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Monoterpenoids' insecticidal properties and use as acaricides

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Monoterpenoids' insecticidal properties and use as acaricides

by

Justin Adam Grödnitzky

A thesis submitted to the graduate faculty in

partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Toxicology

Major Professor: Joel R. Coats

Iowa State University

Ames, Iowa

2001
Graduate College
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This is to certify that the Master's thesis of

Justin Adam Grodnitzky

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
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CHAPTER 1: GENERAL INTRODUCTION

Thesis Organization

This thesis is organized into five chapters. Chapter 1 is a general introduction with background information about each of the other chapters. The second chapter “Using Classic and Quantum Parameters to Determine Monoterpenoids’ Insecticidal QSARs” has been accepted as a chapter in Synthesis and Chemistry of New Potential Agrochemicals. Chapter 3 “Monoterpenoids’ QSARs are used to Predict Toxicity to House Flies” and Chapter 4 “Varroa Mite Control Using Monoterpenoids and their Derivatives” will be submitted to the Journal of Agricultural and Food Chemistry. Chapter 5 consists of a general conclusion and direction for future research.

Introduction

Monoterpenoids are compounds found in essential oils in many higher order plants. These compounds are responsible for giving essential oils their unique odors. For example, limonene is responsible for the scent in oranges and thymol gives thyme its unique odor. Monoterpenoids have also found their way into commercial markets. Some monoterpenoids are used in perfumes, cosmetics, medications and others are used as food additives (1-2).

Monoterpenoids are secondary plant metabolites that seem to serve no major role in the physiological functioning of the plant. These secondary metabolites, are usually synthesized from two isoprene units, and therefore are 10-carbon molecules. Biosyntheses of monoterpenoids are accomplished via the mevalonic acid pathway (3). Monoterpenoids are further processed by the plant through various oxidation steps. This
process starts with the condensation of two acetyl-CoA molecules to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). HMG-CoA synthase is the enzyme that catalyses this reaction. HMG-CoA reductase then catalyses the reduction of HMG-CoA to form mevalonate. This NADPH-dependent reaction is the essential step in regulating terpene and steroid biosynthesis. Mevalonate is then phosphorylated by ATP-dependent mevalonate kinase to form mevalonate 5-phosphate. Mevalonate 5-phosphate is further phosphorylated by phosphomevalonate kinase to form mevalonate 5-diphosphate. The next step in the synthesis of monoterpenoids is the decarboxylation of mevalonate 5-diphosphate, causing the formation of isopentenyl diphosphate (IPP). Mevalonate 5-diphosphate decarboxylase is the ATP-dependent enzyme that catalyses this reaction. IPP isomerase isomerizes isopentenyl diphosphate to form dimethylallyl diphosphate (DMAPP). The next step of this process involves the alkylation of DMAPP and IPP using IPP prenyltransferase. This reaction usually produces one monoterpenoid precursor. Various isomerization steps are needed to form other acyclic monoterpenoids. The formation of simple cyclic monoterpenoids involves isomerization of GPP into (3s)-(+) -linalyl diphosphate and then the further isomerization and cyclization reactions are catalyzed by a single enzyme. Monoterpenoids are further processed by the plant through various oxidation steps.

These compounds seem to play no major role in the metabolic functioning of the plants, and their role is thought to be less critical (secondary). There are several functions for monoterpenoids in the plant. One function is to aid in pollination of the plant by attracting certain insects to the plant. Another function of monoterpenoids is to defend against plant pathogens, herbivores, or competing plant species (4).
Plants and insects have co-evolved for millions of years. Plants have developed the capability to produce secondary metabolites in order to protect themselves against different types of pathogens and herbivores. The pathogens include fungi and bacteria, and the herbivores include insects, birds, mammals, etc. Secondary metabolites, such as monoterpenoids, are potentially good naturally occurring insecticides because of the co-evolution through which they were developed. Monoterpenoids have been shown to be toxic against various insect species. Work in our lab showed monoterpenoids possess both acute and developmental toxicity properties to various insect species (5-8). These species consist of German cockroaches (*Blattella germanica*), house flies (*Musca domestica*), red flour beetle (*Tribolium castaneum*), rice weevil (*Sitophilus oryzae*), mosquito larvae (*Aedes aegypti*), etc. Although some monoterpenoids have toxic effects to insects, other monoterpenoids show very little acute toxic effects to insects. Some monoterpenoids have been shown to be selectivity toxic to some insects with little effects on other; because of monoterpenoids' selective toxicity, apiculturalists have turned to these compounds to study possible use to treat honey bees for protection against an ectoparasitic mite.

*Varroa jacobsoni* is the ectoparasitic mite that has been plaguing apiculturalists in the U.S. since its first appearance in 1987 in Wisconsin (9). These mites have caused significant economic and biological damage to the honey bee community. Varroa mite was once an obscure mite of Asian bees that by 1975 caused significant damage to honey bee colonies in Europe, northern Africa and South Africa (10). This reddish-brown, dorsoventrally flattened mite kills or weakens or deforms developing honey bee larvae, which reduces the effective worker bee population. Mated *Varroa jacobsoni* females feed
off the hemolymph of honey bee larvae. These mites enter the brood cell before adult worker bees cap the cell. In the brood cell, offspring emerge from the mated female. These offspring are mated and the female mites feed on the hemolymph of the developing honey bee larvae. This causes the larvae to be damaged or die. The damage that *Varroa jacobsoni* causes usually is deformed or missing appendages, such as wings, and a shrunken abdomen. Due to mite-induced mortality and damage, some beekeepers have suffered tremendous economic losses. Beekeepers have traditionally used pesticides to control mite populations.

Fluvalinate and coumaphos are the only two synthetic acaricides/insecticides approved to control varroa mite population. The use of these acaricides have various drawbacks. One drawback is that acaricide residues can not be present in the honey or bee wax, through accidental tainting of the honey or bee wax. They can also cause a loss of production to apiculturalists. Another draw-back is that fluvalinate-resistant varroa mites have had tremendous impact on controlling their populations (11). Resistant Varroa mites have caused the emergency registration of coumaphos, an organophosphate that has been shown to be extremely toxic to some non-target organisms.

Due to these drawbacks, biopesticides need to be further investigated. One biopesticide is already being used by apiculturalists to treat bee hives for tracheal mites. They use menthol, an essential oils, to treat their colonies. Essential oils consist of secondary metabolites in plants. Monoterpenoids are secondary metabolites found in essential oils that have been used to control varroa mites in experimental situations (12). These compounds have several advantages over the synthetic acaricides. They have little toxicity to non-target systems, which is evident in that they are used in perfumes,
cosmetics, and medications and are used as food additives. There is also a favorable public perception of monoterpenoids to be safe and naturally effective compounds.

In order to test the toxicity of these compounds to pest mites and insects, we need to perform various bioassays for all the monoterpenoids. This shotgun approach to find the most effective compound is very time-consuming and a costly process. In order to determine the most effective insecticidal monoterpenoids, quantitative structure-activity relationships (QSARs) can be developed.

QSAR is a quantitative way of relating certain chemical features of a molecule to their chemical or biological properties. These relationships use chemical or biological properties as the dependent variable and then, using certain molecular descriptors as independent variables, try to find correlations between the dependent variable and the independent variables. After a relationship is found, the model is used to predict certain properties of the dependent variables. Molecular descriptors range from global empirical and non-empirical descriptors that give information about the molecule as a whole, such as dipole moment, Log P, van der wals radius, and connectivity indices. There are also sub-molecular empirical and non-empirical descriptors, which give information about certain atoms or regions within a molecule, such as the highest occupied molecular orbital (HOMO), the lowest unoccupied molecular orbital (LUMO), Mulliken population, and electrotopological state. Using information about global or sub-molecular descriptors, researchers can predict the biological effects of the compounds, and these compounds can be optimized to enhance their activity by making certain modifications to their structures.
Specific parameters were chosen in order to explain toxicity. These parameters were chosen to represent the structural features of molecules that are important in receptor-ligand interactions. Size and shape of a molecule are important for receptor-ligand interactions. If the receptor does not accommodate the molecule because of its size or shape, then the molecule cannot generate its effect on the system. The other important criterion that must be met for receptor-ligand interactions to occur is the adherence of the ligand to the receptor. Molecular interactions can be explained by affinity due to electrostatic interactions, London dispersion forces, and hydrophobic interactions (13).

Many QSAR models have been developed using various descriptors. QSARs have been used to predict the environmental fate of various pesticides and industrial effluent chemicals. These models predict the various fates of these compounds for persistence of organic pollutants, tropospheric degradation, volatilization, and soil sorption (14-17). Using QSAR models allows industries to design chemicals that are environmentally friendlier. Other models are used to enhance activity of pharmacological and agricultural compounds. QSAR models allow companies to save money by not having to screen every compound and allow directed synthesis of potentially therapeutic compounds. I would like to develop QSAR models to help predict bioactivity and develop more potent insecticidal monoterpenoids, including derivatives and analogs.

