Consequences of Chemical Asian Longhorned Beetle Eradication

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An Interactive Qualifying Project Report

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By:

Gregory J Hutchinson

Nicholas Morassi

Da Teng

Yan Yan

Advisors:

David Spanagel
Assistant Professor of History, Worcester Polytechnic Institute

John MacDonald
Associate Professor of Chemistry, Worcester Polytechnic Institute

Sponsor:

John Tycz
Owner GoOrganic LLC

Abstract

This paper focuses on the use of chemical eradication as a response to Asian Longhorn Beetle infestation. Situated firmly in the historical context of the environmentalist movement, as well as the context of chemical pesticides, the project evaluates the use of the chemical pesticide Imidacloprid, used since the beetle's first appearance in Worcester, Massachusetts. Soil samples were collected from two sites and analyzed using gas chromatography for their current levels of Imidacloprid. The group found concentrations ranging on average from 0.00301 to 0.02480 milligrams of Imidacloprid per kilogram of soil at one site; the other site presented undetectable concentrations. According to current EPA standards for pesticide persistence, these results do not pose a threat to living organisms.

Acknowledgements

Marion H. Emmert
Assistant Professor, Chemistry & Biochemistry
Masters Albert – Ludwigs – Universitaet Freiburg, Germany
PhD Westfaelische Wilhelms – Universitaet Muenster, Germany
mhemmert@wpi.edu

John C. MacDonald
Associate Professor, Chemistry
B.A. Bowdoin College 1987
PhD University of Minnesota 1993
jcm@wpi.edu

Paula Moravek
Operations Manager, Chemistry & Biochemistry Department
Pmoravek@wpi.edu

Rhonda Santos
Legislative and Public Affairs
USDA Animal and Plant Health Inspection Service
Asian Longhorned Beetle (ALB) Eradication Program
Rhonda.J.Santos@aphis.usda.gov

David Spanagel
Assistant Professor, History
B.A. Oberlin College, 1982
MSEd, University of Rochester, 1984
PhD Harvard University, 1996
spanagel@wpi.edu

John Tycz
Owner of GoOrganic LLC
agreenthumb@sbcglobal.net

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1.0 Introduction

Since the discovery of the Asian Longhorned Beetle in Worcester, Massachusetts in 2008, over ten thousand trees have been cut down and removed from residential, commercial, industrial, and government properties alike. Hundreds of thousands of trees have been injected with pesticides in an attempt of federal, state, and local government to eradicate the little black beetle which has entire industries living in fear.

One of the greatest risks to an ecosystem is the introduction of a new, foreign species. Since ecosystems develop and stabilize naturally, the addition of new species has the potential to disrupt and unbalance the natural equilibrium. The transportation of these invading species can occur through both natural methods and human interaction, but with international travel and commerce increasing at incredibly fast rates, the later of these is becoming the major determining factor for species transportation.

The success of various agricultural and commercial industries relies on environmental stability. Moreover, society has developed expectations of the environment. While only a small percentage of all invasive species actually cause substantial harm, the few that do can be devastating.

Significant effort has recently been applied towards stopping the transportation of invasive species and increasing public knowledge of invasive species. These forms of preventative maintenance have proven to be the most effective and farthest reaching. Unfortunately, once an invasive species has established a foothold in a new territory, the proper procedures to contain and eradicate it are not as well established.

Historically, at least since the end of the Second World War, chemical applications have been considered essential components to any species eradication program. Pesticides,

fungicides, and herbicides have been used for decades to control the spread of unwanted species and have proved successful at the task they are designed to do. Unfortunately, other results have occurred as well.

The use of chemicals, specifically pesticides, has dominated much of post-World War II America. In many ways, chemical use has improved life standards through providing solutions to many day to day problems. On the other hand, many unknown side effects of chemicals have been discovered and many more are considered as possible. These effects on non-target organisms have raised the question to what point do the costs of these chemicals outweigh the benefits.

There is currently a growing concern and suspicion of chemical use and side effects. Many people have chosen to avoid powerful chemicals and utilize either safer or organic routes to accomplish the same goal. This is the mentality behind the Go Organic LLC landscaping business. Owner and project sponsor John Tycz offers healthy lawn and yard care through the use of 100% organic treatments. The idea is to create proper soil composition, resulting in improved lawn and yard health and increased natural resistance to common threats.

As safer alternatives to dangerous chemicals are being discovered and applied more often, the necessity of chemical use to control invasive species is being questioned more and more. On one hand, invasive species pose an immediate and significant threat to the agricultural and commercial economy, requiring fast and effective eradication, a job that chemical application can easily achieve. But on the other, the premature use of strong chemicals without proper testing can result in various side effects to non-target organisms. Where, then, is the line drawn between eliminating a pest and using potentially harmful

chemicals? At what point do costs override the benefits for chemical applications? In order to answer this, a deeper understanding of invasive species, chemical use, and history must be analyzed. With newspapers throughout Worcester County, Massachusetts reporting the devastation of eradication efforts and widespread clear cutting, concerned citizens carefully weigh the consequences of less aggressive chemical eradication methods but fear potential unknown long-term effects.

2.0 Background

2.1 The Advent of Environmentalism

For most of recorded history, and all of prehistory, humans lived in a symbiotic relationship with nature. People understood nature, and knew how to read her invisible signs. Humans developed crafts, rituals, and myths which imitated the Earth's and nature's ways [Tokar 1987, 9]. Nature was not seen as inferior, as it is today, but was seen as a big part of humanity, "a part of themselves" [Tokar 1987, 9]. Insect infestation, however, has always been a component of the challenges of agriculture, but with the advent of modern developments in chemistry and biology, it became possible to develop technologies that would lead humans to imagine they control nature rather than live in some kind of negotiated balance with it [Tokar 1987, 19]. According to Tokar, and others who share his sensibility, this exploitative arrogance has resulted in a human-dominated, or anthropocentric, view of the world that placed a diminishing value on biocentrism by endangering the environment, from vegetation, wildlife, and landscape, to the detriment of humanity.

2.2 A Shift in Balance from Biocentrism to Anthropocentrism

With the advent of the Industrial Revolution, nature began to become expendable as people cleared forests and landscapes to build huge manufacturing facilities which often allowed toxic waste products and garbage to spill into rivers and oceans [Reynolds 2006, 1]. However, as technology improved, circumstances often only got worse for nature. During World War II, scientists began work on chemical means to use as weapons against human enemies. Observing that these weapon-grade chemical compounds could be used on a domestic scale to fight everyday pests that were plaguing people's daily lives, soon led

to the development of synthetic pesticides, which were allegedly safer and more powerful than the previously used farmers' poisonous pesticides [Orfano 2012]. As is still true today, the synthetic pesticides developed during this time period were used for the control of any pests that humans determined to be invasive or intrusive to the detriment human welfare [EcoSmart 2008]. Of the synthetic pesticides developed at that time, one of the most dangerous and widely used was the insecticide Dichloro-Diphenyl-Trichloro-Ethane, or DDT. In 1873, DDT was developed by an Austrian student by the name of Othmar Zeidler, however, the capabilities of the chemical got little attention during that era [Medical Discoveries 2012]. It was not until 1940, after four years of vigorous work, that DDT was reproduced and patented by Paul Müller, only this time, Müller knew of the potent potential of the chemical properties. Some of DDT's uses were the eradication insects, like flies, lice, the Colorado beetle, and mosquitoes [Nobelprize.org 1948]. DDT was also used to combat "other insect-borne human diseases…and for insect control in crop and livestock production, institutions, homes, and gardens" [EPA 2012a]. Inexpensive to purchase, DDT use became very popular and widespread [Orfano 2012], however no one knew its hazardous effects on both nature and the human race. In 1962 a woman by the name of Rachel Carson wrote and published the book, *Silent Spring*, which enlightened the public to the startlingly facts of the hazards of insecticides.

2.3 Emerging Public Awareness of the Dangers of Synthetic Insecticides

In her landmark 1962 book, *Silent Spring*, expert wildlife biologist and popular science author Rachel Carson painted a picture of America's dangerous dependence on insecticides, like DDT, with the goal of awakening the general public to the hazards of

synthetic insecticides. Carson divided the various arrays of synthetic insecticides into two major carbon-based chemical groups, known as chlorinated hydrocarbons and organic phosphates [Carson 1962, 18]. Within the chlorinated hydrocarbons, hydrocarbons can be easily altered to form different substances, which could result in deadlier chemicals being formed, like DDT or heptachlor [Carson 1962, 20]. Most, if not all, chlorinated hydrocarbons can be stored in the body, more specifically in organs which contain a lot of fatty substances [Carson 1962, 21]. Organic phosphates, however, are even more poisonous than the afore-mentioned chlorinated hydrocarbons [Carson 1962, 27]. Organic phosphates, like nerve gas, were developed for use in chemical warfare on humans in the late 1930's by German chemist, Gerhard Schrader. Additionally, organic phosphates were used as insecticides aimed at the nervous systems of "insects or warm-blooded animals" [Carson 1962, 28]. Organic phosphates destroy the enzyme cholinesterase in the nervous system. Cholinesterase counteracts acetylcholine, a transmitting chemical necessary to pass impulses across nerves in the nervous system. Without the enzyme cholinesterase present, acetylcholine will build up in the body, causing uncontrollable muscular failure leading to death [Carson 1962, 28-29]. An example of an organic phosphate is parathion, or ethyl-parathion, which was very widely used in the early and mid-twentieth century [Carson 1962, 29]. Deathly toxic and paralytic, Carson described how two Florida children quickly died from parathion poisoning after using an empty bag which once contained parathion to fix a broken swing, and how two other children died in Wisconsin from parathion poisoning, one by touching the sprayer, the other from breathing the spray that drifted in from a nearby field [Carson 1962, 28].

Equally important, Carson wrote about the harm insecticides caused to the soil by leaching into the ground in treated areas, as the toxins entered the soil through either direct contact as a result of insecticide distribution, or through rain water and irrigation. As the synthetic insecticide penetrated the soil, the insecticide's poison targeted the pest earmarked for eradication and killed the species. Unfortunately, these synthetic insecticides were not species-specific, and in turn killed off other essential insects in the soil needed to help plants get life-giving nutrients, like the earthworm. Additionally detrimental to the plants these insecticides were supposedly protecting, synthetic insecticides could easily travel the pathways created by the burrowing earthworms for the facilitation of the plants' absorption of nutrients, and get absorbed along with the nutrients into the plants' root systems, causing both toxicity and genetic damage [Carson 1962, 56]. In some plants, like beans or wheat, insecticides could hinder root development or seedling growth [Carson 1962 60]. While most people tried replanting their dying or dead plants, the fact of the matter was the insecticides was still contaminating the soil, since various synthetic insecticides' toxins had large half-life measurements in years, translating to decades before decontamination would be achieved.

One example of a soil-contaminating synthetic insecticide of the chlorinated hydrocarbon variety would be heptachlor, which caused the afore-mentioned hindrance of root development or seedling growth. Heptachlor has a half-life of nine years, scientifically meaning it takes nine years for half of the chemical to breakdown [Carson 1962, 58]. In the bigger picture, this means the amount of time it would take heptachlor to completely degrade from the soil would be sequentially: nine years for fifty percent; nine years for twenty-five percent; nine years for twelve and a half percent, and so on, continuing in the

same pattern until the heptachlor is ridden from the soil, so the plants would be able to grow in uncontaminated soil. And this would be after only one application of the pesticide. To understand the longevity of soil contamination by a synthetic pesticide, if one application of heptachlor occurred in 1952, it would take 90 years for the chemical to degrade to about 0.1%, meaning it would still be present in the soil of a treated area until 2042. Any subsequent applications would make the process of soil decontamination exponentially longer.

