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Effect of Sublethal Concentrations of Malathion on Coturnix Quail*

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Malathion and other organophosphorus compounds are widely used. The toxicity of malathion and other organosphosphorus compounds is mainly through inhibition of cholinesterase (ChE) in insect pests and other animals (DAHM 1971). ChE inhibition results in accumulation of acetylcholine (ACh) at neuromuscular endplates which then causes prolonged muscle contraction and decreased physical activity.

Wildlife living on croplands may be subjected to low concentrations of organophosphorus compounds several times during a growing season. Repeated absorption of these toxicants may result in an inability to escape from predators or to seek food and water.

No research has been undertaken on the sublethal inhibition of brain AChE caused by malathion, and subsequent reduced physical ability of birds. Insufficient consideration has been given to this insecticide in land areas where contamination of food and water resources of birds is possible. Therefore, research was performed to determine the effects of repeated exposure of coturnix quail (Coturnix coturnix japonica) to sublethal concentrations of malathion and the time for recovery of physical ability of birds in relation to brain AChE and malathion dosage.

MATERIALS AND METHODS

One hundred and twenty mixed strain and mixed sex coturnix quails were used in this experiment. Three-day-old birds were divided into four groups, with thirty birds in each group. One group was used as a control group (Group A). Three groups (B, C, and D) were treated. Each group was randomly distributed and artificial light was furnished for twelve hours per day.

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The quail chicks received locally prepared commercial turkey starter ration for one week and grower ration for the remainder of the experiment.

Commercial malathion (56.5% active ingredient) was used in corn oil and quantitatively force fed to each experimental bird (in a preliminary experiment free choice feeding of toxicant mixed with diet was not satisfactory). Birds in Group A were fed the maximum amount of corn oil received by the other groups of birds, but no malathion. Birds in Groups B, C and D received 20, 40 and 75 mg of malathion per day, respectively. Birds in each group were weighed at each feeding time and proper volumes of the corn oil-malathion mixture calculated and injected into the crop or proventriculus with a syringe and ball-point needle.

Two birds were killed from each group after 5, 10 and 15 days, and 6 birds after 21 days of treatment. The brain of each bird was removed immediately and frozen with dry ice. AChE activity analysis was completed on the same day the animals were killed.

The colorimetric method of GARRY and ROUTH (1964), with some modification, was used for determination of brain AChE activity. One-percent quinidin sulfate was used as the inhibitor in this experiment instead of 0.5%. Brain homogenate was prepared in DTNB-buffer and 0.2 ml of homogenate was used in tests instead of 0.02 ml of blood serum suggested by GARRY and ROUTH (1964).

Force feeding with malathion mixtures was discontinued and the physical ability of each group of birds was measured when the brain AChE activity in groups B, C, and D had declined to approximately 70, 50 and 30 percent of normal, respectively, when compared to the control group.

Physical ability was evaluated by performing a "flap test" on eight or nine birds from each group. The flap test procedure was to first weigh the bird, then place it on its back on the table (Figure 1a). The bird was then allowed to return to the standing position by using its wing and leg muscles (Figure 1b). The number of times the bird could regain its feet was counted for each bird and recorded. The test was completed when the bird was fatigued and unable to gain its feet. Physical ability was also determined for each group of birds at ten and twenty days after force feeding of the toxicant was discontinued.

Brain AChE activity was determined at ten, twenty, and thirty days after the cessation of force feeding with malathion. Six birds from each group were killed at each time interval. Brain AChE activity and physical ability were compared with the same parameters in birds from the control group (Group A) to estimate the time of recovery from the effect of malathion.

