# POLYMER FORMULATIONS OF MOSQUITO LARVICIDES VI. LABORATORY AND FIELD EVALUATION OF SELECTED POLYETHYLENE FORMULATIONS OF CHLORPYRIFOS [1]

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ABSTRACT. Laboratory evaluations were conducted for 8 weeks to compare residue levels maintained in water treated with eight chlorinated polyethylene pellet formulations of chlorpyrifos [o,o-diethyl-o (3,5,6-trichloro-2-pyridyl) phosphorothioate]. The eight formulations were of four particle sizes, each at two chlorpyrifos concentrations. All pellets were cylindrical in shape, had a specific gravity >1.0, and each was evaluated at dosages of 1.0 ppm and 2.0 ppm in laboratory test jars. Two of the eight formulations were evaluated for 18 weeks, at dosages of 0.1 ppm, 0.5 ppm, and 1.0 ppm, in artificial field pools. In the laboratory tests, average weekly residues for all formulations increased significantly through week 4, leveled off through week 7, and showed a second significant increase at week 8; and the 2.0 ppm dosage resulted in significantly higher residues than the 1.0 ppm dosage, except at week 1 after treatment. For each formulation there were no significant differences due to percent chlorpyrifos therein. In the field tests, both formulations were ineffective at the 0.1 ppm dosage, and only marginally effective at the 0.5 ppm dosage. Both were considered effective at the 1.0 ppm dosage, both from the standpoint of larvicidal activity and relative total amounts of formulation which would be required on a hectare basis.

Studies reported by Wilkinson *et al.* (1971), Miller *et al.* (1973a, 1973b), and Roberts *et al.* (1973) have demonstrated the feasibility of obtaining long-term control of larval mosquito populations slow-release formulations of chlorpyrifos through single applications of various slow-release formulations of chlorpyrifos [o,o-diethyl-o-(4,5,6-trichloro-2-pyridyl) phosphorothioate]. In all of these studies, however, the large amount of formulation (kg/hectare) required for control may be prohibitive from the standpoint of large-scale larviciding operations. Therefore, tests were conducted to explore ways of improving the efficiency of chlorinated polyethylene formulations of chlorpyrifos, and thus diminishing the amount of formulation required on a hectare basis.

Stockman *et al.* (1970) reported the theoretical effects of pellet size on the release rate of tributyltin oxide from rubber, and showed that the concentration released from formulations of different pellet size increased geometrically with decreasing pellet size (i.e., increasing surface to volume ratio).[5] Stockman *et al.* (1970) also reported a linear relationship between percent concentration of pesticide in a polymer and release rate. Studies conducted at this Agency (Nelson *et al.*, 1970) showed that increases in percent composition of polyvinyl chloride formulations of chlorpyrifos caused a nearly linear increase in release rates, and residue levels maintained in water. Additional studies were conducted at this Agency to determine if the same theoretical relationships applied to chlorinated polyethylene formulations of chlorpyrifos. Laboratory studies were undertaken to determine the effect of particle size, percent composition, and dosage on the efficiency of chlorinated polyethylene formulations

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[5] Surface to volume ratio: hereinafter referred to as S/V.

of chlorpyrifos in maintaining residues in water, and field studies were conducted to determine the larvicidal effectiveness of chlorinated polyethylene formulations of chlorpyrifos applied at various dosages which might be acceptable from the standpoint of large-scale larviciding.

## MATERIALS AND METHODS

FORMULATIONS. Eight chlorinated polyethylene pellet formulations (4 particle sizes, each at two concentrations of chlorpyrifos) were evaluated in this study. All pellets were cylindrical in shape and had specific gravities >1.0. Other pertinent physical characteristics are shown in Table 1. The two formulations with the largest particle size (M–3460 and DB–1–4758–114) were evaluated in both laboratory and field tests, since the method used in their manufacture was such that larger quantities could be provided for field testing, should they prove to be effective. The other six formulations, all of smaller particle size, were suitable for the laboratory tests concerning effects of particle size and percent composition on residues maintained in water. However, their method of preparation was such that the production of other than relatively small test quantities was not practical.

