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EVALUATION OF *AEDES AEGYPTI* LARVICIDES IN VARIOUS BREEDING CONTAINERS[1]

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INTRODUCTION. Increasingly widespread resistance of *Aedes aegypti* (L.) to insecticides formerly effective in the Caribbean area has necessitated development of bioassay methods suitable for evaluating newer compounds against larvae under field conditions. The method described in this paper was developed in order to evaluate further compounds considered promising alternatives under the World Health Organization's screening program. The methods used and provision for observations on gradual degradation or loss in toxicity to *aegypti* larvae in the several types of common breeding habitat under field conditions may prove effective in other geographical areas or against other species.

DDT and dieldrin, used in this evaluation, are insecticides to which most Jamaican populations of *aegypti* tested recently have been found to be resistant (Zwick, 1962–63), and were included as standards to which the other three compounds were compared. Fenthion[3] has been tested (Kellett and Gilkes, 1961) and utilized under some situations against insects of public health importance. Malathion[4] is employed against a variety of household and agricultural pests and carbaryl[5] is currently used principally as an agricultural insecticide but has been evaluated by Schoof *et al.* (1962) against adult mosquito species.

MATERIALS AND METHODS. The five compounds selected for testing were in the form of water dispersible powders and were procured from commercial sources. The percentages actual active ingredient of each insecticide were as follows:

DDT—50%
dieldrin—50%
fenthion—40%
malathion—25%
carbaryl—80%

The insecticides were suspended in a minimal amount of water and added to tap water to give a concentration of 10 p.p.m. of the technical compound in all 35 test containers.

Containers utilized, one set of seven per insecticide, included easily procurable types commonly encountered as breeding foci by PASB *aegypti* eradication field personnel in the Caribbean area. These consisted of the following container types with the amounts[6] of tap water added to each:

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[3] o,o-dimethyl o-[4-(methylthio)-*m*-tolyl] phosphorothioate.

[4] S-[1,2-bis (ethoxycarbonyl) ethyl] o,o-dimethyl phosphorodithioate.

[5] (Sevin) 1-napthyl methylcarbamate.

[6] United States gallons employed throughout.

1) Vehicle tire 7-1/2 gallons. Used truck tires braced upright with water contained in the lower portion up to the tire bead.

2) Steel drum, 10 gallons. Rusty 55-gallon steel drums cut in half perpendicularly to the long axis. Rust flakes accumulated as the test progressed and the water was quite rusty-colored at the conclusion of the experiment.

3) Wooden barrel, 10 gallons. Uncharred, hardwood barrels used previously for aging rum, tops removed, and the bung holes sealed. Organic compounds leached into the water during the test and imparted a greyish color and putrid odor to the container after the second week.

4) Painted steel can, 10 gallons. Open cylindrical steel cans were given one heavy coat of a water-emulsion type flat finish household paint which contained an acrylic polymer binder and a fungicide of phenyl mercuric succinate. Water was added after the paint had dried.

5) Tin-plated steel lard can, 5 gallons. Purchased new, these cans, minus tops, rusted along the soldered seams as the test progressed and imparted a rusty-orange color to contained water by the end of the testing period.

6) Glass acid carboy, 5 gallons. Restricted opening of 1-1/2 inches reduced the evaporation of water compared to all other containers.

7) Unglazed fired clay pot, 10 gallons, manufactured from native red Jamaican clay. These pots are used as water coolers in rural areas and the water was rapidly evaporated through the semi-porous sides and bottom.

All containers were tested for residual toxicity after being scrubbed and rinsed with tap water prior to addition of the insecticide. Several gallons of water were added to each and left to stand several days after which fourth instar *aegypti* larvae were added to undiluted aliquots in the laboratory and a 24-hour mortality test run. No mortality was observed in the 42 containers employed.

The containers were situated beneath shade trees on the isolated grounds of a Jamaican Government hospital site. Polyethylene tarpaulins were stretched over them to prevent dilution by rainwater and contamination by falling leaves. However, two severe rain and wind storms resulted in an estimated dilution by one gallon of rainwater in several containers and falling leaves, which blew in under the tarpaulins, were constantly removed during the period of the test.

Sampling of the insecticide-treated water in each container was carried out at 1, 2, 4, 6, 8, 11 and 13 week intervals. Samples consisted of 800-ml. portions dipped out with a disposable one-quart plastic-coated paper carton after prior agitation of the breeding container water. Dilutions of the container water were accomplished in the laboratory using a 1000-ml. glass graduate, one per container, as the mixing vessel. To each 250-ml. aliquot of container water 500 ml. of tap water was mixed and five serial three-fold dilutions were made: 1/1, 1/3, 1/9, 1/27, 1/81 and 1/243. These dilutions produced the following calculated test concentrations of larvicides for testing:

Container water diluted	Calculated insecticide concentration in ppm
1/1	10
1/3	3.3
1/9	1.1
1/27	0.37
1/81	0.12
1/243	0.04

A 500-ml. portion of each serial dilution was divided into two 250-ml. replicates for the standard WHO 24-hr. mosquito larvae insecticide test. The test containers were of the cold-drink plastic or waxed surface disposable 16-oz. size which eliminated extensive cleanup of laboratory glassware. The 1000-ml. graduates were washed in a water-detergent solution and run through a potassium dichromate-sulphuric acid bath prior to subsequent use with other insecticides.