DDT, another example of a chlorinated hydrocarbon synthetic insecticide, has a half-life range of about 2-15 years in soil, meaning DDT's degradation could take even longer [Pesticide Management Education Program 2008] than that of heptachlor. For comparison, an example of the organic phosphate, parathion, has a half-life ranging from six weeks to sixteen years [American Bird Conservancy 2010].

Beyond the specific hazards that might pertain to any given chemical, Carson used her extensive knowledge of wildlife ecology to call serious attention to more complex interactions and negative consequences that no purveyors of industrial pesticide products would necessarily have known about or anticipated in the 1950s when these products first began to be used on a massive scale. Examples of these previously poorly understood effects included persistence and migration of harmful chemicals up through the food chain, and evolutionary development of resistance to certain chemicals by targeted pest species populations. Because insecticides remain in the soil for numerous years, the plants grown in these treated areas absorb the insecticide, contaminating the produce and food products people ingested, even if those crops were not sprayed with an insecticide, resulting in soil

that would need to be tested before future planting to establish toxicity levels [Carson 1962, 59].

Carson also noted that once the targeted insect was eradicated, often another even more harmful insect or organism thrived in the absence of the original pest both on land and within the soil. Often the originally-deemed pest insect was actually a predator to another insect far worse a threat for the crops [Carson 1962, 57]. With the eradication of the targeted species due to the synthetic insecticide application, those insects on which the eradicated species fed were free to run rampant without threat of a natural predator, and proliferated uncontrolled. Increases in soil organisms to harmful levels after insecticide application in which a natural predator was destroyed caused what was once considered prey to become a pest.

Carson's argument really excited public concern because she connected all of these ecosystem consequences directly to matters of mammal health. One of the most important to note was the damage synthetic insecticides caused to the oxidative cycle in mammals. Some insecticides are inhibitors of enzymes in the mitochondrion of human cells used for oxidation. The oxidative cycle is important for producing ATP, or energy, for the cells. When a substance inhibits an enzyme, specifically coenzyme Q, the cycle comes to a halt, due to the fact that the enzymes cannot transfer electrons from one complex to another. Without the electron transfer, the complexes in the membrane of the mitochondria cannot release protons necessary for helping the ATP synthase enzyme to produce ATP. Worse still, if this happens, this inhibition will rob cells of oxygen, making the cells unable to use oxygen [Carson 1962, 202-204,]. This cell damage and lack of oxygen usage within cells can cause cells to become cancerous, and developing embryos to have abnormalities and

congenital defects [Carson 1962, 204]. Oxygen is the final piece needed to help hydrogen molecules form into water to complete the "biological circuit" within the inner mitochondrial membrane. But, with an insecticide inhibiting the coenzyme Q, preventing the transfer of electrons between two complexes, the complexes cannot undergo conformational changes, resulting in the complexes not being pushed into the inter-membrane space. Because of the damage caused by the insecticide to the cell, the hydrogen protons cannot go through the ATP synthase, so therefore cannot produce energy. With the lack of ATP, there are no spent hydrogen protons to combine with oxygen to form water.

Shortly after *Silent Spring* was published, some readers had praised Carson on her findings, and bringing to light the consequences of using pesticides and insecticides. However, other readers, like chemical industries, whose income was based on the products Carson referred to in her book, vilified Carson for the damage soon to be done to these industries. These chemical industries tried to use the media to criticize Carson's book in order to have the book banned, so as to keep their pockets lined, and have people still buy the poisonous chemicals. However, numerous scientists stood by her side and backed up Carson's data, validating her work. The United States President at the time, John F. Kennedy, ordered an investigation to see if Carson's claims and were correct. Silent Spring findings prevailed in the court of public opinion, partly as a result of scientific investigation and corroboration of many of Carson's claims [Reynolds 2006, 2]. The pesticide DDT was later banned in the United States in 1972 [Pesticide Management Education Program 2008], with the effects of other chemical pesticides additionally examined to see the damage they caused to both humans and the environment [Reynolds 2006, 2].

2.4 The Birth of Environmental Protection Agency

Rachel Carson's *Silent Spring* awakened the public to the damaging effects of synthetic insecticides on the environment, and the risks to both humans and wildlife. This call to action and accountability started the modern environmentalist movement [Reynolds 2006, 2-3]. In 1969, the Nixon Administration began a "Cabinet-level Environmental Quality Council" [Lewis 1985] and a "Citizen's Advisory Committee on Environmental Quality" [Lewis 1985], however, critics viewed these committees as having little power. By the end of 1969, Congress sent the National Environmental Policy Act, or NEPA, to President Nixon, which outlined promoting peace between man and nature, promoting efforts to protect the environment and well-being of humans, and enriching public knowledge about important ecological issues plaguing nature and the country's natural resources. The bill also wanted the formation of a Council on Environmental Quality, or CEQ, to advise the President on ecological matters. On January 1, 1970, President Nixon signed the National Environmental Policy Act into law [Lewis 1985].

The signing of NEPA promoted the public to be more active about environmental protection. President Nixon encouraged environmental protection even more when he declared on January 22, 1970, in his speech to both congressional houses, "the 1970s a historic period when, by conscious choice, [we] transform our land into what we want it to become" [Lewis 1985]. As the United States' celebrated the first "Earth Day" on April 22, 1970, public concern and awareness rallied around the importance of environmental issues. The success of Earth Day gave greater impetus to a report submitted a week prior to Earth Day by Roy L. Ash, from the President's Commission on Executive Reorganization, to President Nixon, arguing for a new agency to organize the Administration's new

ecological proposals. On July 9, 1970, President Nixon sent Congress "Reorganization Plan No. 3" [Lewis 1985], which called for a new agency that would help monitor and control environmental issues like pollution by setting standards, providing research, and creating new policies to protect the environment [Lewis 1985].

This agency, soon to be known as the Environmental Protection Agency, or EPA, would take, from other departments, like the Department of the Interior and the Department of Agriculture, components of different programs, like part of the pesticide research department and pesticide registration respectively, and form one whole unit. The first EPA Administrator, William D. Ruckelshaus, had the difficult responsibility to unite the various departments in each organization under the newly formed organization of the EPA. Ruckelshaus was a then 38-year old Assistant Attorney General, who was nominated for the EPA position on November 9, 1970, by President Nixon. Ruckelshaus rose to the occasion of his position, accepting all responsibilities, and the EPA was opened on December 2, 1970 [Lewis, 1985]. During Ruckelshaus' first term, he managed to organize the agency, as well as ban the pesticide, DDT, from being used [EPA 2012i].

2.5 Integrated Pest Management

In 1969, the phrase Integrated Pest Management, or IPM, was coined by the United States Academy of Sciences, and in 1972, IPM was adopted as a policy by President Nixon [Aglearn 2004]. The main purpose of IPM is to manage presumed pests while maintaining ecological balance [Orfano 2012]. How IPM programs manage to control the balance is by using various artificial modifications to the ecosystem that start with the least harmful and have the lowest damaging impact on the environment. These include biological control,

which is taking an enemy of the pest and letting nature take its toll in respect to predator against prey; cultural and physical control, which is changing the way one plants certain crops to render pests helpless, such as fencing or netting crops; chemical control, which is choosing the least harmful pesticide, and using the chemical at certain times instead of on a regular basis; plant choice, which is setting up a variety of plants to fend off certain pests from harming one's crop, like planting marigolds, tansy, or genetically modified, pest-resistant crops; genetic control, which is used to limit the amount of offspring the insects produce; and pheromone control, which is a chemical compound secreted as a sexual lure to entice insects into plants [NSW Government 2012].

Integrated Pest Management programs are usually organized into four stages, each of which involves an assessment protocol and an implementation decision point. The first stage of the IPM programs deals with an evaluation of the pest population to determine whether or not the pest is causing a critical threat to an area. Once the threat is identified as critical, the next step is identifying what kind of pest is in the area. This will help determine what kind of control one will need to rid the pest threat. The third step is preventing the insect in the area, using methods that have little to no risk to anyone, including the environment, such as plant choice, an example of which would be not to plant maple trees in an area infested with Asian Longhorn Beetles. The final step, which should be used only if the first three steps are futile, is control. Less risky yet effective methods are chosen first, like pheromone control or genetic control. However, if these fail, chemical control is the last approach taken [EPA 2012b].

2.6 Environmentalism in the 21st Century

Silent Spring's powerful legacy had numerous, long-reaching effects on the environmentalist movement, such as the formation of an international meeting known as Earth Summit. The meeting first began in 1972, and occurs every ten years, with the most recent meeting happening in the summer of 2012. The goal of these meetings is to address environmental effects on the world in order to better the planet on which humans live [Reynolds 2006, 6].

As Carson noted in *Silent Spring*, insecticides were readily available in every store: supermarkets, hardware stores, and garden-supply stores [Carson 1962, 176]. Despite being poisonous, the chemicals were commonly available to consumers who were not aware of the potential threats the chemicals posed. Now people are aware of the dangers caused by the chemicals, thanks to Carson. Nonetheless, while DDT was prohibited for domestic use a few years after the publication of *Silent Spring*, numerous new chemical pesticide products continued to enter into the marketplace every year.

The Environmental Protection Agency now evaluates new pesticides that come onto the market, looking for potential dangers to non-target organism [EPA 2012e]. In addition, the EPA evaluated existing pesticide products in 2008 to make sure any pesticide registered before November 1984 met certain standards. The EPA finished a review and reregistration of older pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act, or FIRRA. The reregistration took into consideration all effects on humans and nature, to reduce the risk of the substances marketed as pesticides. The EPA also evaluated and lowered the risk of pesticide use in food crops, to meet the goals recognized by the Food Quality Protection Act of 1996 [EPA 2012f]. But still harmful, non-registered pesticides

remain. One example is "Chalk," also known as "Miraculous Chalk" or "Chinese Chalk," which bears a resemblance to blackboard chalk [EPA 2012c: EPA 2012d]. Other examples include the products "Tres Pasitos," which contains a very deadly toxin Aldicarb and "Moth Balls," which are a danger to children because of its likeness to candy. Official EPA statements insist that its enforcement efforts are targeted in order to protect the public in situations where these substances may be finding their way into particular companies' products or particular regional ecosystems [EPA 2012c].

2.7 Invasive Species

Just as industrialization triggered a significant uptick in the scale of pollution that could result from the cumulative effects of human society, the increasing penetration and density of human travel and settlement patterns around the world during the same period of rapid commercial growth had other significant consequences for the natural state of evolution. Prior to industrialization and the emergence of a global economy, ecosystems developed in relative isolation from one another, ultimately causing organisms within a particular ecosystem balance out one another. Whenever a nonindigenous species is introduced into an ecosystem and threatens biodiversity, it becomes an invasive species [Myers 2000, 316]. In fact, invasive species are the second greatest threat to biodiversity, second only to habitat destruction [Cory and Myers 2000].

Invasive species are most often the result of accidental transportation. Human interactions, such as trade and transportation, have increased the spread of species throughout the world. Many species introduced into new areas do not become established. Of those that do, only about 1% become pests [Cleland 2001, 5446]. These pest invasive

species have caused either significant damage to or complete die offs of native species, either through out competing them or preying on them. Sometimes a species can be introduced into a new environment but remain at a small population until other factors enable it to explode. This is called the lag effect [Cleland 2001, 5446]. Nonetheless, it is estimated that "over the last 500 years, invasive species have come to dominate 3% of the Earth's ice-free surface [Cleland 2001, 5446]. In the United States alone, a little less than half of the species that are listed as threatened or endangered are considered to be at risk primarily due to competition with or predation by non-indigenous species [Morrison 2004, 273].

