A COMPARATIVE STUDY OF THE TOXICITY OF MALATHION AND MALATHION PLUS PIPERONYL BUTOXIDE BY TOPICAL APPLICATION ON THE GERMAN COCKROACH, BLATTELLA GERMANICA (L.), AND THE MADERA COCKROACH, LEUCOPHEAE MADERAE (F.)

by

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INTRODUCTION

To a large extent, the value of entomology is based on insect control. Insect control involves anything or any measure taken against the insect to eliminate or reduce the damage inflicted on man and his materials.

The struggle between man and insects began long before the dawn of civilization, and has continued without cessation to the present time. If man is to succeed in his constant battle against insects, he must consider the decided advantage insects have in their ability to multiply with incredible speed and adapt themselves to diverse environmental conditions. Being aware of these dangers, he must bring into play every type of weapon he can muster and a planned strategy to combat his prime enemy.

From the standpoint of the entomologist, the best method of insect control is through the aid of natural factors and influences. Although the entomologist may suggest the use of some chemicals and insecticides to combat an insect menace, his strongest point is to attack the insect before the insect is able to do any damage. The use of insecticides and other chemicals are necessary when, through lack of action or ability, other means fail.

Although the use of insecticides to control insects is not a new discovery, the method does not present an ideal situation. The initial success obtained with chemicals like DDT in the control of many serious insect pests gave grounds for the hope that certain insect control problems need never again get out of hand if proper suppressive measures were used.

Various insecticidal chemicals belonging to different groups have been discovered and their applications against various insects have been studied. Examples of these chemicals that have been used in pest control are botanicals, chlorinated hydrocarbons, inorganic compounds, and more recently, organic phosphorus compounds.

Despite the seemingly insurmountable barriers confronting man in his fight against insect pests, encouraging results seem to be forthcoming through research. In his search for newer and better chemicals and insecticides, two very important questions must be answered by the investigator in the course of his research: would this new chemical be useful as an effective insecticide; and how would this new compound compare with others that have been used before both as a toxicant and as an effective means of control?

Rai (1956) conducted a series of experiments to study "the effects of piperonyl butoxide on the action of malathion in DDT susceptible and resistant houseflies, Musca domestica (L.), at various temperatures." In this work he found that "there was a statistically significant level of antagonistic action between malathion and piperonyl butoxide in both strains of houseflies at all the four temperatures" in which the experiments were run.

To determine whether or not this antagonistic behavior was a specific response of houseflies, two species of cockroaches were selected as the test animals. Malathion was chosen as the test insecticide with piperonyl butoxide as synergist.

LITERATURE REVIEW

Since the findings in these experiments were a follow-up of Rai's work (1956) with houseflies, and involved a study on the toxic action of malathion and a combination of malathion with piperonyl butoxide, much of the literature reviewed here follows those in line with Rai's work. Only a handful of workers have done similar work with roaches, probably the most helpful among those were by Roan and Babers (1954), Butts and Davidson (1955), Fisk and Isert (1953), Guthrie (1950), McGovran and Fales (1950), Mitlin and Konecky (1955), Beard (1949), Sumerford (1954), and Rai (1956).

Campbell and Moulton (1943), Bliss (1935), Menusan (1948), and McGovran and Fales (1950), provided basic information on test procedures and laboratory rearing technique.

The insecticide application methods used in these experiments were not entirely new. Several workers have used similar devices to the microapplicator used. An excellent review of the literature covering the use of such a device has been included in papers by Heal and Menusan (1948), Butts and Davidson (1955), Roan and Maedar (1953), and Brook (1950).

The literature reviewed in connection with the insecticide (malathion) and synergist (piperonyl butoxide) used in these experiments covered those written by Earle (1952), Wachs (1947), Bourcart (1913), Rodda (1955), Gersdoff (1954), Chamberlain (1950), Babers and Pratt (1951), and many others.

"Synergism, literally a working together, is a cooperative action encountered in biologically active combinations in which the combined activity of two like-acting components exceeds the sum of activity of the components used alone" (Summerford, 1954). Antagonism as used in this thesis is exactly opposite to this.

