THE EFFECT OF SERIAL AGITATED DILUTION ON THE MORTALITY RATE IN HONEY BEES (*APIS MELLIFERA* L.)

By

SONDRA L. NOLF

Bachelor of Science in Counseling

Baptist Bible College

Clarks Summit, PA

2003

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
December, 2008

THE EFFECT OF SERIAL AGITATED DILUTION ON THE MORTALITY RATE IN HONEY BEES (*APIS MELLIFERA* L.)

Thesis Approved:

Dr. Charles I. Abramson

Thesis Adviser

Dr. Jennifer Byrd-Craven

Dr. James Grice

Dr. A. Gordon Emslie

Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to thank all those who made the completion of this project possible. Thanks to John Bauman, Joshua Clutter, and Landon Massoth for preparing the dilutions of both the pesticide and the sucrose. I would also like to thank T. Andrew Mixson for his help in collecting and running the subjects this summer. Thanks also to Drs. Charles Abramson, James Grice and Jennifer Byrd Craven for serving as members of my thesis committee. Lastly, I would like to thank Dr. James Grice for his patience and invaluable assistance in the statistical analysis of this experiment.

I would like to acknowledge the continued work and support of my mentor Dr. Charles Abramson on this paper. His wisdom and guidance have been a huge part of the success of this project.

TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION 1

Significance of Proposed Study 1

II. REVIEW OF LITERATURE 3

History of Homeopathy 3
Hahnemann's Findings 3
Davenas Study 5
Replications of Serial Agitated Dilutions 7
Serial Dilution Affect Applied to Animal Behavior 10
Current Study 11

III. METHODOLOGY 12

Subjects 12
Materials 13
Procedure 14
Capturing and harnessing *Apis mellifera* L. 14
Dilution of Pesticide, Sucrose and Water 14
Experiment 1: Pesticide 15
Experiment 2: Sucrose 16

IV. FINDINGS 17

Experiment 1 17
Experiment 2 18

V. CONCLUSION 20

Experiment 1 20
Experiment 2 21
Future Direction and Conclusion 22

REFERENCES 25

APPENDICES 29

LIST OF TABLES

Table Page

Table 1. Overview of studies investigating the effects of homeopathic solutions on human basophils and methodologies used 7

Table 2. Research studies investigating biological effect of ASDs 9

Table 3. Frequencies 18

Table 4. Frequencies 19

CHAPTER I

INTRODUCTION

Significance of the Proposed Study

In 2002, the defense-spending bill (HR 3338) approved over $300 million for the United States Department of Agriculture (USDA) to support agroterrorism investigations, upgrade facilities, and improve border control (National Research Council, 2003). In total, approximately $22 billion has been funded for civilian biodefense from 2001-2005 (Schuler, Fitzgerald, Inglesby, & O'Toole, 2004). This large amount of funding is a clear indication of the emphasis that the United States directs toward research on biological defense. With this renewed emphasis on use of biological agents as weapons, a question comes to mind: can bioterrorism occur in front of us without it being traceable or noticeable? Davenas et al. (1988) obtained results that suggested that a completely diluted chemical can still have biological effects on a living organism. These results suggest alarming outcomes, which if abused, could be devastating.

The current study investigated the use of homeopathic principles to create detrimental chemical solutions that could go undetected and be untraceable. The two principles that were investigated in this study were: the effects of dilution and serial agitation.

The first goal of the study was to determine if the effects dilution are influenced by agitation. The second was to investigate the effect of an agitated, diluted solution on the health of whole organisms, namely honey bees.

CHAPTER II

REVIEW OF LITERATURE

History of Homeopathy

Homeopathy (from the Greek *homeo* meaning similar and *pathos* meaning suffering) is a complementary disease treatment technique in which the patient is given minute doses of natural drugs derived from plants, minerals, or animals to stimulate the natural healing response of the body. If given larger doses of the natural drug to healthy individuals, symptoms of the disease itself will appear (Cook, 1989; Vallance, 1998; Shelton, 2004; Lange, 2002).

Hahnemann's Findings

Though controversial, the idea of homeopathy has been in existence for approximately 200 hundred years. In 1796 a German physician, Samuel Hahnemann developed the theory of homeopathy in his work with syphilis. Hahnemann noticed that too much mercury (used at that time to treat syphilis) produced the same symptoms of the disease itself. He also noted the same similarities in the Peruvian cinchona tree and malaria. Ingesting high doses of the bark of the Peruvian cinchona tree elicited malaria symptoms in healthy individuals who did not have malaria. Hahnemann began to investigate these symptoms by ingesting the cinchona bark himself. Consequently, he began to develop symptoms very similar to malaria patients. This discovery led

Hahnemann to develop his first law, the Law of Similars (Cook, 1989; Shelton, 2004; Vallance, 1998).