In this M.S. thesis I investigate the toxicity of monoterpenoids and their derivatives in order to develop QSAR models. Monoterpenoid derivatives in these studies consisted of esters and ethers synthesized from their parent alcohols or phenols. Toxicity bioassays were performed for all the monoterpenoids and their derivatives on two insect species and one arachnid, *Musca domestica* (house fly) and *Apis mellifera* (honey bee) LC$_{50}$
values were used to develop QSAR models. House flies are a standard test species for entomologists. Because of the ease of handling and rearing, I chose to use them as our primary test organism. *Apis mellifera* and *Varroa jacobsoni* (varroa mite, a parasite of the honey bee) were used to test the selective nature of monoterpenoids to an epidemic pest in order to test these compounds in a real-world application. The models develop used various descriptors to help explain electronic and steric parameters. Empirical descriptors HOMO, LUMO, dipole moment, polarizability, Log P, and Mulliken populations were used to try to explain toxicity. Non-empirical descriptors such molecular connectivity, molar refractivity, valence connectivity, shape indices, electrotopological state and GETAWAY descriptors were also used to capture information about the structural requirements that are important in their toxicity. Linear and multiple linear regressions were performed using SAS™ to assess the quality of each regression model. We evaluated the models using the square of the correlation coefficient ($r^2$), and cross-validation ($cv^2$). Regressions with $r^2 > 0.80$ were used to develop QSARs. Validation of our models were performed using the leave-one-out cross validation method. The leave-one-out cross validation method is based on the following equations [1]-[2]:

$Cross\text{-}validation\ q^2 = 1-(PRESS/SSTO) \ [1]$

where

$PRESS = \sum_y (Y_{pred} - Y_{actual})^2 \ [2]$  

Cross-validation values greater then 0.60 have been used to imply a non-random relationship (18). These models were used to allow assessment of the important structural moieties that monoterpenoids or their derivatives possess to exert their toxic effect.
References


6) Karr, L. L., Coats, J. R., J. Econ. Entomol, 1992, 85, 424


Monoterpenoids are naturally occurring plant compounds that are found in higher-order plants. These compounds are secondary metabolites; they are usually synthesized from two isoprene units, and are therefore 10-carbon molecules. Biosyntheses of monoterpenoids are accomplished via the mevalonic acid pathway. Monoterpenoids are further processed by the plant through various oxidation steps. These compounds seem to play no major role in the metabolic functioning of the plants, and their role is thought to
be less critical (secondary). There are several functions for monoterpenoids in the plant. One function is to aid in pollination of the plant by attracting certain insects to the plant. Another function of monoterpenoids is to defend against plant pathogens, herbivores, or competing plant species.

Plants and insects have co-evolved for millions of years. Plants have developed the capability to produce secondary metabolites in order to protect themselves against different types of pathogens and herbivores. The pathogens include fungi and bacteria, and the herbivores include insects, birds, mammals, etc. Secondary metabolites, such as monoterpenoids, are potentially good naturally occurring insecticides because of the co-evolution through which they were developed. Some monoterpenoids have shown insecticidal activity, and a few of these compounds are used as commercial pesticides (d-limonene, menthol, citronellal, and linalool) (1). Although, these monoterpenoids are being used commercially, the mode of action is still unknown. In addition, no quantitative structure-activity relationships (QSARs) have been determined up to this point.

We examined four monoterpenoids (phenols and alcohols) and their ester derivatives. We tested linear monoterpenoids (geraniol), cyclic monoterpenoids (carveol), and aromatic monoterpenoids (carvacrol, thymol) to find a relationship between the monoterpenoids and their toxicity. By using toxicity to house flies, we tried to correlate toxicity with various classical and quantum parameters. Specific parameters were chosen in order to help explain toxicity. These parameters were chosen to represent the features of molecules that are important in receptor-ligand interaction. Size and shape of a molecule is extremely important for receptor-ligand interactions. If the receptor does not
accommodate the molecule because of its size or shape, then the molecule cannot generate its effect on the system. In our case, its effect would be toxicity to house flies. To discern if shape and size of the monoterpenoids are important for their toxicity, we examined several classical parameters. These independent variables were molecular connectivity indices (0, 1, 2), valence connectivity indices (0, 1, 2), shape indices (1, 2, 3), and molar refractivity.

The other important criterion that must be met for receptor-ligand interactions to occur is the adherence of the ligand to the receptor. Molecular interactions can be explained by affinity due to electrostatic interactions, London dispersion forces, and hydrophobic interactions. We examined classical and quantum parameters to help explain these interactions. Log P and molar refractivity are the classical parameters chosen to represent hydrophobic interactions and London dispersion forces. The quantum parameters were chosen to represent both electrostatic interactions and London dispersion forces. Highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), dipole moment, polarizability, and Mulliken population are the quantum parameters chosen to represent receptor-ligand interaction, which can ultimately cause mortality to house flies.

**Synthesis of monoterpenoid esters**

Monoterpenoid parent alcohols or phenols, carveol, geraniol, thymol, and carvacrol, (1 mole) were added to their corresponding anhydride or acid chloride (2 moles) to form ester derivatives in the presence of a catalytic amount of pyridine (2-5 drops). Methylene chloride was used as the solvent, and the reaction was allowed to stir for 24-48 hr at room
temperature. The reactions were monitored by thin-layer chromatography using a 9:1 hexane:acetone mobile phase and developed by vanillin spray (8g vanillin, 1.25ml sulfuric acid brought up to 250 ml with methanol). The reaction was worked up with four (NaHCO₃ and water) washes. Methylene chloride was removed using a rotary evaporator. Compounds were purified using silica gel-column clean up, using a 19:1 hexane:acetone solvent system. Identities of the esters were determined using TLC, comparing Rf values of the parent alcohols or phenols against reaction products. Identities were confirmed using ¹H-NMR 300 Mhz. A total of 25 monoterpenoids were used in this study, which includes the four parent molecules and 21 esters (Fig. 1-2). Four geranyl esters were made from geraniol, and five esters were made from each of the remaining monoterpenoids (thymol, carvacrol, and carveol).

**House fly toxicity testing**

**LD₅₀** values were obtained for all 25 monoterpenoids. Topical application was used to apply 1 µL of various concentrations of monoterpenoid to the pronotum of *Musca domestica* (house fly). We placed 10 treated house flies in a jar and for each concentration, three replications of monoterpenoid were used. At the end of the 24-hr exposure, mortalities of the house flies were recorded. **LD₅₀**s of all the monoterpenoids were calculated using the Spearman-Karber method (3). **LD₅₀** values are shown (Fig 1) (Fig 2). Some compounds’ **LD₅₀** values were previously report from our lab (4).

These showed a range of toxicity to house flies, ranging from **LD₅₀** of 0.17 µmol/fly to 2.35 µmol/fly. The two monoterpenoids which have the greatest toxicity are geranyl
chloroacetate with a LD50 value of 0.17 µmol/fly and thymol with a LD50 value of 0.22 µmol/fly. There is no obvious structural reason why these two compounds have the most insecticidal activity. Geranyl chloroacetate is a derivative of an acyclic monoterpenoid, and thymol is an aromatic monoterpenoid. In the thymol group, thymol was more toxic than its derivatives; however, in the geraniol group, all the derivatives were more toxic than geraniol. Also for the carveol group, carveol was one of the least toxic compounds within that group (similar to the other aromatic compound, thymol). Carvacrol, on the other hand, was one of the most toxic compounds within its group. There was no obvious trend in structure that helps explain toxicity of these compounds. To help clarify what moieties of the molecules are responsible for their toxicity, we examined classical and quantum parameters to try to explain their toxicity.

**Monoterpenoid QSAR analysis**

The classical parameters mentioned previously were, molar refractivity, molecular connectivity indices (0,1,2), valence connectivity indices (0,1,2), shape indices (1,2,3), and Log P, were calculated by CAChe™ (Oxford Molecular). The quantum parameters, highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), dipole moment (magnitude and direction), Mulliken population, and polarizability, were calculated in GAMESS. Geometry and energy of all the molecules were optimized using a split valence basis set and a polarization function (6-31*d) calculation using GAMESSTM. Hessian runs were performed using 6-31*d calculations using GAMESSTM to show that all the molecules tested were at an energy-minimum.
conformation. Classical and quantum parameters were plotted against house fly LD_{50}s. All regression analyses were fitted using Microsoft Excel™.