The discovery of compounds which enhance the efficiency and effectiveness of pyrethrins in fly sprays has provided a stimulus for intensive research on synergism in insecticides. Perry and Hoskins (1951) in their work on synergistic action with DDT toward resistant houseflies reported that perhaps "the first clear case was Pearson's (1935) discovery that pine oil increased the efficiency of pyrethrum cattle spray." Eagleson (1940) in his work with sesame oil found that the oil had a decided effect on the efficiency of pyrethrin solutions on houseflies, and Haller et al. (1942) finally isolated sesamin as an active ingredient.

While there have been other outstanding workers in the field of synergism, there have been many different ideas conceived about synergists. Rodda (1945) believed that the addition of rotenone to pyrethrum in a kerosene spray is the first example of a synergized insecticidal combination used. However, it was not until in the 1930's that industries began to realize the significance and value of synergists in various insecticides, and n-isobutylundecylenamide became the first important synergist commercialized for pyrethrum in 1938. Weinman and Decker (1949) found that aldrin synergized DDT against grasshoppers, but Dicke and Paul (1951) found that the same chemical showed antagonism in the housefly.

The existence of synergism is not always clear cut, and its action has not yet been fully understood. Gnadinger (1945) suggested that synergists have a high degree of specificity depending on the insecticide involved, and further, depends on the insect to be controlled, the type of formulation used, and the manner in which the contact is made between insect and synergized agent, however, this statement or suggestion drew a lot of controversy "as to the prevalence of synergism and in the terms used to describe it" (Summerford, 1954).

Probably the most widely used synergist today is piperonyl butoxide. Piperonyl butoxide has been known as an effective synergist since it was developed by Wachs in 1947. Piperonyl butoxide has been widely used in aerosol, oil-base household sprays, and other types of formulations.

The site and mode of action of piperonyl butoxide in insects are still questionable. Almost all workers seem to think that when combined with other insecticides of the same or related groups, it affects the nervous system. Of the several theories on the mode of synergism that of Page and Blackith (1949) lent itself best to in vitro physical studies. Page and Blackith suggested that "the formation of a loose molecular complex consisting of pyrethrins and synergists combined in a 1:1 mole ratio," and further reported that "this complex to be approximately three times as effective in producing paralysis of Aedes aegypti (L.) mosquitoes as pyrethrins alone."

Chamberlain (1950) proposed that the synergistic effect of piperonyl butoxide was due to its ability to inhibit the detoxication mechanism of houseflies, but further found that it activates the pyrethrin esters by inhibiting lipase, the enzyme responsible for detoxication by hydrolysis. This inhibition was neither non-competitive, nor irreversibly competitive, but seemed to be either of a freely reversible competitive type or due to some unknown physical factors resulting from use of a two-phase system.

In their work, "Synergist is action of piperonyl butoxide fractions and observations refuting a pyrethrins-butoxide complex," Miller, Pellegrini, et al. (1952) went to some finer details in evaluating the physical constants of piperonyl butoxide fractions.

Despite the seemingly endless controversies about synergists and their modes of action, basically all workers seem to agree that most synergists increase the effectiveness of toxicants. With some, synergists greatly increase effectiveness, while with others, there was little or no synergistic effect.

PURPOSE AND SCOPE

The purpose of these experiments was to investigate the comparative toxic effects of malathion and malathion plus piperonyl butoxide by topical applications on the German cockroach and the Madera cockroach, and to compare these findings with the toxic effects of combinations of these insecticides and piperonyl butoxide on DDT resistant and non-resistant strains of the housefly as found by Rai (1956).

MATERIALS AND METHODS

The test insects used in these experiments were the German cockroach, Blattella germanica (L.), and the Madera cockroach, Leucopheae maderae (F.). Only the male adult roaches of each species were used. The female roaches were either placed back in the rearing tubs for the continuation of rearing procedures, or else were discarded after sexing, depending on their maturity and other biological factors. A few of the females that were used for experimental purposes either showed no response whatsoever to the insecticides used on them, or else gave inconsistent results. The male roaches of each species used in these tests were one- to two-week-old adults.

Chemicals and Solvents

The chemicals used in these experiments were malathion as the main insecticide, piperonyl butoxide as a synergist, and acetone as solvent. The malathion was 99.6 percent pure, and was obtained from the American Cyanamid Company of New York.

