POLYMER FORMULATIONS OF MOSQUITO LARVICIDES[1]

III. EFFECTS OF A POLYETHYLENE FORMULATION OF CHLORPYRIFOS ON NON-TARGET POPULATIONS NATURALLY INFESTING ARTIFICIAL FIELD POOLS

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ABSTRACT. Field tests were conducted in artificial pools for 13 weeks at Edgewood Arsenal, Maryland, to determine the effects of chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate], formulated as a slow-release polymer and as a water emulsion, on populations of nymphal and adult gerrids (*Gerris* spp.), adult midges (*Chironomus* spp. and *Calopsectra* spp.), larval chaoborids [*Chaoborus punctipennis* (Say)], and larval dytiscids (*Laccophilus fasciatus* Aube). In the pools treated with the water emulsion, a 5-week reduction in gerrid populations and a 2-week reduction in larval dytiscid populations were noted, while populations of adult chironomids and larval chaoborids appeared unaffected. The slow-release polymer appeared to reduce populations of gerrids and larval chaoborids for 9 weeks, while larval dytiscid populations showed a reduction for 11 weeks. The emerging adult chironomid populations were also reduced in pools treated with the slow-release polymer.

Studies by Wilkinson *et al.* (1971), Miller *et al.* (1973), and Roberts *et al.* (1973) showed that single applications of various slow-release formulations of chlorpyrifos [0,0-diethyl-(3,5,6-trichloro-2-pyridyl) phosphorothioate] could be used to effectively control mosquito larvae for periods up to 6 months. Although no quantitative data were presented, Wilkinson *et al.* (1971) indicated that the slow-release formulations of chlorpyrifos had less harmful effects on non-target organisms than did emulsifiable concentrate formulations of the same material. Similarly, McDonald and Dickens (1970) indicated that treatments with plaster briquettes containing chlorpyrifos were not harmful to aquatic insects other than midges and mosquitoes. This conclusion was based on observation rather than on the collection of quantitative data. Quantitative data were presented by Mulla (1970) to demonstrate that populations of several species of non-target aquatic insects were adversely affected by repeated applications of chlorpyrifos emulsifiable concentrate at mosquito larvicidal dosages. Most of the non-target populations thus affected (Mulla, 1970) made partial recoveries in the interim between treatments, and nearly complete recoveries following the cessation of treatments. The information presented by Mulla (1970) leaves little doubt as to the ecological effects of the application of emulsifiable concentrate formulations of chlorpyrifos. However, the work reported by Wilkinson *et al.* (1971) and McDonald and Dickens (1970) indicated that the use of the same material, chlorpyrifos, in a slow-release formulation has less effect on non-target populations. The present study, therefore, was initiated to compare natural populations of non-target insects in artificial pools treated with slow-release or emulsifiable concentrate formulations of chlorpyrifos.

MATERIALS AND METHODS

EXPERIMENTAL SETUP. The test area, artificial pools, insecticide formulations, and dosage levels were identical to those reported by Roberts *et al.* (1973).

BIOSSAYS. Laboratory bioassays of field-collected specimens were conducted to determine chlorpyrifos susceptibility levels

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for adult *Notonecta undulata* (Say) (Hemiptera, Notonectidae: backswimmers); larval and adult *Laccophilus fasciatus* Aube (Coleoptera, Dytiscidae: predaceous diving beetles); 4th instar larvae of *Chaoborus punctipennis* (Say) (Diptera, Chaoboridae: midges); nymphal and adult *Gerris* spp. (Hemiptera, Gerridae: water striders); adult *Sigara* spp. (Hemiptera, Notonectidae); and nymphal and adult *Velia* spp. (Hemiptera, Veliidae: "small water striders"). Laboratory susceptibility tests were also performed on both laboratory-reared and field-collected 4th instar larvae of *Culex pipiens quinquefasciatus* Say. All non-target insects, except *N. undulata* and *Gerris* spp., were exposed for 24 hours to replicate 250-ml aqueous solutions of each test concentration. Because of their larger physical size, the *N. undulata* and *Gerris* spp. were exposed for 24 hours to replicate 2000-ml aqueous solutions. To avoid possible toxicity due to the use of acetone or ethanol for dilutions, a primary standard of chlorpyrifos was prepared with acetone, and water dilutions of the standard were used to make all test concentrations. Data from laboratory bioassays were subjected to probit analysis to determine the LC_{90} of the test organisms.

