

POLYMER FORMULATIONS OF MOSQUITO LARVICIDES

I. EFFECTIVENESS OF POLYETHYLENE AND POLYVINYL CHLORIDE FORMULATIONS OF CHLORPYRIFOS APPLIED TO ARTIFICIAL FIELD POOLS¹

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ABSTRACT. Field tests were conducted for 24 weeks at Edgewood Arsenal, Maryland, to compare the larvicidal effectiveness of a water emulsion and three polymer formulations of chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl phosphorothioate)] applied to artificial field pools which were either shaded or unshaded, and each with or without a soil bottom. The effectiveness of the formulations was monitored weekly by residue analysis and by in-pool bioassay with 4th instar laboratory-reared *Culex pipiens quinquefasciatus*

Say larvae. Single applications of the water emulsion were effective for 1 to 2 weeks. Single applications of each of the polymer formulations provided effective control of mosquito larvae (93.8-100 percent) during the 24-week test. For the three polymer formulations, 24-week average residue levels were lower in pools containing soil than in those without soil, while the average residues in shaded pools were higher than in pools without shade.

INTRODUCTION

The literature pertaining to the use of slow-release formulations of mosquito larvicides has been extensively reviewed by Whitlaw and Evans (1968), Nelson *et al.* (1970), and Wilkinson *et al.* (1971). Such formulations offer the advantage of extending the effective life of non-persistent larvicides by slowly releasing the materials into the water. In the studies by Wilkinson *et al.* (1971), polyethylene or charcoal formulations of chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate] were effective against mosquito larvae for up to 6 months as measured by field bioassays. This study reports results of field tests to determine the larvicidal effectiveness of, and residue levels maintained by, three polymer formulations of chlorpyrifos which were applied to a series of artificial field pools.

MATERIALS AND METHODS

TEST POOLS. Pools used in this study were polyethylene-lined (6 mil, black), square, wooden boxes measuring 91.4 cm on a side and 60.9 cm in depth, with a total liquid capacity of 509.4 liters. Pools were placed at ground level in a block design to accommodate eight rows of eight pools each. The distance between rows, and between pool positions within a row, was 2.4 meters. To simulate four different aquatic habitats 15 each of the following four types of pools were constructed: pools with a 5-cm base of soil and with 91.4 x 91.4-cm plastic shades suspended 25.4 cm above the top; pools with a 5-cm base of the same soil, but without plastic shades; pools without soil, but with 91.4 x 91.4-cm plastic shades 25.4 cm above the top; and pools with neither soil nor plastic shades. Since only 60 pools were used, four positions within the possible 64-pool block were selected at random to be without pools. With the exception of these four blank positions, each row contained two each of the four types of pools. Initially, the pools were filled (April 1970) to half capacity with hydrant water to a depth of 30.4 cm (254.7 liters) to allow for the containment of increases in water volume. For those pools with shades it was necessary to place perforations in the

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plastic to drain precipitation into the pools. Each pool was provided with a 0.6-cm diameter drain spout 5 cm below the top of the pool. Drains on pools to be treated with the floating pellets were fitted with a 16-mesh screen to prevent loss of floating pellets due to overflow of water from the edges of the pools. Soil added to pools was analyzed by the University of Maryland Soil Testing Laboratory and found to be a loam type with a pH 6.2 and an organic matter content of 14.4 per cent. The organic content is considered very high for Maryland soil.

