THE EFFECT OF AGITATED SERIAL DILUTIONS ON WHOLE ORGANISMS: ROMA TOMATOES (*LYCOPERSICON LYCOPERSICUM*), HONEY BEES (*APIS MELLIFERA* L.) AND ROSY RED MINNOWS (*PIMEPHALES PROMELA*).

By

BRENDA L. MORALES

Bachelor of Science in Psychology

University of Texas – Pan American

Edinburg, Texas

2002

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2006

THE EFFECT OF AGITATED SERIAL DILUTIONS ON WHOLE ORGANISMS: ROMA TOMATOES (*LYCOPERSICON LYCOPERSICUM*), HONEY BEES (*APIS MELLIFERA* L.) AND ROSY RED MINNOWS (*PIMEPHALES PROMELA*).

Thesis Approved:

Thesis Advisor

Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to thank all those who made the completion of this project possible. First of all my advisor Dr. Charles I. Abramson for his time, patience and shared knowledge, if it were not for his great and curious mind I would have not embarked on this research project. Second I would like to thank Dr. Melanie C. Page and Dr. Bill Scott for their time and efforts to be a part of my committee. Additional thanks to Dr. Page for all her extra help, support and guidance. Thanks to Charles Schakelford and TLC Florist and Greenhouses Inc. for their help and the use of their facilities. I would also like to acknowledge the great help of Ms. Sebrina Mathews, who very enthusiastically began this project during her year at Oklahoma State University. Her strenuous work was an essential element for the completion of this project.

I also want to thank all those who motivated me to continue with my academic goals. To the two wonderful people who gave me unconditional love and encouraging words through this arduous project, my parents Hector J. and Yolanda Morales. To a great role model of a women, my sister, Chely Flores. Thanks to those wonderful friends that gave me not only words of encouragement but the support to continue, Brenda L. McDaniel and Patricia Alexander. To my family as a whole thanks for believing in me. This is dedicated to my grandparents Morales and Dávila, I love you all.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

September 11, 2001 was a devastating day for the history of the United States, with the fall of the World Trade Center towers and the attack on the Pentagon. These terrorist attacks frightened the country and displayed a vulnerability that many considered implausible. In the following months, fears rose again when a biological form of an attack began to appear. Several letters containing anthrax were received by several governmental and news media offices in the United States. These biological attacks brought about a great fear and concern for national public safety and health. They raised awareness of the need for emergency preparedness, flexible and sustainable public health infrastructure, and the importance of linkages between environmental exposures and health outcomes (Marmagas, King, & Chuk, 2003). According to O'Toole and Inglesby (2000), biological weapons have a similar potential to cause suffering and death as nuclear weapons do. The possibilities of events such as these are always present and because of this, it is believed by the Central Intelligence Agency (CIA) that there are at least a dozen countries that either possess biological weapons or are actively pursuing offensive bio-weapons programs. The most significant problem, however, is not the existence of biological weapons, but the possibility of them being introduced into a country in an undetectable manner and then being used against that country.

Nature of the Problem

Due primarily to the anthrax attacks, attempt for better screening in post offices and airports has occurred. What happens, however, when someone passes through an airport with a bottle of water? A bottle of water is not a matter of concern; but in fact it might be, if it carries an undetectable agent within it. Molecular biologist Christopher Aston stated that current technology is limited when trying to foil "black biology" (Larkin, 2001). "Black biology" is the use of bacteria, viruses and toxins for the purpose of creating weapons of mass destruction (Breithaupt, 2000). Susan Hallowell, chief of explosives and weapons in the security division of the US Federal Aviation Administration, noted that "computed tomography and electron beam tomography, techniques used by radiologists, are being used to detect 'book bombs' and other small, concealed objects that would be missed with current screening" (Larkin, 2001, p. 1708). Current scanning machines are concerned with using basic X-ray screening for firearms or other weapons. New advanced equipment, however, is starting to be used by more and more airports. The new apparatus used in airports produces clear cross-sectional images that help detect chemicals or material used in bombs and other weapons. Most of this new equipment is focused on identifying conventional weapons, as well as other explosives by measuring the density of a bag's contents (Pimentel & Evangelista, 2001). However, none of these new methods are used to search for agrochemical or aqueous solutions of potential hazard. In fact, even with all this new equipment, it is still possible that other forms of bio-weapons could be smuggled into the country with relative ease.

Historical Overview

Throughout history, there have been events of bioterrorism in which disease and sudden epidemics decimated armies, cities and entire civilizations. As noted by Turvey, Mafoua, Schilling, and Onyango (2003), many of the first recorded incidents were considered natural or accidental causes; several however, were really used by the military as a way to defeat their opponents. One of the first recorded deliberate uses of biological agents as a weapon was during the pre-Christian era. Scythian archers used arrowheads that had been dipped in manure and rotting corpses in order to increase the deadliness of their arrows. In 1340, while Kaffa (Ethiopian kingdom) was under siege, the invading army catapulted plague victims at their enemies, and this is thought to have contributed to the second European plague epidemic. In 1763, during the French and Indian War, the British forces in Pennsylvania distributed blankets that had been used by smallpox victims to the Delaware Indians in an effort to reduce the number of rival forces, causing a smallpox epidemic among the Native Americans (National Research Council, 2003; Turvey et al., 2003).

With the advancements in medicine and technology came new methods of using biological agents as weapons of mass destruction. During World War I, the Germans used both anthrax and glanders (a contagious, usually fatal disease of horses and other equine species) to kill pack animals that were being shipped to Britain and France. Subsequently, during World War II the United States, Great Britain, and other countries created biological-warfare programs directed against plants or animals. During the Biological and Toxic Weapons Convention of 1972, the United States, the United

Kingdom, and Russia agreed to ban offensive biological warfare programs (Microsoft Encarta Reference Library, 2005). Unfortunately, in domestic as well as foreign terrorism, the use of chemical and biological agents has not ceased. Since the 1972 Convention, there have been at least 23 recorded incidents of deliberate use of biological agents by either military or terrorists, against other countries or as domestic terrorism (National Research Council, 2003; Turvey et al., 2003).

One of the largest incidents of bioterrorism in the United States was in 1984 when the Bhagwan Shree Ragneesh cult plotted and executed a salmonella attack in The Dalles, Oregon. By spraying salmonella-laden liquid on the salad bars of ten local restaurants, 796 people got sick of which 45 were hospitalized (Miller, Engelberg, & Broad, 2001). Another case of domestic terrorism was seen in 1996 in the state of Wisconsin. Brian Lea, an owner of an animal-food processing facility, purposely used chlordane feed products to contaminate goods distributed by National By-Products Inc. to Purina Mills (Turvey et al., 2003). As a form of domestic violence in Japan, sarin, a poisonous liquid utilized as nerve gas, was put in a Japanese subway by the Aum Shinrikyo sect in 1995 killing 12 people and making thousands ill. A year later, a Japanese hospital worker introduced Shigella dysenteriae into the food eaten by 13 co-workers which caused amongst other things bloody diarrhea, leaving nine of them requiring strict medical treatment. These events illustrate the damaging effects biological agents can have. It is even more chilling to contemplate that damage can be done with the use of undetectable deadly agents.

Significance of the Proposed Study

In 2001, approximately $40 million was allocated to the United States Department of Agriculture (USDA) by the federal government for agroterrorism (Cain, 2001). In 2002, the defense-spending bill (HR 3338) approved over $300 million for the USDA to support agroterrorism investigations, upgrade facilities, and improve border control (National Research Council, 2003). This large amount of funding is a clear indication of the emphasis that the United States directs to research on biological weapons.

