt , 1969, Perry-Richardson et al., 1990; Ford, 1981; Mori et al., 1996; Arnold, 1984). Members of the genus *Thamnophis* are one of the most common and widespread snakes of North America, making their overlap with atrazine usage inevitable (Rossman et al., 1996; Ruthven, 1908). The checkered gartersnake, in particular, is known to inhabit a wide range of habitats from arid to semi-aquatic but are rarely far from a water source, making this species even more likely to come into contact with contaminated systems (Rossman, et al. 1996; Ernst and Ernst, 2003). *Thamnophis marcianus* are known to feed on a variety of different prey, including tadpoles, frogs, fish, earthworms, and lizards (Rossman et al., 1996; Ernst and Ernst, 2003, many of which are known to be affected by atrazine contamination (Hayes et al., 2002; Hayes et al., 2003; Graymore et al., 2001; Macek et al., 1976). Ingestion, therefore, is most likely one of the major pathways of atrazine contamination for these snakes.

The objective of this work is to determine whether ingestion of environmentally relevant doses of atrazine affects foraging, antipredator, thermoregulatory, and/or reproductive behavior of checkered gartersnakes. These behaviors are vital components of the natural history of snakes and therefore, alterations to any of these traits could have detrimental effects on wild populations exposed to this chemical. Furthermore, this study will be one of the few experiments examining the influence of atrazine on reptiles and one of the first to examine its effects on snakes.

Methods

Animal Care and Handling

This study was conducted with 120 specimens of *Thamnophis marcianus* purchased from a commercial breeding facility. All individuals were maintained following IACUC protocol #2009-003 under Dr. John S. Placyk, Jr. Individuals were housed separately in size-appropriate translucent plastic storage boxes, each filled with 3-5cm of aspen bedding with water available *ad libitum*. Snakes were maintained on a 12:12 light:dark cycle at 27.5°C to simulate a photoperiod and temperature regime commonly experienced in their Texas range during their active season and when they reached adult size were hibernated at 14°C for 7 weeks to stimulate reproductive behavior (Rossman et al., 1996). Snakes were systematically divided by sex and litter into four groups, one control group and three treatment groups, each receiving different doses of atrazine, 1000µg/kg, 100µg/kg and 10µg/kg. These concentrations were chosen to reflect a range of environmentally relevant concentrations (Graymore et al., 2001; De Solla et al., 2006; Hayes et al., 2002). Dilutions were made from combining pure powdered atrazine with 95% ethyl alcohol. Once a month, weights were recorded for each individual. Mean weights were used to determine total volume of solution used for injections, which were adjusted as needed. Therefore, concentrations of atrazine remained constant while total volume of solution increased accordingly per unit snake body weight. The control group received the same total volume of solution as the other treatment groups; however, this volume consisted completely of 95% ethyl alcohol. Food consisted of size-appropriate frozen mice. Once a week, mice were injected with atrazine dilutions before being fed to corresponding treatment groups. Sheds and feeding success

were recorded after each feeding along with any noticeable abnormalities related to feeding.

Behavioral Testing

Behavioral testing focused on four main behaviors: 1) foraging, 2) antipredator, 3) thermoregulatory, and 4) reproductive. To avoid observer error and to increase interobserver reliability, all testing was performed under the supervision of Katie Chamberlain.

Foraging Behavior

Foraging behavior was examined via two different behavioral tests, one focusing on the use of chemical cues in identifying potential prey and the other allowing the use of both chemical and visual cues.

Foraging Behavior: Chemosensory Tests

Standardized chemical extract tests (Burghardt, 1969) were conducted 2-3 days after feeding to avoid variation in appetites. Trials took place within each individual's own container so as to provide as little disturbance as possible. Each individual was exposed to one of two cotton swabs randomly soaked in either distilled water for a negative control or a surface wash (Burghardt, 1969) stimulus of house mice (*Mus musculus)* for a period of 30 sec. After initial trials were completed for each individual, the alternate stimulus was then presented for the same amount of time. A period of at least 60 min occurred between each stimulus. Behavioral measures recorded include

number of tongue flicks and latency to attack. If a swab is bitten, the trial was ended and time of bite was recorded.

From the tongue-flick and latency to attack data, the tongue-flick attack score (TFAS) was calculated for each individual as per Cooper and Burghardt (1990). This measure incorporates both tongue-flicks and latency to attack into a single index. The equation used is as follows:

$$TFAS = TFmax(i) + (TL - latency_i)$$

TFmax(i) is the maximum of tongue-flicks emitted by individual i in any trial, TL is trial length in sec in the absence of an attack, and latencyi is the latency of attack by an individual i.

Repeated measures analyses of variance (ANOVA's) were performed to determine if there was a difference in TFAS within each treatment group between the three trial periods. This was done for both stimulus and control swab trials. If there was no significant difference, TFAS was averaged for the three trials. One-way ANOVA's were then performed to determine if there was a difference in TFAS for stimulus or control swabs between each treatment group.

Foraging Behavior: Prey Handling Tests

All prey handling tests also took place within each individual's own container. One frozen/thawed mouse was placed in each container and observations were made by viewing through the sides in order to decrease disturbance. Two variables of prey handling were recorded (modified from Mori, 1991): direction of prey at ingestion (head or tail first) and swallowing duration (from the moment the snake grasped the mouse until

it was no longer visible and the snake's mouth was able to close). If an individual failed to consume its prey within 45 min, the trial was ended and was attempted the following week for a maximum total of 3 consecutive weeks.

Direction of prey ingestion was analyzed using Chi-square tests due to low numbers of individuals ingesting prey during the allotted time. Separate chi-square tests were performed for each of the three trial periods to determine if there was a difference in the proportion of individuals consuming prey head or tail first between each treatment group.

Swallowing duration was analyzed ANOVA's. Repeated measures ANOVA's were first performed to determine if there was a significant difference in swallowing duration between the three times within each treatment groups. Swallowing duration for each individual was also averaged for the three trials and a one-way ANOVA was used to determine if there was a difference in swallowing duration between the treatment groups.

Antipredator Behavior

For standardized antipredator behavioral tests (modified by Mori et al., 1996), test arenas consisted of a 33 X 30 X 15 cm obtuse plastic container sterilized with Alconox, a concentrated clinical anionic detergent powder. To begin trials, snakes were left undisturbed for 30 sec, after which time the experimenter's finger was brought slowly within 2 cm of the snake's snout and left sedentary for 60 sec (non-moving stimulus session). If the snake crawled away during this time, it was followed, with the extended finger in front of the snake. The snake was then given an additional 30 sec undisturbed period. Afterwards, the snake was again followed by slowly extending a forefinger within

2 cm of the snake's snout. This time, however, the experimenter moved his/her finger back and forth at the rate of three to four oscillations per sec for another 60 sec duration (moving stimulus session). Lastly, after another 30 sec undisturbed period, the snake was gently tapped (head to tail) once every sec for this final 60 sec period (tapping stimulus session). During these trials, behavioral measures such as tongue-flicking, fleeing, striking, biting, tail-wagging, head-hiding, anal gland discharge (musking), defecation, urination, and body flattening were observed and recorded with the usage of counters for each time they occurred (Table 1). Number of tongue-flicks and number of flee attempts for the three stimuli (non-moving, moving, and tapping) were summed for each individual to represent one comprehensive value for each behavior. Due to low frequencies of occurrence, presence and absence of striking, biting, tail wagging, head hiding, musking, defecation, urination, and body flattening was pooled for the three stimuli.

ANOVA's were performed for analysis of tongue flicking and fleeing behavior. Initially, for each of these behaviors, repeated measures ANOVA's were used to determine if there was a difference in number of tongue-flicks or flees between the three trials within each treatment group. To determine if there was a difference in number of tongue-flicks between the four treatment groups, number of tongue-flicks in the three trials were averaged for each individual and a one-way ANOVA was performed. To determine if there was a difference in the number of flee attempts between the treatment groups, number of flees in the three trials were averaged for each individual and a one-way ANOVA was performed.

Chi-square tests were performed to analyze the remaining antipredator behaviors which had low frequencies of occurrence. These behaviors included striking, biting, tail wagging, head hiding, musking, defecation, urination, and body flattening. Chi-square tests were first used to determine if there was a difference in the number of individuals that displayed each behavior between the three trials. Behaviors that were significantly different between the trials were further analyzed using chi-square tests for each trial independently; these include tail-wagging, musking, urination, and flattening. If there was no difference between the three trials, presence and absence of each behavior (striking, biting, head hiding, and defecation) was pooled for each individual. A chi-square analysis was performed to determine if there was a difference in the presence/absence of each of these behaviors between the treatment groups.

Thermoregulatory Behavior

Thermoregulatory trials took place in a rectangular thermoregulation gradient with inside dimensions measuring 0.18 X 0.18 X 2.2 m. Trials in this gradient took place in an environmental chamber set at 14.7 °C. The temperature gradient was achieved using 6 heating pads lining the bottom of the chamber and set at gradual declining temperatures. The coldest side of the gradient had no heating element. One inch of sand was spread across the heating pads to make the temperature changes of the gradient more gradual and to add texture to aid in the movement of the snakes. Temperatures on the surface of the sand ranged from 14.7°C. to 42.7°C. The only lighting used in the chamber was from red lights in order to prevent disturbing the snakes.

Fourteen individuals (7 male and 7 female) from each treatment group were systematically selected to be used for thermoregulation trials. A cloacal thermocouple thermometer with a k-type wire probe was used to measure body temperatures. A bead of paraffin wax was placed at the end of the wire to prevent injury to the cloaca. A duct-tape patch, which aids in the adhesion of the probe, as described by Lutterschmidt and Lutterschimidt (2002), was secured to the thermocouple wire. The patch and wire were then attached using NexCare flexible first aid tape. For each trial, after the thermocouple wire was secured, each individual was placed in the center of the gradient. Individuals were kept in the gradient for an hour and temperatures were recorded every min. If an individual ejected the thermocouple wire, the wire was replaced and the trial started over.

Because gartersnakes are highly active and rarely rest at a stable temperature in thermoregulation gradients, average temperature for each individual throughout the one-hour period was calculated to determine individual temperature preference. A one-way ANOVA was performed to determine if there was a significant difference in temperature preference between the four treatment groups.

Temperature variance over the one hour trial period was also calculated for each individual. This was used to represent the range of body temperatures for each individual. A one-way ANOVA was used to determine if there was a difference in the variation of body temperatures between the treatment groups.