SAMPLING OF TEST POOLS. Adult and immature aquatic insects were sampled using the following methods. A 125-mesh plankton net with a removable 23-ml vial at the tip was passed along the same side of each pool through approximately 66 liters of water per pool. Weekly samples were collected from 15 May to 15 September, preserved in formalin, and later identified in the laboratory. The four corners of each pool were sampled with a 440-ml enamel dipper. Samples were collected each week from 1 June to 14 August. Emerging adult insects were sampled each week with floating traps of the type described by Roberts *et al.* (1973). Surface-dwelling species that could not be readily collected were visually counted and their numbers recorded each week. Data for naturally-occurring non-target species were subjected to 2-way analysis of variance. The significance of differences attributable to insecticide treatment, week after treatment, or the interaction of these two factors, was determined by calculating a least significant difference (lsd) at the 0.05 level of probability (Steel and Torrie, 1960). Residue levels in the chlorpyrifos treated 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 as reported by Miller, *et al.* (1973). Sensitivity of the gas chromatograph used during the present study was 0.1 ppb chlorpyrifos.

RESULTS

INSECTICIDE RESIDUE LEVELS. The average chlorpyrifos residues in all pool types are summarized on a weekly basis (Figure 1) for comparison with the average weekly populations of non-target organisms. The average residues in pools treated with WE-48 were above levels toxic to mosquito larvae (0.9 ppb) for 2 weeks after treatment, while the residues in pools treated with M-3570 were at or above 0.9 ppb for 13 weeks following treatment.

POPULATION SUMMARIES. Preliminary examination of the data on non-target insects indicated that natural infestation of the test pools had not occurred to the extent anticipated. Generally, insect 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. This being the case, weekly populations of non-target insects were calculated to represent average numbers present in each of the three types of test pools. Gerrids (nymphal and adult *Gerris* spp.), adult chironomids (*Chironomus* spp., *Calopsectra* spp.), larval chaoborids (*C. punctipennis*), and larval dytiscids (*L. fasciatus*) were the only non-target insects whose

average populations in the three types of test pools were considered large enough to permit quantitative interpretation of the effects of the insecticide treatments. Average weekly populations of these four insects, as observed in all types of test pools are presented in Figures 2 through 9.

NYMPHAL AND ADULT GERRID POPULATIONS. Pretreatment gerrid populations were statistically similar in control pools and in pools scheduled to be treated with the various insecticide formulations. Following treatment, gerrid populations in pools treated with WE-48 (Figure 2) were lower than those in control pools for 4 weeks, although the reduction was not significant. Thereafter, populations in treated