As international trade increases, more and more invasive species are being spread. In many cases, species hitchhike on trade goods to their new environment. In others, the creation of trade infrastructure (such as manmade canals) has connected previously unconnected bodies of water, causing a flood of invasive species.

2.8 The Asian Longhorned Beetle

The Asian Longhorned Beetle (*Anoplophora glabripennis*) is an invasive insect, known for its destructiveness to the hard wood trees which host it. The Asian Longhorned Beetle, or ALB, is a large glossy black beetle with distinct white to yellow patches. These irregular patches led to the ALB being nicknamed the Starry Sky Beetle, as the patches resemble stars in the night sky. Full grown Asian Longhorned Beetles are between 1 and 1 ½ inches in length (17 to 40 mm) and have a pair of long antennae. For male ALB, the antennae, on average, are double the length of the body, while antennae for females are usually just less than one and a half times the length of the body [USDA 2008; Haack 2010,

522]. Adult Asian Longhorned Beetles can fly, although normally only travel short distances.

Figure 2-1 Adult Asian Longhorned Beetle (USDA)

2.8.1 Life Stages and Reproduction Rate of the Asian Longhorned Beetle

Asian Longhorned Beetles have an average life span of one year. On average, nine months of the ALB's life are spent in the larvae and pupa stages within the host tree, and three months as an adult beetle outside [Alsop 2009]. To reproduce, an adult female ALB will burrow into a host tree creating an "oviposition pit" and deposit a single egg [Haack 2010, 526]. Females lay thirty five to ninety eggs over the course of their life span, one at a time [Alsop 2009]. The eggs are oblong, five to seven millimeters in length, and are white [Haack 2010, 22]. Once the ALB larva hatches, it grows to between 30 and 50 millimeters in length. For the next few months, the ALB larva bores through the host tree, feeding off of and cutting through the cambium, "the tissue that ferries the trees nutrients" before moving into the trees heartwood [Alsop 2000]. The ALB larva next develops into a pupa, ranging from 27 to 38 millimeters long [Haack 2010, 522]. From here, the pupa will become an adult, tunneling to the surface of the host tree and exiting. The adult ALB leaves an alarmingly perfect circular exit hole that is three eighths of an inch in diameter, which is one of the specific signs that the tree is a host for Asian Longhorned Beetles. Despite being able to fly, Asian Longhorned Beetles tend to stay on a host tree for multiple generations. Adult ALBs feed off of the host tree, eating twigs and leaf veins [Haack 2010, 523]. Once a host tree dies and no longer can provide the ALB with food, it moves on to infest another host tree.

Figure 2-2 Asian Longhorned Beetle Larva (USDA)

2.8.2 Why the Nature of the Asian Longhorned Beetle is Particularly Dangerous

The nature of the ALB makes it particularly hard to spot. The damage caused by the ALB occurs inside the host tree, taking place out of view and making recognizing and tracking the infestation difficult. Furthermore, the destruction process is slow and the infestation spreads faster than clear signs of a problem become visible. It takes years for a full grown tree to die from the damage caused by ALB larvae and for homeowners and individuals who do not know what signs to look for, recognizing the presence of the ALB is difficult. In addition, adult Asian Longhorned Beetles live in the canopy of host trees, elevated and out of sight. When people do see them, the beetle is often not realized as the ALB. Knowledge of the presence and potential threat of the ALB is not common, as the ALB

only recently came to the United States. While the relatively large and uniquely marked beetle may stand out as more than an average bug, the ALB is one of 350,000 known species of beetles, and can easily be overlooked as one of its nonthreatening close relatives [Alsop 2009].

2.8.3 Host Trees for the Asian Longhorned Beetle

Arguably the most dangerous characteristic of the Asian Longhorned Beetle is the wide range of tree species that it can infest. It is highly polyphagous, meaning there is a large variety of tree species that will host it [Haack 2010, 526]. While many invasive insects (as well as fungi and diseases) infest and damage plant populations, most threaten a single or small number of species. Uniquely, the Asian Longhorned Beetle infests dozens of tree genus that are common throughout the world. Tree species susceptible to ALB infestation are deemed host species. Common ALB host trees include the *Acer*, *Aesculus*, *Betula*, *Salix* and *Ulmus* genuses which include the Maple, Elm, Birch, and Boxelder species [USDA 2008, 30]. Less common ALB host trees include the *Albizia*, *Fraxinus*, *Plantanus*, *Populus* and *Sorbus* genuses, which include the Mimosa, Silk Tree, Ash, London Plane Tree, Poplar and European Mountain Ash species [USDA 2008, 30].

2.8.4 The Asian Longhorned Beetle Invasion of the United States

The Asian Longhorned Beetle was first identified in the United States in the borough of Brooklyn, New York in August 1996. Almost two full years later in July 1998, the ALB was discovered in Chicago, Illinois. The discovery of the ALB in Chicago not only meant another infestation that required eradication but “also brought the ALB, and the issue of invasive pests in general, into the national spotlight,” as it was now quite clear that “the

beetle was no longer simply an isolated East Coast problem" [Antipin 2004]. More infestations of the Asian Longhorned Beetle were discovered in Hudson County and in both Middlesex and Union Counties New York in 2002 and 2004, respectively. Four years would pass before the Asian Longhorned Beetle was discovered in Worcester, Massachusetts in July 2008. The infestation in Worcester, Massachusetts was the largest infestation and was estimated to be eight to ten years old at the time of discovery.

2.8.5 Why Urban Areas

The Asian Longhorned Beetle infestations of the United States have all occurred in urban environments. This is because urban environments are at the greatest risk.

First and foremost, the human interaction is the mechanism most responsible for the spread of the ALB. The number one method of ALB transportation is the commercial distribution of goods packed in larva infested crates. Since urban areas contain the largest concentration of commercial and industrial businesses that ship and receive goods, and the packing material that comes with them, the ALB spread primarily among urban areas.

In addition, the conditions in urban environments aid in the destruction caused by the Asian Longhorned Beetle. "Trees in more populated areas are subject to greater environmental stress to begin with, making them easier prey for exotic insects and more likely to succumb to damage" [Antipin 2004]. To put it simply, on average, urban trees infested with the ALB die quicker. While adult beetles do move from tree to tree, the Asian Longhorned Beetle has been known to remain on a host tree for generations; a characteristic that naturally limits the spread of the beetle. But when the host tree dies, the

ALB migrates to a new tree. Since infested trees die quicker in urban areas, the natural spread of the ALB increases.

2.8.6 Threat of the Asian Longhorned Beetle

The extensive threat posed by the Asian Longhorned Beetle can be categorized into economic, social, and environmental repercussions.

The economic threat of the ALB is extremely alarming when the complete spectrum of affected industries is taken into consideration. If the ALB infestation were to spread from urban areas to woodland areas, the ALB has the potential to devastate any industry that utilizes or relies on host trees. Immediately affected industries would range from the timber and maple syrup industries to tourism based business [Stefan 2000, 1].

In addition, the Asian Longhorned Beetle has the potential to inflict serious social consequences to the United States. Interaction with nature is a valued part of the lives of many Americans and because of this, society has invested in maintaining a degree of nature. People enjoy parks in cities and the tree cover in suburban yards. The Asian Longhorned Beetle will decimate these landscapes and alter terrain, a process that has already occurred in infested areas. Without eradication efforts, the Asian Longhorned Beetle infestation would flourish, killing all host trees as it spread. Even the eradication effort itself is devastating in infested areas. The widespread removal of infested host trees has left streets, parks and yards bare of hundreds of thousands of trees and the cost to replace these trees is staggering. “The total value of tree resources at risk in the cities of Chicago and New York is $2.1 billion and $2.3 billion, respectively” [Stefan 2000, 2]. As

costly as these figures are, they are nowhere near the $669 billion dollar potential national impact estimate for urban areas alone [Stefan 2000, 2].

Lastly, the environmental threat of the Asian Longhorned Beetle is immense. In urban areas, aside from the aesthetic value of host trees, host trees provide other benefits, including "cleaning the air of pollutants, microclimate effects, diminution of storm water runoff, reduction in street noise, and enhancement of local wildlife populations" [Stefan 2000, 2].

2.8.7 USDA Eradication Program Worcester

When the Asian Longhorned Beetle was discovered in Worcester, Massachusetts, the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) already had an established operating procedure to contain, assess, and eradicated the Asian Longhorned Beetle which it was already implementing at the other infestations in New Jersey, New York, and Illinois.

Immediately surveying was initiated and collaboration between federal, state, and town level personnel began. A quarantine area was established in September 2008. Originally 33 square miles, the quarantine area was later increased to 62 square miles in November 2008. Regulation prevented transportation of wood from within the quarantine area to outside. By the fall of 2012, the Worcester quarantine area reached 110 square miles.

2.8.8 Surveying

A four level survey system was implemented to survey host trees for possible infestation. The first level, called the Intensive Core Survey, consists of annually surveying

all host trees visually within a ½ mile radius from a confirmed sighting. The second level, or Delimiting Survey, is a biennial survey of all host trees at a range of ½ mile to 1 ½ miles. The High Risk Site Detection Survey is the third level and consists of regulatory inspections of tree care companies and related wood handling industries that either conduct business in or are located in the regulated area. The fourth level, or Area Wide Detection Survey, is an inspection of selected locations up to 25 miles from known infested areas.

2.8.9 Public Reactions in Worcester

When the beetle was first mentioned being in Worcester back in 2008, the USDA believed there was an error made on the observer's part. But, when a picture was sent to the USDA, proving that the beetle was indeed the Asian Longhorned Beetle, USDA officials began to worry. With the beetle in the Worcester area, the beetle could reach the Northern hardwood forest, which starts in Worcester and spreads out to Canada and the Great Lakes, the reason for the immediate quarantine [Alsop 2009, 1].

The USDA was not the only group of people who were getting scared over the announcement of the ALB. With news of the ALB invading Worcester, maple syrup producers began to worry as well, since the beetle eats maple trees. With the beetle destroying the maples, less syrup would be supplied, and the maple syrup industries in New England may go out business. New England produced half of the maple syrup made in the United States in 2007. Residents were also worrying about the effects of the ALB. With the beetles making their homes in the maples on the streets, what would happen to citizens property values? Action was taken to inspect the trees, using smokejumpers and tree climbers. After the investigation, the plan to begin eradication of the beetle was to cut

down the trees. However, the trees would have to wait to be cut down until the upcoming frost killed adult beetles. Then, the trees would be cut and ground up, to kill any leftover eggs or larvae living inside the maples [NBCNews 2008].

As soon as word spread that trees were going to be cut down, many Worcester citizens became angry, similar to how the citizens of Chicago reacted about ten years earlier. As a result of the invasion, an ALB research program was launched at Clark University. The research program was set up to investigate how the beetle altered urban life for Worcester, as well as analyzing the successfulness of response methods conducted by individuals in charge of solving the crisis. Resulting knowledge could then be applied to future problems in order to prevent future infestation [Herwitz 2012].

2.9 Introduction to Imidacloprid

Imidacloprid is a neurotoxic insecticide which belongs to the class of the neonicotinoid pesticides. It was discovered in 1984 at Nihon Bayer Agrochem in Japan. It is very lethal against insects and slightly toxic to humans and mammals [Tomlin 1994]. Another advantage is Imidacloprid trends to stay in plants under normal conditions, instead of leaching to deeper soil layers or evaporating into the air [Rouchaud et al. 1994; Tomlin 2000; Hellpointner and Krohn 2002]. Studies indicate that this compound does not accumulate in the field by annual application [Schad 2001; as reviewed in Hellpointer and Krohn 2002].

