EFFECTS OF MIREX ON POPULATIONS OF *AEDES TRISERIATUS* SAY [1]

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ABSTRACT. The effects of mirex on all life stages of *Aedes triseriatus* Say were tested in the laboratory. First instar mosquito larvae were subjected to the mirex in water and the adults were subjected to mirex in a 10% sucrose solution.

Mirex caused mortality of the mosquito larvae in excess of 80% for concentrations of 0.1 ppm and above, but only 7.2% mortality of an adult population resulted from exposure to the same concentration. Percent mortality of progeny from mirex exposed parents resulted in a decrease in mortality when compared to percent mortality of progeny from untreated parents. Data indicated that adult weight was affected slightly if at all by mirex. No physical deformities were noticed in any life stages.

INTRODUCTION. Environmental pollution has remained one of the most pertinent and controversial issues of today. The chlorinated hydrocarbon pesticides are the most frequent offenders, due to long residual properties and accumulation of plants and animals in the food chain of man (Shapley, 1971).

Currently the imported fire ants, *Solenopsis richteri* Forel and *Solenopsis invicta* Buren, are under scrutiny in the South with an active control program in Mississippi utilizing mirex bait. Mirex is not easily broken down by water or sunlight and may remain many years in the environment. Van Valin, *et al.* (1968) reported that at recommended rates, mirex residues in soil, water and vegetation were relatively constant for over 300 days. The awareness that the terminal elements in the food chain are subject to biological magnification of pesticide residues has focused attention on the importance of the invertebrate fauna in metabolism and transfer of pesticides through food chains. However, very little work has been performed on the effects of mirex on invertebrate populations (Johnson, 1968). The importance of the invertebrate fauna in metabolism and in transfer of pesticides through food chains has been reported by Holden (1965); Kallman, *et al.* (1962); Keith (1964); Sparr, *et al.* (1965) and others. Aquatic invertebrate populations have been studied relative to DDT residues (Bridges, *et al.*, 1963; Grzenda, *et al.*, 1964; Hunt and Bischoff, 1960).

Many OCI insecticides (DDT, dieldrin, heptachlor and others) have been shown to cause deleterious effects to nontarget organisms (Graham and Scott, 1958; Harrington and Bidlingmayer, 1958; Cottam and Higgins, 1946; and others). Arant, *et al.* (1958) and Ferguson (1963) reported that efforts to eradicate the fire ant with heptachlor showed disastrous effects upon frogs, fish, lizards, turtles and snakes. DDT, endrin, toxaphene and heptachlor used as water treatments or as widespread aerial sprays have resulted in residues in mud of ponds and lakes (Cope, 1965). Johnson (1963), Graham and Scott (1958), Harrington and Bidlingmayer (1958), Cope (1961), and Hoffman and Olive (1961) reported instances of reduction of bottom dwelling invertebrates after applications of DDT and dieldrin. Cope (1965) stated that the number of invertebrates returned to normal usually within 3–6 months.

Fox (1967) reported that when aldrin, dieldrin or heptachlor were applied to test

[1] Publication No. 2670. Mississippi Agricultural and Forestry Experiment Station. This research was partially supported by Service Contract No. 12-14-140-2262-81, USDA, ARS.

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plots at 6 lbs/acre significant reductions in springtails, suborder Arthropleona, were noted the first year of application. However, springtails, Suborder Symphypleona, and mites were not significantly affected. Chlordane or lindane applications did not significantly affect the number of springtails and mites.

Field populations of striped earwigs, *Labidura ripana* (Pallas) treated with mirex or heptachlor were found to be resistant to these insecticides (Gross and Spink, 1969). It was shown that neither mirex nor heptachlor killed the earwigs when the chemicals were applied at rates recommended for control of imported fire ants.

Claims have been made that mirex causes cancer in mice, kills shrimp and crabs, damages reproduction of fish, fowl and mice (Vaughn, 1971). However, in all cases reported the test animals were subjected to dosages much higher than the recommended rate for fire ant control.

The purpose of this investigation was to determine the effect of mirex at low concentration levels on laboratory-reared invertebrate populations. The selected species of insect reared and treated was the treehole mosquito, *Aedes triseriatus* (Say).