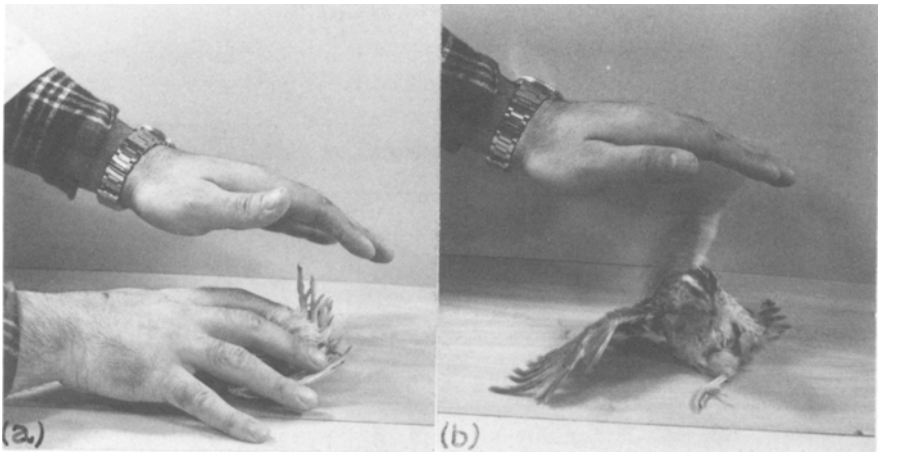

Figure 1. The flap test was performed by first placing the bird on its back (a), then allowing it to return to a standing position using wing and leg muscles (b).

RESULTS

Twenty-one days of administration of malathion at different dosages to the experimental groups of birds was necessary to reduce the average brain AChE activity to 31.7% for Group D, 55.8% for Group C and 75.3% for Group B, as compared to Group A, the control group (Table 1). The mean sulfhydryl (-SH) groups liberated from acetylthiocholine by the activity of brain AChE in one mg of brain homogenate in one minute is also given in Table 1. The brain AChE activity of birds in the three treated groups approached the normal level twenty days after treatment was discontinued.

Comparison of flap test means between treated groups and the control group after 21 days of force feeding of malathion indicated that groups B, C, and D were 65, 47 and 37 percent of the control group, respectively (Table 2). The mean number of times birds from each group could regain a standing position (flaps) after 21 days of treatment, and ten and twenty days after cessation of treatment with malathion, are also given in Table 2.

A definite linear correlation was demonstrated between brain AChE activity and flap test data following 21 days of malathion administration (Figure 2).

TABLE 1

Coturnix Quail Brain AChE Activity at Different Times Following Malathion Administration.

Group	Dosage mg/kg/da	Days[a] 0		10		20		30	
		-SH[b]	%	-SH	%	-SH	%	-SH	%
A	0	1.36	100	1.09	100	1.11	100	1.14	100
B	20	1.01	74.3[c]	0.73	66.6	1.60	100	1.14	100
C	40	0.76	55.8	0.66	60.1	1.40	100	1.15	100
D	75	0.43	31.7	0.50	41.8	1.11	99	1.17	100

[a] days following 21 days of malathion administration.

[b] mean-SH groups released from acetythiocholine in one minute by one mg brain tissue.

[c] mean percent of brain AChE activity as compared to nonexposed (Group A) birds.

TABLE 2

Physical Activity of Coturnix Quail at Different Times Following Malathion Administration

Group	Dosage mg/kg/da	Days[a] 0		10		20		24
		Flap[b] No	%	Flap No.	%	Flap No.	%	%
A	0	243	100	183	100	172	100	-
B	20	158	65[c]	151	82	181	100	-
C	40	115	47	121	65	185	100	-
D	75	90	37	109	59	156	91	100[d]

[a] days following 21 days of malathion administration.

[b] mean number of times birds could regain their feet after being placed on their backs (flaps).

[c] mean percent of physical activity as compared to the physical activity of nonexposed (Group A) birds.

[d] calculated from linear regression.

The flap test revealed the same pattern as the brain AChE activity level. There was no significant difference in the number of flaps or brain AChE activity in birds from Groups B, C or D compared with control birds after treatment was stopped ($P < 0.1$). The birds whose physical ability was less depressed returned to normal sooner than those whose physical ability was more impaired.

