EXPOSURE OF BOBWHITE QUAIL AND COTTONTAIL RABBITS TO METHYL PARATHIONa

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Abstract: Bobwhite quail (*Colinus virginianus*) and cottontail rabbits (*Sylvilagus floridanus*) collected before, during and after spraying operations using methyl parathion and toxaphene on cotton showed significant decreases in brain acetylcholinesterase (AChE) activity levels, as determined by one-way analysis of variance. Inhibition from the pre-spray mean was 9.0 to 68.3% for quail brains, and 7.0 to 32.4% for rabbit brains. Results for plasma activities were similar, but generally more variable than brain values. Activities were also more variable during the spray season than either before or after.

Proc. Ann. Conf. S.E. Assoc. Fish & Wildl. Agencies 32: 326-334

Bobwhite quail and cottontail rabbits are both popular game animals in North Carolina. In recent years biologists of the North Carolina Wildlife Resources Commission, as well as hunters in the state, have expressed concern that populations of both species are declining. No accurate census data are available, nor have any causes of the suspected decline been identified. However, since both quail and rabbits are found extensively in agricultural regions of the state, determining the extent and severity of the species' exposure to commonly used pesticides was of interest.

Pesticide use in North Carolina is estimated to total nearly 2.3 million kg annually for corn, cotton, soybeans, and tobacco (W. H. Hensley, N.C. Dept. Agr., pers. comm.). Cotton, although not grown as extensively as in earlier years, accounts for a large percentage of the state's total pesticide usage. Cotton pesticides are usually applied aerially, and many treatments are required each season for control of the cotton bollworm (*Heliothis spp.*) and boll weevil (*Anthanomus grandis*). During the 1976 growing season in North Carolina, farmers averaged nearly 11 applications of pesticides to their cotton crops (Grube and Carlson 1978).

Toxaphene and methyl parathion are often the materials chosen for cotton insect control. The organophosphate methyl parathion is highly toxic to vertebrates (Tucker and Crabtree 1970), and is probably the most widely used pesticide in North Carolina (J. R. Bradley, NCSU Dept. Entomol., pers. comm.), while toxaphene is the most heavily used organochlorine in the United States today (Casida et al. 1974).

This study was initiated in an attempt to (1) determine exposure of quail and rabbits to methyl parathion by use of acetylcholinesterase activity analysis, and (2) determine whether any significant mortality occurred as a result of pesticide exposure. Toxaphene and methyl parathion residue data and their statistical interpretation, which were also objectives of this study, will be reported elsewhere.

The authors wish to thank the North Carolina Wildlife Resources Commission and the North Carolina Agricultural Experiment Station for financial and technical support of this project (Pittman-Robertson Project No. W-57), and H. McLeod for allowing use of his land for the study area. The authors also express appreciation to D. W. Hayne, who reviewed this manuscript and provided helpful suggestions regarding data analysis. Special thanks also go to R. Brame, who provided assistance in collections and quail crop analysis.

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[a]Paper number 5818 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, NC.

METHODS AND MATERIALS

The Study Area

An area of about 400 ha (300 ha cleared) in Scotland and Robeson Counties of the North Carolina Coastal Plain was chosen for study during the 1976 growing season. Approximately 200 ha of the cleared areas were planted in cotton with the remainder in corn. The terrain was nearly flat and the soil of a sandy loam type, interspersed with elliptical depressions of undetermined origin known as "Carolina bays," where the soil contained more organic matter.

The uncultivated portions of the study area included several habitat types, most of which provided suitable food and cover for wildlife. Major types were: open stands of loblolly pine (*Pinus taeda*), open stands of longleaf pine (*P. palustris*), brush piles and windrows from recently cleared land, road and woods edges, Carolina bays, mixed hardwood stands, and closed-canopy stands of loblolly pine.

The understory vegetation was burned periodically, which promoted wildlife food plant growth in addition to the main purpose of reducing overwintering populations of boll weevils. Most of the potential wildlife habitat was in areas immediately adjacent to sprayed fields, and an extensive system of field roads provided ready access to the area for collection purposes.