Reproductive Behavior

Monthly SVL measurements of adult checkered gartersnakes were used to calculate growth rates. Minimum SVL of female checkered gartersnakes at sexual

maturity range from 34.5 cm in southern Texas to 51.5 cm in southern Arizona (Rossman et al., 1996). Typically males of this species reach sexual maturity at shorter SVL's than females (Rossman et al., 1996) Individuals were given enough time to enable most females to reach 50 cm SVL. In December, 2010, all individuals were placed in a 14° C. dark hibernation chamber. Hibernation lasted a period of 7weeks. Reproductive behavior trials began 2 days post hibernation and continued every 3-4 days for a period of 3 weeks.

Reproductive behavior trials took place in clear 37.85 L aquaria with a light layer of aspen bedding on the bottom to avoid stress and to help the snakes move more easily. Females were randomly paired with males in their corresponding treatment groups and placed in aquaria together for 1 h. Courtship behaviors were recorded and given corresponding courtship scores (Table 2). The initial time of each courtship score, highest courtship score achieved, and if mating occurred, the duration of copulation, was also recorded. After a male achieved copulation with a female, the female was removed from further trials. Some males were repeated for trials in order to achieve the maximum number of copulations.

One-way ANOVA's were performed to determine if there was a difference in the highest courtship score reached between the treatment groups during each of the trial periods. Tukey-Kramer Multiple-Comparison post hoc tests were performed for each trial period with a significant difference. Chi-square tests were also performed for each of the trial periods to determine if there was a difference in the presence/absence of copulation at each time between each treatment group.

Further analyses were performed for only individuals that copulated. To determine if there was a difference in the number of days post-hibernation that copulation

occurred for each individual, a one-way ANOVA was performed. A one-way ANOVA was also performed to determine if there was a difference in the duration of copulation between each treatment group.

Additionally, individuals that copulated and did not skip any step in the courtship scores were analyzed using a Multivariate Analysis of Variance (MANOVA) and a Discriminant Function Analysis (DFA). The MANOVA was used to detect a difference between the treatment groups in the amount of time it took to reach each of the courtship scores 1-5. The DFA was used to determine the number of trials that the timing of the courtship scores did not match the predicted values within each treatment group. This was further represented by calculating the percent that were correctly classified into their corresponding treatment groups.

Results

Foraging Behavior

Foraging Behavior: Chemosensory Tests

Within each treatment group, there was no statistically significant difference in TFAS between the three trials for both the stimulus and control scented swabs. For both the prey stimulus (Table 3, Appendix A; $F_{3,114} = 2.40$, $P = 0.07$) and the control (Table 3,Appendix A; $F_{3,114}=0.48$, $P=0.70$), no significant difference in TFAS was found among the four experimental groups.

Foraging Behavior: Prey Handling Tests

For each of the three trial periods, there was no significant difference in the proportion of individuals consuming prey head or tail first between each treatment group (Table 4, Appendix B; Trial 1: χ_3^2 =3.46, P = 0.33; Trial 2: χ_3^2 = 0.28, P = 0.96; Trial 3: χ_3^2 = 4.15, P = 0.24). There was also no significant difference in swallowing duration between the three trial periods within each treatment group. This allowed for the swallowing durations to be summed for each individual. The summed swallowing duration was not significantly different among the treatment groups (Table 5, Appendix C; $F_{3,49}$ = 0.67, P = 0.57).

Antipredator Behavior

No difference was found in number of tongue-flicks within each treatment group among the trials. After the number of tongue-flicks was averaged for each individual, there no statistically significant difference found in the number of tongue-flicks among the treatment groups (Table 6, Appendix D; $F_{3,110}$ = 1.91, P = 0.13).

For fleeing behavior, there was no significant difference in flee attempts among the three trials. After the number of flee attempts for each individual was averaged, there was also not a statistically significant difference in the number of flees among the four treatment groups (Table 6, Appendix D; $F_{3,110}$ = 0.40, P = 0.75).

There was a statistically significant difference in four of the eight remaining behaviors among the three trial periods (Table 7). These include tail wagging, musking, urination, and flattening. The treatment group receiving 100µg/kg atrazine (χ_2^2 = 7.39, P = 0.02) and the control group (χ_2^2 = 9.97, P = 0.006) tail-wagged a significantly different

number of times among the three trials. The presence/absence of musk was significantly different over the three trials for treatment group receiving 100µg/kg atrazine (χ_2^2 = 10.61, P = 0.004). The presence/absence of urine was significantly different over the three trials for the treatment group receiving 1000µg/kg atrazine (χ_2^2 = 6.69, P = 0.03). The presence/absence of body flattening was significantly different across the three trials for all four treatment groups (1000µg/kg: χ_2^2 = 17.10, $P \leq 0.0001$; 100µg/kg: χ_2^2 = 5.47, P = 0.06; 10µg/kg: χ_2^2 = 9.87, $P \leq 0.01$; Control: χ_2^2 = 10.70, $P \leq 0.01$).

When analyzing each of these behaviors independently for each trial period, only two of the behaviors (tail-wagging and flattening) were significantly different among the experimental groups (Table 7). Each of these behaviors was also only significantly different between the treatment groups for one trial period. There was a statistically significant difference in the presence/absence of tail-wagging among the treatment groups for Trial 1 (Table 7, Figure 4; χ_3^2 = 9.68, P = 0.02). Specifically, the 1000µg/kg and the control group tail-wagged significantly more than the group receiving 10µg/kg. There was also a statistically significant difference in the presence/absence of flattening among the treatment groups for Trial 2 (Table 7, Figure 5; χ_3^2 = 7.16, P = 0.07). Specifically, the group receiving 10µg/kg atrazine flattened significantly more than the group receiving 100µg/kg and the control group.

Four antipredator behaviors (striking, biting, head-hiding, and defecating) were not significantly different between the three trials. Because there was no difference between the trials, each of these behaviors was pooled for each individual. There was no statistically significant difference in the presence/absence of any of these behaviors

among the four experimental groups (Table 8; Strike: $\chi_3^2 = 2.43$, $P = 0.48$; Bite: $\chi_3^2 = 0.66$, $P = 0.88$; Head-hiding: $\chi_3^2 = 0.39$, $P = 0.94$; Defecation: $\chi_3^2 = 0.12$, $P = 0.99$).

Thermoregulatory Behavior

There was no statistically significant difference in the average body temperature among the four experimental groups (Table 9, Appendix E; $F_{3,52} = 0.59$ $P = 0.06$). Temperature variation was also not significantly different among the treatment groups (Table 9, Appendix E; $F_{3,52} = 0.86$, $P = 0.47$).

Reproductive Behavior

During only one of the seven trial periods, 13 days post-hibernation, there was a statistically significant difference in the highest courtship scores achieved among the treatment groups (Table 10, Figure 6, $F_{3,41} = 4.18$, $P = 0.01$). Specifically, significantly more individuals in the group receiving 100µg/kg atrazine copulated than in the group receiving 10µg/kg atrazine (Tukey-Kramer CV = 3.79, df = 41). There was also a statistically significant difference in the presence/absence of copulations among the treatment groups at 13 days post-hibernation (Table 11, Figure 7; $\chi_3^2 = 9.75$, $P = 0.02$). None of the other trial periods had a significant difference in the presence/absence of copulations among the four experimental groups.

For individuals that copulated, there was not a statistically significant difference among the treatment groups in the number of days post hibernation that copulation occurred (Table 12, Appendix F; $F_{3,39} = 2.28$, $P = 0.09$). There was also not a statistically

significant difference in the duration of each copulation among the treatment groups (Table 13, Appendix F; $F_{3,41} = 1.14$, $P = 0.35$).

For individuals that copulated and did not skip any of the courtship scores, there was not a significant difference among the treatment groups in the timing of each courtship score 1-5 (Wilks' Lambda = 0.66, $F = 0.75$, $P = 0.73$, df = 3). Individuals from all four of the treatment groups were incorrectly classified into predicted groups based on the timing of each courtship score (Table 14). Classifications into predicted groups appeared to be random showing no trends. Percents correctly classified are as follows: 1000µg/kg = 28.6%, 100µg/kg = 42.9%, 10µg/kg = 55.6%, Control = 40.0%, Total = 42.4%.

Discussion

In general, the effects of atrazine on foraging, antipredator, thermoregulatory and courtship behavior appear to be negligible in the checkered gartersnake (*Thamnophis marcianus*). These results do not agree with results of behavioral trials on other taxa. Some differences, however, were found among experimental groups in some specific trials.

None of the foraging behaviors examined appeared to be affected at the dosages given. There was a difference among the experimental groups in two of the antipredator trials, though these differences did not follow any noticeable trends. In the first of the three antipredator trials, the group receiving 1000µg/kg and the control group tail-wagged significantly more than the group receiving 10µg/kg. In the second of the three antipredator trials, the group receiving 10µg/kg atrazine flattened significantly more than

the group receiving 100µg/kg and the control group. Presence/absence of tail-wagging and body flattening were the only two antipredator behaviors that were different among the experimental groups. The differences in both of these behaviors were only detected in one of the three trials. Furthermore, the trials in which they were detected were different for these two behaviors. Also, there was no consistent trend in which experimental groups differed. If atrazine is having minor effects on antipredator behavior, this supports the idea that atrazine is not affecting these behaviors in either a dose-dependent or hormetic fashion.

Two measures of courtship behavior were different among the experimental groups, highest courtship score and presence/absence of copulation. Both of these differences occurred at 13 days post-hibernation. Specifically, significantly more individuals in the group receiving 100µg/kg atrazine copulated than in the group receiving 10µg/kg atrazine. This is most likely attributed to over half of the group receiving 100µg/kg atrazine copulating this day. Also, the difference in highest courtship score achieved at this time most likely reflects the large percentage of the copulations in the group receiving 100µg/kg atrazine at 13 days post-hibernation. Because this difference between experimental groups only occurred one day and there was no difference in the number of days post-hibernation that courtship occurred between the groups, this can most likely be attributed to a random occurrence. The remaining measures of courtship behavior indicated no effect of atrazine.

Average body temperatures and variation in these temperatures were not different among the experimental groups; therefore thermoregulation in *Thamnophis marcianus* does not appear to be affected by environmentally relevant doses of atrazine. Individuals

neither displayed differences in temperature preferences nor variation in these temperatures. This indicates that even if metabolic enzymes are being differentially regulated, it may not be occurring at high enough levels in which these gartersnakes need to alter their thermoregulation behavior. Also, none of the foraging behaviors appeared to be affected at the dosages given.

