on a small scale to achieve long-term control of mosquito larvae. However, the large amount of formulation required on a hectare basis may make them impractical for large-scale operations at the dose rates tested in this study.

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POLYMER FORMULATIONS OF MOSQUITO LARVICIDES

II. EFFECTS OF A POLYETHYLENE FORMULATION OF CHLORPYRIFOS ON *Culex* POPULATIONS NATURALLY INFESTING ARTIFICIAL FIELD POOLS [1]

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ABSTRACT. Field tests were conducted in artificial pools for 21 weeks at Edgewood Arsenal, Maryland, to determine the larvicidal effectiveness of chlorpyrifos [o,o-diethyl o-(3,5,6-trichloro-2-pyridyl)phosphorothioate] formulated as a slow-release polymer and as a water emulsion. The effectiveness of the formulations was monitored on a weekly basis by measuring naturally-occurring populations of *Culex pipiens quinquefasciatus* Say and *Culex restuans* Theobald in the pools, by insecticide residue analysis of water samples collected from the pools, and by in-pool bioassay with 4th instar laboratory-reared larvae of *C.p. quinquefasciatus*. A single application of the water emulsion gave 3 weeks of effective mosquito control, while a single application of the slow-release polymer gave effective control throughout the 21-week study. Neither of the formulations produced excessive residues.

In studies reported by Wilkinson *et al.* (1971), mosquito larvae were effectively controlled for up to 26 weeks in artificial pools treated with charcoal or polyethylene formulations of chlorpyrifos [o,o-diethyl o-(3,5,6-trichloro-2-pyridyl) phosphorothioate]. The effectiveness of these formulations was determined primarily by in-pool bioassays with laboratory-reared mosquito larvae, although observations of naturally-occurring mosquitoes were made. In subsequent studies by Miller *et al.* (1973), polyethylene and polyvinyl chloride formulations of chlorpyrifos were shown to be effective for up to 24 weeks under a variety of environmental conditions. The effectiveness of these formulations was based on in-pool bioassays with laboratory-reared mosquito larvae and corresponding residue analysis of the treated water. In the present study, all stages of the mosquito life cycle were surveyed in a quantitative evaluation of the effects of a polyethylene formulation of chlorpyrifos on natural mosquito populations. In-pool bioassays

[1] The opinions contained herein are those of the authors and should not be construed as official or reflecting the views of the Department of the Army. Mention of proprietary products is for the purpose of identification only and does not imply endorsement by the Department of the Army. Address reprint requests to: Commander, USAEHA, Aberdeen Proving Ground, Maryland 21010.
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and residue analysis of treated water were also conducted as part of the present study.

MATERIALS AND METHODS

TEST AREA. The test area was located at Edgewood Arsenal, Maryland. Natural mosquito breeding sites included a wet depression 40 meters west and a swampy area 260 meters east-southeast of the test pools. Surveys of these areas indicated that an adequate natural mosquito population was present and that natural infestation of the test pools could be expected.

TEST POOLS. Square, plastic lined, plywood boxes, 1.2 x 1.2 x 0.8 meters (L x W x H), served as test pools. The pools were placed at ground level, 3.3 meters apart, in a randomized block design. All pools were filled with approximately 970 liters of hydrant water between 6 and 10 April 1970. A polyethylene drainage tube was inserted 13 cm below the top of each pool to prevent water from overflowing the tops of the pools during the study. To simulate 3 types of aquatic habitats, the following 3 types of pools were set up: pools containing only water (non-organic pools); pools containing a 5 cm base of soil (organic pools); and pools containing a 5 cm base of soil plus 500 grams of Wayne® Rabbit Ration pellets (high organic pools). Subsequently, 70 grams of rabbit pellets were added weekly to each of the high organic pools to maintain a relatively high level of organic matter. The soil added to the pools was the same as that reported by Miller, *et al.* (1973). All pools were allowed to stabilize for 7 weeks prior to treatment with insecticides.

INSECTICIDE TREATMENT. The two formulations of chlorpyrifos (M-3507 and WE-48) were the same as those described earlier by Miller, *et al.* (1973). Treatments of each formulation were replicated 3 times for each type of pool (i.e. non-organic, organic, and high organic pools). Three untreated pools of each type served as controls. Pools were treated on 1 June 1970. The M-3570 was dosed at a rate of 2.5 ppm, equivalent to the addition of 21.0 grams of pellets (containing 2.4 grams actual chlorpyrifos) to each pool. The WE-48 was dosed within the recommended range (Anonymous, 1970) at 9.0 ppb[4], equivalent to the addition of 22 ml of the 0.48 percent emulsion (containing 10.5 mg of actual chlorpyrifos) to each pool.

