

Subchronic Dietary Toxicity of Strychnine: Bobwhite Quail Are Less Sensitive than Mallard Ducks

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Abstract. Separate, 28-day, subchronic studies of strychnine dietary toxicity were conducted using northern bobwhite quail (*Colinus virginianus*) and mallard ducks (*Anas platyrhynchos*). Five groups (five males five females/group) of 29-week-old quail were fed Purina® Game Bird Breeder Layena® diets containing mean (\pm SD) 484.2 (\pm 17.0), 972.6 (\pm 54.0), 1,870.8 (\pm 176.1), 3,516.7 (\pm 68.0), and 6,083.3 (\pm 269.6) μ g/g strychnine; whereas five groups of 27-week-old mallards (five males five females/group) were fed similar diets containing mean (\pm SD) 18.8 (\pm 1.3), 91.1 (\pm 27.3), 235.0 (\pm 33.8), 484.2 (\pm 17.0), and 972.6 (\pm 54.0) μ g/g strychnine. Separate "vehicle control" (0.0 μ g/g strychnine) groups (five males, five females/group) were included in each study. Strychnine toxicity was much less pronounced in quail; no observed effect concentrations (NOECs) were 972.6 (\pm 54.0) and 91.1 (\pm 27.3) μ g/g strychnine for quail and ducks, respectively. Several possible explanations for the species effects are offered, and some practical issues affecting the conduct of long-term, dietary toxicity studies are discussed.

Strychnine has been used for over 300 years as an acute rodenticide (Buck 1991; Gratz 1973). While it remains one of the most widely used rodenticides in the world (Ramey *et al.* 1994), the broad-spectrum response of diverse avian/mammalian species, coupled with unsightly convulsive effects, are the chemical's nemesis. Current registrations limit strychnine use to below-ground applications (*e.g.*, bait pocket gopher, *Thomomys/Geomomys* spp., burrows to prevent mound building and open holes on rangeland)—a strategy intended to mitigate nontarget exposures (Federal Register 1983).

This research was carried out while the investigators were at the Denver Wildlife Research Center (DWRC), Denver, CO; DWRC was closed on August 4, 1997.

References to trade names and commercial products do not imply endorsement by the federal government.

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Strychnine is generally more toxic to mammalian than to avian species (Hudson *et al.* 1984; USDA 1994). However, although numerous acute (\leq 24 h) data exist, subchronic ($>$ 24 h) and chronic ($>$ 10% of life span) toxicity data for nontarget species are lacking (see Hudson *et al.* 1984; Brown 1988).

This research was designed to quantify 28-day (subchronic) toxicity and mortality effects in two nontarget avian species fed diets varying in strychnine concentration—northern bobwhite quail (*Colinus virginianus*) and mallard ducks (*Anas platyrhynchos*). While the likelihood that these species would ingest long-term, sublethal doses of strychnine in field situations is remote, these data are essential for understanding the pharmacological action of the poison and for conducting reproductive or other long-term toxicology studies (Pedersen *et al.* in preparation).

Materials and Methods

Strychnine Alkaloid

The strychnine alkaloid (CAS #57-24-9; C₂₁H₂₂N₂O₂; lot 0910003082-UN 1692) was manufactured by Deccan Phytochemicals (Pradesh, India); it was obtained through Nott Manufacturing Co., Inc. (Pleasant Valley, NY) via Pocatello Supply Depot (Pocatello, ID). The chemical was stored in opaque plastic jars at room temperature until mixed into diets. Chemical assays consistently indicated a mean purity of \geq 99.3% (Goodall 1990); all strychnine and dietary analyses were conducted in Analytical Chemistry Laboratories at The Denver Wildlife Research Center (DWRC).

Quail and Ducks

A total of 350 (198 males and 252 females) bobwhite quail and 232 (116 males and 116 females) mallard ducks were purchased from Oak Ridge Game Farm (Gravette, AR) and Whistling Wings, Inc.® (Hanover, IL), respectively. The in-life phases of the studies were conducted at Bio-Life® Associates, Ltd.

