INHIBITION OF *CULEX PIPIENS* (L.) (DIPTERA:CULICIDAE) EMERGENCE WITH METHOPRENE

by

Stuart Graham McCausland

Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Entomology

APPROVED:

E. C. Turner, Chairman

William Robinson

William Allen

January, 1977

Blacksburg, Virginia

ACKNOWLEDGEMENTS

The author wishes to thank Mrs. Linda Hope McCausland for her aid in conducting the tests in Virginia Beach and for her excellent typing of this manuscript. Thanks are also extended to Mr. Butch Palmer of the Zoecon Corporation for providing necessary information and materials, and to Mr. Don Simonet for his invaluable assistance in the statistical procedure applied herein. Drs. E. C. Turner, Jr., W. H. Robinson, and W. A. Allen deserve thanks for editing the manuscript. A special thanks is due Mr. R. E. Dorer, Director of the Bureau of Solid Waste and Vector Control (Virginia Health Department) for his encouragement of both the research and the author's degree pursuit.

LM/MRJ 6/6/97

TABLE OF CONTENTS

INTRODUCTION AND LITERATURE REVIEW

Mosquito Importance

Mosquitoes are known vectors of over 50 human diseases (James and Harwood 1969). Malaria, a disease carried by anopheline species, causes over 1,000,000 human deaths annually (Natl. Acad. Sci. 1973). In the Eastern United States mosquito borne St. Louis Encephalitis caused 95 human fatalities during the summer of 1975 (Center for Disease Control 1976). To hold mosquito populations below a health or nuisance level, Virginia has 27 state-aided mosquito control commissions which annually spend over $2 million.

Methoprene

In June 1973, the United States Environmental Protection Agency issued an experimental sales permit to the Zoecon Corporation in California for the manufacture and sale of Altosid®. Altosid, or methoprene (isopropyl (2E, 4E) - 11 - methoxy - 3, 7, 11 - trimethyl - 2, 4 - dodecadienoate), formerly known as ZR-515, is an insect growth regulator (IGR) for use against mosquitoes. After testing for two more years in 37 states, a full registration was issued for the use of Altosid SR-10 against the larvae of floodwater mosquitoes (Environmental Protection Agency 1975). Several chemical companies including Zoecon, Thompson-Hayward, Monsanto, Hoffman-La Roche, and Stauffer,

as well as the World Health Organization are developing experimental programs on IGR's (Menn and Pallos 1975, Mulla et al. 1974b).

Methoprene is presently the only IGR registered for use as a mosquito control agent. Its empirical formula is $C_{19}H_{34}O_3$ with a molecular weight of 310. Technical methoprene is an amber liquid with a specific gravity of 0.9261 g/ml at 20°C, a water solubility of 1.39 ppm, and a vapor pressure of 2.37×10^{-5} mm Hg at 25°C (Zoecon Corporation 1975). Methoprene synthesis has been outlined by Henrick et al. (1973). According to Schaefer and Dupras (1973) only the trans geometrical isomer is biologically active.

Altosid SR-10 was the only EPA labeled (EPA Reg. No. 20954-1-AA) formulation of methoprene available for the experiments herein documented. Altosid SR-10 is a liquid suspension of polymer-methoprene particles with an average particle diameter of one micron. The technical methoprene is encapsulated in an amide polymeric matrix and the formulation is 10% by weight, which is 0.8579 lb./gal. or 0.1028 kg/l (Schaefer et al. 1974a). The environmental degradation of methoprene has been investigated by numerous workers (Quistad et al. 1974, Quistad et al. 1975a, Quistad et al. 1975b, Quistad et al. 1975c, Schooley et al. 1975a, Schooley et al. 1975b). The half-life of technical methoprene in the field may vary from 2 hours in water (Schaefer and Dupras 1973) to over 10 days on sandy loam (Schooley et al.

1975b). Methoprene can be detected in water at 0.4 ppb and in soils at 1 ppb by gas-liquid chromatography (Miller et al 1975).

Staal (1972, 1975) has reviewed the effects of juvenile hormone mimicing insect growth regulators on insects. Their effects include delay in pupation, delay or failure in emergence, delay in egg hatch, delay in oogenesis, premature adult death, male mosquito sterility (due to non-rotation of genitalia after emergence), breaking of diapause, and possible physiological effects (failure to secrete pheromones). Several theories exist to explain the physiochemical or genetic basis of these effects (Schneiderman 1972, Slama et al. 1974, Williams and Kaftos 1972). Hsieh and Steelman (1974) found the major results of methoprene administered to 12 species of mosquitoes at their respective LC_{90}'s to be: dead, often unmelanized pupae (74%); dead larvae (19%); larval-pupal, and pupal-adult intermediates (2%); and abnormal adults (2%). As a group mosquitoes are most sensitive to methoprene during the late 3rd and 4th larval instars (Noguchi and Ohtaki 1974).

The effect of methoprene on the emergence of various mosquito species is summarized in Table 1. Effects on non-target organisms have also been reported by various workers (Menn and Pallos 1975, Miura and Takahashi 1973, Mulla 1974, Steelman et al. 1975, Takahashi and Miura 1975, Wright 1974). Norland (1974) determined that at 0.1 ppm, metho-

prene would prevent adult emergence of several species of Culicidae, Chironomidae, Ephemeroptera, and Dytiscidae. Other workers have found methoprene to be active against Chironomidae at 0.1 lb. a.i./A (0.1 pound actual ingredient per acre) and 0.2 ppm (Mulla and Darwazeh 1975a, Mulla et al. 1974a).

Resistance to methoprene in the field has not been documented. Schaefer and Wilder (1973) found no resistance after 20 generations in Aedes aegypti (L.) pressured at 0.003 ppm. However, Georghiou et al. (1974) produced resistance in Culex tarsalis Coq. after 12 generations of a 70.8% mortality pressure. Brown and Brown (1974) produced a 13-fold resistance in Culex pipiens pipiens L. after 8 generations of a 50% mortality pressure. Some of the resistant C. pipiens pipiens were found to have a 1.7 fold cross resistance to the organophosphate mosquito larvicide malathion. Georghiou (1974) found some carbamate resistant and organophosphate resistant strains of Musca domestica L. to be cross resistant to methoprene. Schaeffer and Wilder (1972) found some organophosphate resistant strains of Aedes nigromaculis (Ludlow) and C. tarsalis which were a little less sensitive to methoprene than susceptible strains

The objectives of the research herein documented were: (1) the development of data on practical insecticide application techniques, (2) the small scale field testing of Altosid SR-10 against the predominant nuisance mosquito

in Virginia Culex pipiens L., and (3) the development of personal skills in the laboratory handling of mosquitoes

METHODS AND MATERIALS

Six tests were conducted between the summer of 1974 and the fall of 1975. Each test is discussed separately. Analyses of variance of total adult emergence were conducted for replicated experiments (Table 8).

Preliminary Trials

Trial I. - Culex pipiens L. larvae treated with methoprene (Altosid® 10W). (August through September 1974): - Two clean, translucent white plastic 0.24 l bowls were placed approximately 0.6 m apart on the wooden floor of a metal shed in Virginia Beach, Virginia. Approximately 25 field collected C. pipiens larvae (1st to 4th instar) were added to both bowls containing 0.12 l of field collected (ditch) water. Treatment was made using two Tru-Test® Standard 1.5 gallon hand pump sprayers, equipped with #615 TT adjustable nozzles. Each sprayer was filled with 0.947 l of tap water, and 0.02959 l of methoprene (Altosid 10W) was added to one sprayer. One bowl was sprayed with the tap water and one with the methoprene mixture. Treatment was made by making two quick passes of the nozzle approximately 15 cm above the bowl simulating the field application of approximately 2.7 lb. a.i. of methoprene per acre. Additional field collected C. pipiens larvae (2nd to 4th instar) along with 0.0888 l of the field collected water were added to each bowl 23 days later. Pupae were trans-

ferred daily to clean, 0.12 l glass baby food jars by means of two large plastic pipettes. The baby food jars were covered with a small piece of fine mesh nylon and secured on the jar with a rubber band. The jars were stored under temperature conditions ranging from 20 to 35°C, and an average D-L cycle of 7-17. The emerged adult mosquitoes were removed, counted, and recorded daily.

