R. H. 1965. Evolution and speciation within the *maculipennis* complex of the genus *Anopheles*. (in press).

MASON, G. F., and BROWN, A. W. A. 1963. Chromosome changes in insecticide-resistance in *Anopheles quadrimaculatus*. Bull. Wld. Hlth. Org. 28:77–81.

RAI, K. S., and CRAIG, G. B. 1961. A study of the karyotypes of some mosquitoes. Genetics 46:891.

STAHLER, N., and TERZIAN, L. A. 1961. Comparison of mating and biting behavior in two laboratory strains of *Anopheles quadrimaculatus*. Ann. Ent. Soc. Amer. 54:453–459.

EFFECTIVENESS OF VARIOUS DOSAGES OF DICHLORVOS AGAINST *AEDES AEGYPTI* IN CISTERNS, ST. THOMAS, V. I.[1]

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Of the myriad of man-made breeding sources for *Aedes aegypti*, the water storage cistern presents one of the most difficult in which to exercise control of breeding. The necessity of preventing contamination of water by foreign substances, the often limited access to areas to be treated, and the diversified types of cisterns frequently restrict the use of standard insecticidal techniques. Under these highly restrictive conditions, a logical approach is that of the sustained release of insecticidal vapor within the cistern. This method can operate in a wide variety of cistern configurations, can treat the inaccessible areas of the cistern, and avoid the necessity of the direct addition of a foreign substance to the water. Work on the residual fumigant technique for the control of *Culex* in confined spaces, *i.e.*, catch basins, (Maddock *et al.*, 1963 and Brooks *et al.*, 1963) indicated possible usage of this approach against adult *Ae. aegypti*. The objective of this study was to establish dosage levels of this residual fumigant that could be used to control *Ae. aegypti* in cisterns in the Virgin Islands.

MATERIALS AND METHODS. Eighteen cisterns infested with *Ae. aegypti* were selected for treatment in Charlotte Amalie, St. Thomas. The cisterns chosen were of the exposed type located exterior to the residence (Figure 1). All cisterns were

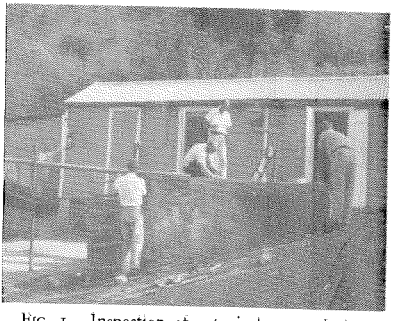

FIG. 1.—Inspection of a typical external cistern on the island of St. Thomas, U. S. Virgin Islands.

rectangular in shape ranging in size from 3′ 0″ x 6′ 6″ to 9′ x 11′ 3″. They were constructed of concrete with either a wood or metal access hatch in the top. Capacities of individual cisterns varied from 127 cubic feet to over 1,128 cubic feet. Water depth varied from 6 inches to more than 7 feet, while the distance between the water line and the roof of the cistern ranged from 3 inches to 10 feet. All cisterns contained rain water collected by roof gutter systems attached to adjacent buildings.

The dispensers utilized in this study were of the 20 percent dichlorvos in resin-

[1] From the Biology/Chemistry Section, Technology Branch, Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Ga.

plastic formulation.[2, 3] The 10″ dispensers (measuring 3/16″ x 2½″ x 10″) were cut to obtain the dosages of less than 1 dispenser. Installations were made with ¼, ½, 1, 2, and 3 dichlorvos resin strips per cistern. One dispenser contained 20 g. of technical dichlorvos.

Attachment of the dispensers within each cistern was accomplished by means of a number 14 galvanized construction wire threaded through one end of the dichlorvos strip and secured. The opposite end of the wire was then wrapped tightly to the lid hinge or another firm projection. The wire was adjusted to place the dispenser unit 3″ to 12″ below the cistern roof.

In the first treatment cycle, units of ¼, ½, and 1 dispenser strip were used to treat 6, 5, and 3 cisterns, respectively. Four additional cisterns were added a short time later, two treated with a single 10″ dispenser and two with two 10″ dispensers each. In a second test series, the original 14 cisterns were used, but the maximum dosage was increased to three units. Treatments of ½, 1, 2, and 3 dispensers were mounted in 2, 4, and 4 cisterns, respectively.

The technique used for evaluation of the effectiveness of the dichlorvos vapor against adults was similar to that used by Brooks and Schoof, 1964, in catch basins. A rectangular screen wire cage ⅜″ x 4″ x 6″ was adapted for these exposures rather than the 4″ cylindrical cage used in earlier studies. In the second test series, to insure more comparable exposures among the cisterns, the exposure cage was fastened to a polystyrene float and the entire unit suspended at the end of a dowel (¼″ diameter x 6′) from the dispenser attachment wire. By this method all test insects were held equidistant from the dichlorvos vapor source. Mortality determinations were made immediately after exposure. To assess the 3-hour exposure of adults, the time in weeks at which the average adult mortality dropped below 70 percent and failed to recover was selected as the criterion of effectiveness.