Following quarantine and acclimation, 60 (30 males, 30 females) 29-week-old quail and 60 (30 males, 30 females) 27-week-old ducks were both selected and assigned randomly to six diet groups (5 males and 5 females/group). These groups were housed in communal wire pens. Quail pens (53 × 61 × 38 cm) were located in a thermostatically

maintained room having a mean (\pm SD) daily temperature and relative humidity (RH) of 24°C (\pm 4°C) and 67% (\pm 10%), respectively. Duck pens (122 × 122 × 122 cm) were located in a room with mean (\pm SD) daily temperature and RH of 22°C (\pm 4°C) and 80% (\pm 14%), respectively. A 10 h on, 14 h off light schedule was maintained for both species using Daylite™ fluorescent tubes (General Electric Corp., Cyrus, OH). Quail and duck diets were presented *ad libitum* in 1-kg (B & F Machine Shop, Neillsville, WI) and 10-kg (Nasco Farm & Ranch, Ft. Atkinson, WI) poultry-type feeders, respectively. Water was provided *ad libitum* to the quail in a 1.2-L plastic jar fount (Kuhl Corp., Flemington, NJ) attached to each cage; ducks received water *ad libitum* via an automatic tube-dispenser system attached to each cage (Kuhl Corp., Flemington, NJ).

Diet Preparations and Analyses

Diet Preparations: Each week, 16-kg batches of nominal 0 (control), 500, 1,000, 2,000, 3,500, and 6,000 μ g/g strychnine diets were prepared for the quail, and 0 (control), 20, 75, 250, 500, and 1,000 μ g/g strychnine diets were prepared for the ducks. Test diets supplemented appropriate amounts of strychnine with <2 parts propylene glycol: 98 parts Purina® Game Bird Breeder Layena® by weight. Control diets (0 μ g/g) consisted of two parts propylene glycol: 98 parts Layena by weight. The Layena was sieved before mixing using commercial 1-mm² (#16) screen wire; only feed particles >1 mm² (\approx 80%) were retained for diets.

Diet Uniformity: On diet preparation, \approx 50 g of each test diet plus \approx 250 g of control diet was sampled in roughly equal portions from the top, middle, and bottom of the mixing bowl. Each sample was placed into a wide-mouthed plastic or glass specimen jar, frozen, and shipped in dry ice to DWRC.

On arrival, samples were thawed, ground, and refrozen. At the time of analysis, the ground samples were removed from the freezer and thawed; three 1.5-g quantities were then weighed into 50-ml plastic screw-cap tubes. Analytical method 25A was used to perform the extraction of strychnine from the Layena using a 70%, 2.5 mM heptanesulfonic acid/30% acetonitrile solution (Furcolow 1990). The samples were extracted three times with \approx 20, \approx 15, and \approx 15 ml of the solution. The extracts were combined and brought to a final volume of 50 ml. The 50-ml extract solution required dilution for feed sample concentrations over 500 μ g/g. Filtered samples were injected into a high-performance liquid chromatograph (Hewlett-Packard Model 1090M equipped with an ultraviolet/visible diode array detector and an HP work station).

Quality control (QC) samples were assayed with each set of diets. Three samples of control diet were fortified with a known quantity of strychnine to approximate each of the test concentrations being analyzed that day. The observed strychnine concentrations were adjusted for recovery based on the QC sample data. The chromatographic response from a fortified sample containing \approx 7 μ g/g strychnine was used to estimate the strychnine concentration required to produce a signal corresponding to three times the baseline noise observed in the chromatogram from a control diet (*i.e.*, method limit of detection; MLOD).

To improve uniformity of diets, a \pm 10%-of-nominal criterion was used to determine whether an alternate batch of diet was to be mixed. That is, if analysis for the first batch mix yielded a strychnine value within 10% of nominal, these batches were fed to the respective groups of quail or ducks; otherwise, a second batch of diet was formulated and fed to the respective group of birds. Time and logistics allowed only one analysis of each batch, with the second batch fed to the birds without a strychnine analysis.