The *aegypti* larvae in these tests were

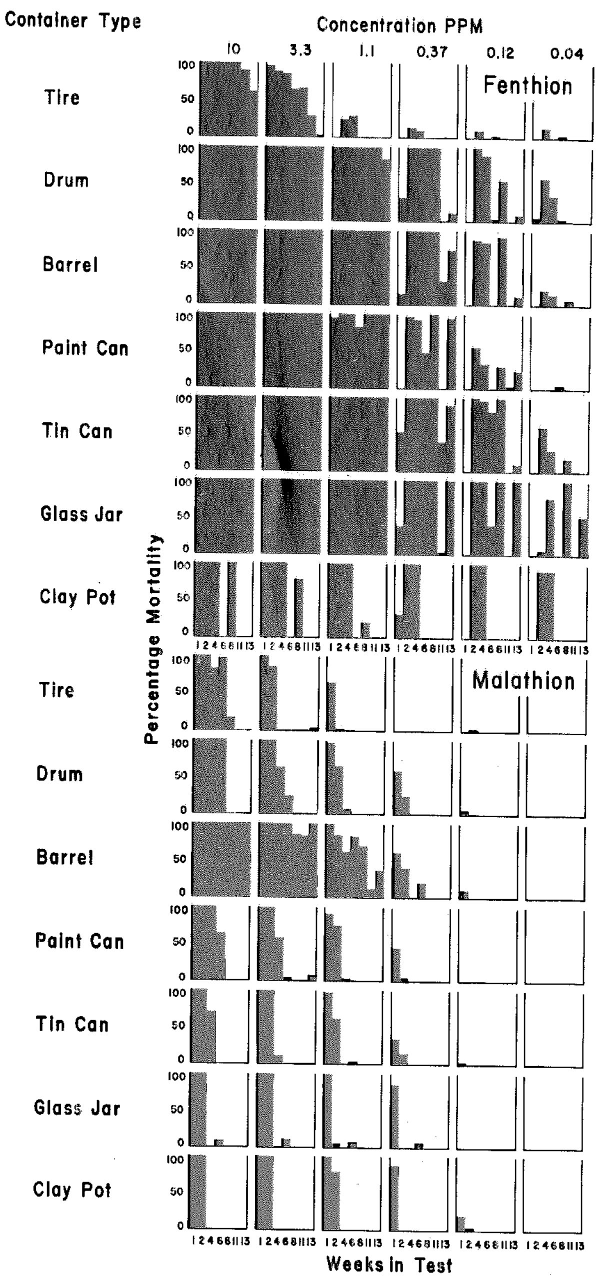
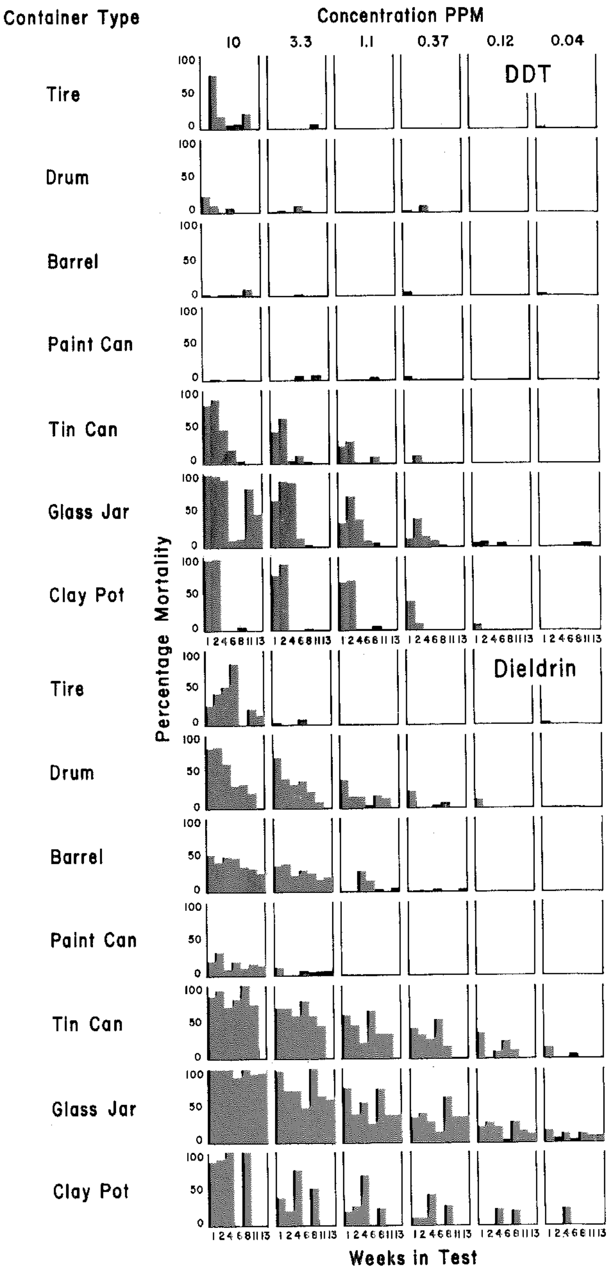


Fig. 1. (a)—Percentage mortality of Lionel Town, Jamaica F_2 strain *Aedes aegypti* larvae to calculated concentrations of five insecticides in various containers over a thirteen-week period.