LABORATORY TESTS. The eight formulations evaluated in the laboratory were tested at dosages of 1.0 and 2.0 ppm active ingredient based on a theoretical total initial release. Treatments were replicated three times in individual glass jars containing 3 liters of distilled water (pH 6.5) at room temperature (22.2° C). Three replicate jars containing only distilled water served as controls. Jars were sealed with aluminum foil liners and screw-cap lids to minimize loss of water due to evaporation.

Water samples (10 ml), water temperature, and pH measurements were taken from 1 through 8 weeks after treatment. Each 10-ml water sample was subjected to hexane extraction and electron capture gas chromatographic analysis as described by Miller *et al.* (1973). The minimum detectable quantity measured in the water during the present study was 0.1 ppb chlorpyrifos.

FIELD TESTS. The two formulations evaluated in the field were each tested at dosages of 0.1, 0.5, 1.0 ppm active ingredient (Table 2) in 378-liter, galvanized metal pools measuring approximately 1.2 x 0.6 x 0.6 meters (L x W x H). Each pool was lined with black polyethylene and raised 0.2 meter above the ground on four cinder blocks. The pools were filled with approximately 190 liters of hydrant water. Five cm of soil similar to that used by Miller *et al.* (1973a), were placed on the bottom of each pool and all pools were allowed to stand for 4 weeks before treatments were applied. Twenty such pools, located at Aberdeen Proving Ground, were randomly treated as follows: 9 treated with M-3460 (3 dosages x 3 replicates); 9

TABLE 1.—Physical characteristics of chlorinated polyethylene pellet formulations containing chlorpyrifos.

| Formulation[a] | Percent chlorpyrifos | Diameter (mm) | Length (mm) | Volume ($mm^3$) | Surface ($mm^2$) | S/V |
|---|---|---|---|---|---|---|
| M–3460 | 9.4 | 1.59 | 3.17 | 6.37 | 19.92 | 3.12 |
| DB–1–4758–114 | 15.0 | 1.59 | 3.17 | 6.37 | 19.92 | 3.12 |
| DB–4758–116A | 9.4 | 1.59 | 1.59 | 3.19 | 11.98 | 3.75 |
| DB 4758–116D | 15.0 | 1.59 | 1.59 | 3.19 | 11.98 | 3.75 |
| DB–4758–116B | 9.4 | 1.59 | 1.02 | 2.02 | 9.12 | 4.50 |
| DB–4758–116E | 15.0 | 1.59 | 1.02 | 2.02 | 9.12 | 4.50 |
| DB–4758–116C | 9.4 | 1.59 | 0.79 | 1.58 | 7.96 | 5.00 |
| DB–4758–116F | 15.0 | 1.59 | 0.79 | 1.58 | 7.96 | 5.00 |

[a] Coded designations provided by Dow Chemical Company, Midland, Michigan.

TABLE 2.—Dosages for M-3460 and DB-1-4758-114 applied to artificial field pools.

| Dosage (ppm) | Equivalent kg formulation/hectare | | Equivalent kg chlorpyrifos/hectare | |
|---|---|---|---|---|
| | M-3460 | DB-1-4758-114 | M-3460 | DB-1-4758-114 |
| 0.1 | 2.7 | 1.7 | 0.24 | 0.24 |
| 0.5 | 13.5 | 8.4 | 1.26 | 1.26 |
| 1.0 | 27.1 | 16.9 | 2.54 | 2.54 |

treated with DB-1-4758-114 (3 dosages x 3 replicates); and 2 untreated control pools.

In-pool bioassays were conducted weekly, using 4th instar laboratory-reared larvae of *Culex pipiens quinquefasciatus* Say, using the techniques described by Miller *et al.* (1973a). Water volumes were calculated and recorded weekly. Water temperatures were measured each week approximately 10 cm below the surface of the center of the pool. Water samples (10-ml) for residue analysis were collected weekly from treated and control pools. Collections were taken approximately 10 cm below the surface at the center of the pool and immediately returned to the laboratory for extraction and analysis by the same procedures described by Miller *et al.* (1973a).