Diet Stability: Stability of the diets was assessed during week 4 of each study. Quantities of the 500, 3,500, and 0 μ g/g quail diets and the 20, 500, and 0 μ g/g duck diets were held in a freezer and in an open-air

feeder in the respective quail/duck rooms. Approximately 50-g samples of freezer- and quail/duck room-stored test diets, plus \approx 250 g of 0 μ g/g diet were obtained on days 21, 25, and 28 of each study; these were labeled, frozen, shipped, and processed/analyzed at DWRC in the same manner as the uniformity samples.

Procedures

Each study involved the continuous 28-day presentation of the diets to the separate, communally housed groups of quail and ducks, respectively. Diets were replenished two or three times per week, but feeders were checked daily. No group of birds ever went without diet. Diet remaining at the end of each week was discarded and replaced with feed from a newly mixed batch.

Weekly diet consumption by each group of quail or ducks was determined gravimetrically. Daily observations of the quail or ducks were performed to record clinical signs, mortality, and diet spillage. The body weight of each quail or duck was obtained on days 1 (start), 7, 14, 21, and 28.

Necropsies were performed on all birds that died during the studies and on four surviving birds (2 males and 2 females) in each group at the end of the study. Examinations focused on the gastrointestinal (GI) tract, liver, kidneys, heart, reproductive organs, and spleen.

Statistical Analyses

The uniformity and stability of diets (μ g/g strychnine) among week 1–4 batches and day 21, 25, and 28 (week 4) samples, respectively, were analyzed using one-way, independent-groups analyses of variance (ANOVA) via SAS PROC ANOVA (see Winer 1971; SAS Institute 1989). Separate ANOVAs were computed for each diet using the three strychnine determinations made for each batch or day. Separate ANOVAs were computed among batches, days, and relative to nominal concentrations; *post hoc* Duncan multiple-range tests were used to assess specific mean differences for significant ANOVA effects (Duncan 1955). (For data summarization, actual mean μ g/g values of the three replicate dietary assays are presented accurate to the nearest 0.1 μ g; this should not be construed as the accuracy of the analytical procedure—MLODs typically were only accurate to \leq 2 μ g/g.)

Mortality, gross pathology, clinical sign, and diet consumption (g/bird/day) were characterized using descriptive statistics (*e.g.*, frequencies, $\bar{x} \pm$ SD), with individual estimates of diet intake derived as [(total weekly diet consumption per group \div days) \div (number of live quail/ducks per group)].

Body weights (g) of each quail and duck were analyzed as mixed model ANOVAs using SAS PROC MIXED, with quail and ducks considered random effects (Winer 1971; SAS Institute 1992). These ANOVAs involved three-way, independent-groups designs (6 diet groups \times 2 genders \times 5 weeks), with weeks considered a repeated measures factor (Winer 1971). Probit analysis (SAS PROC PROBIT) was used to compute the median lethal concentration (LC₅₀) of strychnine diet associated with mortality of each species (SAS Institute 1989).

Results

Strychnine Diet Uniformity: Quail and Ducks

Mean (\pm SD) strychnine in the four weekly batches of nominal 500, 1,000, 2,000, 3,500, and 6,000 μ g/g diets fed to quail were 484.2 (\pm 17.0), 972.6 (\pm 54.0), 1,870.8 (\pm 176.1), 3,516.7 (\pm 68.0), and 6,083.3 (\pm 296.6) μ g/g, respectively (Table 1). Mean (\pm SD) strychnine in the nominal 20, 75, 250, 500, and

Table 1. Mean (\pm SD) $\mu\text{g/g}$ of strychnine alkaloid in diet samples prepared for bobwhite quail and mallard ducks—uniformity

