SUMMARY. The five ULV nonthermal aerosol generators operated by the Commission in 1971 provided adult mosquito control equal to that experienced with thermal aerosols in past years, except in dense ground cover sites, where thermal aerosols offered a marginal advantage. The public acceptance, safety and efficiency of operation, environmental advantages, and cost per acre, however, were markedly superior with applications of ULV nonthermal aerosol generators versus thermal aerosol generators.

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FIELD APPLICATIONS OF ULTRA LOW VOLUME MALATHION TO THREE ANIMAL SPECIES

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ABSTRACT. Goldfish, mice and quail were exposed to ground applications of ULV malathion. The effects of two rates of malathion exposure on these animals were studied. One rate was according to label recommendations (1.5 fl. oz./min); a second rate was applied at 10 times the recommended level. After 20 separate applications, within a 34-day period, all exposed animals failed to exhibit any detectable neurotoxic symptoms. Tests for red cell cholinesterase inhibition on mice and quail, 24 hours after the last exposure, were all negative.

INTRODUCTION. For over a decade Maryland has been using malathion to control adult mosquitoes. Mist sprays and thermal fogs have been effective with no apparent adverse effects on man, pets, fish and wildlife. Although ground Ultra Low Volume (ULV) aerosols offer advantages over other methods, the application of highly concentrated insecticides has been approached with caution. Despite all precautions, there is a possibility that dosages exceeding recommended rates for adult mosquito control may be applied which could create hazards to fish and wildlife. The purpose of this study was to evaluate the effects of repeated ULV malathion applications on three species of nontarget animals.

METHODS AND MATERIALS. Malathion (Cythion® ULV Concentrate 95%) was applied with a truck-mounted Leco Model HD ULV cold aerosol fog generator. A remote control instrument panel was installed in the truck cab. The Leco unit was calibrated before, during and after the study at various operating temperatures.

Test animals were goldfish, white mice and bob-white quail (*Colinus virginianus*). Unsexed 2- to 3-inch goldfish were purchased from a local pet store. Sex-separated white mice and quail were obtained from local sources. Goldfish containers were 1 gallon plastic tanks equipped with aerator-filters. Five fish were placed in each of 12 tanks. Mouse and quail cages

were made of ¼ inch mesh galvanized wire. Mouse cages measured 12 x 12 x 4 inches and quail cages 18 x 18 x12 inches. Five mice were placed in each of 12 cages and five quail in each of 14 cages.

Malathion treatments consisted of weekly applications and daily applications. Within each treatment some animals were exposed to normal (1X) and others to a ten times normal (10X) dosage. Weekly treated 1X and 10X animals were sprayed for 5 consecutive weeks. Daily treated 1X and 10X animals were sprayed for 20 exposures within a 34-day period. Two tanks of goldfish, 2 cages of mice and 2 cages of quail were placed in an open field 50 feet downwind from the path of the ULV unit. Animals were placed in groups of 10 in all tests except for one group of 20 quail exposed weekly to a 10X dosage level. Two sets of control animals (one per treatment) were used. Each set of controls consisted of 2 tanks of fish, 2 cages of mice and 2 cages of quail. Control animals were handled the same as the test animals.

Fish tanks were covered to prevent spillage during transportation to test site. Food and water containers in cages were removed prior to the treatment. Fish and mice were held in the laboratory between exposures while quail were kept outdoors.

At the test site the cages and fish tanks were placed on the ground. The discharge nozzle was directed off the side of the truck and the aerosol aimed directly at the confined animals. At 5 mph a single pass with the Leco was made for the 1X treatment at a discharge rate of 1.5 fl. oz. malathion/minute. Five passes were made for the 10X treatment at a dosage rate of 3.0 fl. oz. malathion/minute/pass. Based on a 300 ft. swath the dosages resulting from 1X and 10X treatments corresponded to .038 and 0.38 lb. actual toxicant per acre treated. Animals remained at the test site until the insecticide had drifted from the area and then were returned to the laboratory for observation. Observations were made daily for diarrhea in mice and quail. Food consumption was also used as a measure of normal behavior. All animals were observed for alertness, morbidity and mortality 6 and 24 hours after exposure and daily thereafter.

