POLYMER FORMULATIONS OF MOSQUITO LARVICIDES V. EFFECTS OF CONTINUOUS LOW-LEVEL CHLORPYRIFOS RESIDUES ON THE DEVELOPMENT OF *CULEX PIPIENS QUINQUEFASCIATUS* SAY POPULATIONS IN THE LABORATORY [1]

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ABSTRACT. Laboratory tests were conducted to determine the effects of chlorpyrifos [0,0-diethyl o-(3,5,6-trichloro-2-pyridyl) phosphorothioate] on the development of populations of *Culex pipiens quinquefasciatus* Say when such populations were exposed to various continuous low-level concentrations from the time of hatching until adult emergence. The low-level concentrations were maintained below the LC_{90} (0.9 ppb) by treating laboratory test jars with various numbers of polyvinyl chloride (PVC) pellets containing 0.1 percent chlorpyrifos. Control of the laboratory populations averaged 47.3 percent in jars containing average chlorpyrifos residues of <0.10 ppb, 76.2 percent in jars containing average residues of 0.14 ppb, and 99.7 percent in jars containing average residues of 0.23 ppb. These levels of control were higher than expected, based on established dosage-mortality data for 4th instar larvae of the laboratory colony of *C.p. quinquefasciatus* used in the tests. Although control of the laboratory populations was achieved at levels below the established LC_{90} (0.9 ppb), the continuous low-level residues (<0.10 —0.23 ppb) were not considered sufficiently low to be significant from the standpoint of possibly reducing necessary field dosages.

In the field studies reported by Wilkinson *et al.* (1971) and Miller *et al.* (1973) mosquito larvae were effectively controlled for extended periods of time by polymer formulations of chlorpyrifos [0,0-diethyl o-(3,5,6-trichloro-2-pyridyl) phosphorothioate] which maintained continuous residues at or abive the LC_{90} of the test insects.

The biological effectiveness of residues maintained continuously at or above the LC_{90} level was determined by 24-hour bioassay with 4th instar mosquito larvae. This means of biological evaluation provided no information on the possible

The biological effectiveness of residues on other life stages, such as eggs or first instar larvae, and more importantly, no information on possible chronic effects of residues maintained continuously at a level below the LC_{90}. Such information was considered valuable from the standpoint of possibly reducing field dosages to a level where control if mosquito larvae could be achieved by chronic rahter than acute toxicity.

Tests were initiated to determine the effects of continuous low-level chlorpyrifos concentrations on the development of mosquito populations in the laboratory. The use of the term "low-level" hereinafter refers to chlorpyrifos residues below the LC_{90} of the test population (i.e., <0.9 ppb). Below this level, specific chlorpyrifos residues were determined either by bioassay with mosquito larvae or by gas chromatographic analysis.

The tests were of two types. Initially, it was not known whether continuous low-level chlorpyrifos concentrations ($<LC_{90}$) would have a significant biological effect on the development of *Culex pipiens quinquefasciatus* Say populations. Therefore, preliminary tests were conducted to determine the magnitude of a biological response by such populations when reared in water known to contain low-level

[1] The opinions contained herein are those of the authors and should not be construed as official or reflecting the view 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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chlorpyrifos residues. Once a biological response was demonstrated, additional tests were conducted to compare chlorpyrifos residue levels with biological response. The purpose of these tests was to evaluate the effect of chlorpyrifos on the development of populations of *C. p. quinquefasciatus* when such populations were exposed to continuous low-level concentrations in the laboratory from the time of hatching until adult emergence.

MATERIALS AND METHODS

TEST FORMULATION. The polymer formulation used to maintain continuous low-level residues during this study consisted of polyvinyl chloride (PVC) pellets containing 0.1 percent analytical grade chlorpyrifos. The pellets weighed an average of 84.0 mg, had a specific gravity >1.0, and were prepared at this Agency according to the methods reported by Miller *et al.* (1973).

PRELIMINARY BIOLOGICAL RESPONSE TESTS. Eighteen glass jars, each containing 3000 ml of distilled water, were set up in an environmental chamber that maintained the water temperature at 27.5° C. Dosages for the various test jars are shown in Table 1.