Malathion is a coined name for the insecticidal chemical having the molecular formula of $C_{10}H_{19}O_6PS_2$, and the structural formula corresponding to O, O-Dimethyldithiophosphate of diethyl mercapto succinate, the pure product has a boiling point of 156° to 157°C. at 0.7 mm., with slight decomposition, and is only slightly soluble in water (145 ppm.), but miscible with most organic solvents. This material was first introduced for experimental purposes by the American Cyanamid Company in 1950 as Insecticide 4049. The piperonyl butoxide (technical grade) used as a synergist with malathion was supplied by the U. S. Industrial Chemicals Company, Division of National Distillers Products Corporation, New York. Piperonyl butoxide is a coined name for the chemical having the molecular formula of $C_{19}H_{30}O_5$, and the structural formula corresponding to 3:4-methylenedioxy-6-propylbenzyl n-butyl diethyleneglycol ether.

Apparatus

The apparatus used for the application of the insecticides to the roaches was essentially a 0.25 cc. tuberculin syringe. This particular syringe was the B-D Yale S.851 tuberculin syringe No. ¼ YT Double Scale ¼ minim and ¼ cc. by volume, produced by Becton, Dickinson and Company, Rutherford, New Jersey. The needle for the syringe was a rustless steel, ½ inch, 26 gauge B-D Yale-Lok needle. The syringe was calibrated to eject 1.07 ul plus or minus 0.05 ul per .005 inch movement of the micrometer.

The loaded syringe was mounted on a microapplicator designed and described by Roan and Maeda (1953) and Butts and Davidson (1955). The microapplicator was set to deliver a known amount of insecticide per operational movement.

Other equipment and apparatus used, other than those used in the rearing procedures, included pipettes for the pipetting of solutions, glass vials for the solutions, beakers for drawing and discarding of acetone and insecticides, a chemical balance for accurate measurements of weights of both roaches and insecticides, a five lambda pipette for topical administration of insecticides where the microapplicator was not used, and half-pint glass jars with lids to receive treated roaches, and used as recovery jars.

Rearing

Both the German roaches and the Madera roaches used in these experiments as test animals were reared in a constant temperature rearing room. The original stock of the roaches had no known treatment in their history, and have been considered as untreated specimens. The room in which the roaches were reared was maintained at between 74° and 82°F., with a relative humidity of 60 percent plus or minus five percent. The roaches were exposed to constant and equal amounts of incandescent light.

Cultures of the two species were started in leak proof, rust proof "Dura-Zink-Alloy" tubs No. 62, manufactured by the Wheeling Corrugating Company, Wheeling, West Virginia. Each tub was

20 inches wide, 20 inches long, and 11 inches deep. A top made out of wooden frame and chicken wire mesh each measuring 22 inches square was constructed and used as cover for the tubs.

The bottom of each tub was covered with sawdust upon which was placed a fabricated wooden framework topped by wire screen to provide hiding place for the developing and maturing roaches. On top of this framework was placed a trough of water made out of inverted quart glass jar and large tender dish in which a wad of cellucotton was placed to provide direct drinking surface for the roaches, and to prevent the roaches from drowning. Feed, consisting of dog meal, was placed in two to three paper cups each holding about seven ounces of dog meal, were left in each tub. Escape of the roaches was prevented by a thin ring of vaseline smeared inside each tub near the top. The roaches were inspected daily, and fed and watered at regular intervals of from three to four days. Dead roaches were always removed from the tubs. As far as was possible, each species of roach was kept in a separate tub and prevented from escaping from the rearing tubs. Runaway roaches were caught by special traps set in the room.

Insect Manipulation, Sexing, and Counting

Since only one- to two-week-old male roaches were used in these experiments, it was necessary to sex them and to isolate them from immatures. In both the German and Madera roaches the distinguishing taxonomic characteristic existing between the males and the females was the number of cerci. Male roaches carry an additional pair of rudimentary cerci on the last abdominal tergite, while females have only the one standard pair.

