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The comparative toxicity of selected organic phosphate and carbamate insecticides to the honey bee.

Robert Edward Grahame
University of Massachusetts Amherst

THE COMPARATIVE TOXICITY OF SELECTED ORGANIC PHOSPHATE AND CARBAMATE INSECTICIDES TO THE HONEY BEE

A Dissertation Presented

By

Robert E. Grahame Jr.

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Robert E. Grahame Jr.

Approved as to style and content by:

G. B. Shaw
(Chairman of Committee)

M. A. McKenzie
(Head of Department)

H. E. Ware
(Member)

W. E. Tomlinson Jr.
(Member)

March 1967
(Month) (Year)

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Table of Contents

Introduction

It has now become a necessity to test newly developed pesticides, not only against potential pest species, but against beneficial species as well. This aids in the general recommendations for its use, and in the development of selective pesticides that can adequately control harmful organisms without destroying their natural controls, or related organisms that may benefit man.

Heading the list of beneficial insects is the honey bee, Apis mellifera Linnaeus. This insect has gained widespread acceptance as an economically important pollinator, and its exposure to the more recently developed insecticides can endanger this important function.

Phosphate and carbamate insecticides are particularly important as they are now utilized in pest control far more than other groups of compounds. A large amount of work has been done on the testing of these insecticides on honey bees in the laboratory and in the field. However, laboratory tests have not always forecast results obtained when an insecticide was applied as a control measure.

Field testing of insecticides on honey bees has become more widely used in recent years. This may be done

as an experiment in itself, or in combination with tests on pest organisms. However, problems can also be encountered in this method of testing. Since field conditions are variable, newly developed insecticides can not accurately be compared unless all are tested at one time. Unfortunately, facilities, time, and finances normally prevent this from being accomplished. Also, bee poisoning experiments conducted in one area may give different results when the same compounds are tested elsewhere.

While it will always be necessary to analyze insecticide results in the field before final conclusions on their toxicity to honey bees can be drawn, the possibility of an improvement in laboratory testing methods should be examined.

A partial solution to the problem would be a greater degree of standardization of the investigations performed. A comprehensive series of laboratory experiments should be devised, and performed by a central agency, for each insecticide now in use. The end result of this would be an evaluation of the overall toxicity to the honey bee. Also determined, would be the effects on bees of the residues, the toxicity as a contact spray or dust, stomach poison, and fumigant. At the present time a multitude of test methods are used. Also, all modes of action may not be

investigated at one time. Under these circumstances, a comparison of experimental results becomes difficult.

The purpose of the problem attempted here was twofold. First, to review the literature of phosphate and carbamate insecticide bee poisoning, emphasizing the methods used in experimentation. Second, the testing of a select group of insecticides by a combination of methods derived from this review.

The results were then compared with previous laboratory and field investigations of these compounds. Where possible, conclusions were drawn relative to the toxicity of the compounds tested and the reliability of methods used in testing.

Literature Review

The literature review is divided into four sections.

Section one is a listing of those phosphate and carbamate insecticides whose toxicities to honey bees have been evaluated. This listing includes the following:

(1) The accepted name of the compound, as given by Kenaga (1966), or as designated by Billings (1965), or Johansen (1966).

(2) The class of the compound:

C=Carbamate

Ch=Chlorinated Hydrocarbon

P=Phosphate

SC=Systemic Carbamate

SP=Systemic Phosphate

(3) The primary uses of the compound:

A=Acaricide

F=Fumigant

H=Herbicide

I=Insecticide

N=Nematocide

(4) The United States and foreign manufacturers.

(5) Bee contact is listed as "yes" if the compound is presently used in situations where it will come in contact with sizeable numbers of field bees. This would occur if it were used on fruit insects, grain insects, legume and grass insects, or vegetable insects.

A "no" is listed if the compound is commercially used, but only on livestock or stored-products. In these situations, it would have little or no contact with significant numbers of field bees.

If neither a "yes" or a "no" is listed, it indicates that the compound was not used, as of 1966, on a commercial scale. This list was compiled from Anonymous (1965a, 1965b, 1965c, and 1966).

(6) The page number refers to the page on which the literature review of the compound can be located.

Section two reviews the 7 compounds covered in the series of tests conducted. A detailed review of the literature, relative to honey bee poisoning, is given for each compound with particular emphasis on the test methods utilized by various investigators.

Section three is a less detailed review of the toxicity of other phosphate and carbamate insecticides to the honey bee.

Section four lists those compounds for which no toxicity data, published in the English language, was reviewed. However, summaries of the toxicity of these compounds to honey bees are given by Johansen (1966), and by Anderson and Atkins (1966). In section four, if a compound is included in the review by Johansen, an Arabic numeral 1 appears after its name. If it is included in the review by Anderson and Atkins, an Arabic numeral 2 appears after its name.

Phosphate and Carbamate Insecticides

Compound	Class	Use	Manufacturer	Bee Contact	Page No.
azinphosmethyl	P	A,I	Chemagro FFB	Yes	11
azinphosethyl	P	A,I	Chemagro FFB	--	83
Azodrin	SP	I	Shell	--	83
Banol	C	I	Upjohn	--	83
Bayer 39007	C	I	Chemagro FFB	--	44
Bayer 41831	P	I	Chemagro FFB Sumitomo	--	83
Bidrin	SP	I	Shell	Yes	83
Bomyl	P	A,I	Allied	--	44
carbaryl	C	I	Union Carbide	Yes	16
carbophenothion	P	A,I	Stauffer	Yes	45
Chlorthion	P	I	Chemagro	--	84
Ciodrin	P	I	Shell	No	84
coumophos	P	A,I	Chemagro FFB	No	46
demeton	SP	A,I	Chemagro FFB	Yes	47
diazinon	P	A,I	Geigy	Yes	24
dicapthon	P	A,I	American Cyanamid	--	49
dichlorvos	P	F,I	Shell	No	49

Compound	Class	Use	Manufacturer	Bee Contact	Page No.
dimethoate	SC	A,I	American Cyanamid	Yes	50
dimetilan	C	I	Geigy	--	84
dioxathion	P	A,I	Hercules	Yes	51
disulfoton	SP	I	Chemagro FFB	Yes	53
endothion	P	I		--	53
EPN	P	A,I	E. I. Dupont	Yes	54
ethion	P	A,I	FMC	Yes	55
famphur	P	A,I	American Cyanamid	--	84
fenthion	SP	A,I	Chemagro FFB	No	84
Imidan	P	A,I	Stauffer	--	56
Isolan	C	I	Geigy	--	85
isopropyl parathion	P	I		--	85
malathion	P	I	American Cyanamid Sumitomo	Yes	29
Matacil	C	I	Chemagro	--	85
menazon	SP	A,I	Imperial	--	85
Mesurol	C	A,I	Chemagro	--	57
Metacide	P	I		--	85
methyl demeton	P	I	Chemagro	Yes	57

Compound	Class	Use	Manufacturer	Bee Contact	Page No.
methyl parathion	P	I	American Potash Monsanto Shell Stauffer Sumitomo Velsicol	Yes	59
Methyl Trithion	P	A,I	Stauffer	--	59
mevinphos	SP	I	Shell	Yes	60
naled	P	A,I	Chevron	Yes	61
NPD	P	A,I	Stauffer	--	63
oxydemetonmethyl	SP	A,I	Chemagro FFB	Yes	86
paraoxon	P	I		--	63
parathion	P	A,I	American Cyanamid American Potash Monsanto Shell Stauffer Sumitomo Velsicol	Yes	64
phorate	SP	A,I	American Cyanamid	Yes	69
phosalone	P	A,I	Chipman	--	42
phosphamidon	P	I	Chevron	Yes	70
Phostex	P	A,I	FMC	--	73
Potasan	P	I		--	74
Pyramat	C	I		--	86
ronnel	SP	A,I	Dow	No	74

Compound	Class	Use	Manufacturer	Bee Contact	Page No.
schradan	SP	A,I	Centerchem Murphy	No	75
Temik	SC	A,I, N	Union Carbide	--	86
tepp	P	I	American Potash Miller Stauffer	Yes	77
Tetram	P		Chipman	--	86
Thiocron	SP	I		--	87
trichlorfon	P	I	Chemagro FFB	Yes	79
Zectran	C	I	Dow	Yes	81
Zinophos	P	N,I	American Cyanamid	--	87

Additional Compounds

Compound	Class	Use	Manufacturer	Bee Contact	Page No.
chloropropylate	Ch	A	Geigy	--	23
methoxychlor	Ch	I	E. I. Dupont Geigy	Yes	42

Literature Review of the Compounds Tested

Azinphosmethyl

Azinphosmethyl is also known as Azinphos-methyl, Bayer 9027, Bayer 17147, Gusathion, Guthion, and 17/147. Its chemical designation is: O,O-dimethyl S-4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl phosphorodithioate.

Laboratory Tests

Anderson and Atkins (1958) tested azinphosmethyl on honey bees at 100, 200, and 400 mg dosages of a 1% dust by a vacuum bell-jar method of testing. Nine replicates, of at least 20 bees/replicate, were used in the tests conducted. Treated bees were held in clean cages at 80°F constant temperature and 65% RH and fed a 50% honey-water solution. Mortality counts were taken at 4, 6, 24, 48, and 72 hr after treatment. The results of the 24 and 72 hr mortality counts are as follows:

Honey Bee Percentage Mortality

Dosage (mg)	Elapsed Time after Treatment 24 hr	72 hr
100	98	99
200	99	100
400	98	100

Control mortality was less than 3%.

Johansen (1961) tested azinphosmethyl to determine its contact action on honey bees. A small mist chamber was utilized in these tests. About 25 bees were placed through an opening into a cone shaped cage and the opening closed with a cork. Azinphosmethyl, diluted with analytical grade acetone, was applied as a 2 ml dosage of spray to the test bees. Controls were sprayed with acetone. After spraying, the cork was replaced with a wad of cotton soaked with 50% sugar-water syrup. Test cages were held at 72°F and mortality counts taken after 72 hr.

Each test series was corrected for control mortality by the use of Abbott's formula. The average mortality data, for the 4 replications of insecticide at each concentration, was then plotted on log-probit paper. The mean LD 50 and LD 95 was 0.005 and 0.006% concentration, respectively. It was regarded as being highly toxic to honey bees.

Graves and Mackensen (1965) examined the response of worker honey bees to azinphosmethyl applied to the thorax, and compared this with the response obtained when it was applied to the abdomen. Individual bees were treated with 2 uliters of test solution.

Following treatment, bees were caged at 80°F and fed a 50% sugar-water syrup. Mortality counts were taken

after 24 hr. The LD 50 obtained, when azinphosmethyl was applied to the abdomen, was 0.14 ug/bee. Values of 0.17 and 0.16 ug/bee were obtained when azinphosmethyl was applied to the thorax.

Field Tests

Johansen (1960b) placed package bee cages, containing 50-100 honey bees apiece, in a field of red clover. Tests took place in 1958 near Pullman, Washington. Plots, 1/10 acre in size, were sprayed with azinphosmethyl at a rate of 1.0 lb/acre. Four replicates were treated. After the application, cages were removed to a holding room having an approximate temperature of 75°F. All bees were fed a 50% sugar-water syrup.

Mortality counts were taken after 24 hr. The mortality of bees exposed during application was 100%, compared with a control mortality of 0.2%. Residual tests were also conducted. At 5 hr, and at 1, 2, 4, and 7 days after spraying, bouquets of treated clover were placed in unexposed package-bee cages containing 50-100 honey bees apiece. The 5 hr residual test killed 43% of the confined bees within 24 hr. Within this same period of time, the 1 day residual test killed 8%, the 2 and 4 day residual tests killed 4%, and the 7 day

residual test killed 0.3%. Control mortalities were 0% except for the 2 day residual test, in which case it was 0.7%.

Shaw and Fischang (1962) applied azinphosmethyl to samples of at least 100 caged honey bees. These were placed in apple trees in Massachusetts. After testing, cages were removed to a dark room. All bees were fed a 50% sugar syrup. The LT 50 was determined. Bees were also subjected to dried pesticide residues by exposing them for 30 min to treated foliage. By contact action, azinphosmethyl caused 50% mortality within 0.5 days. Erratic results were obtained in tests of its residual action. With the exception of one replicate, bee mortality did not differ appreciably from control mortality.

Tests conducted in 1961, utilized azinphosmethyl as a 25% wettable powder at 1.5 lb/acre. Contact treatment gave 100% mortality within 6 hr. Erratic results were again obtained when bees were confined on the day of testing, and 5 days after testing, to treated foliage.

Hays (1965) placed 10x10 in. screen cages, containing 20 bees each, in a crimson clover seed field in Alabama. He then applied azinphosmethyl dust, at 0.75 lb/acre,

with a rotary hand duster. Treatments were replicated four times. Mortality counts were made at 2, 4, and 12 hr after the application.

Residual tests were conducted using treated blooms collected at 2 and 24 hr after application. Twenty bees were placed in a bell-jar, containing 20 clover blooms, and left in contact with them for 2 hr. Mortality counts were taken 30 min after confinement with the blooms and following the conclusion of the 2 hr confinement period. Azinphosmethyl killed 100% of the bees by contact action within 2 hr of application. Within 2 hr, residual tests produced 90% mortality on treated blooms 2 hr old, and 0% mortality on treated blooms 24 hr old.

Johansen (1965) applied azinphosmethyl, as a 25% wettable powder, at the rate of 1.5 lb/acre. Plots of white Dutch clover 1/100 acre in size, at Pullman, Washington, were used in these tests. Applications were made with a hand sprayer and the treatment was replicated four times. Samples of foliage, with 4 hr old residues on them, were then placed in unexposed package-bee cages containing 25-50 honey bees. All cages were held at 75°F and the bees were fed a 50% sugar-water syrup. Mortality was 18% after 24 hr.

Carbaryl

Carbaryl is also known as Sevin and Union Carbide 7744. Its chemical designation is: 1-naphthyl methylcarbamate.

Laboratory Tests

Anderson and Atkins (1958) tested a 2.5% carbaryl dust at 100, 200, and 400 mg dosages in a bell-jar type duster. All tests were made with 9 replicates, of at least 20 honey bees/replicate, of each material. Treated bees were held in clean cages, provisioned with a 50% honey-water solution, at a temperature of 80°F and a relative humidity of 65%. Observations and mortality counts were made at 4, 6, 24, 48, and 72 hr. The results of the 24 and 72 hr mortality counts are as follows:

Honey Bee Percentage Mortality

Dosage (mg)	Elapsed Time after Treatment 24 hr	72 hr
100	29	41
200	72	72
400	96	96

Control mortality was less than 3%.

Johansen (1961) tested carbaryl, as a spray, in a small mist chamber and determined its LD 50 and LD 95 by contact action. He placed approximately 25 bees in a disposable cone shaped cage, constructed from 18 mesh wire screen, and applied 2 ml of spray to the cage. The insecticide was diluted with analytical grade acetone. Controls were sprayed only with acetone. Four replications of carbaryl, at each dilution, were conducted. After removing the cage from the mist chamber, it was held at 72°F and the bees were fed a 50% sugar-water syrup. Mortality counts were taken 72 hr after application of the pesticide. The mortality curve indicated that carbaryl, with an LD 50 and LD 95 of 0.020 and 0.028% concentration respectively, fell into the moderate toxicity group.