Further assessment of the effectiveness of the treatments was based on visual observations of the presence or absence of larvae and living and/or dead adults in the cistern, as well as weekly adult and larval bioassays of the dichlorvos concentrations in the cisterns. Chemical analyses for the presence of dichlorvos in the water were carried out weekly.

The specimens used in the test cages were *Ae. aegypti* from two sources. During test series I, all adult females were reared from larvae collected near the test site. Since this method failed to yield uniform age test insects, caged adult female specimens were then shipped to St. Thomas from the insectary at Savannah, Georgia. These insects were the progeny of a colony established from specimens originally obtained at St. Thomas several months earlier. In each shipment 1-day-old adult females were anesthetized and caged in quantities of 25 per cage. The required number of cages, separated by a paper towel wetted with 10-percent honey water, were stacked within a polyethylene bag. This bag was then placed in a corrugated cardboard carton and air mailed to the test site. As shipment required 2 to 3 days, the specimens at the time of testing were 3 to 4 days old.

The bioassay test utilizing third instar larvae was run concurrently with adult exposures during the second test series. Water samples were withdrawn from two cisterns at each dosage prior to the beginning of each adult exposure. Five hundred ml. of water were set aside for the bioassay and 500 ml. for chemical analysis. Twenty-five larvae, reared from eggs of the Savannah colony, were exposed in 150-ml. aliquots of cistern water for 24 hours and percent mortality recorded. Short exposures of 1, 2, 3, and 4 hours were also run as a check against excessive

[2] Furnished through the courtesy of Shell Chemical Company, Union, New Jersey.

[3] Use of commercial product is for identification purposes only and does not constitute endorsement by the U. S. Department of Health, Education, and Welfare.

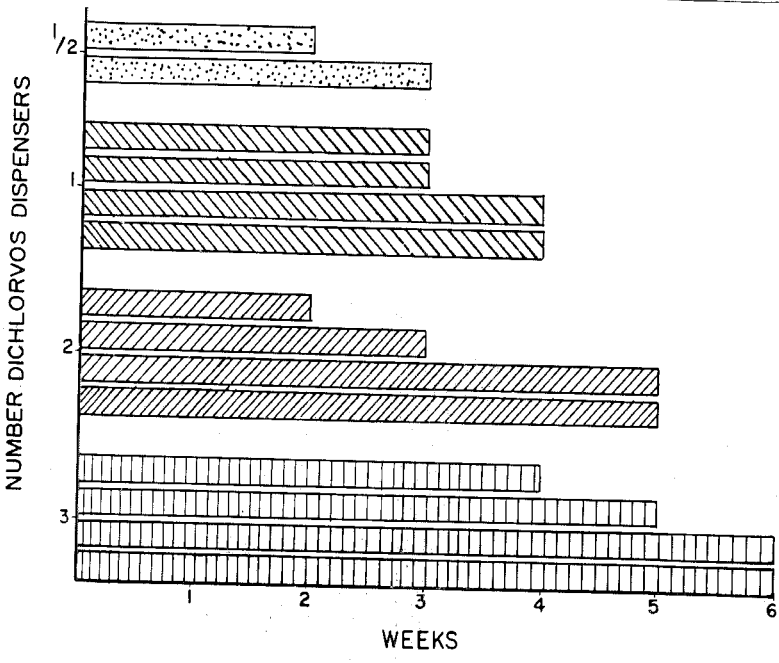

FIG. 2.—Weeks effective kill of adult *Aedes aegypti* in cisterns, St. Thomas, V. I., 1964.

buildup of dichlorvos concentrations.[4] Percentage kills were then compared to the mortalities obtained from standard dichlorvos solutions of 2.5, 1.0 and 0.5 p.p.m. run concurrently.

For chemical analyses 500 ml. of cistern water were washed with high grade carbon tetrachloride for the extraction of organic phosphates. These samples were then analyzed for dichlorvos at the laboratory in Savannah. The colorimetric method of phosphate determination as described in Chemical Memo number 7, Technical Development Laboratories, was used as the procedure for analysis.

RESULTS. Three-hour exposure of caged adult *Ae. aegypti,* in the 14 cisterns treated with ¼, ½, and 1 dispenser strips indicated that lethal dichlorvos concentrations did not persist at any dosage for as long as 5 weeks. Chemical analysis for dichlorvos in the water yielded a maximum reading of 0.014 p.p.m. over 5 weeks of vapor exposure in cisterns treated with ¼ and ½ dispenser units. Cisterns treated with one full strip failed to show more than 0.013 p.p.m. during the series.

In the second test series, the two cisterns treated with ½ unit indicated satisfactory kills for 2 and 3 weeks, respectively, (Figure 2). The 2-dispenser treatment showed unsatisfactory kills in one of four cisterns on week 3, and both the 1- and 2-dispenser treatments showed unsatisfactory kills in two of the four cisterns on week 4. With the 3-dispenser installations, the four cisterns yielded kills above 90 percent on week 3, and on week 4 mortalities were between 76 and 96 percent. On week 5 kills failed to rise above 8 percent in 3 of the 4 cisterns[5] with 1-dispenser treatment, and 20 percent in the four cisterns with two dispensers. The 3-dispenser series yielded kills of 0 and 4 percent in

[4] Mortalities were negative for all short exposure tests run.