Knowledge of potential use of biological agents brings up a major concern and a question: can bioterrorism occur in front of us without it being noticeable or even traceable? Davenas et al. (1988), in what became a very controversial piece of research, obtained results which suggested that a completely diluted chemical can still cause a reaction in a living organism. These results suggest alarming outcomes that if abused, could be devastating. The current study explored the potential use of homeopathic principles to create detrimental chemical solutions that might be undetectable and untraceable. The two principles that were investigated in this study were the effects of dilution and agitation. The goals of the study were twofold. The first goal was to investigate the effect of agitating a solution on the health of three different organisms. The second goal was to determine if the effects of agitation are influenced by dilution.

CHAPTER II

REVIEW OF LITERATURE

History of Homeopathy

In 1796, Samuel Hahnemann, a German physician and chemist, developed what came to be known as homeopathy. Homeopathy is an alternative form of medicine that has been practiced for over 200 years and is used by an estimated 2.5 million people each year in the United States alone (Microsoft Encarta Reference Library, 2005). It is defined by the Merriam-Webster's Collegiate Dictionary (2002) as a medical technique that treats a disease by the administration of minute doses of a remedy that would in healthy people produce symptoms similar to those of the disease. This form of treatment is based on two principles: law of infinitesimals (also known as potentization and succussion) and law of similars.

The law of infinitesimals states that the more a substance is diluted, the more potent it becomes, consequently creating a so-called molecular memory of the original substance (Microsoft Encarta Reference Library, 2005; Shelton, 2004; Vallance, 1998). The resulting diluted solutions are known as potencies. Depending on the dilution factor, the potencies are denoted as either 'X' (decimal for a 10-fold dilution) or 'C' (centesimal for a 100-fold dilution). Vallance (1998) acknowledged the law of infinitesimals as a vital part of homeopathy. Avogadro's Law states that the number of atoms or molecules in a mole of any substance is equal to 6.02×10^{23}. Therefore, homeopathic dilutions beyond

10^{24} are mathematically unlikely to contain a single molecule of the original compound. Moreover, during Hahnemann's first uses of his homeopathic remedies, he noticed that the antidotes that he carried in his saddle bags during his travels seemed to be more effective than those which he administered to his patients while at his office. He assumed it was the jiggling of the bottles during the travels that made the remedies more potent. From then on, Hahnemann did not just shake them when diluting the solutions, but agitated them by thumping the remedy bottle repeatedly against a leather-bound book. This process, which he believed made the solutions more potent, he called succussion. Hahnemann concluded that homeopathy required not only the dilution of a solution but also agitation at each dilution step. This process that includes both the dilution and agitation of solutions is referred to as potentization (Shelton, 2004).

Although homeopathy has been used for a long time, it is not without its critics.

In 1988, Davenas and twelve other scientists from around the world published what immediately became a controversial article in the British journal *Nature* (Davenas et al., 1988). Although Davenas was the first author, it was one of the co-authors, Benveniste, who is most associated with this work. These thirteen researchers conducted a series of experiments in which they studied immunological responses in humans and examined the tendency of basophils to degranulate when there is an allergic reaction. A basophil is a type of white blood cell that is involved in the body's response to an allergic reaction and the granules released are involved in inflammation, thus the process is called degranulation. Degranulation is a crucial indicator of whether an immunological response has occurred in an individual because of an allergic reaction. When the granules are stained with a dye such as toluidine blue after degranulation, if granules were released

(i.e., degranulation occurred), the granules will be a bright blue color and can be easily identified under a microscope. Davenas et al.'s (1988) experiments showed that solutions diluted to contain very low concentrations of a substance affected the organism. Davenas and colleagues used the homeopathic process also referred to as Agitated Serial Dilutions (ASD), in which concentrations of an original substance are diluted to extremely low concentrations, for his experiments. According to the authors, "Transmission of the information depended on vigorous agitation, possibly inducing a sub-molecular organization of water or closely related liquids" (Davenas et al., 1988, p. 818). Agitation or vigorous shaking applies to the rapid inversion or shaking of a solution and is more than a mere stirring of the solution. The conclusions drawn from this series of experiments came to be referred to as "water memory." These findings, although controversial and unlikely to most scientists, supported homeopathy and its principles.

Rationalization for ASD's Ability to Produce Biological Responses

An issue faced by Davenas, Benveniste and their colleagues was having to explain how their findings came about. The success of their results were due to the idea that vigorous shaking enabled the solutions to maintain the ability to yield a biological effect on the samples; this is compatible with the methods used in homeopathy (i.e., succussion). The theory of water having memory has interested researchers from many different fields. Physicists have attempted to explain how water memory is possible. Quantum physics specialists Del Guidice, Perparata, and Vitiello (1988) found that water was able to form consistent domains with diameters so minute that they are measurable in

units of nanometers. They demonstrated that continuous vibrations of water particles eventually led to particles being 'locked' into a stable position. Further physical analysis has led to findings that support the idea that as a solution becomes more diluted it actually becomes more powerful. Samal and Geckeler (2001) found that in a series of dilutions, the particles dissolved in water tended to form clusters with each dilution. They also found, when analyzing the properties of water and other polar solvents such as alcohols and ketones (any solvent with high dielectric constant) that clusters had increased by 0.55mm in diameter each time the solution was diluted. Therefore, the more diluted a solution, the larger the size of the molecule clusters.

Replication of ASD Research

As a result of Davenas' controversial findings, many investigators proceeded to explore this water memory phenomenon. More than a dozen researchers have attempted to reproduce the findings that Davenas and colleagues achieved with human basophil degranulation tests. Guggisberg, Baumgartner, Tschopp, and Heuseer (2005) discussed 14 articles that achieved positive results when investigating the effects of homeopathic solutions on human basophils (see Table 1). They noted that *p* values were not great and dilutions that were significant were not always consistent with the dilutions in Davenas' studies. For example, dilutions 10^{2} and 10^{11} were found to be significant in both Davenas' study as well as in research that replicated it. However; there were solutions such as 10^{12} and 10^{60}, that were significant in research that replicated the original study but were not found significant in the original study. Therefore even though replication studies found

solutions to be significant, their findings were not consistent with prior results (Guggisberg et al., 2005; Hirst, Hayes, Burridge, Pearce, & Foreman, 1993). There are other investigations in which these inconsistencies did not occur. Sainte-Laudy and Belon (1993) replicated Davenas' research and obtained precisely the same results. In other research they have also modified the methodologies used in their investigations in order to eliminate as many confounding variables as possible and still attained findings that supported the original conclusions. It is interesting to note that the majority of studies that found results consistent with Davenas' actually used Histamine and not Anti-IgE as the homeopathic agent and the majority used Flowcytometry rather than visual counting to assess the outcome. Histamine is a physiologically active amine that is released from mast cells as part of an allergic reaction, whereas anti-IgE is a class of antibodies which are responsible for allergic reactions. The different methods of assessment were used to see if subsequent research could improve upon the method of simply counting the degranulating cells.

Table 1

Overview of studies investigating the effects of homeopathic solutions on human basophils and methodologies used.