Following treatment, the water in each of the jars was bioassayed weekly with 20 fourth instar *C. p. quinquefasciatus* larvae. When mortality was no longer observed in the bioassay, the jars were considered to contain low-level chlorpyrifos residues.[4] *C. p. quinquefasciatus* egg rafts (each <12 hours old and containing a known number of eggs) were added to the jars, along with small quantities of ground Wayne® Rabbit Ration pellets. Thereafter, small quantities of ground or whole rabbit pellets were added daily throughout the study.

Since all of the eggs in a given egg raft were not necessarily fertile, a determination of the number of eggs per raft was not always a valid measure of the number of larvae that actually hatched into the test jars. Therefore, the following method was used to estimate the number of larvae hatching into the test jars. The total number of eggs in each of the rafts was counted. The egg rafts were placed in the test jars and kept there for 48 hours to permit hatching, since it was known that hatching occurred subsequent to the 36 hours after oviposition. The rafts were then removed from the jars and the total number of eggs recounted. This second count was necessary because in some instances eggs became detached from the rafts and the total raft was not retrieved from the jars. The percent hatch, therefore, was based on the number hatched divided by the total number from the second counting, and the number of larvae hatched into the jars was estimated by multiplying the total number of eggs originally in the raft by the percent hatch.

[4] Since 20 larvae were used in each replicate test jar, the minimum detectable mortality was 5.0% (LC_{05}).

TABLE 1.—Dosages for test jars used to determine preliminary biological response.

Type of test jar	Average weight of pellets[a] (mg)	Average weight of chlorpyrifos[b] (mg)	Average dosage (ppm chlorpyrifos)
Control w/32 pellets	2695.5	...	...
1 pellet	82.5	.08	.02
4 pellets	334.4	.33	.11
8 pellets	672.9	.67	.22
16 pellets	1366.4	1.36	.45
32 pellets	2698.4	2.69	.89

[a] Average weight of the total number of pellets added to each of three replicate jars.
[b] Average weight of chlorpyrifos contained in the total number of pellets added to each of three replicate jars.

Larvae which hatched into the jars were allowed to complete their development and the effect of the low-level chlorpyrifos residues was measured by determining the relative numbers reaching the pupal and adult stages. To compensate for evaporation, quantities of distilled water were added to the jars daily to maintain the level at 3000 ml. These preliminary biological response tests were conducted twice by introducing one set of egg rafts into the test jars when low-level chlorpyrifos residues were present, allowing the larvae to complete their development, and then introducing a second set of egg rafts into the same test jars. Data concerning percent hatch of egg rafts, percent pupation, and percent adult emergence were analyzed by Analysis of Variance at the .05 level of probability.

COMPARATIVE CHLORPYRIFOS RESIDUE AND BIOLOGICAL RESPONSE TESTS. Fifteen glass jars, each containing 3000 ml of distilled water, were set up in an environmental chamber that maintained the water temperature at 27.5° C. Dosages for the various test jars are shown in Table 2. Following treatment, the water in each of the jars was bioassayed weekly with 20 fourth instar *C. p. quinquefasciatus* larvae. When mortality was no longer observed in the bioassays, the jars were considered to contain low-level chlorpyrifos concentrations.[4] Small quantities of ground rabbit pellets were added about 24 hours before 100 first instar larvae (<2 hours old) were introduced into each of the jars. Small quantities of ground or whole pellets were added daily thereafter. The larvae were allowed to complete their development and effects of the low-level chlorpyrifos residues were measured by determining the relative numbers reaching the pupal stage. Beginning with the day the first instar larvae were added to the jars, chlorpyrifos residues in the test jars were determined by electron capture gas chromatography as reported by Miller et al. (1973). The minimum detectable quantity measured in water during the present study was 0.1 ppb chlorpyrifos. To compensate for both the removal of water samples and evaporation, quantities of distilled water were added to the jars daily to maintain the level at 3000 ml. These comparative tests were conducted three times by introducing an initial set of 100 first instar larvae into each of the test jars, followed by two successive sets of 100 first instar larvae, each set being introduced into the same test jars after the previous set had completed development.