In the case of the Madera roach, the males were picked out from the rearing tubs and placed in quart jars which were smeared with vaseline on the inside near the neck. This prevented the roaches from escaping. A vial of water plugged up with cotton to allow for the easy flow of the water, and dog food pellets were placed in each of the jars to provide food and water for the roaches. Each jar was then covered with a lid, the top of which was made out of screen wire mesh. These tubs were maintained for the culture of these roaches, and each culture was arranged in varying sequence according to the age of the roaches.

The German roaches were a bit more difficult to handle since they move fast. These roaches were anaesthesized with carbon dioxide to facilitate handling. Males were picked out and placed in jars similar to those used for the Madera roaches, and females were either placed back in the rearing tubs or else disposed of by drowning them in either alcohol or acetone or carbon tetrachloride.

Since all experiments were replicated, a total of 90 roaches in each case was required for one experiment, 80 for the four replicated concentrations required in each set of treatment with four different concentrations of insecticide, and 10 set aside as control.

Since no roach was treated on the day it was sexed and removed from the rearing room, it was necessary to precondition them to the test temperature 24 hours before they receive treatment. It was found necessary to treat all roaches throughout the experiments at one standard temperature in order to eliminate any possible error due to temperature variation. The roaches were, therefore, preconditioned at room temperature of 80°F. plus or minus 2°F.

Weighing

Weighing of the roaches was made before each treatment to determine the LD_{50} based on the amount of the insecticide administered and the body weight of the roaches. At the beginning of these experiments, the weight of each of the species of the roaches was unknown, and the only guide as to the approximate weight of each roach was an estimation based on the weight of a housefly. German roaches were thought to be about five or six times heavier than houseflies, while the Madera roaches were thought to be between twenty-five to thirty times the weight of the German roaches.

Roaches were weighed with an analytical balance to insure accuracy. The preconditioned roaches were again anaesthesized with carbon dioxide in the quart size jars, and 10 in each case were selected at random and placed in a glass planchet dish and weighed. The weight of the roaches was calculated as being the difference in weight of the planchet while empty and the planchet while containing ten roaches. From the calculated weight of 10 roaches, the average weight of one roach was then determined.

After weighing, roaches were returned to the jars to await treatment time.

Solution Preparation

Thirty to 40 minutes before treatment time the insecticide stock solutions were measured and weighed. Clear glass vials measuring 6.5 cm. long with diameter of 1.5 cm. were first weighed on an analytical balance. The required weight of the insecticide was set on the balance and the vials were then filled with the insecticide to the set weight.

From these weighed-out samples desired dosages were made out using glass distilled acetone as solvent. For each treatment, four different dosages were made and calculated on the basis of micrograms per microliter. These dilutions were then used almost immediately after each value had been determined to avoid any possible deterioration or decomposition of the insecticide. This was especially more urgent where piperonyl butoxide was used as synergist with malathion.

Dosage Volume Calculation

Although in most experiments of this nature, treatment of the test animal was based on the tolerance of the test animal to certain volume of insecticides and solvents, this factor did not apply in the case of these experiments. Heal (1948) found the American cockroach, Periplaneta americana (L.), could tolerate from 0.002 ml. to 0.004 ml. per 0.1 gram of insect body weight, but these figures were not used in these experiments. The basis of the dosage volume calculation in these experiments was on the microgram per microliter of the insecticide by volume calculated on the gram body weight of the roach after the final reading of the experiment had been plotted.

Dosage Application

Prior to the application of the insecticide to the roaches, recovery jars were prepared in which the treated roaches were later placed to recover from the treatment. Recovery jars were half-pint jars with perforated lids to allow air in the jars while keeping the treated roaches inside. In each of the recovery jars was placed a small vial containing water, and plugged with cotton so that the water does not run out, but allow free flowing enough to water the roaches, and a large pellet of dog food. The vial of water and the dog food pellet were suspended from the lid of the jar, through one of the perforations by copper wire.

The preconditioned roaches were then anaesthesized with carbon dioxide, 40 at a time for one treatment, and were then treated.

The German roaches were treated differently from the Madera roaches. The microapplicator was used in the case of the German roaches, while a 5-lambda pipette was used for the Madera roaches.