Trial II. C. pipiens larvae in natural potholes treated with methoprene at 0.135 lb. a.i./A. (7 May through 12 May, 1975): - Three irregularly shaped potholes containing C. pipiens larvae were located in a field near an abandoned sewage facility in Virginia Beach, Virginia. All were approximately 8 to 15 liters in capacity and situated within 5 meters of each other. Two potholes were treated with methoprene (Altosid SR-10) at a rate of 0.135 lb. a.i./A, and one with tap water, on 7 May, 1975. Treatments were made using Tru-Test Standard 1.5 gallon hand pump sprayers on which the #615 TT nozzle was adjusted to medium fan spray. Pupae were collected from 8 May to 12 May. Adult collection, rearing and counting were conducted as described in Trial I.

Trial III. C. pipiens larvae in abandoned sewage facility treated with methoprene at 0.135 lb. a.i./A. (11 May through 20 May, 1975): - A concrete tank (approximately 3 x 1.5 x 1 m) located in Virginia Beach, Virginia was found to contain several centimeters of water and C.

pipiens (all stages). Approximately 20 larvae (3rd to 4th instar) were collected and placed in a 0.12 l glass baby food jar. The tank was then sprayed with methoprene (Altosid SR-10) at 0.135 lb. a.i./A in a manner similar to that described in Trial II. A day later, approximately 20 larvae were collected. Both treated and untreated larvae were fed approximately 1 g of pulverized Purina® Dog Chow. Pupae were held until death or adult emergence as described in Trial I.

Replicated Experiments

Experiment I. Culex pipiens L. larvae in small plastic containers treated with methoprene at 0.135 lb. a.i./A. (12 May through 6 June, 1975): - Eight, 0.946 l white translucent plastic freezer containers were placed, in pairs, around a private residence in Virginia Beach, Virginia. Four were placed in the grass and exposed to the sun. Two were placed on a window sill in a carport. Two were placed inside a utility room on a shelf. Approximately 0.473 l tap water, 30 field collected C. pipiens larvae (1st and 2nd instar), and approximately 1 g of pulverized Purina® Dog Chow were added to each. One of each pair of containers was treated (as described in Trial I) weekly with methoprene (Altosid® SR-10) at 0.135 lb. a.i./A, and the other was sprayed with tap water. Pupae were collected daily, reared and counted as in Trial I.

Experiment II. C. pipiens larvae in plastic buckets treated with methoprene, temephos, or diflubenzuron at various dosages. (8 June through 1 September, 1975): - This experiment consisted of 3 variants. In the first variant (8-24 June), 20 translucent yellow plastic buckets, with a capacity of 13.25 l, were arranged in 5 rows underneath a 3.5 m tall sweetgum (Liquidambar sp.) tree in the backyard of a residence in Virginia Beach, Virginia. Each bucket was filled with 7.57 l tap water and placed about 15 cm apart. Fifty, field collected C. pipiens larvae (2nd to 4th instar) were placed in each bucket. Using Tru-Test® Standard 1.5 gallon hand pump sprayers with a #615 TT nozzle on medium fan spray, two different rates of methoprene were applied in addition to controls of tap water. Methoprene (Altosid SR-10) was applied weekly at 0.135 lb. a.i./A and at 0.027 lb. a.i./A to each of 5 buckets. The tap water controls were applied in a similar manner. The remaining 5 buckets were each treated once with a 6.7 x 5 x 5 cm briquette of 1% temephos, 0, 0' - (thiodi - 1, 1 - phenylene) 0, 0, 0', 0' - tetramethyl bis (phosphorthioate) or Abate® 4E, in plaster-of-Paris. The briquettes had been previously manufactured by mixing 0.5326 l Abate 4E (a 43% EC) with 11.355 l of water and 15 kg of plaster-of-Paris. The four treatments were randomized and replicated as shown in Table 2. Pupae were collected daily with plastic pipettes or a syringe and placed in 0.12 l baby food jars.

In the second variant (29 June - 20 July), 100 C. pipiens larvae (1st and 2nd instar) were placed in each of 20 buckets, and the buckets were placed approximately 1 m apart. Ten of these buckets were treated weekly with methoprene (Altosid SR-10) at 0.27 lb. a.i./A. At the start of the run a 1% temephos briquette was placed in each of 5 buckets. The remaining 5 buckets were sprayed weekly with tap water.

In the third variant (17 August - 1 September), 5 buckets were treated weekly with methoprene (Altosid SR-10) at 0.27 lb. a.i./A. Five buckets were treated weekly with diflubenzuron, 1 - (4 chlorophenyl) - 3 - (2, 6 - difluorobenzoyl) urea (Dimilin® 25% WP) at 0.003 lb. a.i./A. Five buckets were treated with 1% temephos briquettes and five with tap water. The buckets were placed approximately 1 m apart.

Experiment III. Aedes aegypti (L.) larvae in laboratory treated with methoprene, temephos, or diflubenzuron at various dosages. (20 October through 3 December, 1975): - This experiment consisted of two variants conducted at VPI and SU in Blacksburg, Virginia. Materials and dosages are shown in Table 3. In the first variant, 13 standard watch glasses (10.5 x 4.5 cm) were lined with Saran Wrap™. Twelve were filled with 200 ml softened well water and one with 200 ml distilled water. To achieve a 0.027 lb. a.i./A rate of methoprene, 0.00025 ml methoprene was removed

weekly from a freshly diluted solution and placed into each of three glasses with a 1 ml pipette. To achieve a 0.03 lb a.i./A rate of diflubenzuron, 0.0001 g of the 25% WP was added weekly to each of 3 glasses by pipetting 0.1 ml of a freshly diluted solution into the glasses. For a standard treatment, a 1 g chip of 1% temephos briquette was added to each of 3 glasses one time. The remaining 4 glasses received 1 ml distilled water each once a week.

Ae. aegypti larvae were vacuum hatched from month old eggs (from the VPI colony) at 500 mm Hg for 2 hours. Fifty larvae (1st instar) in approximately 2 ml tap water were added to each watch glass (Peters et al. 1969). Ten milligrams of dry powder brewers yeast (Fisher Scientific Co.) were added to each glass. The glasses were then placed in an environmental chamber (112 x 75 x 152 cm) lighted by a 58.4 cm 20 watt, Cool White, Westinghouse fluorescent bulb situated in the upper front corner and maintaining a 12-12 D-L cycle by an Intermatic time switch model T101B (International Register Co.). Temperature was maintained at 26.6 ± 2°C. The treatments were randomized and arranged in 3 rows of 4 on the chamber floor so that the row nearest the chamber door was 94 cm from the light, the second row was 97 cm from the light, and the third row was 107 cm from the light. The glass with distilled water was placed in the middle, approximately 95 cm from the light. A battery jar of water was placed on a shelf

in the chamber to retard evaporation from the watch glasses. Larvae were fed brewers yeast daily according to the following schedule: Day 0 - 0.2 mg per larva; Day 1 - 0.2 mg per larva; Day 2 - 0.3 mg per larva; Day 3 - 0.4 mg per larva; Day 4 and after - 0.6 mg per larva (Gerberg 1970). A section of fine mesh nylon hose was secured over the top of each glass as soon as the first pupa was noted. Two or 3 drops of ethyl ether were placed on the nylon to anesthetize the emerged adults for removal and counting.