Study	Method	Homeopathic dilution	Measured parameter
Maddox J., Randi J., and Stewart W.W.	Visual counting	Anti-IgE	% Degranulated basophils
Poitevin B., Davenas E., and Benveniste J.	Visual counting	Lung histamine, *Apis mellifica*	% Degranulated basophils

Benveniste J., Davenas E., Ducot B., Cornillet B., Poitevin B., and Spira A.	Visual counting	Anti-IgE, *Apis mellifica*	% Degranulated basophils
Ovelgonne J. H., Bol A. W., Hop W. C., van Wijk R.	Visual counting	Anti-IgE	% Degranulated basophils
Hirst S. J., Hayes N. A., Burridge J., Pearce F. L., and Foreman J. C.	Visual counting	Anti-IgE	% Degranulated basophils
Sainte-Laudy J. and Belon P.	Visual counting	Histamine	% Inhibition of basophil degranulation
Sainte-Laudy J. and Belon P.	Flowcytometry (*anti-IgE*+/CD63)	Histamine	% Inhibition of basophil CD63 expression
Sainte-Laudy J. and Belon P.	Flowcytometry (*anti-IgE*+/CD63)	Histamine	Basophil CD63 expression
Belon P. et al.	Visual counting	Histamine	% Inhibition of basophil degranulation
Sainte-Laudy J.	Flowcytometry (*anti-IgE*+/CD63)	Histamine, Histidine	% Activation of basophil degranulation
Brown V. and Ennis M.	Flowcytometry (*anti-IgE*+/CD63)	Histamine	% Inhibition of basophil CD63 expression
Lorenz I., Schneider E. M., Stolz P. Brack A., and Strube J.	Flowcytometry (*anti-IgE*+/CD63)	Histamine	% Inhibition of basophil CD63 expression
Lorenz I., Schneider E. M., Stolz P. Brack A., and Strube J.	Flowcytometry (*anti-IgE*+/CD63)	Histamine	Test stability, basophil CD63 expression, basophil CD63 expression
Belon P., Cumps J., Ennis M, Mannaioni P. F., Roberfroid M., Sainte-Laudy J., et al.	Visual counting	Histamine	% Degranulated basophils

Many other studies have copied the methodology used in Davenas' original investigation to replicate the findings as well as using it for research that does not target basophil degranulation (Table 2). These studies focus on the effects of ASD on different organisms. In the literature, only one study was found not to support the positive findings of ASDs. In that particular investigation, Labadie and Bollinger (1990) used heat to measure the mixing enthalpies of water and other aqueous solutions. Labadie and Bollinger reported that memory effects of water could not be observed; however within their methods for succussion of each dilution they agitated the solutions 15 seconds, while Davenas et al. (1988) agitated their solutions 10 seconds.

In contrast to the research reported above, eight studies reported results that were consistent with Davenas' original findings. For example, Endler, Pongratz, Kastberger, Wiegant, and Schutle (1994) observed the effect of highly diluted agitated thyroxine (iodine-containing hormone that increases the rate of cell metabolism and regulates growth) on the climbing activity of frogs. They noticed that there was less climbing in frogs treated with dilution thyroxin 10^{30} than those in the control group which were treated with pure water. Findings such as these support Davenas' results, even when looking at different animals. There have been other organisms such as plants that have been looked at using these same methods and have been found to also support the initial findings. For example, in several fruit studies it was demonstrated that homeopathic drugs have fungicidal properties on fruits infected with tomato fruit rot, guava fruit rot, or mango fruit rot (Khanna & Chandra, 1976, 1977, 1978). All of the studies that found positive results had the law of similars in common, meaning that the chemical diluted produces the same symptoms in healthy organisms as those with the disease. It thus

appears that law of similars is very important when investigating the effects of homeopathy.

Ever since Davenas' original study, there have been similar studies supporting the 'water memory' theory, yet no one really knows how it happens though. Since there is no exact explanation for this phenomenon, much of the scientific community is still in disbelief. Even though researchers have explored and begun to find new ways of explaining this phenomenon, it will still take many years before it is accepted, as it defies logic. Current research is exploring different ways to better explain how 'water memory' can occur.

Table 2

Research studies investigating biological effect of ASDs.

Researcher	Year of publication	Area of study	Supports the ASD's effectiveness
Khanna K. K., and Chandra S. *	1976	Control of tomato fruit rot caused by *Fusarium roseum* with homeopathic drugs.	Yes
Khanna K. K., and Chandra S. *	1977	Control of guava fruit rot caused by *Pestalotia psidii* with homeopathic drugs.	Yes
Khanna K. K., and Chandra S. *	1978	A homeopathic drug controls mango fruit rot caused by *Pestalotia mangiferae.*	Yes
Labadie, M. and Bollinger J. C.	1990	Micro-calorimetric study for the successive dilutions of aqueous solutions	No
Endler, P. C., Pontegratz, W., Kasberger, G., Wiegant, F. A. C. and Schulte, J.	1994	The effect of highly diluted agitated thyroxine on the climbing activity of frogs.	Yes

Jonas, W. B. and Dillner, D. K.	2000	Protection of mice from Tularemia infection with ultra-low serial dilutions prepared from *Francisella tularensis* infected tissue.	Yes
Schwartz, S.	2000	Use of herbal remedies to control pet behavior.	Yes
Baumgartner, S. M., Shah, D., Heusser, P. and Thurneysen, A.	2000	Homeopathic dilutions: is there a potential for application in organic plant production?	Yes
Brack, A., Strube, J., Stolz P. & Decker, H.	2003	Effectiveness of agitated serial dilutions on the luminescence of bacterium.	Yes

Note. * Research done prior to Davenas et al., 1988

Current Study

In the past most research has focused on exploring homeopathic effects at the cellular level. There has been a very limited amount of research done to explore the effects of homeopathy on organisms as a whole. Studies such as most of the ones in Table 2 support homeopathy at the cellular level therefore the intention of this study is to take on a more holistic approach. This study explored the potential use of homeopathic principles to create detrimental chemical solutions that might potentially be used as a form of bioterrorism. There was one hypothesis and one research question proposed when exploring the homeopathic effects of detrimental chemicals. Hypothesis 1 was that the ASD solutions were expected to have more of a detrimental effect on the organisms' behavior than the equivalent SD solutions. As a follow-up research question to the hypothesis, the ASD and SD difference was explored at each concentration level.

CHAPTER III

METHODOLOGY

The general strategy taken for this project was to dilute chemicals known to be poisonous to an organism and determine the effect of each diluted solution. In addition to investigating 10 serial dilutions, the effect of agitation was examined. Thus at each dilution level, there was an agitated solution (ASD) or non-agitated solution (SD). The three organisms used were chosen because of their importance to agriculture; these organisms include plants (*Lycopersicon lycopersicum*), insects (*Apis mellifera* L.), and fish (*Pimephales promela*).

Lycopersicon lycopersicum

Lycopersicon lycopersicum, a species of the Roma tomato was selected due to its sensitivity to pesticides. Any biological effects accrued should be observed instantaneously due to its sensitivity (Pfleger & Gould, 2001). Moreover the importance of investigating the effects of homeopathic solutions on agricultural species is of vital importance due to possible damage to crops.

Subjects

The tomatoes used were *Lycopersicon lycopersicum* L. Variable Roma VF. Six plants were used for each solution, ten ASD solutions and ten SD solutions, for a total of 120 tomato plants. TLC Florist & Greenhouses Inc., a commercial greenhouse located in Oklahoma City, was the location for this portion of the investigation. Seeds were planted in early February and were approximately 28 days old at the beginning of the experiment. The plants were grown and tested in a quiescent, double poly and hunt style greenhouse with ventilation. The light/dark cycle was of natural daylight. The temperature in the greenhouse was maintained at 55ºF or above during the day with natural ventilation; the heater turned on at 55ºF. Watering schedule was as needed.

Materials

The tomato plants were planted in a soil mix of TLC Premium potting soil containing approximately 60% Sphagnum peat moss, 26% composted bark, and 14% Fine Perlite. Perlite is a natural form of silicous rock. It is used in horticulture and is one of the ingredients in the soil that supplies aeration and optimum moisture retention for better-quality plant growth. One of the benefits of perlite is its neutral pH and the fact that it is sterile so there is no contamination of the soil. Perlite is also light which makes it ideal for use in container growing. Approximately 50g of soil was used and the plant was potted in a TLC 18 Web pot.

The pesticide used was Ortho ® Concentrate Weed Be Gon – Lawn Weed killer. The active ingredients are Mecoprop, dimethylamine salt (10.60%), 2, 4-D, dimethylamine salt (3.05%), Dicamba, dimethylamine salt (1.30%), and other ingredients

(85.05%). Mecoprop, 2, 4-D and Dicamba as a mixture are all chemicals that control broadleaf plants such as the Roma plant. The three active ingredients are synthetic herbicides that are absorbed into plant cells and disrupt plant growth. Plants affected overgrow, which is observed by the curling of the leaves and stem as well as the browning of the leaves. To reduce the experimenter bias factor, all chemicals were prepared by one person (who was not subsequently involved in the experiment) and were then coded and placed in containers that were identifiable only by randomly assigned letters and numbers.