RESULTS

PRELIMINARY BIOLOGICAL RESPONSE TESTS. Estimated numbers of 1st instar larvae which hatched into the test jars are shown in Table 3. The presence of low-level chlorpyrifos residues had no apparent effect on percent hatch, and it was decided that subsequent tests would be conducted by introducing newly-hatched 1st instar larvae, rather than egg rafts, into the jars. Table 4 shows that the

TABLE 2.—Dosages for test jars used to compare chlorpyrifos residue levels and biological response.

Type of test jar	Average weight of pellets[a] (mg)	Average weight of chlorpyrifos[b] (mg)	Average dosage (ppm chlorpyrifos)
Control w/o pellets	.....	...	...
Control w/16 pellets	1346.6	...	...
4 pellets	330.0	.33	.11
8 pellets	661.5	.66	.22
16 pellets	1332.1	1.33	.44

[a] Average weight of the total number of pellets added to each of three replicate jars.

[b] Average weight of chlorpyrifos contained in the total number of pellets added to each of three replicate jars.

TABLE 3.—Estimated numbers of 1st instar *C. p. quinquefasciatus* larvae hatched into test jars.

Test jar type	Total number of eggs[a]			Estimated percent[b] hatched	Estimated larvae[c] hatched
	Originally added	Recounted	Hatched on on recount		
Control w/pellets	1208	1082	956	88.3	1066
1 pellet	1199	1102	1039	94.2	1129
4 pellets	1037	933	731	78.3	812
8 pellets	1092	947	580	61.2	668
16 pellets	991	934	714	76.4	757
32 pellets	1065	1032	913	88.4	941

[a] Total of two tests of three replicates each.
[b] Estimated Percent Hatched=Total Eggs Hatched on Recount/Total Eggs Recounted.
[c] Estimated Larvae Hatched=Total Eggs Originally Added×Estimated Percent Hatched.

relative numbers reaching the pupal and adult stages were each inversely proportional to the dosage (i.e., the number of 0.1 percent chlorpyrifos pellets, and presumably the chlorpyrifos concentration, in subsequent tests, a determination of the relative number pupating would, in itself, be adequate to measure the effects of the low-level chlorpyrifos residues.

COMPARATIVE CHLORPYRIFOS RESIDUE AND BIOLOGICAL RESPONSE TESTS. Average numbers pupating were inversely proportional to the number of 0.1 percent chlorpyrifos pellets in the jars (Table 5). Average chlorpyrifos residues in the

TABLE 4.—Pupation and adult emergence by *C. pipiens quinquefasciatus* reared in test jars containing various numbers of 0.1% chlorpyrifos PVC pellets.

Test jar type	Estimated larvae hatched	Total Number pupating	Total number of adults emerging		
			Male	Female	Both
Control w/32 pellets	1066	817	405	355	760
1 pellet	1129	892	398	385	783
4 pellets	812	388	186	180	366
8 pellets	668	229	103	122	225
16 pellets	757	66	37	29	66
32 pellets	941	0	0	0	0

[a] All data represent two tests of three replicates each.

the jars). At each dosage, there were no significant effects on sex ratio and the differences between numbers pupating and numbers emerging as adults were not significant. It was decided that in

TABLE 5.—Pupation by *C. pipiens quinquefasciatus* reared in test jars containing various low-level chlorpyrifos residues.[a]

Test jar type	Total larvae added	Total number pupating	Average chlorpyrifos residue (ppb)
Control w/o pellets	900	875	....
Control w/16 pellets	900	838	....
4 pellets	900	442	<0.10
8 pellets	900	198	0.14
16 pellets	900	2	0.23

[a] All data represent three tests of three replicates each.

treated jars were proportional to the number of pellets (Table 5). A detailed tabulation of average daily residues during the three tests is shown in Table 6. Residues were below the LC_{90} (<0.9 ppb) on all test days, with none exceeding 0.5 ppb chlorpyrifos. All values of <0.1 ppb (low limit of detection by gas chromatograph) were transformed to 0.00 to permit computation of an average residue for all three tests.