SAMPLING OF TEST POOLS. Weekly in-pool bioassays were conducted in all pools from 8 June to 28 October with 4th instar laboratory-reared *Culex pipiens quinquefasciatus* Say larvae. The LC_{90} for the laboratory-reared larvae (0.9 ppb chlorpyrifos) and the bioassay technique were the same as reported by Miller, *et al.* (1973).

To determine relative oviposition in the test pools, counts of *Culex* spp. egg rafts were made daily from 20 April to 7 September. After counting, the egg rafts in each pool were placed in floating, 15-cm by 15-cm, Styrofoam® enclosures with 20-mesh cloth screen on the tops. These enclosures prevented recounting of the egg rafts on subsequent days but allowed larvae to hatch and disperse into the pools. The 20-mesh screens prevented oviposition into the enclosures.

Mosquito larvae and pupae were sampled weekly from 15 May to 4 September using 125-mesh plankton net with a removable 23-ml vial at the tip. A single net sample consisted of passing the net through approximately 60 liters of water on the same side of each pool. Samples were preserved in formalin and later identified and grouped as to first and second instar larvae, third and fourth instar larvae and pupae.

Emerging adult mosquitoes were sampled with floating emergence traps which were 30.5 by 30.5 by 20.3 cm. The top and side of each trap consisted of 20-mesh cloth screen stapled to a square

[4] The reference cited in this paper concerning dosages for chlorpyrifos (Anonymous, 1970) recommends 0.0125–0.5 pounds AI/acre without reference to water depth. As a point of clarity, all references to dosage hereinafter, whether stated as ppm, ppb, or kg AI/hectare, are, in fact pounds AI/acre-foot equivalents.

wooden frame. The bottom was left open so that pupae could enter the trap. Weekly samples were obtained from 12 May to 20 August by placing one trap in each pool and collecting the adult mosquitoes that emerged in the traps during a 24-hour period.

Residue levels in all the pools were monitored on a weekly basis. Water samples (10.0 ml) were collected from each pool and returned to the laboratory for extraction and gas chromatographic residue analysis using the procedures described by Miller *et al.* (1973). Sensitivity of the gas chromatograph used during the present study was 0.1 ppb chlorpyrifos.

Data for each naturally-occurring mosquito stage were subjected to 2-way analysis of variance. The significance of differences was determined by calculating a least significant difference (lsd) at the 0.05 level of probability by the method of Steel and Torrie (1960).

RESULTS AND DISCUSSION

IN-POOL BIOASSAYS AND RESIDUE LEVELS —M-3570—In organic and non-organic pools treated with M-3570, 100 percent mosquito larval mortality was observed in the bioassays on all post-treatment weeks, except week 21 in the organic pools and week 16 in the non-organic pools. The 21-week average mosquito larval mortality in both organic and non-organic pools was 99.9 percent. In the high organic pools treated with M-3570, mosquito larval mortality was below 100 percent for a total of 7 weeks, but the 21-week average was 94.6 percent. Minimum chlorpyrifos residues in the various test pools were as follows: 0.4 ppb in the non-organic pools at week 21; 0.1 ppb in the organic pools at weeks 20 and 21; 0.1 ppb in the high organic pools at week 15. Residues in high organic pools were <0.1 ppb from weeks 16 through 21. Maximum chlorpyrifos residues observed in the various test pools were as follows: 2.9 ppb in the non-organic pools at weeks 5 and 15; 2.3 ppb in the organic pools at week 5; and 3.6 ppb in the high organic pools at week 1. The 21-week average chlorpyrifos residue was 1.9 ppb in the non-organic pools, 1.1 ppb in the organic pools, and 1.0 ppb in the high organic pools.

IN-POOL BIOASSAYS AND RESIDUE LEVELS —WE-48. In pools treated with WE-48, mosquito larval mortalities during the 1st week after treatment were as follows: 50 percent in the non-organic pools, 90 percent in the organic pools; and 33 percent in the high organic pools. Subsequent to week 1, no significant larval mortality was observed in bioassays of the pools treated with this formulation. Minimum chlorpyrifos residues in the various pools were as follows: 0.3 ppb in the non-organic pools at week 4; 0.1 ppb in the organic pools at week 5; and 0.2 ppb in the high organic pools at week 5. Maximum chlorpyrifos residues were 1.8 ppb for the non-organic pools and 2.4 ppb for the organic pools, each observed at week 1. Contamination of samples precluded residue determinations of water from the high organic pools during the first 3 weeks. Therefore, maximum residues in the high organic pools could not be determined. Subsequent to week 5, residues in all pool types treated with the WE formulation were <0.1 ppb.