TEST FORMULATIONS AND DOSE RATES. The four formulations tested were: polyethylene pellets containing 9.9 percent chlorpyrifos, weighing an average of 9.9 mg, having a specific gravity <1 and designated M-3409; polyvinyl chloride pellets containing 10.0 percent chlorpyrifos, weighing an average of 45.6 mg, having a specific gravity >1 and designated PVC-10; polyethylene pellets containing 11.5 percent chlorpyrifos, weighing an average of 15.8 mg, and having a specific gravity >1 and designated M-3570; and chlorpyrifos emulsifiable concentrate, formulated as a 0.48 percent water emulsion, and designated WE-48. The M-3409, M-3570, and WE-48 formulations were supplied by the Dow Chemical Company, Midland, Michigan. The PVC-10 formulation was prepared at this Agency by thoroughly mixing di(2-ethylhexyl)phthalate with the PVC resin Geon® 135 at a ratio of 1:1 (w:w). Analytical grade chlorpyrifos was added to the resulting plastisol and the mixture was poured into aluminum pellet molds and cured at 120–125 °C for 30 minutes. Each of the four formulations was placed in 12 separate pools consisting of three replicates of each of the four pool types (i.e., shaded/no soil, shaded/soil, unshaded/no soil, and unshaded/soil pools). The remaining 12 pools were untreated controls. The polymer formulations were hand broadcasted onto the surface of the pools, while the water emulsion was dispersed over the surface using a pipette. The WE-48

was dosed within recommended range (Anonymous, 1970) at 9.0 ppb (0.028 kg AI/hectare).⁴ The three polymer formulations were each dosed at 5.0 ppm (15.5 kg AI/hectare), since it was known that a relatively high dose rate (Nelson, *et al.* 1970) was required to reach and maintain a toxic concentration in the water.

DATA COLLECTION AND TREATMENT. All pools were monitored weekly through 24 weeks post treatment according to the following procedures. Water samples for residue analysis (10 ml) were taken approximately 10 cm below the surface at the center of the pool. Collections were made at approximately 0800 hours and immediately returned to the laboratory for analysis. Chlorpyrifos was extracted from each water sample by solvent partitioning with Nanograde® hexane. Samples were subjected to three successive partitionings of 10, 5, and 5 ml. Concentrations of chlorpyrifos in the combined hexane fractions were determined by electron capture gas chromatography without additional purification. Percent recovery obtained with the above extraction technique based on the addition of a known quantity of chlorpyrifos was 99 percent. A Tracor® Model MT-150 gas chromatograph equipped with a ⁶³Ni electron capture detector was used for analysis of the hexane extracts. The instrument was fitted with a 182.4 cm x 0.63 cm glass column packed with 80/90-mesh Chromosorb® W coated with 3 percent OV-1. The operating parameters were: oven temperature 200 °C; inlet temperature 235 °C; outlet temperature 240 °C; detector temperature 250 °C; attenuation 10² x 16; carrier gas N₂; column flow rate 60 cc/min; and chart speed 0.32 cm/min. The retention time for chlorpyrifos relative to aldrin as unity was 0.89. The minimum

⁴ The reference cited in this paper concerning dosages for chlorpyrifos (Anonymous, 1970) recommends 0.0125–0.05 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.

detectable quantity measured in the water under the above conditions was 0.1 ppb chlorpyrifos.

In-pool bioassays were conducted using 4th instar laboratory-reared larvae of *Culex pipiens quinquefasciatus* Say. The bioassay cups were 470-ml, unwaxed, cardboard cups with the bottoms removed and replaced with a double layer of 12-mesh cheesecloth. A cup was attached to the inside of each pool so that the bottom of the cup was submerged 3-5 cm with the larvae exposed to 250-300 ml of pool water. Ten larvae were placed in each cup at approximately 1000 hours and the 24-hour percent mortality recorded. Water volumes in the pools were calculated by measuring water depth. Water temperatures were measured between 1500-1600 hours approximately 10 cm below the surface at the center of the pools.

Since laboratory-reared *C. p. quinquefasciatus* larvae were used for all in-pool bioassays, it was necessary to compare their susceptibility to chlorpyrifos with that of field-collected larvae of the same species. Therefore, dosage-mortality tests were conducted on both laboratory-reared and field-collected larvae and LC₉₀ values were computed by probit analysis.