A relationship was not found between all the monoterpenoids (and their derivatives) and their toxicity to house flies. We did find relationships within sub-groups such as, thymol and its derivatives. Thymol compounds, carveol compounds, and carvacrol compounds showed no correlation between classical parameters and toxicity. Log P is often used to explain chemical uptake and hydrophobic interactions between ligand and a receptor. The lack of correlation between Log P and the toxicity of monoterpenoids indicates that changing the ester group does not have a dramatic effect on uptake or hydrophobic interactions. No correlations were found for thymol compounds, carveol compounds, and carvacrol compounds, but there were correlations found between the toxicity of geraniol compounds and molar refractivity, molecular connectivity indices (0,1,2), valence connectivity (0,1,2), and shape indices (1,2,3) (Fig 3). However, the correlation between toxicity and the previously mentioned parameters was a parabolic relationship using only five data points. The parabolic relationships suggest that there is an optimal region for toxicity of that series of derivatives. More data points should be added to verify this relationship. No correlations were found between toxicity and molar refractivity, molecular connectivity indices (0,1,2), valence connectivity (0,1,2), or shape indices (1,2,3), for thymol, carveol, and carvacrol compounds, which indicates that modifying the esters at the –OH position of the monoterpenoids does not seem to have a major effect on toxicity. The size or shape of the esters does not seem to be a major factor on toxicity to house flies.
Only one quantum parameter (Mulliken population) showed a correlation between toxicity of thymol, carveol, and carvacrol compounds. Geraniol compounds showed no correlation between their toxicity and any of the quantum parameters. We obtained a correlation between toxicity and Mulliken population within the thymol, carveol, and carvacrol groups. Our study revealed a linear trend of increasing toxicity within the various groups to Mulliken population of certain atoms within that group. Thymol and its derivatives showed a relationship between toxicity and the Mulliken population on three atoms. Thymol compounds revealed that as the Mulliken population around atom 13 increases, toxicity of the compound decreases. The numbers on the atoms for thymol, carvacrol, and carveol correspond to the order the atoms were added to the Z-matrix to construct the molecules (Fig. 4). Atom 12 of the thymol compounds showed that as Mulliken population decreased toxicity increased. Atom 11 of the thymol compounds revealed the inverse relationship of atoms 13 and 12. It showed that as Mulliken population increased, toxicity also increased. We obtained an $r^2=0.96$ for atom 13 with $n=6$ (Fig. 5). Atom 11 had an $r^2=0.83$ with $n=6$ (Fig. 6), and atom 12 had an $r^2=0.92$ with $n=6$ (Fig. 7). We also obtained a linear correlation with toxicity and Mulliken population within the carvacrol group. Two atoms within the carvacrol group (6 and 12) showed a relationship between Mulliken population and toxicity. As Mulliken population increases around these atoms, their toxicity also increases. We obtained an $r^2=0.78$ for atom 6 with an $n=6$ (Fig. 8), and for atom 12 we obtained an $r^2=0.86$ with $n=6$ (Fig. 9). The carveol group of compounds also had a relationship between toxicity and Mulliken population. As the Mulliken population around atom 6 increased, toxicity also increased ($r^2=0.86$;
n=6) (Fig. 10). These correlations demonstrate that the electronic effects of thymol, carveol, carvacrol compounds are important for explaining toxicity.

Conclusion

No relationship was found between parameters for all the monoterpenoids (and their derivatives) and their toxicity; however we did find relationships for the structural characteristics of sub-groups and their toxicity. Since the sub-groups are not as large or diverse as the whole group the monoterpenoids, further compounds are needed to truly test the validity of these relationships. These smaller sets of relationships give us a good starting point to develop more robust QSARs and also can be used to increase the insecticidal effectiveness of compounds within the sub-groups.

Geraniol compounds were the only set of monoterpenoids to show a relationship between toxicity and the classical parameters studied. Those classic parameters all encoded information on size and shape of the ester functional group. If these correlations hold true when more compounds are added, we will know that there is an optimal size and shape requirement for that part of the molecule that must be met for the compound to exert its toxic effect on house flies. Since there is a parabolic relationship, we can already predict the optimum toxicity for these compounds. To increase geraniol compounds' toxicity, other regions of the molecules need to be modified.

For thymol, carveol, and carvacrol compounds, Mulliken population around certain atoms in the molecules showed a strong correlation with their toxicity. Mulliken population, which represents the probability of electron population around the atoms in the molecule, may explain electrostatic interactions of the monoterpenoids to a receptor.
Regardless of the actual mechanism, the electronic effects of the molecule are important for their toxicity. The classical parameters revealed no correlation with these compounds' toxicity nor any structural parameter examined. This indicates we can modify the –OH region of the molecule. Because size and shape of that part of the molecule does not seem to be important for toxicity, we may be able to add a functional group at that part of the molecule to change the Mulliken population around certain atoms to increase toxicity. In the future, more compounds with different functional groups need to be examined in order to truly validate these QSARs.

Acknowledgment

This paper was presented in the symposium on Synthesis and Chemistry of New Potential Agrochemicals at the Fall 2000 Annual Meeting of the American Chemical Society, August 20-24, 2000, in Washington, D.C. The authors are grateful for the technical assistance of Kim Hoover and Erica Simbro in the laboratory. This chapter is journal paper No. J-19195 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA 50011.

Reference


Thymol Compounds:

![Chemical structures of thymol compounds]

- Thymol: LD₅₀ = 0.22 (0.20-0.24)
- Thymyl pivalate: LD₅₀ = 0.34 (0.22-0.42)
- Thymyl propionate: LD₅₀ = 0.49 (0.40-0.62)

- Thymyl acetate: LD₅₀ = 0.49 (0.44-0.54)
- Thymyl trichloroacetate: LD₅₀ = 0.62 (0.56-0.69)
- Thymyl chloropivalate: LD₅₀ = 1.12 (0.98-1.27)

Carvacrol Compounds:

![Chemical structures of carvacrol compounds]

- Carvacryl dichloroacetate: LD₅₀ = 0.39 (0.41-0.53)
- Carvacrol: LD₅₀ = 0.42 (0.40-0.43)
- Carvacryl trifluoroacetate: LD₅₀ = 0.46 (0.41-0.53)

- Carvacryl trichloroacetate: LD₅₀ = 0.47 (0.43-0.51)
- Carvacryl acetate: LD₅₀ = 0.55 (0.50-0.61)
- Carvacryl propionate: LD₅₀ = 0.65 (0.64-0.66)

Figure 1. Structures and LD₅₀ (µmole/fly) of thymol and carvacrol compounds. 95% confidence intervals of LD₅₀ values in parentheses.
Geraniol Compounds:

- **Geranyl chloroacetate**
  - $L_D^{50}=0.17$ (0.15-0.19)

- **Geranyl pivalate**
  - $L_D^{50}=0.39$ (0.37-0.41)

- **Geraniol**
  - $L_D^{50}=0.84$ (0.61-1.01)

Carveol Compounds:

- **Carvyl pivalate**
  - $L_D^{50}=0.37$ (0.35-0.40)

- **Carvyl acetate**
  - $L_D^{50}=0.57$ (0.54-0.61)

- **Carvyl chloropivalate**
  - $L_D^{50}=0.96$ (0.85-1.09)

- **Carvyl propionate**
  - $L_D^{50}=0.99$ (0.92-1.104)

- **Carvyl acetate**
  - $L_D^{50}=1.85$ (1.64-2.09)

- **Carvyl trichloroacetate**
  - $L_D^{50}=2.35$ (2.32-2.39)

Figure 2. Structures and $L_D^{50}$ (µmole/fly) of geraniol and carveol compounds. 95% confidence intervals of $L_D^{50}$ values in parentheses.
Figure 3. Relationships of geraniol compounds' toxicity with connectivity index (0), molar refractivity, valence connectivity index (0), and shape index (1).
Figure 4. Numbering of the atoms for thymol, carvacrol, and carveol compounds. These numbers correspond to the order they were placed into the $Z$-matrix.
Figure 5. Linear correlation between thymol compounds’ house fly toxicity and Mulliken population around atom 13.

Thymol Compounds

\[ y = 15.32x - 85.51 \]

\[ R^2 = 0.97 \]
Figure 6. Linear correlation between thymol compounds' house fly toxicity and Mulliken population around atom 11.
Figure 7. Linear correlation between thymol compounds' house fly toxicity and Mulliken population around atom 12.
Figure 8. Linear correlation between carvacrol compounds’ house fly toxicity and Mulliken population around atom 6.
Figure 9. Linear correlation between carvacrol compounds' house fly toxicity and Mulliken population around atom 12.
Carveol Compounds

\[ y = -312.63x + 1948.25 \]

\[ R^2 = 0.86 \]

Figure 10. Linear correlation between carveol compounds’ house fly toxicity and Mulliken population around atom 6.
CHAPTER 3: MONOTERPENOIDS’ QSARS ARE USED TO PREDICT TOXICITY TO HOUSE FLIES

A paper to be submitted to the Journal of Agricultural and Food Chemistry

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Monoterpenoids are naturally occurring plant compounds that are found in higher-order plants. These compounds are secondary metabolites that seem to play no major role in the metabolic functioning of the plants. One function of monoterpenoids is to defend against plant pathogens, herbivores, or competing plant species. Using these compounds as leads to make more effective insecticides, we hope to develop a safer and more ecologically friendly insecticide. To accomplish these goals, we developed quantitative structure-activity relationships (QSARs) in order to predict toxicity of monoterpenoids and derivatives that have not been synthesized or experimentally tested. Correlations were found between toxicity and certain chemical parameters. We found a linear relationship between LD₅₀ values and Mulliken populations in aromatic monoterpenoids. Multiple linear regression of E-State descriptors and GETAWAY descriptors also showed a relationship with house fly toxicity for a wide range of monoterpenoids.

Introduction

Monoterpenoids are components of essential oils found in many higher-order plants. These compounds give plants their own unique odoriferous properties. For example, limonene is primarily responsible for the scent in oranges, and thymol gives thyme its unique odor. These compounds are often found in perfumes and other cosmetics and are commonly used as food additives.
Monoterpenoids are secondary plant metabolites that consist of two isoprene units. These compounds contain 10 carbons and seem to play no major role in the basal metabolic functioning of the plant. However, monoterpenoids are important to plants because they attract beneficial insects to plants, which aids in pollination, and they can help plants defend against pathogens. The natural insecticidal properties of monoterpenoids is the reason we chose to investigate these compounds.