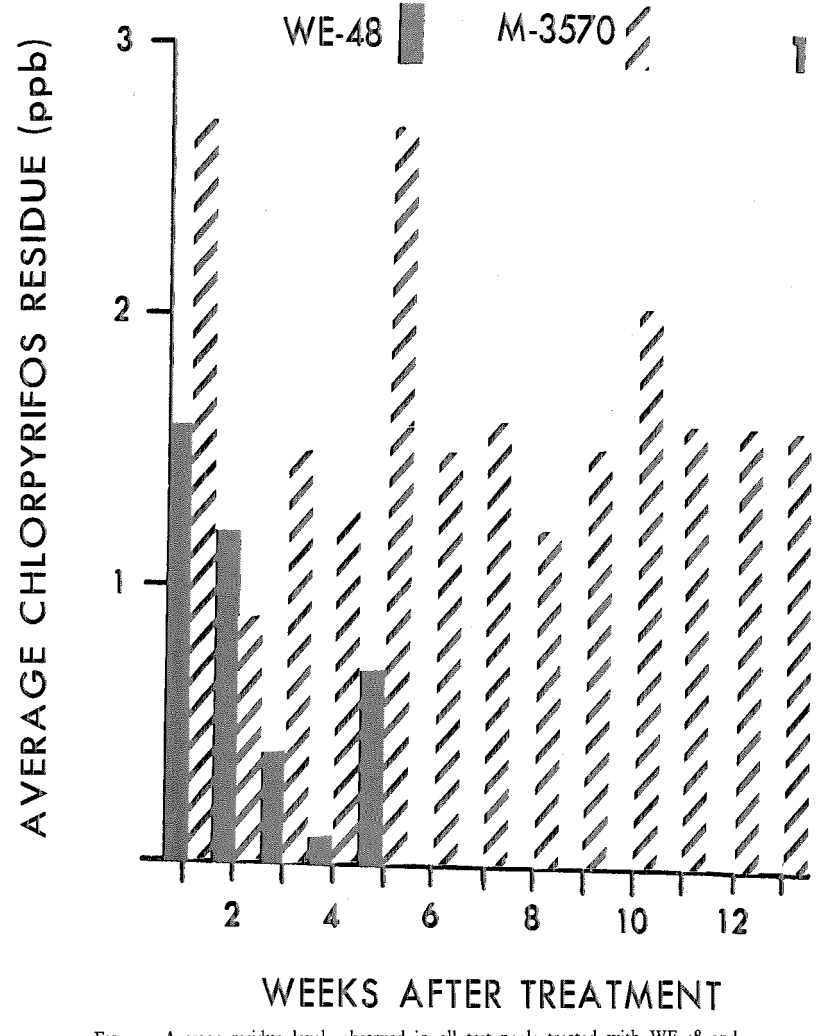
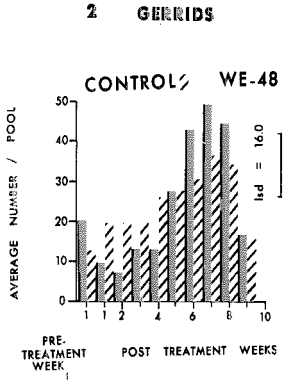
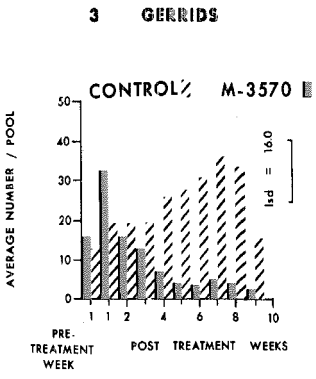


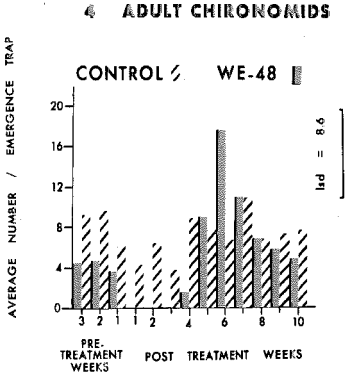
FIG. 1.—Average residue levels observed in all test pools treated with WE-48 and M-3570 formulations.

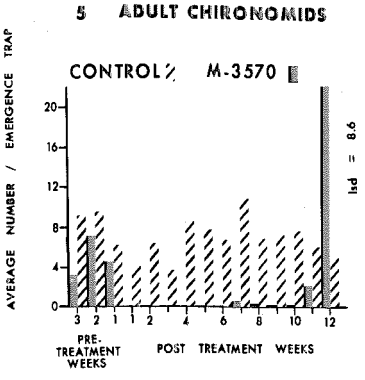
pools were equal to or greater than those in control pools. In pools treated with M-3570 (Figure 3), gerrid populations were below control pool populations by the 2nd week and remained significantly lower through the 8th week.

