F. Thomas of the U. S. Environmental Protection Agency, Beltsville, MD, analyzed the spray formulation. Mr. Daniel Cirelli of our branch provided the automated data processing and analysis. Dr. E. W. Cupp of the Entomology Department at Cornell University critically reviewed the manuscript.

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EVALUATION OF SEVERAL INSECTICIDES FOR THE CONTROL OF LARVAL *AEDES SIERRENSIS* (LUDLOW)

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ABSTRACT. Chlorpyrifos, temephos, fenthion, and propoxur were applied to naturally occurring treeholes at the rate of 1 gm AI liter of treehole water. Inspections for reinfestation and bioassays of water samples from the treeholes were performed at intervals of approximately 1 year. Field infestations of *Ae. sierrensis* occurred during the 2nd year in trees treated with propoxur. Temephos and fenthion treated trees were infested during the 4th year. Larvae of *Ae. sierrensis* were found in treeholes treated with chlorpyrifos during the 6th year. Fenthion, temephos, and propoxur began to fail in bioassay at 2 years and chlorpyrifos at 5 years.

Aedes sierrensis, the western treehole mosquito, is a ubiquitous pest throughout many residential and wooded areas of California from near sea level to the high mountains. It is an exceedingly annoying biter in outdoor shade. Additionally, the adults often are so small that they are able to crawl through ordinary window screen and attack people indoors. During late spring and early summer, the species is responsible for many complaints received by mosquito control agencies. Weinmann and Garcia (1974) have discussed the potential of *Ae. sierrensis* as a vector of canine heartworm, *Dirofilaria immitis* (Leidy), a parasite found frequently in dogs but rarely in humans.

Ae. sierrensis breeds almost exclusively in treeholes, usually a rothole formed at the site of a broken-off or pruned limb and filled during the fall and winter by rainwater. Permanent control can be achieved by providing a drainage outlet for the hole or removing the tree. Filling the hole without proper surgery may provide only temporary control since the tree may continue to rot around solid filling material. Soft fillings, such as sand, may be dug out by animals or washed out by rain. In situations where these measures are impractical or impossible, chemical control becomes the method of choice. Aerosols are of limited value, especially in densely wooded habitats. Effective control may be accomplished by introducing a persistent insecticide into the breeding site to kill the larvae as they hatch from freshly flooded eggs.

DDT, with its extraordinary residual qualities, was effective as a tree-hole larvicide, e.g., Portman and Hall (1961), Brannan (1964). However, many of the chlorinated hydrocarbons fell into environmental disfavor during the 1960's, and DDT was eventually banned.

Early in 1970, we initiated a study to evaluate the efficacy of mosquito larvicides which might be candidates to replace DDT and other chlorinated hydrocarbons. A progress report was presented by Oldham et al. (1972). Coincidentally, in 1969 Lewis and Christenson (1975) initiated a similar study on the longevity of various insecticides in fabricated treeholes. We report here the results of observations made during the period 1970–76 on the utility of chlorpyrifos, fenthion, temephos, and propoxur to control larval *Ae. sierrensis* in naturally occurring treeholes.

MATERIALS AND METHODS. The area selected for the study was an oak forest in rolling hills near Red Bluff, Tehama County, typical of *Ae. sierrensis* habitat in much of California. The predominant tree is the deciduous blue oak *Quercus douglasii;* the interior live oak *Quercus wislizenii* occurs in scattered patches. For each of the 4 insecticides to be tested and for an untreated check, 10 blue oaks containing treeholes were marked and numbered with red paint. Selection of a treehole was based on its apparent ability to hold water for the season and the presence of a large *Ae. sierrensis* larval population.

The volume of water in each treehole was measured by siphoning or pumping as much water as possible into a calibrated container. The volume was recorded and the water returned to the hole. Volumes ranged from 0.2 liter to 20 liters with a median of 2 liters. Amounts of insecticide were added to achieve a dosage of 1 gm AI/liter treehole water. Commercial formulations were used as follows: Baygon® 70% WP, a wettable powder containing 70% propoxur; Abate® 4E, an emulsifiable concentrate containing 4 lb/gal temephos; MAD Special Mosquitocide, an emulsifiable concentrate containing 7 lb/gal fenthion (Baytex®); and Dursban® M, an emulsifiable concentrate containing 4 lb/gal chlorpyrifos. Treatments were made during March 1970.

The 1st posttreatment check was made at 9 months (0.75 year) and the 2nd at 1 year. Sampling was repeated at approximate yearly intervals thereafter. Evaluation of effectiveness of the treatments was determined by visual observation for the presence of live larvae and by bioassay of water samples from the trees. Presence or absence of larvae was determined by inspecting water siphoned from the treeholes into a white enamel pan. At 3 years posttreatment, pH was measured on a sample from each treehole.

