LARVICIDAL EFFECTIVENESS OF A CONTROLLED-RELEASE FORMULATION OF CHLORPYRIFOS IN A WOODLAND POOL HABITAT[1]

E. S. EVANS, JR., J. H. NELSON, N. E. PENNINGTON AND W. W. YOUNG
U. S Army Environmental Hygiene Agency, Aberdeen Proving Ground, Maryland 21010

ABSTRACT. Twelve randomly-selected, naturally-occurring woodland pools were treated with a chlorinated polyethylene pelletized formulation of 10.6 percent chlorpyrifos at levels of 0.25, 0.50, and 1.00 parts per million (based on a theoretical total initial release of all toxicant). Effectiveness of the formulation as a mosquito larvicide was evaluated for 22 post-treatment weeks utilizing in-pool bioassays and natural mosquito infestation rates. Results of in-pool bioassays indicated sufficient chlorpyrifos residues were present to effectively control mosquitoes (mortality≧80 percent) within 1 week in two of three pools dosed at 1.00 ppm and remained effective throughout the 22-week study. Natural mosquito infestation rates were significantly lower in treated versus control pools indicating sufficient residues were present at all three dosage rates to be lethal to newly hatched first instar larvae.

INTRODUCTION. An insecticide formulation which would allow a controlled release of toxicant into an aquatic environment at levels sufficient to effectively reduce or eliminate larval mosquito activity without causing undo adverse environmental effects would be a major contribution to present-day mosquito abatement programs. Following extensive laboratory and simulated field evaluations conducted by the U.S. Army Environmental Hygiene Agency (USAEHA) in the area of slow-release insecticides, as reviewed by Nelson, et al. (1974), actual field tests were deemed necessary in order to develop guidelines for recommending the use of slow-release insecticides in military vector control programs, if through Federal registration these formulations become a reality.

Although investigations in this study, as described by Evans et al. (1974), included environmental characterization of the study areas, evaluation of the effects on nontarget organisms, elucidation of effects of various physico-chemical parameters on release rates of the toxicant and determination of toxicant residues in water and sediment, only the effectiveness of the experimental formulation as a mosquito larvicide will be reported at this time.

MATERIALS AND METHODS. Two study sites [Turner (T) and New Dover (ND)] located in the north-central section of Middlesex County, New Jersey were utilized. Within the study sites, 12 naturally-occurring temporary woodland pools were selected on the basis of size and ranged from the smallest pool, 17.7 m x 6.8 m, to the largest 42.4 m x 26.2 m in surface area. Average water depths ranged from 17.0 cm to 19.4 cm with calculated water volumes at the time of treatment ranging from a high of 642,632 *l* to a low of 28,070 *l*.

Treatments were made in March 1973 with a chlorinated polyethylene (CPE) pelletized formulation of 10.6 percent chlorpyrifos prepared and supplied by Dow Chemical Company, Midland, Michigan. The cylindrically shaped pellets averaged 1.55 mm x 1.37 mm in size, 2.4 mg (±1.0 mg) in weight and had a specific gravity greater than 1.0. Randomly selected treatments of 0.25, 0.50 and 1.00 ppm (based on a theoretical total initial release of all toxicant) were replicated three times and three untreated pools served as controls. The formulation was evenly distributed

[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, U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, Maryland 21010.

by a hand-held Horn[2] Seed Sower. Weekly determinations of larvicidal effectiveness included natural mosquito infestations and in-pool bioassays which were conducted for 22 post-treatment weeks.

Both larval and adult mosquitoes indigenous to the study area were surveyed. Larvae were collected with a 440 ml white enamel dipper while 15-minute biting counts were conducted at dusk for the adult survey.

Natural larval mosquito populations in the study pools were sampled prior to treatment and weekly following treatment using a 440 ml white enamel dipper. Ten dips were made while traversing the periphery of each test pool. Collections were counted and sampled periodically for identification purposes.