Two separate solutions were made: one set was for the ASDs or the vigorously shaken solutions and the other set was the serially diluted solutions with no vigorous shaking (SDs). The solutions were prepared by taking 5ml of the Ortho ® Concentrate Weed Be Gon – Lawn Weed killer solution and making this up to a volume of 420ml. This was the stock solution that was used for the serial dilutions. The initial percentage of the active ingredients in the stock solution was 0.18%. For the vigorously prepared solutions, 70ml was taken from the stock solution and made up to a volume of 420ml with distilled water, giving a 1:6 dilution factor, or a sixth of the initial mixture. The solution was then vigorously stirred using a Thermolyne Model No. SPA1025B magnetic stirrer hot plate for 10 seconds in accordance to the method used by Davenas et al (1988). The first dilution thus results in the second test solution which contained 0.0296% of the active ingredients. The remaining solution (350ml) is not discarded, but kept for the first test solution. From this second solution, 70ml was taken and made up to a volume of 420ml with distilled water. The solution was then vigorously stirred for 10 seconds. This gave the third solution with 0.0049 or 4.9 E-03 percent of active ingredient present. The

dilution and shaking steps were repeated six times giving a total of nine solutions of the following percentages of active ingredient: 1.78 E -01, 2.97 E -02, 4.94 E-03, 8.24 E -04, 1.37 E -04, 2.2 E -05, 3.81 E -06, 6.36 E -07, and 1.06 E -07 all in order of successive dilutions (or concentration). There was also a tenth solution which was solely water, this was considered the control solution. Each of the dilution steps were carried out a second time for the SD solutions. These solutions were not vigorously stirred.

Procedure

The pesticide was applied by spraying to glisten, also referred to as "spray to moisten" (as opposed to spray to drip). In this method of spraying, only the amount of solution necessary to make the leaves moist (rather than making solution drip from the leaves) is sprayed on the leaves. Each plant was photographed daily, thus one hundred twenty pictures were taken each day of the individual plants (there were ten sets of dilutions for each of the two test solutions, each containing six plants). The condition of the plants or their health was rated on a daily basis by three individual experimenters, and the final ratings were by consensus. There were circumstances in which the ratings were initially not all the same for all three researchers. In those occurrences the three observers discussed reasoning for their rating and arrived at a consensus. The healthiness of the plants was rated on a scale from 1 to 5: one signifying that the plant was dead and five meaning the plant was healthy. There were eleven days of observation made on the plants. The observations were made at approximately 3:00 p.m. each day to control for any external variables. All tomato plants were used only once.

Apis Mellifera L.

Apis mellifera L. have been found to be affected indirectly by pesticide distribution (Abramson, Aquino, Ramalho, & Price, 1999; Abramson, Squire, Sheridan, & Mulder, 2004; Stone, Abramson, & Price, 1997). The pesticides effects are measured by the honeybees' proboscis extension. When the antennae of a bee are stimulated with a sucrose solution of sufficient concentration, the bee reflexively extends its proboscis in expectation of food, therefore if a honeybee's normal behavior would be affected there would be no proboscis extension and would likely die of hunger. The effect of the chemical was tested by proboscis extension response (PER).

Subjects

The honey bees (*Apis mellifera* L*)* were maintained in a hive in the open air in conditions that were completely natural. Twenty-five bees were used per solution for both ASDs and SDs, making the total sample 500 honey bees.

Materials

All materials utilized were sterilized prior to each experiment. A 10µl micro syringe was used to administer the chemical being fed to the honey bees. A 500ml volumetric flask, plastic pipettes, and 100ml measuring cylinders were utilized to dilute the chemical used. For the solutions in the ASD condition, a Thermolyne Model No. SPA1025B magnetic stirrer hot plate was employed.

The pesticide used was Sevin ® whose active ingredient is carbaryl. The chemical carbaryl works by disrupting an insect's nervous system and may be toxic if touched or eaten. Carbaryl also has a temporary effect on the nervous system. The procedure for the preparation of the solutions was the same as in the previous experiment, with several amendments. One of changes was the use of 5ml of the Sevin® Concentrate Bug Killer made up to a volume of 250ml. This gave a 1:50 dilution of the original concentrate form of the insecticide. This insecticide had a much larger initial dilution than the weed killer because of its creamy consistency, and so it was diluted to a mixture which would allow sufficient serial dilutions to be made without having a distinguishable color.

Following the initial dilution, 15ml of the mixture was taken and made up to a volume of 60ml with distilled water. For the ASD solutions, this was then vigorously stirred using a Thermolyne Model No. SPA1025B magnetic stirrer hot plate for 10 seconds. This was the first solution made containing 0.45% of the active ingredient carbaryl. From the 60ml of the mixture, 15ml was taken and put in a separate measuring cylinder; this was then made up to a volume of 60ml with distilled water. The solution was then vigorously stirred for 10 seconds. This gave the second solution a 1.1 E-01 percent of active ingredient. The dilution steps were repeated a further seven times giving a total of nine solutions of the following percentages of active ingredient: 4.50 E -01, 1.12 E -01, 2.81 E-02, 7.03 E -03, 1.75 E -03, 4.39 E -04, 1.09 E -04, 2.75 E -05, and 6.90 E -06 all in order of successive dilutions (or concentration). The tenth solution was the control solution which consisted of pure distilled water.

The same method was used for preparation of the non-vigorously prepared test solutions. The only amendment was no agitation of the solutions was done between each

dilution step. The percentage of active ingredients remained the same as in the preparation of the ASDs.

Procedure

The experiment was carried out by randomly collecting the bees 24 hours prior to the day of trials. The honey bees were collected either at the entrance of the hive (the guard honey bees) or on departure from the hive (these are the honey bees that go out and search for nectar - foragers). Approximately 60 different honey bees were captured each day for two weeks using vials with perforated lids for ventilation. The honey bees were placed in ice to immobilize them for transferring from the vials to the harnesses. The honey bees were not immersed in the ice for a long period as this may result in nerve damage or death. The harnesses are comprised of a partially cut bullet and a strip of duct tape, which is used to secure the subject in the harness. The tape was placed between the subject's head and thorax and the tape went straight across from one side to the other. This method was designed by Bitterman, Menzel, Fietz, and Schafer (1983). The honey bees were then left for 15 to 30 minutes to fully regain consciousness and were fed 1.8M sucrose solution until the bees no longer stuck out their proboscis to feed. This feeding occurred 24 hours before the test session. The feeding of the subjects 24 hours prior to the day of the experiment helps to control for any variations in the feeding responses of the subjects when given the test solution, or to ensure a proboscis extension response (due to being hungry) when stimulated by sucrose.

On the day of the experiment, the subjects to be used were screened to determine which would be used for the experiment. The prescreening was done by touching the

antenna of the bees with the 1.8M sucrose solution and looking for any proboscis extension, but the subjects were not fed the sucrose. The bees that did not respond were released while the subjects that responded to the sucrose were divided into the different test groups. Approximately 60 subjects were tested each day, throughout the day. The subjects that showed a response when prescreened were treated with one of the solutions. This was done by feeding the subjects 1μ of the test solution, and to ensure that the bees fed on the solution, an antenna was touched with the sucrose solution so that there was proboscis extension whilst the bees were held over the test solutions, of which they fed upon. Ten minutes were given as the feeding period (Stone et al., 1997). After the ten minutes were over, the first trial was initiated. The PER of each bee was rated by one individual experimenter for each session. Each subject was tested by touching the antenna with the sucrose solution and one the response was then measured based on whether there was extension of the proboscis or not. A healthy bee will stick out its proboscis when stimulated with a sucrose solution. Each subject had antennal stimulation for approximately five seconds. There was an inter-trial interval of 15 minutes with a total of four intervals meaning that the bees were given a stimulus every 15 minutes running to a total of an hour. Following the experiment, the subjects treated with the pesticide were eliminated, no bee was used twice.

