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## TOXICITIES OF THIOTEPA, TEPA, METEPA AND HEMPA TO LATE AQUATIC STAGES OF *CULEX NIGRIPALPUS* THEOBALD<sup>1</sup>

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**ABSTRACT.** Toxicities of aqueous concentrations of four chemosterilants to 3rd and 4th instar larvae and pupae of *Culex nigripalpus* were determined, preparatory to attempts at chemosterilization. The LC<sub>50</sub>'s (ppm) for the respective stages listed were 3.5, 3.75, and 8,300 for

thiotepa; 9.5, 11.9, and 31,250 for tepa; 80, 103, and 9,700 for metepa; and 267, 360, and 8,500 for hempa. More than 90% of larvae were killed when treatment concentrations exceeded 15 ppm of thiotepa, 25 ppm of tepa, 150 ppm of metepa, and 800 ppm of hempa.

**INTRODUCTION.** The mosquito, *Culex nigripalpus* Theobald, was shown to be the most important vector of St. Louis encephalitis in the Florida counties surrounding Tampa Bay during the epidemic of 1962 (Chamberlain *et al.*, 1964; Dow *et al.*, 1964). Previously it had not been known to be associated with epidemics of this disease. Probably this mosquito becomes important only under certain favorable epidemiological and ecological situations which favor its abundance, its infiltration of suburban sections, and the presence of susceptible hosts. The species is found in the southeastern United States, Mexico, Central, and northern South America.

Due to its low numbers during the late winter and spring (Provost, 1969), the

possibility of effecting a high degree of control or near eradication in localized areas at that time by application of sterility techniques appeared feasible. This view was supported by the eradication of a related species, *Culex pipiens quinquefasciatus* Say, by dissemination of sterilized males in a recent demonstration (Patterson *et al.*, 1970).

Thiotepa, tepa, metepa, and hempa are known to be effective chemosterilants of related species (Saito and Hayashi, 1967; Pillai and Grover, 1969; Patterson *et al.*, 1970). As no chemosterilants had been tested on *C. nigripalpus*, toxicological data on stages believed most susceptible to treatment with the four chemosterilants chosen was desirable.

**MATERIALS AND METHODS.** All specimens tested were from a laboratory colony of *C. nigripalpus* maintained at the University of Florida mosquito laboratory since March, 1970. This colony was established from egg rafts obtained from the State Board of Health's West Florida Arthropod Research Laboratory at Panama City, Florida.

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In each larval test, 100 third or fourth instar larvae were placed in an enamel pan 14" x 10" x 2" containing 100 ml of aged tapwater (pH 7.8). Then 400 ml of the test solution were added to produce the concentration desired. The larvae were fed daily until they died or pupated. Dead larvae were removed and their numbers recorded daily. Larvae which failed to pupate in 168 hours were counted dead. Pupae were rinsed and placed in fresh tapwater containers in screened cages for emergence counts. When adult eclosion was completed, counts were made of dead pupae, and living and dead adults. Three replicates of 100 larvae each were used for each concentration tested along with an untreated control of 100 larvae. Results were corrected for control mortality by Abbott's formula.

In each pupal test, 20 pupae 0-6 hours old were placed in a 35-ml plastic cup with an organdy cloth bottom. This cup was then deposited in a similar cup with a solid bottom and containing 25 ml of test solution. Exposure was for 24 hours.

The net-bottom cup containing the treated pupae was then removed, rinsed by 3 dips in flowing tapwater, and transferred to a cup containing 25 ml of fresh tapwater. These containers were put into an ice cream carton (1-pint capacity) covered by nylon netting where they remained until adult emergence was completed. The numbers of dead pupae, and dead and living adults were then recorded. Mortality was calculated as the total number of pupae treated minus the number of adults capable of flying at the end of eclosion. A control of 20 pupae was used with each test and 4 replicates were used for each concentration tested, the results being corrected for mortality by Abbott's formula. All test containers were held on shelves in the insectary where the temperature was  $80^{\circ} \pm 2^{\circ}$  and relative humidity 90 percent  $\pm 6$  percent.

Probit analyses of the data were attempted but were found inapplicable because mortality increases per unit of dosage concentration were too great, resulting in extremely narrow effective