Field Techniques

During the latter part of the growing season, cotton insect pest populations increase to economically damaging levels. In the study area cotton insect scouts search each field at least once weekly, and spraying is initiated when their reports indicate damaging levels of insects. In 1976 methyl parathion and toxaphene were aerially applied at the rate of 1.1 kg of each active ingredient per ha. In addition, Fundal (chlordimeform) was applied at the rate of 0.28 kg/ha but was discontinued in early September. In general, pesticide applications follow a definite schedule for several weeks, after which applications are made as determined by scouting reports of insect population levels.

In 1976 quail and rabbits were collected from the study area by shooting. Collections were made before spraying started; 24 hours after the first, third, fifth, ninth, and final sprays; and 3 weeks after the final spray (Table 1). At the time of collection, blood samples were obtained from the heart in heparinized micro hematocrit tubes, and stored on ice. All animals were sexed, tagged, wrapped in aluminum foil, and stored on ice in plastic bags until time of analysis.

Four transects were selectively established along field borders, and were searched for dead animals during each collection trip. Habitat types and transect lengths were: (1) road and woods edge habitat, 1,265 m; (2) Carolina bay habitat, 425 m; (3) closed canopy loblolly and road edge habitats, 380 m; and (4) closed canopy loblolly habitat, 365 m.

In addition, cotton insect scouts were instructed to make observations of wildlife in fields, and to report any mortality. Routes taken through fields varied, but included at least 1 field edge and 1 line directly through the field, and all fields were visited at least once per week.

Laboratory Techniques

Quail food habits—Quail crops were removed, air-dried, and the contents identified and measured volumetrically. Plant material was identified to species when possible, and insect or other animal matter to family. This was done in order to find any possible correlation between animal matter in the diet and acetylcholinesterase activity depression; *i.e.*, whether secondary poisoning might be a factor in the quail's pesticide exposure.

Acetylcholinesterase activity analysis—Within 48 to 60 hours after collection, acetylcholinesterase (AChE) activity of both brain and blood plasma of collected animals

Table 1. Methyl parathion-toxaphene spray schedule and animal collection dates on cotton growing area in Scotland and Robeson counties, N.C., 1976.

Date		*Spray Number*[a]	*Collection Number*
July	10-11	Pre-spray	1
	28	1	--
	29-30	--	2
	31	2	--
August	4	3	--
	5-6	--	3
	7	4	--
	11	5	--
	12-13	--	4
	14	6	--
	19	7[b]	--
	23-24	8	--
	26	9	--
	27-28	--	5
	31	10	--
September	7	11	--
	13	12[b]	--
	22-30	13[c]	--
October	1-2	--	6
	22-23	--	7

[a]Except as noted, the entire area was covered each time with 1.1 kg active ingredient/hectare each of methyl parathion and toxaphene.

[b]Partial coverage of area.

[c]Partial coverage of area, spray at 0.55 kg active ingredient/hectare.

was determined using the method of Ellman et al. (1961), and either a Beckman DK-2 or Beckman DB spectrophotometer. The method was slightly modified from the original as follows. Two hundred μl of brain homogenate (10 mg/ml in pH8 phosphate buffer) or 10 μl of plasma, and 200 μl of dithiobisnitrobenzoate solution (0.01 M in pH7 phosphate buffer) were added to 6.0 ml of pH8 buffer. Three (3.0) ml were placed in the sample cuvette, and the remaining solution in the reference cell. Twenty μl of acetylthiocholine iodide solution (0.075 M in pH7 phosphate buffer) were added to the sample cell, and the reaction was recorded on the spectrophotometer for 4 to 5 minutes at 412 nm.

For all animals, duplicate samples of both brain and plasma were analyzed with the exception of a few plasma samples which were sufficient for only 1 analysis.

As a check on the method and instrumentation, standard bovine erythrocyte AChE (Sigma Chemical Company) was analyzed on both spectrophotometers, and results compared with the standard activity as reported by Sigma. Standard activity on the DB spectrophotometer was 87.6% of the theoretical activity, and on the DK-2 was 88.2% of theoretical.

Statistical Analysis—Means of brain and plasma AChE activity from each collection were compared to the pre-spray collection mean using a two-tailed t' test (Snedecor and Cochran 1967:114). One-way analysis of variance was performed on log-transformed brain AChE data.