Although populations of naturally-occurring mosquito larvae in the pools were not monitored during the entire 24-week study, samples were taken with an enamel dipper on pretreatment week 1 and post treatment weeks 1 through 4 to determine the immediate effects of the treatments applied to the pools. Control of mosquito larvae was considered to have failed if weekly bioassays from three replicate treatments averaged less than 90 percent. Residue levels were considered inadequate for control if they averaged less than 0.9 ppb, the established LC₉₀ for 4th instar laboratory-reared larvae of *C. p. quinquefasciatus*. Residue levels were considered excessive if above 18.0 ppb (0.06 kg AI/hectare), the recommended (Anonymous, 1970) maximum dose rate.

Residue data were subjected to analysis of variance. The significance of differences was determined by calculation of a

least significant difference (lsd) at the 0.05 level of probability (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

COMPARATIVE SUSCEPTIBILITY OF MOSQUITO LARVAE. Laboratory tests with 4th instar larvae of *C. p. quinquefasciatus* showed that the laboratory-reared specimens were slightly more susceptible to chlorpyrifos than the same species in the field. Based on observations of 360 larvae, the LC₉₀ for the laboratory population was 0.90 ppb, while the LC₅₀ was 0.54 ppb. Tests utilizing 480 field-collected larvae resulted in LC₉₀ and LC₅₀ values of 1.20 ppb and 0.98 ppb, respectively. These differences were not significant at the .05 level of probability. As a result, it was felt that the use of laboratory-reared larvae throughout this study gave an adequate measure of the larvicidal activity of the formulations being tested.

UNTREATED CONTROL POOLS. Pretreatment sampling of all control pools showed an average of >25 naturally-occurring *Culex restuans* Theobald larvae per dip. Throughout the 24-week observation period these pools remained active breeding sites for both *C. restuans* and *C. p. quinquefasciatus*. In-pool bioassays of control pools produced no significant mortality during the entire test period. No chlorpyrifos residue was detected in water samples taken from the control pools during the 24-week test period.

WE-48 FORMULATION. Table 1 presents a summary of the data relating to this formulation. Pretreatment sampling of the four types of pools treated with the WE-48 revealed an average of >25 naturally-occurring *C. restuans* larvae per dip. On post treatment days 1 through 7 no larvae were observed in the pools. Residue information is not available for week 1. The low level of mortality observed in the bioassays indicated that, while the original population had been eliminated, no residual effect was achieved. The 2nd week residues were <0.1 ppb and bioassay mortalities continued at a low level. Lar-

Table 1.—Summary of 24-week residue levels and mosquito larval mortalities in test pools treated with various chlorpyrifos formulations.

Type of pool	24-week average residue (ppb) ^a	Maximum residue (ppb)	Week maximum residue observed	Number of weeks with residue >18.0 ppb	24-week average bioassay mortality (%)
WE-48					
Shaded no soil	<0.1	1.2	3	0	17.6
Shaded soil	<0.1	1.5	4	0	14.6
Unshaded no soil	<0.1	0.6	7	0	12.2
Unshaded soil	0.1	4.7	3	0	16.6
All pool types	<0.1	1.0	3	0	15.3
M-3409					
Shaded no soil	17.2 ^a	31.9	7	10	100
Shaded soil	16.5 ^a	28.5	7	5	100
Unshaded no soil	15.1 ^a	27.5	7	4	100
Unshaded soil	15.9 ^a	33.4	7	9	100
All pool types	16.2	26.9	7	8	100
PVC-10					
Shaded no soil	2.1 ^a	6.2	4	0	97.7
Shaded soil	0.9 ^b	4.3	4	0	86.1
Unshaded no soil	1.6 ^{ab}	5.1	4	0	97.7
Unshaded soil	1.1 ^b	5.5	4	0	93.0
All pool types	1.4	4.5	4	0	93.8
M-3570					
Shaded no soil	3.8 ^a	12.1	4	0	100
Shaded soil	1.5 ^b	6.3	4	0	100
Unshaded no soil	2.5 ^c	5.4	8	0	98.2
Unshaded soil	1.8 ^b	8.0	4	0	100
All pool types	2.4	4.5	4	0	99.5

^a Data for water emulsion formulation not subjected to analysis. For each polymer formulation, averages followed by the same letter do not differ significantly at the 0.05 level of probability.

vae and pupae of *C. restuans* were observed in the pools at the 2nd week.