In-pool bioassays were conducted using early 3rd instar laboratory-reared *Aedes triseriatus* (Say). As available, bioassays were conducted using field-collected *Aedes vexans* (Meigen) larvae. Bioassay containers were made from 470 ml, unwaxed, cardboard cups by removing the bottoms and replacing with a single layer of unbleached muslin. The cups were fitted with three evenly spaced circular pieces of styrofoam held in place with a rubber band. These styrofoam "floats" were adjusted so that the cups were submerged 2–5 cm and the larvae were thus exposed to approximately 250–300 ml of water. Each cup was attached to a wooden stake by a 12-inch string to maintain stability. Twenty larvae were placed in each cup immediately following treatment and mortality was recorded at 24, 48, and 72 hours. Thereafter, weekly bioassays were conducted and mortality recorded after 24 hours. Bioassay cups were used only once.

RESULTS. A total of 12 mosquito species indigenous to the woodland pool study habitat were collected (Table 1). With the exception of *Aedes atlanticus* Dyar and Knab, all other species were found in the larval stage indicating that the study area provided suitable habitats for those mosquito species considered as breeders in woodland pools, fresh-water swamps, treeholes, and in those areas subjected to intermittent flooding.

Table 1. Indigenous mosquito populations Woodland Pool habitat study sites.

Species	Stage collected*	Habitat group†
Aedes canadensis	L, A	WP
A. vexans	L, A	FW
A. trivittatus	L, A	FW
A. atlanticus	A	FW
A. triseriatus	L, A	T
Culex territans	L	FWS
C. restuans	L	W
C. pipiens	L	PW
Psorophora ferox	L, A	FW
Toxorhynchites sp.	L	T
Uranotaenia sapphirina	L	FWS
Anopheles punctipennis	L	FWS

* L=Larvae.
A=Adult.
† WP=Woodland Pool.
FW=Flood Water.
FWS=Fresh Water Swamp.
T=Treehole.
PW=Polluted Water and Artificial Container.

Results of natural mosquito infestations monitored in the woodland pool habitat study pools are presented in Table 2 and Figure 1. Pre-treatment surveys indicated that *Aedes canadensis* (Theobald), a mosquito found in woodland pools in late winter and early spring, was present in all but three of the study pools (T3, T6, and ND12). By the third week following treatment with the chlorpyrifos formulation, only two of nine treated pools had larvae present (ND10, ND11) while two of three control pools (T2, ND12) indicated the presence of larvae. Through 22 post-treatment weeks, a direct correlation was exhibited between numbers of mosquitoes collected per 10 dips and the dosage rate at which each study pool was treated. Pools treated at 1.00 ppm had an overall average of 0.1 mosquito larvae per 10 dips followed by 0.4 in pools treated at 0.50 ppm and 1.6 larvae in those pools treated at 0.25 ppm. Untreated pools had

[2] Horn Seed Sower is a registered trademark of Horn Seed Company, Urbana, Indiana.

Table 2. Natural larval mosquito populations—woodland pool habitat.

Weeks post-treatment			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	$\bar{x}$
Treatment received (ppm)	Pool number	Pre-survey	Number in 10 dips																						
0.25	T7	9	..	..	0	0	0	3	0	0	0	17	2	D	D	D	12	0	D	D	D	D	D	D	3.1
	T8	9	..	..	0	0	0	4	0	0	0	1	0	D	D	D	6	D	D	D	D	D	D	D	1.1
	ND11	3	..	..	1	0	0	1	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0.7
	$\bar{x}$	7	..	..	0.3	0	0	3	0	0	0	6	0.7	0	0	0	10	0	0	0	0	0	0	0	1.6
0.50	T4	1	..	..	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	1	0	4	0	0	0.55
	T6	0	..	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
	ND9	32	..	..	0	2	0	3	0	0	0	0	1	0	D	0	2	0	0	0	0	2	0	0	0.53
	$\bar{x}$	11	..	..	0	0.7	0	1	0	0	0	0	0.3	0	0	0	0.7	2	0	0.3	0	2	0	0	0.36
1.00	T1	3	..	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
	T5	7	..	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
	ND10	14	..	..	3	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.35
	$\bar{x}$	8	..	..	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.12
Control	T2	4	..	..	1	0	0	8	6	0	6	5	167	D	D	D	300	750	D	D	D	D	D	D	113.0
	T3	0	..	..	0	0	0	0	0	0	0	0	0	0	0	16	57	30	0	0	D	19	D	151	14.4
	ND12	0	..	..	3	15	4	8	5	11	18	0	0	0	0	6	9	8	1	12	4	11	44	36	9.8
	$\bar{x}$	1	..	..	1	5	1	5	4	4	8	2	56	0	0	11	122	263	0.5	6	4	15	44	63	45.7

..=No data.
D=Dry.