For the German roaches, each roach received treatment on the mesothorax. The blunt needle of the syringe which was attached to the microapplicator was brought into contact with the mesothorax of the anaesthesized roach and the prescribed amount of the toxicant was dropped through the needle by the manipulation of the microapplicator. Each roach was left touching the needle for a few seconds to allow for the proper dispersal of the toxicant on the thoracic region. The roaches were placed in the recovery jar, and mortality readings were taken following observations at regular intervals for a period of 24 hours.

In the case of the Madera roach, treatment was effected by the use of a 5-lambda pipette, and application was made under the wings of each roach.

Ten roaches were used for each dilution. Therefore, for the four dilutions prepared, 40 roaches were used and 10 more set aside untreated as control. Two replications involving the same number of roaches were made for each experiment of four dilutions.

With the microapplicator, it was necessary to check the syringe and needle for any possible obstruction. The syringe was filled to its ¼ cc. volume with the insecticide, and all the air bubbles expelled. It was then mounted on the microapplicator and fixed in position by a screw. Thus the possibility of treating a roach with air was greatly reduced. As a double check, a few movements of the microapplicator handle were made to clear the apparatus.

The 5-lambda micropipette was accurate in itself since the micro device attached to the pipette reduced all probability of drawing air together with the toxicant, but it was necessary to clean the outside of the pipette to make sure that no more than 5-lambda of the insecticide solution was being applied to the roach.

Both the micro-pipette and the syringe were thoroughly washed in acetone after treatment with each of the dilutions. This was necessary to insure that dilutions were not mixed, and also to minimize contamination. Lower concentrations were applied to the roaches first, the strength of the insecticide increasing with each successive dosage of higher value. Thus the first set of 10 roaches were treated with the weakest concentration until the highest concentration was reached with the last set of 10 roaches concluding a particular experiment.

The syringe and pipette were then thoroughly flushed with acetone and set aside for future use.

Observation

After treatment the roaches were placed in the recovery jars and left at the same temperature and location they had been in during the preconditioning time. Observations were made at regular intervals during that period, and dead roaches were counted and recorded. The most significant factor at this stage was the consideration of what was termed "dead." In these experiments, a roach was considered dead when there was no form of movement whatsoever from the roach. Movement included any movement of the mouthparts, the antennae, any of the abdominal segments, wings, legs, or any other part of the roaches' anatomy. To a great extent, the roaches were teased to see if they showed any movement or not, lack of response after teasing led to the conclusion that they were dead.

RESULTS AND DISCUSSION

The data obtained from 640 Madera roaches treated with malathion alone, and 640 of the same species treated with malathion plus piperonyl butoxide with replications of each were used to plot the dosage mortality curves for the two chemicals used. The same number of German roaches were also tested first with malathion alone and with malathion plus piperonyl butoxide. When malathion was used together with piperonyl butoxide, the concentration was kept constant throughout the experiments at the ratio of one part of malathion to 10 parts of piperonyl butoxide by weight volume. The amount of each chemical used and the percentage mortality for each dosage level for each replication is presented in Tables 1 and 2.

The eye-fitted regression lines were plotted from the average mortality of the two replications for each chemical (Plates I and II).

Although there are few data on the toxicity of malathion on roaches, several workers have tested the chemical on other animals other than roaches and flies. Hazleton and Holland (1953) stated that the acute oral toxicity of malathion for mammals appeared to vary inversely with the degree of its purity. Litchfield and Wilcoxon (1949) working with various species of animals provided a summary for the LD_{50} on malathion with the animals

Table 1. Percent mortalities of male German roaches, Blattella germanica L., at various dosage levels of malathion and malathion plus piperonyl butoxide (at the ratio of 1:10).

Dosage administered: **ug/ul per roach	Malathion alone: Total % mortality	Malathion alone: Replications	Malathion alone: Average % mortality	Malathion & piperonyl butoxide: Total % mortality	Malathion & piperonyl butoxide: Replications	Malathion & piperonyl butoxide: Average % mortality
2	410	5	82	570	6	95
1.5	630	8	78.7	830	9	92.2
1	660	10	66	830	10	83
.5	450	9	50	660	10	66
.3	190	5	38	160	4	40
.2	150	5	30	110	4	25