In view of the short-term control observed in pools treated with the WE-48, the pools were retreated 2 weeks after the initial treatment. The second treatment was applied at the same rate as the initial treatment, 0.009 ppm. The second treatment resulted in the elimination of *C. restuans* populations and an additional 2 weeks without reinfestation. Thereafter, residue levels dropped to <0.1 ppb, in-pool bioassays produced no significant mortality, and the pools, like the untreated control pools, became active breeding sites for *C. restuans* and *C. p. quinquefasciatus*.

The larvicidal activity of the WE-48 did

not appear to be influenced by the presence or absence of soil in the pools or by the presence or absence of shade. Residue levels averaged <0.1 ppb under all conditions (Table 2).

M-3409. Pretreatment sampling of the pools treated with the M-3409 indicated an average of >25 *C. restuans* larvae per dip. No mosquitoes were observed in these pools during the post-treatment period.

Residue analyses showed the chlorpyrifos concentration in all pools was always >0.9 ppb, with the 24 week average for all pool types being 16.3 ppb. Residues were >18.0 ppb during 10 weeks in the shaded pools without soil, 5 weeks in shaded pools with soil, 4 weeks in un-

Table 2.—Summary of separate effects of soil and shade on chlorpyrifos residues.

Formulation	24-week average ppb chlorpyrifos observed in all test pools with ^a			
	Shade	No shade	Soil	No soil
WE-48	<0.1	<0.1	<0.1	<0.1
M-3409	16.9 ^f	15.5 ⁿ	15.8 ⁿ	16.6 ⁿ
PVC-10	1.5 ⁿ	1.3 ^{nb}	1.0 ^b	1.9 ^f
M-3570	2.6 ⁿ	2.1 ^b	1.6 ^c	3.1 ^d

^aData for water emulsion formulation not subjected to analysis. For each polymer formulation, averages followed by the same letter do not differ significantly at the 0.05 level of probability.

shaded pools without soil, and 9 weeks in unshaded pools with soil (Table 1).

The various simulated habitats had no consistent effect on maximum residues (Table 1). Maximum residues were observed at 7 weeks after treatment as follows: 31.9 ppb in a shaded pool without soil; 28.5 ppb in a shaded pool with soil; 27.5 ppb in an unshaded pool without soil; and 33.4 ppb in an unshaded pool with soil. Certain overall effects on average residues were attributable to the habitats (Table 2). Disregarding the presence or absence of soil, 24-week average residues were slightly higher in shaded pools (17.1 ppb) than in unshaded pools (15.5 ppb). Disregarding the presence or absence of shade, 24-week average residues were slightly lower in pools with soil (16.2 ppb) than in those without soil (16.4 ppb). None of the differences in residue levels were significant ($p < .05$), probably due to the high residue maintained in all test pools.

PVC-10. Pretreatment sampling of the pools treated with the PVC-10 formulation showed an average of >25 *C. restuans* larvae per dip. The treatment effectively eliminated the population and no mosquitoes were observed in these pools throughout the 24-week period.

Although the 24-week average residue for all pools was 1.4 ppb, the chlorpyrifos concentration was not maintained above 0.9 ppb in all pools through 24 weeks after treatment. The residue did not exceed

18.0 ppb in any of the pools at any time during the test period.

The various habitats had no consistent effect on maximum residues (Table 1). Maximum residues were observed at 4 weeks after treatment as follows: 6.2 ppb in a shaded pool without soil; 4.3 ppb in a shaded pool with soil; 5.1 ppb in an unshaded pool without soil; and 5.5 ppb in an unshaded pool with soil. Average residue levels were affected by the habitats (Table 2). Disregarding the presence or absence of soil, 24-week average residues in shaded pools (1.5 ppb) and in unshaded pools (1.3 ppb) did not differ significantly. Disregarding the presence or absence of shade, 24-week average residue levels were significantly higher in pools without a soil base (1.9 ppb) than in pools with a soil base (1.0 ppb).