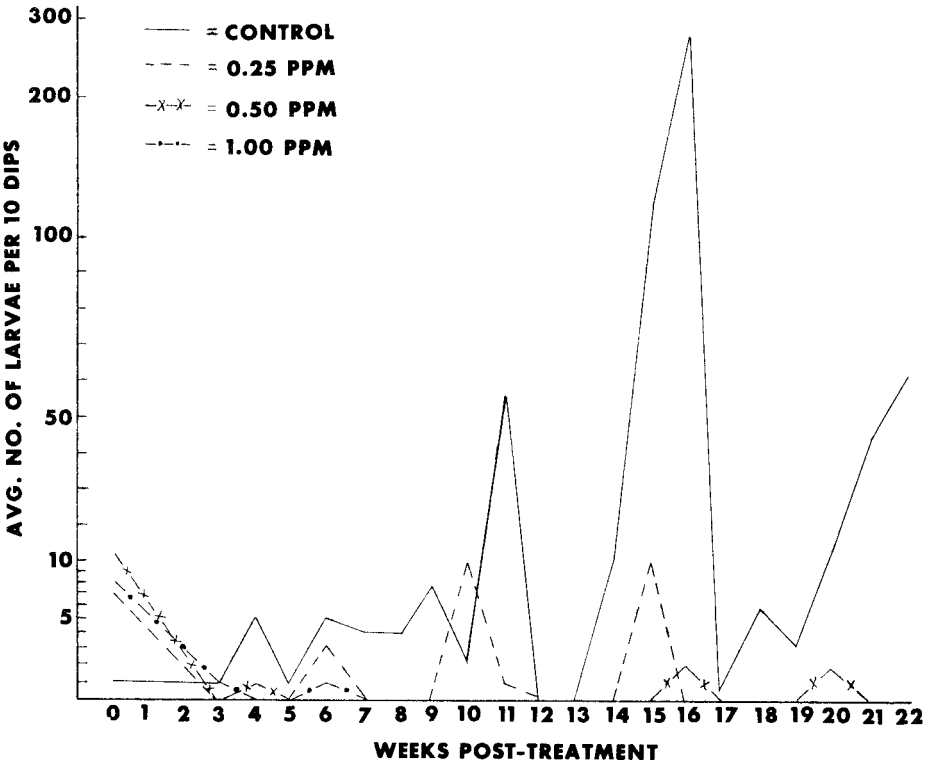

Fig. 1 Natural Mosquito Infestations of Woodland Pools

the highest overall average of 45.7 mosquito larvae collected per 10 dips. Analysis of variance indicated variability between dosages in terms of infestation rate of natural mosquito populations to be significant at the .05 level. Variability between weeks and a dosage times weeks interaction was not significant at the .05 level.

From post-treatment weeks 1 through 10, *A. canadensis* was the primary mosquito species present in control pools and in those few treated pools in which natural mosquito infestation was evident; however, on post-treatment weeks 11, 15, 16, and 22 a sizable hatch of *A. vexans* occurred in control pools T-2 and T-3. *A. vexans* larvae were also present on post-treatment week 15 in all pools treated at 0.25 ppm and periodically from weeks 15 through 20 in two of three pools treated at 0.50 ppm (T4 and ND9). Subsequent to week 13, *Culex territans* Walker and *Culex pipiens* Linn. were the two predominant species infesting the control pool ND12 indicating that this larger, more permanent pool was not a conducive habitat for such floodwater mosquito species as *A. vexans.*

Results of those in-pool bioassays using 3rd instar laboratory-reared *A. triseriatus* are presented in Table 3. Mortality first appeared at 48 hours post-treatment and continued through the 72-hour check. Average percent mortality for the 72-hour period ranged from a low of 8 percent in control pools to 18 percent in those pools treated at 0.50 and 1.00 ppm. Mean mortalities over 22 weeks were 9, 6, and 64 percent in pools treated at 0.25, 0.50, and

Table 3. Results of field bioassay—Woodland Pool habitat.