DISCUSSION

The effectiveness of continuous low-level chlorpyrifos residues is shown in Figures 1 through 4 as the percent of the various test populations reaching the pupal or adult stage, and as the corrected percent control[5] of the pupal or adult populations.

In both the preliminary tests (Figure 2) and the comparative tests (Figure 3) the number of individuals reaching the pupal or adult stages was inversely proportional to the number of 0.1 percent chlorpyrifos pellets. Figure 3 shows that 0.2 percent of the test population survived in jars containing an average chlorpyrifos residue of 0.23 ppm, 22.0 percent survived in jars containing an average of 0.14 ppb chlorpyrifos, and 49.1 percent survived in jars containing an average chlorpyrifos residue of <0.10 ppb.

In terms of corrected percent control, the preliminary tests (Figure 1) showed that the control of pupal and adult populations was proportional to the number of 0.1 percent chlorpyrifos pellets. In the comparative tests (Figure 4) control of pupal populations averaged 47.3 percent in jars containing average chlorpyrifos residues <0.10 ppb, 76.2 percent in jars containing average chlorpyrifos residues of 0.14 ppb, and 99.7 percent in jars containing average chlorpyrifos residues of 0.23 ppb.

These levels of population control are

TABLE 6.—Average residues observed in test jars with various numbers of 0.1 percent chlorpyrifos PVC pellets

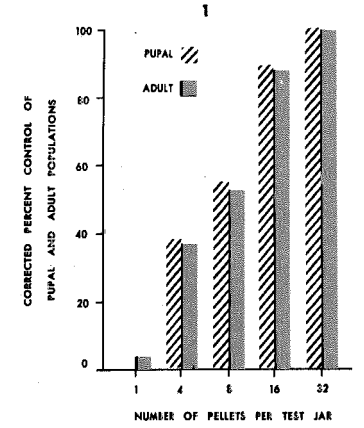
Test day[a]	Average[b] chlorpyrifos residue (ppb) in test jars containing 4 Pellets	8 Pellets	16 Pellets
	1st Test		
1	<0.10	0.23	0.47
2	<0.10	0.10	0.13
3	<0.10	<0.10	0.33
4	<0.10	<0.10	0.43
5	0.13	0.23	0.37
6	<0.10	<0.10	0.23
7	<0.10	0.13	0.17
8	0.10	0.20	0.33
9	<0.10	<0.10	0.27
10	<0.10	0.20	0.33
	2nd Test		
15	<0.10	0.23	0.23
16	0.13	<0.10	0.33
17	<0.10	0.20	0.45
18	<0.10	0.50	0.40
19	<0.10	<0.10	0.30
20	<0.10	0.20	0.27
21	<0.10	0.17	0.20
22	<0.10	0.20	<0.10
23	<0.10	0.33	0.43
24	<0.10	<0.10	<0.10
25	<0.10	<0.10	<0.10
26	<0.10	0.17	<0.10
27	<0.10	<0.10	<0.10
	3rd Test		
30	<0.10	0.43	<0.10
31	<0.10	<0.10	<0.10
32	0.20	<0.10	0.27
33	0.20	<0.10	<0.10
34	<0.10	<0.10	<0.10
35	<0.10	0.17	0.30
36	<0.10	<0.10	<0.10
37	<0.10	<0.10	<0.10
38	<0.10	<0.10	<0.10
39	<0.10	<0.10	<0.10
40	<0.10	<0.10	<0.10
41	<0.10	<0.10	<0.10
Average	<0.10[c]	0.14	0.23

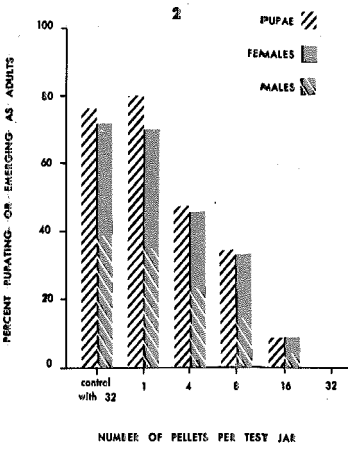
[a] Starting with the day after residues were determined by bioassay to be $<LC_{05}$, and continuing through three successive introductions (1st, 2nd, 3rd tests) of 1st instar larvae into the same test jars.