Pimephales promela

Pimephales promela, commonly known as rosy red minnows, were selected for the experiment because they have been used in agriculture as a test species for water

toxicity. It has also been found that with this particular species, the insecticide Spectracide Bug Stop® Home Insect Killer is highly toxic. In particular, one of the active ingredients in this insecticide, lambda cyhalothrin is toxic to fish (Mueller-Beilschmidt, 1990).

Subjects

The subjects were *Pimephales promela*, commonly known as rosy red minnows, or Fathead minnows. Twenty-five were used for each solution, ten ASD solutions and ten SD solutions, for a total of 500 rosy red minnows. Purchased from Wal-Mart, the fish were selected to be relatively of the same size. The fish were maintained in a tank that had constant filtration and the tank was partially cleaned every two or three days. The temperature of the water was maintained at room temperature and only freshwater was used.

Materials

All bowls used were sterilized prior to the experiments. Twenty individual 325ml glass bowls were used to house the fish for the duration of the experiment. For the dilution process plastic pipettes and measuring cylinders of 100ml each were used. Stop watches were utilized to measure the inter-trial interval time. The pesticide used was Spectracide Bug Stop® Home Insect Killer active ingredients are Lambda-Dyhalothrin (0.03%) and other ingredients (99.97%). A preliminary experiment was carried out to determine the dependent variables to use for recording the effects that the active ingredient lambda cyhalothrin had on the subjects. In order to discern which dependent

variables were to be measured, fish were given the pesticide with the highest concentration and were observed for the behavior patterns relating to dire health. The behavior patterns with easily observable characteristics were chosen.

The protocol for preparation of the solutions was the same as that of experiment one, with several amendments. 50ml of the Spectracide solution was made up to a volume of 200ml with purified water (giving a 1:4 dilution). For the ASD solutions, the mixture was stirred using a magnetic stirrer for a maximum of 10 seconds (i.e., it was shaken vigorously). This was the stock solution that was used for the serial dilutions. The initial percentage of the active ingredients in the stock solution was 7.5 E-03 percent. From this solution, 50ml was transferred into another volumetric flask and made up to a volume of 200ml with water. The mixture was shaken vigorously, giving the second solution a 1.8 E -03% of active ingredient. The process as above was then repeated a further eight more times to get a potency of nine different concentrations of the following percentages of active ingredient: 7.50 E -03, 1.87 E -03, 4.69 E-04, 1.17 E -04, 2.93 E -05, 7.32 E -06, 1.83 E -06, 4.58 E -07, and 1.14 E -07 all in order of successive dilutions (or concentrations). The same method was used for preparation of the non-vigorously prepared test solutions. The only amendment is that there is no agitation of the solutions involved between each dilution step.

Procedure

The experiment was carried out by randomly collecting an individual fish from the tank and transferring it straight from the tank using a fishnet into the test bowl. Immediately after transferring the subject into the test bowl, a ten second observation was

made to measure the three dependent variables and these were recorded on a data sheet. The dependant variable of each fish was rated by one individual experimenter for each session. The dependant variables measured were tremors, breathing, and fin movement. The dependent variables were operationally defined as follows:

Tremors: This was assessed by recording the twitching, or swimming behavior of the fish. The observations were recorded by a rating from 0-3. 0 was no movement, 1 was infrequent swimming or slight movement, 2 was general swimming and more frequent shaking, while 3 was constant tremors. A tremor was defined as erratic body movement or convulsions/seizures and its presence is an indicator of poor health.

Breathing: This was assessed by recording if the minnows were breathing and this was recorded as either a yes (1) or no (0).

Fin movement: This was assessed by recording either a yes (1) or no (0) for whether the fish were moving their fins or not (movement of the fins on either side of the body was observed; movement of fins was good, meaning the fish was behaving normally).

The inter-trial interval was 2 minutes. Two minutes following the first observation, the second observation began and the dependent variables were measured again. The experiment ceased either when the subject died, or when the total duration of the experiment was reached (200 minutes). The fish that survived the experiment were put in a separate tank and were not used again.

CHAPTER IV

FINDINGS

Overview

All hypotheses were tested with mixed design ANOVAs. Time was a within subjects factor, agitation was a between subjects factor [2 levels, agitated (ASD) or not (SD)], and solution was a between subjects factor (10 levels, nine dilution levels and one control). The first hypothesis stated that the effects of the ASD solution would be more detrimental on the organism's behavior than the SD solution. This hypothesis was tested by examining the main effect for agitation. The research question concerned if the ASD and SD solutions were different at each concentration level. This was tested by running a multiple comparisons analysis with a Bonferroni adjustment. Because the results for the other effects tested in this design were not of primary interest in the current study, these results are only presented in the Appendix. Results for time and time by solution were all significant yet results for the time by agitation and time by solution by agitation interactions were mixed. The results for the agitation by solution effect were mixed as well.

Hypothesis 1

Hypothesis 1 stated that the ASD solution effects would be more detrimental on the organism's behavior than the SD solution. The main effect of agitation was examined for all three organisms in order to test this hypothesis.

Lycopersicon lycopersicum. The observations of the plants were made over a total of 11 days and the dependent variable was the overall health of the plants (range 1-5; higher scores represent better health). Although the main effect of agitation [$F(1, 100) = 45$, $p < .001$, *partial eta-squared* = .31, *power* = 1.00] was found to be statistically significant, hypothesis 1 was not supported being that the means showed that the SD solutions were more detrimental than the ASD solutions ($M_{ASD} = 3.50$ and $M_{SD} = 3.48$). If the mean for the ASD solutions would have been lower than that of the SD solutions more of the plants in the ASD condition would have died and this would be attributed to the agitation of the solutions and therefore Hypothesis 1 would have been supported.

Apis mellifera L. Proboscis extension responses (PER) of honey bees were observed and recorded as 0 (no extension response) or 1 (extended proboscis). When observing for the main effects of agitation, it was found to be non-significant [$F(1, 480) = 2.91$, $p = .09$, *partial eta-squared* = .006, *power* = .398], thus not supporting Hypothesis 1. Even though the difference between agitations was found not significant, there were more honey bees that did not extend their proboscis when treated with ASD solutions ($M = .802$) than those treated with the SD ($M = .847$), which was the hypothesized direction. This meant that for the honey bees the ASD solutions did have a more detrimental effect than the non agitated solutions but not enough to be statistically significant.

Pimephales promela. The dependent variables were tremors, breathing, and fin movement for the rosy red minnows. Tremors were measured by frequency of occurrence (high numbers indicate poor health), while breathing and fin movement were denoted by a yes (1) or no (0). The results were consistent across all three variables. When looking at tremors, the main effect of agitation was found to be statistically significant [$F(1, 475) =$

5.84, $p = .02$, *partial eta-squared* = .012, *power* = .674]. Specifically, it was found that the fish placed in the agitated solutions condition had more tremors ($M_{ASD} = 3.03$) than the fish in the non agitated solutions ($M_{SD} = 2.91$), thus Hypothesis 1 was supported. Breathing was then observed and the main effect of agitation shown to be statistically significant [$F(1, 480) = 4.09$, $p = .04$, *partial eta-squared* = .008, *power* = .523]. As evidenced by the means ($M_{ASD} = .373$; $M_{SD} = .406$), Hypothesis 1 was supported again being that the agitated solutions caused more fish to die than the fish placed in the SD solutions. Finally, fin movement, like the other two variables observed, had a main effect of agitation that was statistically significant [$F(1, 480) = 99.73$, $p < .001$, *partial eta-squared* = .172, *power* = 1.00]. Once again Hypothesis 1 was supported since there was less occurrence of fin moment in the ASD group ($M = .247$) than in the SD group ($M = .406$).