Nominal Concentration ($\mu\text{g/g}$)	Diet Bait			
	Week 1	Week 2	Week 3	Week 4
<i>Quail</i>				
500	498.3 (3.2)	479.3 ^b (3.8)	498.0 (7.5)	461.0 ^b (8.5)
1,000	1,023.3 (5.8)	1,016.7 ^b (20.8)	949.7 ^b (11.6)	900.7 ^b (11.9)
2,000	2,040.0 (26.5)	1,980.0 (36.1)	1,853.3 ^b (32.1)	1,610.0 ^{a,b} (65.6)
3,500	3,536.7 (32.1)	3,503.3 (25.2)	3,593.3 (51.3)	3,433.3 (32.1)
6,000	6,363.3 ^b (58.6)	6,130.0 (98.5)	5,676.7 ^{a,b} (61.1)	6,163.3 ^{a,b} (65.1)
0	<MLOD	<MLOD	<MLOD	<MLOD
<i>Ducks</i>				
20	19.3 (1.5)	17.3 (0.6)	18.7 (1.5)	19.7 ^b (0.6)
75	77.0 (1.7)	79.1 ^b (0.6)	136.0 ^{a,b} (3.6)	72.0 ^b (1.0)
250	260.0 (26.4)	253.0 (5.2)	183.7 ^{a,b} (5.6)	243.3 (7.0)
500	498.3 (3.2)	479.3 ^b (3.9)	498.0 (7.6)	461.0 ^b (8.5)
1,000	1,023.3 (5.8)	1,016.7 (20.8)	949.7 ^b (11.6)	900.7 ^b (11.9)
0	<MLOD	<MLOD	<MLOD	<MLOD

^a Diet was remixed (first exceeded $\pm 10\%$ of nominal criterion)

^b Duncan multiple-range tests indicated \bar{x} $\mu\text{g/g}$ significantly different from nominal value

1,000 $\mu\text{g/g}$ batches fed to ducks were 18.9 (± 1.24), 91.1 (± 27.3), 235.0 (± 33.8), 484.2 (± 17.0), and 972.0 (± 54.5) $\mu\text{g/g}$, respectively. All batches of control (0.0 $\mu\text{g/g}$) diet were essentially free of strychnine, with MLODs ≤ 2 $\mu\text{g/g}$.

Overall, weekly batches for 9:10 test diets fed to the quail and ducks averaged between 93.5% and 101.4% of nominal strychnine (Table 1). Only the mean 91.1 $\mu\text{g/g}$ diet fed to ducks deviated greatly— $\pm 21.5\%$ of 75 $\mu\text{g/g}$.

The ANOVAs for the mean $\mu\text{g/g}$ strychnine concentrations used in the quail study yielded significant batch main effects relative to nominal: 484.2 $\mu\text{g/g}$ ($F = 24.62$, $df = 3/8$, $p < 0.0002$), 972.6 $\mu\text{g/g}$ ($F = 54.97$, $df = 3/8$, $p < 0.0001$), 1,870.8 $\mu\text{g/g}$ ($F = 59.39$, $df = 3/8$, $p < 0.0001$), 3,516.7 (± 68.0) $\mu\text{g/g}$ ($F = 4.18$, $df = 3/8$, $p < 0.046$), and 6,083.3 $\mu\text{g/g}$ ($F = 47.84$, $df = 3/8$, $p < 0.001$). For ducks, significant batch main effects occurred for four test diets: 91.1 $\mu\text{g/g}$ ($F = 631.56$, $df = 3/8$, $p < 0.0001$), 235.0 $\mu\text{g/g}$ ($F = 18.07$, $df = 3/8$, $p < 0.0006$), 484.2 $\mu\text{g/g}$ ($F = 24.62$, $df = 3/8$, $p < 0.0002$), and 972.0 $\mu\text{g/g}$ ($F = 54.97$, $df = 3/8$, $p < 0.0001$). *Post-hoc* Duncan multiple-range tests for these effects showed no consistent pattern across weeks of mixing. The significant ANOVA terms probably reflected the precision of the chemical analyses more than meaningful variation among weekly mixes in strychnine concentrations.