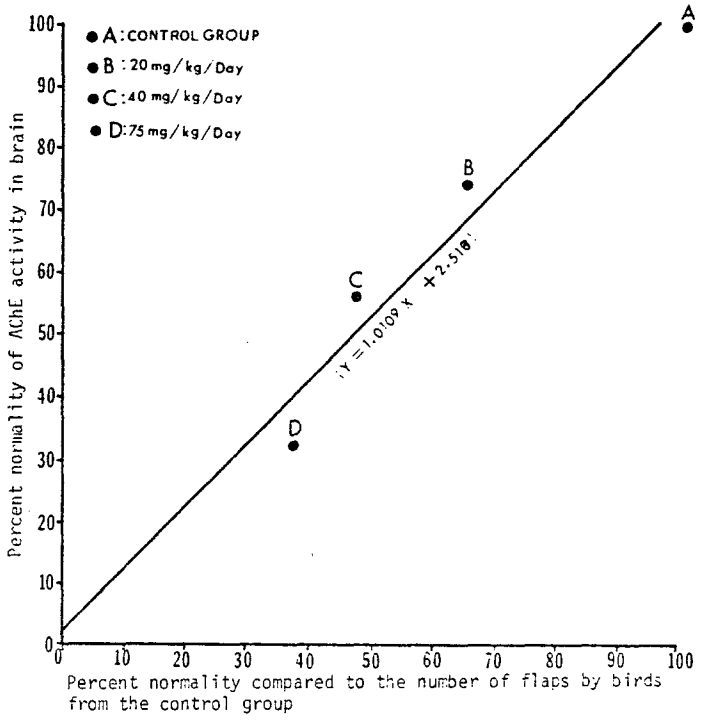

Figure 2. Relationship of brain AChE activity to the number of flaps in coturnix quail, 21 days after administration of malathion with different dosages in treated groups.

DISCUSSION

The blood ChE activity was found to be depressed in several species of birds after experimental exposure to organophosphorus compounds (CRABTREE 1965). No two species reacted identically to any one compound. Results of the experiment reported here indicated that blood serum AChE was not a realiable indicator of the sublethal effect of malathion. Brain tissue, however, was a more reliable indicator of AChE activity, and reduced the variation of results among individual birds from the same groups.

VILLENUVE (1971) described several methods for measuring AChE activity, but the colorimetric method of GARRY and ROUTH (1969), with some modification, was found to be acceptable. The method was initially in the blood, but had been adapted for use of brain tissue by POST and LEASURE (1974).

The flap test used to evaluate physical ability of birds exposed to malathion was found to be satisfactory. The variation in the physical abilities among birds demonstrated an inverse relationship between body weight and physical ability. The distribution of birds of different weights among the groups was similar. Considering that all birds were kept under similar conditions and the only difference was the dosage of malathion received by the treated groups.

There was a direct relationship between the dosage of malathion, inhibition of brain AChE activity and decline in physical ability. These results verify those of DIETER (1974) which demonstrated quantitative inhibition of plasma ChE activity by corresponding increased dosages of malathion to coturnix quail. The results also follow those of POST and LEASURE (1974) which not only demonstrated inhibition of brain AChE activity after an increased concentration of malathion in the environment, but also found reduced physical ability in three species of salmonid fishes.

Alteration of physical ability as demonstrated by this study, have a direct application to wild bird populations when sublethal concentrations of malathion are applied to the habitat. Several sublethal applications of AChE inhibiting pesticides may indirectly alter bird populations. Chronic exposure of bird populations to malathion and/or other organophosphorus compounds may cause an inability to survive because of physical activities necessary to escape predation or seek food and water. There has been no restriction and no waiting time enforced between applications of malathion for controlling insect pests (HANTSBARGER 1977), and the frequent exposure of birds to sublethal concentrations is possible. Direct application of the sublethal alteration of physical ability of wild ranging

birds from environmental organophosphorus contamination should be investigated.

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