MATERIALS AND METHODS. The *Aedes triseriatus* (Say) strain was obtained from Insect Control and Research, Inc., Baltimore, Maryland. A laboratory culture was maintained throughout the study.

Rearing Procedure. The adult mosquito rearing area was conditioned and maintained at 80° F and 80% RH; a photoperiod was set at 14 hours of light and 10 hours of dark. The adult mosquitoes were held in two 60 x 60 x 60 cm screen cages. Larval and adult rearing was modified from Gerberg (1970). The rearing procedure was as follows:

(1) Egg papers were flooded with tap water (27° C) and stimulated to hatch by deoxygenating the water; a pinch of a mixture of ground lab chow and dry activated yeast or nitrogen was added to the water.

(2) First instar larvae were seeded in metal trays at a density of 400 larvae/500 ml of water at a depth of approximately 1.5 cm.

(3) Larvae were fed at a rate of 100 mg of lab chow/400 larvae for 5 days; at this time the rate was increased to 150 mg/400 larvae until pupation had occurred.

(4) Pupae were removed daily and placed in emergence bowls in the adult rearing area.

(5) Upon emergence the adults were offered a 10% sucrose solution in cotton balls placed on the top of each cage. A bloodmeal (laboratory rabbit) was offered to the females 48 hours after emergence. Bloodmeals were offered at weekly intervals until the egg supply was at a surplus. Bloodmeals were then offered only when new eggs were needed.

(6) Forty-eight hours after the bloodmeal, pint plastic cups lined with paper toweling and half filled with colored water (red and green food coloring) were placed in the adult cage for oviposition sites.

(7) The cups were left in the cage for 5 days; the egg papers were then covered and aged for 8 days. After aging, the eggs were conditioned for 24 and 36 hours at 80° F and 80% RH.

(8) The egg papers were then placed in moistened paper toweling and put in pint plastic freezer bags and sealed. The eggs were stored at room temperature until needed.

Population Studies. Mirex (95% technical grade) was dissolved in acetone and concentrations calculated on a weight:volume basis. The stock solution and subsequent serial dilutions were made on a volume:volume basis to give the necessary concentrations, in parts per million (ppm). A total of 120 larvae/150 ml of tap water were placed in pint plastic cups for each test group. Ground lab chow was added daily at a rate of 25 mg/cup. Mirex solutions were added to each cup with a 0.5 cc tuberculin syringe; 0.15 cc of each mirex solution was added to each container to obtain the desired concentration. Five individual tests were run with each test hav-

ing 4 replicates per concentration. Dead larvae and pupae were removed daily. Equal numbers of larvae were placed in cups as controls. Mortality was recorded daily until all individuals of a cup were extracted because of death or pupation. Observed mortalities in treatment were corrected for control mortality using Abbott's formula (Abbott, 1925) and the 2, 4, 8, 16 and 24-day LC_{50} values and 95% confidence intervals (95% C.I.) were computed by log probit analysis by Mississippi State University computer center.

Surviving pupae of the previous test were used to determine the percent emergence. Five hundred pupae were subjected to concentrations of 0.01 ppm, 0.005 ppm, 0.0025 ppm and 0.001 ppm. One hundred seventy-nine and 402 pupae were used per 1.0 and 0.1 ppm, respectively. Mortality counts were made daily until all had emerged or the pupae died. The LC_{50} was calculated on 72-hour mortality data. Surviving adults were then held for 25 days to observe for any behavioral abnormalities. Dead adults taken from cages were observed under a stereomicroscope for physical deformities.

The effect of mirex on the adult mosquitoes was also tested. Four mirex concentrations were prepared and added to 10% sucrose to give the desired final concentrations. The 4 concentrations used in testing were 0.5 ppm, 0.1 ppm, 0.01 ppm and 0.001 ppm. Six cotton balls, each containing 2.5 cc of the appropriate solution were placed on top of each cage; the cotton balls were changed daily. Each test group was replicated 3 times with 100 adults/cage for each replicate; equal numbers of untreated adults were held as controls. Mortality was recorded daily until the test was terminated at 25 days. LC_{50} values were computed for the 4, 8, 12, 16 and 24-day intervals. Bloodmeals were offered to the females on a weekly basis and reproductive effects observed.