Strychnine Diet Stability: Quail and Ducks

Regarding the week 4 stability data, significant ANOVA effects occurred among days for the 484.2 $\mu\text{g/g}$ ($F = 64.63$, $df = 2/6$,

Table 2. Mean (\pm SD) $\mu\text{g/g}$ of strychnine alkaloid in diet samples used to assess stability during week 4 of bobwhite quail and mallard duck toxicity study

Test Conditions	Nominal Concentration ($\mu\text{g/g}$)	Day		
		Day 21	Day 25	Day 28
Freezer-stored	20	22.7 (0.6)	21.3 (0.6)	20.7 (0.6)
	500	526.6 ^a (3.1)	494.0 (5.3)	498.7 (2.3)
	3,500	3,713.3 ^a (15.3)	3,243.3 ^a (28.9)	3,703.3 ^a (15.3)
	0	<MLOD	<MLOD	<MLOD
Quail room-exposed	500	522.7 ^a (7.2)	518.7 ^a (6.1)	505.3 (2.3)
	3,500	3,826.7 ^a (70.2)	3,793.3 (170.4)	3,723.3 ^a (30.6)
	0	<MLOD	<MLOD	<MLOD
Duck room-exposed	20	20.6 (0.6)	19.3 (0.6)	20.7 (0.6)
	500	488.3 (16.6)	492.0 (5.3)	494.0 (7.2)
	0	<MLOD	<MLOD	<MLOD

^a Duncan multiple-range tests indicated \bar{x} $\mu\text{g/g}$ significantly different from nominal value

$p < 0.0001$) and 3,516.7 $\mu\text{g/g}$ ($F = 499.15$, $df = 2/6$, $p < 0.0001$) freezer-stored diets, as well as for the 484.2 $\mu\text{g/g}$ ($F = 7.80$, $df = 2/6$, $p < 0.0124$) quail room-exposed diet (Table 2). *Post-hoc* Duncan multiple-range tests revealed that the day 25 and 28 (week 4) strychnine concentrations were decreased relative to the day 21 mean for each diet. The 3,516.7 $\mu\text{g/g}$ ($F = 0.72$, $df = 2/6$, $p < 0.5256$) quail room-exposed diet yielded no day effect. Control diet samples were free of strychnine (<MLOD).

For the duck diets, among-day ANOVA effects were significant for the 18.9 $\mu\text{g/g}$ ($F = 9.83$, $df = 2/6$, $p < 0.0128$) and 484.2 $\mu\text{g/g}$ ($F = 64.43$, $df = 2/6$, $p < 0.0001$) freezer-stored diets, plus the 18.9 $\mu\text{g/g}$ ($F = 9.95$, $df = 2/6$, $p < 0.0124$) duck room-exposed diet. *Post-hoc* mean comparisons showed that the day 21 strychnine concentrations were greater than those for Days 25 and 28. The strychnine in the 484.2 $\mu\text{g/g}$ ($F = 0.21$, $df = 2/6$, $p < 0.8165$) duck room-exposed diet did not differ across days. All samples of control diet contained <MLOD strychnine across storage conditions and days.

Quail Toxicity

Mortality/Gross Pathology: Deaths of 10 quail occurred during the 28-day study—five quail (3 males and 2 females) each in the 3,516.7 and 6,083.3 $\mu\text{g/g}$ mean diet groups (Table 3). Regarding the temporal sequence of these deaths, mortalities of quail in the 3,516.7 $\mu\text{g/g}$ mean strychnine group occurred on day 4 (male), 8 (female), 13 (male), 24 (female), and 25 (male). Deaths of quail fed 6,083.3 $\mu\text{g/g}$ mean strychnine occurred on days 9 (male), 13 (female) and 18 (male). No mortality occurred in the 0.0, 484.2, or 972.6 $\mu\text{g/g}$ diet groups.