The theory of homeopathy is based on two principles, the law of similars or "like cures like" and potentization (dilution). The law of similars holds that a disease can be treated by giving extremely diluted substances that at higher concentrations would produce effects in healthy people that are very similar to the symptoms of the disease (Shelton, 2004; Lange, 2002). Potentization originates from the fact that homeopathic remedies are toxic and therefore need to be diluted to avoid unwanted side effects. Hahnemann was concerned about the toxicity and strong side effects of most of the remedies and was inclined to use the smallest effective dose. With dilution, the side effects decreased, but so did the curative power of the remedy. Shelton (2004) notes an interesting finding made by Hahnemann in his travels: While Hahnemann was traveling to patients' houses, he noticed that the remedies he used during travel seemed to be more potent and effective than remedies given in the laboratory. Hahnemann investigated the reason for this change in potency/effectiveness and realized that the remedies were being agitated in his saddlebags. Through this incident, Hahnemann began what is now known as succussion. He would repeatedly pound the remedy bottle against a leather-bound book, at least ten times, between each dilution. This repeated agitation of the dilution caused it to become more potent. Hahnemann finally concluded that neither dilution nor vigorous shaking alone were enough to eliminate side effects and increase healing power, or potency. The two must be done together, and this combined process in now known as potenization (Shelton, 2004).

Homeopathic remedies often extend into what is called ultra-high diluted substances (Vallance, 1998). These remedies are prepared using a strict procedure of standardized serial dilutions of various plants, minerals or animal matter with vigorous shaking in between (succussions). Serial dilutions are performed as either 1:10 (noted as "x" dilutions) or 1:100 (noted as "c" dilutions). The “x” dilutions are known as decimal series, or 10 fold dilutions, and “c” dilutions are centesimal series, or 100-fold dilutions. These dilutions are then called potencies. For example, for a 1X potency, the researcher would take one part of the mother tincture (or raw substance) and add nine parts of alcohol or water and succuss (Cook, 1989; Shelton, 2004; Vallance, 1998).

Avagadro’s Law states that the number of atoms or molecules in one mole is 6.02 X 10^{23}. Therefore, homeopathic dilutions beyond 10^{24} or 24X/12C are statistically unlikely to contain a single molecule of the mother tincture (Cook, 1989; Vallance, 1998). A common potency for homeopathic remedies is 30C, so the dilutions still yield biological effects, though it is highly unlikely that the dilution contains any molecule of the original substance (Shelton, 1998; Davenas et al., 1988). How is this possible? Benveniste in Davenas (1988) concluded that this phenomenon required some “interaction between the original molecules and water, thus yielding activity capable of specifically imitating the native molecules” (p 818). This phenomenon has become known as the “memory of water” hypothesis (Schiff, 1995).

The Davenas Study

The violation of Avogadro’s Law and the hypothesis of the memory of water have made homeopathy the object of criticism in medicine for years. In 1988, Dr. Jaques Benveniste, a highly respected immunologist, sparked major controversy when he

published a paper with 12 other authors in the well-respected journal *Nature* (Davenas et al., 1988). The paper advocated for the existence of ultra-high dilutions (UHD) and is now viewed as the paradigm study of UHD research (Vallance, 2004). The study explored allergic sensitivities utilizing IgE-dependent basophil degranulation. A basophil is a type of white blood cell that is involved in the body's release of histamine during an allergic reaction. When basophil granules release histamine, the process is called degranulation. Before degranulation is complete, cells are stained with a dye, toluidine blue. After degranulation is complete, the cell will lose its color, providing evidence that the process has occurred. This evidence is easily seen under a microscope. The Davenas et al. group used the homeopathic technique of serial agitated dilutions, or potentization, to dilute antiserum to the level of ultra-high dilution. Solutions were diluted down to the level of 60X. To make sure agitation took place, the potencies were vortexed for 10 seconds between each dilution. The authors concluded that ultra-high dilutions (10^{-60} or 10^{-120}) of succussed anti-Ige were still capable of inducing basophil degranulation (Davenas et al., 1988). An issue faced by Benveniste and his colleagues was to explain how the findings came about. The success of their results was due to the idea that vigorously shaking the solutions enabled them to maintain the ability to yield biological effects; this is compatible with the methods used in homeopathy (i.e. succussion) and could be explained by this mysterious "memory of water."