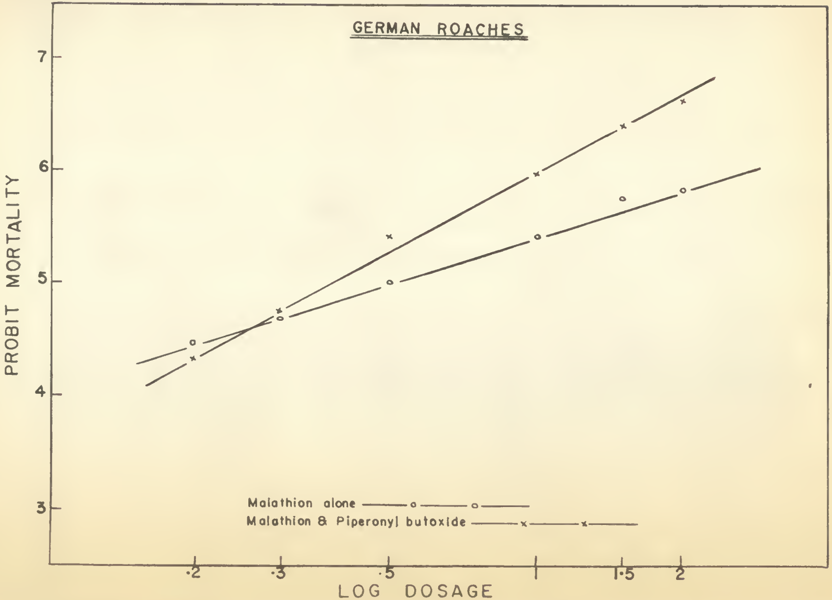
** ug/ul calculated on each dilution of the compounds used. Actual amount of insecticides administered to each insect was 1.07 times more than these values, since a calibrated syringe ejecting 1.07 ul of insecticide delivery was used.

EXPLANATION OF PLATE I

Regression lines for the average 24 hour percent mortalities of the German cockroach, *Blattella germanica* L., with malathion and malathion plus piperonyl butoxide.

Log dosage values are calculated on micrograms per gram of roach body weight.

PLATE I


GERMAN ROACHES
7
6
5
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3
PROBIT MORTALITY
·2
·3
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1·5
2
LOG DOSAGE
Malathion alone
Malathion & Piperonyl butoxide

Table 2. Percent mortalities of male Madera roaches, Leucopheae mederae F., at various dosage levels of malathion and malathion plus piperonyl butoxide (at the ratio of 1:10).

Dosage administered: **ug/ul per roach	Malathion alone: Total % mortality	Malathion alone: Replications	Malathion alone: Average % mortality	Malathion & piperonyl butoxide: Total % mortality	Malathion & piperonyl butoxide: Replications	Malathion & piperonyl butoxide: Average % mortality
20	440	5	88	340	5	68
15	340	5	68	260	5	52
12	310	5	62	190	5	38
10	250	5	50	150	5	30

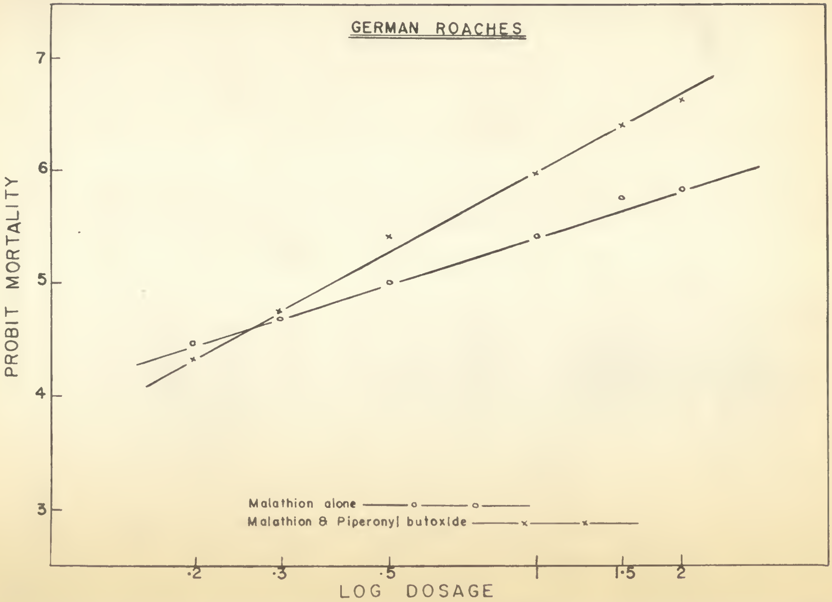
** ug/ul calculated on each dilution of the compounds used. Actual amount of insecticide administered to the insects was five times more than these values, the five lambda micro-pipette being the means of application.