The second variant was similar to the first except the amounts of test chemicals (methoprene, diflubenzuron, and temephos briquette) were reduced to 10% of that tested in the first variant, only 3 controls were used, and only distilled water was utilized as the substrate.

RESULTS AND DISCUSSION

Preliminary Trials (see Table 4).

Trial I. Culex pipiens L. larvae treated with methoprene (Altosid® 10W): - Altosid 10W was an experimental 10% liquid formulation of methoprene impregnated charcoal and has since been discarded. It was tested because it was the first methoprene product available to this worker. Zoecon Corporation recommended 0.27 lb. a.i./A against Culex mosquito larvae. Through a computational error, the test was conducted with approximately 2.7 lb. a.i./A. At this rate, 100% emergence inhibition resulted for at least 23 days.

Trial II. C. pipiens larvae in natural potholes treated with methoprene at 0.135 lb. a.i./A: - The recommended dosage of methoprene against Culex larvae is 0.27 lb. a.i./A. However, in this test, a 0.135 lb. a.i./A rate was used. The results are suspect since for unknown reasons one of the treated potholes failed to produce any pupae. Consequently the same or other unknown factors could have affected the remaining two potholes. However, the 90% emergence inhibition (Table 8) is consistent with results expected from other experiments (Table 1) and from the manufacturer.

Trial III. C. pipiens larvae in abandoned sewage facility treated with methoprene at 0.135 lb. a.i./A: - In this test, methoprene was again applied at half the

recommended dosage for Culex larvae. The results were similar to the above trial.

Replicated Experiments

Experiment I (see Table 5). Culex pipiens L. larvae in small plastic containers treated with methoprene at 0.135 lb. a.i./A: - The 100% emergence inhibition occurring in this test from the application of half the recommended dosage of methoprene to Culex larvae was unexpected in view of the previous trials. One explanation might be that the methoprene was absorbed by the plastic containers and re-released into the water as the level of methoprene in solution fell due to biochemical breakdown and rainwater dilution (Dunn et al. 1974). The absence of pupae in containers 4 and 8 (Appendix 8) resulted from the accidental disturbance of these containers.

Experiment II (see Table 6). C. pipiens larvae in plastic buckets treated with methoprene, temephos, or diflubenzuron at various dosages: - The methoprene performed in a manner consistent with previous tests. Diflubenzuron was tested against C. pipiens larvae (1st and 2nd instar) at 10 times less than the recommended dosage (as stated on the experimental label secured from the Thompson-Hayward Chemical Company), and resulted in 96% emergence inhibition. Temephos in plaster-of-Paris briquettes (1% by weight) is an effective larvicide employed by several mosquito control commissions throughout Virginia. It is

general practice to use one temephos briquette per storm sewer catch basin, which would hold approximately the same amount of water as the buckets employed in these tests. Complete data from the 3 variants in this experiment are given in Appendices 9, 10, and 11.

Experiment III (see Table 7). Aedes aegypti (L.) larvae in laboratory treated with methoprene, temephos, or diflubenzuron at various dosages: - These tests were conducted using the standard test animal Ae. aegypti, although this mosquito is no longer endemic to Virginia (Gladney and Turner 1969). Methoprene at both 0.027 lb. a.i./A and 0.0027 lb. a.i./A rates resulted in 100% emergence inhibition of Ae. aegypti. These results are consistent with those presented by other workers (Table 1). Diflubenzuron also resulted in 100% emergence inhibition at both the recommended and 10 times less than the recommended dosages. Temephos in plaster-of-Paris was again used as a standard for comparison and resulted in 100% emergence inhibition. Appendices 7 and 12 present complete data from these tests.

General Discussion

Determining the effects of methoprene on targets in such a way that results of various assays are comparable is difficult because some elements of the experiment were uncontrolled. Mosquito species and strains can be differentially sensitive to methoprene (Georghiou et al. 1974,

Hsieh and Steelman 1974). The manufacturer states that methoprene is ineffective against all but the 2nd, 3rd and 4th instars, with the late 3rd and early 4th being the most sensitive (Noguchi and Ohtaki 1974). Georghiou and Lin (1974) recommended scoring only those larvae which pupate 10 to 30 hours after exposure to methoprene in order to standardize results. Mulla and Darwazeh (1975b) have shown that water temperature is important in determining the length of effective control with methoprene. The formulation is also of importance (Jakob 1972). Technical methoprene tends to remain at the surface (Schaefer and Dupras 1973) whereas the SR-10 formulation tends to settle out (Schaeffer et al. 1974b). Some mosquito species may also show a differential methoprene sensitivity between sexes (Jakob and Schoof 1971).

There are several ways to test a hormonomimetic insect growth regulator (Gilbert and Schneiderman 1960, Riddiford and Truman 1974, Trost 1972), but the most practical way to determine its field potential is to use the inhibition of adult emergence as the scoring criterion (Slama et al. 1974, Staal 1972). Such a criterion was employed in these tests. If an adult mosquito was completely free of its pupal case, it was scored as having emerged.

CONCLUSION

Although the tests herein documented were relatively small in scale, most of them were designed to simulate full scale field application of the test materials against the most abundant nuisance mosquito in Virginia, Culex pipiens L. Results of these tests as summarized in Table 8 indicate the methoprene formulation Altosid® SR-10 can effectively control these mosquitoes under field conditions. This material has no mammalian toxicity (Beadles et al. 1975, Pest Control 1975); it is exempt from residue tolerances in drinking water and many crops (Environmental Protection Agency 1975); and it also has no documented field resistance to mosquitoes, thus making it a logical candidate for use in Virginia. However, only the full scale use of this material by a mosquito control agency can accurately determine the cost-benefit ratio as compared to other available mosquito control methods.

Table 1. Percent emergence inhibition of various mosquito species exposed to methoprene (compiled from several literature sources).

Mosquito Species	Methoprene Rate or Concentration	% Emergence Inhibition**	References
Aedes aegypti (L.)	0.027 lb. a.i./A	100	*
	0.00014 ppm	100	Henrick et al. 1973
	0.04 ppm	100	Schaefer and Wilder 1973
	0.25 ppm	95	Jakob 1972
	0.7799 ppm	90	Hsieh and Steelman 1974
Ae. canadensis (Theobald)	0.054 lb. a.i./A	100	*
Ae. cantator (Coquilett)	0.054 lb. a.i./A	91-99	*
Ae. communis (De Geer)	0.054 lb. a.i./A	100	*
Ae. dorsalis (Meigen)	0.02 lb. a.i./A	100	*
	0.05 lb. a.i./A	100	Wagstaff and Minson 1975
Ae. excrucians (Walker)	0.027 lb. a.i./A	90	*
Ae. melanimon Dyar	0.0004 ppm	100	Schaefer and Wilder 1973
	0.0125 lb. a.i./A	90	Schaefer et al. 1974a
	0.02 lb. a.i./A	40-100	Schaefer et al. 1974a, *

Table 1 Continued. Percent emergence inhibition of various mosquito species exposed to various concentrations of methoprene.

Mosquito Species	Methoprene Rate or Concentration	% Emergence Inhibition**	References
Ae. melanimon Dyar	0.025 lb. a.i./A	95-100	Schaefer et al. 1974a, *
	0.029 lb. a.i./A	95-100	*
	0.05 lb. a.i./A	100	*
	0.1 lb. a.i./A	100	Schaefer and Wilder 1973, *
Ae. nigromaculis (Ludlow)	0.0125 lb. a.i./A	90-100	Schaefer et al. 1974a, Schaefer and Wilder 1973
	0.014 lb. a.i./A	80-97	*
	0.016 lb. a.i./A	90-95	*
	0.020 lb. a.i./A	40-100	*
	0.025 lb. a.i./A	50-100	Schaefer et al. 1974a
	0.027 lb. a.i./A	89-100	*
	0.029 lb. a.i./A	95-100	*
	0.033 lb. a.i./A	70-100	*
	0.05 lb. a.i./A	100	*
	0.1 lb. a.i./A	100	*

Table 1 Continued. Percent emergence inhibition of various mosquito species exposed to various concentrations of methoprene.