Figure 2-3 Tree Trunk Imidacloprid Application (USDA)

Imidacloprid is a colorless crystal with a melting point range of 136.4–143.8 °C [Tomlin, 2000]. It has the solubility of 0.61 g/L at 20 °C in water [Tomlin, 2000]. Absorption is the main process for Imidacloprid in soil [Sabbagh et al. 2002]. Imidacloprid has a medium to high absorption tendency for soil [Tomlin, 2000; Hellpointer and Krohn 2002]. Absorption intensity for Imidacloprid and its metabolites is influenced by soil type and depends largely on organic carbon content [Cox et al. 1998; Capri et al. 2001]. In summary, persistence of Imidacloprid in soil is affected by various factors, including temperature, organic matter of the soil, and whether the field is cropped or not. The time required for 50% of the field-applied Imidacloprid to dissipate [DT50] can range anywhere from approximately 80 days to two years. Assuming typical DT50s of 1 to 2 years, PMRA

has classified Imidacloprid as persistent in soil based on the classification scheme of Goring et al. [1975].

Despite high water solubility, if used correctly (e.g., at recommended rates, without irrigation, and when heavy rainfall is not predicted), Imidacloprid does not characteristically leach into the deeper soil layers [Rouchaud et al. 1994; Tomlin 2000; Hellpointner and Krohn 2002]. In a series of field trials conducted by Rouchaud et al. [1994, 1996] in which Imidacloprid was applied to sugar beet plots, no detectable leaching of Imidacloprid to the 10-20 cm soil layer occurred. Imidacloprid was applied to a corn field in Minnesota, and no Imidacloprid residues were found in sample column segments below the 0 - 15.2 cm depth segment [Rice et al. 1991; as reviewed in Mulye 1995].

Imidacloprid's low vapor pressure of 4 x 10-10 Pa at 20 °C results in a very low potential for volatilization [Tomlin 2000]. The geometric mean of five studies, conducted under laboratory conditions at 20°C, produced a DT50 of 156 days (SD of 40) [Hellpointer and Krohn 2002]. The DT50 determined from 11 bare soil field trials in Northern and Southern Europe was 96 days (SD=38), after mathematical adjustment to equivalent temperature (20°C) [Schad 2001 as reviewed in Hellpointer and Krohn 2002]. Hellpointner and Krohn suggest that there is little potential for accumulation as a result of repeated annual application.

Imidacloprid is a systemic insecticide that is taken up by plants, primarily through the roots, and transported within the vascular system of the plant where it can kill plant-feeding pests to protect the plants. After contacting with insects, Imidacloprid causes a swift death by shutting down the insects' central neural system [Tomlin 2000].

2.9.1 Human Exposure

Although Imidacloprid affects species differently, it is relatively safe to humans. Three case reports of attempted suicides described signs of toxicity including drowsiness, dizziness, vomiting, disorientation, and fever. Oral toxicity for Imidacloprid is designated as moderately toxic, while dermal and inhalation are designated as having a low toxicity.

2.9.1.1 Oral

Imidacloprid is moderately toxic to mammals via the oral route of exposure [PMRA 2001]. In a two-year rat study, the oral NOAEL for thyroid toxicity was determined to be 100 mg a.i./kg diet (i.e., 5.7 mg a.i./kg bw/day for males and 7.6 mg a.i./kg bw/day for females], with a LOAEL of 300 mg a.i./kg diet (i.e., 16.9 mg a.i./kg bw/day for males and 24.9 mg a.i./kg bw/day for females] [U.S. EPA 1995].

2.9.1.2 Dermal Exposure

Imidacloprid exhibits low toxicity via the dermal route of exposure [PMRA 2001]. The LD50 for dermal application of Imidacloprid to rats was reported as >5000 mg a.i./kg bw [Mulye 1996]. A dermal rat LD50 of >2000 mg a.i./kg bw has been reported for an end-use product containing Imidacloprid, Admire 240F [Mulye 1996].

2.9.1.3 Inhalation

Imidacloprid exhibits low toxicity via the inhalation route of exposure [PMRA 2001]. The inhalation 4-h LC50 for rats was reported as >0.069 mg a.i./L air (as aerosol] and >5.3 mg a.i./L air (as dust) [Mulye 1996]. Signs in rats exposed through inhalation to Imidacloprid included slightly labored breathing, decreased motility, piloerection, slight tremors, and decreased body-weight gain [Mulye 1996]. Rats exposed to Imidacloprid

through inhalation for 6 hours/day, 5 days/week for 4 weeks showed increased liver weight, increased coagulation time, and clinical chemistry changes at an exposure rate of 0.191 mg a.i./L air/day (i.e., 51.9 mg a.i./kg bw/day] [PMRA 1995].

	Very Low Toxicity	Low Toxicity	Moderate Toxicity	High Toxicity
Oral (mg/kg)	≤ 50 mg/kg	> 50 – 500	> 500 – 5000	> 5000
Inhalation (mg/L)	≤ 0.05	>0.05 – 0.5	> 0.5 – 2.0	> 2.0
Dermal (mg/kg)	≤ 200	> 200 - 2000	>2000 – 5000	> 5000

Figure 2-4 Imidacloprid Toxicity Classification

2.9.2 Plant Exposure

Imidacloprid is absorbed and distributed throughout plants acropetally (moves from base to new growth) [Tomlin 2000]. Imidacloprid has been found to translocate in a variety of crops and plants [Mukherjee and Gopal 2000; Dikshit et al. 2003]. After administration to soil or to seed, Imidacloprid has excellent root-systemic properties. In experiments using wheat and barley, researchers found that Imidacloprid applied as a seed treatment, alone and in combination with various fungicides, was not deleterious to plant growth based on plant stand, tillers produced, or plant height at a concentration of 2.5

g/seed [Pike et al. 1993]. When Imidacloprid was sprayed on tomato plants at two to four times the recommended application rate (4 x 80 g a.i./ha), phytotoxic symptoms were not observed [Dikshit et al. 2003].

2.9.3 Biochemical Consequences of Imidacloprid

Since Imidacloprid is a synthetic compound, it does not occur naturally. Due to its chemical properties, the introduction of Imidacloprid into the natural environment may cause some adverse effects to different substances and living creatures.

Imidacloprid is likely to absorb into soil, where it remains until it decomposes. As the organic content increases in soil, Imidacloprid will bind with soil and have an increased immobility. Although it is soluble in water, applying Imidacloprid in high organic matter soil will have a low probability of leaching or causing pollution in underground water. Since Imidacloprid has a low volatility, the potential risk for living creatures to breathe in Imidacloprid from the air will be very low [Liu, et.al. 2006].

Due to the characteristics of Imidacloprid, the major concern for animals is the influence of Imidacloprid in soil. Soil is the habitat of some animals and is the base for plants, the food source of other animals.

2.9.3.1 Earthworm

Earthworms are one of the most important species in the world. They form the base of the food chain and play an important part in the history of the world. Earthworms usually live underground, influence soil structures, and act as natural decomposer organisms. They ingest and decompose substances to humus and nutrition, and their translocation continuously relocates soil, which benefits the growth of plants' roots and

seeds to a huge extent, just as Darwin wrote “The plough is one of the most ancient and most valuable of man's inventions; but long before he existed the land was in fact regularly ploughed, and still continues to be thus ploughed by earth-worms.” [Darwin 1892]

However, the presence of Imidacloprid in soil can also have sub-lethal or even lethal effects on earthworms. The concentration of a chemical that can kill half of a tested animal after 48 hours is the LC50 value. The LC50 value for earthworms and Imidacloprid is 200mg/L [Feng et al. 2004]. In addition, Imidacloprid has effects on living earthworms. The behavior of earthworms can be hugely influenced under a very low concentration (0.5 and 1 ppm) of Imidacloprid, which results in making shorter, fewer, narrower, and more sinuous burrows. Since oxygen, water, and carbon dioxide can go through burrows created by earthworms, and the burrows can also make soil porous, the fertility of the soil and the quality of the plants living on that soil will heavily depend on burrows. The change in burrowing behavior of earthworms will hugely impact the normal function of the soil and the growth of the plants [Capowiez, et al. 2003, Capowiez, Bastardie, and Costagliola 2006]

Other aspects of earthworm health can also be affected by pesticide. When living in dry soil at concentrations of 0.5 and 1 mg/kg, earthworms decrease in weight significantly [Capowiez, et al. 2005]. When adding leaves containing Imidacloprid at a concentration of 3 mg/kg to microcosms of earthworms, feeding rate reduced [Kreutzweiser, et al. 2008].

Earthworm reproduction might also be influenced. After Imidacloprid is applied at a concentration higher than 0.5mg/kg dry soil, sperm deformity and DNA damage of earthworms emerge [Luo, et al. 1999].

2.9.3.2 Soil Respiration

Applying Imidacloprid may also affect the function of microorganisms in soil, which can be revealed by the change in soil respiration. By calculating the releasing rate of carbon dioxide from soil, one study shows that at higher concentration of Imidacloprid, the decrease in soil respiration will be more significant; although after 13 days the releasing rate of carbon dioxide of the soil with high Imidacloprid concentration (100μg/g) increases, which means Imidacloprid may have some excitatory effect on soil respiration. After one month the respiration of test soil returns to normal [Huijun, Wei, and Weiping. 2001]. Soil itself can be affected as well. According to related studies, the activity of soil enzymes can be altered by applying Imidacloprid, and the influence on the activity of catalase in soil is directly proportional to the concentration of Imidacloprid applied [BAM 2008]. Since catalase acts as a catalyst in soil metabolism, and the change in activity of catalase may influence the nutrition release in soil, applying Imidacloprid may have the potential to affect plant growth [Wei, HuiJun, and WeiPing. 2000]. However, research also shows that plants can continuously take up and help with degradation of pesticides, thus reducing the concentration of Imidacloprid in soil [Ishii, Y., et al. 1994]. At the same time, the hydrolysis and photolysis products of Imidacloprid will in turn have a lower influence on the soil [Wei, HuiJun, and WeiPing 2000; Huijun, Wei, and Weiping 2001].

2.9.3.3 Plant Germination

Imidacloprid is synthesized for killing pests and thus protects plants. In some cases, however, Imidacloprid can affect the normal growth of certain plants. Rice plants are one example. After applying Imidacloprid to rice plants, researchers found that the physiology

and biochemistry in plants may be altered [Wu, and Xu. 2003]. Although Imidacloprid can help get rid of pests of rice plants such as the brown planthopper, at the same time it can induce the susceptibility of rice to that pest [Cheng, et al. 2012]. When rice plants are subjected to foliar spray and root treatment of Imidacloprid, zeathins riboside contents, a substance that regulates growth, development, physiology, and biochemistry of rice plants, can be significantly reduced in plants' bodies [Qiu, et al. 2004]. Another study shows although Imidacloprid may cause no negative influence on rice plants under well-handled applying, if seeds are continuously under exposure during germination stage, some adverse effects might show up including reducing in normal germination [Stevens, et al. 2008]. Although there are few studies related to the influence of Imidacloprid on plant growth other than rice, when applying on agricultural lands, there is still a potential risk for Imidacloprid to cause a reduction in production of grain and other food supplies, and more research is needed to be done in this field.

2.9.3.4 Bee Colonies

Honey bees are the largest and one of the most important species that can be easily affected by Imidacloprid. Due to the commercial value of honey and the benefits that bee pollination habits offer, the influence of Imidacloprid on bees is under huge concern.

Since Imidacloprid is a systemic pesticide, when seeds of plants or soil near plants receive treatment, Imidacloprid can translocate into the plant's tissue. After these plants grow up, Imidacloprid may appear in the plant's nectar, the liquid produced by flowers. Honey bees heavily rely on nectar for survival [Krischik, Landmark, and Heimpel 2007]. When bees acquire Imidacloprid from nectar, behavior alteration may occur, causing a

reduction in foraging ability, impaired orientation, and even death [Krischik, Landmark, and Heimpel 2007].