Research Question 1

Research question 1 explored if the ASD versus SD effects would be seen at every concentration level. Observing the same behaviors as the ones used to test Hypothesis 1, multiple comparisons were examined to test Research Question 1 (see Table 3).

Lycopersicon lycopersicum. When observing plant health, it was found that most dilutions were statistically significant; however, only three ASD solutions had a more detrimental effect than SD solutions. The ASD dilutions that had a more detrimental effect were dilutions 1.78 E -01, 1.37 E -04, and 2.2 E -05 which were not the least concentrated solutions [$F(1,100) = 245{,}000$, $p < .001$, *partial eta-squared* = 1.00, *power* = 1.00, $F(1,100) = 296{,}450$, $p < .001$, *partial eta-squared* = 1.00, *power* = 1.00 and F

(1,100) = 72,200, $p < .001$, *partial eta-squared* = .99, *power* = 1.00 respectively]. Not only were these solutions not the least concentrated solutions they were also in no particular order to which a hypothesis could be composed.

Table 3

Table of Means for Agitation by Solution Interaction for Lycopersicon lycopersicum

	Agitation	
Solution concentration	ASD	SD
1.78 E -01*	1.45	4.63
2.97 E -02	1.36	1.36
4.94 E -03*	4.72	1.36
8.24 E -04*	4.45	3.09
1.37 E -04*	1.36	4.86
2.29 E -05*	3.18	4.90
3.81 E -06*	4.90	4.64
6.36 E -07*	4.45	4.18
1.06 E -07*	4.36	1.36
0*	4.73	4.45

Note: * = $p < .001$(Adjusted for multiple comparisons: Bonferroni)

Apis mellifera L. When observing the multiple comparisons analysis for the honey bees' PER it was found that only one dilution was statistically significant (see Table 4 for means). The dilution that was found to be significant was also one in which the ASD had a more detrimental effect than the SD counterpart. As with the *Lycopersicon lycopersicum* the bees did not have any evidence of a hypothesis forming pattern for Research question 1.

Table 4

Table of Means for Agitation by Solution Interaction for Apis mellifera L.

	Agitation	
Solution concentration	ASD	SD
4.50 E -01	.930	.860
1.12 E -01	.870	.900
2.81 E -02	.720	.760
7.03 E -03	.850	.810
1.75 E -03	.820	.840
4.39 E -04	.800	.840
1.09 E -04 *	.720	.950
2.75 E -05	.600	.710
6.90 E -06	.830	.870
0	.880	.930

Note: * = $p < .001$ (Adjusted for multiple comparisons: Bonferroni)

Pimephales promela. Once again the last organism observed was the Rosy Red Minnow**.** When looking at tremors, the multiple comparisons showed that dilutions 7.32 E -06 and 1.83 E -06 were significant; yet these dilutions were not the least concentrated solutions (see Table 5). The solutions that had a more detrimental ASD effect were solutions from the middle of the sequence; however, since there were only two significant solutions it was not considered a profitable finding for Research question 1. If more dilutions would have been found significant and they would have been either all in high concentration, all in low concentration or all in the middle this would have created a pattern which would have let us see there was a particular occurrence besides a difference

with agitation. Breathing was then observed and as with tremors multiple comparisons showed dilutions 7.32 E -06 and 1.83 E -06 to be significant once again and again not being enough to reveal a pattern in dilution and agitation effects. Finally, fin movement, unlike the other two variables observed in the fish had five of the ten dilutions that were found significant and the least diluted ASD solutions were more detrimental than the SD solutions. This pattern can therefore lead us to hypothesize that highly diluted ASD solutions would have worse effects than highly diluted SD or any of the least diluted solutions. This coincides with homeopathic principles which state the more a chemical is diluted the more potent it becomes.

Table 5

Table of Cell Means for Pimephales promela Dependant Variables

Solution Concentration	Tremor		Breathing		Fin movement	
	ASD	SD	ASD	SD	ASD	SD
7.50 E -03	3.82	3.80	.051	.055	.031	.055
1.87 E -03	3.72	3.72	.082	.084	.043	.084
4.69 E -04	3.60	3.61	.131	.128	.076	.128
1.17 E -04	3.57	3.52	.167	.165	.122	.165
2.93 E -05	3.47	3.44	.215	.211	.132	.211
7.32 E -06	3.32*	2.85	.255*	.434	.165*	.434
1.83 E -06	2.98*	2.62	.414*	.530	.219*	.530
4.58 E -07	2.23	2.14	.702	.771	.485*	.771
1.14 E -07	2.10	2.04	.743	.729	.486*	.729
0	1.43	1.40	.969	.952	.712*	.952

Note: * = $p < .001$ (Adjusted for multiple comparisons: Bonferroni)

CHAPTER V

CONCLUSIONS AND DISCUSSION

This research explored the potential use of homeopathic principles to create detrimental chemical solutions that could potentially be used for bioterrorism. With the growing concern for terrorism and public safety, this investigation was deemed timely and important. Literature reviewed showed that there are not many investigations that focus on the effects of homeopathic dilutions on animals or living organisms as a whole, hence the importance of this particular research.

Lycopersicon lycopersicum

The first organism studied was a species of the Roma tomato, *Lycopersicon lycopersicum*. The experiment tested the sensitivity of the plant to Ortho® Concentrate Weed Be Gon - lawn weed killer. The three active ingredients Mecoprop, 2, 4-D and Dicamba are, as already mentioned, synthetic herbicides that increase the rate at which the plants grow, and so the plants in a matter of speaking 'grow to death'. The signs of the effects are curling of the leaves and bending of the stalk. The results showed that there was a significant difference in health, sometimes as quick as a day after application of the solution. However, the declining health of the plant did not only occur with ASD solutions but with SD solutions as well. The only ASD solutions that had more detrimental effects than the SD solutions were 1.78 E -01, 1.37 E -04 and 2.2 E -05. Solution 1.78 E -01 is the least diluted solution of the series while solutions 1.37 E -04

and 2.2 E -05 are in the middle. According to homeopathic principles agitating a solution dilution should be more effective than the same dilution when not agitated. The results from the *Lycopersicon lycopersicum* experiment do not support this idea which was stated in Hypothesis 1 because more plants died when they were in the SD condition than those in the ASD condition. After observing the multiple comparisons for the Roma tomato plant there was no consistent pattern of results and therefore nothing that stood out as pertinent to Research Question 1.

There are two possibilities of why the results were inconsistent. The first is potential rater problems. Although the raters did come to a consensus on their ratings, we did not obtain inter-rater reliability nor did we record the number of times there were disagreements. There are a few cells in which the ratings are inconsistent with the pattern of the data. For example, in the most concentrated SD solution, the plants were rated very healthy and in the next two subsequent concentrations, they are almost all dead. Another possible explanation is just random variation in the health of the plants. Although 6 plants were used at each concentration to try and account for this, there was a significant difference between the SD and the ASD solutions in the control condition which was just water. If all the plants were the same or the raters were rating the plants consistently, then there should not have been a difference between these two conditions. It is thus unclear exactly what happened in the plants, but what is clear is that the ASD versus SD effects seen are more than likely due to chance and not homeopathy.

Apis mellifera L.

In the second experiment, honey bees were fed the different diluted solutions and the PER was observed and measured. Results which tested both the hypothesis and research question led to the rejection of one and no note worthy observation of the other. In the *Apis mellifera* L., like with the plants, ASD solutions did not have a more detrimental effect than the equivalent SD solutions and the highly diluted ASD solutions did not cause more damage than the other solutions. Abramson et al (2004) found that the agricultural pesticides that were referred to as being not harmful to honey bees had an effect on their learning behavior. This was then found to affect their foraging abilities. In the current study however, the chemical used does not appear to have affected PER. It could also be that this particular chemical affects a particular physiological response of the honey bee, but not one dealing with its abilities to forage. With the honeybees as with the Roma tomato plants an inter-rater reliability test was not conducted. In this case there was only one rater per bee; however this could still be a reason to why there was no effect found in the honey bees PER behavior.