Although populations of naturally-occurring mosquitoes were not monitored during the entire study, natural larval populations were sampled with an enamel dipper 1 week before and 1 week after treatment to determine the immediate effects of the treatments. Control of mosquito larvae was considered to have failed on any given week if bioassay mortality from three replicate treatments averaged <90 percent. Residue levels were considered inadequate for control if <0.9 ppb, the established $LC_{90}$ for 4th instar laboratory-reared larvae of *C. p. quinquefasciatus* (Miller *et al.*, 1973a).

## RESULTS

LABORATORY TESTS—FORMULATIONS AND DOSAGES. Results of treatments with the series of 9.4 percent chlorpyrifos formulations are shown in Table 3. At both dosages, average 8-week residues increased with each increase in S/V. At the 1.0 ppm dosage, the 9.4 percent formulations with S/V equal to 3.75 and 4.50 produced average 8-week residues which did not differ from each other, but which were significantly higher than those produced by the 9.4 percent formulations with S/V equal to 3.12. The 9.4 percent formulation with S/V equal to 5.00 produced an average 8-week residue which was significantly higher than all other formulations. At the 2.0 ppm dosage, the 9.4 percent formulations with S/V equal to 3.12, 2.75, and 4.50, showed average 8-week residues which increased significantly with each increase in S/V. The average 8-week residue for the 9.4 percent formulation with S/V equal to 5.00 was significantly higher than all other 9.4 percent formulations, except the one with S/V equal to 4.50.

Results of treatments with the series of 15.0 percent chlorpyrifos formulations are shown in Table 3. Each increase in S/V resulted in an increase in average 8-week residue. At the 1.0 ppm dosage, the 15.0 percent formulations with S/V equal to 3.12, 3.75, and 4.50 produced average 8-week residue which increased significantly with each increase in SV. The average 8-week residue for the 15.0 percent formulation with S/V equal to 5.00 was significantly higher than all other 15.0 percent formulations, except the one with S/V equal to 4.50. At the 2.0 ppm dosage, average 8-week residues increased significantly with each increase in S/V.

For each formulation (both the 9.4

TABLE 3.—Effects of formulation and dosage on 8-week average residues maintained in water treated with polymer formulations of chlorpyrifos.

| | 8-week average residue[b] | | |
|---|---|---|---|
| Formulation[a] | 1.0 ppm dosage | 2.0 ppm dosage | S/V |
| | | 9.4 percent series | |
| M-3460 | 38.0 ab | 57.8 cd | 3.12 |
| DB-4758-116A | 57.3 cd | 91.3 f | 3.75 |
| DB-4758-116B | 64.4 de | 115.6 h | 4.50 |
| DB-4758-116C | 104.1 g | 122.1 hi | 5.00 |
| | | 15.0 percent series | |
| DB-1-4758-114 | 32.2 a | 43.9 b | 3.12 |
| DB-4758-116D | 48.4 bc | 72.2 e | 3.75 |
| DB-4758-116E | 69.4 e | 93.7 fg | 4.50 |
| DB-4758-116F | 71.7 e | 126.8 i | 5.00 |

[a] Coded designations provided by Dow Chemical Company.
[b] Values represent ppb chlorpyrifos; those followed by a common letter do not differ significantly at the 0.01 level of probability.

and the 15.0 percent series) the average 8-week residue at the 2.0 ppm dosage was significantly above the average for the 1.0 ppm dosage.

LABORATORY TESTS—PERCENT COMPOSITION. At equivalent pellet sizes, the average 8-week residues produced by the 9.4 percent and 15.0 percent formulations did not differ significantly from each other (Table 4). However, at each pellet size, the average residue for the 15.0 percent formulations was always below that of the 9.4 percent formulations.