Mosquito Species	Methoprene Rate or Concentration	% Emergence Inhibition**	References
Ae. nigromaculis (Ludlow)	0.2 lb. a.i./A	100	Wagstaff and Minson 1975
	0.35 lb. a.i./A	100	Schaefer et al. 1973
Ae. sollicitans (Walker)	0.0013 ppm	90	Hsieh and Steelman 1974
	0.027 lb. a.i./A	75-100	*
	0.04 lb. a.i./A	91-100	*
Ae. squamiger (Coquillett)	0.027 lb. a.i./A	100	*
Ae. stimulans (Walker)	0.027 lb. a.i./A	90	*
Ae. taeniorhynchus (Wiedeman)	0.004 ppm	100	Schaefer and Wilder 1973
	0.1121 ppm	90	Hsieh and Steelman 1974
	0.020 lb. a.i./A	100	Mulrennan 1975
	0.027 lb. a.i./A	95	Giglioli 1975
	0.05 lb. a.i./A	99-100	*
	0.1 lb. a.i./A	100	*

Table 1 Continued. Percent emergence inhibition of various mosquito species exposed to various concentrations of methoprene.

Mosquito Species	Methoprene Rate or Concentration	% Emergence Inhibition**	References
Ae. triseriatus (Say)	0.6472 ppm	90	Hsieh and Steelman 1974
Ae. vexans (Meigen)	0.054 lb. a.i./A	91-99	*
Anopheles albimanus Wiedeman	0.0025 ppm	95	Jakob 1972
An. crucians Wiedeman	0.027 lb. a.i./A	100	*
An. quadrimaculatus Say	0.4964 ppm	90	Hsieh and Steelman 1974
	0.054 lb. a.i./A	100	*
An. stephensi Liston	0.05 ppm	95	Jakob 1972
Culex pipiens L.	0.01 ppm	18-100	Schaefer and Wilder 1972
	0.1 lb. a.i./A	100	Wagstaff and Minson 1975
	0.25 lb. a.i./A	65	Wagstaff and Minson 1975
C. pipiens pallens Coquillett	1 ppm	100	Noguchi et al. 1974
	0.0006 ppm-0.03 ppm	50	Noguchi and Ohtaki 1974
C. pipiens pipiens L.	1 ppm	100	Hoppe et al. 1974

Table 1 Continued. Percent emergence inhibition of various mosquito species exposed to various concentrations of methoprene.

Mosquito Species	Methoprene Rate or Concentration	% Emergence Inhibition**	References
C. pipiens pipiens L.	0.004 ppm	50	Brown and Brown 1974
C. pipiens quinquefasciatus Say	0.1936 ppm	90	Hsieh and Steelman 1974
	0.025 ppm	95	Jakob 1972
	0.05 ppm	100	Schaefer et al. 1973
	0.25 ppm	99-100	Schaefer et al. 1973, Jakob 1972
	0.1 ppm	99-100	Schaefer et al. 1973, Jakob 1972
	1 ppm	99-100	Schaefer et al. 1973, Jakob 1972
C. quinquefasciatus Say	50 ppb	50-90	Coombes and Meisch 1973
	0.1 lb. a.i./A	100	*
C. salinarius Coquillett	1.2388 ppm	90	Hsieh and Steelman 1974
	0.027 lb. a.i./A	100	*
C. tarsalis Coquillett	0.0325 ppm	90	Hsieh and Steelman 1974
	0.027 lb. a.i./A	100	Lewallen 1974

Table 1 Continued. Percent emergence inhibition of various mosquito species exposed to various concentrations of methoprene.

Mosquito Species	Methoprene Rate or Concentration	% Emergence Inhibition**	References
C. tarsalis Coquillett	0.1 lb. a.i./A	96-100	Mulla and Darwazeh 1975b, Sschaefer et al. 1974a.
	0.2 lb. a.i./A	70	Schaefer and Wilder 1973
C. tritaeniorhynchus Giles	0.0006 ppm-0.03 ppm	50	Noguchi and Ohtaki 1974
Culiseta inornata (Williston)	1.6357 ppm	90	Hsieh and Steelman 1974
	0.1 lb. a.i./A	100	Mulla and Darwazeh 1975b
Psorophora confinnis (Lynch Arribalzaga)	0.1007 ppm	90	Hsieh and Steelman 1974
	0.025 lb. a.i./A	100	Mulla and Darwazeh 1975a
	0.027 lb. a.i./A	100	*
	0.05 lb. a.i./A	90-100	*
	0.054 lb. a.i./A	100	*
	0.1 lb. a.i./A	100	*
P. ferox	0.0007 ppm	90	Hsieh and Steelman 1974
P. varipes (Coquillett)	0.002 ppm	90	Hsieh and Steelman 1974

Table 1 Continued. Percent emergence inhibition of various mosquito species exposed to various concentrations of methoprene.

---

* Information from Zoecon Corporation, Palo Alto, California.

** Percent EI = ((% emerged from controls - % emerged from test) / % emerged from controls) x 100 (Mulla and Darwazeh 1975b).

Table 2. Experimental design of Experiment II - Culex pipiens L. larvae in plastic buckets treated with methoprene, temephos, or diflubenzuron at various dosages.

Treatment	Material	Formulation	Dosage	No. Replicates
		First Variant		
1	methoprene	Altosid® SR-10	0.135 lb. a.i./A	5
2	methoprene	Altosid SR-10	0.027 lb. a.i./A	5
3	temephos	Abate® 4E	1% briquette	5
4	--	Tap Water	--	5
		Second Variant		
1	methoprene	Altosid SR-10	0.27 lb. a.i./A	10
2	temephos	Abate 4E	1% briquette	5
3	--	Tap water	--	5
		Third Variant		
1	methoprene	Altosid SR-10	0.27 lb. a.i./A	5
2	diflubenzuron	Dimilin® 25% WP	0.003 lb. a.i./A	5
3	temephos	Abate 4E	1% briquette	5
4	--	Tap Water	--	5

Table 3. Experimental design of Experiment III - *Aedes aegypti* (L.) larvae in laboratory treated with methoprene, temephos, or diflubenzuron at various dosages.

Treatment	Material	Formulation	Dosage	No. Replicates
		First Variant		
1	methoprene	Altosid® SR-10	0.027 lb. a.i./A	3
2	temephos	Abate® 4E	1 g of 1% briquette	3
3	--	Tap Water	--	3
4	diflubenzuron	Dimilin® 25% WP	0.03 lb. a.i./A	3
5	--	Distilled Water	--	1
		Second Variant		
1	methoprene	Altosid SR-10	0.0027 lb. a.i./A	3
2	temephos	Abate 4E	0.1 g of 1% briquette	3
3	--	Distilled Water	--	3
4	diflubenzuron	Dimilin 25% WP	0.003 lb. a.i./A	3

Table 4. Percent emergence of Culex pipiens L. adults in preliminary trials.

Treatment and Formulation**	# Pupae in test	# Males Emerged	# Females Emerged	% Emergence*
		Trial I		
methoprene (Altosid® 10W) @ 2.7 lb. a.i./A	29	0	0	0
Tap water	24	15	8	96
		Trial II		
methoprene (Altosid SR-10) @ 0.135 lb. a.i./A	40	2	2	10
methoprene (Altosid SR-10) @ 0.135 lb. a.i./A	0**	0	0	0
Tap water	12	6	6	100
		Trial III		
Before treatment	4	2	2	100
methoprene (Altosid SR-10) @ 0.135 lb. a.i./A	22	1	0	4.5

* Percent emergence is calculated from the number of pupae.