Monoterpenoids have been shown to possess insecticidal activity, and a few of these compounds are currently being used commercially as pesticides (d-limonene, menthol, citronellal, and linalool) (1). Although these monoterpenoids are being used commercially, the mode of action of monoterpenoids is still not well understood. In addition, quantitative structure-activity relationships (QSAR) have not been determined, so the chemical basis for their insecticidal properties is not yet known. These compounds have very low mammalian toxicity, which could make them a very effective alternative insecticide.

In this paper, we examine the toxicity of natural monoterpenoids and their derivatives to Musca domestica (house fly). The toxicities of these compounds were then used to develop QSARs. In developing these relationships, we hope to design more effective insecticidal monoterpenoids and gain insight about the structural properties that are responsible for their toxicity. We investigate a variety of parameters that help explain receptor ligand interactions. If the molecule does not interact with a receptor because of its size or shape, then the molecule cannot generate its effect on the system. These interactions are responsible for their toxicity to house flies. Steric and electronic descriptors were used to help encode information about the important characteristics of
monoterpenoids that are responsible for their toxic effects. Geometry, topology and atomic weights assembly descriptors (GETAWAY) were used to capture information about the three-dimensional structure of the molecules and encode information about the steric requirements of these compounds (2). This descriptor gives higher values for atoms that are distal from the molecule's center than for atoms near the molecule's center. The higher values can be interpreted as the atom's accessibility to outer interactions, which encodes information about the molecule's size and shape. We used two descriptors to explain electronic interaction of these compounds to a receptor. Mulliken populations and electrotopological state descriptors were used to represent electron density around certain atoms in the molecule. Electrotopological state descriptors have been shown to be highly correlated with Mulliken population and encode information about the electron accessibility for each atom in the molecule (3). These descriptors have been useful in other QSARs to help explain the importance of electronic properties of molecules for various biological and physicochemical effects (4-7). In this study, these descriptors were used to develop an effective model to describe highly insecticidal monoterpenoids and their derivatives.

Material and Methods

Synthesis of monoterpenoid esters. A total of 30 monoterpenoids and their derivatives were examined in this study. All parent monoterpenoids were alcohols or phenols, and synthesized derivatives were esters and ethers.

Esters: Parent alcohols or phenols (1 mole) were added to the corresponding anhydride or acid chloride (2 moles) to form ester derivatives in the presence of a
catalytic amount of pyridine (2-5 drops). Methylene chloride was used as the solvent, and the reaction was allowed to stir for 24-48 hr at room temperature. The reactions were monitored by thin-layer chromatography using a 9:1 hexane:acetone mobile phase and developed by vanillin spray (8g vanillin, 1.25ml sulfuric acid, brought up to 250ml with methanol). The reaction was worked up with four (NaHCO₃ and water) washes. Methylene chloride was removed using a rotary evaporator. Compounds were purified using silica gel-column clean up, using a 19:1 hexane:acetone solvent system. Identities of the esters were determined using TLC, comparing Rf values of the parent alcohols or phenols against reaction products and confirmed using ¹H-NMR Varian VXR 300 Mhz.

Ethers: Alkyl ether synthesis reactions were carried out using thymol and its corresponding alkyl halide in the presence of the phase-transfer catalyst, benzyltributylammonium bromide (BTAB). Thymol (0.10 mol) was dissolved in 50 ml of CH₂Cl₂ together with the alkyl halide. The reaction was allowed to stir for two weeks. The reactions were monitored by thin-layer chromatography plates (using a 9:1 hexane:acetone mobile phase) that were developed using the vanillin spray. The reaction was worked up with four (NaHCO₃ and water) washes. Methylene chloride was removed using a rotary evaporator. Compounds were purified using silica gel-column clean up, using a 19:1 hexane:acetone solvent system. Identities of the esters were determined using TLC, comparing Rf values of the parent alcohols or phenols against reaction products and confirmed using ¹H-NMR Varian VXR 300 Mhz.

LD₅₀ values were obtained for 30 monoterpenoids (Table 1, 2). Topical application was used to apply 1 µL of various concentrations of monoterpenoid to the pronotum of house flies. Ten treated house flies where placed in a jar. For each concentration, three
replications of 10 monoterpenoid treated flies were used. Controls were run for each of the treatments. One µL of solvent was applied to the pronotum of the flies. At the end of the 24-hr exposure, mortalities of the house flies were recorded. LD_{50}s of all the monoterpenoids were calculated using the Spearman-Karber method (8). Some compounds' LD_{50} values were previously reported from our lab (9). LD_{50} values were expressed as µmol/fly.

Descriptors: Mulliken population was calculated in GAMESS™. Geometry and energy of all the molecules were optimized using a split valence basis set and a polarization function (6-31*^d) calculation using GAMESS™. Hessian runs were performed using 6-31*^d calculations using GAMESS™ to show that all the molecules tested were at an energy-minimum conformation. Electrotopological state descriptors were calculated using E-calc™. GETAWAY descriptor HATS2p was calculated using Dragon™.

Linear and multiple linear regressions were performed using SAS™. The toxicity data was expressed as log (1/C), where C is the concentration that produces 50% mortality in house flies. The quality of each of the regression models was evaluated using the square of the correlation coefficient (r^2), and cross-validation (q^2). We examined only regressions with r^2 > 0.80. To evaluate the validated of our models, we used the leave-one-out method. It is calculated based the following equations [1]-[2]:

\[
\text{Cross-validation } q^2 = 1-\frac{(PRESS/SSTO)}{\text{PRESS}}
\]

where

\[
\text{PRESS} = \sum_y (Y_{\text{pred}} - Y_{\text{actual}})^2.
\]

And SSTO is sum of squares total.
Cross-validation values greater than 0.60 have been used to imply a non-random relationship (10).

Results

Various monoterpenoids and derivatives were examined; among these compounds, we obtained two relationships. One of these relationships was developed for ten thymol compounds (Fig 2).

Due to the diverse nature of the monoterpenoids in this study, we separated the monoterpenoids into two groups: aromatic thymol compounds and non-aromatic (aliphatic) monoterpenoids (Fig 1). The aliphatic monoterpenoids had additional requirements that had to be met in order for a monoterpenoid to be a candidate for this QSAR. Compounds had to contain a methyl-cyclohexene backbone with a double bond present in the 2 position (Fig 4). Monoterpenoids that possessed a bicyclic structure were included in the QSAR, if they met the basic structural requirements of the model.

Twenty aliphatic monoterpenoids were used to construct our QSAR model. An electronic and steric descriptor were used to predict toxicity of monoterpenoids and their derivatives to the house fly. The GETAWAY descriptor was used to account for size and shape requirements that are essential for monoterpenoid toxicity. The electrotopological state (E-state) descriptor on atom 3 was used to represent the important electronic properties necessary for monoterpenoids to exert their toxic effects. We obtained a good linear correlation between GETAWAY and electrotopological state descriptors with house fly toxicity \( (n = 20, \ s = 0.11, \ F = 32.59, \ r^2 = 0.86 \) and \( q^2 = 0.72 \). \) The model obtained is as follows:
\[
\log(1/\text{LD}_{50}) = -30.7(\pm 4.9) + 15.1(\pm 2.4)[\text{E-state}] + 213.8(\pm 36.4)[\text{GETAWAY}] - 105.8(\pm 17.6)[\text{interaction}] \] [3].

Experimental and calculated \(\log (1/\text{LD}_{50})\) values are shown in Table 1. Our model showed a good correlation between descriptors and their insecticidal activity. From equation 3 we found that as the electron population or electronic accessibility increases, the monoterpenoid’s toxicity increases, and as GETAWAY values increase, toxicity also increases. In this model, there is an interaction effect between the two descriptors. There is a balance between GETAWAY and electrotopological state descriptors due to the negative interaction term. This indicates that if both of these descriptors values get too large, toxicity will decrease. If one of the descriptors yields a smaller value, it allows the other descriptor to have a large value and still exert a toxic effect.

The other QSAR that was developed consisted of thymol and nine of its derivatives. Two of the derivatives were ethers and the other seven were esters. Thymol and the two ether derivatives had the greatest toxicity. We obtained a linear relationship between toxicity of thymol compounds and the Mulliken population (electron density) around atom 13 (\(n = 10, s = 0.08, F = 68.52, r^2 = 0.90, q^2 = 0.84\)). The numbers on the atoms of thymol compounds correspond to the order in which the atoms were added to the Z-matrix to construct the molecules (Fig. 3). Experimental and calculated \(\log (1/\text{LD}_{50})\) values are shown in Table 2. Our model showed a good correlation between Mulliken population and toxicity. Our model for thymol compounds is presented in equation [4]:

\[
\log (1/\text{LD}_{50}) = 65.3(\pm 7.9) - 11.6(\pm 1.4)[\text{Mulliken population}] \] [4]
This relationship shows that toxicity decrease as the Mulliken population increases.

**Conclusion**

We developed two new QSAR models of monoterpenoids. In both models, the electronic characteristic plays an important role in toxicity. The non-aromatic model used electrotopological state descriptors to represent the electronic properties of the molecules. As the electron population or electron accessibility increased, toxicity also increased. These relationships might be due the electrostatic interaction of these compounds to a receptor and as electron accessibility for the monoterpenoids increases, binding affinity also increases. Electronic properties were also the essential component in the thymol QSAR. However, its effect was the inverse of the aliphatic QSAR. In both models, the electronic properties seem to be important.

