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Effects of Northern Bobwhite (*Colinus virginianus*) Age and Weight on Results of the Avian Dietary Toxicity Test

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The question of test animal age is not new in wildlife toxicology. Hill and Camardese (1981) addressed this question in their three part study using Japanese quail (Coturnix japonica). They concluded that "subacute toxicity data were significantly affected by the age of the Japanese quail during the first three weeks after hatching". These researchers presented examples of potential age-related factors for changes in response including: (1) maturation of detoxicating enzyme systems, (2) increases of target enzymes activity, (3) the improved efficacy of the hepatic and renal clearance process, and (4) development of a blood-brain barrier against some compounds. The LC_{50} values increased with increased age for all nine chemicals they tested. Earlier work on the question (Hudson et al., 1972) found that age affected the results of mallard (Anas platyrhynochos) dietary tests in a biphasic way. For example, ducklings both younger and older than seven days of age had lower LC_{50} values for some chemicals and higher values for others.

Researchers at the U.S. Environmental Protection Agency (EPA), Environmental Research Laboratory, Corvallis (ERL-C), Oregon (Bennett, pers. comm.) have noted that LC_{50} values also increased with test animal weight. Hill and Camardese (1981) reported that food consumption in proportion to body weight decreased with increased age and that this natural reduction in food consumption could reduce potential exposure by 60%. They did not attribute this phenomenon to differences in body weight among different aged birds. Rather, they attributed it to maturation of biochemical and physiological processes associated with increased age. This study investigated how age and body weight and the interaction of these two factors of northern bobwhite affected results of the avian dietary toxicity test.

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MATERIALS AND METHODS

The following chemicals were selected: dicrotophos [3-(dimethyloxyphosphinyloxy)-N,N-dimethyl-cis-crotonamide; purity = 90.0%, lot G141], dieldrin [1,2,3,4,10,10,-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo,1,4,5,8-dimethanonaphthalene; purity = 99.7%, lot A807], and methiocarb [3,5-dimethyl-4-(methylthio)phenyl methylcarbamate; purity = 99.6%, lot E350]. Dietary concentrations for each chemical were selected based on the knowledge of previously determined LC_{50} values (Bennett, 1989; Hill et al., 1975; Hill and Camardese, 1981; Kononen et al., 1986) and then geometrically spaced above and below these values. Six dietary concentrations including 0 (control) were used in each test. Chemicals were dissolved in corn oil and mixed 2:98 (w/w) with Purina(R)* gamebird starter diet. Control feed contained corn oil in the same proportion. Dietary concentrations were verified by gas chromatography.

The study was conducted as a factorial design with animal age, weight, and dietary concentration as independent variables. Northern bobwhite were used for all tests at the Wildlife Research Facility (WRF), ERL-C. Birds in the dieldrin and methiocarb tests originated from ERL-C while birds in dicrotophos tests were purchased from GQF Manufacturing Company, Savannah, GA, as eggs. All eggs were incubated and hatched at the WRF. Birds were 10 days or 17 days of age at the beginning of each treatment period. These ages represented the extremes recommended in the Avian Dietary Test guidelines (EPA, 1982a; 1982b). Three days prior to treatment, birds were removed from communal brooders, wing tagged for identification, and weighed. For each age, birds were separated into three

Table 1. Mean initial body weight for each age and weight group.[1]

Weight Group	10 days-old	17 days-old
	x ± sd	x ± sd
LO	10.9 ± 0.2	26.1 ± 1.7
MED	13.1 ± 0.2	30.9 ± 1.7
HI	15.0 ± 0.4	35.7 ± 1.3

[1] Means were calculate using mean weight from each of the three tests.

* Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

sequential weight groups hereafter identified as LO, MED, and HI. Mean initial weights are presented in Table 1.

Birds were randomly assigned to one of six dietary concentrations (with regard to age and weight) and placed five per brooder. There were 36 separate age-weight-dietary concentration groups, each replicated twice for each chemical tested. Approximately 360 birds were used for each chemical tested. Following guidelines for avian dietary toxicity tested used by the EPA (1982a; 1982b), birds were exposed to treated feed for five days, and then presented untreated feed for three days.

Feed consumption, clinical signs of toxicity, and mortality were recorded daily during the 3-day pre-treatment period, the 5-day treatment and the 3-day post-treatment periods. Feed consumption (corrected for spillage) was calculated as mean grams consumed per bird-day and mean grams consumed per bird-day per 100 g of body weight. Chemical consumed was calculated using nominal concentrations and reported as mean micro-grams per gram of body weight per day. The number of birds alive each morning was used to calculate consumption.