** All larvae and pupae in this pothole died for undetermined reasons.

Table 5. Percent emergence of Culex pipiens L. adults from 1st and 2nd instar larvae exposed to methoprene @ 0.135 lb. a.i./A in Experiment I.

Treatment and Formulation**	# Pupae in test	# Males Emerged	# Females Emerged	% Emergence*
methoprene (Altosid® SR-10)	61	0	0	0
Control	32	21	7	87.5

* Percent emergence is calculated from the number of pupae.

** Two of the containers, one treated and one untreated were accidentally disturbed resulting in the absence of pupae.

Table 6. Percent emergence of Culex pipiens L. adults from larvae exposed to three chemicals in Experiment II.*

Chemical and Formulation	lb. a.i./A	# Larvae in test	# Pupae developed	# Males Emerged	# Females Emerged	% Emerg. from pupae	% Emerg. from larvae
methoprene (Altosid® SR-10)	0.027	250	160	4	3	4.4	--
	0.135	250	161	1	7	5.0	--
	0.27	1500	104	0	0	0	--
diflubenzuron (Dimilin® 25% WP)	0.003	500	10	3	1	--	1.0***
temephos (Abate® 4E)	Ca. 5 cc**	1250	0	0	0	--	0***
Tap water	--	1250	342	129	122	73.4	21

* Chemicals were applied weekly until all immature mosquitoes were dead or developed into adults.

** Temephos briquettes never completely dissolved. The concentration of temephos in the water was not determined.

*** Diflubenzuron and temephos kill larvae before the pupal stage.

Table 7. Percent emergence of *Aedes aegypti* (L.) adults from 1st instar larvae exposed to three chemicals in Experiment III.*

Chemical and Formulation	lb. a.i./A	# Larvae in test	# Males Emerged	# Females Emerged	% Emerged from larvae
methoprene (Altosid® SR-10)	0.0027	150	0	0	0
	0.027	30	0	0	0
diflubenzuron (Dimilin® 25% WP)	0.003	150	0	0	0
	0.03	30	0	0	0
temephos (Abate® 4E)	1 g**	150	0	0	0
	0.1 g	30	0	0	0
Well water	--	150	22	10	21.3
Distilled water	--	80	36	12	60.0

* Chemicals were applied weekly until all immature mosquitoes were dead or developed into adults.

** Temephos chips were never completely dissolved. The concentration of temephos in water was not determined.

Table 8. Summary of all experiments showing percent emergence inhibition (EI) of Aedes aegypti (L.) and Culex pipiens L. when larvae exposed to various chemicals.*

Test	Chemical and Formulation	lb. a.i./A	C. pipiens EI	A. aegypti EI	Confidence level (%)	# insects in test
Trial I	methoprene (Altosid® 10W)	2.7	100	--	--***	53
Trial II	methoprene (Altosid SR-10)	0.135	90	--	--***	52
Trial III	methoprene (Altosid SR-10)	0.135	95.5	--	--***	26
Exp. I	methoprene (Altosid SR-10)	0.135	100	--	95-99	93
Exp. II	methoprene (Altosid SR-10)	0.027	94	--	99	250
	methoprene (Altosid SR-10)	0.135	93	--	99	250
	methoprene (Altosid SR-10)	0.27	100	--	95-99	1500
	diflubenzuron (Dimilin® 25% WP)	0.003	96	--	95-97.5	500
	temephos (Abate® 4E)	5 cc**	100	--	95-99	1250

Table 8 Continued. Summary of all experiments showing percent emergence inhibition (EI) of Aedes aegypti (L.) and Culex pipiens L. when larvae exposed to various chemicals.*

Test	Chemical and Formulation	lb. a.i./A	C. pipiens EI	A. aegypti EI	Confidence level (%)	# insects in test
Exp. III	methoprene (Altosid SR-10)	0.0027	--	100	< 95	150
	methoprene (Altosid SR-10)	0.027	--	100	> 99	30
	diflubenzuron (Dimilin 25% WP)	0.003	--	100	< 95	150
	diflubenzuron (Dimilin 25% WP)	0.03	--	100	> 99	30
	temephos (Abate 4E)	1 g**	--	100	99	150
	temephos (Abate 4E)	0.1 g**	--	100	95	30

* Percent emergence inhibition is ((% emerged in controls - % emerged in test) / % emerged in control) x 100 (Mulla et al. 1974b).

** 1% temephos briquette (Abate 4E in plaster-of-Paris) of indicated size utilized.

*** Analyses of variance were not calculated due to lack of replicates.

REFERENCES CITED

Beadles, M. L., J. A. Miller, W. F. Chamberlain, J. L. Eschle and R. L. Harris. 1975. The Horn Fly: Methoprene in drinking water of cattle for control. J. Econ. Entomol. 68: 781-90.

Brown, T. M. and A. W. A. Brown. 1974. Experimental induction of resistance to a juvenile hormone mimic. J. Econ. Entomol. 67: 799-801.

Center for Disease Control. 1976. Vector Topics No. 1 Control of St. Louis Encephalitis. 35 pp. U. S. DHEW, PHS, CDC, Bureau of Tropical Diseases, Vector Biology and Control Division, Atlanta, Georgia. 30333.

Coombes, L. E. and M. V. Meisch. 1973. Control of mosquito larvae with an insect growth regulator in various water types. Arkansas Farm Res. 22: 4.

Dunn, R. L., T. J. Case, and R. K. Washino. 1974. Mosquito control studies in Northern California with juvenile hormone analogues: a progress report. Proc. Calif. Mosq. Contr. Assoc. 42: 153-6.

Environmental Protection Agency. 1975. Mosquito pesticide registered for use. EPAlert, No. 20, 11 April. U. S. EPA, Philadelphia, Pennsylvania.

Georghiou, G. P. 1974. Cross-resistance to juvenile hormone analog in insecticide-resistant strains of Musca domestica L. Pesticide Science. 5: 759-67.

Georghiou, G. P. and Chi S. Lin. 1974. Time-sequence response of Culex tarsalis following exposure to insect growth regulators. Proc. Calif. Mosq. Contr. Assoc. 42: 165-6.

Georghiou, G. P., C. S. Lin, C. S. Apperson and M. E. Pasternak. 1974. Assessment of potentiality of Culex tarsalis for development of resistance to carbamate insecticides and insect growth regulators. Proc. Calif. Mosq. Contr. Assoc. 42: 117-8.

Gerberg, E. J. 1970. Manual for mosquito rearing and experimental techniques. Amer. Mosq. Contr. Assoc., Inc., Bull. #5. Pages 53-5.

Giglioli, M. E. C. 1975. Low dosage aerial applications of insect growth regulators against Aedes taeniorhynchus through dense mangrove canopy. Trans. R. Soc. Trop. Med. Hyg. 69: 17 (Abst.).

Gilbert, L. I. and H. A. Schneiderman. 1960. The development of a bioassay for the juvenile hormones of insects. Trans. Amer. Microsc. Soc. 79: 38-67.

Gladney, W. J. and E. C. Turner, Jr. 1969. The Insects of Virginia: No. 2. Mosquitoes of Virginia (Diptera:Culicidae). Research Div. Bull. 49, Virginia Polytechnic Inst. and State Univ., Blacksburg, Virginia. 24 pp.

Henrick, C. A., G. B. Staal, and J. B. Siddall. 1973. Alkyl 3, 7, 11 - trimethyl-2, 4 - dodecadienoates, a new class of potent insect growth regulators with juvenile hormone activity. Agric. Food Chem. 21: 354-9.

Hoppe, T., H. Isler, and W. Vogel. 1974. Biological activity of juvenile hormone analogues against larvae of Culex pipiens pipiens tested in small-scale field trials. Mosq News. 34: 293-6.

Hsieh, M. Y. G. and C. D. Steelman. 1974. Susceptibility of selected mosquito species to five chemicals which inhibit insect development. Mosq. News. 34: 278-82.