NATURAL MOSQUITO POPULATIONS—SUMMARIES. Generally, mosquito populations were very low in the non-organic pools, intermediate in the organic pools, and relatively high in the high organic pools. Data for populations observed in the non-organic and organic pools were not considered sufficient to stand alone. Therefore, weekly mosquito populations are summarized to represent average numbers present in the 3 types of test pools, and possible interactions between type of pool and insecticide treatment were not determined. Average weekly populations of naturally-occurring *Culex* spp., predominantly *C.p. quinquefasciatus* and *Culex restuans* Theobald, were grouped according to life stage (i.e. egg rafts, first

and second instar larvae, third and fourth instar larvae, pupae, or adults). Average chlorpyrifos residues in all types of pools were summarized through 12 weeks after treatment (Figure 1) for comparison with

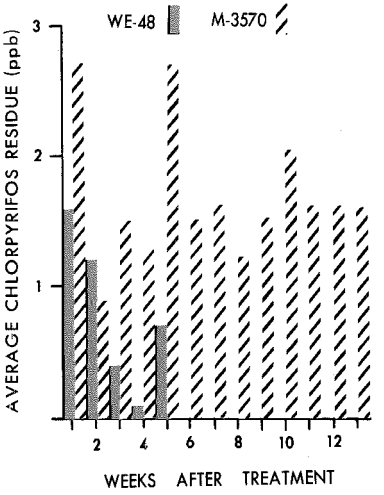


Fig. 1.—Average residue levels observed in all test pools treated with WE-48 and M-3570 formulations.

the average weekly mosquito populations in the chlorpyrifos treated pools.

NATURAL MOSQUITO POPULATIONS—M-3570. Populations of *Culex* spp. egg rafts in treated and control pools did not differ significantly either before or after treatment (Figure 2).

Populations of first and second instar *Culex* spp. larvae were similar in pools scheduled for treatment and in control pools during the pre-treatment period (Figure 4). Following treatments, populations were reduced to near zero through 13 weeks. During this same period, populations in control pools increased and were significantly higher than treated pool populations during weeks 1 through 3 and weeks 5 and 6.

Populations of third and fourth instar *Culex* spp. larvae in pools scheduled for treatment and in control pools were similar during the pretreatment period (Figure 6). Following treatment, populations in treated pools were reduced to near zero for 13 weeks. Control pool populations increased and were significantly higher than treated pool populations during weeks 1 through 4 after treatment.

Populations of *Culex* spp. pupae in treated and control pools did not differ significantly either before or after treatment (Figure 8) although the average number of pupae in the control pools was consistently higher than that of the treated pools for most of the study.

Pretreatment populations of *Culex* spp. adults were significantly higher in pools scheduled to be treated than in control pools (Figure 10). Following treatment, the number of adults emerging from treated pools was reduced to near zero for 9 weeks. Adult emergence from control pools during this same period increased and was significantly higher than the treated pools during weeks 1 through 3.

Average residues in pools treated with M-3570 were at or above 0.9 ppb through 13 weeks after treatment (Figure 1).

NATURAL MOSQUITO POPULATIONS—WE-48. The average number of *Culex* spp. egg rafts observed in treated pools did not differ significantly from those in control pools, except during weeks 5 and 6 after treatment (Figure 3) when oviposition was higher in treated pools.