Although there are many studies demonstrating that atrazine has detrimental effects on behavior, reproduction, physiology, and many more aspects of the life history of organisms (Goldman, 1999; Benotti et al., 2009; Forney and Davis, 1981; Victor-Costa et al., 2010; Hayes et al., 2002; De Solla et al., 2006; Dewey, 1986; Flynn and Spellman, 2009; Ottinger et al., 2008; Biradar and Rayburn, 1995), there have also been studies indicating that at environmentally relevant concentrations, this chemical has negligible effects (Lutz et al., 2009; Storrs and Semlitsch, 2008; LaFiandra et al., 2008; Jaeger, 1999). Because this is one of the first studies examining the effects of atrazine on reptiles, it is difficult to compare these results with that of similar research, in particular snakes.

Most of these studies on reptiles, however, indicate that atrazine effects life-history of these organisms. Map turtles (*Graptemys ouachitensis* and *Graptemys pseudogeographica*) exhibited decreased hatch rate and hatchling success (Neuman-Lee and Janzen, 2005). De Solla et al. (2006) also found changes in snapping turtle gonadal development in response to different concentrations of this herbicide. This research and many studies performed on behavior of other taxa, yielded results notably different than those in this study. Other studies on the effects of atrazine on behavior of other taxa have revealed changes in habitat preferences and swimming behavior of Zebrafish (Steinberg et al., 1995), reduced mobility and balance in Rainbow trout (Steinberg et al., 1995), and

decreased mussel aggregations (Flynn and Spellman, 2009). Because this study is one of the first to examine the effects of atrazine on snakes, a few hypotheses can be inferred: atrazine may have no effect on snakes in general, it may have negligible effects on only a few species of snakes including *Thamnophis marcianus*, or it may only have negligible effects on the behaviors examined in this study. In order to have a more complete understanding of the effects of atrazine on snakes, additional species need to be examined. In addition, exposure pathways and developmental exposure of this chemical should be examined.

The main goal of this study was to examine the effects of atrazine on the behavior of checkered gartersnakes; it did not, however, examine the pathway of atrazine through these snakes. To begin with, this study only examined exposure of atrazine through ingestion. In the wild, gartersnakes have the potential for multiple routes of atrazine exposure including ingestion of contaminated water or animals at lower trophic levels, respiration, and absorption through their skin. These other pathways should also be explored in order to better determine possible effects of this chemical in the environment. Also, examining levels of atrazine throughout the snakes' bodies would aid in determining the pathway of atrazine. There is a probable potential that only low levels of atrazine or no atrazine at all is actually being absorbed through their bodies. The majority of this chemical may simply be excreted, which is one possible explanation of the negligible effects detected. If this was true, we could determine that checkered gartersnakes are not susceptible to atrazine exposure through ingestion because their bodies are able to excrete the chemical.

Early exposure to atrazine seems to have the most significant effects on organisms (Storrs and Semlitsch, 2008; Wilhelms et al., 2006; Dewey, 1986; Macek et al., 1976; Graymore et al., 2001; Ottinger et al., 2008; Rohr and Palmer, 2005; Neuman-Lee and Janzen, 2005). The individuals used in this study began receiving doses of atrazine after birth. Atrazine therefore, may potentially have more detrimental effects on development of body systems during embryonic development. If development is hindered during these early stages, major effects could arise in these organisms later in life. This could affect any number of aspects of life history including those examined in this study. Therefore, studies of long-term exposure including during embryonic development and the examination of second and third generations, may result in more significant effects of atrazine.

It would also be beneficial to examine a variety of reptile species, in particular snakes to better understand the ecological implications of this chemical. Even closely related species have been shown to have drastic differences in tolerance to atrazine exposure (Shimabukuro, 1967; Solomon et al., 1996). The tolerance of a species to atrazine is dependent on that particular species' ability to metabolize, detoxify, and excrete the chemical (Solomon et al., 1996, Shimabukuro, 1967). Even if checkered gartersnakes appear to have negligible effects, similar organisms may have completely different susceptibility to this chemical.

If atrazine does not influence behavior or other life-history characteristics of snakes, major environmental implications could be made from this knowledge. This chemical would no longer be a concern for conservation of periled snake species. Also, if snakes are able to metabolize atrazine, higher-level organisms in food webs that eat

snakes (i.e., large raptors, carnivorous birds of prey, and carnivorous and scavenger mammal species) will not be contaminated through this ingestion pathway.

Further studies are needed, however, to determine how atrazine is potentially having negative effects, which organisms are at risk, and how these negative effects may affect ecosystem and community structures. If atrazine is causing detrimental effects to humans or wildlife, the usage of this chemical may need to be modified. Application methods may be altered, or restrictions made on allowable concentrations and volumes of the chemical that can be applied. Alterations could be made to the structure of atrazine to make it more easily broken down in the environment or make it more selective toward target weed species.

References

Arnold, S. J. 1984. Behavioural variations in natural populations. III: Antipredator displays in the garter snake *Thamnophis radix*. Animal Behaviour 32: 1108-1118.

Barr, D. B., P. Panuwet, J. V. Nguyen, S. Udunka, and L. L. Needham. 2007. Assessing exposure to atrazine and its metabolites using biomonitoring. Environmental Health Perspectives 115:1474-1477.

Beaupre, S. J. and L. Douglas. 2009. Snakes as Indicators and Monitors of Ecosystem Properties. In S. J. Mullin and R. A. Siegel (Eds.), *Snakes: Ecology and Conservation.* (pp 244-261). Ithaca, NY: Comstock Publishing Associates.

Benotti, M. J., R. A. Trenholm, B. J. Vanderford, and J. C. Holady. 2009. Pharmaceuticals and endocrine disrupting compounds in US drinking water. Environmental Science and Technology 43: 597-603.

Biradar, D. P. and A. L. Rayburn. 1995. Chromosomal damage induced by herbicide contamination at concentrations observed in public water supplies. Journal of Environmental Quality 24: 1222-1225.

Cooper, W. E. and G. M. Burghardt. 1990. A comparative analysis of scoring methods for chemical discrimination of prey by squamate reptiles. Journal of Chemical Ecology 16: 45-65.

Burghardt, G. M.. 1969. Comparative prey-attack studies in newborn snakes of the genus *Thamnophis*. Behaviour 33:77–114.

Campbell, K. R. and T. S. Campbell. 2009. A logical starting point for developing priorities for lizard and snake ecotoxicology: A review of available data. Environmental Toxicology and Chemistry 21: 894-898.

De Solla, S., P. Martin, K. Fernie, B. Park, and G. Mayne. 2006. Effects of environmentally relevant concentrations of atrazine on gonadal development in snapping turtles (*Chelydra serpentina*). Environmental Toxicology and Chemistry 25: 520-526.

Dewey, S. L. 1986. Effects of the herbicide atrazine on aquatic insect community structure and emergence. Ecology 67: 148-162.

Ernst, C. and E. Ernst. 2003. Snakes of the United States and Canada. Washington and London: Smithsonian Institution.

Flynn, K. and T. Spellman. 2009. Environmental levels of atrazine decrease spatial aggregation in the freshwater mussel, Elliptio complanata. Ecotoxicology and Environmental Safety 72:1228-1233.

Ford, N. B., 1981. Seasonality of pheromone trailing behavior in two species of garter snake, *Thamnophis* (Colubridae). The Southwestern Naturalist: 26: 385-388.

Ford, N. B., and J. P. Karges. 1987. Reproduction in the Checkered Garter Snake, *Thamnophis marcianus*, from Southern Texas and Northeastern Mexico: Seasonality and Evidence for multiple clutches. The Southwestern Naturalist 32: 93-101.

Forney, D. R. and D. E. Davis. 1981. Effects of low concentrations of herbicides on submersed aquatic plants. Weed Science 29:677-685.

Gleason, John. 2006. *Mobility of Herbicide Active Ingredients: Atrazine and 2, 4 D*. City of Austin. Web. 17 Apr 2011. <http://www.ci.austin.tx.us/growgreen/downloads/atrazine_mobility.pdf>.

Goldman, J., T. Stoker, and R. Cooper. 1999. Neuroendocrine and reproductive effects of contemporary-use pesticides. Toxicology and Industrial Health 15: 26-36.

Graymore M., G. Allinson, M. Allinson, F. Stagnitti, Y. Shibata, and M. Morita. 1999. Environmental fate of pesticides used in Australian viticulture: V. Behavior of atrazine in the soils of the South Australian Riverland. Toxicology and Environmental Chemistry 70:427-439.

Graymore, M., F. Stagnitti, and G. Allinson. 2001. Impacts of atrazine in aquatic ecosystems. Environment International 26:483-495.

Hayes, T. B., A. Collins, M. Lee, M. Mendoza, N. Noriega, A. Stuart, and A. Vonk. 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Ecology 99:5476-5480.

Hayes, T. B., K. Haston, M. Tsui, A. Hoang, C. Haeffele, and A. Vonk. 2003. Atrazine Induced hermaphroditism at 0.1 ppb in American Leopard Frogs (*Rana pipiens*): Laboratory and field evidence. Environmental Health Perspectives 111:568-575.

Jaeger, J., I. Carlson, and W. Porter. 1999. Endocrine, immune, and behavioral effects of aldicarb (carbamate), atrazine (triazine) and nitrate (fertilizer) mixtures at groundwater concentrations. Toxicology and Industrial Health 15:133-151.

Johnston, I. A. and A. F. Bennett (Eds.). 1996. Animals and Temperature: Phenotypic and Evolutionary Adaptation. Oxford University Press. Oxford.

Kiely, T., D. Donaldson, and A. Grube. 2004. Pesticide industry sales and usage: 2000 and 2001 market estimates. EPA-733-R-04-001. U.S. Environmental Protection Agency, Washington, D.C., USA.

Krohmer, R. W. 2004. The male red-sided garter snake (*Thamnophis sirtalis parietalis*): Reproductive pattern and behavior. Institute for Laboratory Animal Research Journal 45:65-74.

LaFiandra, E. M., K. J. Babbitt, and S. A. Sower. 2008. Effects of atrazine on anuran development are altered by the presence of a nonlethal predator. Journal of Toxicology and Environmental Health 71:505-511.