Jakob, W. L. 1972. Additional studies with juvenile hormone-type compounds against mosquito larvae. Mosq. News. 32: 592-5.

Jakob, W. L. and H. F. Schoof. 1971. Studies with juvenile hormone type compounds against mosquito larvae. Mosq. News. 31: 540-3.

James, M. T. and R. F. Harwood. 1969. Mosquitoes. Pages 167-222 in M. T. James and R. F. Harwood, eds. Herms's Medical Entomology, Sixth Edition, Collier-MacMillan Ltd., London.

Lewallen, L. L. 1974. Summary of Results with methoprene (Altosid^{R} SR-10) applied at three-to-four day intervals for control of Culex tarsalis. Zoecon Field Reports, Zoecon Corp., 975 California Ave., Palo Alto, California.

Menn, J. J. and F. M. Pallos. 1975. Development of morphogenetic agents in insect control. Environ. Lett. 8: 71-88.

Miller, W. W., J. S. Wilkins, and L. L. Dunham. 1975. Determination of Altosid insect growth regulator in waters, soils, plants, and animals by gas-liquid chromatography. Journal Assoc. Offic. Anal. Chem. 58: 10-8.

Miura, T. and R. M. Takahashi. 1973. Insect development inhibitors. 3. Effects on non-target aquatic organisms. J. Econ. Entomol. 66: 917-22.

Mulla, M. S. 1974. Laboratory and field evaluation of insect growth regulators against mosquitoes. Proc. Calif. Mosq. Contr. Assoc. 42: 175-6.

Mulla, M. S. and H. A. Darwazeh. 1975a. Evaluation of insect growth regulators against chironomids in experimental ponds. Proc. Calif. Mosq. Contr. Assoc. 43: 26-9.

Mulla, M. S. and H. A. Darwazeh. 1975b. Activity and longevity of insect growth regulators against mosquitoes. J. Econ. Entomol. 68: 791-4.

Mulla, M. S., R. L. Norland, T. Ikeshoji, and W. L. Kramer. 1974a. Insect growth regulators for the control of aquatic midges. J. Econ. Entomol. 67: 165-70.

Mulla, M. S., H. A. Darwazeh and R. L. Norland. 1974b. Insect growth regulators: Evaluation procedures and activity against mosquitoes. J. Econ. Entomol. 67: 329-32.

Mulrennan, J. A. 1975. Application of Altosid SR-10 as a mosquito larvicide by use of hand sprayers. Florida Dept. of Health and Rehabilitative Services, Division of Health, Jacksonville, Florida. Memorandum No. 249, 17 July.

National Academy of Sciences. 1973. Mosquito Control. Some perspectives for developing countries. Washington, D.C. 63 pp.

Noguchi, Y. and T. Ohtaki. 1974. Difference in the susceptibility of Culex larvae against methoprene and its slow-release formulation A by various stages of the development. Jap. Journal Sanit. Zool. 25: 185-9. In Jap., Engl. Sum.

Noguchi, Y., T. Ohtaki and K. Mori. 1974. Lethal effects of synthetic juvenile hormone analogues against larvae of Culex pipiens pallens. Jap. Journal Sanit. Zool. 24: 257-60. In Jap., Engl. Sum.

Norland, R. L. 1974. Impact of an insect growth regulator on aquatic ecosystem - development of techniques for evaluation. Dissert. Abstr. Intern. B 34 1: p. 6040 B.

Pest Control. 1975. Growth regulator cuts hazards. Pest Control. 43: 20, 22, 37.

Peters, T. M., B. I. Chevone, M. C. Greenough, R. A. Callahan and P. Barbosa. 1969. Intraspecific competition in Aedes aegypti (L.) larvae: Equipments, Techniques and Methodology. Mosq. News. 29: 667-74.

Quistad, G. B., L. E. Staiger and D. Schooley. 1974. Environmental degradation of the insect growth regulator methoprene (isopropyl (2E, 4E)-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate). I. Metabolism by alfalfa and rice. J. Agric. Food Chem. 22: 582.

Quistad, G. B., L. E. Staiger and D. A. Schooley. 1975a. Environmental degradation of the insect growth regulator methoprene (isopropyl (2E, 4E)-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate). III. Photodecomposition. J. Agric. Food Chem. 23: 299-303.

Quistad, G. B., L. E. Staiger and D. A. Schooley. 1975b. Environmental degradation of the insect growth regulator methoprene. V. Metabolism by houseflies and mosquitoes. Pestic. Biochem. Physiol. 5: 233-41.

Quistad, G. B., L. E. Staiger, B. J. Bergot and D. A. Schooley. 1975c. Environmental degradation of the insect growth regulator methoprene. VII. Bovine metabolism to cholesterol and related natural products. J. Agric. Food Chem. 23: 743-9.

Riddiford, L. M. and J. W. Truman. 1974. Hormones and insect behavior. Pages 286-96 in L. B. Brown, ed. Experimental Analysis of Insect Behavior. Springer, New York.

Schaefer, C. H. and E. F. Dupras, Jr. 1973. Insect developmental inhibitors. 4. Persistence of ZR-515 in water. J. Econ. Entomol. 66: 923-5.

Schaefer, C. H., E. F. Dupras and W. H. Wilder. 1973. Pond tests with ZR-515: Biological and chemical residues. U. of Calif. Mosq. Contr. Research Lab., 5545 East Shields Ave., Fresno, California.

Schaefer, C. H., T. Miura, F. S. Mulligan III and E. F. Dupras, Jr. 1974a. Insect development inhibitors: Formulation research on Altosid®. Proc. Calif. Mosq. Contr. Assoc. 42: 140-5.

Schaefer, C. H. and W. H. Wilder. 1973. Insect developmental inhibitors. 2. Effects on target mosquito species. J. Econ. Entomol. 66: 913-6.

Schaefer, C. H., W. H. Wilder, F. S. Mulligan III and E. F. Dupras, Jr. 1974b. Insect development inhibitors: Effects of Altosid®, TH 6040 and H24108 against mosquitoes (Diptera:Culicidae). Proc. Calif. Mosq. Contr. Assoc. 42: 137-9.

Schneiderman, H. A. 1972. Insect Hormones and insect control. Pages 3-28 in J. J. Menn and M. Beroza, eds. Insect Juvenile Hormones. Academic Press, New York.

Schooley, D. A., B. J. Bergot, L. L. Dunham and J. B. Siddall. 1975a. Environmental degradation of the insect growth regulator methoprene (isopropyl (2E, 4E)-11-methoxy-3, 7, 11-trimethyl-2, 4,-dodecadienoate). II. Metabolism by aquatic organisms. J. Agric. Food Chem. 23: 293-8.

Schooley, D. A., K. M. Cresell, L. E. Staiger and G. B. Quistad. 1975b. Environmental degradation of the insect growth regulator isopropyl(2E, 4E)-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate (methoprene). IV. Soil metabolism. J. Agric. Food Chem. 23: 369-73.

Slama, K., M. Romanuk and R. Sorm. 1974. Insect Hormones and Bioanalogues. Wien: Springer, Austria. 477 pp. Library of Congress Card Catalogue No. 72-94418.

Staal, G. B. 1972. Biological Activity and Bioassay of Juvenile Hormone Analogs. Pages 69-94 in J. J. Menn and M. Beroza, eds. Insect Juvenile Hormones. Academic Press, New York.

Staal, G. B. 1975. Insect growth regulators with juvenile hormone activity. Ann. Rev. Entomol. 20: 417-60.

Steelman, C. D., J. E. Farlow, T. P. Breaud and P. E. Schilling. 1975. Effects of growth regulators on Psorophora columbiae (Dyar and Knab) and non-target aquatic insect species in rice fields. Mosq. News. 35: 67-76.