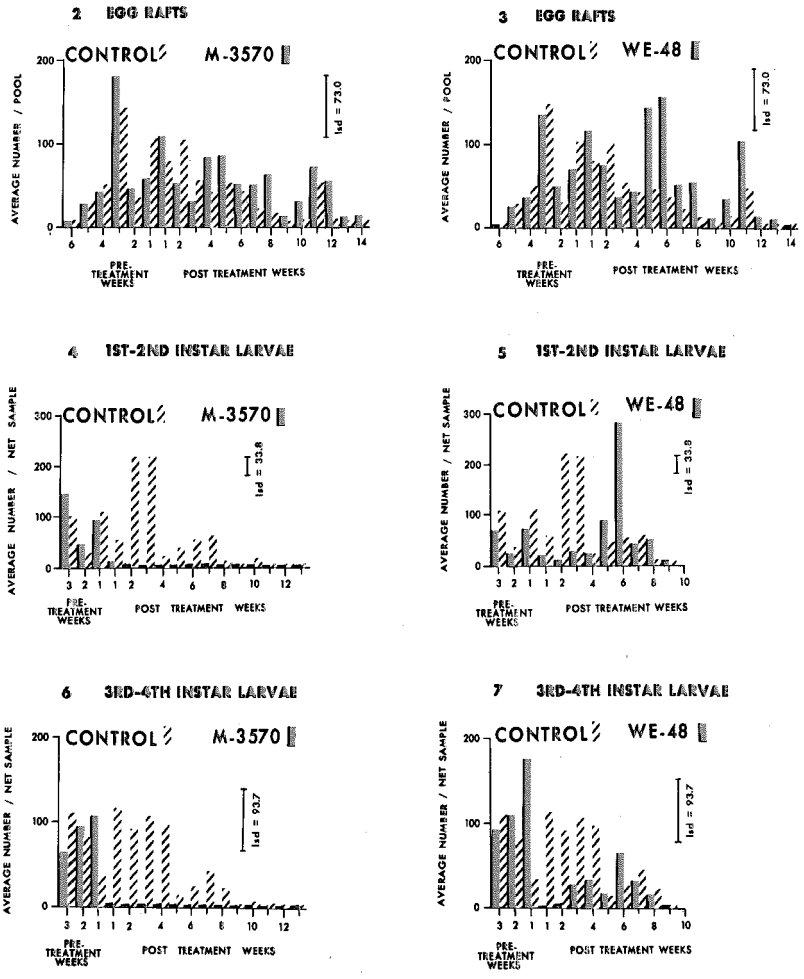
During the pretreatment period, populations of *Culex* spp. first and second instar larvae were slightly higher in control pools than in those scheduled for treatment (Figure 5). During post treatment weeks 1 through 3, first and second instar larvae increased significantly in control pools, while they remained at a low level in treated pools. However, by post treatment weeks 5 and 6, populations of first and second instar larvae were significantly higher in treated pools than in control pools.

Populations of third and fourth instar *Culex* spp. larvae were significantly higher in pools scheduled for treatment than in control pools on week 1 pretreatment (Figure 7). Following treatment the population was significantly lower in

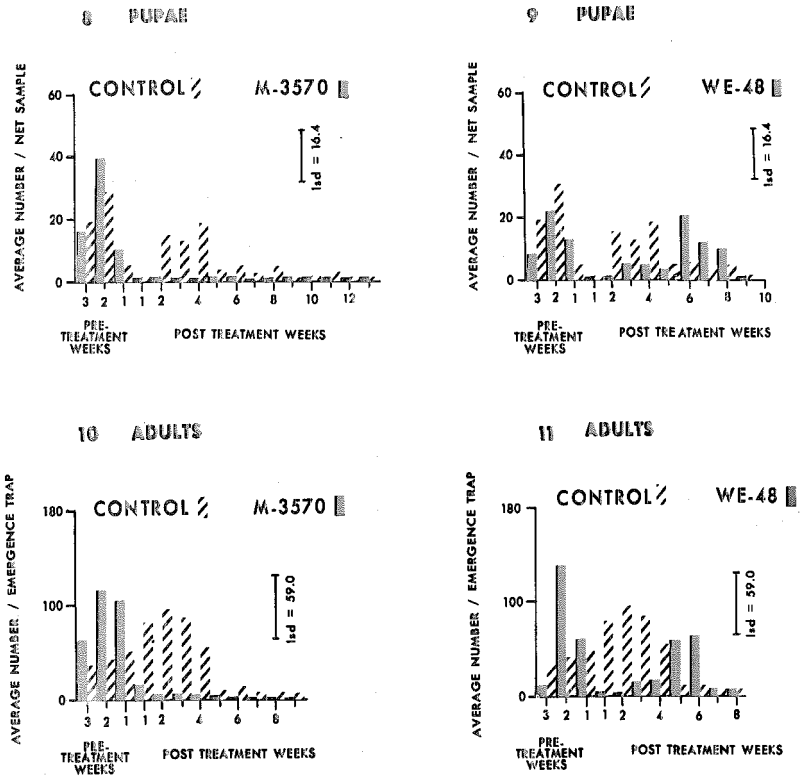
treated pools than in control pools for 3 weeks. Thereafter, populations were similar in both treated and control pools.