The theory of the memory of water proposed by this controversial article continues to be a major stumbling block for the acceptance of potentization and homeopathy as a science. There have been many papers dedicated to just the topic of the memory of water (Rao, Roy, Bell, & Hoover, 2007; Isbell & Kane, 1997; Chaplin, 2007; Samal &

Geckeler, 2001; Tschulakow, Yan & Klimek, 2005). Quantum physics specialists Del Guidice, Perparata, and Vitiello (1988) found that continuous vibrations of water particles eventually led to particles being 'locked' into a stable position. Other findings 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, 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.

Replications of Serial Agitated Dilutions

In a previous study done in the Laboratory of Comparative Psychology and Behavioral Biology, Oklahoma State University, Morales (2006) found that more than a dozen researchers have also 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 and negative results when investigating the effects of homeopathic solutions on human basophils (see Table 1).

Table 1

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

Study	Method	Homeopathic Dilution	Measured Parameters
Maddox, Randi, and Stewart, 1988*	Visual counting	Anti-IgE	%Degranulated basophils
Poitevin, Davenas, and Benveniste, 1987	Visual counting	Lung histamine, *Apis mellifica*	%Degranulated basophils

Benveniste, Davenas, Ducot, Cornillet, Poitevin, and Spira 1991	Visual counting	Anti-IgE, *Apis mellifica*	%Degranulated basophils
Ovelgonne J. H., Bol A. W., Hop W. C., van Wijk 1992*	Visual counting	Anti-IgE	%Degranulated basophils
Hirst, Hayes, Burridge, Pearce, and Foreman, 1993*	Visual counting	Anti-IgE	%Degranulated basophils
Sainte-Laudy and Belon, 1993	Visual counting	Histamine	%Inhibition of basophil degranulation
Sainte-Laudy and Belon, 1996	Flowcytometry (anti-IgE+/CD63)	Histamine	%Inhibition of basophil CD63 expression
Belon et al. 2004	Visual counting	Histamine	%Inhibition of basophil degranulation
Sainte-Laudy 1987	Flowcytometry (anti-IgE+/CD63)	Histamine, Histidine	%Activation of basophil degranulation
Brown and Ennis 2001	Flowcytometry (anti-IgE+/CD63)	Histamine	%Inhibition of basophil CD63 expression
Lorenz, Schneider, Stolz, Brack, and Strube 2003	Flowcytometry (anti-IgE+/CD63)	Histamine	%Inhibition of basophil CD63 expression
Lorenz, Schneider, Stolz, Brack, and Strube, 2003	Flowcytometry (anti-IgE+/CD63)	Histamine	Test stability, basophil CD63 expression, basophil CD63 expression
Belon et al. 2004	Flowcytometry	Histamine	%Degranulated basophils

* Studies that found negative results and did not support Davenas' original findings

Morales (2006) found many other studies that had copied the methodology used in Davenas' original investigation to replicate the findings. She also found papers that used

the same methodology for research that did not target basophil degranulation (Table 2). These studies focused 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) changed the methods of succussion in the preparations of their solutions. They agitated the solutions 15 seconds, while Davenas et al. (1988) agitated their solutions 10 seconds.

Table 2

Research studies investigating biological effect of ASDs.

Researcher	**Year of publication**	**Area of study**	**Supports the ASD's effectiveness**
Khanna, and Chandra. *	1976	Control of tomato fruit rot caused by *Fusarium roseum*	Yes
Khanna, and Chandra. *	1977	Control of guava fruit rot caused by *Pestalotia psidii* with	Yes
Khanna, and Chandra. *	1978	A homeopathic drug controls mango fruit rot caused by *Pestalotia mangiferae*.	Yes
Labadie, and Bollinger	1990	Micro-calorimetric study for the successive dilutions of aqueous solutions	No
Endler, Pontegratz, Kasberger, Wiegant, and Schulte	1994	The effect of highly diluted agitated thyroxine on the climbing activity of frogs.	Yes
Jonas and Dillner	2000	Protection of mice from Tularemia infection with ultra-low serial dilutions prepared from *Francisella tularensis* infected tissue.	Yes
Baumgartner, Shah, Heusser, and Thurneysen	2000	Homeopathic dilutions: is there apotential for application in organic plant production?	Yes
Brack, Strube, Stolz & Decker	2003	Effectiveness of agitated serial dilutions on the luminescence of bacterium.	Yes

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

In contrast to the study mentioned above, the remaining seven studies reported results that were consistent with Davenas' original findings. Endler, Pongratz, Kastberger, Wiegant, and Schutle (1991, 1994) observed the effect of highly diluted agitated thyroxine 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, and extend theses results to 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 has been shown 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). The Khanna studies are of particular interest to this study because of the way the homeopathic drugs were utilized. The drugs acted as a pesticide or fungicide toward the fungi that were attacking the tomato, guava and mango plants. We are aiming to look at the same research in honey bees.