Meanwhile, when exposed to sub-lethal doses of Imidacloprid, bees along with other insects such as termites do not demonstrate behavioral aversion to things containing Imidacloprid, resulting in sudden death [Thorne, and Breisch. 2001]. In other words, Imidacloprid will eventually kill bees rather than repel them when bees are exposed to sub-lethal amount of Imidacloprid over a long period of time. Imidacloprid has a very high oral toxicity to honeybees [Schmuck, et al. 2001]. In one study, only 60 ng/bee can cause acute intoxication, and even at doses 6,000 lower than the dose providing acute intoxication will also have chronic toxicity to bees, which leads to death after 72 hours [Suchail, Guez, and Belzunces. 2001]. According to another study, the LD50 value was 4 ng/bee for oral and 8 ng/bee for contact [Bonmatin et. al. 2010]. Because of this high toxicity, when a novel bee malady emerged in France during the 1990's, beekeepers accused Imidacloprid of causing illness or death of the bees.

However, in one field study, no impact on either duty performance or reproduction was observed of bees exposed to Imidacloprid residue concentrations of 0.02 mg/kg. Future research rejects the interpretation that the bee malady in France was caused by Imidacloprid [Schmuck, et al. 2001]. Another study shows that mixing Imidacloprid and syrup to feed bees will only increase the activity of bees and the number of capped brood cells. After Imidacloprid is no longer applied, the behavior of those bees returns back to normal level [Faucon, et al. 2004]. The study asserting the exact value of LD50 for bees also shows that the mean level of Imidacloprid is 2-3 ng/g in the pollen of corn and sunflowers,

which does not reach the level of either the oral or contact LD50 values established earlier [Bonmatin et. al. 2010].

In conclusion, if the concentration reaches a certain extent, Imidacloprid in habitats of bees will cause some abnormal behavior or mortality in bees. However, whether controlled applications of Imidacloprid threaten local bee colonies is still under debate.

2.9.3.5 Other Insects

The effect of Imidacloprid on other insects in nature should also be considered. Since Imidacloprid is an insecticide, it may kill some other non-target insects at the same time and breaking the balance of nature may lead to unwanted consequences. Predator insects that help control populations of pests may be killed, and thus the pest insects that are present in insignificant numbers may suddenly grow into huge populations. Due to the short life cycle of insects, insects may develop resistances toward Imidacloprid-like pesticides, requiring the synthesis of new pesticides. Once these situations emerge, they might become a much huger problem than the trouble brought from one kind of pest.

Although Imidacloprid has a low influence on beneficial insects, some research indicates that Imidacloprid may reduce population sizes [Rogers 2008]. It was observed that one kind of coccinellid predator, the *Coleomegilla maculate*, experienced reduced general mobility, survivorship, and reproduction rate when confined with Imidacloprid-treated sunflowers [Smith and Krischik 1999]. In a study of cornfields using Imidacloprid seed treatment to prevent wireworm and cutworm damage, data collected over five years showed that although most insect species were not affected in population, *Staphylinidae* and *Heteroptera* were drastically reduced in number. On the contrary, *ostrinia nubilalis*,

another pest of sweet corn, increased in number over those years, most likely from the reduction of *heteroptera*, a predator of *ostrinia nubilalis* [Albajes, López, and Pons 2003]. Another study revealed an increase in the population of spider mites, a pest on plants, when Imidacloprid was applied to soil. The cause of this is most likely the high mortality rate of this mite's predator, *Orius tristicolor* [Sclar, Gerace, and Cranshaw 1998].

Resistance may also be built by insects against Imidacloprid. Although Imidacloprid is a new pesticide and there is no real case report on the ineffectiveness of Imidacloprid on pests which can be killed, one experiment revealed the possibility of targets species developing resistance. Only after 15 generations, a high level of resistance in silverleaf whitefly, *Bemisia argentifolii*, had developed, continuing into further generations. In further research a low level of cross-resistance to some other insecticides was also observed [Prabhaker et al. 1997].

2.9.3.6 Amphibians

Amphibians are an animal species that can live in both water and land in the adult stage, but undergo metamorphosis in water. They are the predators of many insects, meaning they help reduce the pest population, and can act as ecosystem indicators of ecosystem restoration success due to their sensitivity to changes in systems, easiness of samplings, and anticipatory ability of impending changes in the whole system [Waddle 2006].

When Imidacloprid is applied into aquatic and agricultural areas, amphibians living in local fields may have the risk of suffering from alternation in DNA structure in erythrocytes of their bodies. When using 50 mg/L concentration of Imidacloprid to test on

tadpoles, nearly 10% of the tadpoles will die in three days. Since the concentration of Imidacloprid in nature aquatic areas should be far less than this concentration, it is unlikely for tadpoles to experience eradication due to Imidacloprid. However, this concentration indicates that the acute toxicity on tadpoles is very low. When testing for sub-lethal effects at concentrations of about 32 mg/L of Imidacloprid, chromosomal damage takes place. For the erythrocytes of frogs, only 0.05 mg/L for Imidacloprid may have a chance to induce DNA damage, which is a low concentration that may be easily reached in the field by overuse of the pesticide. Since frogs are predators of insects and mites, the sub-lethal amount of pesticide obtained by prey may be digested by frogs, which increases the quantity of pesticides present in the bodies of the frogs [Feng, et al. 2004].

2.9.3.7 Birds

Imidacloprid also brings health danger to birds, and the effect varies with species. Birds ingest Imidacloprid either by eating seeds and parts of plants that contain Imidacloprid, or preying on other animals or insects that have high concentrations of pesticides in their bodies. When pigeons feed on Imidacloprid-coated seeds, Imidacloprid will act on their tissues and organs, and when anatomizing dead pigeons, pesticide residues are detected in their liver [Berny et al. 1999]. One study presents that Imidacloprid is toxic to birds with a LD50 value of 25-50mg/kg [Pflueger and Schmuck 1991]. The normal functioning of organs in Japanese quails will be disturbed by Imidacloprid, and in one paper it reveals that histopathological changes take place in Japanese quails' liver and testis [Eissa 2004]. In another study, when red-winged blackbirds (*Agelaius phoeniceus*) and brown-headed cowbirds (*Molothrus ater*) were fed rice seeds with 1870 ppm Imidacloprid,

some of the birds experienced ataxia and retching, although those adverse effects were only transitory [Avery et al. 1993].

Fortunately, it is showed that Imidacloprid is an effective bird repellent pesticide. In a study of evaluating the response of red-winged blackbirds (*Agelaius phoeniceus*) to rice seeds treated with Imidacloprid, blackbirds will always avoid choosing treated seeds or seeds with high concentration of Imidacloprid for food [Avery, Decker, and Fischer 1994]. This result shows that the characteristic of avian repellency for Imidacloprid can minimize the adverse effect to birds, and thus Imidacloprid poses a relative insignificant threat to birds.

2.9.3.8 Mammals

Since Imidacloprid is a relatively new pesticide, there are a limiting number of research papers focusing on the negative effect of Imidacloprid on mammals. Although Imidacloprid is a very effective pesticide, according to many studies, it does not cause significant harm to mammals. Furthermore, Imidacloprid has even been applied in medicine for parasite treatment in mammals [Johnson et al. 2010]. The selectivity of Imidacloprid is largely due to its low binding affinity with nicotinic acetylcholine receptors (nAChRs] in bodies of mammals. Although nAChRs are present in both mammals and insects, mammals also possess resistant nicotinic receptor subtypes in their bodies which can stop the mechanism for Imidacloprid to enact [Wu, Lin, and Cheng 2001]. Also, the presence of the blood-brain barrier in mammal bodies does not allow Imidacloprid to penetrate, thus the central nervous system of mammals is protected. Because of these differences, when mammals touch, breath, or even eat Imidacloprid in relative small

amounts, the Imidacloprid is only absorbed, metabolized in the liver, and excreted via urine without causing poisoning. Related studies also show that Imidacloprid is not carcinogenic [Thyssen and Machemer 1999].

It is unlikely for people to acquire Imidacloprid from water and air, thus the most probable way for people to take in Imidacloprid is by ingestion with food. Studies show that people occasionally ingest Imidacloprid and the fraction ingested is 10^{-2} kg (ingested)/kg (applied) on average, a level far lower than the dose that induces adverse reactions in humans. Furthermore, washing fruits and plants with water before cooking can easily remove Imidacloprid residues [Juraske et al. 2009]. Thus, the effect of accidental Imidacloprid ingested by humans in normal cases is very small.

There are few documented case reports related to severe human poisoning caused by Imidacloprid. In most cases, patients intended to ingest Imidacloprid in large doses intentionally, rather than by accident. Studies show that Imidacloprid will cause specific neuro-toxicological symptoms at moderate to large doses, resulting in respiratory arrest in a few cases [Thyssen and Machemer. 1999]. In several case reports related to the ingestion of Imidacloprid, the pesticide was taken with suicidal intent. One report indicated a man consuming 50 mL of Imidacloprid of 17.8% SL (17.8% active ingredient per gallon) concentration recovered within one month after receiving medical treatment [Panigrahi, Subrahmanyam, and Mukku 2009]. Another fatal case indicated that Imidacloprid leads the central nervous system to perform abnormally. Symptoms included central nervous system depression and gastrointestinal irritation, symptoms consistent with nicotine poisoning [Shadnia and Moghaddam 2008]. In another case related to Imidacloprid ingestion with alcohol, the patients also suffered from acute multiple organ failure, which put forward the

possibility that Imidacloprid may also cause heart, kidney, and other organ damage besides symptoms described above [Yeh, Lin, and Hwang 2010].

Several studies have been conducted in regard to the effects of the application of Imidacloprid on mammals other than humans. In one study, applying 10.0% Imidacloprid-0.08% ivermectin in ivermectin-sensitive Collies did not cause any side effect to Collies, although the dose used is five times the proposed maximum therapeutic dose [Paul et al. 2004]. Yet in another case study, after drinking water from a pond contaminated with Imidacloprid used for spraying plants, eight buffaloes died after exhibiting symptoms related to the dysfunction the of central nervous system. Researchers indicated that other mammals, such as cats and dogs, were also observed to perform the same symptoms when taking Imidacloprid. By analyzing dead buffaloes, researchers found that the ailment and death of buffaloes were caused by exposure to Imidacloprid, although other than the accumulation of acetylcholine, there was no significant change in either the hematological parameters or the histopathology of vital organs in the buffaloes. The treatment used, Dextrose Normal Saline Therapy, which helps to dilute blood toxins and acts as an energy resource, and led the sick buffaloes to recover in about three days only [Shridhar 2010].

Although most studies indicated that Imidacloprid at high concentrations mainly only influences the central nervous system of mammal's body, in one study on rats, researchers pointed out that at concentrations greater than 1 μM, Imidacloprid and nicotine, a neurotoxin, produced similar excitatory effects towards mammalian nAChRs. Since nAChRs are important for brain development, applying Imidacloprid doses at concentrations greater than 1 μM may cause effects similar to those of nicotine [Kimura-Kuroda et al. 2012]. As a result, the potential influence on brain development of mammals

caused by Imidacloprid should be well recognized before applying it near residential areas, especially areas in which pregnant women live, although the lack of research in this field could not let us tell whether Imidacloprid will perform the same hazardous effect towards the human brain.

2.9.3.9 Additional Potential Risks: Inert Ingredient in Commercial Pesticides

Although the pure Imidacloprid compound may not induce serious poisoning to mammals, inert ingredients, or other substances added to the final commercial insecticide product, may have severe adverse effects to mammals. Crystalline quartz silica and naphthalene are identified in Imidacloprid products, and both of them are regarded as carcinogens to humans by the National Toxicology Program, and naphthalene can even cause chromosome damage [Buffin 2003, 22]. When assessing the side effects of Imidacloprid towards humans, the influence of inert ingredients must also be investigated. However, due to limitations in this project, the focus of investigation will be on pure Imidacloprid.