Post-treatment Treatment received (ppm)	Time / Pool No.	Hours* 28	48	72	X	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X̄**
		Mortality (percent)***																										
0.25	T7	0	5	20	8	20	0	0	5	0	0	0	5	5	0	5	D	D	D	0	0	D	D	D	D	D	D	3
	T8	0	25	45	23	5	5	0	58	30	15	10	10	75	65	80	D	D	D	90	D	D	D	D	D	D	D	16
	ND11	0	10	15	8	10	20	5	0	5	25	0	0	15	5	10	0	5	0	0	5	10	5	70	5	0	0	9
	x̄	0	13	27	13	12	8	2	21	12	13	3	5	32	23	32	0	5	0	30	3	10	5	70	5	0	0	9
0.50	T4	0	10	55	22	0	0	5	0	0	0	0	10	10	5	0	5	5	0	0	0	0	0	0	5	0	10	3
	T6	0	50	35	28	20	5	5	35	42	15	10	10	5	20	5	5	0	0	5	10	5	..	10	5	0	5	11
	ND9	0	0	10	3	10	0	0	5	0	5	0	30	0	0	0	0	D	0	0	0	0	0	D	0	0	5	3
	x̄	0	20	33	18	10	2	3	13	14	7	3	17	5	8	2	5	2	0	2	3	2	0	5	3	0	7	6
1.00	T1	0	25	10	12	80	5	60	95	100	95	100	95	90	100	90	90	90	95	100	100	100	75	100	100	80	100	88
	T5	0	30	40	23	80	50	..	85	95	60	95	20	65	95	90	70	100	100	80	35	95	80	100	100	D	100	81
	ND10	0	35	20	18	20	0	15	25	0	20	0	10	20	5	10	10	95	55	0	5	65	20	100	0	5	25	23
	x̄	0	30	23	18	60	18	37	68	65	58	65	42	58	67	63	57	95	83	60	47	87	58	100	67	43	75	64
Control	T2	5	0	20	8	5	0	0	5	5	10	0	0	5	0	0	D	D	D	5	5	D	D	D	D	D	0	3
	T3	0	5	35	13	3	0	.:	5	5	0	0	5	5	0	0	0	0	0	0	0	0	0	0	5	D	0	2
	ND12	0	5	5	3	20	0	20	0	0	0	0	0	10	5	5	5	15	0	0	5	10	5	0	0	0	0	5
	x̄	2	2	20	8	10	0	10	3	3	3	0	2	7	2	2	3	8	0	2	3	5	3	0	3	0	0	3

D=Dry.

* Mean mortality over 72 hr. period.

** Mean mortality weeks 1–22 only.

*** Figures rounded to next whole number.

1.00 ppm, respectively. A two-way analysis of variance indicated significant variability between dosages (.01 level) and between weeks (.05 level). There was no significant dosage times weeks interaction (.05 level). Variability between average percent mortality for individual study pools within a given treatment was evident. Significantly greater percent mortalities (.01 level) for pools T8 (0.25 ppm) and T6 (0.50 ppm) and significantly lower values (.01 level) for ND10 (1.00 ppm) occurred. While mortality was 80 percent or greater for 19 weeks in T1 and 17 weeks in T5, only 2 of the 22 weeks had at least 80 percent mortality in ND10 (all treated at 1.00 ppm). Average mortality was 84 percent for 22 weeks if only pools T1 and T5 were considered. No definitive explanation can be given for the lack of effective mosquito control observed in pool ND10. Although the possibility exists that some physico-chemical parameter may have been influencing release rates of chlorpyrifos, in all probability an underestimate of the total volume of water at the time of treatment caused the pool to be misdosed. There were no significant differences (.05 level) between individual control pools.