Necropsies of the 10 quail found dead revealed strychnine-related abnormal anatomical conditions in each carcass (Table 4). At discovery, five carcasses displayed torpor, with the

Table 3. Mortalities of bobwhite quail and mallard ducks across days for the respective strychnine dietary exposures

Day	Quail Diets ($\mu\text{g/g}$)						Duck Diets ($\mu\text{g/g}$)						
	0	500	1000	2000	3500	6000	0	20	75	250	500	1000	
1	—	—	—	—	—	—	—	—	—	—	—	—	1 ♂ 1247
2	—	—	—	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	—	1 ♂ 0743
4	—	—	—	—	1 ♂ 0820 ¹	—	—	—	—	—	1 ♂ 0905	—	—
5	—	—	—	—	—	—	—	—	—	—	—	—	1 ♀ 0900
6	—	—	—	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	1 ♀ 1015	—	—	—	—	—	—	—	—
9	—	—	—	—	—	1 ♂ 1100	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—	—	—	—
13	—	—	—	—	1 ♂ 1000	1 ♂, 2 ♀ 1000	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—	—	—	—	—	—
18	—	—	—	—	—	1 ♂ 1535	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—	—	—	—	—	1 ♀ 1040
20	—	—	—	—	—	—	—	—	—	—	1 ♀ 0815	—	—
21	—	—	—	—	—	—	—	—	—	—	1 ♀ 0800	—	—
22	—	—	—	—	—	—	—	—	—	—	—	—	1 ♂ 1300
23	—	—	—	—	—	—	—	—	—	—	—	—	1 ♂ 0920
24	—	—	—	—	1 ♀ 0830	—	—	—	—	—	—	—	—
25	—	—	—	—	1 ♂ 0920	—	—	—	—	—	1 ♂ 0800	1 ♀ 0800	—
26	—	—	—	—	—	—	—	—	—	—	—	—	—
27	—	—	—	—	—	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—	—	—	—	—	—

¹ Times based on 24-h clock; birds checked regularly at 0800–0900 and 1500–1600 (other times involved staff nearby that noticed deaths)

legs stretched behind the body, and five carcasses had evidence of internal hemorrhages (*i.e.*, three in the 3,516.7 and two in the 6,083.3 $\mu\text{g/g}$ groups, respectively).

Necropsies of four arbitrarily selected surviving quail from each group at the end of the study indicated gross pathology in three carcasses. Intestinal hemorrhage was evident in one sacrificed quail from both the 972.6 and 6,083.3 $\mu\text{g/g}$ diet groups, while a mottled liver was found in a sacrificed quail fed the mean 1,870.8 $\mu\text{g/g}$ strychnine diet.

Clinical Sign: Signs of ataxia, muscle spasms, wing-beat convulsions, torpor, tremors, and sitting posture occurred in quail fed $\geq 1,870.8$ $\mu\text{g/g}$ strychnine; no signs were noted for quail fed 0.0 (control), 484.2, and 972.6 $\mu\text{g/g}$ mean strychnine during the 4 weeks. Additionally, signs were sporadic, irregular, and inconsistent precursors of death in bobwhite quail; of the nine deaths, only one displayed any sign of toxicosis (day 19, muscle spasms) prior to death.

Diet Consumption/Body Weight: Quail fed diets containing $\geq 3,516.7$ $\mu\text{g/g}$ mean strychnine showed a ≤ 8 g/quail/day intake as early as the first week of the study, with eventual adjustment to quantities similar to the other groups by week 4 (Figure 1). Quail fed $\leq 1,870.8$ $\mu\text{g/g}$ strychnine displayed no apparent decreased intakes, with control birds actually displaying $\approx 33\%$ increased consumption during the course of the study—a probable growth effect.

Body weight changes in quail occurred as a function of diets, gender, and weeks, with the following main and interaction

effects significant: diets: $F = 3.59$, $df = 5/48.4$, $p < 0.0077$; gender: $F = 6.41$, $df = 1/48.5$, $p < 0.0147$; weeks: $F = 54.84$, $df = 4/166$, $p < 0.0001$; diets \times gender: $F = 3.83$, $df = 5/48.4$, $p < 0.0053$; diets \times weeks: $F = 6.15$, $df = 20/166$, $p < 0.0001$; and diets \times genders \times weeks: $F = 2.27$, $df = 20/166$, $p < 0.0025$. Only the genders \times weeks interaction was not significant.