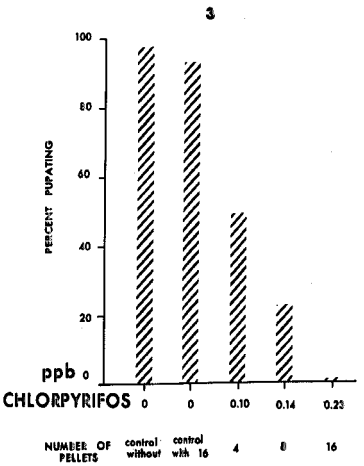
[b] Average of three replicates.

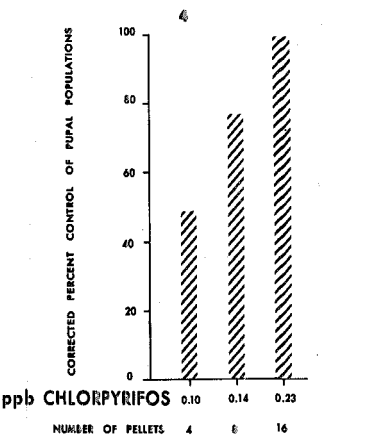
[c] Computed by transformation of <0.10 to 0.00.

[5] Corrected % Control = $\frac{\text{\% Surviving in Control w/Pellets} - \text{\% Surviving in Test}}{\text{\% Surviving in Control w/Pellets}} \times 100$









FIGS. 1–4.—1. Corrected percent control of *C. p. quinquefasciatus* populations reared in test jars containing various numbers of 0.1% chlorpyrifos PVC pellets. 2. Percent pupation and adult emergence by *C. p. quinquefasciatus* populations reared in test jars containing various numbers of 0.1% chlorpyrifos PVC pellets. 3. Percent pupation by *C. p. quinquefasciatus* populations reared in test jars containing various continuous low-level concentrations of chlorpyrifos. 4. Corrected percent control of *C. p. quinquefasciatus* populations reared in test jars containing various continuous low-level concentrations of chlorpyrifos.

higher than those which would be expected at the respective residue levels. Based on dosage-mortality information for the laboratory colony of *C. p. quinquefasciatus* used in this study, residues of <0.10, 0.14, and 0.23 ppb chlorpyrifos would be expected to produce 24-hour percent mortalities of 0.0, 1.2, and 5.0 respectively. Therefore, it appears that such levels of population control are attributable to the chronic rather than the acute effects of the chlorpyrifos.

Larval populations could not be determined on a daily basis, due to the inaccuracy involved in counting 1st and 2nd instar larvae in the test jars. It was observed that the population reductions were not due solely to the larvae succumbing in the early instars. Many of the larvae reached the 3rd, or even the 4th instar, but died before pupating. With specific regard to those larvae reaching the 3rd or 4th instars, it is possible that a single acutely toxic residue could have occurred during the periods between the routine daily residue determinations. However, data shown in Table 6 indicate that this was not the case because daily residue levels for jars treated with sixteen 0.1 percent chlorpyrifos PVC pellets were always below 0.5 ppb, and residues in jars treated with fewer pellets were proportionally lower.

CONCLUSIONS

Various levels of control were achieved in the laboratory when *C. p. quinquefasciatus* populations were reared in water containing continuous low-level concentrations of chlorpyrifos: 47.0 percent control at <0.10 ppb; 76.2 percent control at 0.14 ppb; and 99.7 percent control at 0.23 ppb. Although control of the laboratory populations was achieved by exposure to the various continuous low-level concentrations, the residue levels (<0.10–0.23 ppb) were not considered sufficiently below the established LC_{90} (0.9 ppb) to be significant from the standpoint of possibly reducing necessary field dosages.

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NOTICE

The 18th Annual Livestock Insect Work Conference will be held in Madison, Wisconsin, on July 9 through 12, 1974.

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