EXPLANATION OF PLATE II

Regression lines for the average 24 hour percent mortalities of the Madera cockroach, *Leucopheae medera* F., with malathion and malathion plus piperonyl butoxide.

Log dosage values are calculated on micrograms per gram of roach body weight.

PLATE I


GERMAN ROACHES
PROBIT MORTALITY
7
6
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1·5
2
LOG DOSAGE
Malathion alone
Malathion & Piperonyl butoxide

they worked. Their data indicated that rats were more susceptible than mice, and that in either species the toxicity decreased with increased purity. Martin (1955) gave the acute oral LD_{50} to mice

Table 3. LD_{50} values for malathion and malathion plus piperonyl butoxide at 24 hours for the German roach and the Madera roach by topical applications.

Compound	Micrograms per gram of roach body weight	
	German roach LD_{50}	Madera roach LD_{50}
Malathion alone	89.4	271.6
Malathion plus piperonyl butoxide	67.1	521.3

and rats ranging from 480-5800 mg/kg; and rats receiving 1000 p.p.m. of technical malathion for 92 weeks showed normal weight gains.

Working with DDT-resistant and non-resistant houseflies, Rai (1956) found the LD_{50} values of malathion to be 20.83 for non-resistant strain, and 32.72 for the resistant strain, while malathion plus piperonyl butoxide showed values of 34.66 with the non-resistant strain, and 50.79 with the resistant strain. Rai's values were calculated on the basis of microgram per gram body weight of the flies, and his malathion plus piperonyl butoxide was in the ratio of one part malathion to 10 parts piperonyl butoxide by weight.

It has already been mentioned elsewhere in this work that piperonyl butoxide in itself has not insecticidal value, but used with other insecticides it "enhances toxicity." Martin (1955) stated that piperonyl butoxide is practically non-toxic and non-irritating to warm-blooded animals, but has an oral LD_{50} of about 7.5 mg/kg for both rats and rabbits.

Eddy et al. (1954) and Hoffman et al. (1954), working with a number of organic phosphorus compounds with many candidate synergists, reported that piperonyl butoxide when combined with certain compounds gave quite a high level of synergism, while with others it did not.

The data presented in Table 1 and Plate I, seem to indicate that there was not much difference between the toxic action of malathion when used alone or when used with piperonyl butoxide on the German roaches. The deviation of the regression lines derived from the average mortality of the two replications of each of the combinations used might be sttributed to experimental error though these have not been established statistically.

In the case of the Madera roaches a definite trend toward antagonism has been observed from the results of the experiments. It was found that it required at least twice as much malathion in malathion-piperonyl butoxide combination than malathion alone to kill 50 percent of the population.

The mechanisms of the toxicity of these two compounds have not been studied, but Rai (1956) attributed it to either the reduction in the intoxication rate or to increased rate of detoxication, or both in the antagonism shown in the flies he worked with. The claims of other workers in this respect have been mentioned elsewhere in this work.

It would be interesting to find out whether or not the place of application is a factor in the general outcome of the experiments. Insecticides were applied to the mesothorax in the German roaches, while the site of application on the Madera roaches was under the wings, and whether or not the same results would be obtained in both species if a standard application apparatus is used for the treatment.

SUMMARY AND CONCLUSIONS

In this study it was found that the action of malathion and malathion plus piperonyl butoxide was similar in the German cockroach, Blattella germanica (L.), If there were any tendencies towards either synergism or antagonism, the results of these studies showed a slight tendency towards synergism. A statistical analysis was not made, and this assumption could, therefore, not be confirmed.

In the Madera roaches, Leucopheae maderae (F.), it was evident that there was a difference in the toxic mechanism of the action of malathion and malathion plus piperonyl butoxide. It required at least twice as much malathion plus piperonyl butoxide to kill 50 percent of the population as it would malathion alone, thereby showing a definite trend toward antagonism.