Georghiou and Atkins (1964) obtained carbaryl in a pure crystalline form and dissolved it in acetone on a weight to volume basis. Bees were anesthetized with CO_2 and treated on the notum, by a micrometer driven syringe, with 1 uliter of test solution/bee. After treatment, bees were held in screen wire cages supplied with 20% honey solution. Groups of test bees were confined at 60°, 80°, and 90°F at approximately 60% RH. Controls, treated only with acetone, were also kept at each

temperature. Mortality counts were taken at 4, 8, 16, and 24 hr after testing. It was found that the temperature after treatment affected the toxicity of carbaryl, which was 3.86 times as toxic at 60°F as at 90°F. Data indicated that, in spite of the high toxicity of carbaryl to the honey bee, a detoxification mechanism for this compound is present which is sensitive to temperature.

Graves and Mackensen (1965) examined the response of worker honey bees to carbaryl, applied to the thorax, and compared this with the response obtained when it was applied to the abdomen. Individual bees were treated with 2 uliters of the test solution applied to the dorsal surfaces of these areas. Following treatment, bees were caged in groups of 15 at 80°F and fed a 50% sugar-water syrup. Mortality counts were taken after 24 hr. The LD obtained, when carbaryl was applied to the abdomen, was 0.96 ug/bee. A value of 0.78 ug/bee was obtained when carbaryl was applied to the thorax.

Field Tests

Shaw (1959) exposed cages, containing variable number of bees, to carbaryl. A 50% wettable powder at 1 lb and 2 lb/100 gal and a 36% mull, containing 2.5

lb of carbaryl/gal in an oil and emulsifier carrier, were tested. Tests were conducted in an orchard located in Massachusetts, utilizing both hydraulic and air blast sprayers. Residual effects were observed by exposing bees to treated foliage for 30 min.

Following treatment, cages were brought into the laboratory and the bees were placed in clean holding cages. These were placed in a dark room and the bees were fed a 50% sugar-water syrup. Mortalities were observed for the first 6 hr, and then daily, until conclusion of the experiment. All treatments indicated that carbaryl was highly toxic as a contact spray. Residual tests, at 1 lb actual/100 gal, indicated that the toxicity of carbaryl varied depending on application procedures. Air blast machine residues were found to produce a greater toxicity. These were highly toxic after 24 hr, but decreased greatly in toxicity after 96 hr.

Johansen (1960a) treated alfalfa plots with carbaryl in the form of an 85% powder at 1.5 lb actual/acre, and combined with 10 fl. oz of R-874 repellent plus 1 fl. oz of Atlox 1045A emulsifier. Two hives of bees were placed near the center of each test plot, 1-5 days before treatment of a site, near Pullman, Washington. Each hive was fitted with a dead-bee-pollen trap, and a dead-bee pan, to allow for recovery of bees

discarded from the hive. A large number of dead bees were collected for several days at the hives in fields treated with carbaryl alone. However, only normal numbers were collected at those fields treated with carbaryl plus repellent R-874.

Johansen (1960b) placed package bee cages, containing 50-100 bees apeice, in clover plots in Eastern Washington. He then applied 2 lb of carbaryl/acre with a truck mounted, horizontal boom sprayer.

After treatment, the cages were removed to a holding room having an approximate temperature of 75°F. All bees were fed a 50% sugar-water syrup. Mortality counts were taken after 24 hr. Residual tests were also conducted. At 5 hr, and at 1, 2, 4, and 7 days after spraying, bouquets of red clover from each plot were placed in unexposed package bee cages. Each cage contained 50-100 honey bees. These cages were held under the same conditions as those used in the contact tests. The results of these tests are as follows:

Honey Bee 24 Hour Percentage Mortality

Sprayed during Application	Caged with Treated Foliage Age of Residues				
	5 hr	1 day	2 days	4 days	7 days
69	23	9	15	13	4

Control mortality was 0.7% or less.

Morse (1961a) examined randomly placed bee colonies, in New York State, after an aerial application of carbaryl at 1¼ lb/acre. He found that colony mortality was abnormally high for up to 3 weeks following application. It was hypothesized that this occurred because pollen, contaminated with carbaryl in the field, was collected and stored without immediate effect upon the pollen collecting bees. Later, this could cause an abnormal loss of bees for up to 3 weeks. In this experiment, the majority of colonies recovered well. It was also determined that direct application to hives was less important than contamination of either the field area, or plants, visited by bees.

Morse (1961b) also noted results when 1 lb/acre of carbaryl was applied to an open field containing two story colonies during an aerial spraying, for gypsy moth, in New York State. It had been suggested that the screening of colonies to prevent foraging gave protection against loss of bees. In this test, colonies were screened for 24 and 48 hr. While screening can be dangerous, keeping the bees from foraging did greatly reduce the number of bees killed. However, carbaryl was still able to kill large numbers of foraging bees after 48 hr.

Shaw and Fischang (1962) placed cages, containing at least 100 honey bees apiece, in apple trees located in Massachusetts. These trees were sprayed with carbaryl alone, and in combination with various fungicides. Carbaryl was applied as a 50% wettable powder, at 2 lb/100 gal of water, using a Hardy airblast machine. Bees were transferred to holding cages immediately after spraying, fed a 50% sugar syrup, and kept in a dark room. Results showed that carbaryl, either alone or in combination with the tested fungicides, caused 50% mortality within 15 hr when sprayed on bees.

Morse et al. (1963) examined residues of carbaryl in bees and pollen. They reported that bees could collect contaminated pollen in the field, return to the hive, and survive long enough to store this pollen. By the analysis of dead bees taken from immediately in front of colonies which had suffered losses as a result of a recent aerial spraying with carbaryl, it was found that dead bees, taken within 24 hr of treatment, showed a residue of 0.020, 0.054, and 0.044 ug/bee.

Morse (1964) undertook a series of tests, in New York State, to determine what effects the contamination of hive parts would have on bee mortality. This would indicate if there was any value in covering hives during aerial spraying. An aircraft was used to reproduce the droplet size and spray distribution pattern produced

under field conditions. The spray coverage obtained was equivalent to 1 kg of actual carbaryl/hectare. Tests indicated that any contamination on the outside of the hive would have little effect on the colony. It would therefore be of little value to merely cover hives to prevent their external contamination.

Morse (1965) reported that he had checked honey from colonies located in areas sprayed with carbaryl and had never found it in the honey. He believes, since most plant nectaries lie deep within the flower, very little carbaryl ever comes in contact with nectar.

Chloropropylate

Chloropropylate is also known as Acaralate and G 24163. Its chemical designation is: isopropyl 4,4'-dichlorobenzilate.

Apparently, little laboratory or field testing on the toxicity of this compound, to honey bees, has been conducted. However, Johansen (1966) lists it as having a low toxicity when applied as a spray in the field. He also lists its residual toxicity, to honey bees in the field, as being less than 1 day.

Diazinon

Diazinon is also known as Basudin and G-24480. Its chemical designation is: O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate.

Laboratory Tests

Atkins and Anderson (1954), using a vacuum bell-jar method of testing, applied a 5% diazinon dust to bees at 100, 200, and 400 mg dosages. Prophyllite was used as a diluent. At least 9 replicates, of 20 bees each, were treated at each dosage. After dusting, bees were transferred to clean holding cages and fed a 50% honey-water solution. Treated bees were held at 80°F and 65% RH. It was noted that diazinon caused test bees to regurgitate. This was more pronounced at the highest dosage. A bee was considered dead if no movement was observed for several seconds. The results of these tests are as follows:

Honey Bee Percentage Mortality

Dosage (mg)	Elapsed Time after Treatment 24 hr	72 hr
100	98	100
200	100	100
400	99	100

Control mortality was 4% after 24 hr and 9% after 72 hr.

Palmer-Jones (1958) undertook a series of laboratory tests with several insecticides, including diazinon. He confined bees to 3x3x1½ in. deep cages with nylon-mesh sides. Bees were fed a 33 1/3% (w/v) sucrose solution. Cages were held at 30°C and 25% RH. CO_2 was used as an anesthetic in handling the bees. The diazinon was formulated in a 16% solution (w/v) with an emulsifier and xylene.

To determine the effects of diazinon taken internally, test bees were starved for 1 hr and then placed singly in small vials having a feeding tube at one end. This tube contained 0.02 ml of a sucrose solution in which a known amount of pesticide had been dissolved. Bees that regurgitated, or did not consume the pesticide, were discarded. The remaining bees, usually 16 to 20, were placed in an observation cage and mortality counts taken after 24 hr. The LD 50 for diazinon was obtained by feeding bees a series of concentrations. This was calculated to be 0.24 ug.

In testing insecticides as contact poisons, groups of 50 bees were anesthetized with CO_2 and

spread over a 5 in. filter paper contained in a petri dish. One ml of the test solution was then applied to the bees by use of a DeVilbiss No. 15 atomizer. This had been adapted to fit into the top of a graduated cylinder. Four replicates were sprayed, after which they were placed in cages and fed. Mortality counts were taken after 24 hr. Diazinon, as a 0.1% (w/v) solution, produced 100% mortality within 24 hr in all replicates.

In evaluating residual action, an Aerograph MP spray gun was used to cover one side of a piece of tin foil, 600 sq. in. in area, with pesticide. By adding dye to the solution, an even application was obtained. The foil was weighed before the spray application and after it had been dried in an oven at 33°C. This treated foil was used to completely line the inside of a square wire cage with a press-on lid, after which 50 bees were caged for 1 hr with this residue. Air was sucked through the cage, by means of a tube attached to a water pump, to prevent any fumigant action by the pesticide. After treatment, test bees were kept in a cage and fed sugar solution. Mortality counts were taken after 24 hr.

Diazinon was found to be highly toxic in these tests at an equivalent field application rate of 2 oz or higher/acre of actual insecticide. Two oz/acre produced 94% mortality

and sprays equivalent to 7, 8, 63, and 108 oz/acre all produced 100% mortality.

Circular holders, having a diameter of 4 in. and a depth of 2.5 in., were constructed to study fumigant effects. Ths tops and bottoms of these were made of wire gauze. Each holder was constructed with a flange that could be slid over the top of a tin, in which was placed a petri dish containing a 3.5 in. piece of filter paper. The filter paper was soaked with 1 ml of the pesticide undergoing testing and the holder, containing 50 bees, was fitted onto it. Bees were kept in this container for 1 hr and then transferred to holding cages. Mortality was observed after 24 hr. Each dilution of a pesticide was tested on 4 groups of bees. It was found that bees which had fed lightly before exposure had a lower mortality than those which had fed heavily. In each group of 4 replicates, 2 were fed sugar syrup for 25 min only, and 2 were fed the same syrup for a minimum of 1.5 hr. This was intended to represent light and heavy feeding. Bees that fed lightly on 0.1%, 0.5%, and 1.0% diazinon had mortality counts of 0 and 2%, 100 and 32%, and 60 and 70%, after 24 hours, respectively. Bees that fed heavily had 100% mortality at all concentrations.

Field Tests

Johansen (1954) exposed package bee cages, containing 50-100 honey bees, to a 4% diazinon dust. This was applied with a power duster, at 40 lb/acre, to an alfalfa field located in Washington. Residual toxicity was evaluated by confining bees with bouquets of alfalfa blooms at 1 and 3 days after application of the pesticide. All caged bees were held at a temperature varying from 75-80°F. Five replicates were conducted on $\frac{1}{4}$ acre plots. In these tests, diazinon caused 100% mortality within 30 min as a contact poison. It produced 100% mortality after 1 day, and 0% mortality after 3 days, by a residual mode of action.

Johansen (1965) discussed past tests, conducted on bee poisoning, in the state of Washington. In 1962 and 1963, experimental plots of white Dutch clover seed were treated with diazinon. Four replicates were used at each concentration tested. Package bee cages, containing 50-100 honey bees apiece, were placed at each plot just before spraying. Samples of foliage from these test plots were utilized in conducting residual tests. The samples, large enough to fill the lower 2/5 of the cage, were placed in unexposed package bee cages at intervals after application. Each cage contained 25-50 honey bees. Bees were held at 75°F and fed 50% sugar-water syrup. Mortality counts were taken after 24 hr.

In 1962, diazinon, at 0.5 lb/acre, produced 3% mortality when bees were caged with 1 day old foliage. Tests in 1963, in which diazinon was applied at 1.0 lb/acre, produced 100% mortality to bees exposed during application. Mortality to bees, caged with treated foliage, was 100% with 4 hr old foliage and 67% with 1 day old foliage.

Malathion

Malathion is also known as American Cyanamid 4049 and Malathon. Its chemical designation is: diethyl mercapto= succinate, S-ester with O,O-dimethyl phosphorodithioate.

Laboratory Tests

Anderson and Tuft (1952) utilized a hand duster to treat bouquets of Lippia Lanceolata flowers with a 1% malathion dust. They then placed treated bouquets in 10x10x14 in. screen cages. At least 20 bees, collected from Lippia flowers in the field, were also placed in each cage. Similar numbers of bees were caged with undusted flowers as controls. Sugar-water solution was fed the bees and mortality counts were taken at intervals of several minutes to several hours. Six replicates were treated with malathion. It was found that 100% of the test

bees died within five hr after confinement with the treated blooms. The control bees experienced 12% mortality in this same period.

A series of tests were also conducted utilizing a vacuum bell-jar duster. A 0.25 g charge of 1% malathion dust was applied to varying lots of bees confined to 3 x 6 in. cylindrical screen cages. The dust was allowed to settle for 30 sec, after which the cages were removed from the dusting chamber.

After testing, bees were transferred to clean cages and treated as in the preceeding series of tests. Nine replicates were conducted. Within 20 min, 100% mortality had occurred. In this same period, no mortality occurred among the control bees. It was concluded that malathion was highly toxic to bees in both series of tests.

Atkins and Anderson (1954), using the vacuum bell-jar method of testing, dusted malathion on honey bees at 100, 200, and 400 mg dosages. A 2% malathion dust was applied to groups of 20 bees. At least 9 replicates were used in each test. After dusting, bees were transferred to clean holding cages, provisioned with a 50% honey-water solution, and held at 80°F and 65% RH. The results of the 24 hr and 72 hr mortality counts are as follows:

Honey Bee Percentage Mortality

Dosage (mg)	Elapsed Time after Treatment 24 hr	72 hr
100	99	99
200	100	100
400	100	100

Control mortality was 4% after 24 hr and 9% after 73 hr.

Wiese (1962) conducted a detailed series of laboratory tests on malathion, combined with parathion and two grades of mineral oil. While malathion alone was not tested, the methods used in testing are of interest.

Bees were inactivated with CO_2 prior to treatment. During the exposure period, bees were kept in the dark to avoid undue excitement. Natural mortality was corrected for by the use of Abbott's formula.

Tests were conducted at 5 temperature-humidity combinations, ranging from 65-95^{o}F and 55-85% RH.

Stomach feeding tests were conducted using batteries of micro-pipettes. Each test honey bee was fed 20 uliters of a 20% (w/v) sucrose syrup containing pesticide. Test bees were placed in a dark room at the proper temperature-humidity combination for 3 hr. After this period, bees

that had consumed the test solution were enclosed singly in 4½ in. petri dishes. The remaining bees were discarded. All petri dishes were supplied with queen cell cups filled with a 20% sucrose solution and closed with wire gauze lids. Mortality counts were made at 3, 6, 12, and 24 hr after testing. It was found that mortalities were lower at the lower temperature-humidity combinations than at the higher combinations.