3.0 Methodology

3.1 Overview of Treated Sites

The United States Department of Agriculture Animal and Plant Health Inspection Service Asian Longhorned Beetle Eradication Program used Imidacloprid to chemically treat host trees within a half of a mile radius of known infested trees. In the spring of 2010, host trees in seven areas were treated in Worcester County, Massachusetts. Surveying conducted afterwards showed movement in the infestation. Treatment boundaries were adjusted according to survey results before treatment began in 2011. Two new areas of infestation were added to the 2011 treatment lineup, while one previously treated area was removed.

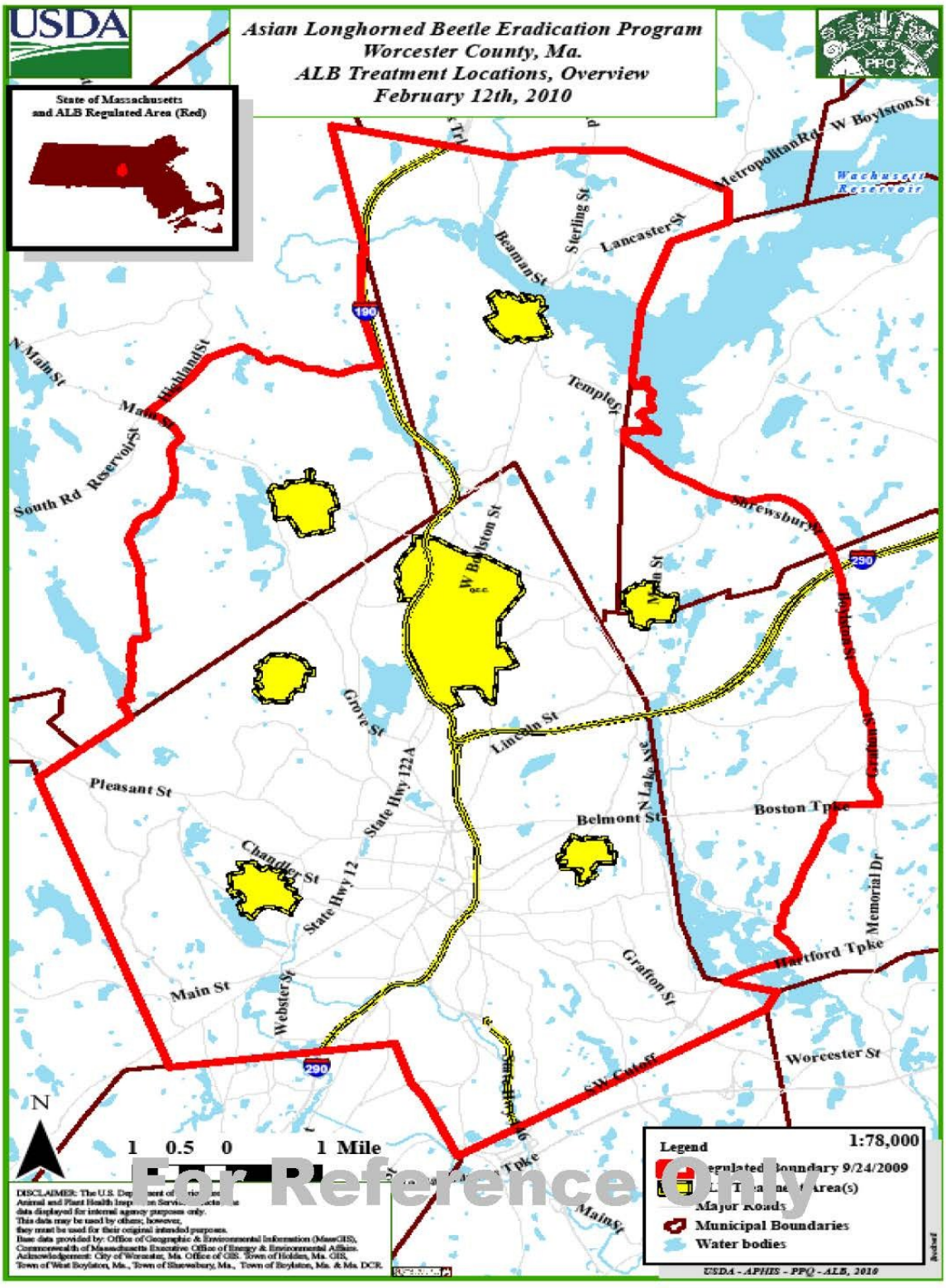

Figure 3-1Map of 2010 Imidacloprid Treatment Areas (USDA)

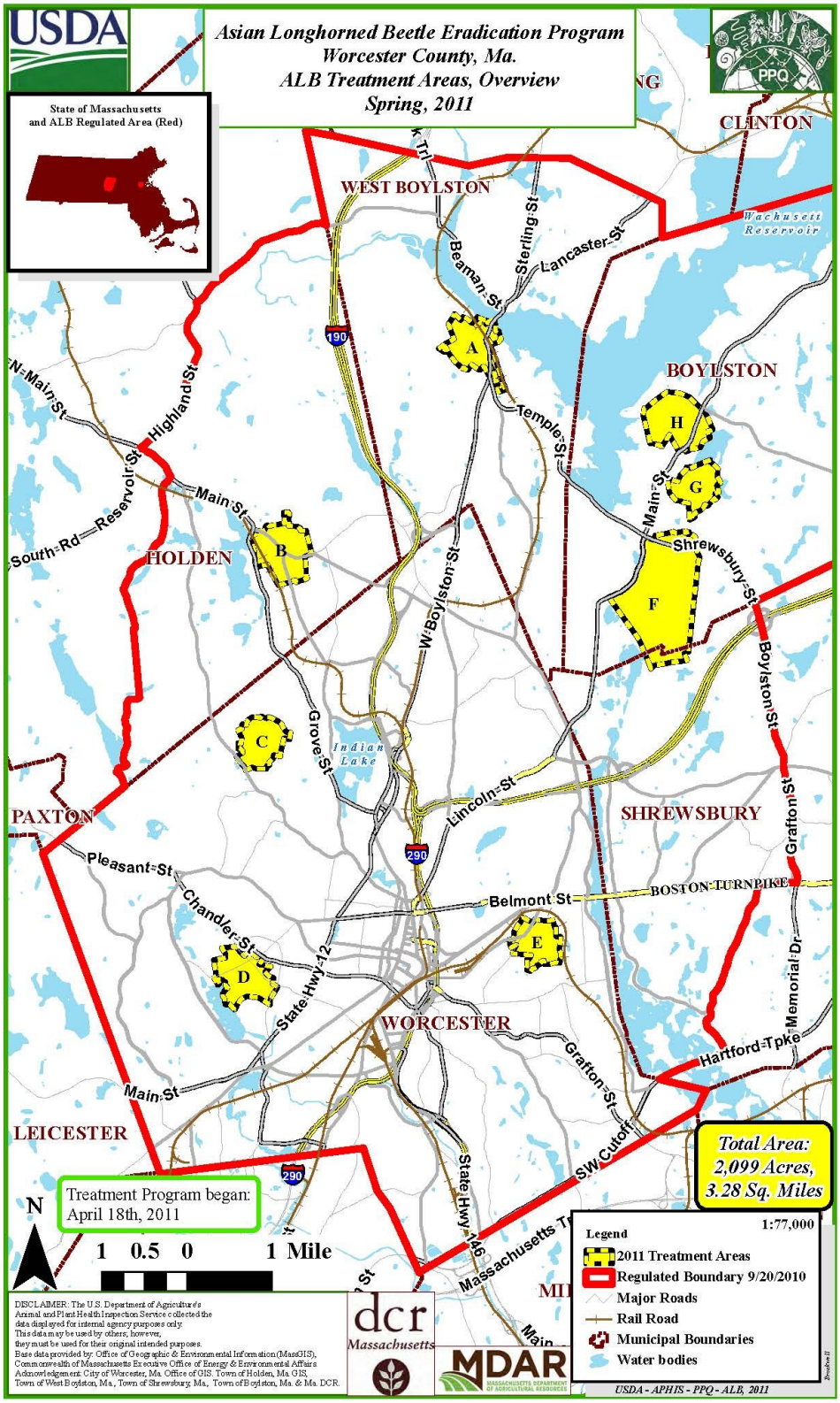

Figure 3-2 Map of 2011 Imidacloprid Treatment Areas (USDA)

3.2 Why We Chose Our Sites

Due to the physical and chemical properties of Imidacloprid, compounded by the lack of information on how treatment was carried out, concentrations of Imidacloprid could be affected by a variety of factors. For example, Imidacloprid is water soluble, meaning it dissolves in water. Exposure to the elements could cause soil concentrations of Imidacloprid to decrease as runoff water could carry the pesticide elsewhere. In addition, Imidacloprid naturally breaks down into smaller, less toxic compounds over time. It is factors like these that dictate concentrations.

One of the most probable factors that could influence Imidacloprid concentration in soil is the frequency of Imidacloprid application. The areas treated with Imidacloprid applications in Worcester fall under two frequency categories; those that received two years of Imidacloprid application and those that received one year of Imidacloprid application. To clarify, due to adjustments made to treatment boundaries between Imidacloprid applications, certain sections of the areas treated in 2010 were not treated again in 2011. These 2010 only sites received only one application. On the other hand, the bulk of the areas treated in 2010 received a second treatment in 2011, giving these areas two years of Imidacloprid exposure. Finally, the two areas added after the first year of treatment received one application of Imidacloprid in 2011.

A second possible factor affecting Imidacloprid concentrations in soil is the time elapsed since the most recent application. The Imidacloprid treatment in Worcester provides for two categories of elapsed time since the last application: sites that last received Imidacloprid treatment in 2010 and sites that last received Imidacloprid

treatment in 2011. This factor is significant due to the fact that Imidacloprid decomposes, meaning concentrations will become weaker as time, since the last treatment, increases.

In order to accommodate these two factors into a sampling plan, samples were originally going to be taken from a site that received Imidacloprid treatment in 2010 only, a site that received Imidacloprid treatment in 2010 and 2011, and a site that received Imidacloprid treatment in 2011 only. Public access sites would be the preferred locations to sample, as owner permission would be need to be obtained in order to take samples at private sites.

3.3 Procedures for Collecting Samples

Before any field work took place, all supplies needed for the sampling procedure were collected or purchased. These supplies included nitrile gloves, a stainless steel trowel, glass containers, paper towels, water, and denatured alcohol. In addition, a handheld Garmin GPS device was obtained for the purpose of recording the exact longitudinal and latitudinal coordinates of each sample.

Next, steps were taken to prepare and de-contaminate all items that would eventually come with soil sample material. All glass containers and their metal tops were rinsed with denatured and then emptied and allowed to air dry. Once dry, all tops were carefully reinstalled and a label noting the sample site and sample number was placed on the outside of each glass container before being neatly covered over with a piece of clear packing tape to protect the label from moisture and dirt in the field.

The exact locations where each individual sample would be taken were determined on site. Sample locations not only needed to adequately cover the sampling site to provide

a representation of the entire site, but also needed to be taken from areas where there was a high probability to find Imidacloprid. Trees treated with Imidacloprid received a metal identification tag upon treatment, and since Imidacloprid soil concentrations should be the highest at the base of these treated trees, samples were taken from center points of clusters of treated trees.