Progeny from the previous test were used to determine the effect on the offspring of adults which had been subjected to insecticide treatment. Mortality counts were taken daily until all had died or had pupated. LC_{50} values were computed at 2, 4, 8, 16 and 24-day intervals. Adult weights were taken to determine any deleterious effect on weight development. Mirex solutions of 1.0 ppm, 0.1 ppm, 0.01 ppm, 0.005 ppm, 0.0025 ppm, and 0.001 ppm were used in treating 120 larvae/cup. Within 12 hours after emergence the weights of males and females were taken separately. Ten mosquitoes were used per weighing and average weight recorded. Five replications were made for each concentration.

RESULTS AND DISCUSSION. The results obtained from mortality counts made daily on larvae and pupae exposed to mirex from the 1st instar through pupation showed that mirex exposure consistently exhibited $>80\%$ mortality at 1.0 and 0.1 ppm. A $>80\%$ mortality occurred at 1.0 ppm at the 4-day post-treatment period; however, $>80\%$ mortality for 0.1 ppm did not occur until day 16. Furthermore, mortality did not exceed 10% in the 0.01, 0.005, 0.0025 and 0.001 ppm levels up to 24 days post-treatment. Adults which emerged from the pupae surviving exposure to mirex at 0.001 to 0.01 ppm exhibited no noticeable physical deformities. This differs from the findings of physical deformities in vertebrates (fish and mice) by Van Valin, *et al.* (1968) and Gaines and Kimbrough (1970). Their studies which indicated physical abnormalities in vertebrates involved much higher dosages and longer exposures than recommended for fire ant control (Vaughn, 1971).

LC_{50} values were computed at the 2, 4, 8, 16 and 24-day intervals (Table 1); each time interval mortality count yielded a significant regression. Results indicated that a 3.4-fold difference in LC_{50} levels occurred between days 2 and 4. Furthermore, fold differences between days 4 and 8 (1.95-fold), 8 and 16 (1.26-fold), and 16 and 24 (1.16-fold) decreased as the time interval increased.

The percent emergence of surviving pupae at 1.0 and 0.1 ppm from the previous test differed significantly when compared

TABLE 1.—LC_{50} values expressed in ppm mirex at indicated day intervals for *Aedes triseriatus* larvae and pupae.

Time interval (Days)	LC_{50}	95% C.I.[a] Lower	Upper	Slope
2	0.489	0.229–1.546		1.22
4	0.142	0.007–0.375		1.38
8	0.073	0.027–0.249		1.60
16	0.058	0.020–0.164		1.56
24	0.050	0.021–0.145		1.48

[a] Confidence intervals at 95% probability.

to control percent emergence (Table 2). The percent mortality of surviving pupae was greater than 60% at both the 1.0 and 0.1 ppm levels. However, the data indicated that at 0.01 ppm and lower levels the percent emergence was greater than 80%. Emergent adults (1.0 ppm and 0.1 ppm) were very weak flyers; many could only move about the cage by crawling. The last males died on the 6th and 8th day at 1.0 ppm and 0.1 ppm levels, respectively. The females lived longer; the last females died on the 11th and 15th days at 1.0 ppm and 0.1 ppm levels, respectively. Two blood meals were offered to the females at the 2nd and 9th days. Blood was taken by the females of both 1.0 ppm and 0.1 ppm at first blood meal, but blood was taken by the females at the 0.1 ppm level during the last feeding; no eggs were collected from the females of 1.0 ppm and 0.1 ppm. Eggs were collected from the females exposed to lower concentrations. The eggs were aged and stimulated to hatch to check if embryonation had occurred. Progeny were collected from eggs of all the four concentrations. An LC_{50} value of 0.091 (0.024–3.475 (95% C.I.)) was determined for pupae prior to emergence.

Results indicated a nonsignificant regression on adults exposed to mirex concentrations of 0.5, 0.1, 0.01 and 0.001 ppm (Table 3). This was probably due to low mortality obtained at all concentrations. There was no percent corrected mortality at the lowest level of exposure, and at the highest level of exposure the percent corrected mortality was less than 12. Thus, mirex had no significant effects on the adult population at levels used.