ADULT CHIRONOMID POPULATIONS. Adult chironomids emerging from control pools and from pools scheduled to be treated with the various insecticide formulations were statistically similar during the pre-treatment period. Following treatment with WE-48, adult chironomid emergence did not differ significantly from the number emerging from control pools through 10 weeks. Although there was an obvious population reduction for 4 weeks after treatment, the differences between treated and control pool populations were not significant. In pools treated with the M-3570 (Figure 5), the adult chironomid population was near zero for 10 weeks. However, due to fluctuations in the control pool populations, this reduction was statistically significant only at week 7. By week 12 the number of adults emerging







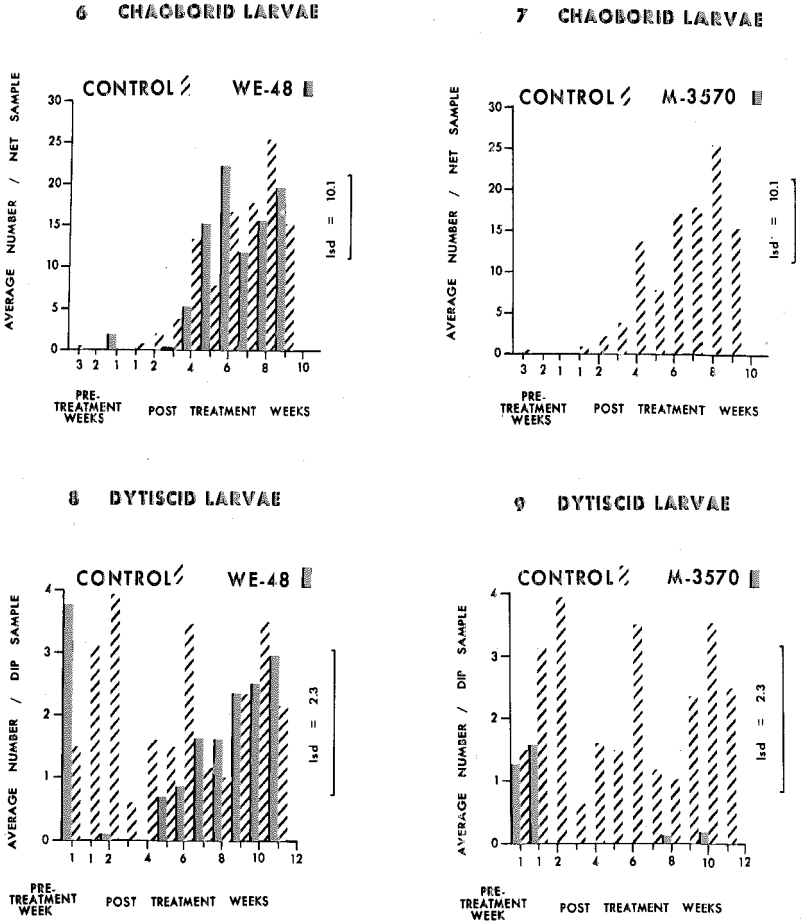


Figs. 2–5.—Non-target insects observed in test pools treated with WE-48 and M-3570 formulations. 2. Gerrids in WE-48 pools. 3. Same, M-3570 pools. 4. Adult chironomids in WE-48 pools. 5. Same, M-3570 pools.

from the treated pools increased and was significantly higher than that of control pools.

LARVAL CHAOBORID POPULATIONS. During the pretreatment period, larval chaoborid populations were near zero in control pools and all pools scheduled to be treated with insecticide. By the first week after treatment, the chaoborid population began to increase in control pools and reached a peak at the 8th week. During the same period (weeks 1 through 8) the chaoborid populations in pools treated with WE-48 (Figure 6) showed a similar increase and did not differ significantly from the control pool populations. In pools treated with M-3570 (Figure 7), the chaoborid populations remained at zero through the 9th week.

LARVAL DYTISCID POPULATIONS. During the pretreatment period, larval dytiscid populations in control pools were statistically similar to populations in pools scheduled for treatment with the M-3570



Figs. 6–9.—Non-target insects observed in test pools treated with WE-48 and M-3570 formulations. 6. Chaoborid larvae in WE-48 pools. 7. Same, M-3570 pools. 8. Dytiscid larvae in WE-48 pools. 9. Same, M-3570 pools.

formulation. Larval dytiscid populations in pools to be treated with WE-48 were significantly higher than control pool populations prior to treatment. Following treatment with WE-48 (Figure 8) the larval dytiscid population dropped off significantly for 2 weeks. Thereafter, the population in treated pools increased steadily through 11 weeks and did not differ from the control pool population. Following treatment with M-3570 (Figure 9) the larval dytiscid population was near zero for 11 weeks. However, due to fluctuations in the control pool populations, the number of larval dytiscids in treated pools was not always significantly lower than the controls.