Size and shape of the molecule were implicitly expressed in the GETAWAY descriptor. The non-aromatic monoterpenoid QSAR used a dynamic range of compounds, ranging from monocyclic to bicyclic monoterpenoids. Because of this dynamic range, our model used the GETAWAY descriptor to account for required shape and size of these compounds. The use of this descriptor infers that there is an optimum shape and size requirement that monoterpenoids must possess to fit into some receptor. In the thymol QSAR, the size and shape of the structure had very little effect on toxicity. This is probably due to the fact that these compounds are very closely related in structure, and that the slight changes in structure has little effect on toxicity. This information can be
used to aid in the development of a more effective thymol derivative. Also, because changing the size and shape of the structural moiety at atom 13 has little effect on toxicity, groups can be substituted on that atom to influence the electronic properties of the molecules in order to increase their toxicity.

As previously noted, the modes of action of monoterpenoids are not well understood. However, by developing our models individually (aromatic, aliphatic), we believe that the monoterpenoids included in each model exert the same mode of action. However, the structural requirements for each model makes it unclear whether the monoterpenoids in both models have the same mode of action, and suggests that the compounds in the aliphatic model may have a different mode of action than the aromatic compounds. We hope that these models can be used in the future to develop very effective alternative insecticides.

References

2) Rice, P. J., Coats, J. R., Pestic Sci. 1994, 41, 195


Figure 1. Structure of all aliphatic compounds studied
Figure 2. Structure of thymol and its derivatives.
Table 1. LD₅₀ house flies, predicted and residual values for all aliphatic monoterpenoids and their derivative

<table>
<thead>
<tr>
<th>Chemical Sample</th>
<th>Experimental LD₅₀ (µg/mol)</th>
<th>Experimental log(1/LD₅₀)</th>
<th>Predicted log(1/LD₅₀)</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carveol</td>
<td>1.03 (0.78-1.46)</td>
<td>-0.01</td>
<td>-0.11</td>
<td>0.10</td>
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<tr>
<td>Carvyl acetate</td>
<td>0.57 (0.54-0.61)</td>
<td>0.24</td>
<td>0.16</td>
<td>0.08</td>
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<tr>
<td>Carvyl propionate</td>
<td>0.99 (0.92-1.06)</td>
<td>0.00</td>
<td>0.23</td>
<td>-</td>
</tr>
<tr>
<td>Carvyl 3-chloropropionate</td>
<td>1.43 (1.33-1.54)</td>
<td>-0.16</td>
<td>-0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>Carvyl trichloroacetate</td>
<td>2.70 (2.38-3.02)</td>
<td>-0.43</td>
<td>-0.42</td>
<td>-</td>
</tr>
<tr>
<td>Carvyl pivalate</td>
<td>0.37 (0.35-0.40)</td>
<td>0.43</td>
<td>0.39</td>
<td>0.04</td>
</tr>
<tr>
<td>Carvyl chloropivalate</td>
<td>0.96 (0.85-1.09)</td>
<td>0.02</td>
<td>0.18</td>
<td>-</td>
</tr>
<tr>
<td>Carvomenthen-4-ol</td>
<td>0.71 (0.67-0.75)</td>
<td>0.15</td>
<td>0.12</td>
<td>0.02</td>
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<tr>
<td>Carvomenthen-4-yl pivalate</td>
<td>0.16 (0.13-0.20)</td>
<td>0.79</td>
<td>0.74</td>
<td>0.05</td>
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<tr>
<td>Carvone</td>
<td>1.12 (0.68-1.42)</td>
<td>-0.05</td>
<td>-0.20</td>
<td>0.15</td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>1.29 (0.86-1.45)</td>
<td>-0.11</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Perillyl alcohol</td>
<td>0.38 (0.32-0.45)</td>
<td>0.42</td>
<td>0.31</td>
<td>0.11</td>
</tr>
<tr>
<td>α-Terpinene</td>
<td>0.86 (0.62-1.20)</td>
<td>0.07</td>
<td>0.24</td>
<td>0.17</td>
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<tr>
<td>Limonene</td>
<td>0.37 (0.34-0.58)</td>
<td>0.43</td>
<td>0.31</td>
<td>0.13</td>
</tr>
<tr>
<td>Myrtenol</td>
<td>0.64 (0.57-0.73)</td>
<td>0.19</td>
<td>0.16</td>
<td>0.03</td>
</tr>
<tr>
<td>Myrtenal</td>
<td>1.54 (1.47-1.62)</td>
<td>-0.19</td>
<td>-0.16</td>
<td>-</td>
</tr>
<tr>
<td>Myrtenyl acetate</td>
<td>0.36 (0.31-0.41)</td>
<td>0.45</td>
<td>0.45</td>
<td>0.00</td>
</tr>
<tr>
<td>Verbenyl acetate</td>
<td>0.59 (0.54-0.67)</td>
<td>0.23</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>Verbenol</td>
<td>1.51 (1.45-1.56)</td>
<td>-0.18</td>
<td>-0.18</td>
<td>0.00</td>
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<tr>
<td>α-Pinene</td>
<td>0.82 (0.62-1.20)</td>
<td>0.09</td>
<td>0.09</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 2. LD$_{50}$ of house flies, predicted and residual values for thymol and its derivatives.

<table>
<thead>
<tr>
<th>Chemical Sample</th>
<th>Experimental $LD_{50}$ (µg/mol)</th>
<th>Experimental log(1/$LD_{50}$)</th>
<th>Predicted log(1/$LD_{50}$)</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymol</td>
<td>0.22 (0.20-0.24)</td>
<td>0.66</td>
<td>0.62</td>
<td>0.04</td>
</tr>
<tr>
<td>Thymyl acetate</td>
<td>0.49 (0.44-0.54)</td>
<td>0.31</td>
<td>0.38</td>
<td>-0.07</td>
</tr>
<tr>
<td>Thymyl propionate</td>
<td>0.49 (0.40-0.62)</td>
<td>0.31</td>
<td>0.37</td>
<td>-0.06</td>
</tr>
<tr>
<td>Thymyl pivalate chloride</td>
<td>0.34 (0.22-0.42)</td>
<td>0.47</td>
<td>0.47</td>
<td>0.00</td>
</tr>
<tr>
<td>Thymyl dichloroacetate</td>
<td>1.12 (0.98-1.27)</td>
<td>-0.05</td>
<td>-0.05</td>
<td>0.00</td>
</tr>
<tr>
<td>Thymyl chlorodifluoroacetate</td>
<td>0.47 (0.31-0.68)</td>
<td>0.33</td>
<td>0.21</td>
<td>0.12</td>
</tr>
<tr>
<td>Thymyl trichloroacetate</td>
<td>0.90 (0.70-1.60)</td>
<td>0.05</td>
<td>0.11</td>
<td>-0.06</td>
</tr>
<tr>
<td>Thymyl ethyl ether</td>
<td>0.62 (0.56-0.69)</td>
<td>0.21</td>
<td>0.21</td>
<td>0.01</td>
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<tr>
<td>Thymyl isopropyl ether</td>
<td>0.27 (0.21-0.37)</td>
<td>0.57</td>
<td>0.66</td>
<td>-0.09</td>
</tr>
<tr>
<td>Thymyl propionate</td>
<td>0.23 (0.18-0.32)</td>
<td>0.64</td>
<td>0.51</td>
<td>0.13</td>
</tr>
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</table>
Figure 3. Numbering of the atoms for thymol compounds. These numbers correspond to the order in which they were placed into the Z-matrix.

Figure 4. The methyl-cyclohexene carbon skeleton with a double bond present in the 2 position is a structure requirement a molecule needs to fit an aliphatic QSAR.
Figure 5. Plot of calculated versus observed toxicity values for aliphatic monoterpenoids and their derivatives
Figure 6. Plot of calculated versus observed toxicity values for thymol and its derivatives
CHAPTER 4: VARROA MITE CONTROL USING MONOTERPENOIDS AND THEIR DERIVATIVES

A paper to be submitted to the Journal of Agricultural and Food Chemistry

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Abstract

Monoterpenoids have been tested against Varroa jacobsoni with moderate success. In our investigation we tested a wide variety of monoterpenoids to determine the most effective acaricidal monoterpenoids. Functional-group transformations were performed on monoterpenoid parent alcohols and phenols in an attempt to increase their selective toxicity to Varroa jacobsoni. A QSAR model was then developed to help predict the toxicity and selectivity of monoterpenoids and their acetate derivatives.

Introduction

Honey production and crop pollination in the U.S. have been reduced since the presence of an ectoparasitic mite that first arrived in this country in 1987 (1). The varroa mite (Varroa jacobsoni) is the ectoparasitic mite that was first discovered in the USA in Wisconsin while inspecting migratory honey bee colonies. The varroa mite has caused significant economic impact and biological damage (2).

Developing honey bee pupae die or develop deformed wings when affected by the varroa mite. Mite infestation reduces the number and vigor of the worker bee population. If this infestation is left untreated, the colonies die off (3). Pesticidal control of varroa mites has relied almost totally on fluvalinate and coumaphos. These acaricides/insecticides are the only two synthetic acaricides registered for varroa mite control.
control. The use of these acaricides has various drawbacks. One drawback is that acaricide residues cannot be present in honey or bee wax, so accidental tainting of the honey or bee wax can cause loss of product to apiculturist. Another drawback is fluvalinate resistant varroa mites have caused a serious impact on control of their populations (3). Formic acid has also been registered recently for control of Varroa mites.