Lillywhite, H. B. "Temperature, Energetics, and Physiological Ecology." *Snakes: Ecology and Evolutionary Biology*. Ed. Richard A. Seigel, Joseph T. Collins, and Susan S. Novak. New York: McGraw-Hill, 1987. Print.

Lutterschmidt D. I. and W. Lutterschmidt. 2002. Herpetological Review 33(2):110-112.

Lutz, K. W., T. Springer, H. Krueger, L. Holden, and A. Hosmer. 2009. Does atrazine influence larval development and sexual differentiation in *Xenopus laevis*? Toxicological Sciences 107:376-384.

Macek, K. J., R. S. Buxton, S. Sauter, S. Ginilka, J. W. Dean. Chronic toxicity of atrazine to selected aquatic invertebrates and fishes Washington DC: Environmental Protection Agency, 1976 (Ecological Research Series 600/3-76-047).

Mori, A. 1991. Effects of prey size and type on prey-handling behavior in *Elaphe quadrivirgata.* Journal of Herpetology 25:160-166.

Mori, A., D. Layne, and G. M. Burghardt. 1996. Description and preliminary analysis of antipredator behavior of *Rhabdophis tigrinus tigrinus*, a colubrid snake with nuchal glands. Journal of Herpetology 16:94-107.

Mori, A. and G. M. Burghardt. 2004. Thermal effects on the antipredator behaviour of snakes: A review and proposed terminology. Herpetological Journal 14: 79-87.

Neuman-Lee, L. A. and F. Janzen. 2011. Atrazine exposure impacts behavior and survivorship of neonatal turtles. Herpetologica 67: 23-31.

Ottinger M. A., E. Lavoie, N. Thompson, A. Barton, K. Whitehouse, M. Barton, M. Abdelnabi, M. Quinn Jr., G. Panzica, and C. Viglietti-Panzica. 2008. Neuroendocrine and behavioral effects of embryonic exposure to endocrine disrupting chemicals in birds. Brain Research Review 57: 376-385.

Perry-Richardson, J. J., C. W. Schoefield, and N. B. Ford. 1990. Courtship of the Garter Snake, *Thamnophis marcianus*, with a Description of Female Behavior for Coitus Interruption. Journal of Herpetology 24:76-78.

Pough, F. H. 1980. The advantages of ectothermy for tetrapods. American Naturalist 115:92-112.

Rohr, J. R. and B. D. Palmer. 2005. Aquatic herbicide exposure increases salamander desiccation risk eight months later in a terrestrial environment. Environmental Toxicology and Chemistry 24:1253-1258.

Rossman, D., N. Ford, and R. Siegel. 1996. The Gartersnakes: Evolution and Ecology. Norman, OK: University of Oklahoma Press.

Ruthven, A. G.. 1908. Variations and Genetic Relationships of the Garter-snakes. Washington, U.S.A.: Government Printing Office.

Salgio P. and S. Trijasse. 1998. Behavioral responses to atrazine and diuron in goldfish. Environmental Contamination and Toxicology 35:484-491.

Shimabukuro, R. H. 1967. Atrazine metabolism and herbicidal selectivity. Plant Physiology 42: 1269-1276.

Solomon, K. R., D. Baker, R. P. Richards, K. R. Dixon, S. J. Klaine, T. W. La Point, R. J. Kendall, C. P. Weisskopf, J. M. Giddings, J. P. Giesy, L. W. Hall, Jr., and W. M. Williams. 1996. Ecological risk assessment of atrazine in north American surface waters. Environmental Toxicology and Chemistry 15:31-76.

Sparling, D. W., G. Linder, and C. A. Bishop (Eds.). 2000. Ecotoxicology of amphibians and reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Storrs S. and R. Semlitsch. 2008. Variation in somatic and ovarian development: Predicting susceptibility of amphibians to estrogenic contaminants. General and Comparative Endocrinology 156: 524-530.

Streit, B. and H. M. Peter. 1978. Long-term effects of atrazine to selected freshwater invertebrates. Archives of Hydrobiology Supplement 55:62-77.

Victor-Costa, A. B., S. M. C. Bandeira, A. G. Oliveira, F. A. B. Mahecha, and C. A. Oliveira. 2010. Changes in testicular morphology and steroidogenesis in adult rats exposed to Atrazine. Reproductive Toxicology 29: 323-331.

Wauchope, R. D., T. M. Buttler, A. G. Hornsby, P. W. Augustijn-Beckers, and J. P. Burt. 1992. The SCS/ARS/CES pesticide properties database for environmental decision-making. Rev Environ Contam Toxicol. 123:1-155.

Wilhelms, K. W., K. F. Fitzpatrick, C. G. Scanes, and L. L. Anderson. 2006. In ovo exposure to a triazine herbicide: Effects of atrazine on circulating reproductive hormones in gonadal histology in young Japanese quail. Archives of Environmental Contamination and Toxicology 51: 117-122.

Table 1. Antipredator behaviors exhibited by checkered gartersnakes, *Thamnophis marcianus,* during standardized antipredator testing (modified from Mori et al., 1996).

Antipredator Behavior	**Description**
Tongue-flicking	The snake exposes and retracts any portion of the tongue.
Flee	The snake rapidly crawls away from the stimulus object. The snake may move around inside the arena or attempt to climb up the walls of the arena.
Anal gland discharge (musking)	The snake expels products of the cloacal glands.
Defecation	The snake expels feces from the cloaca.
Urination	The snake expels uric acid from the cloaca.
Strike	The snake, with either closed or open mouth, rapidly orients the head toward the stimulus object or straightens the anterior body to strike the stimulus. Actual contact with the stimulus object may or may not occur, but the snake does not hold on to the stimulus with hits jaws.
Bite	The snake strikes the stimulus and holds on to it with its jaws.
Head-hiding	The snake hides its head under part of its body.
Tail-wagging	The snake slowly or rapidly vibrates its tail.
Flatten	The snake dorsoventrally flattens part of its body or its head.

Table 2. Graduated scale of male courtship in the checkered gartersnake (*Thamnophis marcianus*).

Courtship score	Behavioral description
0	Male either fails to investigate female or investigates female only briefly.
1	Male tongue-flicks and/or chin-rubs female after initial investigation.
2	
3	Male aligns with and covers the female's back while moving slowly with female; male cannot generally be distracted from courting.
4	Male presents all of the characteristics of 2 but also rapidly and repeatedly traverses the length of the female's body and/or contractile waves along the length of his body.
5	Male behaves as 3 but also attempts cloacal apposition by maneuvering his body and/or tail under female's body and/or tail.
	Male achieves intromission.

Modified from Krohmer, 2004. The male red-sided garter snake (*Thamnophis sirtalis parietalis*): Reproductive pattern and behavior. Institute for Laboratory Animal Research Journal 45:65-74.

Table 3. Mean TFAS ± 95% CIs in *Thamnophis marcianus* presented with prey stimulus and control swabs. TFAS was averaged for three trials. Individuals had been exposed to varying doses of atrazine.

Atrazine Treatment	**Mean TFAS Stimulus**	**Mean TFAS Control**
1000µg/kg	23.600 ± 2.836	22.088 ± 2.815
100µg/kg	20.643 ± 2.197	21.322 ± 3.121
10µg/kg	20.426 ± 2.937	20.690 ± 2.879
Control	18.265 ± 3.519	19.766 ± 2.918

∗ denote TFAS significantly different between the treatment groups ($P < 0.05$).

Table 4. Number of *Thamnophis marcianus* individuals that consumed prey head or tail first during 3 separate trials. Individuals had received varying doses of atrazine. "H" represents individuals that consumed prey head first and "T" represents individuals that consumed prey tail first.

	Direction of Prey Ingestion							
Atrazine Treatment	**1000 µg/kg**		**100 µg/kg**		**10 µg/kg**		**Control**	
	H	**T**	**H**	**T**	**H**	**T**	**H**	**T**
Trial 1	12	8	11	8	10	8	14	3
Trial 2	9	7	9	7	8	7	10	6
Trial 3	8	7	9	5	10	2	6	7

* denote treatment groups that are significantly different from each other ($P < 0.05$).

Table 5. Mean swallowing duration of prey in seconds (s) ± 95% CIs by *Thamnophis marcianus*. Swallowing duration is the average of three trials. Individuals had been exposed to varying doses of atrazine.

Atrazine Treatment	**Swallowing Duration (s)**
1000µg/kg	292.643 ± 67.535
100µg/kg	344.642 ± 81.341
10µg/kg	282.917 ± 80.633
Control	292.000 ± 72.022

∗ denote behaviors significantly different between the treatment groups ($P < 0.05$).

Table 6. Mean number of tongue-flicks and flees ± 95% CIs by *Thamnophis marcianus* receiving varying doses of atrazine. Total number of tongue-flicks for three stimuli (non-moving, moving, and tapping) was averaged for three trials.

Atrazine Treatment	Mean Tongue-flicks	Mean Flees
1000µg/kg	132.000 ± 13.050	50.467 ± 6.707
100µg/kg	111.821 ± 13.921	47.714 ± 6.636
10µg/kg	123.500 ± 9.785	46.536 ± 4.305
Control	114.929 ± 16.830	46.750 ± 5.531

∗ are located above treatment groups that are significantly different from each other ($P < 0.05$).

Table 7. Presence/absence of antipredator behaviors in *Thamnophis marcianus* receiving varying levels of atrazine treatment. Presence and absence was pooled for each individual exposed to three stimuli (non-moving, moving, and tapping). Data is from the first of three trials. "P" indicates presence of a behavior and "A" indicates absence of a behavior during a trial.

Presence and Absence of Antipredator Behaviors

Atrazine Treatment	**1000 µg/kg**		**100 µg/kg**		**10 µg/kg**		**Control**	
	P	**A**	**P**	**A**	**P**	**A**	**P**	**A**
Trial 1								
Tail-wagging *	28	2	25	5	19	10	27	3
Musking	17	13	21	9	16	13	19	11
Urination	9	21	13	17	10	19	13	17
Flatten	8	22	15	15	9	20	16	13
Trial 2								
Tail-wagging	25	5	20	9	18	10	23	6
Musking	15	15	8	21	9	19	11	18
Urination	14	16	10	19	12	16	10	19
Flatten *	20	10	12	17	19	9	12	17
Trial 3								
Tail-wagging	23	7	14	14	15	14	16	13
Musking	18	12	14	14	15	14	11	18
Urination	19	11	14	14	14	15	12	17
Flatten	23	7	20	8	19	10	24	5

∗ denote behaviors significantly different between the treatment groups ($P < 0.05$).