Figure 3-3 Tree Identification Tag at the Sterling Road Site

Once an individual sample location was determined, and the surface leaves were carefully removed, the stainless steel trowel was rinsed with denatured alcohol and allowed to air dry. Using the trowel, approximately 200g of soil from the surface to the depth of about three inches, was collected in a "core" or cylindrical matter. The sample was placed into a clean glass container and sealed shut. The sample location was then taken using the GPS device and recorded in a note book along with a brief description of the

terrain, soil, and tree cluster. The stainless steel trowel was then wiped clean with soapy water, rinsed with denatured alcohol, and allowed to dry. This process was repeated for the rest of the samples.

Figure 3-4 Imidacloprid Warning Tag at Sterling Road Site

Figure 3-5 Sample Location following Removal of Sample

3.4 Mapping Out the Sample Sites

The coordinates of each sample location were recorded using a GPS device for experimental continuity purposes. These coordinates also provide a visual representative of the sampling spread. The program, Google Earth, provides overlays of map coordinate grids on satellite images, allowing the sample locations to be precisely mapped.

Five samples were collected from the Sterling Street site, which was treated in both 2010 and 2011. In addition, three samples were taken from the Spruce Pond site, which was treated in 2011 only. No tagged trees were found at the Clinton Street and Kendrick Field sites, which were treated in 2010 only. No samples were taken from these sites.

The Sterling Street site was filled with hundreds of trees marked as treated with Imidacloprid. The soil was dark and filled with organic matter in the form of sponge like little roots. The Sterling Street site sloped slightly from the actual power line clearing towards the Wachusett Reservoir. Treated tree size varied from saplings to full grown trees and non-treated evergreen trees lined the sides of the treated area.

Figure 3-6 Google Earth Map of Sample Locations at the Sterling Road Site

The Spruce Pond site, while very dense with vegetation, only contained a couple sapling size treated trees. These treated trees were scattered sparsely over the site, outnumbered by non-host Oak trees. The site had a rolling terrain but angled to the pond. All treated trees were on steep hill faces with the exception of one which was located in a small valley. The soil was extremely sandy and very light in color.

Figure 3-7 Google Earth Map of Sample Locations at the Spruce Pond Site

3.5 Sample Analysis

The amount of Imidacloprid in each soil sample was determined by Gas Chromatography (GC), a process that determines concentrations of volatile compounds. Unfortunately, with a saturated vapor pressure of 4×10^{-10} Pa at 20^{o}C, Imidacloprid is not a volatile compound. Therefore, in order to determine Imidacloprid concentration levels using Gas Chromatography, the Imidacloprid had to be hydrolyzed under a mild basic condition and transferred to a volatile compound, which could then be analyzed through the GC process.

50g of soil sample and deionized water were mixed and treated in a supersonic bath for 15 min. The mixture was filtered twice and the volume was adjusted to 250ml by deionized water. 0.4g Sodium Hydroxide was added to the solution. The solution was heated to 85 ℃ for 15 min, then cooled to 20℃ and neutralized with 0.01mol of Hydrochloric Acid. The volume was adjusted to 250ml and extracted with chloroform twice. The sodium sulfate dehydrate was added to the organic phase and the solution was condensed to 1ml through rotary evaporation followed by micro-snyder column. 200µl of solution was spiked with Anthracene (4µl, 3µg/ml) as internal standard. The final solution was diluted and shot into the GC machine.

A calibration curve was made using a known amount of Imidacloprid with Anthracene. The concentration of Imidacloprid in each sample was calculated using the integrated area compared to the calibration curve [Vilchez et al. 1996]. The concentrations were compared to the standard and the toxicity levels were determined according to the procedures outlined by the standard works on chromatography techniques [Vilchez, et.al. 1996].

4.0 Results & Discussion

4.1 Results

Our initial data, derived from our laboratory testing, is directly summarized in Figure 4-1 and Figure 4-2. Furthermore, we obtained the average concentration of Imidacloprid of the three subsamples in each sample and summarized the data in Figure 4-4.

We collected five samples from the Sterling Street Sample Site and three samples from the Spruce Pond site. We divided the samples taken from each site into three subsamples and tested the Imidacloprid concentration of each individual subsample. For the Sterling Street site, the average Imidacloprid concentrations in the five samples are 0.01506, 0.02580, 0.01100, 0.00301, 0.01894 mg Imidacloprid/kg soil, respectively. For the Spruce Pond site, no detectable Imidacloprid concentrations were observed, indicating that the Imidacloprid concentration was below the detection limit of GC.

Sterling Street Sample Area	
Test Sample (Area Sample # – Trial Round)	Concentration (µg/50g)
1-A	1.8276
1-B	0.2593
1-C	0.1721
2-A	0
2-B	0.7581
2-C	2.9621
3-A	0.7006
3-B	0.4487
3-C	0.5001
4-A	0
4-B	0
4-C	0.4509
5-A	1.8678
5-B	0
5-C	0.9728

Figure 4-1 Sterling Street Area Imidacloprid Concentration Table

Spruce Pond Sample Area	
Test Sample (Area Sample # – Trial)	Concentration (µg/50g)
1-A	0
1-B	0
1-C	0
2-A	0
2-B	0
2-C	0
3-A	0
3-B	0
3-C	0

Figure 4-2 Spruce Pond Area Imidacloprid Concentration Table

Figures 4.1 and 4.2 display the Imidacloprid concentration results from both the Sterling Street and Spruce Pond sample areas, respectively. Five samples were taken from the Sterling Street site and three samples were taken from the Spruce Pond site. Each of these samples was then homogenized and three trials of testing were conducted from each sample. These trials are expressed with the letter labels A, B or C. All numerical data in Figures 4-1 and 4-2 is summarized into the diagrammatic form, which is in Figure 4-3.

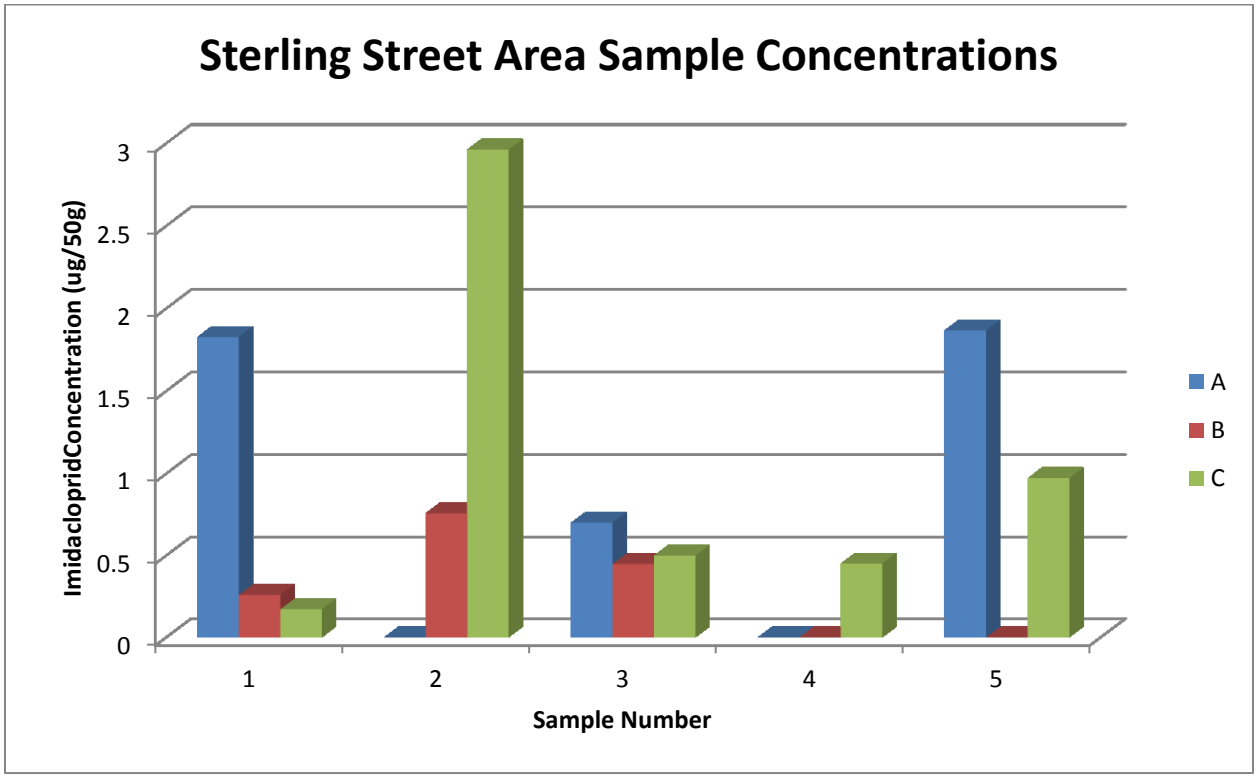

Figure 4-3 Sterling Street Area Imidacloprid Concentration Graph

The concentrations of Imidacloprid obtained by taking the average of the data for the three samples in every subsample site from Figure 4-1 are listed in Figure 4-4.

Sample	Concentration (mg/kg soil)
Sterling Street Sample 1	0.01506
Sterling Street Sample 2	0.02480
Sterling Street Sample 3	0.01100
Sterling Street Sample 4	0.00301
Sterling Street Sample 5	0.01894
Spruce Pond Sample 1	Undetectable
Spruce Pond Sample 2	Undetectable
Spruce Pond Sample 3	Undetectable

Figure 4-4 Sterling Street Sample Imidacloprid Concentration Average Table

All numerical data in Figure 4-3 are summarized into the diagrammatic form, which is in Figure 4-4 below.

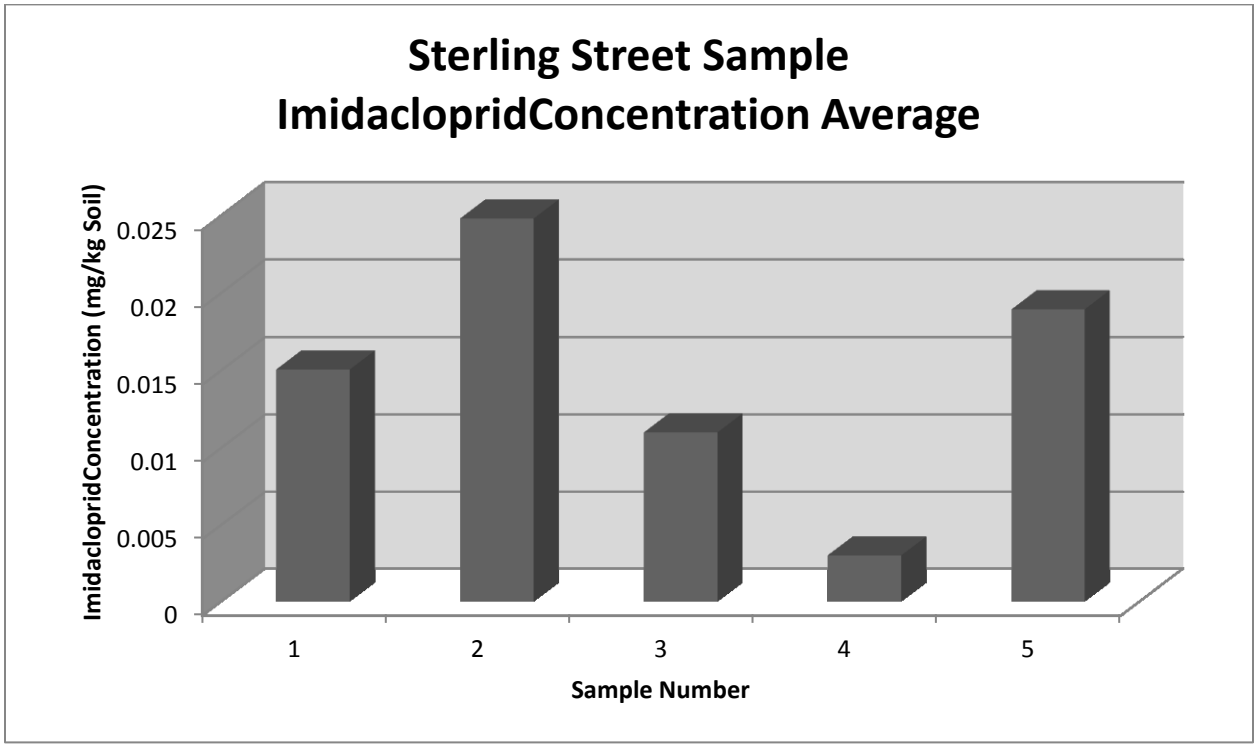

Figure 4-5 Sterling Street Sample Imidacloprid Concentration Average Graph

4.1.1 Explanation of Outlier Data

As presented in Figure 4-1, several values are much bigger or smaller compared with other values in the same subsample plot (for example, the Imidacloprid concentration in the soil sample named Sterling Street: 1A is 1.8276 μg /50g, which is about ten times greater than the concentration in the soil sample of Sterling Street: 2C, 0.1721 μg /50g.), and the standard deviation of the concentration in the same subsample plot is high to about 50% comparing to the average value. These differences may be attributed to incomplete homogenization of soil, since the concentration of Imidacloprid depends heavily on the depth of the soil, as the soil goes deeper, the concentration of Imidacloprid decreases accordingly. When the experimenter took samples from the beakers with soil that

