BRAIN ACETYLCHOLINESTERASE LEVELS IN CHICKENS AND RABBITS EXPOSED TO GROUND APPLICATIONS OF ULV MALATHION

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ABSTRACT. Chickens and rabbits and their feed and water were exposed to 5 levels of ground applied Cythion® ULV concentrate (95% malathion). At the dosage levels used, no signs of organophosphate poisoning were observed. Brain acetylcholinesterase (AChE) determinations showed both animal species capable of tolerating up to 15 min of continuous ULV malathion exposure. At this level, chickens responded with 62.9% inhibition and rabbits with 8.6% inhibition. Chickens were found to be more sensitive to ULV malathion exposure than rabbits.

Major efforts toward the control of adult mosquitoes in Maryland have primarily been directed to the use of high volume mist sprayers. With the advent of ground ultra low volume (ULV) equipment and its inherent advantages, high volume mist sprayers are gradually being phased out of service. The mosquito adulticide used in the ground ULV studies reported here was 95% malathion. Application of this highly concentrated insecticide may present some concern as to its effect on non-target animals. Studies by Joseph et al. (1972) indicated that multiple ULV malathion treatments to mice and quail failed to inhibit red cell cholinesterase. Efforts were continued to further evaluate ULV malathion on other non-target animals. Using more refined techniques to measure cholinesterase inhibition, this paper reports on laboratory and field studies of ULV malathion treated chickens and rabbits.

METHODS AND MATERIALS. Field studies were made with ground applied ULV malathion to evaluate its toxic effect on brain acetylcholinesterase (AChE) activity in mature chickens and rabbits. Rabbits were of the white New Zealand strain and chickens were a commercial meat-type broiler being a cross between a Vantress male on an Arboracres female. Malathion

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ULV concentrate was applied with a truck-mounted Leco Model H.D. cold, aerosol fog-generator. Separate groups of animals, 8 each, were exposed to 5 dosage levels. A dosage level of 1X is defined as a flow rate of 2.1 fl oz/min with a vehicle speed of 5 mph or 4.3 fl oz/min at 10 mph and is the maximum label recommendation for adult mosquito control with malathion. Dosage levels used were 5X, 20X, and 40X at a distance of 50 feet from the sprayer and 5 and 15 min of continuous exposure at a distance of 30 feet.

At 5 mph, 5 passes were made with the sprayer to obtain a 5X treatment at a discharge rate of 2.1 fl oz/min. At the same speed, 20X and 40X treatments were obtained by increasing the flow rate to 4.3 fl oz/min using 10 passes for the 20X and 20 passes for the 40X level. A flow rate of 4.3 fl oz/min was also used in the 5 and 15 min continuous exposure with the vehicle in a fixed and stationary position. All applications were made downwind.

Chickens used for each dosage level were confined in a 6 x 2 x 2 ft cage of ½ in. hardware cloth. Four rabbits were placed in each of 2 cages measuring 2 x 2 x 2 ft which were constructed of heavy gauge 2 x 3 in. mesh wire. Shallow enameled trays containing food and water were placed on the ground next to the confined animals. Three glass plates measuring 8 x 8 in. were also placed in the target area for malathion residue analysis. Ten min after each exposure the animals, treated food, water, and glass plates were returned to the laboratory. During the 24-hr holding period, the animals were fed the treated food and water.

Chickens and rabbits, 8 each, with equal number of sexes, were used in all 5 tests as well as for establishing base line control values. Four chickens and 4 rabbits were also subjected to oral dose levels of malathion to establish high levels of brain AChE depression. These animals were fasted overnight, weighed and given an appropriate amount of technical 95% malathion introduced orally via French feeding tubes. Oral doses given to rabbits were based on the acute oral LD_{50} for male rats which, for malathion, is 1,375 mg/kg (Gaines 1969). Doses given to chickens were based on the acute oral LD_{50} for female mallard ducks of 1,485 mg/kg (Tucker and Crabtree 1970). Two chickens were given ½ LD_{50} and 2 were given ¼ LD_{50}. With rabbits, 2 were dosed with 1 LD_{50} and the remaining 2 with ½ LD_{50}.

All animals were sacrificed 24 hr after exposure, except for the 1 group of chickens and rabbits which were subjected to a time-response study after receiving 5 min of continuous exposure. Two chickens and 2 rabbits were sacrificed 1, 2, 3 and 4 days posttreatment. All AChE assays were made immediately after dissection on freshly prepared brain homogenates. Brain AChE levels were determined by a modification of the electrometric method of Michel (1949) as described by Hawkins and Knittle (1972). Whole brain preparations were used and treated exactly as described by Knittle and Tucker

Table 1. Brain AChE levels in chickens exposed to malathion ULV concentrate.

Dosage level	Mean Δ pH in 45 min	Range	S.D.	% C.V.	Mean % inhibition	Malathion residue lbs A I/acre
5x	1.539	1.424–1.598	0.0341	2.2	0.7	0.0161
20x	1.451	1.232–1.680	0.1268	8.7	6.4	0.0625
40x	1.370	1.255–1.442	0.0698	5.1	11.6*	0.1877
5 min	1.399	1.286–1.589	0.0957	6.8	9.8*	2.6820
15 min	0.576	0.324–0.897	0.1836	31.9	62.9*	2.2350
Controls	1.550	1.506–1.695	0.0573	3.7	....	....

* Significantly different from control.