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A COMPARATIVE STUDY OF THE TOXICITY OF MALATHION AND MALATHION PLUS PIPERONYL BUTOXIDE BY TOPICAL APPLICATION ON THE GERMAN COCKROACH, BLATTELLA GERMANICA (L.), AND THE MADERA COCKROACH, LEUCOPHEAE MADERAE (F.)

by

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The struggle between man and insects ante-dates history, and his struggle for survival against his arch enemy, man has battled all through the ages against insect pests and insect menace.

In recent years insecticides have been discovered and developed to aid man in insect control. The initial success some workers had with certain insecticides has provided the incentive for more work in the field of toxicology, and the development of better insecticides and of more effective ways of using these insecticides in insect control.

Several workers have worked with particular insecticides in studying their modes of action against specific insects. Malathion and a combination of malathion plus piperonyl butoxide when used on DDT-resistant and non-resistant strains of houseflies was found to show a statistically significant level of antagonism between the two compounds in both strains of houseflies at four different temperatures. To determine whether or not this antagonistic behavior was a specific response of houseflies, two species of cockroaches, the German cockroach, Blattella germanica (L.), and the Madera cockroach, Leucopheae maderae (F.), were selected as test animals, and malathion, (O, O-dimethyl-dithiophosphate of diethyl mercaptosuccinate) was used as the test insecticide with piperonyl butoxide (3:4 methylenedioxy-6-propylbenzyl n-butyl diethyleneglycol ether) as synergist.

Only the male adult roaches of each species of the German roach and the Madera roach were used in these tests. These were reared in a constant temperature rearing room in "Dura-Zink-Alloy" No. 62 tubs at temperatures of between 74° and 82°F., with a relative humidity of 60 percent plus or minus five percent. The roaches were sexed 24 hours before treatment time, and preconditioned for treatment at room temperature of 80°F., plus or minus 2°F. Shortly before treatment time, the roaches were weighed and the average weight recorded. The LD_{50} values were determined based on the amount of the insecticide administered and the body weight of the roaches.

Two replications involving a total of 50 roaches of each species per replication were made in each "set" of experiments consisting of four dosage levels. Ten roaches per replication were treated with four different concentrations of insecticides comprosing the four dosage levels, and 10 more were set aside untreated as control. Observations were made and recorded at regular intervals for a period of 24 hours at which the number of dead roaches per level of concentration were counted. Eye-fitted regression lines were plotted from the average mortality of the two replications for each chemical, and the LD_{50} values were then determined.

Malathion alone was used in half the number of the total tests run, and malathion plus piperonyl butoxide at the ratio of one part malathion to 10 parts piperonyl butoxide by weight-volume made the other half. Glass distilled acetone was used as the solvent in each dilution throughout the tests.

Treatment of the roaches was effected by the use of a 0.25 cc. tuberculin syringe carrying a ½ inch, 26 gauge, rustless steel blunt needle and mounted on a microapplicator. The syringe was calibrated to eject 1.07 ul of toxicant per 0.005 inch movement of the micrometer. The roaches were treated topically on the mesothorax. The Madera roaches were treated with the toxicant through a 5-lambda micro-pipette. The treatment was topically under the wings. Roaches were placed in recovery jars for observations.

The data obtained from 640 Madera roaches per each of two replications treated with malathion alone, and 640 of the same species per replication treated with malathion plus piperonyl butoxide were used to plot the dosage mortality curves for the two chemicals used. The same number of German roaches were also tested first with malathion alone and with malathion plus piperonyl butoxide. Eye-fitted regression lines plotted from the average mortality of the two replications were used in drawing conclusions on the presence or absence of synergism in each of the species of cockroaches.

In this study it was found that the toxic action of malathion and malathion plus piperonyl butoxide was similar in the German cockroach, and it was also observed that there was a slight tendency towards synergism. In the Madera roaches, it was evident that there was a difference in the toxic mechanism of malathion and malathion plus piperonyl butoxide. It required at least twice as much malathion plus piperonyl butoxide to kill 50 percent of the population as it would malathion alone, thereby showing a definite trend toward antagonism.