Serial Dilution Effect Applied to Animal Behavior

Two of the articles listed in Table 2 used animal behavior as the dependent variable under investigation. In Endler et al. (1994) the climbing activity of frogs was investigated. This study was a replication of a previous study done by the researchers in 1991. The replication involved adding a cross-over control group to the study. Endler et. al (1994) found that by applying thyroxine to the water of juvenile frogs, they could alter the jumping activity of these frogs. Upon observation, the researchers reported that frogs that were treated with 10^{30} potency of thyroxine jumped less and were significantly less

active than the group treated with pure water.

In the second study, Brack et al. (2003) investigated the effect of serial agitated dilutions of 3,5-Dichlorophenol on the luminescent activity of *Vibrio fischeri*, a common bacterium used to evaluate the toxicity of waste water. Following the standard procedures of serial dilutions, the researchers were able to demonstrate that UHDs of 3,5-dichlorophenol had a weak but significant, inhibitory effect on the luminescence of the bacterium.

Current Study

Most previous studies have dealt with homeopathic principles at a cellular level, and there has been very limited research done on the effects of these homeopathic principles on whole organisms. This study investigated the possible detrimental effects of diluted solutions on the honey bee, *Apis mellifera* L. This study also explored different ways of trying to explain the phenomenon of "memory of water". The first hypothesis of the current study was that serial agitated solutions of pesticides were as effective as the non diluted pesticide in killing honey bees. The second hypothesis of this study was that the serial agitated solutions were more potent than serial non-agitated solutions at each dilution level. The third hypothesis investigated the effects of serially diluted sucrose on the feeding behavior of honey bees. If the solution has become more potent and was effective enough to kill honey bees, then it should have a positive effect, which was investigated by diluting sucrose and observing proboscis extension.

CHAPTER III

METHODOLOGY

The general strategy of this experiment was to dilute two substances, and present the substances to groups of honey bees at two of the steps (24X and 60X) of the dilution process. The first substance was commercial grade Sevin© (Carbaryl: 1-naphthyl methylcarbamate). This insecticide was selected because it can be obtained for pest control at any home improvement store. Also, various concentrations of Sevin© have been shown to lead to learning impairments and eventually death in the honey bee (Abramson, Aquino, Ramalho, & Price, 1999). The second substance was sucrose, a substance known to produce consistent proboscis extension in honey bees. Pure water served as the control for this study. The three substances (pesticide, sucrose and pure water) had three serially diluted levels. The three levels were non-diluted, serially diluted to 24X and serially diluted to 60X. The experimental substances (pesticide and sucrose) had two groups. The first group was serially diluted just by adding water while the second group was agitated according to homeopathic remedy standards.

Subjects

The species of honey bee that was utilized for this study was *Apis mellifera* L. *Apis mellifera* L. have been found to be affected indirectly by pesticides. This indirect affect was seen in negative effect on learning in honey bees (Abramson et al., 1999; Abramson, Squire, Sheridan, & Mulder, 2004; Stone, Abramson, & Price, 1997). The effects of

pesticides on honey bees in the previously mentioned studies were measured by the honey bees' proboscis extension.

The honey bees (*Apis mellifera* L.) that were collected for this study were maintained in a hive in conditions that were completely natural. Twenty honey bees for each solution were used in this study, making the total number of bees equal to 180. Upon running a power analysis using the minimally important difference criteria (Harris & Quade, 1992), the number of bees to obtain approximately 50% power was 90, or 10 bees per cell. To ensure that there was enough power in this study, the number of bees per cell was doubled to 20.

Materials

All materials used in this experiment were sterilized prior to each experiment to ensure that no sediment interfered with the results. A volumetric flask, plastic pipettes, and measuring cylinders were utilized to dilute the pesticide, sucrose, and water (control). A 10μL micropipette was used to administer the solutions to the honey bees. Each bee received only 5μL from the 10μL micropipette. For a truly homeopathic technique, solutions should be "thumped" against a surface that is solid yet has some give, such as a leather bound book. But replication studies have shown that vortexing the dilution produces results very similar to the original method (Guddisberg et al., 2005; Poitenvin, Davenas, & Benveniste, 1988; Sainte-Laudy & Belon, 1993). In order to keep the study as standardized as possible, solutions were vortexed for 10 seconds between each serial dilution. Vortexing is established by placing a test tube containing a solution against the edge of an oscillating, rubber cup. The rapid oscillating motion transfers to the test tube and causes a vortex of liquid in the test tube. This process thoroughly mixes the solution.

Metal tubing, duct tape, glass vials, and an ice bath were utilized to capture and restrain the honey bees. Additionally, 100mm capillary tubes were used to feed the honey bees the sucrose solution.