Progeny were collected from each test group of the previous test and were subjected to the same concentrations as the parents (Table 4). Percent corrected mortality was calculated and compared to that for progeny of untreated parents. At the 0.5 ppm level the mortality of larvae from the mirex treated parents was 78.1% and 85.25% mortality for the larvae from untreated parents. A decrease was also shown at the 0.01 and 0.1 ppm concentration levels. At the 0.001 ppm concentration the percent corrected mortality had shown an increase. Striped earwigs which had been exposed to mirex over successive generations had shown resistance to occur (Gross and Spink, 1969). These data taken on the mosquito were for one generation.

TABLE 2.—Percent corrected mortality[a] and average percent emergence for adult *Aedes triseriatus* when the larval and pupal stages had been exposed to the indicated mirex concentrations.

Concentration	Percent corrected mortality	Percent emergence
Control	.....	94.2
0.001	1.46	92.8
0.0025	5.64	88.8
0.005	9.39	85.2
0.01	14.61	80.2
0.1	69.42	30.2
1.0	63.82	35.8

[a] Corrected mortality=observed mortality 72 hours after the pupae were placed in the adult cage.

TABLE 3.—Percent corrected mortality[a] for *Aedes triseriatus* adults at the indicated concentrations of mirex.

Concentration	Percent corrected mortality
Control	....
0.001	....
0.01	2.2
0.1	7.2
0.5	11.1

[a] Corrected mortality=observed mortality at 25 days of exposure.

TABLE 4.—Percent corrected mortality[a] of progeny from mirex exposed parents. Progeny was subjected to the same concentrations as the parents.

Concentration	Percent corrected mortality	
	Treated parents	Untreated parents
Control	.....	.....
0.001	3.31	2.50
0.01	7.05	8.58
0.1	74.10	80.52
0.5	78.10	85.25

[a] Corrected mortality = observed mortality through the larval and pupal stages.

Long-term studies over many successive generations may yield data to suggest that resistance would occur.

Adult weight of the males was less in all concentrations than the control (Table 5). The females had higher average weights in all concentrations with the exception of 0.005 ppm than the control. These data indicated that adult weight was affected slightly, if at all. If any sex was affected in a detrimental manner it was the male of the species. The males seemed to be weaker in all concentrations, especially 1.0 ppm and 0.1 ppm than were the males of the control group. Weakness in flying of the females only was noticed in adults which had been exposed to 1.0 ppm and 0.1 ppm.

The results observed from all tests showed that mirex would deplete 75% of the larval populations at concentrations of 0.1 ppm and above; but only 7.2% of the adult population died at 0.1 ppm. This study was in agreement with Muncy and Oliver (1963) and Ludke, *et al.* (1971) who showed that adult crayfish appeared not to be affected by mirex while juveniles of 2 species were found to be quite sensitive. Thus, if mirex were magnified to levels up to 0.1 ppm, deleterious effects on mosquito populations would be expected.

TABLE 5.—Adult weight of *Aedes triseriatus*[a] when the larvae and pupae had been exposed to the indicated mirex concentration.

Concentration	Males	Females
Control	1.09	1.96
0.001	1.02	2.02
0.0025	1.08	2.00
0.005	1.02	1.94
0.01	0.99	2.00
0.1	1.03	2.00
1.00	1.02	2.06

[a] Adult weight expressed in mg.

Literature Cited

Abbott, W. B. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265–267.

Arant, F. S., K. L. Hays and D. W. Speake. 1958. Facts about the imported fire ant. Highlights Agr. Res. 5:12–13.

Bridges, W. R., B. J. Kallman and A. K. Andrews. 1963. Persistence of DDT and its metabolites in the farm pond. Trans. Amer. Fish. Soc. 92:421–427.

Cope, O. B. 1961. Effects of DDT spraying for spruce budworm on fish in the Yellowstone River system. Trans. Amer. Fish. Soc. 90:239–251.

Cope, O. B. 1965. Agricultural chemicals and freshwater ecological systems, pp. 115–128. *In* Research in Pesticides. New York: Academic Press.

Cottam, C. and E. Higgins. 1946. DDT and its effect on fish and wildlife. J. Econ. Entomol. 39:44–52.

Ferguson, D. E. 1963. Notes concerning the effects of heptachlor on certain poikilotherms. Copeia 2:441–443.