Contact toxicity was evaluated by applying varying concentrations of insecticide, in a fixed volume of solvent, to the mesonotal region of test bees. Xylene was used as a solvent. After testing, bees were confined in groups of 33, in 3x3 in. cylindrical cages, and fed a 20% sucrose solution. Mortality counts were made at 6 and 12 hr intervals. Again, the mortalities were lower at the lower temperature-humidity combinations.

Spray applications were conducted using a spray tower, together with a DeVilbiss No. 152 atomizer. The anesthetized bees were placed in the base of the tower, dorsal side up, and thoroughly wetted with 5 ml of spray solution. Mortality counts were similar to previously conducted tests.

Fumigant toxicity was studied by constructing an apparatus from 2 wide-mouthed, 1-quart mason jars. Groups

of 33 honey bees were confined in the apparatus. A hand bellows pushed air over an insecticide formulation in one jar. This air then passed into the other jar containing a wire gauze cage of bees. This experiment demonstrated that malathion had no fumigant activity to bees.

Residual tests were also conducted. Insecticide formulations were sprayed on citrus foliage, wax paper, and filter paper, to test the effects of insecticides when applied to various surfaces. Treated surfaces were kept under conditions of continuous light or continuous darkness. This allowed a study of the effects of light on the decomposition of spray deposits. The wax paper and filter paper surfaces were treated, with 5 ml of solution, in a spray tower. To obtain treated citrus foliage, potted citrus trees were wetted with the solution undergoing testing. Cork boxes were then completely lined with each material. After the spray had dried, 33 bees were placed in each box. Exposures to the test substance were made immediately after treatment, and each 24 hr thereafter, until mortality had dropped to a low level. Mortality counts were taken at 12 and 24 hr intervals. Three replicates were tested at each formulation.

In the case of filter paper and waxed paper, the same surfaces were used for repeated exposures. Fresh citrus

leaves were used for each successive exposure, due to drying out of the leaves. It was found that the presence of light was highly significant in reducing the toxicity of malathion deposits. Also, increases in temperature reflect increases in mortalities over the range 65-85°F. Deposits on foliage became more rapidly detoxified than those on wax paper. Filter paper approximated foliage in this respect.

Field Tests

Lieberman et al. (1954) placed hives of honey bees on a small plot, adjacent to an alfalfa field, in Utah. Counts of bees, working in the field, were made at sq yd counting stations. Counts of dead bees were taken on 2-sq yd counting stations located away from the working bee stations.

Malathion, sprayed at 10 oz/acre, was applied between 6 and 7 AM and after 7 PM. During these periods, few bees were visiting the field. In tests conducted in 1952, bees freshly poisoned by malathion were still being recovered in small numbers, 4 days after application. At the concentration used, 46% of the field bees were killed.

Johansen (1954) exposed package bee cages, containing 50-100 honey bees each, to malathion. A 50% emulsifiable

concentrate, at 1 lb/acre actual insecticide, and a 4% dust, at 40 lb/acre, were tested. Mortality counts were made at 30 min and 2 days after application. Residual toxicity was evaluated by caging bees with bouquets of treated alfalfa blooms, cut at varying intervals after the applications. Bees were held at a temperature of 75-80°F and mortality counts taken after 24 hr. The test results are as follows:

Honey Bee Percentage Mortality

Formulation	Sprayed during Application		24 hr mortality of Bees Caged with Treated Foliage Age of Residues		
	30 min	2 days	14 hr	1 day	3 days
1.0 lb/acre EC	0	71	--	22	15
4% dust	0	100	1	--	--

Wolfenbarger (1957) recorded daily colony weights of 1-2 colonies of bees, in Florida, over a 5 year period. Following the application of malathion, at ½ lb/acre actual insecticide, he compared spray dates with daily colony weight changes. He determined that the number of dead bees was not greater after the spray applications than on previous days. Spray applications were also found to have no relationship to honey bee colony weight changes. He concluded that the amount of malathion applied to the colony site, and to nearby areas, was insufficient to produce colony weight reductions.

Anderson and Atkins (1958) sprayed 30 acres of alfalfa, in Southern California, at 8 oz of malathion in 6 gal of water/acre. Treatments were made, by airplane, at 9:30 AM. This field was located adjacent to an apiary and bees were actively working the alfalfa blooms.

In addition to field counts and colony observations, 5x5x5 in. cages, containing an average of 20 bees/cage, were placed in the shade under the plants. Caged bees in the field were provided with a 50% honey-water solution.

Counts made after 24 hr showed 74% mortality in those cages placed in the field prior to treatment and 4% mortality in those cages placed in the field following treatment.

Also placed in the field before spraying were $3\frac{1}{2}$x$4\frac{1}{2}$x3/4 in. screen cages, each containing 25-35 bees. These bees were all dead or affected within 30 hr. The malathion treatment reduced the number of bees in the field to zero within several minutes after treatment. However, bees reappeared in the field within 6 hr and activity was normal by the following morning. Bees working in the field during treatment died in the field, or soon after returning to the hive. Bees in the hives appeared to be little affected by the treatment. However, many of

the bees in each colony were working in areas other than the one treated with malathion. A second field, 25 acres in size, was also sprayed at the same dosage. The honey bee population dropped quickly but was normal by the next morning. Colonies adjacent to the field appeared to have incurred no ill effects from the spraying.

In 1956, malathion at 0.75 lb/acre was applied. This caused 100% mortality among those honey bees placed in the field during treatment and among honey bees placed in the field immediately following treatment, as well as killing all bees placed in the field up to 4½ hr following treatment. Higher dosages and higher temperatures in 1956 than in 1955 may have been responsible for the increased mortality among test bees in this experiment. A moderate kill was obtained in front of the colonies after treatment, but little serious damage was revealed by an examination of the interior of the hives on the following day.

Shaw and Fischang (1962) placed sleeve cages, containing at least 100 honey bees apiece, in unsprayed apple trees. They then sprayed these cages with malathion, using a Hardy Airblast machine. Malathion caused 50% mortality within 0.5 days. Bees exposed to residues of malathion on the day of application, and 3 days after treatment, did not show appreciable mortality above that of control bees.

Hays (1965) applied malathion dust at 1.25 lb/acre, in Alabama, in 1960. Dusts were applied with a rotary hand duster at a rate of 20 lb of dilute dust/acre. Contact toxicity was determined by placing 10x10 in. screen cages, containing 20 honey bees apiece, in the plot to be treated and in a check plot. Treatments were replicated four times.

Mortality counts were taken at 2, 4, and 12 hr after application. Residual toxicity was determined by confining groups of 20 bees to a bell-jar containing clover blooms taken from treated or check plots. Blooms were tested at 2 and 24 hr after spraying. Bees were confined for 2 hr with the treated blooms and mortality counts taken 30 min after confinement and at the end of the 2 hr confinement period. Bees were considered dead if unable to make coordinated movements. Malathion caused 100% mortality within 2 hr when sprayed on bees. Residual tests showed 0% mortality within 30 min and 100% mortality within 2 hr. Control mortality was 0% in both instances.

Johansen (1965) conducted tests with malathion in a 5 lb EC formulation. This was sprayed at a rate of 25 gal/acre, on white Dutch clover plots, in Southeastern Washington. A total of 1 lb/acre actual insecticide was applied. Bees were sprayed in the field during application and samples of foliage were placed in unexposed package bee cages at 4 hr and 1 and 2 days after spraying. Each cage

contained 25-50 honey bees. Cages were placed in a holding room at 75°F and the test bees fed a 50% sugar-water syrup. Mortality counts were taken after 24 hr. In 1963, malathion caused 100% mortality within 24 hr to bees sprayed during application, 100% mortality to bees caged on 4 hr old blooms, 27% mortality to bees caged on 1 day old blooms, and 5% mortality to bees caged on 2 day old blooms.

Johansen et al. (1965) applied 8 fl oz of malathion (low volume concentrate)/acre, by airplane, to alfalfa fields in the state of Washington. Six colonies of honey bees were placed in the field 36 hr before application. Two hives were covered with wet burlap during application. One of these was uncovered after 24 hr, the other after 48 hr. Two hives had their landing boards protected with a piece of wood and the remaining two hives were left unprotected. Two additional colonies, 2.25 miles from the sprayed area, were used as checks. Dead bee pans were fitted to the entrances of all hives immediately after application and daily collections of dead bees were made.

Package-bee cages, each containing 150-200 bees, were placed in the treated field and in an untreated field, 2 and 7 hr after application, and left there for 3 hr. Groups of 25-50 bees were also caged with foliage samples 2 hr, and at 1, 2, 3, 4, 5, and 6 days after application of the pesticide. All bees were kept at 75°F

and fed sugar syrup after testing. Mortality counts were taken after 24 hr. Residual test results are as follows:

Honey Bee 24 Hour Percentage Mortality

Caged with Treated Foliage Age of Residues						
2 hr	1 day	2 days	3 days	4 days	5 days	6 days
100	100	50	32	16	4	4

Control mortality was 11% or less.

Undiluted malathion, sprayed on sprinkler irrigated bloom at the same dosage, produced similar results. Malathion exibited no fumigant action to bees caged in the field after treatment.

Levin (1966) indicated that in tests comparing a standard formulation of malathion with an ultra low volume formulation, higher temperatures extended the period of residual toxicity of the ultra low volume formulation. In tests conducted at Bakersfield, California, above normal dead bee counts were taken for 7 days following application of malathion in the ultra low volume formulation.

Anderson and Atkins (1966) field tested low volume spray concentrations of malathion on honey bees. Treatments were made, by airplane, to 16 acre plots located in fields of seed alfalfa. All fields were in bloom and contained 2 or 3 well established colonies of bees/acre. Treatments were made directly over unprotected colonies. The effects

of spraying were determined from records of kill at the hives, mortality in field cages of bees, colony strength and behavior, and field bee visitation rates to blooms.

The results indicated that airplane sprays of undiluted technical malathion, at 8 oz/acre, caused serious losses to honey bee colonies and seriously reduced bee visitation in the treated area for one week after treatment. This occurred whether malathion was applied as an early morning or as an evening treatment. Malathion, at 8 oz and at 16 oz in 5 gal of water/acre, had no significant effects on colonies or on field visitation by honey bees.

Hitchcock et al. (1966) noted the effects on honey bee colonies when undiluted malathion was sprayed by airplane, in 1964, on 58,000 acres of rangeland in Wyoming. Treatments were at $\frac{1}{2}$ pt/acre of active malathion. Maximum temperatures ranged between 84 and 100°F during the dates that spraying took place. Commercial beekeepers screened their colony entrances to confine bees during spraying, but released them about 2 hr after application of the pesticide.

Preliminary observations indicated that severe poisoning of honey bees had occurred. It was estimated that nearly 600 colonies were seriously affected by the pesticide.

Methoxychlor

Methoxychlor is also known as dianisyl trichloroethane, DMDT, Marlate, and methoxy DDT. Its chemical designation is: 1,1,1-trichloro-2,2-bis(p-methoxyphenyl) ethane.

Johansen (1966) lists methoxychlor as having a low laboratory toxicity and as being moderately toxic when applied as a spray in the field. Its residual toxicity to honey bees is less than 1 day. These conclusions are substantiated by a number of investigations.

With an overall low toxicity to honey bees, this insecticide is regarded as nonhazardous to bees when applied at a time when they are not flying. Methoxychlor was included in the tests conducted, as a standard, to better enable an interpretation of the results obtained.

Phosalone

Phosalone is a newly developed compound which is also known as ENT 27163, RP 11974, and Zolone. Its chemical designation is: (O,O-diethyldithiophosphorylmethyl)-3-chloro-6-benzoxazolone.

Although no published material was reviewed indicating the toxicity of phosalone to honey bees, a summary put out

by its distributors indicates that tests have been conducted on bee toxicity in France, Great Britain, and the United States. Dr. Anderson at Riverside, California, in experiments conducted by dusting, determined the percent of active toxicant needed to give 50% mortality. The conditions under which these tests were conducted are not stated. However, he found the LD 50 for phosalone to be 7.40%. This compared with malathion at 0.64%, carbaryl at 1.20%, and methoxychlor at 19.50%. Other test results mentioned in the above summary, range from a description of phosalone as being nontoxic, to its being moderately toxic to honey bees.

Literature Review of Other Phosphate and Carbamate Insecticides

Bayer 39007

Bayer 39007 is also known as Baygon, Bayer 9010, propoxur, and Unden. Its chemical designation is: o-isopropoxyphenyl methylcarbamate.

Laboratory Tests

Georghiou and Metcalf (1962) determined by a topical application method that the 24 hr LD 50 for Bayer 39007 was 0.08 ug/bee. All honey bees were held at 16°C following treatment.

Bomyl

Bomyl is also known as GC 3707. Its chemical designation is: dimethyl 3-hydroxyglutaconate, dimethyl phosphate.

Field Tests

Johansen (1965) sprayed clover, in Washington, with Bomyl at 0.5 lb/acre and determined its contact toxicity to honey bees, and its residual effects by caging bees

with treated foliage. In tests conducted in 1962, exposure to Bomyl treated foliage, 1 day after application, caused 91% mortality to caged bees. In 1963, this same concentration caused 100% mortality to bees sprayed during an application to clover. Bees sustained 100% mortality when caged with 4 hr old foliage, 61% mortality with 1 day old foliage, and 33% mortality with 2 day old foliage.

Carbophenothion

Carbophenothion is also known as Garrathion, Trithion, and R 1303. Its chemical designation is: S- [(p-chloro= phenylthio)methyl] 0,0-diethyl phosphorodithioate.

Laboratory Tests

Anderson and Atkins (1958) applied carbophenothion to honey bees as a 5% dust. It was found to be less toxic than a standard DDT treatment. By contact action, it produced an average of 2% mortality within 24 hr at a 100 mg dosage, 12% mortality at a 200 mg dosage, and 44% mortality at a 400 mg dosage.

Johansen (1961) applied carbophenothion as a contact spray and determined its 72 hr LD 50 and LD 95 to be 0.003 and 0.047% concentration, respectively. He rated it as being moderately toxic to honey bees.

Field Tests

Anderson and Atkins (1958) conducted tests of carbophenothion on alfalfa in California. At 1.0 lb/acre, it gave a moderate kill of bees caged in the field at the time of application. However, there was almost no kill of honey bees placed in the field immediately following treatment. It was concluded that carbophenothion could be used on blooming alfalfa without seriously affecting honey bee populations.

Johansen (1960b) conducted tests in Washington, at 1.0 lb/acre, and found that carbophenothion killed 100% of those bees sprayed during application. Bees, caged with treated foliage 1 day old, incurred no mortality. Those caged with foliage less than 24 hr old, sustained 79% mortality with foliage 2 hr old, 4 and 8% mortality with foliage 5 hr old, and 3% mortality with foliage 12 hr old.

Coumaphos

Coumaphos is also known as Asuntol, Bayer 21/199, Co-ral, ENT 17957, Muscatox, and Resistox. Its chemical definition is: O-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl) O,O-diethyl phosphorothioate.

Laboratory Tests

Atkins and Anderson (1954) determined that a 5% coumaphos dust at a 200 mg dosage produced 27% mortality to bees within 24 hr and 44% mortality within 72 hr.

Demeton

Demeton is also known as Bayer 8169, E-1059, mercaptophos, and Systox. It is a mixture of O,O-diethyl S-(and O)-2-(ethylthio) ethyl phosphorothioates.