M-3570. Pretreatment sampling of the pools treated with M-3570 showed an average of >25 *C. restuans* larvae per dip. The population was effectively eliminated by the treatment and throughout the 24-week test period immature mosquito larvae were not observed in the pools.

Average residue levels were maintained above 0.9 ppb in all pools, except for pools with soil and shade at week 13. Residues did not exceed 18.0 ppb in any of the pools.

Maximum residues were not consistently affected by the simulated habitats (Table 1). Maximum residue occurred at week 4 in a shaded pool without soil (12.1 ppb), in a shaded pool with soil (6.3 ppb) and in an unshaded pool with soil (8.0 ppb). An unshaded pool without soil showed a maximum residue of 5.4 ppb at week 8. The habitats had some effect on 24-week average residue levels (Table 2). Disregarding the presence or absence of soil, average residues were significantly higher in shaded pools (2.6 ppb) than in unshaded pools (2.1 ppb). Disregarding the presence or absence of shade, pools with soil showed a significantly lower residue (1.6 ppb) than those without soil (3.1 ppb).

OVERALL EFFECTIVENESS OF THE FORMU-

LATIONS. The various simulated habitats produced certain significant differences in the residues maintained by the polymer formulations. However, at the dosage used (5.0 ppm) none of the residues were biologically different, since all were maintained at or above larvicidal levels. Therefore, residue levels and mosquito larvae mortalities observed in the four types of test pools were grouped and averaged, along with average water volumes and average water temperatures, to give an overall picture of the performance of each formulation (Figures 1 through 6).

Based on observations of the elimination of naturally-occurring *C. restuans* populations in the pools, the initial treatment with the WE-48 provided control for approximately 1 week. A second treatment with this formulation, applied 2 weeks after the initial one, prevented reinfestation for an additional 2 weeks and resulted in average bioassay mortalities of 82.5 percent and 65.8 percent, respectively, during the additional 2 weeks. Thereafter, residues dropped to a low level and in-pool bioassays produced no significant mortality for the remainder of the 24-week test period. Due to the short residual life of this formulation, any effects due to fluctuations in water volume or water temperature could not be observed.

A single application of M-3409 provided 100 percent control (based on bioassay) for the 24-week test period (Figure 4). Although residue levels were affected by fluctuations in water temperature and water volume (Figures 5 and 6), residues were maintained well above the LC_{90} during the entire test period.

A single application of the PVC-10 provided an average of 93.8 percent control (based on bioassay) for 24 weeks (Figure 2). Actual control was above 90.0 percent in all cases except during weeks 13, 23, and 24. Although water temperature and water volume fluctuations affected residue levels (Figures 5 and 6), the residues were maintained above 0.9 ppb during all weeks, except 13, 23, and 24.