A plot of the diets \times genders \times weeks cell means revealed much about these weight effects. First, the random assignment of birds to groups, in the absence of a matching procedure, led to sizable differences at the start of the study. Second, neither male nor female quail in groups fed ≤ 972.6 (± 54.0) $\mu\text{g/g}$ mean strychnine declined more than 12 g (5.8%) in weight during the course of the study. Third, pronounced weight loss was evident for male and female quail fed $\geq 1,870.8$ (± 176.1) $\mu\text{g/g}$ strychnine, with most of the observed weight loss evident during week 1.

NOEC/LC₅₀: Based on the mortality/pathology/sign data, the NOEC for bobwhite quail was 972.6 $\mu\text{g/g}$. Probit analysis yielded an LC₅₀ of 4,973.6 $\mu\text{g/g}$ strychnine ($\pm 95\%$ CL between 3,624.5 and 10,706.7 $\mu\text{g/g}$) for this species.

Duck Toxicity

Mortality/Gross Pathology: A total of 11 ducks died during the study—four (2 male, 2 female) and seven (4 male, 3 female) in the mean 484.2 and 972.0 $\mu\text{g/g}$ diet groups, respectively

Table 4. Gross pathology of adult bobwhite quail fed respective diets (nominal) containing strychnine

Quail	Fate	Test Day	Anatomy	Pathology
0 µg/g group				
457 ♂	Sacrificed—Final	PE	NA	NA
462 ♂	Sacrificed—Final	PE	NA	NA
165 ♀	Sacrificed—Final	PE	NA	NA
432 ♀	Sacrificed—Final	PE	NA	NA
500 µg/g group				
452 ♂	Sacrificed—Final	PE	NA	NA
454 ♂	Sacrificed—Final	PE	NA	NA
182 ♀	Sacrificed—Final	PE	NA	NA
431 ♀	Sacrificed—Final	PE	NA	NA
1,000 µg/g group				
451 ♂	Sacrificed—Final	PE	Intestines	Hemorrhagic
465 ♂	Sacrificed—Final	PE	NA	NA
170 ♀	Sacrificed—Final	PE	NA	NA
2,000 µg/g group				
449 ♂	Sacrificed—Final	PE	Liver	Mottled
461 ♂	Sacrificed—Final	PE	NA	NA
157 ♀	Sacrificed—Final	PE	NA	NA
179 ♀	Sacrificed—Final	PE	NA	NA
3,500 µg/g group				
445 ♂	Found Dead	04	Legs	Stretched behind body
156 ♀	Found Dead	08	Legs	Stretched behind body
			Intestines	Blood present; gaseous; dark green-colored contents
467 ♂	Found Dead	13	Backbone	Clotted blood present
			Intestines	Gaseous and slightly hemorrhagic
			Kidneys	Clotted blood in area
180 ♀	Found Dead	25	Intestines	Gaseous
448 ♂	Found Dead	26	Legs	Stretched behind body
			Crop	Blood present
			Intestines	
460 ♂	Sacrificed—Final	PE	NA	NA
474 ♂	Sacrificed—Final	PE	NA	NA
154 ♀	Sacrificed—Final	PE	NA	NA
172 ♀	Sacrificed—Final	PE	NA	NA
6,000 µg/g group				
444 ♂	Found Dead	9	NA	NA
158 ♀	Found Dead	13	NA	NA
178 ♀	Found Dead	13	NA	NA
153 ♀	Found Dead	13	NA	NA
469 ♂	Found Dead	18	NA	NA
464 ♂	Sacrificed—Final	PE	NA	NA
472 ♂	Sacrificed—Final	PE	NA	NA
171 ♀	Sacrificed—Final	PE	NA	NA
176 ♀	Sacrificed—Final	PE	NA	NA

PE = Postexposure; NA = not applicable, no pathology

(Table 3). Deaths occurred on day 4 (male), 20 (female), 21 (female), and 25 (male) for the ducks in the 484.2 µg/g group; whereas, mortalities for ducks fed mean 972.0 µg/g strychnine occurred on day 1 (male), 3 (male), 5 (female), 19 (female), 22 (male), 23 (male), and 25 (female).