Table 8. Presence/absence of antipredator behaviors in *Thamnophis marcianus* receiving varying levels of atrazine treatment. Presence and absence was pooled for each individual exposed to three stimuli (non-moving, moving, and tapping) and for three separate trials. "P" indicates presence of a behavior and "A" indicates absence of a behavior during a trial.

Presence and Absence of Antipredator Behaviors

Atrazine Treatment	**1000 µg/kg**		**100 µg/kg**		**10 µg/kg**		**Control**	
	P	**A**	**P**	**A**	**P**	**A**	**P**	**A**
Striking	12	18	9	19	6	22	8	20
Biting	7	23	7	21	8	20	9	19
Head-hiding	18	12	19	9	18	10	18	10
Defecation	17	13	17	11	16	12	16	12

∗ denote behaviors significantly different between the treatment groups ($P < 0.05$).

Table 9. Mean body temperature (°C) and mean variance in body temperatures ± 95% CIs of *Thamnophis marcianus* individuals in a temperature gradient. Individuals were receiving varying doses of atrazine.

Atrazine Treatment	Mean Body Temperature (°C)	Mean Temperature Variance
1000µg/kg	13.300 ± 5.944	27.542 ± 1.890
100µg/kg	11.036 ± 2.927	28.229 ± 1.423
10µg/kg	8.329 ± 3.592	27.073 ± 1.860
Control	10.464 ± 5.823	26.431 ± 3.050

∗ are located above treatment groups that are significantly different from each other ($P < 0.05$).

Table 10. Mean highest courtship scores ± 95% CIs of *Thamnophis marcianus* receiving varying levels of atrazine treatment.

Days Post-hibernation	Mean Highest Courtship Score			
	1000 µg/kg	100 µg/kg	10 µg/kg	Control
2	2.818 ± 0.784	2.786 ± 0.791	2.429 ± 0.775	2.538 ± 0.469
6	2.800 ± 1.108	2.286 ± 0.766	2.571 ± 0.870	3.230 ± 1.022
9	3.200 ± 0.942	3.214 ± 0.882	2.714 ± 0.731	3.000 ± 1.041
13 *	3.375 ± 0.993	4.308 ± 0.624	2.643 ± 0.923	3.900 ± 0.787
16	2.750 ± 1.596	3.800 ± 0.555	3.091 ± 1.102	3.167 ± 1.808
20	3.333 ± 2.063	3.800 ± 1.360	3.111 ± 1.409	3.250 ± 2.718
23	3.250 ± 3.528	3.000 ± 2.250	3.667 ± 1.838	4.000 ± 4.302

∗ indicate number of days post-hibernation in which highest courtship scores were significantly different between treatment groups ($P < 0.05$).

Table 11. Presence and absence of copulations in *Thamnophis marcianus* receiving varying levels of atrazine treatment. "P" indicates presence of copulation and "A" indicates absence of copulation during a trial.

Days Post-hibernation	Atrazine Treatment							
	1000 µg/kg		100 µg/kg		10 µg/kg		Control	
	P	A	P	A	P	A	P	A
2	1	11	0	14	0	14	0	13
6	1	9	0	14	0	14	3	10
9	1	9	2	12	0	14	1	10
13 *	0	8	8	5	3	11	4	6
16	2	6	0	5	2	9	2	4
20	2	4	1	4	3	6	1	3
23	1	3	0	4	3	3	2	1

∗ represent number of days post-hibernation that presence/absence of copulations is significantly different between the treatment groups ($P < 0.05$).

Table 12. Mean number of days post-hibernation ± 95% CIs that copulation occurred in *Thamnophis marcianus* receiving varying doses of atrazine.

Atrazine Treatment	Mean Days Post-hibernation
1000µg/kg	14.000 ± 6.272
100µg/kg	12.909 ± 1.911
10µg/kg	18.181 ± 2.767
Control	13.615 ± 3.596

∗ are located above treatment groups that are significantly different from each other ($P < 0.05$).

Table 13. Mean duration of copulation (min) ± 95% CIs in *Thamnophis marcianus* receiving varying doses of atrazine.

Atrazine Treatment	Mean Copulation Duration (min)
1000µg/kg	6.195 ± 0.890
100µg/kg	4.939 ± 0.867
10µg/kg	5.549 ± 1.523
Control	5.248 ± 0.667

∗ are located next to treatment groups that are significantly different from each other ($P < 0.05$).

Table 14. Classification count table for Discriminant Function Analysis for timing of courtship scores 1-5 within atrazine treatment groups. Numbers in table represent number of individuals predicted to fall into each treatment group based on the timing each courtship score was achieved.

Timing of Courtship Scores Classification Count Table

	Predicted				
Actual	1000 µg/kg	100 µg/kg	10 µg/kg	Control	**% Correctly Classified**
1000µg/kg	2	1	3	1	**28.6**
100µg/kg	2	3	0	2	**42.8**
10µg/kg	2	0	5	2	**55.6**
Control	1	1	4	4	**40.0**
Total	7	5	12	9	**42.4**

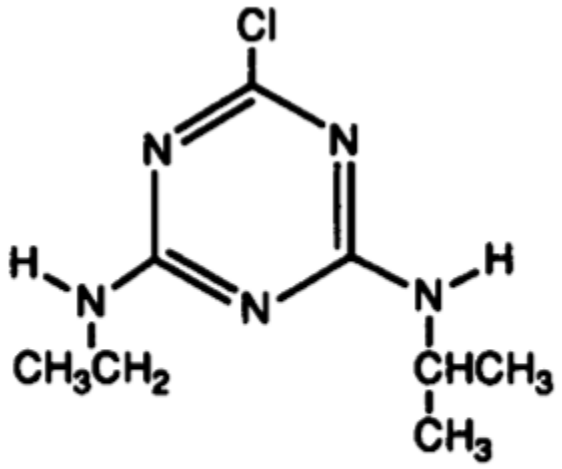


Figure 1. Chemical structure of atrazine (2-chloro-4-ethylamino-6-amino-s-triazine) modified from Eldridge et al. (1992)

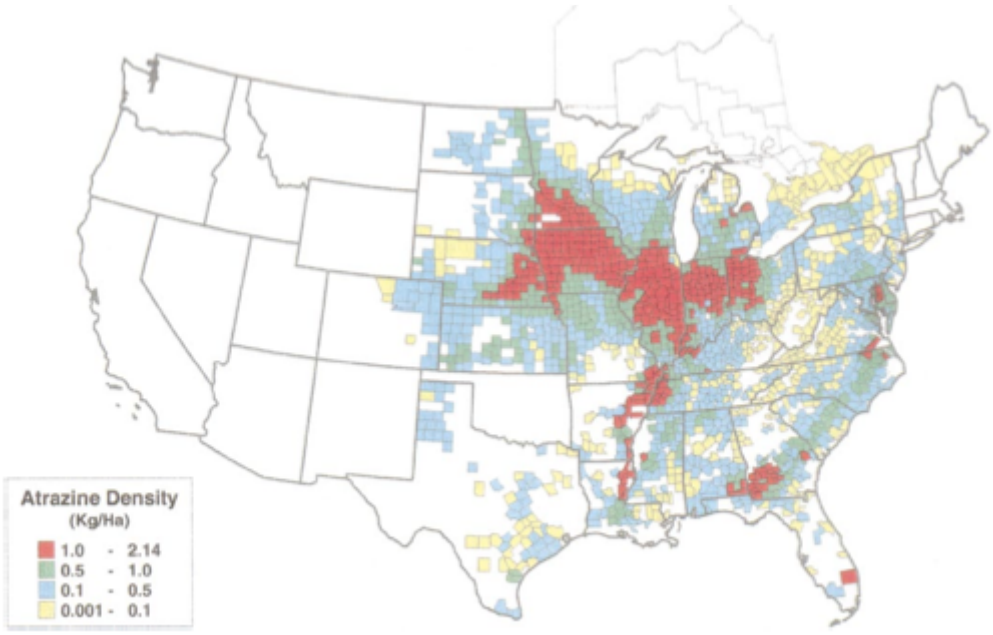


Figure 2. Map of the United States showing atrazine usage on corn per county, 1985-1988. Highest atrazine use occurs in the Midwestern states. From Solomon et al. (1995).

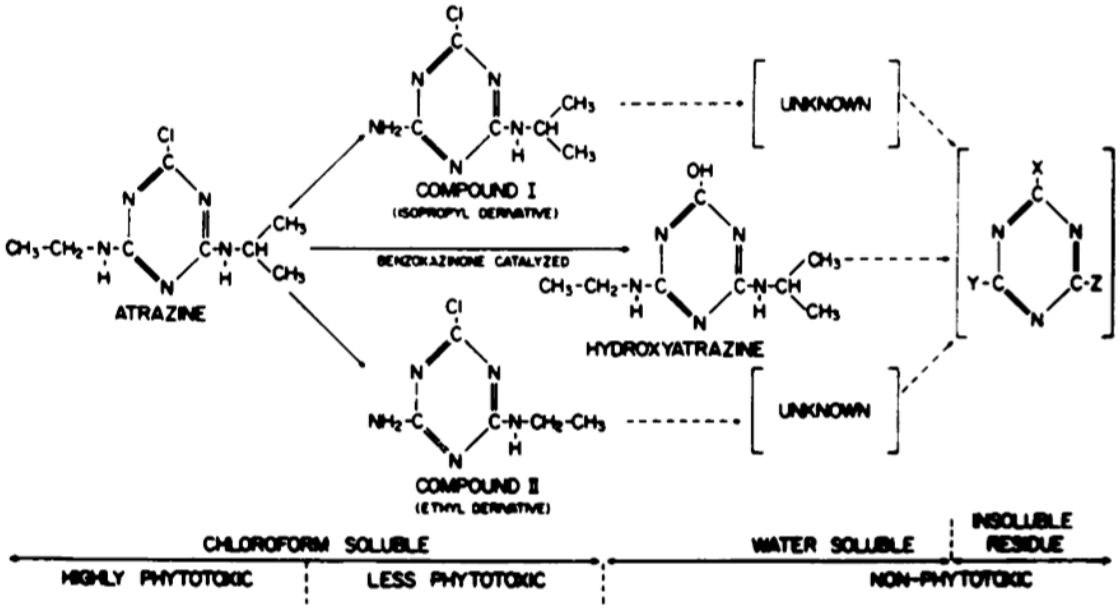


Figure 3. Dealkylation and hydroxylation pathways for atrazine detoxication in higher plants. Broken lines signify unknown portions of pathways. Several unknown intermediates are present before formation of insoluble residue. From Shimabukuro (1967).