Table 2. Brain AChE levels in rabbits exposed to malathion ULV concentrate.

Dosage level	Mean △ pH in 45 min	Range	S.D.	% C.V.	Mean % inhibition	Malathion residue lbs A I/acre
5x	0.737	0.671–0.771	0.0510	6.9	None	0.0161
20x	0.703	0.660–0.736	0.0212	3.0	1.8	0.0625
40x	0.702	0.670–0.743	0.0246	3.5	2.0	0.1877
5 min	0.709	0.691–0.743	0.0163	2.3	1.0	2.6820
15 min	0.654	0.591–0.732	0.0464	7.1	8.6*	2.2350
Controls	0.716	0.690–0.764	0.0267	3.7	...	....

(1970). When the mean △ pH value of a treated group was less than 2 standard deviations from the control mean, the results were considered significant. Malathion residue analyses were conducted on the acetone washes of glass plates exposed to the spray treatments. Each plate was rinsed with approximately 500 ml of acetone. The acetone was concentrated to an appropriate volume for gas chromatographic analysis. Analyses were done on a Packard gas chromatograph equipped with a 1.83 m x 4 mm glass column packed with 10% DC-200 on Gas Chrom Q (100–120 mesh) using the flame photometric detector. Conditions were as follows: Nitrogen carrier gas at 80 ml/min, column temperature 190° C, inlet 240° C, outlet 195° C and detector 165° C. Residue determinations were made on all 3 plates in each of the 5 exposures. Results in each of the 5 plate tests were averaged and are expressed in pounds of active ingredient per acre (lbs A I/acre).

RESULTS. No mortality was observed in any test animal in the 24 hrs after spray treatment at all dosage levels used. During the 24-hr posttreatment period, no signs of organophosphate poisoning were detected. Chickens exposed to 5X, 20X and 40X dosage levels of malathion showed corresponding brain AChE inhibition levels of 0.7, 6.4 and 11.6% respectively (Table 1). Response by chickens to 5 min continuous exposure resulted in AChE inhibition of 9.8%. Chickens exposed to 15 min continuous exposure showed 62.9% brain AChE inhibition. Rabbits treated at dosage levels of 5X, 20X, 40X and 5 min of continuous exposure responded with a slight decrease in brain AChE activity. Continuous exposure for 15 min produced in rabbits 8.6% brain AChE depression (Table 2).

A time-response study was made of the animals treated for 5 min continuous exposure. Maximum chicken AChE inhibition, 14.4%, occurred 3 days posttreatment (Table 3). With rabbits a maximum depression of 2.8% was obtained at the end of 24 hrs. Response by chickens to oral doses of ½ LD_{50} and ¼ LD_{50} produced corresponding enzyme inhibitions of 94.4 and 87.3% respectively (Table 4). Rabbits responded to 1 LD_{50} and ½ LD_{50} oral doses with corresponding activity depressions of 79.2 and 49.6%. Signs of organophosphate poisoning were detected in all orally dosed animals. Mortality occurred in some of the animals (Table 4). At dosage rates of 5X, 20X and 40X malathion residue analysis showed a gradual increase in the deposition of actual toxicant

Table 3. Time response study of brain AChE levels in animals[1] exposed to malathion for 5 minutes.

Day[2]	Animal	Mean △ pH in 45 min	Mean % inhibition
1	Chickens	1.396	9.9*
	Rabbits	0.696	2.8
2	Chickens	1.438	7.2
	Rabbits	0.701	2.1
3	Chickens	1.326	14.4*
	Rabbits	0.733	none
4	Chickens	1.435	7.4
	Rabbits	0.710	0.8

[1] Two chickens and 2 rabbits sacrificed daily.
[2] Days following exposure.

Table 4. Dose-response study of brain AChE levels in animals orally dosed with malathion.

Animal and no. used	Oral dosage	Mortality	Mean △ pH in 45 min	Range	% Inhibition
Chickens 2	1/2 LD_{50}	1	0.087	0.071–0.102	94.4*
Chickens 2	1/4 LD_{50}	0	0.197	0.034–0.359	87.3*
Rabbits 2	1 LD_{50}	2	0.149	0.130–0.167	79.2*
Rabbits 2	1/2 LD_{50}	1	0.361	0.174–0.547	49.6*

from 0.0161 to 0.1877 lbs A I/acre (Table 1). The amount of actual toxicant recovered after 5 min of continuous discharge was 2.6820 lbs A I/acre and for 15 min 2.2350 A I/acre was obtained.

DISCUSSION. When the mean AChE activity of a treated group was more than two standard deviations from the control mean results were considered significantly different. At levels of 40X, 5 and 15 min of continuous exposure significant inhibition of AChE values was obtained on chickens and at 15 min with rabbits. Chickens and rabbits were found to tolerate all 5 dosage levels without showing any outward signs of organophosphate poisoning. Of the 2 animal groups studied, chickens were found to be more sensitive to malathion than rabbits at all dosages used. At a dosage level of 15 min continuous exposure chickens responded with 62.9% inhibition whereas rabbits receiving the same dosage responded with 8.6% inhibition. Chickens responded to an oral ½ LD_{50} dose with 94.4% inhibition, whereas similarly treated rabbits showed 49.6% inhibition. A discrepancy in the malathion residue analysis at the 5 min continuous exposure level is evident. This was not surprising considering the unfavorable weather condtions such as sudden wind changes in direction and velocity during the time of application.

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