Birds were weighed at the start of the pre-treatment period, the start of the treatment period (day 0), at death, at end of treatment period (day 5), and at the end of the test (day 8). Birds surviving the test were asphyxiated with CO_2. Individual body weights and percent of body weight change were used to calculate mean change in body weight and mean percent of day 0 body weight change. Percent weight change at death was determined by subtracting weight at death from weight at day 0.

The median lethal concentrations (LC_{50}) and 95% confidence intervals were calculated by probit analysis using Statistical Analysis System computer software package (SAS Institute, Inc., 1982). LC_{50} values were considered significantly different from each other when the 95% confidence interval (fiducial probability interval) did not overlap (Finney, 1971). Three-way analysis of variance (SAS Institute, Inc., 1982) was used to determine the significance that each of the main effect variables (age, weight, dietary concentration) and their interactions had on the response variables (feed consumption, weight change,amount of chemical consumed).

RESULTS AND DISCUSSION

Table 2. Percent mortality for bobwhite in three dietary tests by age-weight group and dietary concentration of three chemicals.

Age-Weight Group	Percent Mortality by Dietary Concentration (mg/kg)					
	Dicrotophos					
	0 mg/kg	20 mg/kg	28 mg/kg	40 mg/kg	56 mg/kg	80 mg/kg
10,LO	0	100	100	100	100	100
10,MED	0	60	100	100	100	100
10,HI	0	80	60	100	100	100
17,LO	0	30	40	90	100	100
17,MED	0	0	50	70	100	100
17,HI	0	0	50	80	100	100
	Dieldrin					
	0 mg/kg	15 mg/kg	21 mg/kg	30 mg/kg	43 mg/kg	60 mg/kg
10,LO	0	0	10	60	80	100
10,MED	0	0	80	70	80	100
10,HI	0	0	0	60	80	100
17,LO	0	0	10	10	80	100
17,MED	0	0	0	30	80	100
17,HI	0	0	0	20	60	90
	Methiocarb					
	0 mg/kg	590 mg/kg	770 mg/kg	1000 mg/kg	1300 mg/kg	1690 mg/kg
10,LO	0	10	40	90	100	100
10,MED	0	10	50	40	100	100
10,HI	0	0	20	10	70	80
17,LO	0	0	20	30	50	90
17,MED	0	0	0	10	20	50
17,HI	0	0	10	10	0	40

Mortality was 100% for 18 of 36 age-weight-dietary concentration groups in the dicrotophos test (Table 2). Though mortality was excessive, survival generally improved with increased age and weight. Mortalities generally ended by the sixth day (first day of post-treatment). A dietary concentration of 60 mg/kg dieldrin produced 100% mortality in all but the oldest and heaviest age-weight group (17,HI 90%). No mortalities occurred at 0 or 15 mg/kg dieldrin. Unlike

Table 3. Median lethal concentrations (LC_{50}) by age-weight group for bobwhite exposed to three chemicals.

Age-Weight Group	LC_{50} (mg/kg)	95% Confidence Interval	Slope
	Dicrotophos		
10,LO	<12.5[1]		
10,MED	<18.7[1]		
10,HI	15.8	0 - 22.6	3.8
10 Overall[2]	14.0 *[3]	4.0 - 18.4	4.7
17,LO	27.0	21.4 - 32.5	6.5
17,MED	30.8	24.4 - 36.4	7.7
17,HI	29.9	23.8 - 35.0	8.6
17 Overall	29.0 *	25.9 - 31.9	7.2
	Dieldrin		
10,LO	30.2	25.4 - 35.7	7.6
10,MED	24.5	0 -	5.3
10,HI	31.5	26.6 - 36.7	8.9
10 Overall	28.8 *	26.1 - 31.7	6.8
17,LO	35.3	30.2 - 41.9	8.6
17,MED	34.7	29.7 - 40.3	10.5
17,HI	40.0	33.7 - 47.8	7.7
17 Overall	36.0 *	33.6 - 40.0	8.5
	Methiocarb		
10,LO	786 a	693 - 888	11.6
10,MED	867	740 - 1002	7.7
10,HI	1205 a	1038 - 1426	6.9
10 Overall	963 *	886 - 1044	7.1
17,LO	1170 b	1006 - 1387	6.8
17,MED	1795 b	1492 - 2793	5.7
17,HI	1928 b	1538 - 3719	4.8
17 Overall	1575 *	1410 - 1834	5.2

[1] LC_{50} for 10,LO and 10,MED were calculated based on day 7 mortalities, before conditions of probit analysis were violated.

[2] Overall LC_{50}s were calculated for entire age group without regard to weight group.