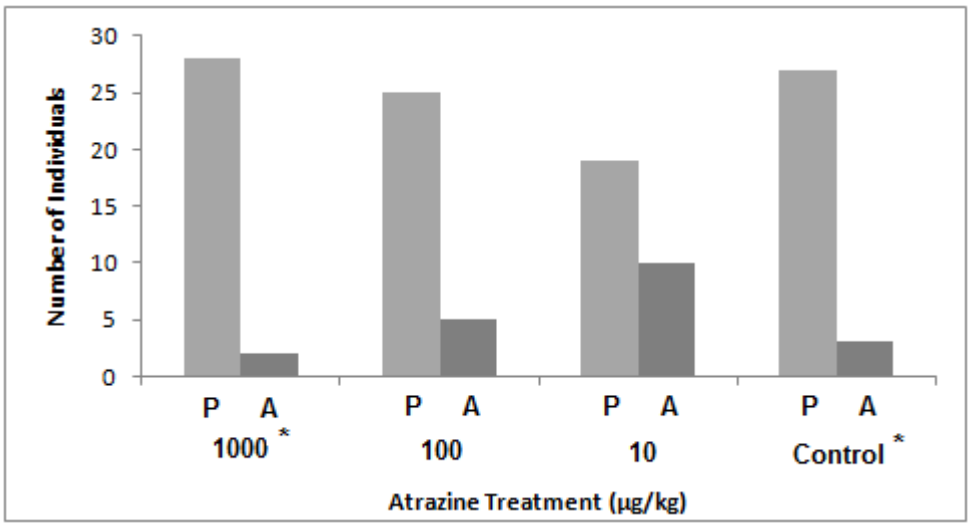


Figure 4. Presence/absence of tail-wagging antipredator behavior in *Thamnophis marcianus* receiving varying levels of atrazine treatment. Presence and absence was pooled for each individual exposed to three stimuli (non-moving, moving, and tapping). Data is from the first of three antipredator behavior trials. "P" indicates presence of tail-wagging and "A" indicates absence of tail wagging behavior during a trial. A significant difference in presence/absence of tail-wagging was found between the treatment groups (χ_3^2=9.68, P=0.0214). * represents a treatment group that was significantly different (P < 0.05) from the group receiving 10µg/kg atrazine.

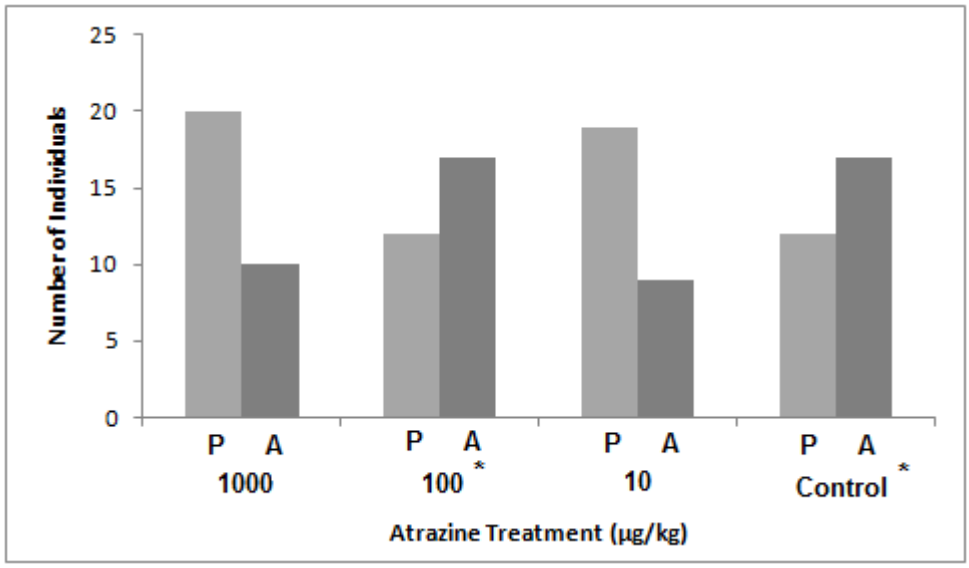


Figure 5. Presence/absence of body flattening antipredator behavior in *Thamnophis marcianus* receiving varying levels of atrazine treatment. Presence and absence was pooled for each individual exposed to three stimuli (non-moving, moving, and tapping). Data is from the first of three antipredator behavior trials. "P" indicates presence of body flattening and "A" indicates absence of body flattening behavior during a trial. A significant difference in presence/absence of body flattening was found between the treatment groups (χ_3^2=7.16, P=0.0669). * represents a treatment group that was significantly different (P < 0.05) from the group receiving 10µg/kg atrazine.

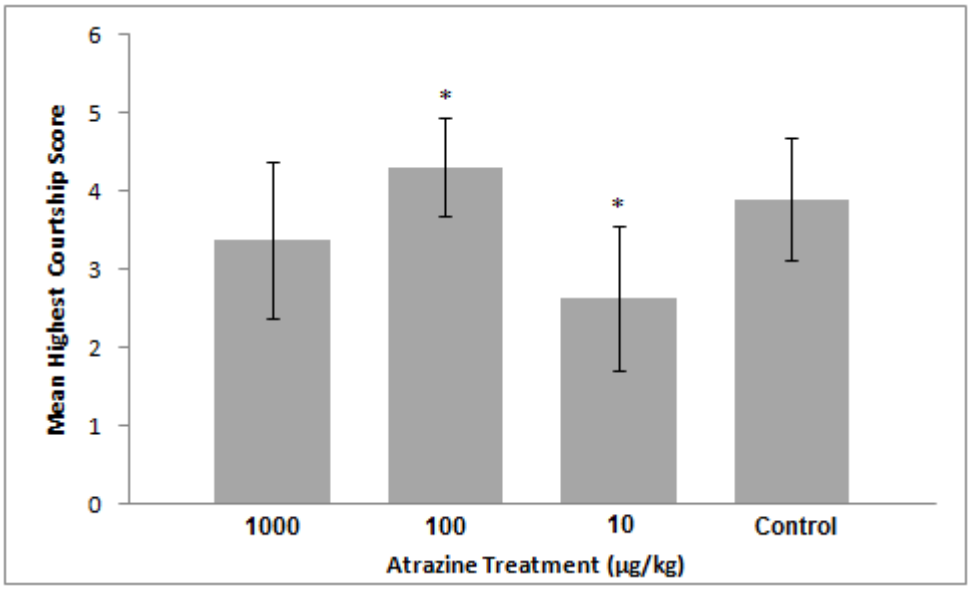


Figure 6. Mean highest courtship scores ± 95% CIs of *Thamnophis marcianus* at 13 days post-hibernation. Individuals were receiving varying levels of atrazine treatment. ∗ are located above treatment groups that are significantly different from each other ($P < 0.05$).

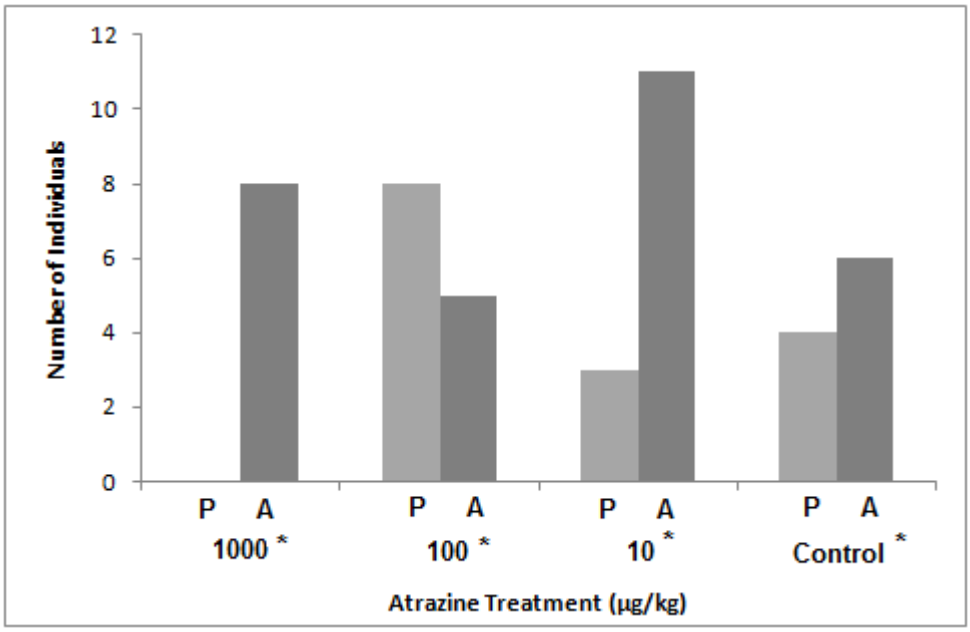
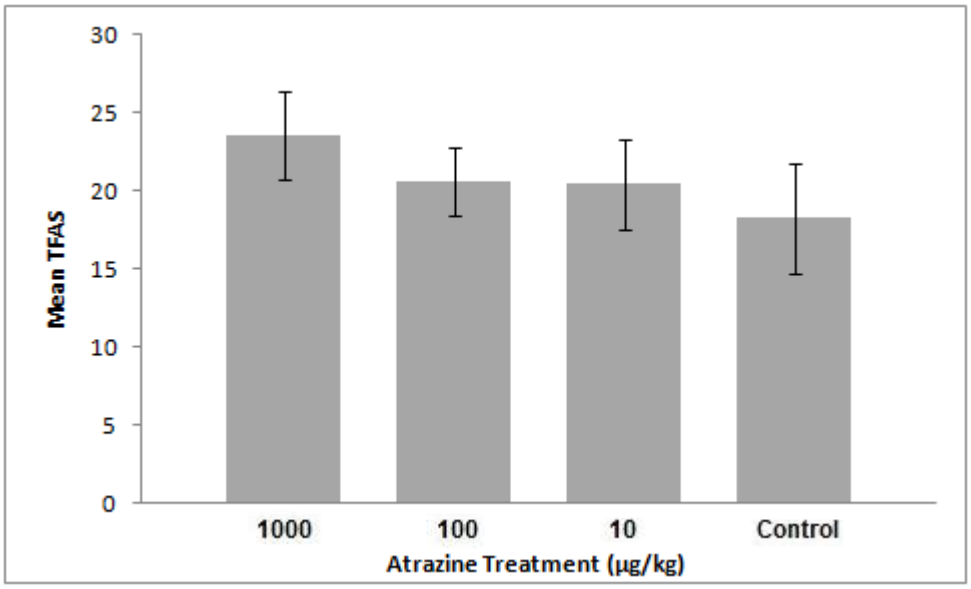


Figure 7. Presence and absence of copulations at 13 days post-hibernation in *Thamnophis marcianus*. Individuals were receiving varying levels of atrazine treatment. "P" indicates presence of copulation and "A" indicates absence of copulation during a trial event. A significant difference in presence/absence of copulations at 13 days post-hibernation was found between the treatment groups (χ^2_3= 9.75, P = 0.0208). * are located next to treatment groups that were significantly different (P < 0.05).