Container Type
Concentration PPM
10
3.3
1.1
0.37
0.12
0.04
DDT
Tire
Drum
Barrel
Paint Can
Tin Can
Glass Jar
Clay Pot
Dieldrin
Tire
Drum
Barrel
Paint Can
Tin Can
Glass Jar
Clay Pot
100
50
0
Percentage Mortality
1 2 4 6 8 11 13
Weeks in Test

FIG. 1. (b).

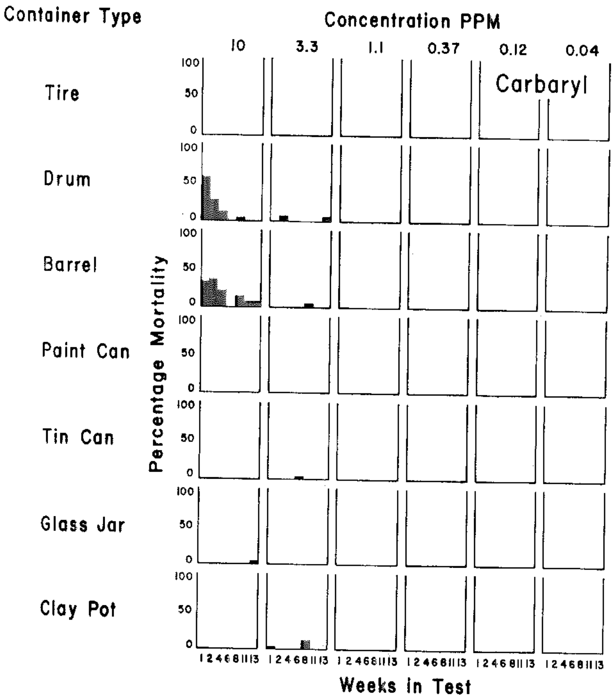


FIG. 1 (c).

FIG. 1.—Percentage mortality of Lionel Town, Jamaica F_2 strain *Aedes aegypti* larvae to calculated concentrations of five insecticides in various containers over a thirteen week period.

second generation three- or four-day old laboratory reared individuals in their third or fourth instar. The strain was collected from Lionel Town, a rural locality on the southern coast of Jamaica, treated intradomiciliarly with DDT and dieldrin in previous years under the country's malaria eradication program. Numerous tests of this strain using the standard WHO larval test solutions indicated the LC_{100} for DDT as >20 p.p.m., and for dieldrin as >5 p.p.m.

During each week's tests, control samples of untreated water from an identical group of containers were employed as a control to determine if toxicity from unknown causes might be influencing mortality in the treated containers. No control mortality was observed over the 13-week period.

RESULTS. Figure 1 (a, b and c) presents the average mortality of the two replicates of 25 larvae tested at each concentration over the 13-week test period. Clay pots held water for only two (DDT, malathion) or four (dieldrin, fenthion, carbaryl) weeks, after which five gallons of tap water were added to each on the sixth week and the eighth week bioassay test was completed.

Comparing the performances of the five compounds, fenthion proved the most toxic at the greatest dilutions and the most persistent of the compounds. Since the Lionel Town strain employed here was known to be highly DDT and dieldrin resistant, lack of 100 percent mortality at all concentrations of the former in all containers and the total mortality of larvae in only three dieldrin containers was not surprising. Malathion was most effective in the wooden barrel for the longest period—in all other container types its toxicity was of shorter duration. Carbaryl was completely ineffective as an *aegypti* larvicide at the highest concentration in all containers.

The effectiveness of the larvicides tested here is influenced greatly by the type of breeding container in which they are utilized. Generally, with all five compounds investigated, the loss in control effectiveness is greatest in the automobile tire followed by the paint can > wooden barrel > rusty steel drum > tin can > clay pot > glass jar.

In decreasing order of effectiveness against chlorinated hydrocarbon resistant *aegypti* larvae the insecticides may be ranked: fenthion > malathion > dieldrin > DDT > carbaryl.

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EDITOR'S NOTES: (1) This number of *Mosquito News* is the largest that we have published. This is partly a result of the inclusion of Part I of the Papers and Proceedings of the 20th Annual Meeting. It also reflects the increasing tendency of authors or their agencies to take advantage of the privilege of having longer papers published, by paying extra charges. We take this opportunity to call the attention of contributors to the regulations on length of papers and number of illustrations and tables, which are printed on Cover 3 of each issue of *Mosquito News.* If an author is tempted to use a long article or one with many illustrations as a precedent or as an example for his own, he should remember that these papers involve extra charges which are paid by the author or his agency.

(2) Part II of the "Proceedings and Papers of the 20th Annual Meeting" will be included in the September number.