LABORATORY TESTS—WEEKS AFTER TREATMENT. Effects of week after treatment on average residue levels are shown in Table 5. For each dosage, average weekly residues increased significantly through week 4, then leveled off through week 7. A second significant increase occurred at week 8 for both dosages. The average 8-week residues for the 1.0 and 2.0 ppm dosages (60.7 ppb and 90.4 ppb, respectively) differed significantly. The average weekly residues for the 1.0 and 2.0 ppm dosages differed from each other during all weeks except week 1. Specific average weekly residues maintained by each formulation, at each dosage, are shown in Figures 1 and 2.

FIELD TESTS—M-3460. Pretreatment sampling of pools scheduled for treatment with M-3460 showed an average of >25 naturally occurring *Culex restuans* Theobald larvae per dip. The natural larval population was eliminated by treatment

TABLE 4.—Effects of percent composition on 8-week average residues maintained in water treated with polymer formulations of chlorpyrifos.

| Formulation[a] | Percent chlorpyrifos | S/V | 8-week average residue[b] |
|---|---|---|---|
| M-3460 | 9.4 | 3.12 | 47.9 a |
| DB-4758-114 | 15.0 | 3.12 | 38.0 a |
| DB-4758-116A | 9.4 | 3.75 | 74.3 b |
| DB-4758-116D | 15.0 | 3.75 | 60.3 ab |
| DB-4758-116B | 9.4 | 4.50 | 90.1 cd |
| DB-4758-116E | 15.0 | 4.50 | 81.6 bc |
| DB-4758-116C | 9.4 | 5.00 | 113.1 d |
| DB-4758-116F | 15.0 | 5.00 | 99.2 cd |

[a] Coded designations provided by Dow Chemical Company.
[b] Values represent ppb chlorpyrifos; those followed by a common letter do not differ significantly at the 0.01 level of probability.