Twenty-four hours after the last daily treatment, mice (four 1X and four 10X) and quail (two 1X and four 10X) were sacrificed for red cell cholinesterase studies. At the same time blood samples from controls, six mice and six quail, were used to establish normal red cell cholinesterase levels. The remaining animals were kept under observation for one week. Red cell cholinesterase studies were performed by the Pharmacopathics Research Laboratory, Laurel, Maryland, using Pfizer diagnostic procedure. Results are reported in AChE-tel units.

RESULTS AND DISCUSSION. Daily observations of the animals receiving 1X and 10X treatments on a weekly and daily schedule indicated normal behavior throughout the exposure period. All animals remained alert and well coordinated with no evidence of neurotoxic symptoms. Food consumption remained normal and there was no diarrhea in the mice or quail.

The results of 5 weekly treatments to goldfish, mice and quail with malathion ULV at 1X and 10X rates are given in Table 1. A 20 percent mortality occurred

TABLE 1.—Effects of weekly ULV malathion exposures on fish, mice and quail.

	Fish	Mice[a]	Quail[a]
Dosage level 1X			
No. of animals	10	10	10
No. of exposures	5	5	5
Mortality—No./%	2/20	0/0	0/0
Dosage level 10X			
No. of animals	10	10	20
No. of exposures	5	5	5
Mortality—No./%	2/20	0/0	0/0
Controls			
No. of animals	10	10	10
Mortality—No./%	2/20	0/0	1/10

[a] Equal numbers of male and female animals were used except fish which were not sex separated.

TABLE 2.—Effects of daily ULV malathion exposures on fish, mice and quail.

	Fish	Mice	Quail
Dosage level 1X			
No. of animals used	10	10	10
No. of exposures	20	20	20
Mortality—No./%	1/10	0/0	5/50
Dosage level 10X			
No. of animals used	10	10	10
No. of exposures	20	20	20
Mortality—No./%	0/0	2/20	0/0
Controls			
No. of animals used	10	10	10
Mortality—No./%	2/20	0/0	5/50

in goldfish at both 1X and 10X dosages, and also in controls. Death in 1X and 10X fish may therefore be due to natural causes. No mortality occurred in treated or control mice. All quail survived both 1X and 10X dosages, but one control quail died.

The results of daily malathion ULV treatments to goldfish, mice and quail at 1X and 10X dosages are given in Table 2. There was a 10 percent mortality in goldfish receiving the 1X application and no mortality in fish at the 10X rate of application. Untreated goldfish had a 20 percent mortality rate which suggested that malathion was not the causative mortality factor in the 1X treated fish.

There was no mortality in 1X mice exposed to 20 applications of malathion. Twenty percent mortality occurred in mice exposed to the 10X dosage level. Both mice were females and death was attributed to fighting. Of the two dead mice, one died after the second application and the other died after the third application. No additional mortality occurred during the following 17 treatments. No mortality occurred in control mice.

Control, 1X and 10X quail showed corresponding mortalities of 50, 50 and 0 percent respectively. Mortality in control and 1X birds occurred after a severe storm of freezing rain, snow and high wind on November 25, 1971. Death in these birds was therefore attributed to adverse weather conditions. Mortality occurred between the 13th and 14th treatment period.

Red cell cholinesterase levels in 1X and 10X mice and quail 24 hours after the final malathion daily treatments failed to show any inhibition. (Table 3.)

SUMMARY. Under the conditions of the test, malathion ULV applications at 1X and 10X rates did not produce any detectable neurotoxic symptoms in test animals treated 20 times within a 34-day period. Animals remained alert, active and well coordinated. Food consumption was normal and no diarrhea was detected in mice and quail. Mortality occurring in test animals was attributed to factors other than malathion treatment. No red cell cholinesterase inhibition was found in mice and quail at the completion of the test. At ten times the normal rate of application, malathion was found to be safe to goldfish, laboratory mice and bob-white quail.

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TABLE 3.—Red cell AChE-tel cholinesterase levels [1] on mice and quail exposed to 20 daily applications of malathion

	No. AChE-tel tests	Avg. No. AChE-tel units	Range AChE-tel units
Mice			
1X	4	44	42–48
10X	4	42	30–51
Control	6	39	24–48
Quail			
1X	2	49	36–63
10X	4	47	42–60
Control	6	58	36–78

[1] AChE-tel units may be converted to International Cholinesterase units by using the following formula:

AChE-tel units $\times$ 0.011 = micromoles of substrate hydrolyzed/minute/ml. of packed red blood cells.