We investigated the use of biopesticides because of the drawbacks of synthetic acaricides. These biopesticides are already being tested by a few apiculturist as an alternative to synthetic acaricides for treatment of varroa mites. Monoterpenoids are secondary metabolites found in some essential oils that have been used against varroa mites in Europe (4). Menthol is a monoterpenoid that is marketed in the USA for control of tracheal mites in honey bees. A few of the monoterpenoids possess unusual selectivity. These compounds are extremely toxic to the Varroa jacobsoni, but not the Apis mellifera. Monoterpenoids have several advantages over the synthetic acaricides. They have little or no toxicity to mammalian systems. Monoterpenoids are registered and used in decongestants, perfumes, and artificial flavorings (5-6) These compounds are considered more biodegradable compared to other pesticides. In this paper, we investigated the toxicity of monoterpenoids and their derivatives to both Apis mellifera and Varroa jacobsoni. Using their toxicological profiles, we develop a quantitative structure-activity relationship (QSAR) in order to develop more effective monoterpenoid biopesticides. In developing these QSARs, we hope to gain insight into the structural requirements needed for these compounds to exert their selective toxicity to the mites.
Materials and Methods

Chemicals:

Monoterpenoids used in this experiment were purchased from Aldrich, Milwaukee, WI, Sigma, St. Louis, MO and Eastman, Rochester, NY.

Synthesis:

Esters: Parent alcohols or phenols were added to their corresponding anhydride or acid chloride (1 mol : 2 mol) to form ester derivatives in 100 ml of methylene chloride. A catalytic amount of pyridine (2-5 drops) was added, and the reaction was allowed to stir for 24-48 hr at room temperature (5). The perillyl acrylate reaction was stirred for 8 hours at 0°C. Thin-layer chromatography (TLC) was used to monitor the reactions, by using a 9:1 hexane:acetone mobile phase; the plates were developed by a vanillin spray (8 g vanillin, 1.25 ml conc. sulfuric acid brought up to 250 ml with methanol). The reaction mixture was worked up with four washes of NaHCO₃ in water. A rotary evaporator was used to remove the methylene chloride solvent. Compounds were purified with silica gel-column clean up, using a 19:1 hexane:acetone solvent system. Esters' identities were determined using TLC by comparing Rf values of the parent alcohols or phenols against reaction products. Identities of the purified monoterpenoid derivatives were confirmed using ¹H-NMR 300 MHz.

The formate ester was synthesized by the following method: Perillyl alcohol and formic acid (1 mol / 2 mol ratio) were added to a 250-ml round-bottom flask. A catalytic amount of concentrated hydrochloric acid was added to the mixture, and the solution was allowed
to reflux for 14 hours. After 14 hours, the reaction was worked up the same way as the other reactions.

Ethers: Ether reactions were carried out using thymol and their corresponding alkyl halide in the presence of a phase-transfer catalyst, benzyltributylammonium bromide (BTAB). Thymol (5 g) was dissolved in 50 ml of CH₂Cl₂ together with the alkyl halide (10 ml) and benzyltributylammonium bromide (0.535 g). Then NaOH (2.0 g) dissolved in H₂O (175 ml) solution was added into the organic layer. The reaction was allowed to stir for two weeks (6). Work-up for this reaction was the same as for the previous reactions. Identities of monoterpenoids were again determined using TLC and ¹H-NMR 300 Mhz.

**Preliminary Screening:**

Preliminary screening was performed to rapidly test for highly efficient monoterpenoids with selective toxicity to varroa mites and not to honey bees. These compounds were then used in a more definitive bioassay to develop accurate bioactivity data which could be used in QSAR models. Two separate screening tests were performed. One set of screening was conducted as a fumigation assay for both *Apis mellifera* and *Varroa jacobsoni* and was performed by Dr. Marion Ellis at the University of Nebraska. The second set of screening used fumigation LT₅₀ bioassay on *Apis mellifera* and a contact assay for *Varroa jacobsoni*, which were performed at Fassbinder Apiaries.

**Screen One Bioassay:**

Worker honeybees were collected from varroa-infested hives located on the University of Nebraska East campus. Bees were placed in 9x9x9-cm cages made of eight-mesh wire and fitted with a smaller sugar syrup dispenser. Caged bees were placed in 5.4-liter plastic containers fitted with sticky boards to collect any mites that fell from the bees.
Test compounds were applied to 3x2x1-cm foam-plastic florist blocks located on top of the cages. One ml of 18.5 ppm solution was evaluated for all the compounds. The containers were closed immediately after treatment and placed in a dark incubator at 32°C. After 24 hours, all mites that fell to the sticky boards were counted. The adult bees were then killed and shaken in a 70% ethanol solution to recover any mites remaining on the bees. The remaining mites on the bees were then counted, and percent mortality was calculated. Structures of the monoterpenoids in this assay are presented in Figure 1.

**Screen Two Bioassays:**

(A) This toxicity test of monoterpenoids on the *Apis mellifera* was a fumigation assay. One ml of each monoterpenoid or derivative was placed on a thick paper towel in a smaller screen container. The small-screened containers were placed inside a 5-liter container to prevent the bees from coming in direct contact with the treatment compound. Thirty bees were placed in each 5-liter container. The container was then sealed. The $LT_{50}$ ratings (Lethal Time to kill 50% of the test organisms, as defined by stopping of all motion in 50% of the organisms) were recorded for each monoterpenoid.

(B) This toxicity test of monoterpenoids on the *Varroa jacobsoni* was by contact. One ml of each monoterpenoids and derivatives was placed on a thick paper towel and then placed in a 5-liter container. Thirty *Varroa jacobsoni* were placed in each container, and placed on the paper towel. The container was then sealed and $LT_{50}$ ratings were recorded for each of the monoterpenoids. Structures of monoterpenoids tested in these assays are shown in Figure 2.
**Fumigation Bioassay:**

Fumigation bioassays were performed to evaluate the toxicities of 14 monoterpenoids and their derivatives. All monoterpenoids solutions used soybean oil as a solvent. 500 µl of each monoterpenoid solution was applied to 70-mm filter paper. Controls used 500 µl of soybean oil on a 70-mm filter paper. The filter paper was then placed in the bottom of a 475-ml glass container. The *A. mellifera* fumigation bioassay used 4-cm long cotton wicks that were saturated with a sucrose solution (50% by volume) and placed inside each specimen chamber. A 1-mm mesh cloth screen was placed on top of each chamber. The specimen chamber was then suspended in the 475-ml large container using a stainless steel wire. After the chamber is suspended, the large container was sealed.

After 20 hours, specimen chambers were emptied, specimens were placed in petri dishes, and then mortality of *A. mellifera* and *V. jacobsoni* was recorded. Each concentration was replicated three times. Thirty *A. mellifera* used for each replication and 25 *V. jacobsoni* were used for each replication. LC50s and their 95% confidence intervals values were obtained by using PROBIT analysis (SAS, 1985). Selectivity ratios were defined as LC50 of *A. mellifera* / LC50 of *V. jacobsoni* and were determined for each of the compounds. Structures of monoterpenoids in this assay are shown in Figure 3.

**QSAR:**

Geometry and energies of monoterpenoids and their derivatives were optimized using an AM1 basis set which was calculated by CAChe™ (Oxford Molecular). Electronic properties examined were Highest Occupied Molecular Orbitals (HOMO), Lowest Unoccupied Molecular Orbital (LUMO), and dipole moment. Classical parameters and other chemometric parameters were calculated using the Dragon™. These
parameters encode information on various physiological effects important for a molecule's toxicity, which includes molar refractivity, Log P, various topological descriptors, and GETAWAY descriptors.

Linear regressions were performed using SAS™. The toxicity data was expressed as nmol/cm³. The quality of the regression models was evaluated using the square of the correlation coefficient ($r^2$). Only regressions with $r^2 > 0.80$ were examined. Cross-validation ($q^2$) was used to validate the model. The leave-one-out cross-validation method was used. It was calculated based on the following equations [1]-[2]:

$$\text{Crossvalidation } q^2 = 1 -(\text{PRESS/ SSTO}) \[1\]$$

where

$$\text{PRESS} = \sum_y (Y_{\text{pred}} - Y_{\text{actual}})^2. \[2\]$$

And SSTO is sum of squares total.

Non-random relationships have been shown for cross-validation that were greater than 0.40 (8).

**Results:**

*Screen One:* Percent mortalities are presented in Table 1. Myrtenyl acetate was the most toxic compound, killing 100% of the mites and having no acute toxic effect to the bees. Thymyl trifluoroacetate had the second greatest toxicity, killing 79% of the mites on bees. However, thymyl trifluoroacetate showed some toxicity to bees killing 15% of them.

Thymyl acetate also possesses efficacy as an acaricide by killing 41% of the mites on the bees with no adverse effect to the bees. Thymyl ethyl ether had high toxicity to the bees (100% mortality) and showed little toxicity to the mites (19% mortality). Using this
preliminary screen to pick compounds to be tested in the more robust bioassay, we chose to test myrtenyl acetate and thymyl acetate because of their highly selective toxicity to mites. Thymyl trifluoroacetate was not chosen because of the potential of mammalian toxicity due to the present of trifluoroacetate.