INSECTICIDE SUSCEPTIBILITY TESTS. Susceptibility levels could not be determined for all species which were tested, either because a sufficient number of field-collected specimens could not be obtained at one time, or because the particular organisms did not survive in the laboratory long enough to complete the tests. For example, the nymphal and adult *Gerris* spp., *Sigara* sp., and *Velia* spp., are simply too active to survive or to be contained in the test vessels for the required time period. The larval *L. fasciatus* are cannibalistic, and even attempts to use as few as five larvae per replicate test resulted in significant mortality in the controls. Despite these difficulties, chlorpyrifos susceptibility levels were determined for a total of five of the species (Table 1).

The chlorpyrifos LC_{90} levels for *L. fasciatus* and *N. undulata* adults, and *C. punctipennis* larvae, were all significantly higher than the LC_{90} values for laboratory-reared and field-collected *C. p. quinquefasciatus* larvae. In addition to the laboratory tests discussed above, a field bioassay was conducted with nymphal and adult *Gerris* spp. by placing five insects in emergence traps on each of the pools treated with M-3570 and respective control pools. No mortality was observed during the 24-hour test period even though chlorpyrifos residents in the pools averaged 2.0 ppb.

DISCUSSION

In pools treated with the WE-48 there was an initial reduction in the numbers of gerrids, but the population in pools treated with M-3570 showed a significant reduction for 9 weeks. In-pool bioassays discussed above indicated that the insecticide concentration in pools treated with M-3570 was not toxic to the gerrids. Since the gerrids are surface-dwelling predators, having the ability as adults to fly from pool to pool, reductions in their populations may be a result of migration from the treated pools after prey populations were reduced by the insecticide treatment.

In pools treated with the WE-48, the emergence of adult chironomids was reduced for about 4 weeks after treatment, but overall the adult population was unaffected. In pools treated with the M-3570, the adult chironomid emergence was near zero for 11 weeks after treatment. However, the large emergence which occurred during the 12th week indicates that populations were not eliminated by treatment with M-3570. The emergence of the adults is in many cases an explosive phenomenon (Iovino and Miner, 1970) taking place during a relatively short period of time with no prior indication of its imminent occurrence. The fact that such large emergences were not observed in the control pools may be a reflection of the sampling technique that was used. All pools were monitored on a weekly rather than a daily basis, and some of the emergences could have been missed. Regardless of any possible shortcomings in sampling techniques, the important observation is that the adult chironomid populations were reduced but not eliminated in treated pools (M-3570) that contained chlorpyrifos concentrations which were toxic to the mosquito larvae.

Larval chaoborid populations appeared unaffected by treatment with the WE-48. In pools treated with the M-3570, establishment of the larval chaoborid population was suppressed through the 9th week. Susceptibility information on this insect

Table 1.—Chlorpyrifos susceptibility levels (ppb) of mosquito larvae and certain non-target organisms.

Insect	LC_{50}	LC_{90}
Culex p. quinquefasciatus larvae [a]	0.5	0.9
Culex p. quinquefasciatus larvae	1.0	1.5
Laccophilus fasciatus adults	2.1	5.2
Chaoborus punctipennis larvae	5.4	15.1
Notonecta undulata adults	35.2	48.8

[a] Laboratory-reared; all others field-collected.

(Table 1) showed the LC_{90} to be 15.1 ppb chlorpyrifos. The maximum residue in pools treated with M-3570 was 2.7 ppb (Figure 1), which is well below the LC_{90} level for chaoborid larvae. However, since these larvae do not have the ability to migrate from the pools, they may have succumbed to the continuous low-level chlorpyrifos residue which was present for 13 weeks following treatment.

In pools treated with WE-48, the dytiscid populations were reduced for 2 weeks, but eventually returned to control pool levels. The M-3570 eliminated the larval dytiscids during virtually all post treatment weeks. These larvae, like the chaoborids do not have the ability to leave the test pools, and their absence may also be attributable to a continuous low-level residue which was maintained for 13 weeks.