For quail, correlation coefficients comparing insects in the crop and brain AChE activity were calculated: (1) using volume of insects in the crop, and (2) using percentage of total volume due to insects.

RESULTS

Field collections and transect searches

Between 10 July and 23 October 1976, 7 collection trips produced a total of 27 quail and 15 rabbits. Sample size for each collection is included in Tables 2 and 3.

No dead animals were found during the transect searches, and cotton insect scouts did not observe any wildlife mortality. However, it should be noted that the limited field searches used in this study were sufficient to detect only very "heavy" mortality.

Quail food habits

Insects, as percentage of total volume in crops, decreased in importance in the quail's diet as the season progressed. Results of correlations between animal matter in the diet and brain AChE activity are reported later in this section.

Acetylcholinesterase Activity

Collection means and standard errors of brain and plasma AChE activity from sample quail and rabbits are found in Tables 2 and 3. Fig. 1 presents brain AChE activity means as percentage of pre-spray mean for quail and rabbits. These results represent brain AChE inhibition from the pre-spray mean in quail of 23.3, 47.4, 57.5, 68.3, and 9.0% for the second through sixth collections, respectively. Brain AChE activity three weeks after spraying ceased was 17.1% above the pre-spray mean.

Table 2. Mean (±S.E.) brain and plasma acetylcholinesterase (AChE) activity[a] of quail collected from Scotland and Robeson counties, N.C., during the 1976 cotton growing season.

Collection	*Brain Sample Size*	*Brain AChE*[a,b]	*Plasma Sample Size*	*Plasma AChE*[a]
1	6	86.87 (±2.42)	5	1231.30 (±156.17)
2	4	66.62 (±8.91)	4	836.50 (±104.16)
3	4	45.70 (±10.10)	---[c]	--
4	4	36.90 (±10.06)	3	577.84 (±127.28)
5	2	27.50 (± 6.03)	1	135.11[d]
6	3	79.02 (±1.30)	2	972.93 (±367.17)
7	4	101.69 (±1.72)	2	1492.98 (±221.44)

[a]Activity is expressed in nanomoles acetylthiocholine iodide hydrolyzed per ml brain homogenate (or blood plasma) per minute.

[b]Significantly different among collections ($p > 0.01$, F-test).

[c]Samples lost.

[d]One sample only.

Rabbits showed less marked inhibition from the pre-spray mean, with brain AChE inhibition of 7.2, 7.0, 14.0, and 32.4% for the second through fifth collections. Lack of rabbit samples from the 2 final collections makes these results somewhat inconclusive, but rabbits apparently receive less exposure than quail, are less susceptible to AChE inhibition, or both.

Table 3. Mean (±S.E.) brain and plasma acetylcholinesterase (AChE) activity[a] of rabbits collected from Scotland and Robeson counties, N.C., during the 1976 cotton growing season.

Collection	*Brain Sample Size*	*Brain AChE*[a,b]	*Plasma Sample Sample*	*Plasma AChE*[a]
1	6	106.58 (±2.74)	6	354.36 (±49.14)
2	3	98.92 (±1.32)	3	213.27 (±59.88)
3	1	99.07[d]	---[c]	--
4	4	91.64 (±4.97)	4	200.01 (±25.20)
5	1	72.06[d]	1	60.00[d]

[a]Activity is expressed in nanomoles acetylthiocholine iodide hydrolyzed per ml brain homogenate (or blood plasma) per minute.

[b]Significantly different among collections ($p < 0.01$, F-test).

[c]Samples lost.

[d]One sample only.