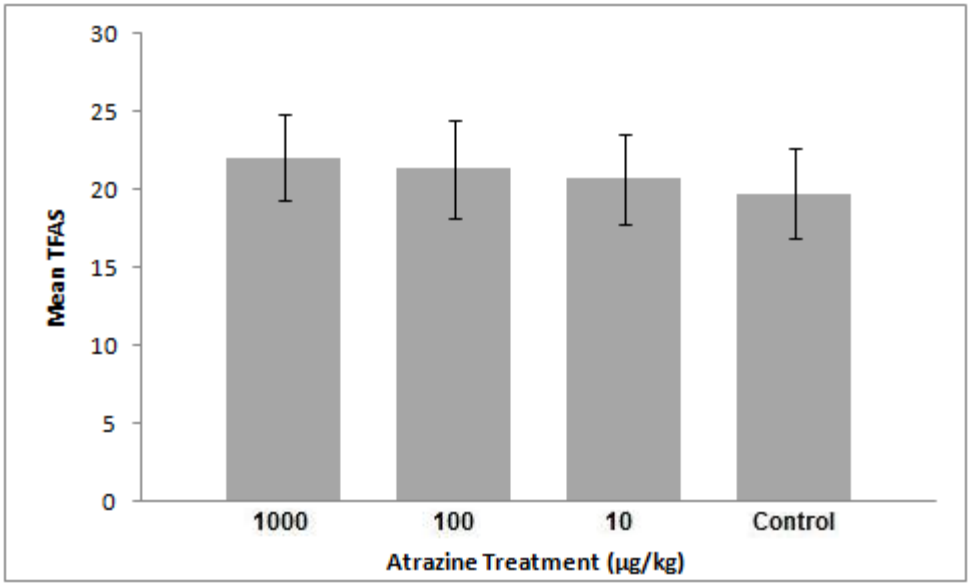
Appendices

Appendix A

(a)



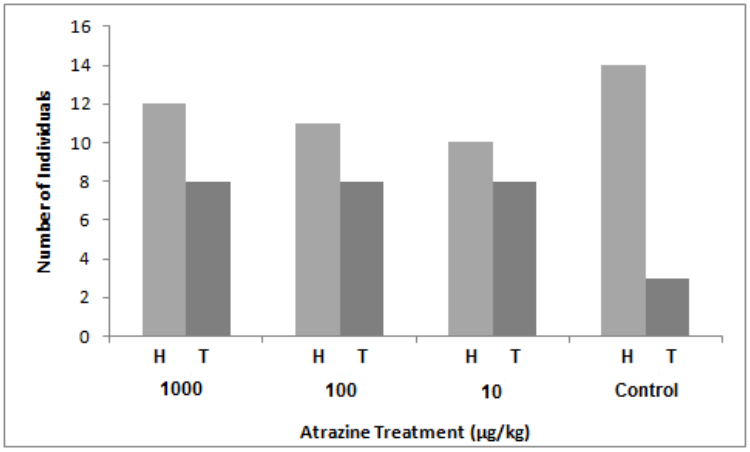
(b)



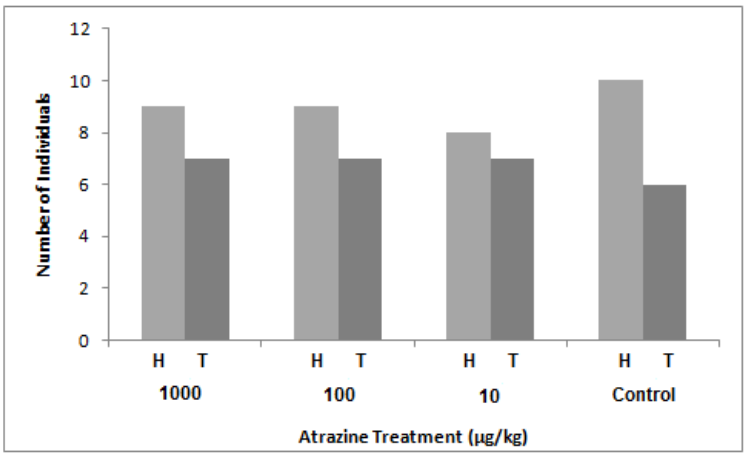
Mean TFAS ± 95% CIs in *Thamnophis marcianus* presented with (a) prey stimulus swabs and (b) control swabs. TFAS was averaged for three trials. Individuals had been exposed to varying doses of atrazine. ∗ denote TFAS significantly different between the treatment groups ($P < 0.05$).

Appendix B

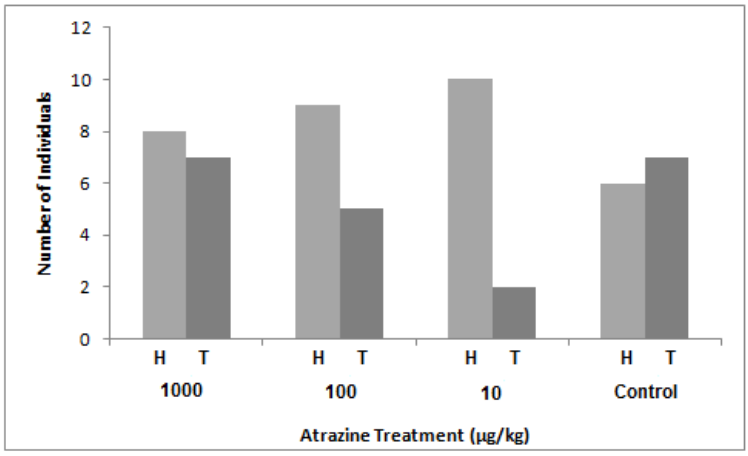
(a)



(b)

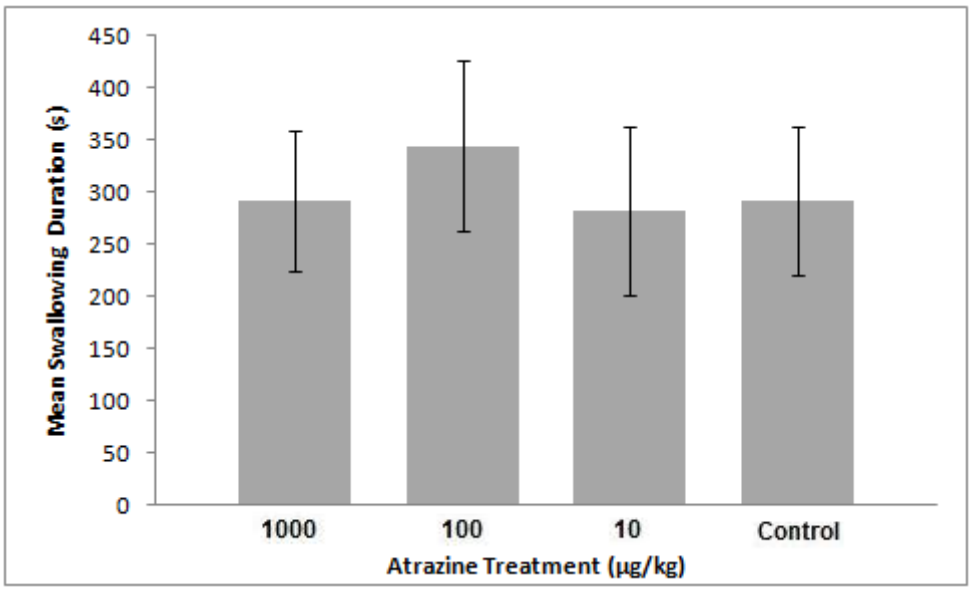


(c)



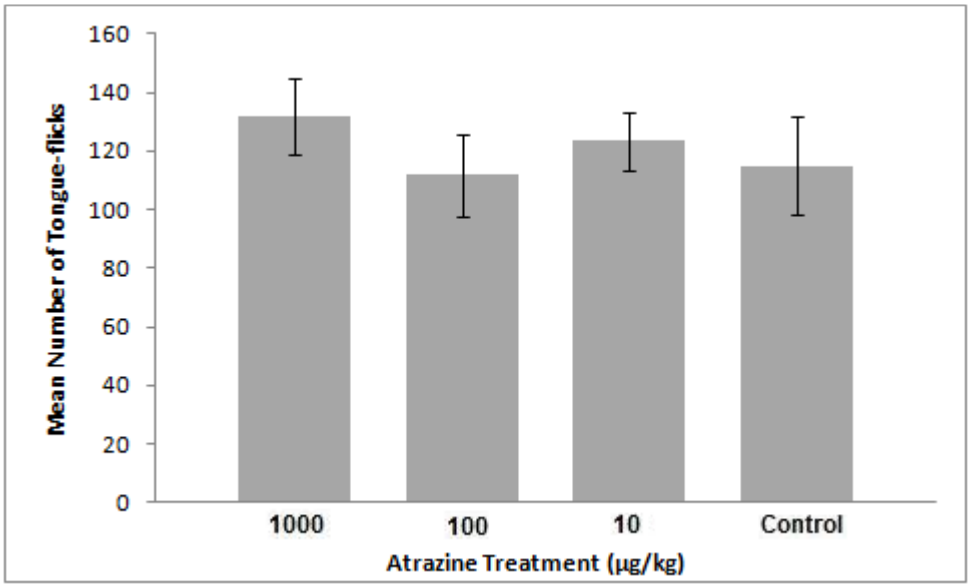
Number of *Thamnophis marcianus* individuals that consumed prey head or tail first during (a) trial 1, (b) trial 2, and (c) trial 3. Individuals had received varying doses of atrazine. "H" represents individuals that consumed prey head first and "T" represents individuals that consumed prey tail first.