[5] No test was made in the 4th cistern because of objection by the householder.

two of the cisterns on week 5, with 72 and 92 percent in the remaining two. Continuation of tests on week 6 gave acceptable mortalities in >50 percent of the cisterns treated with the 1, 2, and 3 dispenser dosages. The reason for the marked drop and then rise in effectiveness on weeks 5 and 6 was not apparent.

The 159 larval bioassays in the second test series showed levels of dichlorvos to be below 0.1 p.p.m. in all cisterns except one treated with 3 dispenser units. In this cistern the dichlorvos level was above 0.1 p.p.m. for samples taken at 5 and 12 days after treatment as indicated by 24-hour mortalities but at 19, 26, and 33 days the level was below 0.1 p.p.m. A second cistern treated at the same dosage level and sampled concurrently gave readings below 0.1 p.p.m.

Of the 72 chemical analyses carried out for these tests, dichlorvos concentrations at or above detectable limits (0.01 p.p.m.) were found on six occasions. One cistern that received the 1-dispenser treatment contained a dichlorvos concentration of 0.1 p.p.m. 1 week after treatment, while five cisterns treated at dosages of ¼, ½ and 1 dispenser contained average concentrations of 0.014, 0.012, and 0.01 p.p.m., respectively, 5 weeks after treatment. Chemical analyses of samples taken at 9 weeks after treatment failed to detect dichlorvos. Thirty-one additional samples failed to yield detectable quantities of toxicant at any of the dosage levels thereafter.

DISCUSSION. Use of general visual observations for evaluation of treatment was suspended early in the tests. Factors such as the low absorption of dichlorvos in the water coupled with continued larval infestation brought about by fluctuating water levels, left no visible evidence of larval reduction directly attributable to treatment. Visual detection of adult kills appeared valid at first observation. However, no means were available to distinguish initial adult mortality from adults which may have succumbed to the vapor at later dates.

Although reason for the marked rise in effectiveness shown on week 6 was not apparent, earlier applications of this formulation in confined spaces displayed this same characteristic behavior by the dispensers when approaching their threshold for maintaining satisfactory vapor concentrations. As this threshold is approached, minor variations in temperature, wind, etc. may result in favorable mortalities 1 day and opposite findings a short time later.

The residual life as reported is based on 3-hour exposures of the test insect to a vapor-containing atmosphere. It may be postulated that under field conditions, individuals of the endemic mosquito population entering the cistern would be exposed for longer periods. Thus, the length of residual action for these tests may reflect a lesser period of effectiveness than would be obtained under natural exposures. Data from these tests show that successful control of adult *Ae. aegypti* may be maintained by the residual fumigant technique. As indicated by both chemical and biological analyses, dichlorvos absorption into the water remained minimal at dosage levels. For field application, the 3-dispenser dosage offers the most promise in the Virgin Islands. Such treatments applied at 6-week intervals gave satisfactory control of adults with the additional advantage of manpower savings over conventional control methods.

SUMMARY. Evaluation of the field use of dichlorvos-resin strips for the control of adult *Ae. aegypti* in water storage cisterns was made on the island of St. Thomas, U. S. Virgin Islands, in 1964. Thirty-two treatments of 20 percent dichlorvos-resin dispensers were installed in 18 cisterns in Charlotte Amalie. Dispenser units of 3/16" x 2½" x 10" were applied in part or in multiple to achieve applications of ¼, ½, 1, 2, and 3 dispensers. Satisfactory mortalities of caged adult female *Ae. aegypti* were sustained for 2 to 6 weeks at these dosages. The installation of three 10" dispensers per cistern gave the most effective and consistent kills of 4 to 6 weeks. Bioassays as well as the chemical analyses of the cistern water on only one occasion indi-

cated dichlorvos levels in the water to be greater than 0.1 p.p.m. with most of the determination at or below detectable limits of 0.01 p.p.m.

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References

BROOKS, G. D., ELMORE, C. M., JR., SCHOOF, H. F., and CARMICHAEL, G. T. 1963. Field evaluation of three types of DDVP dispensers for the control of *Culex pipiens quinquefasciatus* Say in catch basins. Proc. 50th Ann. Meet. N. J. Mosq. Exterm. Assn. and the 19th Ann. Meet. Am. Mosq. Control Assn. March 12–15, 1963. pp. 355–363.

BROOKS, G. D. and SCHOOF, H. F. 1964. Field evaluations of five types of dichlorvos dispensers against *Culex pipiens quinquefasciatus* in catch basins. Mosquito News, 24(2):144–148.

MADDOCK, D. R., ELMORE, C. M., JR., and SCHOOF, H. F. 1963. Preliminary tests with DDVP vapor for the control of *Culex pipiens quinequefasciatus* in catch basins. Mosq. News 23(2):69–74.

U. S. Public Health Service, Technical Development Laboratories, Savannah, Georgia, Chemical Memorandum 7 (March 1961).