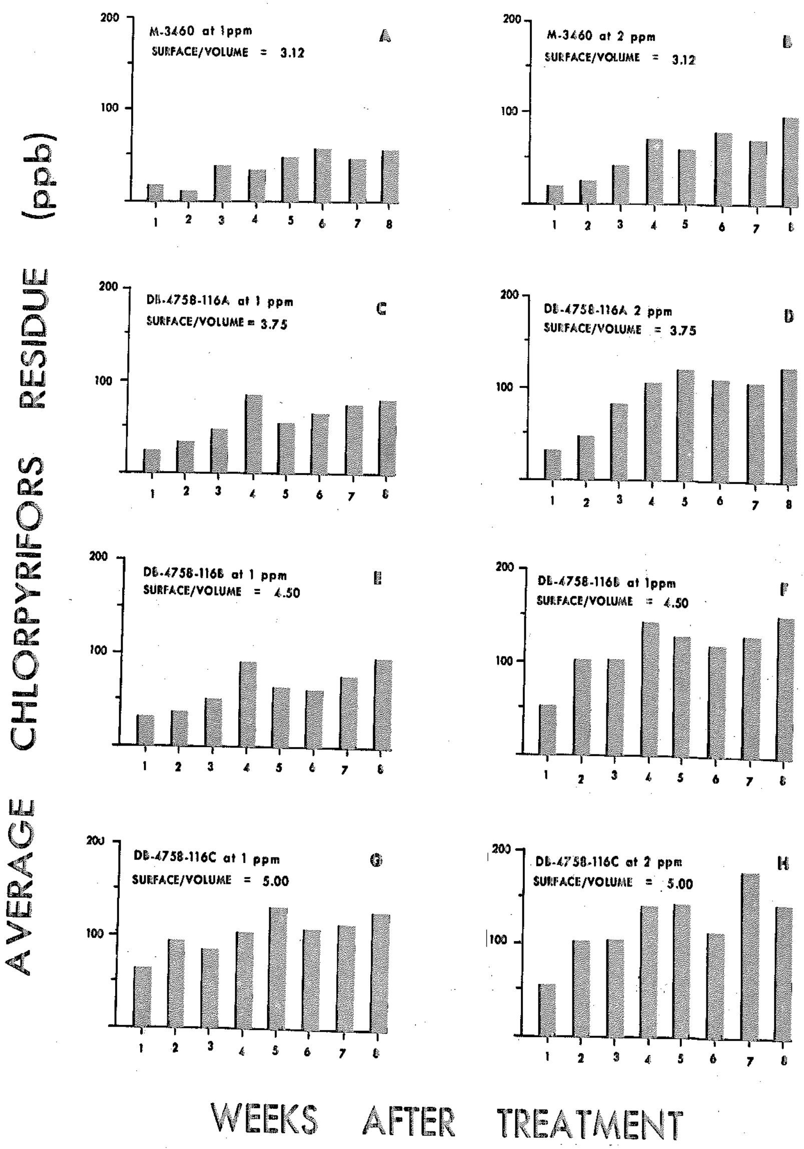


FIG. 1.—Average weekly residues observed in laboratory jars dosed at 1.0 ppm or 2.0 ppm with chlorinated polyethylene pellets containing 9.4% chlorpyrifos and having various surface to volume ratios.

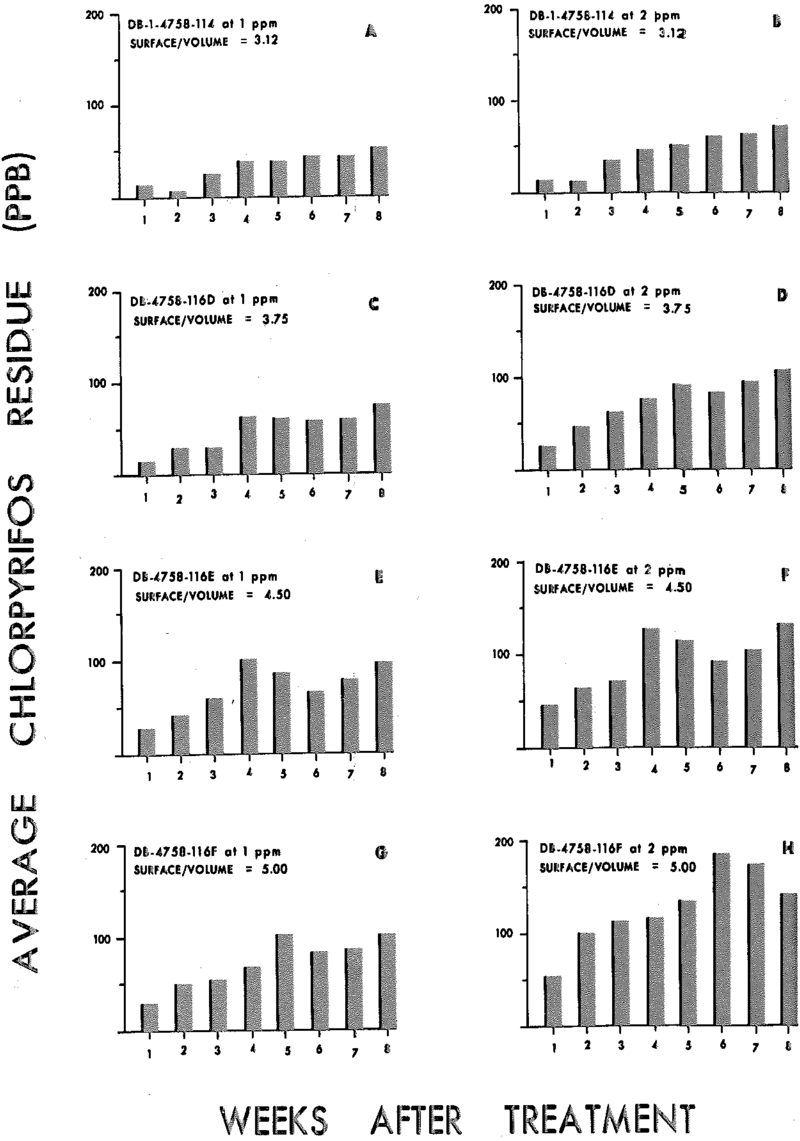


FIG. 2.—Average weekly residues observed in laboratory jars dosed at 1.0 ppm or 2.0 ppm with chlorinated polyethylene pellets containing 15.0% chlorpyrifos and having various surface to volume ratios.