Laboratory Tests

Atkins and Anderson (1954) determined the contact toxicity of a 1% demeton dust to honey bees. Within 24 hr, it produced 4% mortality among honey bees treated with a 100 mg dosage and 5% mortality among bees treated with 200 and 400 mg dosages. It was considered as being safe to honey bees.

Field Tests

Lieberman et al. (1954) estimated that a 6 oz dosage/ acre of demeton, applied to alfalfa in Utah, killed 1% of the field force of bees. It also caused a 0.5% decline in the number of honey bees visiting treated flowers, compared with the number of bees visiting untreated flowers.

This insecticide was determined to be safe to use for controlling harmful insects on alfalfa in bloom, due to this low toxicity to honey bees.

Johansen (1954) sprayed seed alfalfa in Washington, by airplane, with demeton at 0.5 lb/acre actual in 5 gal of water. This treatment produced 18% mortality within 2 days to bees sprayed during application. Mortality was 3% among bees caged with 1 day old treated blooms, and 2% among bees caged with 3 day old blooms. When sprayed, by airplane, at the rate of 1½ pints/10 gal of water/acre, 100% mortality resulted among test bees within 24 hr. Mortality was 2% among bees caged with 3 hr old foliage and 0% among bees caged with 1 day old foliage.

Johansen et al. (1957) determined that honey bees were not adversely affected when demeton was applied to alfalfa in Washington. The amount of honey produced or number of dead bees collected in front of treated hives did not differ from that of the check hives. It was noted that honey bees appeared to be repelled from treated plots for 24 hr.

Johansen (1960b) found that a spray application of 0.5 lb/acre of demeton, to red clover, produced 28% mortality among treated honey bees. Bees, caged with treated clover, incurred 0% mortality with 5 hr old blooms, and 1% mortality with 1 day old blooms.

Dicapthon

Dicapthon is also known as American Cyanamid 4124 and Di-Captan. Its chemical designation is: O-(2-chloro-4-nitrophenyl)O,O-dimethyl phosphorothioate.

Laboratory Tests

Anderson and Atkins (1958) tested a 2% dust of dicapthon on honey bees. Among bees dusted with 100 or 400 mg dosages, mortality was 100% within 48 hr. Bees dusted with a 200 mg dosage sustained 100% mortality within 24 hr.

Dichlorvos

Dichlorvos is also known as DDVP, ENT-20738, Herkol, and Vapona. Its chemical designation is: 2,2-dichlorovinyl dimethyl phosphate.

Laboratory Tests

Anderson and Atkins (1958) tested a 2% dust of dichlorvos on bees at 100, 200, and 400 mg dosages. At the 100 and 200 mg dosages, it caused 100% bee mortality within 72 hr. At the 400 mg dosage, it caused 100% mortality within 48 hr.

Dimethoate

Dimethoate is also known as AC 12880, Cygon, ENT 24650, NC-262, Perfekthion, Rogor, and Roxion. Its chemical designation is: O,O-dimethyl S-(methylcarbamoylmethyl) phosphorodithioate.

Field Tests

Palmer-Jones et al. (1959) applied 16 fl oz of dimethoate (Rogor 40), in 7 gal of water/acre, by airplane. Two acres of flowering turnips, in New Zealand, were sprayed. It was found that dimethoate was highly toxic to field bees although brood in the hives was not affected. The conclusion was that dimethoate should never be applied to flowering crops attractive to honey bees.

Johansen (1960b) determined honey bee mortality when dimethoate, at 1.0 lb/acre, was sprayed on red clover located in Washington. Treated bees experienced 100% mortality within 3 hr. Two hr and 1 day residues produced 100 and 1% mortality, respectively.

Jaycox (1964) determined the effects on honey bees when dimethoate, at 1 lb/acre, was applied in the form of a spray, and as granules, in Utah. Three species of flowering plants were used in the tests conducted, and the possible contamination of nectar and honey was carefully studied.

It was found that bee mortality was still 32-46%, 4 days after application.

Granular applications proved less toxic than sprays, at equal amounts of active ingredient/acre. Results indicated that dimethoate could penetrate floral nectar in quantities large enough to kill honey bees.

Johansen (1965) determined honey bee poisoning when dimethoate was applied to crops of clover and alfalfa in Southeastern Washington. At 0.25, 0.5, and 1.0 lb/acre, 100% of the bees sprayed during application were killed. Residual action was tested by caging bees with treated foliage. Using this procedure, mortality was high at all concentrations for the first 5 hr. The highest honey bee mortality was obtained with dimethoate at 1.0 lb/acre. In one instance, this was 89% when bees were caged with 1 day old treated foliage, and 58% when bees were caged with 2 day old treated foliage.

Dioxathion

Dioxathion is also known as Delnav, ENT 22897, Hercules AC-528, and Navadel. Its chemical designation is: S,S'-p-dioxane-2,3-diyl O,O-diethyl phosphorodithioate (cis and trans isomers).

Laboratory Tests

Anderson and Atkins (1958) treated honey bees with a 2% dioxathion dust. The results of the 24 hr and 72 hr mortality counts are as follows:

Honey Bee Percentage Mortality

Dosage (mg)	Elapsed Time after Treatment 24 hr	72 hr
100	2	3
200	2	5
400	1	3

Control mortality was 1% after 24 hr and 2% after 72 hr.

Field Tests

Johansen (1965) determined the effects of dioxathion on sprayed honey bees, and on bees caged with treated foliage, when this compound was applied at 1.0 and 2.0 lb/acre on clover located in Southeastern Washington. The results of these applications are as follows:

Honey Bee 24 Hour Percentage Mortality

Dosage	Sprayed during Application	Caged with Treated Foliage Age of Residues 3 hr	3-6 hr	24 hr
1 lb/acre	--	--	17	0
2 lb/acre	78	23	--	4

Disulfoton

Disulfoton is also known as Bayer 19639, Di-Syston, dithiodemeton, Dithio-Syston, Frumin AL, Solvirex, and Thiodemeton. Its chemical designation is: O,O-diethyl S-2-(ethylthio)ethyl phosphorodithioate.

Field Tests

Johansen (1960b) found that disulfoton granules, applied at 2.0 lb/acre actual to red clover in Washington, produced 3% mortality among bees caged in the field during application. Mortality was 1% among bees caged with 2 hr old treated foliage, and 2% among bees caged with 1 day old treated foliage.

Endothion

Endothion is also known as AC-18737, Niagara 5767, and Phosphate 100. Its chemical designation is: S-[(5-methoxy-4-oxo-4H-pyran-2-yl)methyl] O,O-dimethyl phosphorothioate.

Field Tests

Palmer-Jones (1959) applied endothion as a spray, by airplane, to a flowering crop of New Zealand rape. Each

acre was sprayed with 30 gal of a 0.33% active aqueous solution of endothion.

Endothion caused no bee mortality even though applied when this crop was very attractive to honey bees. No repellency was exerted by this compound. It was concluded that endothion can be applied to flowering brassica crops without endangering honey bees.

EPN

EPN is also known as EPN-300. Its chemical designation is: O-ethyl O-p-nitrophenyl phenylphosphonothioate.

Laboratory Tests

Eckert (1950) treated bees with EPN by both feeding and spraying methods. He determined that it had a high toxicity to honey bees as a stomach poison and as a contact insecticide. EPN killed bees which came in contact with only minute amounts of it. Beekeepers were advised to keep bees out of areas in which EPN was applied to legumes in bloom, for not less than 1 week following treatment.

Anderson and Tuft (1952) dusted bees with a 2% EPN dust. Mortality was 100% within 40 min. Bees were also confined to cages with bouquets of flowers treated with a 2% dust. Results showed 100% mortality within 5 hr.

Atkins and Anderson (1954) dusted bees with EPN at a 100 mg dosage. In this instance, 100% mortality among test bees occurred within 12 hr. At a 200 mg dosage, 100% mortality occurred within 24 hr, while a 400 mg dosage produced 100% mortality within 48 hr. EPN is considered to be highly toxic to honey bees.

Ethion

Ethion is also known as Niagara 1240 and Nialate. Its chemical designation is: O,O,O'O'-tetraethyl S,S' methylene bisphosphorodithioate.

Laboratory Tests

Johansen (1961) determined the LD 50 and LD 95 of ethion, as a contact poison at 72 hr, to be 0.078 and 0.139% concentration, respectively. This compound has a low toxicity to honey bees.

Field Tests

Johansen (1960b) sprayed plots of red clover, in Washington, with ethion. Groups of honey bees were sprayed in the field, while other groups of bees were caged with treated foliage. The results of these tests are as follows:

Honey Bee 24 Hour Percentage Mortality

Dosage	Sprayed during Application	Caged with Treated Foliage Age of Residues 2 hr	5 hr	24 hr
0.5 lb/acre	2	--	0.9	0.8
1.0 lb/acre	100	1	--	0

Imidan

Imidan is also known as Prolate and R-1504. Its chemical designation is: O,O-dimethyl S-phthalimidomethyl phosphorodithioate.

Field Tests

Johansen (1965) applied Imidan to clover and alfalfa in Washington and studied its effects on honey bees. Emulsifiable concentrate and wettable powder treatments, of 0.5 and 1.0 lb/acre, were tested. Groups of bees were sprayed during application while other groups of bees were caged with treated foliage. Honey bees, sprayed during application of the pesticide, sustained 100% mortality. The results of the residual tests are as follows:

Honey Bee 24 Hour Percentage Mortality

Dosage	Caged with Treated Foliage Age of Residues 4-5 hr	24 hr	72 hr
0.5 lb/acre EC	92	53	0
1.0 lb/acre EC	100	69	13
0.5 lb/acre WP	100	77	0
1.0 lb/acre WP	100	81	4

Mesurol

Mesurol is also known as Bayer H-321, Bayer 9026, Bayer 37344, ENT-25726, and mercaptodimethur. Its chemical designation is: 4-(methylthio)3,5-xylyl methylcarbamate.

Laboratory Tests

Georghiou and Metcalf (1962) determined that the 24 hr LD 50 for Mesurol, by topical application, was 0.11 ug/bee. All bees were held at 16°C following treatment.

Georghiou and Atkins (1964) found that the toxicity of Mesurol varied with temperature. The 24 hr topical LD 50, in ug/bee, was 0.155 ug at 16°C, 0.205 ug at 27°C, and 0.250 ug at 32°C.

Methyl Demeton

Methyl demeton is also known as Bayer 21/116 and Meta-Systox. It is a mixture of O,O-dimethyl S-(and O)-(2-ethylthio) ethyl phosphorothioates.

Laboratory Tests

Palmer-Jones et al. (1957) and Palmer-Jones (1958) discussed the effects of methyl demeton on honey bees. It was found that when bees were forced to run through a gauze tube, moistened with a 1% spray of methyl demeton, 100% mortality occurred after 3 min contact with the gauze. Bees exposed to a vapor of 1% methyl demeton for 1½ hr, all died within 5 hr, while those exposed to a dry film of this compound died only when exposed to very high concentrations. It was concluded that when dry, methyl demeton spray would not be a hazard to honey bees. The 24 hr LD 50, as a stomach poison, was 0.5 mg.

Field Tests

Palmer-Jones et al. (1957) sprayed a crop of chou moellier, in New Zealand, and determined that methyl demeton did not effect young bees or brood. However, a high mortality to field bees was obtained. It was concluded that this compound should never be applied to flowering crops that are attractive to honey bees.

Methyl Parathion

Methyl parathion is also known as Bayer E-601, Dalf, dimethyl parathion, Metron, and Nitrox. Its chemical designation is: O,O-dimethyl O-p-nitrophenyl phosphoro= thioate.

Laboratory Tests

Atkins and Anderson (1954) tested a 2% dust at 100, 200, and 400 mg dosages on honey bees. As a contact poison, methyl parathion caused 100% bee mortality within 24 hr at the 100 and 400 mg dosages. Mortality was 100% within 48 hr at the 200 mg dosage.

Methyl Trithion

Its chemical designation is: S-(p-chlorophenylthio) methyl O,O-dimethyl phosphorodithioate.

Field Tests

Johansen (1960b) observed honey bee mortality when Methyl Trithion was sprayed on red clover in Washington. As an emulsifiable concentrate at 1.0 lb/acre, it caused 100% mortality to bees sprayed during application. Bees caged with 2 hr old treated foliage, averaged 99% mortality.

Bees caged with 1 day old treated foliage, averaged 1% mortality.

Mevinphos

Mevinphos is also known as ENT 22374, OS 2046, and Phosdrin. Its chemical designation is: methyl 3-hydroxy-alpha-crotonate dimethyl phosphate.

Laboratory Tests

Anderson and Atkins (1958) found that a 2% mevinphos dust produced 84% honey bee mortality within 24 hr after application at a 100 mg dosage, and 87% mortality at 200 and 400 mg dosages.

Johansen (1961) applied mevinphos as a contact spray and determined the LD 50 and LD 95, at 72 hr, to be 0.0003 and 0.004% concentration, respectively. He rated mevinphos as being highly toxic to honey bees.

Field Tests

Johansen (1960b) observed honey bee mortality when mevinphos emulsifiable concentrate was sprayed, at 0.5 lb/acre, on red clover in Washington. Mortality counts were taken of bees sprayed during application, and of bees caged with treated foliage at intervals following application.

The results of these tests are as follows:

Honey Bee 24 Hour Percentage Mortality

Year	Sprayed during Application	Caged with Treated Foliage Age of Residues 2 hr	5 hr	12 hr	24 hr
1958 - Test #1	100	--	6	--	0
Test #2	100	--	8	1	0
1959	100	2	--	--	2

Palmer-Jones and Forster (1963) sprayed mevinphos, by airplane, at 0.4 lb of active material in 10 gal/acre. Tests were conducted on white clover seed crops, in full flower, in New Zealand. Mortality among field bees averaged 82% on the day of application and 52% the next morning. No significant mortality occurred among bees collected on the third morning after application. Mevinphos is a systemic compound, and the tests conducted indicated that it killed larger numbers of field bees after it had reached the clover nectaries. Its honey bee toxicity lasted longer than 24 hr, but mortality was confined to field bees. Brood maintenance and hive activities remained unaffected.

Naled

Naled is also known as Dibrom and RE 4355. Its chemical designation is: 1,2-dibromo-2,2-dichloroethyl dimethyl phosphate.

Field Tests

Johansen (1960b) treated red clover, in Eastern Washington, with naled at 1.0 lb/acre. He determined its contact toxicity to honey bees by direct application and its residual action by caging bees with treated foliage at varying intervals after application. The results of these tests are as follows:

Honey Bee 24 Hour Percentage Mortality

Test No.	Sprayed during Application	Caged with Treated Foliage Age of Residues 5 hr	12 hr	24 hr
#1	100	7	--	0
#2	100	7	0.6	0

Shaw and Fischang (1962) sprayed honey bees, in Massachusetts, with an emulsifiable concentrate of naled at 1 pint/100 gal of water. Bee mortality was 100% within 6 hr after treatment.

Shaw and Armstrong (1966) exposed nucleus hives and colonies of bees, in Massachusetts, to a naled fog, such as that used to control mosquitoes and flies. This was dispensed at the rate of 3 quarts/100 gal of fuel oil. Exposure did not result in any appreciable loss of bees. It was cautioned that further tests at higher temperatures be conducted, before final conclusions are drawn.