Appendix C

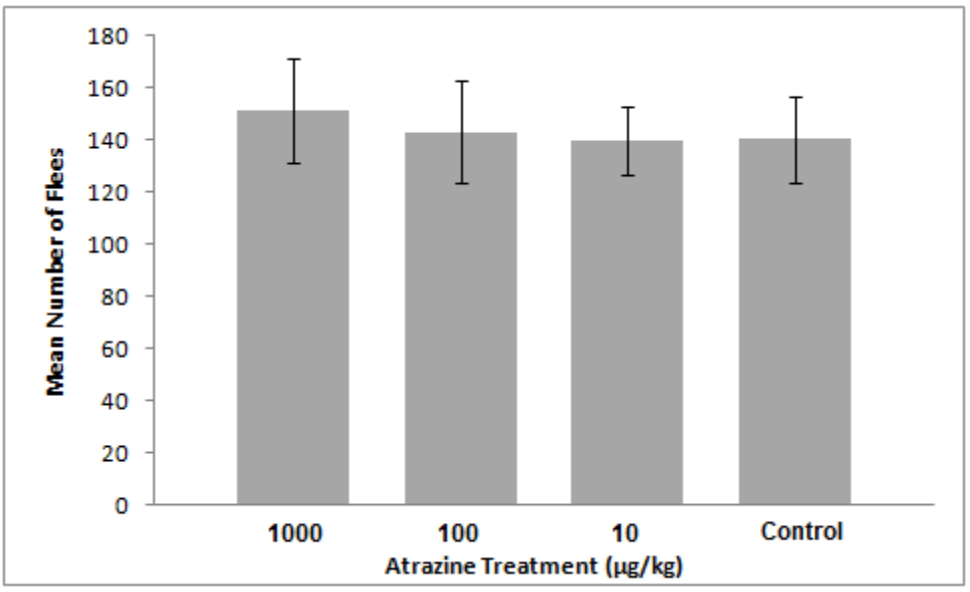


Mean swallowing duration of prey in seconds (S) ± 95% CIs by *Thamnophis marcianus*. Swallowing duration is the average of three trials. Individuals had been exposed to varying doses of atrazine. ∗ denote behaviors significantly different between the treatment groups ($P < 0.05$).

Appendix D



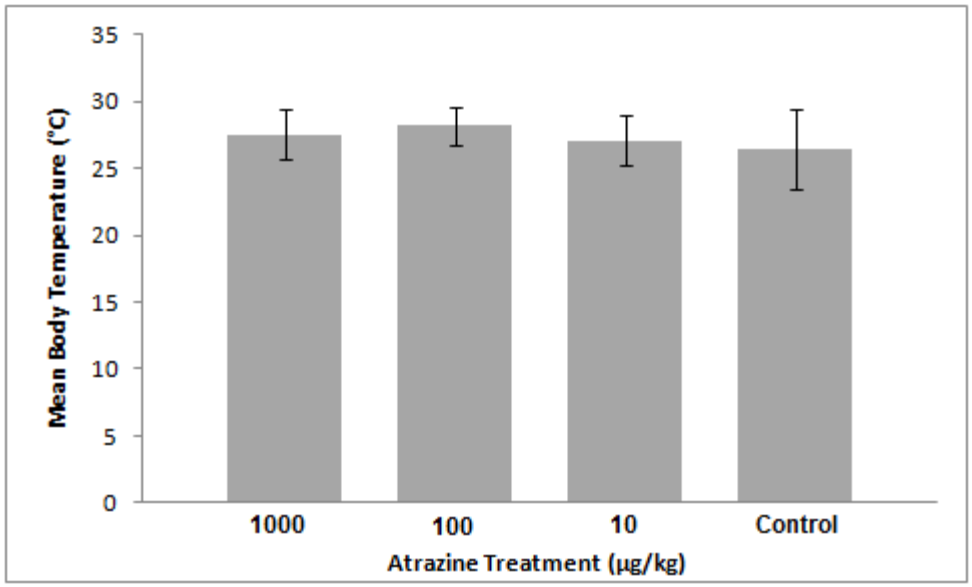
(a)



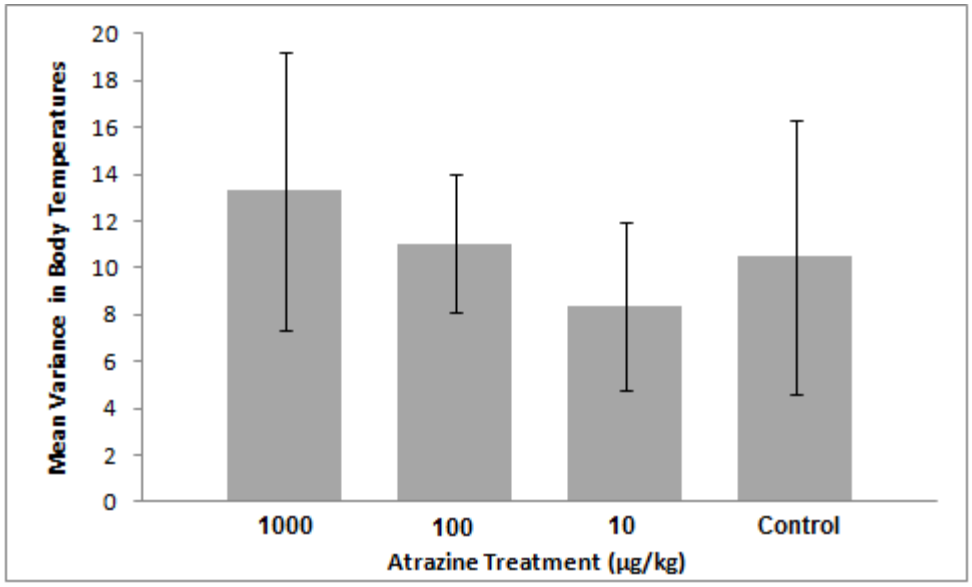
(b)

Mean number of (a) tongue-flicks ± 95% CIs and (b) flees ± 95% CIs by *Thamnophis marcianus* receiving varying doses of atrazine. Total number of tongue-flicks for three stimuli (non-moving, moving, and tapping) was averaged for three trials. ∗ are located above treatment groups that are significantly different from each other ($P < 0.05$).

Appendix E



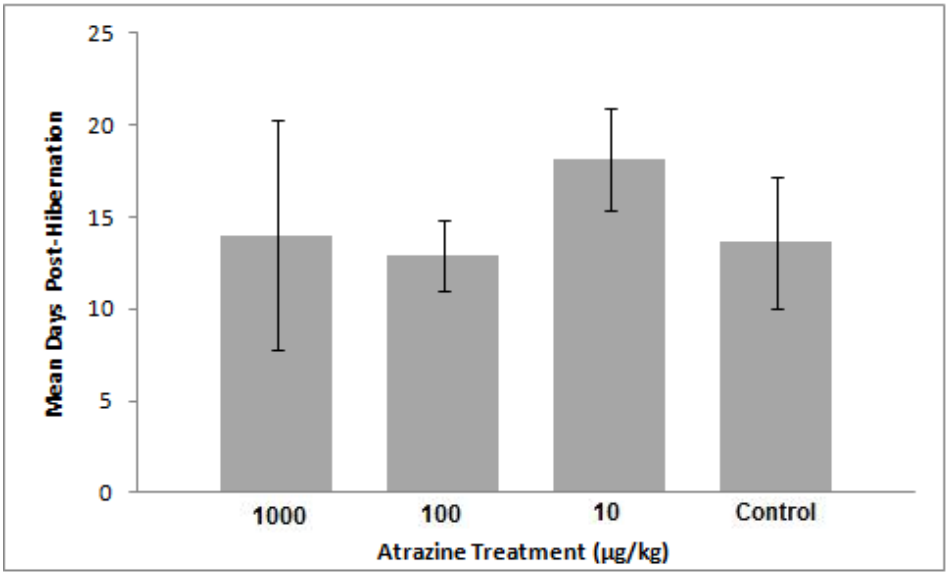
(a)



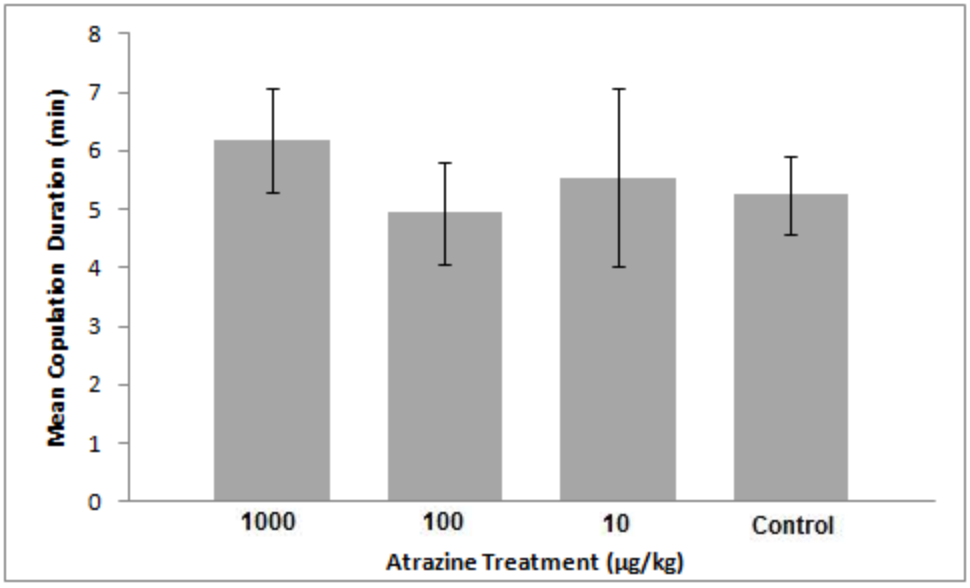
(b)

Mean (a) body temperature (°C) ± 95% CIs and (b) mean variance in body temperatures ± 95% CIs of *Thamnophis marcianus* individuals in a temperature gradient. Individuals were receiving varying doses of atrazine. ∗ are located above treatment groups that are significantly different from each other ($P < 0.05$).

Appendix F



(a)



(b)

Mean (a) number of days post-hibernation and (b) duration of copulation (min) ± 95% CIs that copulation occurred in *Thamnophis marcianus* receiving varying doses of atrazine. ∗ are located above treatment groups that are significantly different from each other ($P < 0.05$).