Procedure

Capturing and Harnessing *Apis mellifera* L.

Three hundred and sixty bees were selected at random from a laboratory hive in Stillwater, Oklahoma and were placed in training harnesses. During the months of June and July (2008) subjects were taken from the laboratory hive around 9 am on the day prior to use. No attempt was made to determine the age of the subjects. To control for calendar variables and fluctuating hive conditions, animals from all experiments were run simultaneously and selected from multiple laboratory hives contained within the apiary.

Foraging honey bees were captured individually in glass vials from laboratory hives, placed in an ice water bath, and while inactive harnessed in metal tubes. Once active, they were fed 1.8 M sucrose solution until satiated and set aside for use approximately 24 hours later. Only those animals that vigorously extended their proboscis to sucrose stimulation during a pretest were used in experiments.

Dilution of pesticide, sucrose and water

The dilution of the three groups; pesticide, sucrose and water followed the strict protocol for serial dilutions that has been laid out in studies previously discussed. The serial dilution procedure started with the stock concentrations of each of the substances (pesticide, sucrose, and water). The potencies were diluted in steps of 1:10. The diluted solution was agitated according to standardized instructions; at every step a sterile bottle

containing one part of the previous solution and nine parts distilled water was vortexed for 10 seconds. The vortex successfully agitated the solution. The two concentrations of 24X and 60X were selected to replicate previous studies. According to Avogadro's Law, 24X should not contain any of the original substance, and 60X is the typical potency used for most homeopathic remedies.

The dilution of the three substances took place at the University of Tulsa and was performed by independent researchers. An individual not participating in the experiments coded all samples by replacing the sample number with a number drawn at random. The list was kept in a sealed envelope for the duration of the experiments. The codes were only disclosed after all data had been collected and analyzed.

Experiment 1—Pesticide

In Experiment 1 we investigated whether consuming various potencies of the diluted pesticide would lead to death. One hundred and eighty bees were divided randomly into 9 groups of 20 for feeding tests. In the feeding tests, each group differed in pesticide potency: water only, water diluted to 24X, water diluted to 60X, pesticide only, pesticide serially agitated to 24X, pesticide serially agitated to 60X, pesticide only (2), pesticide serially diluted to 24X, and pesticide serially diluted to 60X. In order to reach dilutions of 24X and 60X, the original stock solution was diluted with a 1:10 ratio for 60 consecutive times. Again, only the 24X and 60X potencies were used.

Once bees were broken down into their respective groups, they were fed the diluted pesticide. This was done by feeding the subjects 5μL of the test solution, and to ensure that the bees fed on the solution, antennas were touched with the sucrose solution so that there was proboscis extension. The honey bees were then held over the test solutions, of

which they continued to feed upon. It has been shown that once the proboscis is extended, the honey bees will continue to feed on what is placed in front of them (Abramson, et al. 2006). After their consumption, all honey bees were observed for 10 minutes for an effect, and mortality rate was calculated for each group.

Experiment 2—Sucrose

In Experiment 2 we investigated whether serially agitated dilutions of sucrose would still elicit the proboscis extension that is seen in stock strength levels of sucrose. One hundred and eighty bees were divided randomly into 9 groups of 20 for feeding tests. The dependent variable for the second experiment was proboscis extension.

In the feeding tests, each group differed in sucrose potency: water only, water diluted to 24X, water diluted to 60X, sucrose serially agitated, sucrose serially agitated to 24X, sucrose serially agitated to 60X, sucrose stirred, sucrose serially diluted to 24X, and sucrose serially diluted to 60X. In order to reach dilutions of 24X and 60X, the original stock solutions were diluted with a 1:10 ratio for 60 consecutive times.

On the day of the experiment, the subjects were screened by touching the antenna with the 1.8 M sucrose solution and looking for any proboscis extension. The subjects that did not respond were released while the remaining subjects were divided into the different test groups.

Subjects were divided equally into one of the 9 potency groups. Each subject was presented with one of the 9 potencies and proboscis extension was observed. Responses to the sucrose dilutions were visually categorized into one of two states. If the proboscis extended following antenna stimulation, a response was registered; if not, a nonresponse was recorded.

CHAPTER IV

FINDINGS

Experiment 1

A 3-way frequency analysis was attempted in order to test a hierarchical log-linear model of serial agitated dilutions on pesticide treatment in honey bees. The categorical variables were group (experimental shaken, experimental unshaken, control), dilution (no dilution, 24X, 60X) and mortality (yes, no). Only partial results could be obtained from the model, however, due to the low amount of variability across particular conditions in the study design. Chi-square tests of association between pairs of variables were therefore used to model the observed frequencies.