underwent a failed homogenization process, the concentration gradient of Imidacloprid in soil still existed. Since the experimenter took the soil from the top to the bottom of the beaker, the concentration of Imidacloprid in each soil sample will follow the gradient in the original subsamples and thus in one subsample plot the values vary to a great extent. Also the organic components of soil play a crucial role in the concentration. Soils containing roots, fallen leaves, and other plants tissue will retain higher concentrations than the pure soil. Due to the random distribution of these organic components in the soil, the standard deviations of concentrations in different subsamples are rational to be high. Since the experimenter only mixed the soil by physically shaking the beaker, the different components of the soil samples are still not mixed adequately and concentrations of Imidacloprid in each soil sample showed a high standard deviation.

4.1.2 Proposed Reasons for No Results at Spruce Pond Site

Of the three samples taken from the Spruce Pond site, none yielded any concentration of Imidacloprid. There are two possible reasons for this.

First and foremost, there is a possibility that the experiment went wrong. Although Gas Chromatography is an extremely accurate process, there is much room for error in both the experimental preparation of the samples and the process of transferring the solution to the volatile compound. On the other hand, the soil truly may not have contained Imidacloprid.

There are many factors which could have led to the Spruce Pond samples testing negative for Imidacloprid. Unlike the Sterling Street Sample site, the number of treated trees at Spruce Pond was small. In fact, there were so few treated trees that searching for

the small identification tags proved tedious. Because of this, the original goal of sampling from the center of a cluster of treated trees could not be implemented and samples had to be taken at the base of the treated trees.

Of the three trees whose base soil was sampled from, the largest had a diameter of less than eight inches, while the Sterling Street site had treated host trees with diameters greater than 14 inches. In other words, the treated host trees at Spruce Pond were significantly smaller than those at the Sterling Street site.

The treated trees at the Spruce Pond site were also on rough terrain. Two of the three trees from which samples were taken at the base of were on extremely steep slopes. On top of that, the soil at Spruce Pond was very light in color and contained sand, leading to the possibility that the soil contained little organic matter and drained well.

There are many possible reasons for the Spruce Pond site testing negative. Due to the small size of the trees, it is possible that less Imidacloprid was used per tree. This compounded with the fact that there were so few treated trees at Spruce Pond and that they were all spread out, could have kept the soil concentrations of Imidacloprid low. On top of that, with little organic matter in the soil to hold onto the Imidacloprid and good drainage, what little Imidacloprid was in the soil could have been quickly washed away. It is also a possibility that differences in Imidacloprid application could have affected the soil levels. Both soil injections and tree trunk injections were administered. It is possible that one method, most likely soil injection, would cause higher Imidacloprid concentrations in soil.

4.2 Comparison of Data with Official Values

The document *Imidacloprid– Human Health and Ecological Risk Assessment – Final Report* [Anatra-Cordone and Durkin 2005] provides an official basis for data comparisons. This paper was published under the auspices of the U.S. Department of Agriculture and the data presented in this document represent official positions. We compared our data values to the USDA official data, but since our samples were taken from surface of the soil to a depth of three inches, we only focused on the official data for long term concentrations in the central and upper levels of soil [Anatra-Cordone and Durkin 2005].

By comparison, all of our values for Imidacloprid concentration are smaller than any of the values from central and upper level of soil used for risk assessment in official report (our highest concentration: 0.025 mg/kg soil versus the lowest concentration in official report: 0.03 mg/kg soil). The result of comparison shows that the repeated application of Imidacloprid in these two treated areas in Worcester did not result in excessive amount of persistent presence of the chemical two years later.

4.3 Risk Assessment

The three most common ways for living creatures to be exposed to Imidacloprid include inhalation, dermal contact, and ingestion. For most species, the toxicity value from the official reports are assessed in the ways that are in no relationship with the concentration of Imidacloprid in soil, such as to detect the toxicity value for inhalation by measuring the mass of Imidacloprid per unit volume of air that will cause side effects to certain animals, for dermal contact by measuring mass per unit area of skin exposed to Imidacloprid directly, for food consumption by measuring mass taken per animal or per

unit weight of that animal per day, and for water consumption by measuring mass per volume in water consumed by animal. Due to the type of data we obtained (mg/kg soil), it is hard for us to make a direct comparison to the data from official reports, which contain different units. Because of these limitations, the official data we can compare to for health risk assessment is confined into two major types of species: terrestrial animals and soil microorganisms, which are creatures that live in and directly absorb energy resources from soil.

Soil dwellers support the functioning of soil and act as ecological indicators of the health and vitality of the surrounding ecosystem. When concerns about chemical pesticides are raised, these are the organisms that biologists examine for a quantitative assessment of the degree of potential harm. Due to their limited mobility and sensitivity to their living condition, earthworms in one area can be largely influenced by the extent of outside pollution, which includes but is not limited to the application of Imidacloprid only. This influence can be detected by humans through observing their alternation in life expectancy, activity, and quantity in that area, and in other words, it means the behavior and population of earthworms can reflect the health conditions of soil in one area, and people can make judgment on whether one certain place is acceptable for using as a public recreational area by making observation on these species. For example, *Eisenia foetida*, one earthworm species, is studied as a toxicological indicator, since the extent of change in population and life style can indicate the level of toxicity, thus the abundance and normal functionality of these earthworms can be recognized by people as a sign of low pollutant impact in one certain area [Cortet et al., 1999].

The official report indicates that living in a condition with concentrations of Imidacloprid in soil to be 0.5mg/kg soil, one species of earthworm, *Eisenia foetida*, experiences sperm deformity, while the NOAEC (no observed adverse effect concentration) for sperm deformity is 0.1 mg/kg soil [Anatra-Cordone and Durkin 2005, 91]. For the Pheretima group of earthworms, (*Amynthas hawayanus, A. aeroginosus and A. diffringens*) the 24 hour LC50 concentration is 155 mg/kg soil, the 48-hour LC50 is 5.0 mg/kg soil and the seven-day LC50 is 3.0 mg/kg soil [Anatra-Cordone and Durkin 2005, 242]. For terrestrial fungi, NOAEC for sperm deformity is 0.1 mg/kg soil, while the concentration of 10 mg/kg inhibits fungal growth. Through comparison, none of our samples retain concentrations higher than any of the concentrations that can cause adverse effect to either the earthworms or fungi we studied (our highest concentration: 0.025 mg/kg soil versus lowest data: 0.5 mg/kg soil for earthworms), indicating that there should not be any long-term adverse effect on either earthworms or fungi. The half-life of Imidacloprid in soil varies from 38.9 days to longer than one year depending on environmental condition, and by calculating the largest possible original concentration of Imidacloprid, the concentration of 0.5 mg/kg soil is still not reached. This indicates that it is unlikely for Imidacloprid to cause any adverse effect on earthworms and fungi, and if there is any, the effect should be transient [Anatra-Cordone and Durkin 2005, 259].

According to our results, the local ecological system has not been seriously threatened by the Imidacloprid applications carried out since 2010. That being said, studying the effects of Imidacloprid on an ecological indicator, such as the earthworm, would allow for more accurate inferences to be made regarding the effects of Imidacloprid on the environment and ultimately human safety.

5.0 Conclusion

5.1 Project Summary

The issue of chemical safety has been a huge concern to residents since the first emergence of *Silent Spring.* Imidacloprid has been utilized for eradicating the Asian Long-horned Beetle, an invasive species, which causes significant damage and rapid death to trees. Since the persistence of chemical residue as a result of applications has not previously been adequately studied in Worcester County, Massachusetts, we decided to carefully examine the concentration of Imidacloprid to determine the safety of the application. We collected soil samples from two locations in Worcester and determined the Imidacloprid concentrations in each soil sample. Based on the concentrations, the corresponding locations were categorized based on hazard levels to humans and other living organisms.

After the concentration of Imidacloprid in each sample was determined by Gas Chromatography, we compared the resulting data with official documents which indicate standards for safe exposure rates of Imidacloprid concentration. We now have greater confidence that Imidacloprid has not been over applied at the Sterling Street and Spruce Pond locations in Worcester County, Massachusetts and persistent Imidacloprid residues in soil are not harmful to humans.

5.2 Future Direction

5.2.1 Testing Additional Sites

In this project, only two sites in Worcester County, Massachusetts were tested. Because of this, we are unable to present the pattern of the situation in all of Worcester due to the lack of data. For further work, more sites could be tested to collect enough data to

represent the full range of Imidacloprid concentrations in Worcester County, Massachusetts.

5.2.2 Alternate Routes of Testing

In this project, only soil samples were tested. However, several papers indicate that Imidacloprid tends to reside in plants instead of soil, and that Imidacloprid residues can even be detected in newer growing portions of the plants after application ends [Anatra-Cordone and Durkin, 2005: Hellpointner and Krohn 2002: Rouchaud et al. 1994; Tomlin 2000]. Imidacloprid can accumulate in bodies of herbivorous animals through food intake and show negative effects once beyond acceptable concentration, and high concentration of Imidacloprid in plants has potential to cause the collapse of honey bee colonies. Based on these facts, determining the concentration of Imidacloprid in plants and the risk level assigned to plant-related animals would be another very good area of interest.

A safe water system is very important to the health of environment. Since Imidacloprid has a water solubility of 0.61 g/L at 20 °C, further work could focus on the concentration of Imidacloprid in water systems.

5.3 Closing Remarks

On Friday, February 22, 2013, the Worcester Telegram & Gazette reported that the State of Massachusetts declared another quarantine zone, but this time, this quarantine zone is not for the Asian Longhorned Beetle [Lindsay, 2013, A4]. Berkshire County, Massachusetts now hosts an infestation of the Emerald Ash Borer beetle, another invasive species with the potential to negatively impact the environment and economy. Similar to the ALB eradication program, state and federal legislature has been emplaced to regulate

transportation of host materials but this time, the goal is containment, not eradication. Meanwhile back in Worcester County, Massachusetts, a new ALB infestation area of 92 acres in Shrewsbury has been discovered and thousands of trees are scheduled to be removed [Elaine Thompson, 2013]. It is clear that the Asian Longhorned Beetle continues to spread and public concern regarding the removal of trees remains strong. As pesticide treatment continues, residual Imidacloprid concentrations in soil must be monitored throughout the treated areas to ensure public and environmental safety.

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