Most of the non-target organisms were present in the test pools in low numbers. In view of this, it would appear desirable to conduct future studies in natural habitats where adequate non-target organism populations already exist, or to allow artificial habitats to stabilize for more than a single season before testing. Additionally, rather than attempting to sample the entire biological community, certain selected "indicator" organisms, such as *Gerris* spp., *C. punctipennis* or *L. fasciatus* might be used.

For those non-target organisms whose populations were sufficiently high, several situations appear to have existed. In the case of some predatory species, such as the gerrids, their absence from the pools was perhaps a result of the elimination of an adequate prey population, rather than the toxic action of the insecticides. For other predatory and non-predatory species, such as the chaoborid, and dytiscid larvae that do not have the ability to migrate from the treated area, it appears the M-3570 treatment eliminated, or suppressed development of, their populations. However, since some are predators, a disruption in the food chain may have had a partial or total effect on these populations.

CONCLUSIONS

In pools treated with a single application of the WE-48 formulation, at a rate of 0.009 ppm, a 4-week population reduction of gerrids was noted as was a 2-week reduction of the larval dytiscid population. Populations of larval chaoborids and adult chironomids appeared unaffected. A single application of the M-3570 formulation, at a rate of 2.5 ppm, reduced or suppressed establishment of populations of gerrids and larval chaoborids for 9 weeks after treatment, while larval dytiscid populations were reduced for 11 weeks. This formulation may have affected the chironomid population. In general, the particular reason for any given population reduction, whether due to the toxic nature of the insecticide or to some indirect effect on the food chain, could not be clearly elucidated on the basis of these studies. However, it was evident the non-target populations included in the study were adversely affected in one way or another by treatment with the M-3570 formulation.

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POLYMER FORMULATIONS OF MOSQUITO LARVICIDES

IV. LARVICIDAL EFFECTIVENESS OF POLYETHYLENE AND POLYVINYL CHLORIDE FORMULATIONS OF CHLORPYRIFOS DURING AN 18-MONTH FIELD TEST[1]

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ABSTRACT. The long-term effectiveness of three polymer formulations of chlorpyrifos [0,0-diethyl o-(3,5,6-trichloro-2-pyridyl) phosphorothioate] was evaluated in artificial field pools at Edgewood Arsenal, Maryland. The formulations, designated M-3409, PVC-10, and M-3570, differed in percent composition, polymer carrier, specific gravity, and pellet size. Each was applied to the pools at a dosage of 5.0 ppm in May 1970 and chlorpyrifos residues maintained in the treated water were monitored monthly through October 1971. Single applications of the formulations resulted in the following 18-month average chlorpyrifos residues: 14.7 ppb for the M-3409; 1.5 ppb for the PVC-10; and 2.5 ppb for the M-3570. During 18 months in the pools, the formulations released the following percentages of the chlorpyrifos originally contained therein: 91.6 percent for the M-3409; 16.7 percent for the PVC-10; and 28.8 percent for the M-3570. Although each formulation was effective for at least an 18-month period covering two breeding seasons, the large amount of formulation (kg/hectare) required at the 5.0 ppm dosage may make them impractical for large-scale field use.

Studies by Miller, *et al* (1973), evaluated the larvicidal effectiveness of three polymer formulations of chlorpyrifos [0,0-diethyl o-(3,5,6-trichloro-2-pyridyl) phosphorothioate] applied to four types of artificial field pools. Each of the formulations (designated: M-3409, PVC-10, and M-3570) was determined to be effective for 6 months based on weekly in-pool bioassay with laboratory-reared mosquito larvae and weekly gas chromatographic residue analysis of the treated water. In the present study, the same artificial pools were monitored monthly for an additional 12 months by gas chromatographic residue analysis to determine if the three polymer formulations would be effective for a total period of 18 months, covering two mosquito breeding seasons.

METHODS AND MATERIALS. During the first 6 months of this study (May–October 1970), the artificial pools, polymer formulations of chlorpyrifos, and dosage levels were as reported by Miller, *et al.* (1973). During the last 12 months of the study (November 1970–October 1971), all shades

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