TABLE 5.—Effects of week after treatment on average residues maintained in water treated with polymer formulations of chlorpyrifos.

| Week after treatment | Average chlorpyrifos residue (ppb) [a] | |
|---|---|---|
| | 1.0 ppm dosage | 2.0 ppm dosage |
| 1 | 28.0 a | 37.0 ab |
| 2 | 38.0 ab | 62.0 c |
| 3 | 47.8 b | 76.2 de |
| 4 | 72.5 cd | 103.3 f |
| 5 | 73.8 d | 104.7 f |
| 6 | 68.3 cd | 104.8 f |
| 7 | 72.0 cd | 114.6 fg |
| 8 | 85.1 e | 120.8 g |
| Average [b] | 60.7 | 90.4 |

[a] Averages followed by a common letter do not differ significantly at the 0.01 level of probability.

[b] These averages (60.7 and 90.4) differ significantly at the 0.01 level of probability.

with this formulation at all dosages, and no *C. restuans* larvae were observed during the first week posttreatment. At the 1.0 ppm dosage, the M–3460 treatments were monitored for 18 weeks after treatment (Table 6). During this period, the average chlorpyrifos residues were >0.9 ppb during all weeks, except weeks 5, 8, 11, and 12. Weekly bioassay mortalities were >90 percent during all weeks, except week 17. At the 0.5 ppm dosage, the M–3460 treatments were also monitored for 18 weeks (Table 6). During this period, the average chlorpyrifos residue was 0.9 ppb, while the average bioassay mortality was 85 percent. Weekly residues were <0.9 ppb during 9 of the 18 weeks, and weekly bioassay mortalities were <90 percent during 8 of the 18 weeks. At the 0.1 ppm dosage, the M–3460 treatments were monitored for 8 weeks (Table 6). During this period, the chlorpyrifos residue was <0.1 ppb, while average bioassay mortality was 5.0 percent.

FIELD TESTS—DB–1–4758–114. Pretreatment sampling of pools scheduled for treatment with DB–1–4758–114 showed an average of >25 *C. restuans* larvae per dip. This formulation, at all dosages, eliminated the natural mosquito larvae population through posttreatment week 1. At the 1.0 ppm dosage, the DB–1–4758–114 treatments were monitored for 18 weeks (Table 6). During this period the average chlorpyrifos residue was 1.3 ppb, and the average bioassay mortality was 97.6 percent. Weekly residues were >0.9 ppb during all weeks, except 5, 8, and 11, and weekly bioassay mortality was >90 percent during all weeks, except 13 and 17. At the 0.5 ppm dosage the treatments were also monitored for 18 weeks (Table 6). During this period the average chlorpyrifos residue was 0.7 ppb, and the average bioassay mortality was 83.9 percent. Weekly residues were <0.9 ppb during 14 of the 18 weeks and weekly bioassay mortality was <90 percent during 6 of the 18 weeks. At the 0.1 ppm dosage, the DB–1–4758–114 treatments were monitored for 8 weeks (Table 6). During this period, the average chlorpyrifos residue was <0.1 ppb, while average bioassay mortality was 6.6 percent.