Pimephales promela

Tremors, breathing, and fin movement were the dependant variables measured in the Rosy Red Minnows. In all three dependant variables the ASD condition as a whole had a greater detrimental effect than the SD counterpart. This was the only organism that had results which supported Hypothesis 1. However when exploring the multiple comparisons the highly diluted ASD effect did not occur in two of the dependant variables (tremors and breathing), yet in fin movement five ASD solutions were seen to

have a more detrimental effect than the SD. The ASD solutions that had more of an effect than their SD counterpart solutions were 7.32 E -06, 1.83 E -06, 4.58 E - 07, 1.14 E -07, and the control. The results of this particular dependant variable supported one of the homeopathic ideas in that extremely diluted solutions have a greater effect than more concentrated solutions as long as they have been agitated. These findings may lend support to the idea that it is important to assess a variety of dependent variables for each organism. Although the hypothesis was not supported within tremors and breathing, a logical pattern of results occurred. In both conditions as the concentrations get less, the fish do better (fewer tremors, more breathing and fin movement). This makes logical sense in that as the dosage of poison gets less, it has less of an effect.

Strengths, Limitations and Future Direction

A strength of this investigation was that the focus was done on organisms as a whole, rather than individual cellular responses. Although the findings were not as expected, the direction that was taken was innovative. It is interesting to note that in the previous cellular studies that used water as the distillation solution, there might be a confound. Specifically, any cell will degranulate when plain water is added to it because it is not isotonically balanced with the interior of the cell (i.e., the salt concentration of the interior of the cell is higher), so the cell will absorb the water until it explodes. Thus in previous research in which the investigators thought they found evidence for homeopathy, it may have been a phenomenon of cellular biology. This is another reason

why the current study was a more sophisticated test of homeopathic principles as it looked at whole organisms and not cells.

Some of the limitations of the current study are that inter-rater reliability was not calculated for the plant, bee or fish raters. It is also unclear if all of the plants and fish were "identical" and all equal before the solutions were applied. Future research should include pre-test ratings of all the dependent variables to rule out pre-existing differences. Finally, it should be noted though that in this investigation, the solutions were not diluted down to an immeasurable amount. Specifically, the least concentrated solutions were still approximately one microgram per liter when traditionally homeopathy dilutions are 12C or 24X (Shelton, 2004). In the future not only should the solutions be diluted to 24X but follow dilution measures in the literature in order to have a more accurate comparison.

The intent of this study was to investigate the potential use of ASD as a form of agroterrorism. Hypothesis 1 stated that any dilution would have a damaging effect as long as the solution was agitated. This would mean that if anyone took a pesticide or poison and succussed the solution then administered it on crops or livestock these organisms would have effects that could be as simple as damage but as dire as death to the organism. Even with the concern of potential bioterrorism due to the terrorist attacks in 2001 this study gives us knowledge that homeopathy is not of concern for potential bio or agroterrorism. Within the findings from this investigation only one organism found to support homeopathic effects. *Pimephales promela* was the only organism which showed that agitating a solution was more detrimental than the same solution when not agitated; however being that this was the only organism that the ASD affected more than the SD solution this can be partly do to random chance. Therefore with these findings

homeopathic use is not seen as concern for potential use of agroterrorism. Further investigation would have to prove otherwise.

Homeopathy has been used for over 200 years and despite the fact that scientists disagree about its veracity, many people still believe it is highly effective. The results of the current study suggest that the reason homeopathy might be "effective" at times might be due to mere coincidence or perhaps the placebo effect. The "water memory" phenomenon will continue to be a controversial topic for years to come. In this particular investigation however, it was found that water does not have memory when affecting organisms as a whole.

REFERENCES

Abramson, C. I., Aquino, I. S., Ramalho, F. S., & Price, J. M. (1999). Effect of insecticides on learning in the Africanized honey bee (Apis mellifera L.). *Archives of Environmental Contamination and Toxicology*, *37*, 529-535.

Abramson, C. I., Squire, J., Sheridan, A., & Mulder, Jr., P. G. (2004). The effect of insecticides considered harmless to honey bees (Apis mellifera L.): Proboscis conditioning studies using the insect growth regulators Confirm® 2F Tebufenozide) and Dimilin ® 2L (Diflubenzuron). *Environmental Entomology, 33*, 378-388.

Bitterman, M.E, Menzel, R., Fietz, A., Schafer, S. (1983). Classical conditioning of proboscis extension in honeybees (Apis mellifera). *Journal of Comparative Psychology*, *97*, 107-119.

Breithaupt, H. (2000). Toxins for terrorists. *European Molecular Biology Organization Reports*, *1*, 298 – 301.

Cain, S. (2001). *Agroterrorism.* Retrieved September 26, 2005, from Purdue University, Agricultural Economics Web site: http://knxas1.hsdl.org/homesec/docs/dhs/nps08-111303-04.pdf

Davenas, E., Beauvais, F., Arnara, J., Oberbaum, M., Robinzon, B., & Benveniste, J. et al. (1988). Human basophil degranulation triggered by very dilute antiserum against IgE. *United Kingdom: Nature 333*, 816-818.

Del Giudice E., Preparata G., & Vitiello G. (1988). Water as a free electric dipole laser. *Physical Review Letters, 61*, 1085-1088.

Endler, P. C., Pontegratz, W., Kasberger, G., Wiegant, F. A. C., & Schulte, J. (1994). The effect of highly diluted agitated thyroxine on the climbing activity of frogs. *Veterinary and Human Toxicology*, *36*, 56-59.

Guggisberg, A. G., Baumgartner, S. M., Tschopp, C. M., & Heusser, P. (2005). Replication study concerning the effects of homeopathic dilutions of histamine on human basophil degranulation in vitro. *Complementary Therapies in Medicine*, *13*, 91-100.

Hirst, S. J., Hayes, N. A., Burridge, J., Pearce, F. L., & Foreman, J. C. (1993). Human basophil degranulation is not triggered by very dilute antiserum against human IgE. *Nature*, *366*, 525-528.

Khanna K. K., Chandra S. (1976). Control of tomato fruit rot caused by *Fusarium roseum* with homeopathic drugs. *Indian Phytopathology*, *29*, 269-272.

Khanna, K. K., Chandra, S. (1977). Control of guava fruit rot caused by *Pestalotia psidii* with homeopathic drugs. *Plant Disease Report*, *61*, 362-366.

Khanna, K. K., Chandra, S. (1978). A homeopathic drug controls mango fruit rot caused by

Pestalotia mangiferae Henn. Experentia, 34, 1167-1168.

Labadie, M., & Bollinger, J. C. (1990). Microcalorimetric study for the success dilutions of aqueous solutions. *Thermochemica Acta*, *162*, 445-448.

Larkin, M (2001). New technology to combat terrorism set for deployment. *The Lancet*, *358*, 1708.

Marmagas, S.W., King, L.R. & Chuk, M.G. (2003). Public health's response to a changed world: September 11, biological terrorism, and the development of an environmental health tracking network. *American Journal of Public Health*, *93*, 1226.

Merriam-Webster's Collegiate Dictionary. (2002). Springfield, MA: Merriam Webster, Inc.

Miller, J., Engelberg, S., & Broad, W. (2001). *Germs: Biological Weapons and America's Secret War.* NY: Simon & Schuster.

Microsoft Encarta Reference Library. (2005). United States: Microsoft Corporation.

National Research Council of the National Academies. (2003). *Countering Agricultural Bioterrorism*. Washington, DC: The National Academies Press.

O'Toole, T., Inglesby, T.V. (2000). Facing the biological weapons threat. *The Lancet*, *356*, 1128-1129.