NPD

NPD is also known as A-42, ASP-51, and E 8573. Its chemical designation is: O,O,O,O-tetrapropyl dithiopyro=phosphate.

Atkins and Anderson (1954) dusted honey bees with a 4% NPD dust. The results of the 24 hr and 72 hr mortality counts are as follows:

Honey Bee Percentage Mortality

Dosage (mg)	Elapsed Time after Treatment 24 hr	72 hr
100	9	13
200	27	30
400	71	72

Control mortality was 4% after 24 hr and 9% after 72 hr.

Paraoxon

Paraoxon, an oxygen analog of parathion, is also known as E-600 and Mintacol. Its chemical designation is: diethyl p-nitrophenyl phosphate.

Laboratory Tests

Metcalf and March (1949), using a topical application method, determined that the honey bee LD 50 of paraoxon was 0.6 mg/g of body weight.

Parathion

Parathion is also known as Alkron, American Cyanamid 3422, Bayer E-605, Niran, and Thiophos. Its chemical designation is: O,O-diethyl O-p-nitrophenyl phosphorothioate.

Laboratory Tests

Butler and Shaw (1948) sprayed honey bees with various dilutions of parathion. At dilutions between 0.08 and 0.5 lb/100 gal of water, parathion caused 100% mortality within 6½ hr. It was classified as being highly toxic to honey bees at these concentrations.

Eckert (1948) stated that parathion appeared to be the most toxic compound to bees that he had observed. In several tests conducted, he determined that parathion was toxic to honey bees as a contact insecticide, stomach poison, and fumigant.

Beard (1949) determined the effects of parathion on honey bees by various routes of administration. The enteral route ranked first, followed by parenteral, and topical routes.

Eckert (1949) determined the LD 50 for parathion by feeding caged honey bees known quantities in a 20% sugar syrup. The LD 50, in 72 hr, was 0.07 mg/bee. Parathion was found to be highly toxic as a stomach poison, contact poison, and fumigant.

Metcalf and March (1949) determined that the topical LD 50 of parathion, to worker honey bees, was 3.5 mg/g of body weight.

Shaw and Butler (1949) found that parathion produced 100% mortality, within 24 hr, to honey bees as a dust. As a spray, 100% mortality occurred within 2.5 hr. Bees, placed in contact with treated foliage immediately after application and left for 30 min, all died within 24 hr. It was concluded that parathion was highly toxic to honey bees as a spray and as a dust. The danger to bees remains for at least 24 hr after application of the insecticide.

Salkeld (1951) conducted mass feeding tests on groups of honey bees and determined that parathion had an LD 50 of 0.41 mg/bee. Because he considered this method unsatisfactory, feeding tests were conducted on individual bees. These tests showed that parathion had an LD 50 of 0.095 mg/bee.

Anderson and Tuft (1952) found that a 1% parathion dust caused 100% mortality to honey bees within 30 min. Bees caged with treated flowers, all died within 5 hr. Parathion was considered to be highly toxic to honey bees.

Atkins and Anderson (1954) treated honey bees with a 2% parathion dust. At dosages of 100 and 400 mg, 100% mortality occurred among treated bees within 24 hr. At a 200 mg dosage, mortality was 100% within 48 hr. It was

concluded that parathion was highly toxic to honey bees.

Glynne Jones and Connell (1954) determined that when bees remained in contact for 1 hr with a residual film of parathion, sprayed at 2 oz/acre, mortality was 90% within 24 hr. When exposed to vapors for 1 hr, from a residual film of 0.005 mg/sq cm, 100% bee mortality resulted within 24 hr. The 24 hr LD 50, as a stomach poison, was determined to be 0.04 mg.

Johansen (1961) found the 72 hr LD 50 and LD 95 for parathion, by contact action, to be a 0.002% concentration. Parathion was considered to be highly toxic to honey bees.

Wiese (1962) showed that by contact, stomach, and residual modes of action, parathion at lower temperature and humidity combinations is less toxic to honey bees than at higher temperature and humidity combinations. He also demonstrated that the presence of light accelerates the breakdown of this compound.

Field Tests

Todd et al. (1949) found that a 1% parathion dust was very destructive to field bees in Utah. Approximately 40% of the exposed honey bees were killed. The majority of bee mortalities occurred within 2 days following the application.

Ghani and Shaw (1950) found that parathion, as a

0.054% spray or 0.05% dust applied in Massachusetts, gave a very quick knockdown of honey bees. Sprayed bees all died within 24 hr, while dusted bees sustained 100% mortality within 48 hr. It appeared that parathion exerted some repellency to bees and that dusts appeared to be more repellent than sprays.

Knowlton et al. (1950) determined honey bee mortality when parathion was applied to Utah alfalfa fields in bloom, during hours when bees were not foraging. When applied at 0.58 lb/acre active ingredient, 32.5% bee mortality resulted. It was concluded that parathion was too toxic to warrant additional testing on seed alfalfa in bloom.

Robinson (1950 and 1955) observed the effects of parathion on honey bees when a grapefruit grove in Florida was sprayed, with a 15% wettable powder, at the rate of 2 lb/100 gal of water. Spray was applied at 35 gal/tree. Results indicated that parathion would kill sprayed bees, but there would be little danger to bees that later worked in the sprayed grove.

Knowlton (1950a and 1950b) found that when parathion was applied in Utah, to alfalfa in bloom, a sharp increase in dead honey bees at nearby experimental hives always followed. This lasted for 2 days following application with less damage on the third day. It was recommended that, when used on alfalfa, parathion be applied before any bloom develops in the field.

Johansen (1954) applied a 1% dust of this insecticide, to seed alfalfa in Washington, at 35 lb/acre. Bees, dusted during application, all died within 2 days. Bees, caged with dusted blooms 14 hr after testing, were still alive after 24 hr.

Johansen et al. (1957) observed the effects on honey bees when parathion was applied, to alfalfa in Washington, at a rate of 1 quart of 25% solution/acre. Plots were treated at 6:15 AM and 3:45 PM. There were over 6 times as many dead bees in the first 24 hr following afternoon treatments, as there were following morning applications. Honey production was depressed only slightly in the parathion treated plots.

Anderson and Atkins (1958) found that when parathion was sprayed on blooming alfalfa in California, at 0.25 lb/acre, it destroyed the entire field force of bees at the time of treatment. Caged honey bees, placed in the field up to 4-6 hr after treatment, continued to be killed.

Johansen (1965) noted the effects on honey bees when parathion, at 0.5 lb/acre, was sprayed on white Dutch clover in Washington. Bees, sprayed during application, all died within 24 hr. Bees, caged with treated foliage 4 hr old, all died within 24 hr while those caged with 1 day old foliage, sustained 9% mortality in 1962 tests and 79% mortality in 1963 tests.

Phorate

Phorate is also known as AC 3911, L 11/6, and Thimet. Its chemical designation is: O,O-diethyl S-(ethylthio)-methyl phosphorodithioate.

Laboratory Tests

Anderson and Atkins (1958) treated honey bees with a 2% phorate dust at 3 dosages. The results of the 24 hr and 72 hr mortality counts are as follows:

Honey Bee Percentage Mortality

Dosage (mg)	Elapsed Time after Treatment	
	24 hr	72 hr
100	9	12
200	14	22
400	24	35

Control mortality was less than 3%.

Johansen (1961) determined the 72 hr LD 50 and LD 95 for phorate, by contact action, to be 0.004 and 0.031% concentration, respectively.

Field Tests

Johansen (1960b) determined the honey bee toxicity of phorate as 5% granules and as an emulsifiable concentrate. Tests were conducted in Eastern Washington. The results of these applications are as follows:

Honey Bee 24 Hour Percentage Mortality

Dosage	Sprayed during Application	Caged with Treated Foliage Age of Residues 2 hr	5 hr	24 hr
2.0 lb/acre (Granules)	100	2	--	1
0.5 lb/acre EC				
Test #1	100	--	24	0
Test #2	100	--	10	0.3

Anderson and Atkins (1966) observed alfalfa fields, in Southern California, treated by airplane with 16 oz of phorate in 5 gal of water/acre. The effects on honey bees were determined from the kill at hives and in field cages, colony strength and behavior, and field bee visitation rates. Phorate killed approximately 80% of the caged bees, but had little effect on field bee visitation rates. No kill was obtained when caged bees were placed in the field following treatment.

Phosphamidon

Phosphamidon is also known as Dimecron, ML-97, and OR-1191. Its chemical designation is: dimethyl phosphate, ester with 2-chloro-N,N-diethyl-3- hydroxycrotonamide.

Laboratory Tests

Johansen (1961) sprayed honey bees and determined the 72 hr LD 50 and LD 95 for phosphamidon to be 0.005 and 0.007% concentration, respectively. Phosphamidon was considered as being highly toxic to honey bees.

Field Tests

Johansen (1960b) exposed honey bees to a spray of phosphamidon, applied to red clover in Eastern Washington, and caged bees with treated foliage at intervals after spraying. The results of these applications are as follows:

Honey Bee 24 Hour Percentage Mortality

Dosage	Sprayed during Application	Caged with Treated Foliage Age of Residues		
		2 hr	5 hr	24 hr
0.5 lb/acre	76	--	0	0
1.0 lb/acre	100	7	--	1

Jaycox (1964) fed honey bees an emulsifiable concentrate of phosphamidon, in a 50% sugar syrup, and determined that the 72 hr LD 50 oral toxicity was 0.15 ug.

Palmer-Jones (1964) applied 5 oz of active phosphamidon, diluted with water to 12 gal, to a flowering crop of white clover in New Zealand. Field bee mortality averaged 45% on the day of application and 57% on the

following day. Honey bee losses continued for 6 days following treatment. Since phosphamidon is claimed to be a systemic insecticide and penetrates plant tissues rapidly, it appeared that mortality was due to the ingestion of toxic nectar by field bees. It was concluded that phosphamidon was very hazardous to honey bees.

Johansen (1965) conducted tests, in Washington, on honey bee poisoning. Phosphamidon was applied, to white Dutch clover, in 1960 and 1963. The results of these applications are as follows:

Honey Bee 24 Hour Percentage Mortality

Year	Dosage	Sprayed during Application	Caged with Treated Foliage Age of Residues	
			3-6 hr	24 hr
1961	1.0 lb/acre	--	98	0
	1.0 lb/acre	100	100-(3 hr)	7
1962	1.0 lb/acre	--		29
1963	0.5 lb/acre	100	96-(4 hr)	43

Other honey bee poisoning investigations conducted with phosphamidon during 1960-1963 are summarized as follows:

Honey Bee 24 Hour Percentage Mortality

Dosage	Sprayed during Application	Caged with Treated Foliage Age of Residues 2-3 hr	10 hr	24 hr
0.25 lb/acre	100	32	1	2
0.5 lb/acre	100	71	9	5
1.0 lb/acre	100	99	61	42
1.0 lb/acre	100	100	45	36

Phostex

Phostex is also known as Niagara 1137. It is a mixture of bis(dialkoxyphosphinothioyl) disulfides.

Laboratory Tests

Anderson and Atkins (1958) treated honey bees with 200 mg of a 2% Phostex dust. Mortality was 2% within 24 hr and 5% within 72 hr.

Johansen (1961) determined the LD 50 and LD 95 of Phostex, as a contact poison at 72 hr, to be 0.151 and 0.823% concentration respectively. It was classified as having a low toxicity to honey bees.

Field Tests

Johansen (1960b) applied 1.0 lb/acre of Phostex to

red clover in the state of Washington. Caged bees, sprayed during this application, all died within 24 hr. Bees, caged with treated clover 2 hr after spraying, averaged 13% mortality. No mortality was observed among honey bees caged with treated clover 24 hr after spraying.

Potasan

Potasan is also known as E-838. Its chemical designation is: O,O-diethyl O-(4-methyl-2-oxo-2H-1-benzopyran-7-yl) phosphorothioate.

Laboratory Tests

Atkins and Anderson (1954) applied a 2% dust of Potasan to bees at a 200 mg dosage. Mortality was 81% within 24 hr after dusting and 84% within 72 hr after dusting.

Ronnel

Ronnel is also known as Dow ET-14, Dow ET-57, Etrolene, fenchlorphos, Korlan, Nankor, Trolene, and Viozene. Its chemical designation is: O,O-dimethyl O-2,4,5-trichloro= phenyl phosphorothioate.

Laboratory Tests

Anderson and Atkins (1958) treated honey bees with a 2% ronnel dust. At a 200 mg dosage, mortality among test bees was 18% within 24 hr and 24% within 72 hr. At a 400 mg dosage, mortality was 40% within 24 hr and 44% within 72 hr.

Field Tests

Hays (1965) dusted clover, in Alabama, with ronnel at 1.2 lb/acre and observed the effects of this application on honey bees.

Bees were caged in the field during application and on clover blooms 2 and 24 hr after dusting. All bees, dusted during application, died within 12 hr. Those caged with treated blooms, 2 hr old, averaged 10% mortality within 2 hr. No mortality was observed among bees caged with 24 hr old treated blooms.

Schradan

Schradan is also known as E-3314, OMPA, Pestox III, and Sytam. Its chemical designation is: octamethyl= pyrophosphoramide.

Laboratory Tests

Glynne Jones and Thomas (1953) determined that while schradan could kill honey bees as a stomach poison, its contact toxicity was negligible. It was also considered unlikely that bees would ingest lethal amounts from treated plants under normal conditions.

Johansen (1953) fed individual honey bees 10 uliters of schradan. Concentrations ranging from 0.07 to 0.74% were tested in 50% sugar syrup. In all cases, 100% mortality to honey bees occurred within 24 hr. Bees fed contaminated nectar, 5 and 11 days after an application of schradan to plants, died very quickly when compared with control bees. It was concluded that flowers sprayed with schradan may yield poisonous nectar. This compound must be considered as very toxic to honey bees, as a stomach poison, in comparison with other agricultural sprays.

Atkins and Anderson (1954) treated honey bees with a 2% schradan dust. Mortality was 21% after 24 hr and 26% after 72 hr. They classified schradan as being moderately toxic to honey bees.

Field Tests

Johansen et al. (1957) treated alfalfa plots in Washington with 1 quart of 42% schradan/5 gal of water/acre.

It was concluded that honey bees were not adversely affected by treatments. There was no decrease in honey production or increase in the number of dead bees collected in front of hives located in treated plots.

Tepp

Tepp is also known as Tep and Tetron. Its chemical designation is: tetraethyl pyrophosphate. Tepp was discovered to be the actual insecticidal ingredient of HETP, the first organic phosphate insecticide to be manufactured commercially.

Laboratory Tests

Eckert (1948) tested this compound as both HETP and tepp. Dilutions of 1:800 - 1:1,500,000 were tested. It was concluded that this compound was toxic to bees in minute quantities as a contact and as a stomach poison.

Eckert (1949) determined LD 50's for HETP and tepp. By feeding honey bees these chemicals, in a 20% sugar solution, it was determined that the LD 50, in 72 hr, was 0.29 ug/bee for HETP and 0.75 ug/bee for tepp.

Metcalf and March (1949) determined that the topical LD 50 of tepp to worker honey bees was 1.2 ug/gram of body weight.

Glynne Jones and Connell (1954) determined that the LD 50 of tepp, as a stomach poison, to honey bees was 0.065 ug. When left in contact with a residual film of tepp for 1 hr, 8% bee mortality occurred within 24 hr. It was concluded that tepp was highly effective as a stomach or contact poison, but had no measurable effect as a residual film or fumigant.