## DISCUSSION AND CONCLUSIONS

For the formulations evaluated in the laboratory, average chlorpyrifos residues maintained in water increased significantly with time after treatment, and significantly higher chlorpyrifos residue occurred in the water whenever dosage or S/V were increased. The effects of increasing S/V were in agreement with the theoretical considerations discussed earlier (Stockman *et al.*, 1970). The increases in residues which occurred with increased dosage were also expected. The fact that the 9.4 percent formulations consistently maintained residues greater than those for the 15.0 percent formulations may be explained on the basis of the formulations not differing sufficiently in percent composition. At equivalent particle sizes and dosages (i.e., active ingredients) the total amount of 15.0 percent formulation added to the test jars was approximately half the total amount added for the 9.4 percent formulation. This means that the total surface area of the 15.0 percent treatments was also half that of comparative 9.4

TABLE 6.—Larvicidal effectiveness of M–3460 and DB–1–4758–114 applied to artificial field pools at dosages of 1.0, 0.5, and 0.1 ppm.[a]

| Weeks after treatment | 24-hour percent mortality | Insecticide residue (ppb)[b] | Volume of water (liters) | 24-hour percent mortality | Insecticide residue (ppb)[b] | Volume of water (liters) | 24-hour percent mortality | Insecticide residue (ppb)[b] | Volume of water (liters) | Water temperature (° C) |
|---|---|---|---|---|---|---|---|---|---|---|
| | M–3460 at 1.0 ppm | | | M–3460 at 0.5 ppm | | | M–3460 at 0.1 ppm[c] | | | |
| 1 | 100 | 1.6 | 193 | 83 | 0.8 | 189 | 0 | 0.2 | 173 | 27.6 |
| 2 | 100 | 2.0 | 164 | 100 | 0.8 | 173 | 0 | <0.1 | 158 | 26.0 |
| 3 | 100 | 1.8 | 164 | 100 | 0.9 | 173 | 3 | 0.3 | 158 | 26.0 |
| 4 | 100 | 2.1 | 142 | 100 | 1.0 | 142 | 17 | 0.2 | 142 | 25.5 |
| 5 | 100 | 0.3 | 245 | 93 | 0.5 | 252 | 0 | 0.1 | 252 | 26.0 |
| 6 | 100 | 1.3 | 261 | 100 | 0.8 | 268 | 0 | <0.1 | 268 | 27.0 |
| 7 | 100 | 1.3 | 252 | 97 | 0.9 | 252 | 0 | <0.1 | 252 | 24.5 |
| 8 | 100 | 0.3 | 241 | 93 | 0.2 | 236 | 20 | <0.1 | 236 | 25.0 |
| 9 | 100 | 1.9 | 299 | 83 | 0.8 | 315 | .. | ... | ... | 24.0 |
| 10 | 100 | 1.2 | 283 | 80 | 0.9 | 284 | .. | ... | ... | 25.1 |
| 11 | 100 | 0.6 | 241 | 70 | 0.6 | 315 | .. | ... | ... | 22.5 |
| 12 | 100 | 0.8 | 308 | 57 | 0.9 | 331 | .. | ... | ... | 27.6 |
| 13 | 93 | 1.4 | 308 | 43 | 1.0 | 315 | .. | ... | ... | 17.5 |
| 14 | 100 | 1.8 | 315 | 66 | 1.3 | 315 | .. | ... | ... | 19.8 |
| 15 | 100 | 1.2 | 335 | 100 | 0.8 | 347 | .. | ... | ... | 15.6 |
| 16 | 93 | 1.3 | 319 | 100 | 1.0 | 347 | .. | ... | ... | 12.1 |
| 17 | 57 | 3.0 | 362 | 77 | 1.9 | 362 | .. | ... | ... | 15.6 |
| 18 | 93 | 1.1 | 351 | 93 | 0.8 | 362 | .. | ... | ... | 19.4 |
| Average | 96.4 | 1.4 | 265.7 | 85.0 | 0.9 | 277.0 | 5.0 | <0.1 | 205.0 | 22.6 |