Table 3 shows the contingency table for the serially agitated pesticide data. An inspection of the table clearly shows a marked difference between the non-diluted condition and the remaining groups in the study. The non-diluted conditions of pesticide (shaken and unshaken) were the only groups that brought about a high rate of death in the honey bees, with all 40 bees suffering death compared to a zero mortality rate in the control condition, χ^2 (2, n = 60) = 60.00, $p < .001$. The diluted conditions produced a total of only three deaths, each in a different group. Not surprisingly for the 24x [χ^2 (2, n = 60) = 1.03, $p < .60$] and 60x [χ^2 (2, n = 60) = 2.03, $p < .36$] conditions there was not a significant relationship between mortality and the three groups (control, shaken, and unshaken).

Table 3 *Frequencies*

		Dilution			
Group	**Mortality**	*No Dilution*	*24X*	*60X*	**Total**
Experimental Shaken	Yes	20	1	0	21
	No	0	19	20	39
	Total				60
Experimental Unshaken	Yes	20	1	1	22
	No	0	19	19	38
	Total				60
Control	Yes	0	0	0	0
	No	20	20	20	60
	Total				60

Experiment 2

The statistical analyses of Experiment 2 were identical to that of Experiment 1. Table 4 shows the contingency table for the serially agitated sucrose data. An inspection of the table clearly shows no difference between the non-diluted group and the remaining groups in the study. For the dilution conditions non-diluted [χ^2 (2, n = 60) = 2.03, p < .36], 24x [χ^2 (2, n = 60) = .00, p < 1.00], and 60x [χ^2 (2, n = 60) = 2.14, p < .34] there was not a significant relationship between proboscis extension and the three groups (control, shaken, and unshaken).

Table 4 *Frequencies*

Group	Proboscis Extention	Dilution			Total
		No Dilution	*24X*	*60X*	
Experimental Shaken	Yes	20	18	18	56
	No	0	2	2	4
	Total				60
Experimental Unshaken	Yes	20	18	20	58
	No	0	2	0	2
	Total				60
Control	Yes	19	18	18	55
	No	1	2	2	5
	Total				60

CHAPTER V

CONCLUSION

Experiment 1

We began this research by investigating the use of homeopathic principles to create detrimental chemical solutions that could go undetected and be untraceable. The two principles that were investigated in this study were the effects of dilution and serial agitation. The first goal of the study was to determine if the effects of dilution were influenced by agitation. As the results indicate, there were no differences in the two dilutions groups. The results of this study would suggest that agitation played no role in the potency of the diluted pesticide.

Classic homeopathic literature argues that the potency of the substance being diluted intensifies as the dilution extends further into the ultra high dilution levels. With this in mind, the common potency used in homeopathic medicine is 60X.

We hypothesized that the 60X potency would be as effective as the non-diluted pesticide in killing honey bees (*Apis mellifera* L.). As the results show, the exact opposite was found, as there were virtually no deaths in any of the groups, apart from the pure, undiluted pesticide groups. With these results, it will be safe to conclude that diluted pesticide (agitated or unagitated) has no impact on the mortality rate in honey bees. The long-term impact of diluted pesticide in the honey bee was not examined, but this is

something that should be tested in future research. Chronic homeopathic levels of pesticide may interfere with the learning and overall adaptive functioning of the honey bee. Previous research has shown that pesticides do have a negative effect on the behavior and learning of honey bees (Abramson et al., 1999; Abramson, Squire, Sheridan, & Mulder, 2004; Stone, Abramson, & Price, 1997).

With these results, it will be safe to conclude that diluted pesticide (agitated or unagitated) has no impact on the mortality rate in honey bees. The long-term impact of diluted pesticide in the honey bee was not examined, but this is something that should be tested in future research. Chronic homeopathic levels of pesticide may interfere with the learning and overall adaptive functioning of the honey bee. Previous research has shown that pesticides do have a negative effect on the behavior and learning of honey bees (Abramson et al., 1999; Abramson, Squire, Sheridan, & Mulder, 2004; Stone, Abramson, & Price, 1997).

The results of this study are also important at a comparative level. With renewed emphasis in agroterrorism and biodefense, it is important to rule out the effects of the serial dilution process on potentially hazardous chemicals. The first segment of this study potentially rules out the ability of pesticides to be utilized as undetected tools in agroterrorism.