Atkins and Anderson (1954) found that a 1% tepp dust caused 100% mortality to honey bees at dosages of 100, 200, and 400 mg. Tepp was considered to be highly toxic to honey bees.

Field Tests

Lieberman et al. (1954) determined that when tepp was sprayed as a morning application to alfalfa at 6 oz/acre, in Utah, an estimated 63% of the field bees were killed. As an evening application, at the same dosage, only 6% of the field bees were destroyed.

Johansen (1954) applied a 1% tepp dust to alfalfa, in Washington, and observed mortalities among bees caged in the field during application and confined with treated blooms at varying intervals after dusting. The results of these applications are as follows:

Honey Bee 24 Hour Percentage Mortality

Dosage	Dusted during Application	Caged with Treated Foliage Age of Residues 14 hr	24 hr	72 hr
65 lb/acre	100	--	0	0
35 lb/acre (8:00 PM)	100	4	--	--
35 lb/acre (6:45 AM)	40 (after 2 days)	--	--	--

Significant differences between AM and PM applications were noted.

Trichlorfon

Trichlorfon is also known as Bayer L13/59, chlorophos, Dipterex, Dylox, ENT-19763, Neguvon, Trichlorphon, and Tugon. Its chemical designation is: dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate.

Laboratory Tests

Anderson and Atkins (1958) dusted honey bees with 2% trichlorfon at several rates. The results of the 24 and 72 hr mortality counts are as follows:

Honey Bee Percentage Mortality

Dosage (mg)	Elapsed Time after Treatment 24 hr	72 hr
100	2	3
200	1	6
400	2	3

Control mortality was less than 3%. Trichlorfon was considered to be relatively nontoxic to honey bees.

Field Tests

Anderson and Atkins (1958) treated an alfalfa field in California, by airplane, with trichlorfon at 1.0 lb/acre in 5-6 gal of water. At this dosage, there was little effect on the number of bees visiting the field after treatment. Slight kills of honey bees caged in the field during treatment did occur, but there was almost no kill of bees placed in the field after treatment. Counts of dead bees in front of hives were light immediately following treatment. It was concluded that trichlorfon, at 1 lb/acre, had little effect on field populations of bees.

Shaw and Fischang (1962) sprayed caged honey bees under field conditions, in Massachusetts, and subjected other groups of bees to dried residues after spraying. Trichlorfon produced 100% mortality within 6 hr after

the contact treatment. Exposure on the day of application to residues, resulted in significant decreases in honey bee longevity.

Palmer-Jones (1963) applied trichlorfon to white clover in New Zealand. Honey bees, collected on the day of spraying, averaged 9% mortality after 48 hr. Bees collected on the following morning, averaged 2% mortality. No adverse effects were observed on adult bees or brood after the pesticide application. It was determined that trichlorfon was safe to use on white clover crops.

Anderson and Atkins (1966) tested trichlorfon, in Southern California, at 1 lb/acre in 5 gal of water and in 1 quart of water as a low volume treatment. They observed bee visitation to treated alfalfa, bee kills at hives, and mortality among bees caged in the field during and after treatment. Trichlorfon, in both types of treatment, did not kill bees at the hives or reduce field visitation. Caged honey bees, sprayed in the field, averaged between 22 and 45% mortality. No kill was observed when honey bees were caged in the field following treatment.

Zectran

Zectran is also known as Dowco 139 and ENT 25766. Its chemical designation is: 4-dimethylamino-3,5-xylyl methylcarbamate.

Laboratory Tests

Georghiou and Metcalf (1962) determined, by topical application, that the 24 hr LD 50 for Zectran was 0.06 ug/bee. All honey bees were held at 16°C following treatment.

Georghiou and Atkins (1964) found that the toxicity of Zectran varied with temperature. The 24 hr topical LD 50, in ug/bee, was 0.0823 ug at 16°C, 0.129 ug at 27°C, and 0.188 ug at 32°C.

List of Phosphate and Carbamate
Insecticides not Reviewed

Azinphosethyl[1] [2]

Azinphosethyl is also known as Bayer 16259 and Ethyl Guthion. Its chemical designation is: O,O-diethyl S-4-oxo-1,2,3-benzotriazin-3 (4H)-ylmethyl phosphorodithioate.

Azodrin[2]

Azodrin is also known as SD 9129. Its chemical designation is: dimethyl phosphate, ester with cis 3-hydroxy-N-methylcrotonamide.

Banol[1] [2]

Banol is also known as U-12927 and Zok. Its chemical designation is: 2-chloro-4,5-dimethylphenyl N-methylcarbamate.

Bayer 41831[1] [2]

Bayer 41831 is also known as fenitrothion, Folithion, Sumithion, and Sumitomo S-1102A. Its chemical designation is: O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate.

Bidrin[1] [2]

Bidrin is also known as SD 3562. Its chemical designation is: dimethyl phosphate, ester with cis-3-hydroxy-N,N-

dimethylcrotonamide.

Chlorthion[1] [2]

Chlorthion is also known as Bayer 22/190. Its chemical designation is: O-(3-chloro-4-nitrophenyl)O,O-dimethyl phosphorothioate.

Ciodrin[1] [2]

Ciodrin is also known as SD-4294. Its chemical designation is: alpha-methylbenzyl 3-hydroxycrotonate dimethyl phosphate.

Dimetilan[1] [2]

Dimetilan is also known as Dimetilane and GS-13332. Its chemical designation is: 1-(dimethylcarbamoyl)-5-methyl-3-pyrazolyl dimethylcarbamate.

Famphur[1] [2]

Famphur is also known as Famophos, CL 38023, and Warbex. Its chemical designation is: o-p-(dimethylsulfamoyl)=phenyl O,O-dimethyl phosphorothioate.

Fenthion[1] [2]

Fenthion is also known as Bayer 29493, Baytex, Entex, ENT 25540, Lebaycic, S 1752, and Tiguvon. Its chemical

designation is: O,O-dimethyl O-[4-(methylthio)-m-tolyl] phosphorothioate.

Isolan[1]

Isolan is also known as G-23611. Its chemical designation is: 1-isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate.

Isopropyl Parathion[1]

Its chemical designation is: di-isopropyl 4-nitrophenyl phosphorothionate.

Matacil[1 2]

Matacil is also known as aminocarb, arprocarb, Bayer 44646, and ENT-25784. Its chemical designation is: 4-dimethylamino-m-tolyl methylcarbamate.

Menazon[1 2]

Menazon is also known as PP 175, Saphizon, and Saphos. Its chemical designation is: S-(4,6-diamino-s-triazin-2-ylmethyl) O,O-dimethyl phosphorodithioate.

Metacide[1 2]

Metacide is a preparation containing O,O-dimethyl O-p-nitrophenyl phosphorothioate (methyl parathion) and O,O-

diethyl O-p-nitrophenyl phosphorothioate (parathion) in the ratio of 4 to 1.

Oxydemetonmethyl[1 2]

Oxydemetonmethyl is also known as Bayer 21097, demeton-S-methyl sulfoxide, and Meta-Systox-R. Its chemical designation is: S-2-(ethylsulfinyl) ethyl O,O-dimethyl phosphorothioate.

Pyramat[1 2]

Pyramat is also known as G-23330. Its chemical designation is: 6-methyl-2-propyl-4-pyrimidinyl dimethylcarbamate.

Temik[1]

Temik is also known as ENT 27093 and UC 21149. Its chemical designation is: 2-methyl-2-(methylthio) propionaldehyde O-methylcarbamoyl) oxime.

Tetram[1 2]

Tetram is also known as R-6199. Its chemical designation is: S-(2-diethylaminoethyl) diethyl phosphorothiolate hydrogen oxalate.

Thiocron[1]

Its chemical designation is: S-(2-methoxyethyl= carbamoylmethyl) dimethyl phosphorothiolothionate.

Zinophos[1 2]

Zinophos is also known as AC 18133, Cynem, Nemafos, and Nemaphos. Its chemical designation is: O,O-diethyl O-2-pyrazinyl phosphorothioate.

Materials and Methods

Seven pesticides were used in each of the 6 types of tests undertaken. Tests were conducted at the University of Massachusetts apiary. The pesticides tested, and the formulations used in testing, are listed below:

Name	Formulation Used
Azinphosmethyl	EC - 2 lb/gal
Carbaryl	WP - 50%
Chloropropylate	EC - 2 lb/gal
Diazinon	EC - 4 lb/gal
Malathion	EC - 5 lb/gal
Methoxychlor	EC - 2 lb/gal
Phosalone	EC - 3 lb/gal

All materials except chloropropylate, methoxychlor, and carbaryl, are organic phosphates. Chloropropylate and methoxychlor are chlorinated hydrocarbons and carbaryl is a carbamate. Chloropropylate and phosalone are newly developed compounds. Chloropropylate is an acaricide and phosalone is a combination insecticide and acaricide.

All materials were tested at 1.0 lb/100 gal and 0.5 lb/100 gal actual insecticide. Three replicates, of 50 bees each, were tested for a pesticide at each concentration. Controls, consisting of 3 replicates, were included in a test of the 7 pesticides at each concentration.

Honey bees were taken from healthy colonies, above an excluder, with only bees from a single colony used in a days testing. Bees were anesthetized with CO_2 and confined, after testing, to holding cages. Each holding cage consisted of a 1-quart, cardboard ice cream container with a screen top (fig. 1). the container had a hole cut in one side, near the base, in which a cork was inserted. Dead bees were removed through this opening each day, when mortality counts were taken. These were taken daily for a period of 7 days, but due to the rise in control mortality which normally occurred after the fourth or fifth day, it was decided to base an interpretation of test results on the 24 and 72 hr mortality counts.

Bees were held, after testing, in a darkened room at the apiary (fig. 2). Temperature and humidity conditions were recorded on a hygrothermograph.

Confined bees were fed with a 33 1/3% sugar-distilled water solution. This same solution was combined with the pesticides in the feeding tests conducted. The feeding bottle used, consisted of a 2 oz specimen bottle with a metal top. The top had 5 or 6 holes punched in it with a 3/32 in. diameter nail. By inverting the bottle on the screen top of the holding cage, bees were easily able to feed on the sugar solution (fig. 3).

The types of tests conducted were: (1) topical application, (2) mass spraying, (3) individual feeding, (4) mass feeding, and (5) and (6); residual tests of 2 types. All tests were performed in 1966.

Topical Application Test

A topical application machine was used to apply 1 uliter of the test solution, dissolved in acetone, to the dorsal surface of the thorax of each honey bee (fig. 4). Controls were treated with 1 uliter of acetone. This test was conducted on May 17, 21, and 23.

Mass Spraying Test

A spray tower was constructed, using a 38 in. high glass cylinder, having a diameter of 10 in. A DeVilbiss No. 15 atomizer was mounted on top of this (fig. 5). When a 9 in. paper plate was inserted at the bottom of the tube, a uniform spray coverage of the plate was obtained.

Groups of 50 honey bees, that had previously been confined to 2 oz bottles, were anesthetized with a short burst of CO_2, spread out on the paper plate, and placed in the spray tower. One ml of the pesticide undergoing testing was then sprayed over this plate. The spray solution had a drop of red food coloring added to it, to allow for easier observation of the spray pattern obtained. Controls were sprayed with distilled water.

Immediately after spraying, bees were removed from the spray tower and placed in holding cages. The mass spraying test was conducted on July 20.

Individual Feeding Test

Bees were anesthetized with CO_2 and confined individually in 1 dram homeopathic vials. Each vial was fitted with a No. 2 cork having a 1 in. section of No. 3 glass tubing inserted through its center.

After a 4-6 hr starvation period, confined bees were fed 1 uliter of test solution. This was composed of the pesticide being tested, dissolved in the standard sugar solution. Controls were fed only the sugar solution. A drop of red food coloring was added to this solution, to allow for easier observation of the small quantities used. The uliter of solution was dispensed by a topical application machine into the interior opening of the piece of glass tubing (fig. 6).

Sixty confined bees were fed each concentration of pesticide and the first 50 to consume the solution were confined to a holding cage. This test was conducted on April 24 and 25, June 7 and 8, and July 8 and 11.

Mass Feeding Test

Bees were confined, in groups of 50, to holding cages. They were allowed 2 hr for recovery from anesthesia.

This interval also served as a starvation period prior to being fed a pesticide solution.

One ml of a solution, composed of the test pesticide dissolved in the standard sugar solution, was dispensed into a 1 dram vial having a tight fitting plastic cap. Four holes, drilled with a 1/16 in. drill, allowed the bees to feed through the cap (fig. 7). Controls were fed only the sugar solution. To allow for easier observation of the amounts of pesticide solution remaining in the vials, a small amount of red food coloring was mixed with each pesticide. Bees were allowed to feed for 24 hr. After this, the tube was replaced with a feeding bottle of sugar solution. This test was conducted on July 21 and 22.

Foil Residual Test

Kimax petri dish bottoms (100x15 mm) were completely lined with aluminum foil, dull side up. Utilizing a DeVilbiss No. 15 atomizer, 12 dishes were sprayed with each pesticide at each concentration.

Approximately 0.5 ml of the test solution was placed on each dish. Controls were sprayed with water. A small amount of food coloring was added to the distilled water used in mixing the pesticide solutions. Tests were conducted at 2 hr, and at 1, 3, and 5 days after application

of the pesticide. Previously unused, treated dishes were used for each day's test. Foil dishes, not in use, were stored in a darkened room until needed.

Bees were anesthetized, counted out in groups of 50, and confined to 2 oz feeding bottles. After 1 hr, they were anesthetized with a short burst of CO_2 and placed in the treated dishes. Each dish was then covered by a $4\frac{1}{2}$x5 in. piece of screening to contain the bees and allow for ventilation (fig. 8). This prevented any fumigant effect by the pesticides.

Bees would normally recover from the CO_2 in less than 5 min and begin to walk over the treated surface. A 150-w bulb, positioned above the table on which tests were conducted, served to stimulate the bees to activity.

Bees were allowed to remain in contact with the treated surface for 1 hr, after which they were again anesthetized with a short burst of CO_2 and placed in holding cages. During anesthetization, a Buchner funnel was attached to the CO_2 delivery tube to completely cover the petri dish. This test was conducted on August 18, 19, 21, and 23.

Leaf Residual Test

In preparation for testing, oak leaves, of a uniform size, were selected from an unsprayed tree. These were

placed, in groups of 15, with their stems immersed in water. Styrofoam coffee cups were used to contain the leaves.

Each group of 15 leaves was then removed from its container, sprayed with a test pesticide, and replaced in water. A DeVilbiss No. 15 atomizer was used to deposit approximately 0.5 ml of spray on each leaf. The containers were placed on a ledge, near a closed window. Here, the leaves were exposed to light each day.

Tests were conducted at 2 hr, and at 1, 3, and 5 days after spraying. Twelve oak leaves were used during the course of testing. For each day's test, bees were counted out and confined as in the preceding test. Three leaves, from each of the sprayed groups, were then selected and trimmed to fit the petri dish bottoms (fig. 9). A 1 hr exposure to the treated surface was allowed, after which the bees were anesthetized and confined to holding cages. This test was conducted on August 24, 25, 27, and 29.