TABLE 6.—*Continued*

| Weeks after treatment | 24-hour percent mortality | Insecticide residue (ppb) [b] | Volume of water (liters) | 24-hour percent mortality | Insecticide residue (ppb) [b] | Volume of water (liters) | 24-hour percent mortality | Insecticide residue (ppb) [b] | Volume of water (liters) | Water temperature (° C) |
|---|---|---|---|---|---|---|---|---|---|---|
| | DB–1–4758–114 at 1.0 ppm | | | DB–1–4758–114 at 0.5 ppm | | | DB–1–4758–114 at 0.1 ppm [c] | | | |
| 1 | 100 | 1.3 | 173 | 93 | 0.7 | 173 | 0 | 0.2 | 173 | 27.6 |
| 2 | 100 | 1.0 | 173 | 90 | 0.4 | 158 | 3 | <0.1 | 158 | 26.0 |
| 3 | 100 | 1.8 | 173 | 90 | 0.8 | 158 | 0 | <0.1 | 158 | 26.0 |
| 4 | 100 | 1.6 | 142 | 100 | 0.7 | 142 | 33 | 0.1 | 142 | 25.5 |
| 5 | 100 | 0.6 | 252 | 93 | 0.5 | 252 | 3 | <0.1 | 252 | 26.0 |
| 6 | 100 | 1.2 | 268 | 100 | 0.7 | 268 | 0 | 0.3 | 252 | 27.0 |
| 7 | 100 | 1.0 | 252 | 100 | 0.6 | 252 | 0 | 0.1 | 252 | 24.5 |
| 8 | 100 | 0.2 | 236 | 100 | 0.1 | 236 | 17 | 0.1 | 221 | 25.0 |
| 9 | 100 | 1.4 | 299 | 97 | 1.8 | 315 | .. | ... | ... | 24.0 |
| 10 | 100 | 0.9 | 284 | 95 | 0.4 | 284 | .. | ... | ... | 25.1 |
| 11 | 100 | 0.8 | 315 | 63 | 0.5 | 315 | .. | ... | ... | 22.5 |
| 12 | 100 | 1.3 | 315 | 100 | 0.6 | 315 | .. | ... | ... | 27.6 |
| 13 | 87 | 1.6 | 299 | 40 | 1.5 | 315 | .. | ... | ... | 17.5 |
| 14 | 100 | 2.1 | 315 | 67 | 1.2 | 315 | .. | ... | ... | 19.8 |
| 15 | 100 | 1.4 | 347 | 73 | 0.6 | 347 | .. | ... | ... | 15.6 |
| 16 | 100 | 1.6 | 331 | 43 | 1.2 | 331 | .. | ... | ... | 12.1 |
| 17 | 80 | 1.2 | 347 | 67 | 0.7 | 347 | .. | ... | ... | 15.6 |
| 18 | 90 | 1.4 | 347 | 100 | 0.8 | 362 | .. | ... | ... | 19.4 |
| Average | 97.6 | 1.3 | 270.4 | 83.9 | 0.7 | 271.4 | 6.6 | <0.1 | 201.0 | 22.6 |

[a] Values for percent mortality, insecticide residue, and water volume represent the average of 3 replicates. Values for water temperature represent the average water temperature in six randomly-selected pools.
[b] Values represent ppb chlorpyrifos.
[c] Tests at this dosage terminated at 8 weeks.

percent treatments. As discussed earlier, increases in surface area result in geometric increases in release rate, while increases in percent composition result in linear increases in release rate. It appears that, with the two percentages evaluated in this study, relative surface area was more influential than relative percent composition. It also appears, on a theoretical basis, that increases in percent composition would not result in correspondingly higher residues, unless formulations were dosed on the basis of equivalent total amounts rather than equivalent active ingredients. The M-3460 and DB-1-4758-114 formulations were each effective as larvicides for 18 weeks in the field when applied at a dosage of 1.0 ppm. Both formulations were marginally effective at the 0.5 ppm dosage and ineffective at the 0.1 ppm dosage. The relative amounts of total formulation required at the 1.0 ppm dosage, 27.1 kg per hectare for the M-3460 formulation and 16.9 kg per hectare for the DB-1-4758-114 formulation, would be acceptable for large-scale field larviciding.

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