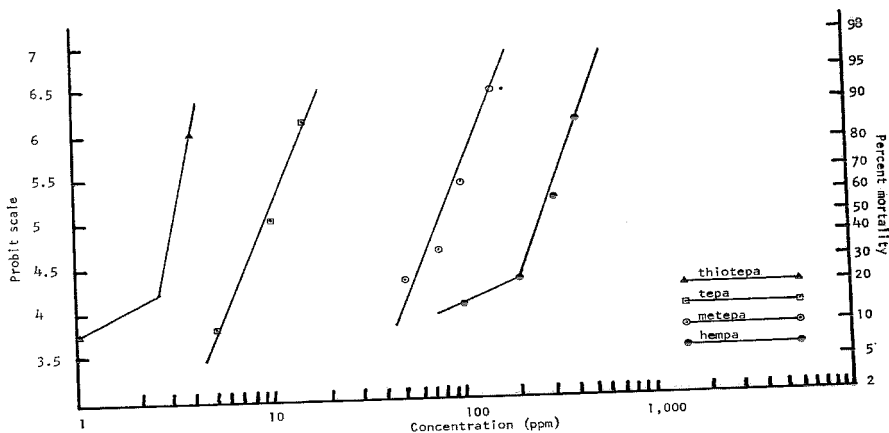


FIG. 1.—Eye-fitted log-concentration/probit regression lines indicating total percent mortality of *Culex nigripalpus* larvae, pupae, and emerging adults after 3rd instar larvae were exposed until pupation in aqueous solutions of thiotepa, tepa, metepa, and hempa. Mortality determined 48 hours after pupation and corrected by Abbott's formula.

dosage ranges. Consequently curves had to be fitted by eye and  $LC_{50}$ 's determined graphically.

**RESULTS AND DISCUSSION.** Figures 1, 2, and 3 show the findings for the tests of 3rd instar larvae, 4th instar larvae, and pupae respectively.  $LC_{50}$ 's in ppm were 3.5, 3.75, and 8,300 for thiotepa; 9.5, 11.9, and 31,250 for tepa; 80, 103, and 9,700 for metepa, and 267, 360, and 8,500 for hempa. Thiotepa showed the highest toxicity to both larvae and pupae. Based on the observation by Olson and O'Brien (1963) that polar agents in aqueous solutions did not penetrate cockroach cuticle until the water evaporated, Seawright *et al.* (1970) believe that the less strongly polarized thiotepa may be attracted to, and may penetrate the waxy cuticle of the pupae more readily than the closely related compound, tepa, which is highly polarized. Hempa, which is not an aziridinyl compound, was the least toxic to larvae and tepa was least toxic to pupae. Pupae were much more resistant than larvae to the effects of the chemosterilants. Many larvae were unable to pupate and

eventually died as a result of the treatments. Highest mortalities occurred in 3rd instar larvae during the first 24 hours after treatment. Otherwise, most deaths were noted during the last larval molt and during emergence from the pupal cases. At least 90 percent mortality was produced in 3rd and 4th instar larvae exposed to more than 15 ppm of thiotepa, 25 ppm of tepa, 150 ppm of metepa, and 800 ppm of hempa. In the larval tests particularly, the increases in mortality per unit of dose concentration rose very rapidly for all the compounds tested. This mortality-dosage relationship is not encouraging since the differences between sterilizing doses and fatal ones may be so close as to negate practical use of such compounds in sterile-male release programs of control.

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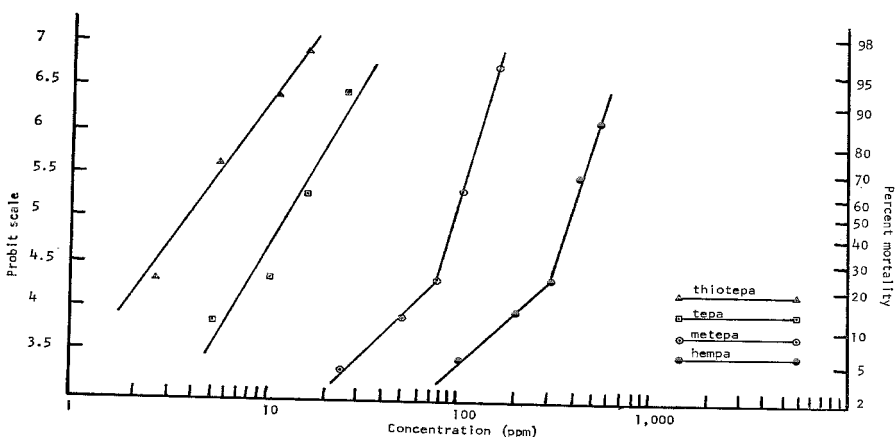


FIG. 2.—Eye-fitted log-concentration/probit regression lines indicating total percent mortality of *Culex nigripalpus* larvae, pupae, and emerging adults after 4th instar larvae were exposed until pupation and corrected by Abbott's formula. Mortality determined 48 hours after pupation.