Populations of *Culex* spp. pupae in control pools and treated pools did not differ either before or after treatment (Figure 9).

Adult populations of *Culex* spp. in pools scheduled for treatment were equal to or greater than those of control pools during the pretreatment period (Figure 11). Following treatment the number of adults emerging from treated pools was reduced significantly over the number emerging from control pools for 3 weeks. Subsequently, the number of adults emerg-



Figs. 2–7.—*Culex* spp. egg rafts, 1st–2nd instar larvae, and 3rd–4th instar larvae observed in test pools treated with M-3570 and WE-48 formulations. 2. Egg rafts in M-3570 pools. 3. Same, WE-48 pools. 4. 1st–2nd instar larvae in M-3570 pools. 5. Same, WE-48 pools. 6. 3rd–4th instar larvae in M-3570 pools. 7. Same, WE-48 pools.



Figs. 8–11.—*Culex* spp. pupae and adults observed in test pools treated with M-3570 and WE-48 formulations. 8. Pupae in M-3570 pools. 9. Same, WE-48 pools. 10. Adults in M-3570 pools. 11. Same, WE-48 pools.

ing from treated and control pools did not differ significantly through 8 weeks post treatment.

EFFECTIVENESS OF THE FORMULATIONS. Neither of the formulations had significant adverse effects on the *Culex* spp. populations as determined by monitoring oviposition or pupal populations. The WE-48 significantly reduced populations of first and second instar larvae, third and fourth instar larvae, and adults for 3 weeks after treatment. This reduction of larval and adult populations for 3 weeks corresponded closely to the duration of toxic residues in the pools (Figure 1). The M-3570 significantly reduced first and second instar larvae for approximately 6 weeks after treatment, third and fourth instar larvae for 4 weeks, and adult populations for 3 weeks after treatment. However, since toxic residues were present for at least 13 weeks and bioassay mortality averaged 94.6 percent for 21 weeks, the effectiveness of this formulation was greater when measured by these criteria than when measured by the monitoring of natural mosquito populations. The fact that significant population reductions in treated pools could not be demonstrated for periods longer than those listed above is probably due to the overall decline in the *Culex* spp. populations in both treated and control pools subsequent to weeks 5 and 6 after treatment.

CONCLUSIONS

A single application of WE-48, at a dosage of 0.009 ppm, gave 3 weeks of effective mosquito control, while a single application of M-3570, at a dosage of 2.5 ppm. gave at least 21 weeks of effective control. The formulations had significant quantitative effects only on larval and adult populations. However, the effectiveness of the formulations could not be adequately evaluated by monitoring only naturally-occurring larval and adult populations, since the overall natural population did not remain at a high level during the entire test period. The monitoring of insecticide residue levels, or the use of mosquito larvae as bioassay organisms, provided a consistent measure of larvicidal effectiveness.

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MALATHION RESISTANCE IN *AEDES AEGYPTI* FROM PRESSURE ON ADULTS[1]

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Detailed information on the development of resistance to malathion in *Aedes aegypti* larvae has been obtained by malathion pressure on larvae (Brown and Pal, 1971), but similar studies with adults, of equal or more importance, have not been made. Brown and Abedi (1960) found that malathion pressure on larvae causes only a small increase in adult resistance.

During the *Aedes aegypti* eradication campaign of 1969 in Puerto Rico, strains resistant to malathion occurred, and the adults of one such strain from the town of Arecibo were resistant by a factor of about five when compared with the most susceptible Puerto Rican strains (Fox and Bayona, 1972). The purpose of the present experiments was to find out (a) whether resistance in the Arecibo strain would increase by pressure on the adults in the laboratory for a dozen generations and (b) whether the Arecibo strain allowed to breed freely in the laboratory over a long period of time away from malathion would lose its resistance.

MATERIALS AND METHODS. Using the World Health Organization standard kit, I tested caged adults of the Arecibo, Puerto Rico strain and saved the survivors to provide eggs for breeding the subsequent generations. For the early generations it was sometimes necessary to save survivors of 0.4 percent and 0.8 percent malathion to get enough eggs, but in later generations survivors of 1.6 percent and 3.2 percent were sufficiently numerous for exclusive use. After pressure on the parents and pressure on each of 12 filial generations, I made comparative tests using the same set of impregnated papers on (a) a malathion suscepti-

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