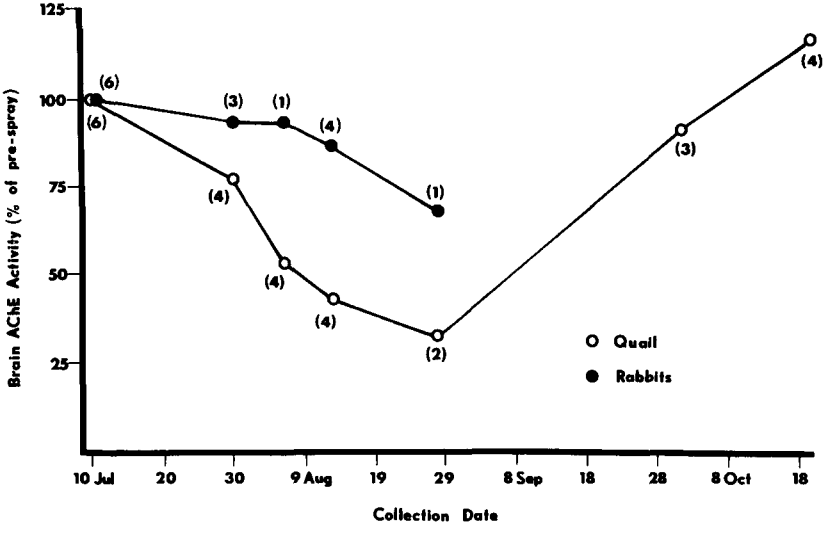

Fig. 1. Quail and rabbit brain acetylcholinesterase activity 24 to 60 hours after aerial application of methyl parathion. Numbers in parentheses indicate sample size. The 10 July collection was made before spraying operations started.

Table 4. Results of two-tailed t' test comparing collection means of quail brain and plasma acetylcholinesterase activity[a] with normal (pre-spray) mean activity. Data from animals collected during 1976 cotton growing season from Scotland and Robeson counties, N.C.

Comparison Collection No.	*t' value* (brain)	*t' value* (*plasma*)
1 *vs* 2	2.19 n.s.	2.10 n.s.
1 *vs* 3	3.97*	---[b]
1 *vs* 4	4.83*	3.24 barely n.s.
1 *vs* 5	9.13 n.s.	---[b]
1 *vs* 6	2.86 n.s.	0.65 n.s.
1 *vs* 7	-4.99**	-0.97 n.s.

n.s. non-significant, $p > 0.05$.

* significant difference, $p < 0.05$.

** significant difference, $p < 0.01$.

[a]Activity is nanomoles acetylthiocholine iodide hydrolyzed per minute per ml brain homogenate (or blood plasma).

[b]Insufficient sample.

Table 5. Results of two-tailed t' test comparing collection means of rabbit brain and plasma acetylcholinesterase activity[a] with normal (pre-spray) mean activity. Data from animals collected during 1976 cotton growing season from Scotland and Robeson counties, N.C.

Comparison Collection No.	*t' value* (*brain*)	*t' value* (*plasma*)
1 *vs* 2	2.52 n.s.	1.82 n.s.
1 *vs* 4	2.63 n.s.	2.79*

n.s. non-significant, $p > 0.05$.

* significant difference, $p < 0.05$.

[a]Activity is nanomoles acetylthiocholine iodide hydrolyzed per minute per ml brain homogenate (or blood plasma).

Collection means of brain and plasma AChE activity were compared using a t' test, and these results are summarized in Tables 4 and 5 for quail and rabbits respectively. Variations in activity level among samples in collections during spray operations caused most differences to be non-significant. Mean brain AChE activities of quail for collections 3, 4, and 7 were the only values significantly different from pre-spray levels. The lack of adequate samples allowed only 2 tests of differences for rabbits, with plasma values from collection 4 giving the single significant difference. In general, brain activities were less variable than plasma activities.

Based upon laboratory studies of Japanese quail (*Coturnix coturnix*), Ludke et al. (1975) suggested that a level of AChE inhibition greater than 50% from normal was indicative of organophosphate induced death. We have used this 50% inhibition as a

reference point in interpreting our field data. We examined the combined records of brain activity for spray-season collections (2 through 6 for quail and 2 through 5 for rabbits). Assuming that the untransformed data approximated a normal distribution, we fitted a normal distribution (Snedecor and Cochran 1967:36) and calculated the percentage of activities expected to fall below the 50% level of pre-season activity. This percentage was 35.2% for quail and 0.01% for rabbits; actually 6 of 17 quail brain activities (about 35%) were below the 50% level, with no rabbit brains approaching this level of inhibition.

The analysis of variance of brain AChE data allowed separation of different components of variability. There were significant differences both among collections ($p < 0.01$, $F = 7.95$) and among samples within collections ($p < 0.01$, $F = 220.00$) for quail. Large differences among samples within collections may indicate differing exposure or susceptibility among animals, a possibility to be discussed in the next section.