Results and Discussion

The results obtained from testing were statistically analyzed by the performance of a three way analysis of variance. Variable No. 1 was the pesticide treatment; variable No. 2 the rates of treatment; and variable No. 3 the types of application. This data was fed into a computer as counts of dead bees which were, in each case, statistically transformed into the square root of the mean. A comparison of transformed means was then conducted using Tukey's test. A significance level of 5% was used in all cases. The analysis of variance performed appears in Tables 9 and 10.

The results of this analysis demonstrated that the differences between pesticide treatments were significant. Methoxychlor and chloropropylate did not differ from each other, or from the control, in respect to overall toxicity at either the 24 hr (Table 11) or 72 hr (Table 12) intervals after treatment. However, all other pesticides were significantly different in overall toxicity from the control and each other. These differences can be used to group them according to their overall toxicity to honey bees.

The 0.5 lb/100 gal and 1.0 lb/100 gal rates of application did not differ significantly in the tests

performed.

While significant differences would likely be found between tests conducted to measure various modes of a pesticide's action, in all cases there were also significant differences between the tests conducted to measure a single mode of action. Thus, the results of the topical application test differed from those of the mass spraying test, and the results of the individual feeding test differed from those of the mass feeding test. In the residual tests, it was found that the results at 2 hr, and at 1, 3, and 5 days after testing, differed for the two types of tests performed. Tables 13 and 14 summarize these results.

Although it was not possible to hold temperature and humidity conditions constant, a record was kept of the conditions under which tests were conducted and under which honey bees were held after testing. These are summarized by type of test in Tables 15-20.

A detailed review of the compounds tested follows. Pesticides are listed in a descending order of toxicity and grouped as to overall toxicity using the transformed 24 hr square root of the mean. The following system was devised to group these compounds.

Toxicity Group	Square Root of the Mean
Extreme	5.000 and above
High	3.500 - 4.999
Moderate	2.800 - 3.499
Low	2.000 - 2.799
Relatively Nontoxic	1.999 and below

The data obtained from the testing of carbaryl appears in Table 1.

Table 1. The Toxicity of Carbaryl to Honey Bees

Test Method	1.0 lb/100 gal[a] 24 hr	1.0 lb/100 gal[a] 72 hr	0.5 lb/100 gal[a] 24 hr	0.5 lb/100 gal[a] 72 hr
Topical Application	48.0	48.3	44.0	44.3
Mass Spraying	31.7	33.0	21.0	22.7
Individual Feeding	47.0	47.7	46.0	46.3
Mass Feeding	50.0	50.0	50.0	50.0
Foil Residual (2 hr)	44.3	44.3	49.0	49.7
(24 hr)	50.0	50.0	48.7	48.7
(72 hr)	47.3	48.0	48.3	49.0
(120 hr)	50.0	50.0	50.0	50.0
Leaf Residual (2 hr)	46.7	46.7	15.0	16.0
(24 hr)	25.7	41.0	8.7	10.7
(72 hr)	37.7	39.3	9.7	10.0
(120 hr)	22.3	25.0	5.7	6.0

(a) Average mortality of three replicates of 50 bees each.

The transformed square root of the mean for carbaryl was 5.985. It was rated as being extremely toxic to bees in the tests performed. This compound was found to be consistently toxic to honey bees in all tests. Its prolonged residual toxicity was particularly noticeable.

The data obtained from the testing of malathion appears in Table 2.

Table 2. The Toxicity of Malathion to Honey Bees

Test Method		1.0 lb/100 gal(a) 24 hr	1.0 lb/100 gal(a) 72 hr	0.5 lb/100 gal(a) 24 hr	0.5 lb/100 gal(a) 72 hr
Topical Application		49.7	50.0	49.7	50.0
Mass Spraying		50.0	50.0	50.0	50.0
Individual Feeding		49.3	49.3	49.9	49.9
Mass Feeding		50.0	50.0	47.0	50.0
Foil Residual	(2 hr)	50.0	50.0	50.0	50.0
	(24 hr)	50.0	50.0	50.0	50.0
	(72 hr)	50.0	50.0	50.0	50.0
	(120 hr)	46.0	46.0	29.7	30.0
Leaf Residual	(2 hr)	50.0	50.0	45.0	45.3
	(24 hr)	0.7	1.7	1.3	1.7
	(72 hr)	3.7	4.0	3.3	4.7
	(120 hr)	0.0	0.3	0.3	0.3

(a) Average mortality of three replicates of 50 bees each.

The transformed square root of the mean for malathion was 5.613. It was rated as being extremely toxic to bees in the tests performed. It gave quicker total knockdown

of test bees than carbaryl and appeared more toxic as both a contact and stomach poison. However, it had less residual toxicity than carbaryl.

The data obtained from the testing of diazinon appears in Table 3.

Table 3. The Toxicity of Diazinon to Honey Bees

Test Method		1.0 lb/100 gal(a) 24 hr	1.0 lb/100 gal(a) 72 hr	0.5 lb/100 gal(a) 24 hr	0.5 lb/100 gal(a) 72 hr
Topical Application		48.0	50.0	49.7	50.0
Mass Spraying		50.0	50.0	50.0	50.0
Individual Feeding		49.0	49.3	48.0	48.3
Mass Feeding		50.0	50.0	42.3	50.0
Foil Residual	(2 hr)	50.0	50.0	50.0	50.0
	(24 hr)	36.3	37.3	0.7	0.7
	(72 hr)	0.7	2.0	1.3	2.0
	(120 hr)	1.3	2.0	0.3	0.7
Leaf Residual	(2 hr)	50.0	50.0	50.0	50.0
	(24 hr)	16.3	19.3	1.0	1.3
	(72 hr)	8.7	9.3	2.7	3.3
	(120 hr)	0.7	0.7	0.0	0.0

(a) Average mortality of three replicates of 50 bees each.

The transformed square root of the mean for diazinon was 4.576. It was rated as being highly toxic to bees in the tests performed. Diazinon was found to be consistently toxic to honey bees as both a contact and stomach poison. Bees were normally knocked down more

rapidly than with the other compounds tested, and usually within ½ hr after feeding or application of the pesticide. However, its residual life was much shorter than either carbaryl or malathion.

The data obtained from the testing of azinphosmethyl appears in Table 4.

Table 4. The Toxicity of Azinphosmethyl to Honey Bees

Test Method		1.0 lb/100 gal[a] 24 hr	1.0 lb/100 gal[a] 72 hr	0.5 lb/100 gal[a] 24 hr	0.5 lb/100 gal[a] 72 hr
Topical Application		47.3	50.0	49.3	50.0
Mass Spraying		1.7	2.7	3.7	6.7
Individual Feeding		2.3	2.7	4.0	5.3
Mass Feeding		50.0	50.0	47.0	50.0
Foil Residual	(2 hr)	50.0	50.0	39.7	39.7
	(24 hr)	50.0	50.0	10.7	12.0
	(72 hr)	50.0	50.0	32.0	32.7
	(120 hr)	42.7	42.7	18.0	18.3
Leaf Residual	(2 hr)	10.0	11.3	0.0	1.7
	(24 hr)	0.7	1.0	0.3	0.7
	(72 hr)	3.7	4.7	2.0	3.0
	(120 hr)	0.3	0.3	0.0	0.0

(a) Average mortality of three replicates of 50 bees each.

The transformed square root of the mean for azinphosmethyl was 3.954. It was rated as being highly toxic to bees in the tests performed. In general, it gave toxicity results dependent upon the test method used.

Small quantities of azinphosmethyl, when ingested, did not cause high mortality among honey bees. However, a large quantity of this compound at the same concentration, caused complete mortality to test bees within 24 hr. It was also demonstrated that mass spraying produced little bee mortality as compared with a smaller amount of this compound administered by topical application methods. However, this technique consistently produced higher mortalities among test bees, probably due to the fact that acetone was used to dissolve the chemicals for this test. Acetone has been shown to aid in the penetration of a pesticide through the cuticle of insects.

The results of the residual tests performed, differed widely. This again was probably due to a lower concentration of pesticide and the possibility of greater absorption of the pesticide in the leaf residual test. It demonstrates that the toxicity of azinphosmethyl was more dependent on dosage than were the other pesticides tested.

The data obtained from the testing of phosalone appears in Table 5.

Table 5. The Toxicity of Phosalone to Honey Bees

Test Method	1.0 lb/100 gal[a] 24 hr	1.0 lb/100 gal[a] 72 hr	0.5 lb/100 gal[a] 24 hr	0.5 lb/100 gal[a] 72 hr
Topical Application	17.7	21.0	3.3	8.3
Mass Spraying	7.7	8.7	1.0	4.3
Individual Feeding	3.0	4.7	1.0	1.0
Mass Feeding	50.0	50.0	46.3	50.0
Foil Residual (2 hr)	12.0	12.3	8.7	11.0
(24 hr)	23.7	24.0	7.0	8.7
(72 hr)	23.7	26.0	40.0	41.3
(120 hr)	15.3	15.7	6.3	6.7
Leaf Residual (2 hr)	1.7	2.0	0.0	1.3
(24 hr)	1.7	2.7	2.3	3.3
(72 hr)	7.3	8.3	5.3	5.7
(120 hr)	0.0	0.0	1.0	1.0

(a) Average mortality of three replicates of 50 bees each.

The transformed square root of the mean for phosalone was 3.082. It was rated as being moderately toxic to bees in the tests performed. It appeared to be toxic to bees only in large quantities as both a contact and stomach poison. In testing its residual action, erratic results occurred in one instance. While it does possess prolonged residual activity, it appears that high dosages are necessary to cause high mortality among bees.

The data obtained from the testing of methoxychlor appears in Table 6.

Table 6. The Toxicity of Methoxychlor to Honey Bees

Test Method		1.0 lb/100 gal(a) 24 hr	1.0 lb/100 gal(a) 72 hr	0.5 lb/100 gal(a) 24 hr	0.5 lb/100 gal(a) 72 hr
Topical Application		3.0	3.7	0.0	1.0
Mass Spraying		2.7	3.3	0.7	2.0
Individual Feeding		2.7	6.3	2.3	5.3
Mass Feeding		0.3	2.0	0.7	2.7
Foil Residual	(2 hr)	1.7	2.0	0.3	0.7
	(24 hr)	1.3	2.0	0.7	0.7
	(72 hr)	0.7	1.7	2.3	4.3
	(120 hr)	1.3	2.3	1.3	2.0
Leaf Residual	(2 hr)	0.0	2.7	0.7	1.7
	(24 hr)	1.7	2.0	2.7	3.0
	(72 hr)	3.7	4.0	4.3	4.7
	(120 hr)	0.0	0.0	1.7	2.0

(a) Average mortality of three replicates of 50 bees each.

The transformed square root of the mean for methoxychlor was 1.501. It consistantly showed little or no toxicity to bees at both 1.0 lb and 0.5 lb/100 gal. It was rated as being relatively nontoxic to bees in the tests performed.

The data obtained from the testing of chloropropylate appears in Table 7.

Table 7. The Toxicity of Chloropropylate to Honey Bees

Test Method		1.0 lb/100 gal(a) 24 hr	1.0 lb/100 gal(a) 72 hr	0.5 lb/100 gal(a) 24 hr	0.5 lb/100 gal(a) 72 hr
Topical Application		0.3	1.0	0.3	2.3
Mass Spraying		0.7	2.0	1.3	2.7
Individual Feeding		1.0	4.3	1.7	2.3
Mass Feeding		0.0	2.0	0.7	1.3
Foil Residual	(2 hr)	0.3	0.3	0.0	0.7
	(24 hr)	2.7	2.7	1.0	1.0
	(72 hr)	1.0	2.3	1.0	2.3
	(120 hr)	1.7	2.0	0.3	1.0
Leaf Residual	(2 hr)	1.7	3.3	2.3	3.0
	(24 hr)	1.3	1.7	2.0	2.3
	(72 hr)	4.0	4.3	4.7	5.7
	(120 hr)	0.3	1.3	0.0	0.0

(a) Average mortality of three replicates of 50 bees each.

The transformed square root of the mean for chloropropylate was 1.433. It was rated as being relatively nontoxic to bees in the tests performed.

The following results were obtained in the control series of tests. These results are included for the sake of completeness in Table 8. Mortality counts were low in all cases.

Table 8. Control Mortality in the Tests Conducted

Test Method	1.0 lb/100 gal[a] 24 hr	1.0 lb/100 gal[a] 72 hr	0.5 lb/100 gal[a] 24 hr	0.5 lb/100 gal[a] 72 hr
Topical Application	0.0	0.7	0.3	1.0
Mass Spraying	1.0	3.0	0.7	2.3
Individual Feeding	0.7	2.7	2.0	2.3
Mass Feeding	0.3	0.3	1.0	1.3
Foil Residual (2 hr)	0.0	0.3	0.7	1.3
(24 hr)	2.3	3.7	0.0	0.0
(72 hr)	0.7	2.0	1.3	3.0
(120 hr)	1.0	1.0	1.3	2.0
Leaf Residual (2 hr)	0.7	2.7	1.0	2.0
(24 hr)	1.0	2.0	2.7	2.7
(72 hr)	3.0	3.0	4.0	5.7
(120 hr)	0.3	0.7	0.7	0.7

(a) Average mortality of three replicates of 50 bees each.

The transformed square root of the mean for the control tests was 1.384.

Summary and Conclusions

The following table is a comparison between the laboratory tests conducted and the summarized laboratory toxicity rating of these compounds as given by Johansen (1966) and the field toxicity rating as given by Anderson and Atkins (1966).

Compound	Transformed Square Root of the Mean (24 hr)	Toxicity Rating	Johansen	Anderson & Atkins
carbaryl	5.985	Extreme	Low-High	High
malathion	5.613	Extreme	Very High	High
diazinon	4.576	High	Very High	High
azinphosmethyl	3.954	High	Very High	High
phosalone	3.082	Moderate	-------	Moderate
methoxychlor	1.501	Relatively Nontoxic	Low	Relatively Nontoxic
chloropropylate	1.433	Relatively Nontoxic	-------	-------

The results of the tests conducted gave an overall toxicity rating to each compound which corresponds well to the toxicity ratings of Johansen and Anderson and Atkins.

While a grouping of compounds as to their overall toxicity was attempted, this was purely artificial. More information regarding relative position in overall toxicity can be obtained from a compound's numerical rank.

Particularly noticeable is the gap in overall toxicity which appears between malathion and diazinon. Thus, while both compounds fall into the same grouping in most summaries of their toxicity to honey bees, malathion is far more toxic to field bees due to its extended residual life. Because of this, it was thought essential to provide an additional category containing compounds which have an extreme toxicity to honey bees.

It was found that not only the mode of action tested, but also how a single test on honey bee toxicity is set up, can influence the results obtained and the relative rating of the compounds tested. Thus, a series of feeding tests, contact tests, and residual tests should be undertaken to fully evaluate a compounds toxicity under various conditions and to determine differences between compounds which can aid in a more precise evaluation of toxicity.

The results also showed that for the compounds tested, there were no significant differences between honey bee mortalities at 1.0 lb/100 gal and mortalities at 0.5 lb/100 gal. It should also be noted that the results obtained from taking dead bee counts at 24 and 72 hr were similar, when statistically analyzed.

A weak point in the tests performed was that temperature could not be kept uniform throughout the series of

experiments. However, all compounds were evaluated at the same time by each method. Thus, their relative position would not be influenced by differences in temperature.