[3] Values with same letter were significantly different within ages and * indicates significant difference between overall values (ages) within treatment.

the dicrotophos test, mortalities continued through the post-treatment period (day 8). As expected, mortality tended to increase with increasing dietary concentration and to decrease with increasing age. Differences among weight groups were not obvious for dieldrin. Mortality patterns for the methiocarb test were similar to those of the other tests in their general tendency toward lower mortality values with increased age and

weight. Mortalities of 100% occurred only in the two smallest age-weight groups at the two highest dietary concentrations. Mortalities did not occur above age-weight group 10,MED at the lowest dietary concentration. As with dicrotophos, mortalities in the methiocarb test stopped by the sixth day (first day of post-treatment). The cessation of mortality was similar to that observed by Bennett (1989), in tests of an organophosphate and two carbamates. In addition to cessation of mortality, the apparent health of birds exposed to either dicrotophos or methiocarb improved dramatically after exposure ended. This was not true of birds exposed to dieldrin where mortalities and apparent illness continued after exposure ended.

The LC_{50} in mg/kg of feed differed significantly between ages in the three dietary tests and within weight groups for methiocarb (Table 3). Differences among weight groups within age in the dicrotophos test could not be tested because of high mortalities and there was no influence of weight on LC_{50} values in the dieldrin study.

Analysis of feed consumption data for the 5-day exposure period demonstrated that age, weight, and dietary concentration significantly influenced feed consumption. In general, as dietary concentration increased within age-weight-groups, feed consumption decreased. Food consumption per bird increased with increasing age and weight. Seventeen-day-old birds consumed significantly more feed than 10-day-old birds and within ages, HI weight birds consumed significantly more feed than LO weight birds. There was not a significant interaction between age and weight on feed consumption.

On a per unit of body weight basis (grams of feed consumed per 100 g of body weight per day), feed consumption decreased with increased age and generally decreased with increased weight group within age. Less feed was consumed per unit of body weight by 17-day-old birds than by 10-day-old birds. Within age, less feed per 100 g of body weight per day was consumed by the HI weight birds than by LO weight birds. Ten-day-old birds consumed significantly more chemical per gram of body weight than did 17-day-olds at each dietary concentration for each chemical tested. Within this pattern, differences among weight groups also occurred, though weight did not contribute significantly to feed consumption (per gram of body weight) in the methiocarb test. Lighter birds generally consumed more chemical per gram body weight than heavier birds within the same age. Hill and Camardese (1981) reported this response for birds of different ages, but they did not consider

differences in body weight within age groups. It is well known that as body weight increases, food intake on the basis of percent of body weight decreases for at least two reasons: 1) energy required to maintain body heat in the larger birds decreases because of a lower surface area to body weight ratio; and 2) less energy is required for growth as birds approach adult weight (Kenaga 1973, Whittow 1976). Disproportionate food consumption rates may have influenced response in the present study. Ten-day-old birds may have suffered more severe effects than their significantly heavier 17-day-old counterparts because they consumed more chemical per unit of body weight.

Birds that died before completion of the test usually lost weight. Mean percent weight loss ranged from 26 to 44%, 0 to 27%, and 9 to 44% for dicrotophos, dieldrin, and methiocarb tests, respectively. Two exceptions occurred in the dieldrin test when birds gained weight. For birds in age-weight-dietary concentration group 10,LO, 21 mg/kg mean percent weight gain was 8.0% and in group 17,LO, 30 mg/kg mean percent weight gain was 1.8%. The relationship of age, weight, and dietary concentration to mean percent body weight change for birds that died before completion of the test was inconsistent for the three chemicals. For the dicrotophos test, weight loss increased with increased age and weight group. Percent weight change did not significantly change with increasing dietary concentration for birds that died before the end of test. In contrast, percent body weight change differed significantly between ages and dietary concentration in the dieldrin test. Percent weight loss was greatest in the dieldrin test among 17-day-old birds, becoming larger as dietary concentration increased. In the methiocarb test, percent body weight change differed significantly between ages only. Seventeen-day-old birds that died before the end of the methiocarb test had the greatest percent weight loss regardless of weight group or dietary concentration. During the post-treatment period weight change was positive. Percent weight change was greatest for the 10-day-old birds and tended to increase with increased dietary concentration for all tests.

The results of this study demonstrated that responses were significantly affected by initial body weight in one of three test chemicals. In addition, this study indicated that responses to all three chemicals were significantly affected by age as shown in a similar study conducted by Hill and Camardese (1981). Age and weight did not interact in a manner that significantly affected measured responses. In general, the results indicate that when interpreting results from dietary

toxicity tests, age and body weight should be considered, noting that responses may vary by as much as 2-fold between extreme ages and weights.

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