Results of the analysis of variance for rabbits showed significant differences among collections ($p < 0.01$, $F = 6.17$). Differences among samples within collections were not significant ($p > 0.05$, $F = 1.92$) in contrast to the large difference for quail. This result may simply be due to the sample taken, but could also indicate more uniform susceptibility among rabbits.

Correlations of volume of insects in the crop *vs.* brain AChE activity, and insects as percentage of total volume in the crop *vs.* brain AChE activity, were both non-significant ($p > 0.05$).

DISCUSSION

Although no mortality was observed, the limited nature of the searches conducted precludes concluding that no pesticide induced mortality occurred. The failure to observe any mortality does, however, suggest that large scale pesticide induced die offs did not occur.

Ludke et al. (1975) reported that in birds brain AChE inhibition exceeding 20% indicated exposure to organophosphates and inhibition greater than 50% was sufficient for diagnosing cause of death. However, the authors noted that some individuals could survive with brain AChE activity lower than 50%. Based on the above findings, the levels of inhibition observed for quail in this study indicate exposure to methyl parathion. Furthermore, the data indicate that approximately 35% of the population from which the samples were drawn could be expected to have an inhibition equal to or greater than 50% of normal. This observation suggests that a substantial portion of the quail population in the study area may have been exposed to a potentially harmful level of methyl parathion. Consistent with this speculation is the observation that 6 of the 17 quail collected exhibited AChE inhibition greater than 50% of the pre-spray mean.

Only 2 rabbits collected during the spray season demonstrated brain inhibition greater than 20% and none of the individual inhibition levels equaled or exceeded 50% inhibition. However, the validity of using the 20 and 50% levels as indicative of exposure and mortality in mammals has not been explored. Variability in normal activity levels and in visible effects at various inhibition levels has been noted for fish (Gibson et al. 1969, Weiss 1958) and for birds (Bunyan et al. 1969, Mehrotra et al. 1967).

AChE inhibition in quail was most severe during the height of spraying operations, and recovered rapidly thereafter. The incomplete data on rabbits showed a less dramatic drop in activity, but the general trend was similar.

AChE activities of quail collected after all spraying ceased were significantly higher than pre-spray levels. This may be an artifact of the sampling or analysis, but there is some evidence for a seasonal variation in enzyme activities of rodents (Shellhammer 1961) and perhaps humans (Gage 1967). If the observed change in pre- *vs.* post-spray activities is in fact a normal seasonal variation, its magnitude is in any case less than the inhibition observed during spray operations. It is also possible that animals overcompensated for enzyme inhibition, and temporarily maintained higher than normal AChE activity levels after exposure ceased.

Animal matter in the quail's diet did not correlate well with enzyme activity, and probably reflected the normal decrease in animal matter consumption as the peak breeding season passed. These results suggest that most of the quail's pesticide exposure was apparently not due to eating poisoned insects, but further work in this area is necessary before any definite conclusions can be made.

The results of the analysis of variance performed on the brain AChE data set indicate that most of the variation in AChE activity was due to collection date (*i.e.*, amount of pesticide applied) and to individual variation among samples. Both before and after spraying operations sample variance of AChE activity was low (C.V. <7%), indicating a rather narrow range of normal activity levels. This low variance in the absence of pesticide pressure, and the high variances associated with quail samples during spray operations (C.V. >40%), support the hypothesis that availability of pesticides differed at different locations in the study area. Alternatively, susceptibility to AChE depression may differ among quail, or a combination of both factors may be present. Variances of rabbit AChE results were smaller, which may indicate more uniform susceptibility among rabbits. The data support this to some extent as inhibition in rabbits was generally slight, although statistically significant in the analysis of variance.

The narrow range of normal activity levels also supports the continued use of AChE analysis in monitoring wildlife populations for suspected organophosphate exposure. Several previous field studies have made use of this technique with apparently good results (Elder and Henderson 1969, Finley 1965, Hamilton and Stanley 1975, Hill et al. 1971, Holland et al. 1967, Seabloom et al. 1973, Williams and Sova 1966, Zinkl et al. 1977). Additional work may reveal whether there are significant population, seasonal, or other differences in normal activity levels, and allow refinement of this promising technique.

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