Fox, C. J. S. 1967. Effects of several chlorinated hydrocarbon insecticides on the springtails and mites of grassland soil. J. Econ. Entomol. 60: 77–79.

Gaines, T. B. and R. P. Kimbrough. 1970. Oral toxicity of mirex in adult and suckling rats. Arch. Environ. Health. 21:7–14.

Gerberg, E. J. 1970. Manual for Mosquito Rearing and Experimental Techniques. AMCA Bull. 5:62–64.

Graham, R. J. and D. O. Scott. 1958. Effects of forest insect spraying on trout and aquatic insects in some Montana streams. Montana Fish and Game Dept. 32 pp.

Gross, H. R. and W. T. Spink. 1969. Responses of striped earwigs following application of heptachlor and mirex, and predator-prey relationships between imported fire ants and striped earwigs. J. Econ. Entomol. 62:686–689.

Grzenda, A. R., H. P. Nicholson, J. E. Teasley and J. H. Patric. 1964. DDT residues in mountain stream water as influenced by treatment practices. J. Econ. Entomol. 57:615–618.

Harrington, R. W., Jr. and W. L. Bidlingmayer. 1958. Effects of dieldrin on fishes and in-

vertebrates of a salt marsh. J. Wildl. Mgmt. 22:76–82.
Hoffman, D. A. and J. R. Olive. 1961. The effects of rotenone and toxaphene upon plankton of two Colorado reservoirs. Limnol. Oceanog. 6:2:219–222.
Holden, A. V. 1965. Contamination of freshwater by persistent insecticides and their effects on fish. Ann. Appl. Biol. 55:332–335.
Hunt, E. G. and A. I. Bischoff. 1960. Inimical effects on wildlife of periodic DDD applications to Clear Lake, California. Fish Game 46:91–106.
Johnson, D. W. 1968. Pesticides and fishes—A review of selected literature. Trans. Amer. Fish. Soc. 97:398–424.
Johnson, H. E. 1963. DDT and trout. Montana Acad. Sci. 23:96–110.
Kallman, B. J., O. B. Cope and R. J. Navarre. 1962. Distribution and detoxification of toxaphene in Clayton Lake, New Mexico. Trans. Amer. Fish. Soc. 91:14–22.
Keith, J. O. 1964. Relation of pesticides to waterfowl mortality at Tule Lake refuge. U.S. Fish Wildlife Serv., Denver Wildlife Res. Center. Ann. Prog. Rept. 14 pp.
Ludke, J. L., M. L. Finley and C. Lusk. 1971. Toxicity of mirex to crayfish, *Procambarus blandingi*. Bull. Environ. Contam. and Toxicol. 6:89–95.
Muncy, R. J. and A. D. Oliver, Jr. 1963. Toxicity of 10 insecticides to the red crayfish (*Procambarus clarki* (Girard)). Trans. Amer. Fish. Soc. 92:428–431.
Shapley, D. 1971. Mirex and the fire ant: Decline in fortunes of "perfect" pesticides. Science 172:358–360.
Sparr, B. I., W. G. Appleby, D. M. DeVries, J. V. Osmun, J. M. McBride and E. L. Foster. 1965. Insecticide residues in waterways from use in agriculture. Manuscript. 21 pp.
Van Valin, C. C., A. H. Andrews and L. K. Eller. 1968. Some effects of mirex on two warm water fishes. Trans. Amer. Fish. Soc. 97:185–198.
Vaughn, W. L. 1971. Mirex: next on "EEK-ology Ban Wagon?" Farm Chemicals 134: 46–48.

NOTICE

The "1971 Global Mosquito and Mosquito-Borne Disease Situation" by Helen Sollers-Riedel is off the press. This Supplement to the New Jersey Mosquito Extermination Proceedings contains 51 pages and is a review of the literature on mosquitoes for 1971. The booklet is divided into sections on taxonomy and distribution; techniques; genetics; behavior, biology and ecology; anatomy, morphology and ecology; arboviruses and other vertebrate viruses; filariasis; malaria; yellow fever; adulticides and larvicides; sterilization methods; biological control; resistance and susceptibility; attractants and repellents. The cost is just $2.50 U.S. It may be obtained from the N. J. Mosquito Extermin. Assoc. Fund No. 25, P.O. Box 19009, Washington, D.C. 20036, USA.