Pimentel, B. & Evangelista, B. (2001, September 17). Tech vs. Terrorism Airports look to new Technologies to beef up security. *San Francisco Chronicle*. Retrieved October 31, 2005, from http://sfgate.com

Pfleger, F. L., & Gould, S. L. (2001). Nonparasitic Disorders of Tomato (University of Minnesota Extension Service). Abstract retrieved March 28, 2005, from

http://www.extension.umn.edu/distribution/horticulture/DG1156.html

Sainte-Laudy, J., & Belon, P. (1993). Inhibition of human basophil activation by high dilutions of histamine. *Agents and Actions*, *38*, C245-C247 Sp. Iss. CI.

Samal, S. & Geckeler, K. E. (2001). Unexpected solute aggregation in water on dilution. *Chemical Communication,* 2224.

Shelton, J. W. (2004). *Homeopathy : How it really Works*. Amherst, NY: Prometheus Books.

Stone, J.C., Abramson, C.I., & Price, J.M. (1997). Task dependent effects of dicofol (Kelthane) on learning in the honey bee (Apis mellifera). *Bulletin of Environmental Contamination and Toxicology*, *58*, 177-183.

Turvey, C.R., Mafoua, E., Schilling, B., & Onyango, B. (2003, July). *Economics, Hysteresis and Agroterrorism*. Paper presented at the meeting of the Canadian Agricultural Economics Society, Montreal, Quebec.

Vallance, A. K. (1998). Can Biological Activity be maintained at Ultra-High Dilution? An Overview of homeopathy, Evidence, and Bayesian Philosophy. *Journal of Alternative and Complementary Medicine*, *4*, 49-76 SPR.

APPENDIX

Analysis of Variance for Homeopathic Effects on Lycopersicon lycopersicum

Source	*df*	*F*	η	power
	Between subjects			
Solution (S)	9	100911*	1.00	1.00
Agitation (A)	1	45*	.310	1.00
S X A	9	128400*	1.00	1.00
S within-group error	100	(.001)		
	Within subjects			
Time (T)	1.24	6930*	.986	1.00
T X S	11.16	497*	.978	1.00
T X A	1.24	179*	.642	1.00
T X S X A	11.57	579*	.981	1.00
T X *S* within-group error	123.97	(.035)		

Note. Values enclosed in parentheses represent mean square errors. *$p < .001$.

Analysis of Variance for Homeopathic Effects on Apis mellifera L.

Source	*df*	*F*	η	power
	Between subjects			
Solution (S)	9	3.30**	.058	.984
Agitation (A)	1	2.90	.006	.398
S X A	9	.954	.018	.479
S within-group error	480	(.349)		
	Within subjects			
Time (T)	2.72	11.78**	.024	.999
T X S	24.49	2.08*	.038	.997
T X A	2.72	1.60*	.003	.401
T X S X A	24.49	1.17*	.021	.900
T X *S* within-group error	1306	(.076)		

Note. Values enclosed in parentheses represent mean square errors. *$p < .05$. **$p \leq .001$.

Analysis of Variance for Homeopathic Effects on Pimephales promela (Tremors).

Source	*df*	*F*	η	power
	Between subjects			
Solution (S)	9	11.83**	.183	1.00
Agitation (A)	1	5.84*	.012	.674
S X A	9	113.70**	.683	1.00
S within-group error	475	(27.05)		
	Within subjects			
Time (T)	25.407	240.92**	.337	1.00
T X S	228.66	13.02**	.198	1.00
T X A	25.41	.784	.002	.719
T X S X A	228.66	1.06	.020	1.00
T X *S* within-group error	12068	(2.90)		

Note. Values enclosed in parentheses represent mean square errors. *$p < .05$. **$p \leq .001$.

Analysis of Variance for Homeopathic Effects on Pimephales promela (Breathing).

Source	*df*	*F*	η	power
	Between subjects			
Solution (S)	9	12.08**	.185	1.00
Agitation (A)	1	4.09*	.008	.523
S X A	9	143.51**	.729	1.00
S within-group error	480	(3.40)		
	Within subjects			
Time (T)	5.68	573.76**	.544	1.00
T X S	51.11	30.71**	.365	1.00
T X A	5.68	1.09	.002	.424
T X S X A	51.11	1.19	.022	.995
T X *S* within-group error	2725.98	(.736)		

Note. Values enclosed in parentheses represent mean square errors. *$p < .05$. **$p \leq .001$.

Analysis of Variance for Homeopathic Effects on Pimephales promela (Fin Movement).

Source	*df*	*F*	η	power
	Between subjects			
Solution (S)	9	14.86**	.218	1.00
Agitation (A)	1	99.73**	.172	1.00
S X A	9	111.18**	.676	1.00
S within-group error	480	(3.20)		
	Within subjects			
Time (T)	11.35	253.80**	.346	1.00
T X S	102.16	12.97**	.196	1.00
T X A	11.35	10.23**	.021	1.00
T X S X A	102.16	1.60**	.029	1.00
T X *S* within-group error	5448.61	(.578)		

Note. Values enclosed in parentheses represent mean square errors. *$p < .05$. **$p \leq .001$.

VITA

Brenda L. Morales

Candidate for the Degree of

Master of Science

Thesis: THE EFFECT OF AGITATED SERIAL DILUTIONS ON WHOLE ORGANISMS: ROMA TOMATOES (*LYCOPERSICON LYCOPERSICUM*), HONEY BEES (*APIS MELLIFERA* L) AND ROSY RED MINNOWS (*PIMEPHALES PROMELA*).

Major Field: Psychology

Biographical:

Education: Received a Bachelor of Science in Psychology with a Minor in French from University of Texas-Pan American, Edinburg, Texas in May, 2002; completed requirements for the Master of Science degree in Experimental Psychology at Oklahoma State University May 2006.

Experience: Graduate assistant in the Department of Psychology, Oklahoma State University, Stillwater, Oklahoma, 2002-2004 and 2005-present; Teaching Assistant in the Department of Psychology, Oklahoma State University, Stillwater, Oklahoma, 2004-2005.

Professional Memberships: American Psychological Association, Sigma Xi, American Zoo & Aquarium Association, Psi Chi Honor Society, Phi Beta Delta Honor Society.

Name: Brenda L. Morales Date of Degree: July, 2006

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: THE EFFECT OF AGITATED SERIAL DILUTIONS ON WHOLE ORGANISMS: ROMA TOMATOES (*LYCOPERSICON LYCOPERSICUM*), HONEY BEES (*APIS MELLIFERA* L.) AND ROSY RED MINNOWS (*PIMEPHALES PROMELA*).

Pages in Study: 46 Candidate for the Degree of Master of Science

Major Field: Psychology

Scope and Method of Study: This study explored the potential use of homeopathic principles to create detrimental chemical solutions that might potentially be used as a form of bioterrorism. The general strategy taken for this project was to dilute chemicals known to be poisonous to an organism and determine the effect of each diluted solution. In addition to investigating 10 serial dilutions, the effect of agitation was examined. The three organisms used were chosen because of their importance to agriculture; these were plants (*Lycopersicon lycopersicum*), insects (*Apis mellifera* L.), and fish (*Pimephales promela*).

Findings and Conclusions: Hypothesis 1 stated that any dilution would have a damaging effect as long as the solution was agitated. This would mean that if anyone took a pesticide or poison and succussed the solution then administered it on crops or livestock these organisms would have effects that could be as simple as damage but as dire as death to the organism. Even with the concern of potential bioterrorism due to the terrorist attacks in 2001 this study gives us knowledge that homeopathy is not of concern for potential bio or agroterrorism. Within the findings from this investigation only one organism found to support homeopathic effects. *Pimephales promela* was the only organism which showed that agitating a solution was more detrimental than the same solution when not agitated; however being that this was the only organism that the ASD affected more than the SD solution this can be partly do to random chance. Therefore with these findings homeopathic use is not seen as concern for potential use of agroterrorism.

ADVISER'S APPROVAL: _____