A single application of the M-3570 formulation gave an average of 99.5 per-

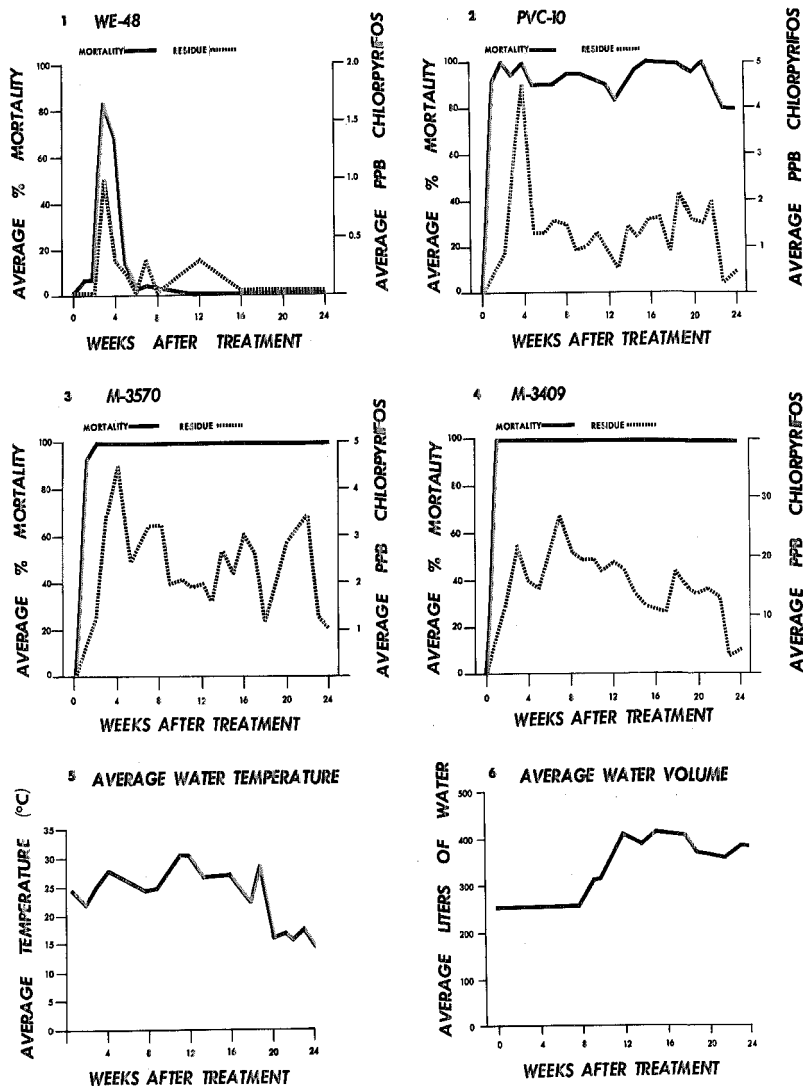
cent control (based on bioassay) for 24 weeks (Figure 3). Residues were maintained above 0.9 ppb for the same period, despite effects due to fluctuations in water volume and water temperature (Figures 5 and 6).

The relative effectiveness of the formulations may be observed by comparing the information presented in Figures 1-4. The PVC-10 and M-3570 formulations were each effective for at least 24 weeks, under a variety of environmental situations, and did not produce excessive residues when applied at a rate of 5 ppm. The M-3409 formulation was effective, under different environmental situations, for at least 24 weeks, but produced excessive residues during one-third of the test period when dosed at a rate of 5 ppm.

Each of the polymer formulations of chlorpyrifos was effective in controlling mosquito larvae. However, the dosages used in the present study would be equivalent on a hectare basis, to the application of 153 kg of PVC-10, 152 kg of M-3409, or 131 kg of M-3570.

CONCLUSIONS

Single applications of WE-48 dosed at a rate of 0.009 ppm provided effective control of mosquito larvae for 1 to 2 weeks. Single applications of PVC-10 or M-3570, each dosed at a rate of 5 ppm, provided effective control of mosquito larvae for at least 24 weeks without producing excessive residues in any of the simulated aquatic environments treated. Single applications of M-3409, dosed at a rate of 5 ppm, provided effective control for at least 24 weeks, but produced excessive residues in some of the treated aquatic environments during 8 of the 24 weeks. The various simulated aquatic habitats had definite and consistent effects (disregarding statistical significance) on the average residues maintained by the polymer formulations. For each formulation, residues were consistently higher in shaded pools than in unshaded pools, and consistently lower in pools with soil than in those without soil. The polymer formulations could be used



Figs. 1-6.—Residue levels, percent mortality, water temperatures, and water volumes for test pools treated with various formulations of chlorpyrifos. 1. Average residue levels and percent mortality for WE-48 treatments. 2. Same, PVC-10 treatments. 3. Same, M-3570 treatments. 4. Same, M-3409 treatments. 5. Average water temperature for all pools. 6. Average water volume for all pools.

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 POOLS¹

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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 [0,0-diethyl 0-(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 [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate]. The effectiveness of these formula-

tions 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

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