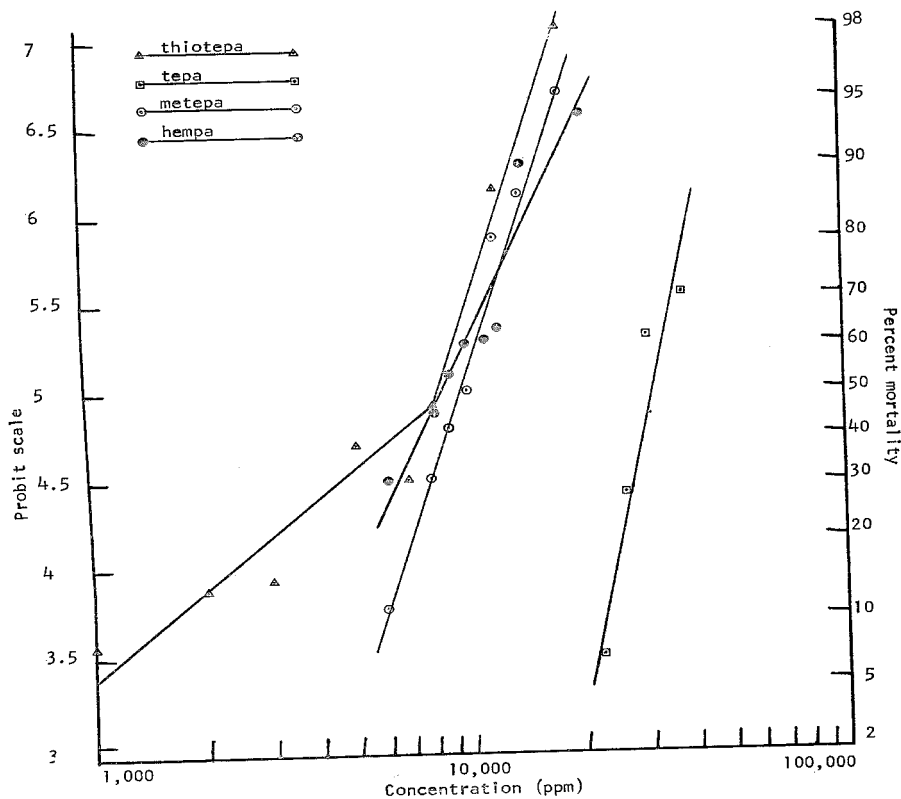


FIG. 3.—Eye-fitted log-concentration/probit regression lines indicating total percent mortality of *Culex nigripalpus* pupae and emerging adults after pupae (0-6 hour-old) were exposed for 24 hours in aqueous solutions of thiotepa, tepa, metepa, and hempa. Mortality determined 24 hours post-treatment and corrected by Abbott's formula.

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## EFFECTIVENESS OF SIX PROMISING INSECTICIDES FOR MOSQUITO CONTROL<sup>1</sup>

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**ABSTRACT.** Six chemicals were evaluated in the laboratory for their effectiveness against larvae of *Anopheles quadrimaculatus* Say and adults of *Aedes taeniorhynchus* (Wiedemann). Chevron RE-11775 (*m*-sec-butylphenyl methyl(phenylthio) carbamate) and Plant Protection PP 511 (*O*-[2-(diethylamino)-6-methyl-4-primidinyl]*O*,*O*-dimethyl phosphorothioate) were the only compounds that showed promise against mosquito larvae. Fisons NC-6897 (2,3-(isopropylidenedioxy)phenyl methylcarbamate) and Chevron

RE-11775 were the most effective of the six against adult mosquitoes. Of the other four, Geigy GS-13006 (*O*,*O*-diethylphosphorodithioate *S*-ester with 4-(mercaptomethyl)-2-methoxy- $\Delta^2$ -1,3,4-thiadiazolin-5-one), Sandoz 52,115 (1-ethyl-1-methyl-2-propynyl crotonate dimethyl phosphate), and Sandoz 52,097 (isopropyl (*E*)-3-hydroxy-crotonate methyl propylphosphoramidate) were slightly more effective and Plant Protection PP 511 was slightly less effective than malathion against the adults.

The evaluation of candidate chemicals against larval *Anopheles quadrimaculatus* Say and adult *Aedes taeniorhynchus* (Wiedemann) is an essential part of the effort of the Insects Affecting Man Investigations Laboratory at Gainesville, Fla. This paper reports the results obtained with six new chemicals in laboratory tests.

**TESTING TECHNIQUE.** Larval susceptibility tests were conducted by placing groups of

25 fourth instar larvae of *A. quadrimaculatus* in glass jars containing 250 ml of distilled water that had been treated with various concentrations of the insecticides in acetone solution. Larvae not exposed to chemicals showed no mortality. Duplicate jars were used at each concentration, and three tests were made with each concentration of each insecticide.

Adult susceptibility tests were conducted by exposing groups of 25 adult female *A. taeniorhynchus* to contact sprays containing a range of concentrations of each insecticide in a wind tunnel. A description of the wind tunnel and procedures used is

<sup>1</sup>This paper reports the results of research. Mention of a pesticide in this paper does not constitute a recommendation of this product by the U. S. Dept. of Agriculture.