Experiment 2

In a simultaneous second experiment, we investigated the effect of serially agitated dilutions of sucrose on the behavior of honey bees. The behavior of interest in this study was the proboscis extension rate. If the potency of a diluted substance increased with each dilution, one would expect ultra high dilutions of sucrose to produce the same

results as that of stock solutions of sucrose. As noted earlier, stock solutions of sucrose reliably will elicit proboscis extension in honey bees. The results of this study showed no difference between any of the groups. This indicates that the honey bees did not discriminate between the sucrose, sucrose dilutions or water. It is interesting to note that the honey bees were reliably extending their proboscis at all antennae stimulation, whether water or sucrose. According to the literature, one would expect them to only respond to the identifiable sucrose solution. It could be that the honey bees were deprived of water in the dry summer months and were responding out of thirst.

Future Direction and Conclusion

A noteworthy aspect of this study is the difficulty that was encountered in the analysis of the data. An investigation of the raw data and the variables that were used would lead one to believe that a log-linear analysis of the data should be used. An a priori power assessment was conducted, and a sample size that ensured high power was chosen for this study. An attempted log-linear analysis using the Hierarchical option in SPSS 15, however, failed to yield interpretable results because of insufficient error variability in the data. This is a limitation of the statistic that suggests new techniques need to be developed to model data with such limited error. The raw data of this study will be provided in Appendix 1 for future analysis of this problem.

This study does have some limits, and further research should be considered. This study only used one pesticide, Sevin© (Carbaryl: 1-naphthyl methylcarbamate). The lack of effect may have been due to using this particular pesticide, so future experiments should investigate the effect of serial agitated dilution on other pesticides. Future studies should also examine the effect of substances beyond sucrose and pesticides. One possible

route could be investigating effect of serially agitated dilutions of certain vitamins on the whole organism. Can the benefit of taking vitamins at the ultra high dilution level match the benefits of taking vitamins at the conventional level?

A third direction of future study would be to investigate the effects of serial agitated dilutions on organisms that are less complex than the honey bee. One of the unique aspects of this study was using the whole organism to examine the effects of serial agitated dilutions. The effect was not found on this complex organism, but the effect may be found in less complex organisms (i. e. planarian) or in single celled organisms such as paramecium.

A final direction of future study would be to investigate the effect ultra-high dilutions of Sevin© as a homeopathic remedy for pesticide exposure in honey bees. Honeybees, *Apis mellifera* L., are ecologically and economically important insects. They ensure the pollination of many wildflowers, and also pollinate many crop plants. Honey bees also contribute economically from hive products such as honey, royal jelly, and wax (Williams, 1994).

Previous research has revealed the negative effect of various concentrations of Sevin© on the health and functioning of the honey bee. This previous research has shown that exposure to Sevin© leads to learning impairments and eventually death in the honey bee (Abramson, Aquino, Ramalho, & Price, 1999). Despite their negative effect on honey bees, the use of insecticides is the primary method used to control pests in agriculture. The honey bee is a nontarget organism that comes in contact with the pesticide through the foragers of the colony (Ramalho, Wanderley, and Santos, 1996). Not only foragers visiting crops are exposed to pesticides though, hive bees and larvae feeding on pollen

and nectar stored in the combs are also exposed.

Future studies should investigate the effect of serially agitated dilutions of pesticides on the health of honey bees previously exposed to pesticides from the environment. The serially agitated dilutions of pesticides could act as a type of "vaccination" to the colonies of honey bees and potentially help reduce the negative impact of pesticides on the health of the colony.

In conclusion, these two studies examined the effects of serial agitated dilutions on the potencies of two dilution levels (24X and 60X). The two substances used in this study were Sevin,© a commercially available pesticide, and sucrose. Neither the pesticide nor the sucrose solutions showed any effects at the 24X and 60X dilution levels. It appears that there is no potency effect caused by agitation in this dilution process.

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.

Cook, T. M., (1989). *Homeopathic Medicine Today.* New Canaan, CT: Keats.

Chaplin, M. F., (2007). The memory of water: an overview. *Homeopathy, 96,* 143-150.

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. (1991). Climbing activity in frogs and the effect of highly diluted successed thyroxine. *British Homeopathic Journal, 80,* 194-200.

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.

Isbell, W., & Kane, S. (1997). Potentization—just a myth? *British Homeopathic Journal, 86,* 156-160.

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 40 *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.

Lange, N. D., (2002). *Getting at the Root: Treating the Deepest Source of Disease.* Berkeley, CA: North Atlantic Books.

Morales, B., (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*). Master's thesis, Oklahoma State University, 2006.

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

Poitenvin, B., Davenas, E., & Benveniste, J. (1988). In vitro immunological degranulation of human basophils is modulated by lung histamine and *Apis mellifica*. *British Journal of Clinical Pharmacology, 25*, 439-444.

Rao, M. L., Roy, R., Bell, I. R., & Hoover, R. (2007). The defining role of structure (including epitaxy) in the plausibility of homeopathy. *Homeopathy, 96,* 175-182.