During the first 2 years. 2 treeholes from each of the insecticide treatment plots were selected for bioassay. Beginning with the 3rd year all tree holes not reinfested were included. Approximately 100 ml of water were withdrawn from each treehole. The water was placed in silanized glass bottles and returned to the Tehama County Mosquito Abatement District laboratory where tenfold serial dilutions were prepared from each sample with aged tap water in waxed paper cups. The resulting series was undiluted, 1/10 and 1/100 of the concentration remaining in the treehole. Greater dilutions were sometimes made but are ignored here. Similar dilutions were prepared from a 100 ml sample of water taken from an untreated treehole. Ten 4th-instar larvae from a *Culex pipiens quinquefasciatus* Say colony maintained in the Sacramento laboratory of the Vector and Waste Management Section were introduced into each cup. This was accomplished by precounting the larvae into another cup containing a small amount of water, then pouring the water and larvae onto a 3-inch square of nylon net. The net was then dipped into the test water and the stranded larvae allowed to swim free. A separate nylon square was used for each cup. Mortality was evaluated at 24 hrs.

RESULTS. Table 1 shows the sequence in which field infestations were found in the treated treeholes. All 10 treeholes treated with propoxur remained uninfested for 1 year; those treated with fenthion or temephos, 3 years; and with chlorpyrifos, 5 years.

Three bioassays were performed during the first 2 years posttreatment. All four materials produced 100% mortality of the *Cx. quinquefasciatus* larvae in the undiluted treehole water at 0.75 and 1 year. Fenthion, temephos, and propoxur began to fail in bioassay at 2 years, as shown by 24-hr survival of some laboratory larvae in the undiluted water from some of the treated treeholes. Chlorpyrifos maintained its effectiveness in bioassay until the 5th year after application.

Bioassay data are presented only for chlorpyrifos, the most persistent insecticide tested (Table 2). During the first 2 years, not only the undiluted samples but the 10-fold dilutions, and in most cases the 100-fold dilutions, produced 100% 24-hour mortality in the laboratory larvae. By the 4th year, the undiluted sample still killed all the larvae but most survived in the 10-fold dilutions. By the 5th year, incomplete 24-hour mortality was seen in 4 of the undiluted samples. At the 6th year, most chlorpyrifos treated trees were reinfested. Of the 3 still free of larval *Ae. sierrensis*, 2 showed evidence of insecticidal activity.

Table 1. Duration of control of *Aedes sierrensis* larvae provided by several insecticides in treeholes treated at the rate of 1 gm AI/liter water. FI indicates that at least one of 10 trees was infested with larvae, except that at pretreatment all trees were infested.

Material	pH range	Date treated	Pre-treatment	Years Posttreatment 0.75	1	2	3	4	5	6
Propoxur	6.3–8.4	3/21/70	FI	–	–	FI	FI	FI	FI	FI
Temephos	6.4–8.4	3/16/70	FI	–	–	–	–	FI	FI	FI
Fenthion	5.6–7.8	3/23/70	FI	–	–	–	–	FI	FI	FI
Chlorpyrifos	6.7–8.5	3/17/70	FI	–	–	–	–	–	–	FI
Checks	5.9–8.2		FI	FI	FI	FI	FI	FI	FI	FI

DISCUSSION AND CONCLUSIONS. Lewis and Christenson (1975) reported temephos and fenthion in fabricated treeholes effective for 3 years at dosage rates comparable to ours. Chlorpyrifos was still 100% effective at the end of 4¾ years, when their observations were terminated. Our tests indicated that chlorpyrifos applied at dosage rates of approximately 1 gm AI/liter could provide residual control of *Ae. sierrensis* larvae in treeholes for up to 5 years. On the basis of this information, we asked the Dow Chemical Company to apply for registration of chlorpyrifos for treehole mosquito control in California. A state label to meet a special local need was granted by the California Department of Food and Agriculture in late 1976.

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Table 2. Percent mortality of *Culex pipiens quinquefasciatus* larvae exposed for 24 hrs to water from treeholes treated with chlorpyrifos at the rate of 1 gm AI/liter water. Figures are for undiluted samples, 10-fold dilutions, and 100-fold dilutions. FI indicates field infestation.

Tree No.	pH	Vol. liters	Years posttreatment						
			0.75	1	2	3	4	5	6
1	7.1	1.1	100,100,100	100,100,100	100,100,77	100,100,95	h e a l e d		
2	7.8	10.5				100,100,64	100,18,0	100,20,0	FI
3	7.2	0.2				100,90,5	100,0,0	10,0,0	FI
4	8.1	1.5				100,100,100	dry	90,50,0	FI
5	8.3	0.35				100,20,0	100,0,0	0,0,0	FI
6	6.7	1.6			dry	100,100,10	100,0,0	90,10,0	0,0
7	8.0	0.4	100,100,90	100,100,100	100,100,100	100,100,76	100,100,5	90,20,0	FI
8	8.5	3.6				100,84,5	100,0,0	88,20,0	FI
9	7.1	2.1				100,100,21	100,0,0	100,90,0	100,0
10	8.2	1.15				100,100,11	100,0,0	10,0,0	80,0,0

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