It is apparent that valuable information on the toxicity of pesticides to honey bees can be obtained from a laboratory testing procedure as outlined here. The commercially used rates of all pesticides, in common use, should be tested on honey bees in such a series of tests to evaluate more precisely their overall toxicity to honey bees. The results obtained could then be analyzed and each compound given a numerical rating. This would serve to group the pesticide with other compounds possessing a similar toxicity to honey bees and to point out differences in modes of action between compounds.

Using these methods, the toxicity of newly developed pesticides could be rapidly evaluated and added to the list of compounds tested. This would facilitate the development of application procedures that would aid in decreasing losses sustained by beekeepers.

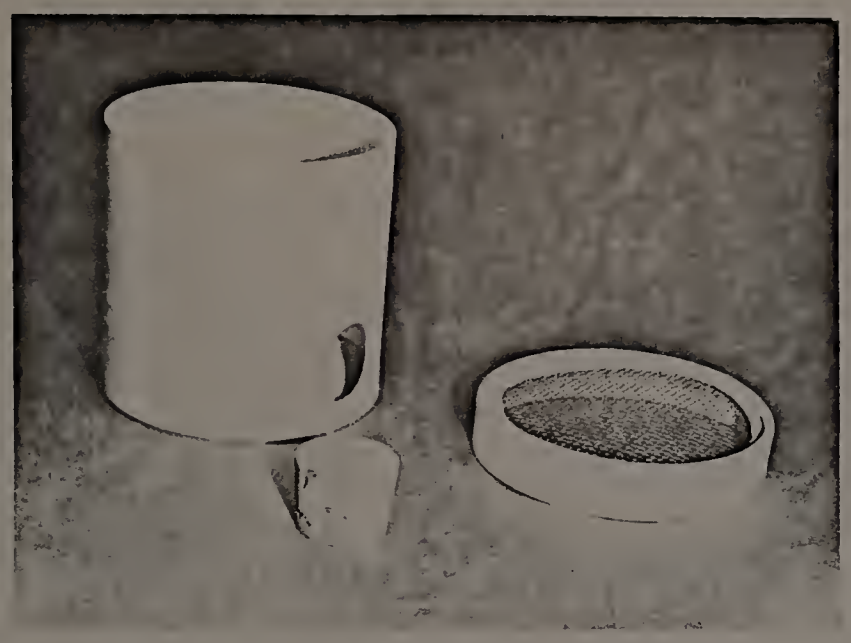

Figure 1. Disassembled holding cage.

Figure 2. Holding room at apiary.

Figure 3. Method used in feeding confined bees.

Figure 4. Method used in topical application test.

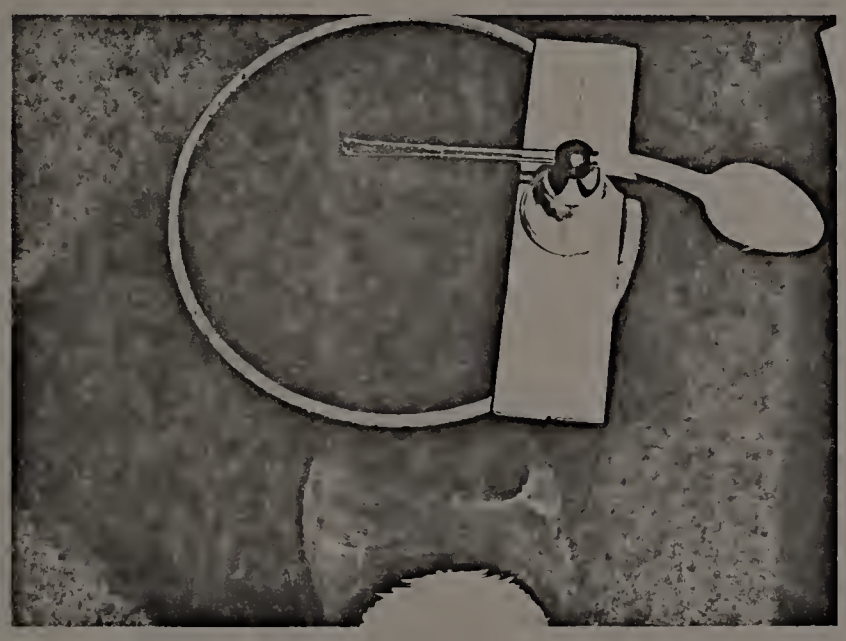

Figure 5. Mass spraying tower.

Figure 6. Method used in dispensing pesticide solution into a piece of glass tubing.

Figure 7. Mass feeding test.

Figure 8. Foil residual test.

Figure 9. Leaf residual test.

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APPENDIX I

Analysis of Data

Table 9. Analysis of Variance - 24 Hour Mortality Counts of Bees

Source	DF	SS	MS	F-Ratio	F at 95%	Significance
Treatments	7	1792.511	256.073	41.187	3.50	Significant
Rates within Treatments	8	49.738	6.217	0.624	1.98	Non-significant
Type of Application	176	1754.636	9.970	29.638	1.22	Significant
Within Replicates	384	129.170	0.336			
Total	575	3726.055				

Table 10. Analysis of Variance - 72 Hour Mortality Counts of Bees

Source	DF	SS	MS	F-Ratio	F at 95%	Significance
Treatments	7	1648.685	235.526	40.375	3.50	Significant
Rates within Treatments	8	46.668	5.833	0.614	1.98	Non-significant
Type of Application	176	1670.906	9.494	24.005	1.22	Significant
Within Replicates	384	151.874	0.396			
Total	575	3518.132				

Table 11. Comparisons of Means - 24 Hour Mortality Counts of Bees

Between Treatments:

$$Sd = \sqrt{2S^2/n} = \sqrt{2(.336)/72} = 0.096$$

From Tukey's Table: t = 3.03

Significant Difference: 3.03 x 0.096 = 0.291

Table of Differences(a):

		Treatment No.							
		1	5	3	4	7	6	2	8
Treatment No.	Mean	5.985	5.613	4.576	3.954	3.082	1.501	1.433	1.384
1. carbaryl	5.985	0	0.372	1.409	2.031	2.903	4.484	4.552	4.601
5. malathion	5.613		0	1.037	1.659	2.531	4.112	4.180	4.229
3. diazinon	4.576			0	0.622	1.494	3.075	3.143	3.192
4. azinphosmethyl	3.954				0	0.872	2.453	2.521	2.570
7. phosalone	3.082					0	1.581	1.649 *	1.698 *
6. methoxychlor	1.501						0	0.068	0.117 *
2. chloropropylate	1.433							0	0.049
8. control	1.384								0

(a) An asterisk placed above a number denotes no significant difference in F at 95%.

Table 12. Comparisons of Means - 72 Hour Mortality Counts of Bees

Between Treatments:

$$Sd = \sqrt{2S^2/n} = \sqrt{2(.396)/72} = 0.105$$

From Tukey's Table: t = 3.03

Significant Difference: 3.03 x 0.105 = 0.318

Table of Differences(a):

		Treatment No.							
		1	5	3	4	7	6	2	8
Treatment No.	Mean	6.111	5.671	4.683	4.114	3.311	1.787	1.705	1.623
1. carbaryl	6.111	0	0.440	1.428	1.997	2.800	4.324	4.406	4.488
5. malathion	5.671		0	0.988	1.557	2.360	3.884	3.966	4.048
3. diazinon	4.683			0	0.569	1.372	2.896	2.978	3.060
4. azinphosmethyl	4.114				0	0.803	2.327	2.409	2.491
7. phosalone	3.311					0	1.524	1.606 *	1.688 *
6. methoxychlor	1.787						0	0.082	0.164 *
2. chloropropylate	1.705							0	0.082
8. control	1.623								0

(a) An asterisk placed above a number denotes no significant difference in F at 95%.

Each test method used has been assigned a number. The following list is to be used in conjunction with tables 13 and 14 which follow:

Treatment Number	Test Method
1.	Topical Application
2.	Mass Spraying
3.	Individual Feeding
4.	Mass Feeding
5.	Foil Residual - 2 Hours after Spraying
6.	Foil Residual - 1 Day after Spraying
7.	Foil Residual - 3 Days after Spraying
8.	Foil Residual - 5 Days after Spraying
9.	Leaf Residual - 2 Hours after Spraying
10.	Leaf Residual - 1 Day after Spraying
11.	Leaf Residual - 3 Days after Spraying
12.	Leaf Residual - 5 Days after Spraying

Table 13. Comparisons of Means - 24 Hour Mortality Counts of Bees

Between Types of Treatment:

$$Sd = \sqrt{2S^2/n} = \sqrt{2(.336)/48} = 0.118$$

From Tukey's Table: t = 3.27
Significant Difference: 3.27 x 0.118 = 0.386
Table of Differences(a):

No.		4	1	5	7	6	3	2	8	9	11	10	12
	Mean	4.836	4.355	4.345	3.965	3.904	3.662	3.438	3.419	3.376	2.555	1.984	1.453
4.	4.836	0	0.481	0.491 *	0.871	0.932	1.174	1.398	1.417	1.460	2.281	2.852	3.383
1.	4.355		0	0.010	0.390 *	0.451	0.693	0.917	0.936	0.979	1.800	2.371	2.902
5.	4.345			0	0.380	0.441 *	0.683 *	0.907	0.926	0.969	1.790	2.361	2.892
7.	3.965				0	0.061	0.303 *	0.527	0.546	0.589	1.410	1.981	2.512
6.	3.904			0	0.242	0.466 *	0.485 *	0.528 *	1.349	1.920	2.451		
3.	3.662				0	0.224	0.243 *	0.286 *	1.107	1.678	2.209		
2.	3.438					0	0.019	0.062 *	0.883	1.454	1.985		
8.	3.419						0	0.043	0.864	1.435	1.966		
9.	3.376							0	0.821	1.392	1.923		
11.	2.555								0	0.571	1.102		
10.	1.984									0	0.531		
12.	1.453										0		

(a) An asterisk placed above a number denotes no significant difference in F at 95%.

Table 14. Comparisons of Means - 72 Hour Mortality Counts of Bees

Between Types of Treatment:

$Sd = \sqrt{2S^2/n} = \sqrt{2(.396)/48} = 0.128$

From Tukey's Table: t = 3.27
Significant Difference: 3.27 x 0.128 = 0.419
Table of Differences(a):

No.		4	1	5	7	6	3	2	9	8	11	10	12
	Mean	5.055	4.586	4.424	4.192	3.967	3.917	3.739	3.638	3.513	2.704	2.256	1.518
4.	5.055	0	0.469	0.631 *	0.863 *	1.088	1.138	1.316	1.417	1.542	2.351	2.799	3.537
1.	4.586		0	0.162	0.394 *	0.619	0.669	0.847	0.948	1.073	1.882	2.330	3.068
5.	4.424			0	0.232	0.457 *	0.507 *	0.685	0.786	0.911	1.720	2.168	2.906
7.	4.192				0	0.225 *	0.275 *	0.453	0.554	0.679	1.488	1.936	2.674
6.	3.967			0	0.050 *	0.228 *	0.329 *	0.454 *	1.263	1.711			2.449
3.	3.917				0	0.178 *	0.279 *	0.404 *	1.213	1.661			2.399
2.	3.739					0	0.101 *	0.226 *	1.035	1.483			2.221
9.	3.638						0	0.125 *	0.934	1.382			2.120
8.	3.513							0	0.809	1.257			1.995
11.	2.704								0	0.448			1.186
10.	2.256									0			0.738
12.	1.518												0

(a) An asterisk placed above a number denotes no significant difference in F at 95%.

APPENDIX II

Physical Factors During Conduct of Experiments

Table 15. Topical Application Test Temperature-Humidity Data[a]

	1.0 lb/100 gal		0.5 lb/100 gal	
Testing Temperature (C)				
Average	28.3		28.3	
Range	27-29		27-29	
Holding Temperature (C)	(24 hr)	(72 hr)	(24 hr)	(72 hr)
Average	28.1	27.1	28.1	27.1
Range	25-32	25-32	25-32	25-32
Holding Humidity (%)				
Average	40.6	43.9	40.6	43.9
Range	29-40	29-52	29-40	29-52

(a) Average or range of three replicates.

Table 16. Individual Feeding Test Temperature-Humidity Data[a]

	1.0 lb/100 gal		0.5 lb/100 gal	
Testing Temperature (C)				
Average	28.7		29.7	
Range	28-29		29-31	
Holding Temperature (C)	(24 hr)	(72 hr)	(24 hr)	(72 hr)
Average	28.2	28.2	29.0	28.6
Range	26-32	26-32	26-34	25-34
Holding Humidity (%)				
Average	54.0	53.7	52.9	55.3
Range	40-64	40-66	44-64	44-64

(a) Average or range of three replicates.

Table 17. Mass Spraying Test Temperature-Humidity Data[a]

	1.0 lb/100 gal		0.5 lb/100 gal	
Testing Temperature (C)				
Average	26.5		26.5	
Range	26-27		26-27	
Holding Temperature (C)	(24 hr)	(72 hr)	(24 hr)	(72 hr)
Average	26.8	27.7	26.8	27.7
Range	25-28	25-30	25-28	25-30
Holding Humidity (%)				
Average	56.5	57.4	56.5	57.4
Range	56-59	56-60	56-59	56-60

(a) Average or range of three replicates.

Table 18. Mass Feeding Test Temperature-Humidity Data[a]

	1.0 lb/100 gal		0.5 lb/100 gal	
Testing Temperature (C)				
Average	29.5		28.5	
Range	29-30		27-28	
Holding Temperature (C)	(24 hr)	(72 hr)	(24 hr)	(72 hr)
Average	28.1	28.7	30.0	30.6
Range	27-29	27-30	28-31	28-31
Holding Humidity (%)				
Average	56.4	58.0	58.8	57.0
Range	56-60	56-60	56-60	56-60

(a) Average or range of three replicates.

Table 19. Foil Residual Test Temperature-Humidity Data[a]

	2 hr	1 day	3 days	5 days
Testing Temperature (C)				
Average	30.5	31.5	29.0	29.5
Range	30-31	31-32	28-30	29-30
Holding Temperature (C) (24 hr)				
Average	32.6	32.8	31.8	30.5
Range	31-34	32-34	31-33	29-31
Holding Temperature (C) (72 hr)				
Average	32.4	32.1	31.1	30.0
Range	30-34	30-34	29-33	29-31
Holding Humidity (%) (24 hr)				
Average	53.7	53.3	55.8	55.4
Range	54-58	48-58	50-56	48-62
Holding Humidity (%) (72 hr)				
Average	55.9	53.7	59.1	53.9
Range	48-58	48-60	50-66	48-62

(a) Average or range of three replicates for concentrations of both 1.0 and 0.5 lb/100 gal.

Table 20. Leaf Residual Test Temperature-Humidity Data[a]

	2 hr	1 day	3 days	5 days
Testing Temperature (C)				
Average	28.3	29.4	29.0	29.0
Range	28-29	29-30	28-30	28-30
Holding Temperature (C) (24 hr)				
Average	30.1	29.6	31.3	31.5
Range	29-31	29-31	30-32	30-34
Holding Temperature (C) (72 hr)				
Average	29.9	30.3	31.1	31.3
Range	29-31	28-32	29-32	30-34
Holding Humidity (%) (24 hr)				
Average	53.9	52.8	52.3	53.2
Range	52-58	46-56	50-55	50-60
Holding Humidity (%) (72 hr)				
Average	52.5	52.1	52.4	52.9
Range	46-58	46-55	50-55	50-61

(a) Average or range of three replicates for concentrations of both 1.0 and 0.5 lb/100 gal.