Takahashi, R. M. and T. Miura. 1975. Insect developmental inhibitors: Multiple applications of Dimilin® and Altosid® to Gambusia affinis (Baird and Girard). Proc. Calif. Mosq. Contr. Assoc. 43: 85-7.

Trost, Barry M. 1972. The Origin of Juvenile Hormone Chemistry. In J. J. Menn and M. Beroza, eds. Insect Juvenile Hormones. Academic Press, New York.

Wagstaff, K. H. and K. L. Minson. 1975. Field observations on a promising growth regulator for mosquito larvae. Proc. Utah Mosq. Abatement Dist. 27: 33-5.

Williams, C. M. and F. C. Kafatos. 1972. Theoretical aspects of the action of juvenile hormones. Pages 24-42 in J. J. Menn and M. Beroza, eds. Insect Juvenile Hormones. Academic Press, New York.

Wright, J. E. 1974. Insect growth regulator: Juvenile hormone analogs for control of the stable fly in marine plants in Florida. Mosq. News. 34: 160-2.

Zoecon Corporation. 1975. Technical Bulletin - Altosid™ (Formerly ZR-515). Zoecon Corporation, 975 California Ave., Palo Alto, California, 94304.

APPENDIX

Items included:

1. Concentration of methoprene in buckets in Experiment II @ 0.027 lb. a.i./A.
2. Concentration of diflubenzuron in buckets in Experiment II @ 0.003 lb. a.i./A.
3. Determination of Altosid® SR-10 amount needed per watch glass in Experiment III @ 0.027 lb. a.i./A rate.
4. Concentration of methoprene in watch glasses in Experiment III @ 0.027 lb. a.i./A rate.
5. Determination of Dimilin® 25% WP needed per watch glass in Experiment III @ 0.03 lb. a.i./A rate.
6. Concentration of diflubenzuron in watch glasses in Experiment III @ 0.03 lb. a.i./A rate.
7. Percent emergence of Aedes aegypti (L.) adults from larvae exposed to three chemicals in Experiment III, first variant.
8. Percent emergence of Culex pipiens L. adults from 1st and 2nd instar larvae exposed to methoprene @ 0.135 lb. a.i./A in Experiment I.
9. Percent emergence of Culex pipiens L. adults from larvae exposed to two chemicals in Experiment II, first variant.
10. Percent emergence of Culex pipiens L. adults from larvae exposed to two chemicals in Experiment II, second variant.

Appendix 1. Concentration of methoprene in buckets in Experiment II @ 0.027 lb. a.i./A.

1. 0.027 lb./A = 0.0003025 mg/cm^2.

2. Surface area of bucket (at 2.5 gallon level) = approximately 3.14 x $(12.5\ cm)^2$ = 490.625 cm^2.

3. Methoprene/bucket = approximately 0.0003025 mg/cm^2 x 490.625 cm^2 = 0.14841 mg.

4. Weight of water/bucket = approximately 9462500 mg.

5. Concentration of methoprene/bucket = approximately 0.148416/9462500 = approximately 1.5684438 x 10^{-8} = approximately 15.68 ppb*.

* This is the calculated initial concentration. Evaporation, rain, and biodegradation would change this concentration.

Appendix 2. Concentration of diflubenzuron in buckets in Experiment II @ 0.003 lb. a.i./A.

1. 0.003 lb./A = 0.0000336 mg/cm^2.

2. Surface area of bucket = approximately 3.14 x $(12.5\ cm)^2$ = 490.625 cm^2.

3. Diflubenzuron/bucket = approximately 0.0000336 mg/cm^2 x 490.625 cm^2 = 0.01648 mg.

4. Weight of water/bucket = 9462500 mg.

5. Concentration of diflubenzuron/bucket = approximately 0.01648/9462500 = approximately 1.74 ppb.*

* This is the calculated initial concentration. Evaporation, rain, and biodegradation would change this concentration.

Appendix 3. Determination of Altosid® SR-10 amount needed per watch glass in Experiment III @ 0.027 lb. a.i./A rate.

1. 0.027 lb. a.i./A = 4 oz. Altosid SR-10/A.

2. 4 oz./A = 0.0000029 ml/cm^2.

3. Diameter of watch glass = 10.5 cm.

4. Surface area of watch glass = 3.14 x (5.25 cm)^2 = 86.55 cm^2.

5. Altosid SR-10/glass = 86.55 cm^2 x 0.0000029 ml/cm^2 = 0.00025 ml.*

* This amount was obtained by mixing 1 ml of Altosid SR-10 with 1 liter of distilled water and then pipetting 0.25 ml of this solution into each glass.

Appendix 4. Concentration of methoprene in watch glasses in Experiment III @ 0.027 lb. a.i./A rate*.

1. Amount of methoprene/glass = 0.000025 ml.

2. Weight of methoprene/ml = 0.27 lb./4 oz. = 1035.3109 mg/ml.

3. Weight of methoprene/glass = 0.000025 x 1035.3109 mg = 0.02588 mg.

4. Weight of water/glass = 200,000 mg.

5. Concentration of methoprene/glass = 0.02588/200,000 = 129 ppb.

* This is the calculated initial concentration. Evaporation, absorption and biodegradation would change this concentration.

Appendix 5. Determination of Dimilin® 25% WP needed per watch glass in Experiment III @ 0.03 lb. a.i./A rate.

1. 0.03 lb. a.i./A = 0.0000003 g a.i./cm^2.
2. Diameter of watch glass = 10.5 cm.
3. Surface area of watch glass = 3.14 x $(5.25\ cm)^2$ = 86.55 cm^2.
4. 0.0000003 g a.i. = 0.0000012 g 25%.
5. Dimilin 25% WP/glass = 86.55 cm^2 x 0.0000012 g/cm^2 = 0.0001 g.*

* This amount was obtained by mixing 1 g of Dimilin 25% WP with 1 liter of distilled water and then pipetting 0.1 ml of this solution into each glass.

Appendix 6. Concentration of diflubenzuron in watch glasses in Experiment III @ 0.03 lb. a.i./A rate.*

1. Amount of diflubenzuron/glass = 0.000025 g.

2. Weight of diflubenzuron/glass = 0.000025 g = 0.025 mg.

3. Weight of water/glass = 200,000 mg.

4. Concentration of diflubenzuron/glass = 0.025/200,000 = 125 ppb.

* This is the calculated initial concentration. Evaporation, absorption and biodegradation would change this concentration.

Appendix 7. Percent emergence of Aedes aegypti (L.) adults from larvae exposed to three chemicals in Experiment III, first variant.

Number	# Larvae in test	# Pupae developed	# Males emerged	# Females emerged	% Pupation	% emerged from pupae	% emerged from larvae
1*	50	--	0	0	--	--	0
2*	50	--	0	0	--	--	0
3*	50	--	0	0	--	--	0
4**	50	0	0	0	0	0	0
5**	50	0	0	0	0	0	0
6**	50	0	0	0	0	0	0
7***	50	23	7	2	46	39	18
8***	50	20	9	4	40	65	26
9***	50	29	6	4	58	34	20
10****	50	0	0	0	0	0	0
11****	50	0	0	0	0	0	0
12****	50	0	0	0	0	0	0
13*****	50	37	26	7	74	89	66

* Methoprene @ 0.027 lb. a.i./A.
** Temephos in plaster-of-Paris. One 1 g chip in each glass.
*** Distilled water. Glasses filled with tap water.
**** Diflubenzuron @ 0.03 lb. a.i./A.
***** Distilled water. Glass filled with distilled water also.

Appendix 8. Percent emergence of Culex pipiens L. adults from 1st and 2nd instar larvae exposed to methoprene @ 0.135 lb. a.i./A in Experiment I.