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

Samal, S. & Geckeler, K. E. (2001). Unexpected solute aggregation in water on dilution. *Chemical Communication,* 2224-2225.

Schiff, M. (1995) *The Memory of Water.* London: Thorsons.

Schuler, A., Fitzgerald, J., Inglesby, T. V., O'Toole, T. (2004). Executive government positions of influence in biodefense: the bio-plum book. *Biosecurity and Bioterorrism: Biodefense Strategy, Practice, and Science, 2,* 328-341.

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.

Tschulakow, A. V., Yan, Y., & Klimek, W. (2005). A new approach to the memory of water. *Homeopathy, 94,* 241-247.

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.

APPENDICES

APPENDIX A

Experiment 1: Pesticide dilutions, agitated and unagitated. Dependent variable is death. 1= die 0=live

Trials	Pesticide								
	59	13	49	86	71	88	33	65	16
1	0	0	0	0	0	0	0	1	1
2	0	0	0	0	0	0	0	1	1
3	0	0	0	0	0	0	0	1	1
4	0	0	0	0	0	0	0	1	1
5	0	0	0	0	0	0	0	1	1
6	0	1	0	0	0	0	0	1	1
7	0	0	0	0	1	0	0	1	1
8	0	0	0	0	0	0	0	1	1
9	0	0	0	0	0	0	0	1	1
10	0	0	0	0	0	0	0	1	1
11	0	0	0	0	0	0	0	1	1
12	0	0	0	0	0	0	0	1	1
13	0	0	0	0	0	0	0	1	1
14	0	0	0	0	0	0	0	1	1
15	0	0	0	0	0	0	0	1	1
16	0	0	0	0	0	0	0	1	1
17	0	0	0	0	0	0	0	1	1
18	0	0	0	0	0	0	0	1	1
19	0	0	1	0	0	0	0	1	1
20	0	0	0	0	0	0	0	1	1

Experiment 2: Sucrose dilutions, agigated and unagitated. Dependent varialbe is proboscis extension. 1= extension 2= no extension

Trials	Sucrose								
	80	22	67	33	47	32	91	86	88
1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1
4	1	1	0	1	1	1	1	1	1
5	1	0	0	0	1	0	1	0	1
6	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	0
9	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1
12	1	0	1	1	1	1	1	1	1
13	1	1	1	1	1	0	1	1	1
14	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	0	1
16	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	0
19	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1

APPENDIX B

Key for Raw Data in Appendix A

65 pesticide unshaken
16 pesticide shaken
80 sucrose shaken
47 sucrose unshaken
33 H2O
22 24X sucrose
32 24X unshaken
71 24X pesticide
13 24X unshaken pest
86 24X H2O
67 60X sucrose
91 60X unshaken sucrose
59 60X pesticide
49 60X unshaken pest
88 60X H2O

VITA

Sondra L. Nolf

Candidate for the Degree of

Master of Science

Thesis: THE EFFECT OF SERIAL AGITATED DILUTION ON THE MORTALITY RATE OF HONEY BEES (*Apis Mellifera* L.)

Major Field: Psychology

Biographical:

Education:
Completed the requirements for the Bachelor of Science in Counseling at Baptist Bible College, Clarks Summit, Pennsylvania in May, 2003.

Completed the requirements for the Master of Science in Psychology at Oklahoma State University, Stillwater, Oklahoma in December, 2008.

Experience:
Graduate Teaching Assistant in the Department of Psychology, Oklahoma State University, Stillwater, Oklahoma, 2006-present; Teaching Introductory Psychology, Social Psychology and Quantitative Methods.

Professional Memberships: Association for Psychological Science

Name: Sondra L. Nolf Date of Degree: December, 2008

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: THE EFFECT OF SERIAL AGITATED DILUTION ON THE MORTALITY RATE OF HONEY BEES (*Apis Mellifera* L.)

Pages in Study: 32 Candidate for the Degree of Master of Science

Major Field: Psychology

Scope and Method of Study:
The present study examined the effect of serial agitated dilutions of Sevin© on the mortality rate of honey bees (*Apis mellifera* L.). In a second experiment, this study looked at the effect of serial agitated dilutions of sucrose on the proboscis extension rate in honey bees. It appears that serially agitated dilutions of pesticides and sucrose have no effect on honey bees.

Findings and Conclusions:
This series of studies examined the effects of serial agitated dilutions on the potencies of two dilution levels (24X and 60X). The two substances used in this study were Sevin,© a commercially available pesticide, and sucrose. Neither the pesticide nor the sucrose solutions showed any effects at the 24X and 60X dilution levels. It appears that there is no potency effect caused by agitation in this dilution process.

ADVISER'S APPROVAL: Dr. Charles I. Abramson