Screen Two: Twenty-two monoterpenoids' and their derivatives' LT50 values are presented on Table 2. Myrtenyl acetate possessed the highest toxicity against *Varroa jacobsoni*, with an LT50 value of 32 sec. Myrtenyl acetate has the greatest toxic effect on *Varroa jacobsoni* in both screens. Thirteen monoterpenoids had LT50 values of less than 10 minutes, with 1,8-cineole, perillyl acetate, pulegone, and thujone showing good toxicity to the mites. Out of these four compounds, perillyl acetate showed high selectivity for the mites and had the slowest toxicity to the bees.

Fumigation studies: In these studies, we examined the best compounds from the screens and their parent compounds to determine the effectiveness of the derivatives. We also examined monoterpenoids that were previously reported to have acaricidal properties. Monoterpenoids' and their derivatives' LC50 values are present in Table 3.

Four monoterpenoid derivatives, linalyl acetate, perillyl acetate, thymyl acetate, and myrtenyl acetate, were shown to be highly toxic to varroa mites. Acetates and propionates are selectively more toxic to varroa mites than their parent alcohols. All four of these derivatives had selective toxicity ratios greater than one. Out of the four most efficient acaricidal monoterpenoids, myrtenyl acetate had the lowest selectivity ratio. Myrtenyl acetate was 3.6 times more toxic to varroa mites than to the honey bee. Linalyl acetate has the largest selective toxicity ratio value. It was 83.3 times more toxic to the varroa mite than to the honey bee.
QSAR: We examined several parameters that have previously been used to help explain the toxicity. However, we found no relationship between HOMO, LUMO, dipole moment, Log P, various connectivity indices, and molar refractivity with toxicity to the honey bee or the varroa mite. However, a linear relationship was found between honey bee toxicity and GETAWAY descriptor H6u (n = 11, $r^2 = 0.95$, and $q^2 = 0.92$). The QSAR model excludes thymol and thymyl acetate, because of their aromatic characteristics, and they were outliers in the model. It is possible that these aromatics compounds might act at a different site of action than aliphatic monoterpenoids. Experimental and predicted LC$_{50}$ values are shown on Table 4. As the H6u values increase, monoterpenoids' and their derivatives' toxicity decrease, as shown in the following equation [3]:

$$LC_{50} = -4.6(\pm0.8) + 22.7 (\pm1.8)(GETAWAY)$$ [3]

**Conclusion:**

Preliminary screens focused on many of the monoterpenoids found in the essential oils that have been used by others to attempt to control varroa mite populations. Some of these monoterpenoids show good toxicity rates to the mite, but they also seemed to be toxic to the bees. We believe both preliminary screens provided a useful way to assess which compounds should be tested in the more robust fumigation bioassay. Acetate derivatives seem to have greater selectivity toxicity than the parent alcohols or phenols. Linalyl acetate, (S)-perillyl acetate, thymyl acetate, and myrtenyl acetate were the most effective acarcides in the fumigation study. Each of these compounds showed selective toxicity great than one. Linalyl acetate showed the greatest selective toxicity ratio and potentially is the best monoterpenoid for varroa mite control. It seems that the
addition of the acetate or propionate group makes monoterpenoids selectively more toxic to the mites.

We were unable to find a QSAR relationship for the varroa mite. A honey bee QSAR model was developed. A relationship between toxicity and GETAWAY descriptor Hu6 was obtained. This model can be used to determine the potential risk of aliphatic monoterpenoids and their acetates to honey bees in order to predict their toxicity. Knowing these compounds’ toxicity to bees allows us to test compounds that are predicted to be safe to the bees.

Reference:


5) Rice, P. J., Coats, J. R., Pestic Sci. 1994, 47, 195


Figure 1. Structures of monoterpenoids tested in Screen One
Figure 2. Structure of monoterpenoids tested in Screen Two.
Figure 3. Structure of monoterpenoids tested in the fumigation assay
Table 1. Percent mortality for both *Varroa jacobsoni* and *Apis mellifera* at 18.5 ppm per hive.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>% Mortality of <em>Varroa jacobsoni</em></th>
<th>% Mortality of <em>Apis mellifera</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Myrtenyl acetate</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Terpinyl acetate</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>(s) Perillyl pivalate</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>(s) Perillyl acrylate</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Myrtenyl propionate</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Thymyl ether ether</td>
<td>19</td>
<td>100</td>
</tr>
<tr>
<td>Thymyl propargyl ether</td>
<td>2.8</td>
<td>10</td>
</tr>
<tr>
<td>Thymyl acetate</td>
<td>4.1</td>
<td>0</td>
</tr>
<tr>
<td>Thymyl propionate</td>
<td>3.4</td>
<td>1</td>
</tr>
<tr>
<td>Thymyl trifluoroacetate</td>
<td>79</td>
<td>15</td>
</tr>
<tr>
<td>Thymyl isopropyl ether</td>
<td>2.6</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2. LT$_{50}$ values for monoterpenoids in Screen Two for both *Varroa jacobsoni* and *Apis mellifera*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>LT$_{50}$ (min) for <em>Varroa jacobsoni</em></th>
<th>LT$_{50}$ (min) for <em>Apis mellifera</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alcohol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citronellol</td>
<td>8</td>
<td>200</td>
</tr>
<tr>
<td>(S)-Perillyl alcohol</td>
<td>9</td>
<td>65</td>
</tr>
<tr>
<td><strong>Phenol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carvacrol</td>
<td>5</td>
<td>120</td>
</tr>
<tr>
<td><strong>Formate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perillyl formate</td>
<td>19</td>
<td>360</td>
</tr>
<tr>
<td><strong>Acetates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menthyl acetate</td>
<td>36</td>
<td>275</td>
</tr>
<tr>
<td>Perillyl acetate</td>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>Myrtenyl acetate</td>
<td>0.32</td>
<td>190</td>
</tr>
<tr>
<td>4-Isopropyl benzyl acetate</td>
<td>20</td>
<td>240</td>
</tr>
<tr>
<td>Carvacryl acetate</td>
<td>49</td>
<td>26</td>
</tr>
<tr>
<td><strong>Propionate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isopropyl benzyl propionate</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>Perillyl propionate</td>
<td>120</td>
<td>298</td>
</tr>
<tr>
<td>Myrtenyl propionate</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td><strong>Aldhyde</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citral</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td><strong>Ether</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td><strong>Hydrocarbons</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limonene</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>Myrcene</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td><strong>Ketones</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Ionone</td>
<td>90</td>
<td>60</td>
</tr>
<tr>
<td>Pulegone</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Thujone</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Verbenone</td>
<td>5</td>
<td>49</td>
</tr>
<tr>
<td>Carvone</td>
<td>7</td>
<td>30</td>
</tr>
</tbody>
</table>
Table 3. LC$_{50}$ values, 95% confidence intervals, and Selectivity Ratio values for *Varroa jacobsoni* and *Apis mellifera*.

<table>
<thead>
<tr>
<th>Compounds</th>
<th><em>Varroa jacobsoni</em> LC$_{50}$ (nmol/cm$^3$)</th>
<th><em>Apis mellifera</em> LC$_{50}$ (nmol/cm$^3$)</th>
<th>Selectivity Ratio: A. mellifera LC$<em>{50}$/V. jacobsoni LC$</em>{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bornyl acetate</td>
<td>3.87 (3.06-5.20)</td>
<td>1.94 (1.53-2.34)</td>
<td>0.50</td>
</tr>
<tr>
<td>Carvyl acetate</td>
<td>3.09 (2.32-6.18)</td>
<td>2.06 (2.01-3.19)</td>
<td>0.67</td>
</tr>
<tr>
<td>Citronellyl acetate</td>
<td>2.77 (2.07-4.49)</td>
<td>15.33 (12.10-22.19)</td>
<td>5.53</td>
</tr>
<tr>
<td>Geranyl acetate</td>
<td>3.41 (2.34-6.32)</td>
<td>4.28 (3.21-8.25)</td>
<td>1.25</td>
</tr>
<tr>
<td>Linalyl acetate</td>
<td>0.15 (0.05-0.36)</td>
<td>12.89 (9.78-19.10)</td>
<td>84.33</td>
</tr>
<tr>
<td>(R)-Myrtenol</td>
<td>0.13 (0.07-0.20)</td>
<td>0.13 (0.07-0.20)</td>
<td>1.00</td>
</tr>
<tr>
<td>(R)-Myrtenyl acetate</td>
<td>0.36 (0.31-0.41)</td>
<td>1.29 (1.03-1.49)</td>
<td>3.57</td>
</tr>
<tr>
<td>(S)-Perillyl alcohol</td>
<td>3.55 (2.69-4.60)</td>
<td>1.44 (112-1.171)</td>
<td>0.41</td>
</tr>
<tr>
<td>(S)-Perillyl acetate</td>
<td>0.15 (0.10-0.26)</td>
<td>3.55 (3.09-4.27)</td>
<td>23.00</td>
</tr>
<tr>
<td>Linalool</td>
<td>N/A</td>
<td>1.56 (1.17-2.14)</td>
<td>N/A</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>N/A</td>
<td>1.17 (0.97-1.36)</td>
<td>N/A</td>
</tr>
<tr>
<td>Thymol</td>
<td>1.20 (1.07-1.33)</td>
<td>5.86 (4.73-8.19)</td>
<td>4.89</td>
</tr>
<tr>
<td>Thymyl acetate</td>
<td>0.31 (0.26-0.36)</td>
<td>1.77 (1.46-2.19)</td>
<td>5.67</td>
</tr>
</tbody>
</table>
Table 4. Experimental and predicted LC$_{50}$ values obtain from the QSAR model.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Experimental LC$_{50}$ (nmol/cm$^3$)</th>
<th>Predicted LC$_{50}$ (nmol/cm$^3$)</th>
<th>Residual (nmol/cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bornyl acetate</td>
<td>1.94</td>
<td>1.99</td>
<td>-0.05</td>
</tr>
<tr>
<td>Carvyl acetate</td>
<td>2.06</td>
<td>-0.29</td>
<td>2.35</td>
</tr>
<tr>
<td>Citronellyl acetate</td>
<td>15.33</td>
<td>15.0</td>
<td>0.35</td>
</tr>
<tr>
<td>Geranyl acetate</td>
<td>4.28</td>
<td>4.56</td>
<td>-0.28</td>
</tr>
<tr>
<td>Linalyl acetate</td>
<td>12.9</td>
<td>11.9</td>
<td>1.01</td>
</tr>
<tr>
<td>(R)-Myrenol</td>
<td>0.13</td>
<td>-0.85</td>
<td>0.98</td>
</tr>
<tr>
<td>(R)-Myrenyl acetate</td>
<td>1.29</td>
<td>2.49</td>
<td>-1.20</td>
</tr>
<tr>
<td>(S)-Perillyl alcohol</td>
<td>1.45</td>
<td>1.76</td>
<td>-0.31</td>
</tr>
<tr>
<td>(S)-Perillyl acetate</td>
<td>3.55</td>
<td>4.51</td>
<td>-0.96</td>
</tr>
<tr>
<td>Linalool</td>
<td>1.56</td>
<td>3.44</td>
<td>-1.88</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>1.17</td>
<td>1.17</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Figure 4. Plot of calculated versus observed toxicity values for monoterpenoids and their acetates.
CHAPTER 5: CONCLUSIONS