Container	# Pupae in test	# Males Emerged	# Females Emerged	% Pupal emergence
1*	4	0	0	0
2*	56	0	0	0
3*	1	0	0	0
4*	0	0	0	0
5**	11	7	4	100
6**	14	10	2	85.7
7**	8	4	1	62.5
8**	0	0	0	0

* Methoprene @ 0.135 lb. a.i./A.

** Tap water.

Appendix 9. Percent emergence of Culex pipiens L. adults from larvae exposed to two chemicals in Experiment II, first variant.

Number	# Larvae in test	# Pupae developed	# Males emerged	# Females emerged	% Pupation	% emerged from pupae	% emerged from larvae
1*	50	33	1	0	66	3	2
2*	50	35	0	2	70	6	4
3*	50	28	0	2	56	7	4
4*	50	29	0	1	58	3	2
5*	50	36	0	2	72	6	4
6**	50	35	13	15	70	80	56
7**	50	37	13	21	74	92	68
8**	50	42	19	17	84	86	72
9**	50	31	12	9	62	68	42
10**	50	28	12	9	56	75	42
11***	50	44	2	1	88	7	6
12***	50	31	0	0	62	0	0
13***	50	31	0	0	62	0	0
14***	50	27	1	1	54	4	2

Appendix 9 Continued. Percent emergence of Culex pipiens L. adults from larvae exposed to two chemicals in Experiment II, first variant.

Number	# Larvae in test	# Pupae developed	# Males emerged	# Females emerged	% Pupation	% emerged from pupae	% emerged from larvae
15***	50	27	1	2	54	11	6
16-20****	250	0	0	0	0	0	0

* Methoprene @ 0.135 lb. a.i./A.

** Tap water.

*** Methoprene @ 0.027 lb. a.i./A.

**** Temephos. Approximately 5 cc (2 cu. in.) temephos in plaster-of-Paris briquette in each bucket.

Appendix 10. Percent emergence of Culex pipiens L. adults from larvae exposed to two chemicals in Experiment II, second variant.

Number	# Larvae in test	# Pupae developed	# Males emerged	# Females emerged	% Pupation	% emerged from pupae	% emerged from larvae
1*	100	3	0	0	3	0	0
2*	100	0	0	0	0	0	0
3*	100	9	0	0	9	0	0
4*	100	18	0	0	18	0	0
5*	100	6	0	0	6	0	0
6*	100	17	0	0	17	0	0
7*	100	16	0	0	16	0	0
8*	100	27	0	0	27	0	0
9*	100	1	0	0	1	0	0
10*	100	0	0	0	0	0	0
11**	100	19	8	4	19	63	12
12**	100	32	13	10	32	72	23
13**	100	49	17	23	49	82	40
14**	100	9	2	3	9	56	5

Appendix 10 Continued. Percent emergence of Culex pipiens L. adults from larvae exposed to two chemicals in Experiment II, second variant.

Number	# Larvae in test	# Pupae developed	# Males emerged	# Females emerged	% Pupation	% emerged from pupae	% emerged from larvae
15**	100	1	1	0	1	100	1
16-20***	500	0	0	0	0	0	0

* Methoprene @ 0.27 lb. a.i./A.

** Tap water.

*** Temephos. Approximately 5 cc (2 cu. in.) temephos in plaster-of-Paris in each bucket.

Appendix 11. Percent emergence of adult Culex pipiens L. from larvae exposed to three chemicals in Experiment II, third variant.

Number	# Larvae in test	# Pupae developed	# Males emerged	# Females emerged	% Pupation	% emerged from pupae	% emerged from larvae
1*	100	8	3	1	8	50	4
2*	100	0	0	0	0	0	0
3*	100	1	0	0	1	0	0
4*	100	1	0	0	1	0	0
5*	100	0	0	0	0	0	0
6**	100	32	0	0	32	0	0
7**	100	6	0	0	6	0	0
8**	100	9	0	0	9	0	0
9**	100	7	0	0	7	0	0
10**	100	14	0	0	14	0	0
11***	100	4	1	0	4	25	1
12***	100	30	12	4	30	53	16
13***	100	4	1	3	4	100	4
14***	100	0	0	0	0	0	0

Appendix 11 Continued. Percent emergence of adult Culex pipiens L. from larvae exposed to three chemicals in Experiment II, third variant.

Number	# Larvae in test	# Pupae developed	# Males emerged	# Females emerged	% Pupation	% emerged from pupae	% emerged from larvae
15***	100	21	5	4	21	43	9
16-20****	500	0	0	0	0	0	0

* Diflubenzuron @ 0.003 lb. a.i./A.

** Methoprene @ 0.27 lb.a.i./A.

*** Tap water.

**** Temephos. Approximately 5 cc (2 cu. in.) temephos in plaster-of-Paris briquette in each bucket.

Appendix 12. Percent emergence of Aedes aegypti (L.) adults from larvae exposed to three chemicals in Experiment III, second variant.

Number	# Larvae in test	# Pupae developed	# Males emerged	# Females emerged	% Pupation	% emerged from pupae	% emerged from larvae
1*	10	--	0	0	--	--	0
2*	10	--	0	0	--	--	0
3*	10	--	0	0	--	--	0
4**	10	0	0	0	0	0	0
5**	10	0	0	0	0	0	0
6**	10	0	0	0	0	0	0
7***	10	--	2	1	--	--	30
8***	10	--	6	4	--	--	100
9***	10	--	2	0	--	--	20
10****	10	--	0	0	--	--	0
11****	10	--	0	0	--	--	0
12****	10	--	0	0	--	--	0

* Methoprene @ 0.0027 lb. a.i./A.

** Temephos in plaster-of-Paris. One 0.1 g chip in each glass.

*** Distilled water. Glasses filled with distilled water.

**** Diflubenzuron @ 0.003 lb. a.i./A.

VITA

Name: Stuart Graham McCausland

Present Residence: 3508 Silina Drive, Virginia Beach, Virginia

Born: February 23, 1948 in Corpus Christi, Texas

Marital Status: Married 1969. Two children.

Education:

1966 - Graduated from Princess Anne High School in Virginia Beach, Virginia.

1970 - Graduated from Old Dominion University in Norfolk, Virginia with B. S. in biology.

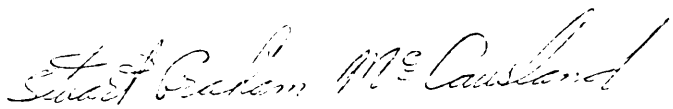
1977 - Graduation from Virginia Polytechnic and State University expected with M. S. in entomology.

Employment:

1970-1972 - Second lieutenant in United States Air Force.

1972-1973 - Biologist for Kempsville-Bayside Mosquito Control Commission in Virginia Beach, Virginia.

1973-Present - Entomologist for Bureau of Solid Waste and Vector Control, Virginia Health Department, in Norfolk, Virginia.



INHIBITION OF *CULEX PIPIENS* L. (DIPTERA:CULICIDAE) EMERGENCE WITH METHOPRENE

by

Stuart Graham McCausland

(ABSTRACT)

Methoprene (isopropyl (2E, 4E)-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate) formulated as Altosid® SR-10 was tested against *Culex pipiens* L. under simulated field conditions and against *Aedes aegypti* (L.) in the laboratory. Rates from 0.0027 to 0.27 lb. a.i./A resulted in 90 to 100% emergence inhibition. In the same tests, diflubenzuron (1-(4 chlorophenyl)-3-(2, 6-difluorobenzoyl) urea), formulated as Dimilin® 25% WP, induced 96 to 100% emergence inhibition at rates from 0.003 to 0.03 lb. a.i./A. Also in the same tests, temephos (0,0'-(thiodi-1, 1-phenylene) 0, 0, 0', 0'-tetramethyl bis (phosphothioate)), formulated as 1% Abate® 4E in plaster-of-Paris briquettes, was applied at normal field dosages and resulted in 100% emergence inhibition.