Results of limited in-pool bioassays using field-collected *A. vexans* are presented in Table 4. Average percent mortality was 74.0, 21.1, and 46.7 for pools treated at 1.00, 0.50, and 0.25 ppm, respectively. Percent mortality for control pools averaged 1.2. A Student t-statistic indicated significant mean differences between dosages. Only those mean differences present between 0.25 and 0.50 ppm pools and between 0.25 ppm and control pools were not significant (.05 level). The higher average percent mortality observed in pools treated at 0.25 ppm as opposed to pools treated at 0.50 ppm is misleading. On post-treatment week 15, 100 percent mortality was observed in pool T8. The almost complete elimination of this pool due to drying at that time no doubt contributed to the increased mortality thereby

Table 4. Results of in-pool bioassays using *Aedes vexans*—Woodland Pool habitat.

		Weeks post-treatment				
		14	15	16	22	$\bar{x}$
Treatment received (ppm)	Pool number	Mortality (percent)				
0.25	T7	..	0	35	D	17.5
	T8	..	100	D	D	100
	ND11	..	0	5	0	1.7
	$\bar{x}$	..	33.3	20.0	0	46.7
0.50	T4	..	0	0	10	3.3
	T6	25	5	45	20	23.8
	ND9	..	0	0	15	5.0
	$\bar{x}$	25	1.7	15.0	15.0	21.1
1.00	T1	100	100	95	100	98.8
	T5	..	95	45	100	80.0
	ND10	..	0	5	100	35.0
	$\bar{x}$	100	65.0	48.3	100	74.0
Control	T2	..	0	0	D	0
	T3	0	5	0	1.7	..
	ND12	..	0	5	0	1.7
	$\bar{x}$	0	1.7	1.7	0	1.2

D=Dry.

increasing the overall average percent mortality for this species in those pools dosed at 0.25 ppm. As was observed in those bioassays conducted using laboratory-reared *A. triseriatus,* two of three pools treated at 1.00 ppm indicated that effective control was being achieved.

DISCUSSION. The evaluation of a mosquito larvicide formulation must be concerned with the type of control desired in a particular aquatic habitat (i.e., short or long-term). Based on the seasonal sequence of events in the woodland pool habitat and the bionomics of the primary mosquito species associated with this habitat type, an ideal larvicide would be one which could be applied at the initial flooding of the area to control the first brood of early season mosquitoes, become inactive during periods of dryness when no breeding is occurring, then again become available following reflooding to control summer floodwater species.

Effectiveness of the formulation in terms of acute toxic effects would depend on rapid release of the toxicant from the pellet following initial treatment of a flooded habitat or upon reflooding following a period of dryness. Effective control (mortality $\geqq$ 80 percent) was attained 1 week following treatment in two of three pools treated at 1.00 ppm (Table 3). Physico-chemical factors such as pH, high organic matter content, as well as water temperatures, no doubt contributed to the time for acute toxic effects to appear. Stockman, et al. (1970) reported that with decreasing temperatures there is a corresponding decrease in the release of insecticide from a polymer formulation. Test applications were made in March when water temperatures averaged 6.2° C. Sufficient chlorpyrifos residues were released following application when early season mosquito larvae first appeared to be lethal within the time frame of the developing larvae. Colder water temperatures encountered in northern woodland pools contribute to extended developmental time of *A. canadensis* with complete development often taking several weeks.

As concerns the effectiveness of the CPE formulation following drying and flooding, conditions were such (no adequate reflooding) that nothing more than generalizations can be made. Of the two woodland pools treated at 1.00 ppm which effectively controlled mosquitoes, only study pool T5 dried completely (post-treatment week 21). Following a substantial summer rain, the pool was again inundated. Results of an in-pool bioassay conducted on week 22 indicated insufficient chlorpyrifos had been released for effective control.

Results of in-pool bioassays and natural infestation rates indicated that persistent or long-term mosquito control was exhibited throughout the study particularly in pools treated at 1.00 ppm. Persistent effects would be particularly important to control those *Culex* spp. breeding in the larger more permanent pools.

Chlorpyrifos residues were insufficient to effectively control third instar *A. triseriatus* in woodland pools treated at 0.25 and 0.50 ppm as evidenced utilizing in-pool bioassays (Table 3); however, natural infestations were significantly lower in all treated pools as compared to controls indicating sufficient residues were present to be lethal to newly hatched first instar larvae (Table 2).

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