In Chapter One, a QSAR model was not developed for the whole group of monoterpenoids (and their derivatives), although closely related subsets of monoterpenoids did demonstrate good QSARs. This data hints to more than one mode of action for monoterpenoids. The inability to obtain a QSAR model for the entire group of monoterpenoids may be due to different sites of action, for example, for aromatic versus aliphatic type of monoterpenoids. If these compounds do act at different sites of action, then the structural requirements for the molecules would be different for those monoterpenoids, and one overall QSAR model would be unattainable. The lack of QSAR as a whole might also be due to other factors that influence toxicity. Factors such as metabolism of monoterpenoids might cause some of these compounds to be activated and cause the detoxification of others. If metabolism occurs rapidly, and those monoterpenoids’ structure is changed due to metabolism before the compound is able to reach the site of action, then QSAR models may not be able to predict toxicity. Uptake rate also plays a major role in insect toxicity. It is our assumption that approximately the same amount of chemical is absorbed into the insect; however this may not be an accurate assumption. Different monoterpenoids have various vapor pressures. This difference in vapor pressure causes various monoterpenoids to evaporate off the cuticle at different rates. Certain monoterpenoids may also pass through the insect’s cuticle at different rates. Different vapor pressures and cuticular penetration rates can thus affect how much of a certain monoterpenoid can exert its effect at the site of action. Potential
penetration and metabolism differences might have prevented the development a single QSAR relationship for the whole group of monoterpenoids.

However, I did find relationships for the structural characteristics of sub-groups of monoterpenoids and their toxicities. Since the sub-groups are not as large or diverse a set of compounds as the whole group of monoterpenoids, further compounds are needed to truly test the validity of these relationships. These smaller sets of relationships gave a good starting point to develop more robust QSARs and could be used to increase the insecticidal effectiveness of compounds within a certain sub-group. Because of sub-group structural similarities, relationships were able to be obtained. I believe that all of the compounds within a sub-group probably have the same mode of action and is the reason I was able to develop QSARs.

Geraniol and its derivatives were the only sub-group of monoterpenoids to possess a relationship between toxicity and classic parameters. Classic parameters captured essential information on the size and shape of the geraniol compounds. Due to the small set of compounds studied in this model, more compounds are needed to validate these QSARs. If these correlations hold true when more compounds are added, I will know that there is an optimal size and shape requirement for that part of the molecule and that ester groups were added for the compound to exert its toxic effect on house flies. These relationships found were parabolic relationships, and because of these types of relationships, I can predict the optimum toxicity for these compounds. To develop more insecticidally active geraniol compounds, other regions of the molecule must to be modified to enhance their toxicity.
This study also showed that Mulliken population around certain atoms of thymol, carveol, and carvacrol compounds showed a strong correlation with their toxicity. Mulliken population represents the electron density around the atoms in a molecule. Mulliken population may represent important electrostatic interactions of monoterpenoids to a receptor. Although the modes of action of these compounds are not completely worked out, it seems that electronic properties of the molecules are an essential component of their toxicity. No correlations were obtained for the classical parameters and the compounds’ toxicities. This indicates we can modify the –OH region of the molecules, because size and shape of that part of the molecule does not seem to be important for its toxicity. I was able to add different ester functional groups at that part of the molecule to change their Mulliken population around certain atoms to enhance their toxicity. Additional compounds will be added in the future, altering various parts of the molecules to obtain a more dynamic set of derivatives. This dynamic set of derivatives is needed in order to examine the true validation of our QSARs models.

In Chapter Two, I developed one new QSAR model and increased the type of functional groups and the number of compounds for the thymol QSAR present in the previous chapter. In both models, the electronic characteristics of monoterpenoids play an important role in their toxicity. The aliphatic model used electrotopological state descriptors to represent the electronic properties of the molecules. Electrotopological state values were used in this model because the speed and the accessibility of the calculations allow a large number of compounds to be screened in a short amount of time. As the electron population or electronic accessibility increased around atom #3, then its toxicity also increased. These relationships might explain binding affinity of these
monoterpenoids to a receptor. These relationships might explain the electrostatic interaction of these compounds to a receptor, and as electronic accessibility for the monoterpenoids increases, binding affinity also increases. Electronic characteristics of the thymol compounds were essential components in thymol insecticidal properties. As thymol compounds' Mulliken population decreased, their toxicity increased. In both models, the electronic characteristics are essential for their toxic effects to house flies.

The molecule size and shape was implicitly expressed in the GETAWAY descriptor. The aliphatic monoterpenoid QSAR used a dynamic range of compounds ranging from monocyclic to bicyclic monoterpenoids. To account for the difference in the size or shape of the monoterpenoids, GETAWAY descriptors were used. This descriptor infers that there is an optimum shape and size requirement that monoterpenoids must possess to fit into some receptor. The thymol QSAR showed that size and shape encoded by the GETAWAY descriptors had very little effect on their toxicity. The lack of size requirements is probably due to the fact that these compounds are closely related in structure, and that the slight changes in structure have little effect on their toxicity. In addition, because influencing the size or shape of the structural moiety at atom 13 has little effect on toxicity, I was able to add groups to atom 13 that can influence the electronic properties of the molecules in order to predict or improve their toxicity.

The modes of action of monoterpenoids are not well understood. However, developing QSAR models will help us separate out different modes of action. When QSAR models are developed, they relate certain structural properties of the molecules to certain biological effects. Molecules that possess the same structural similarities would act at the same site of action, and structures that are dissimilar could act at a different site.
If two separate QSARs are developed for monoterpenoids, it might indicate two different sites of action for these compounds. I believe that these models shed some light on the structural requirements for monoterpenoids to exert their toxic effects on house flies.

In Chapter Three our data showed the highly selective nature of monoterpenoids and their derivatives. Some monoterpenoids were highly toxic to the mite with little acute toxicity to the bees. Acetate derivatives seem to possess the greatest selectivity properties. Parent alcohols showed good toxicity to the mite, but most were also toxic to honey bees. Linalyl acetate, (S)-perillyl acetate, thymyl acetate, and (R)-myrtenyl acetate were the most effective acarcicides in the fumigation assay. All of these compounds had selective toxicity ratios greater than one. Linalyl acetate showed the greatest selective toxicity ratio and potentially is the best monoterpenoid for varroa mite control.

We were unable to find a QSAR relationship for the varroa mite or for the selective toxicity ratios. However, a QSAR model was developed for honey bees. The relationship obtained showed a linear correlation between toxicity and GETAWAY descriptor H6u. This model can be used to assess the potential risk of non-aromatic monoterpenoids and their acetate derivatives to honey bees in order to predict their toxic effect. Being able to predict toxicity of these compounds to bees allows us to synthesize and test only compounds that are predicted to be safe to the bees.

In the future, QSAR models will be used to develop and predict highly insecticidally active compounds. Monoterpenoids or simple derivatives would make ideal biopesticides. These compounds also possess the ability to have low acute toxicity to non-target species. This is reflected by the fact that these compounds are found in various commercial products. As seen in Chapter Three, some monoterpenoids have very
selective toxicity to *Varroa jacobsoni*. In the future, this selective toxicity can hopefully be used to design new monoterpenoids, which are toxic to pest species and have very few non-target effects.