Necropsies of the 11 ducks that died during the study revealed more diverse and numerous pathology than was observed for the quail (Table 5). Strychnine-related abnormal anatomical conditions (*e.g.*, emaciation, blood pooled in lungs, gaseous or hemorrhagic skin/testes/intestines) were found in each carcass. Additionally, poststudy necropsies of ducks from each group indicated that frequencies of pathology were typically more prevalent as mean dietary strychnine levels increased.

Clinical Sign: No signs were observed in ducks fed mean 0.0, 18.9, or 91.0 µg/g strychnine. Compared to quail, ducks displayed considerably more frequent diverse signs; a total of two (female), nine (four male, 5 female), and eight (3 male, 5 female) ducks displayed at least one clinical sign in diet groups fed mean 235.0, 484.2, and 972.0 µg/g strychnine diets. Of the 11 quail that died during the study, three died suddenly and lacked signs but the remainder displayed at least tremors, difficulty walking, or lethargy between 2 and 25 days earlier. Signs of toxicosis included ataxia, asthenia, loss of balance, tipped back on tail feathers, wing-beat convulsions, torpor, lethargy, stumbling, loss of righting reflex, tremors, leg kicking, unsteady gait, convulsions of legs, falling, unkemptness, pushing the body using legs only, and inability to elevate the head.

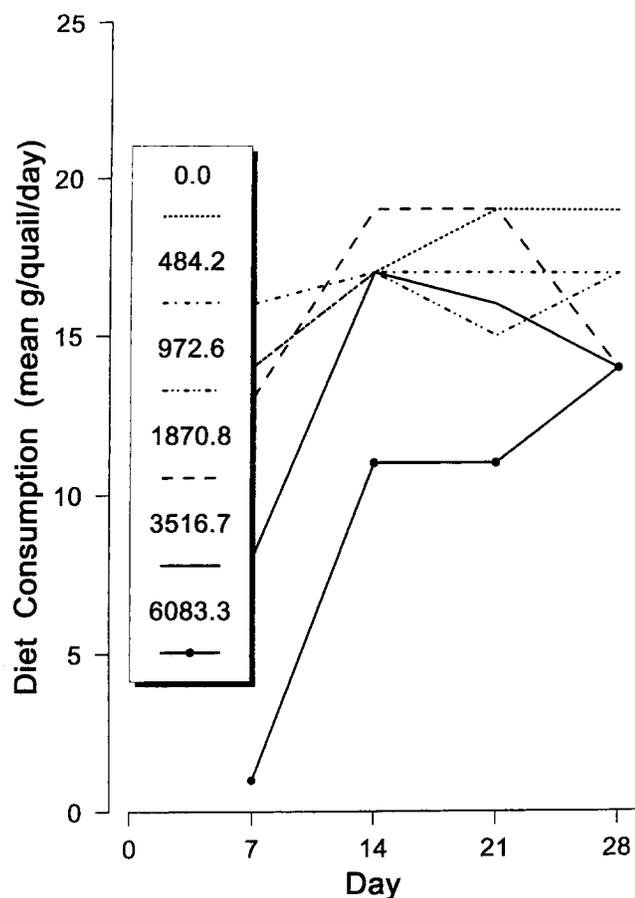


Fig. 1. Mean estimated diet consumption (g/quail/day) for bobwhite quail (note—The estimates reflect total weekly consumption based upon the number of quail surviving in each group of five birds)

Diet Consumption/Body Weight: Ducks fed ≥ 484.2 $\mu\text{g/g}$ diets decreased their food intake dramatically (≈ 50 – 75%) during week 1 relative to other groups (Figure 2), but gradually began to consume the high-concentration strychnine diets. Ducks fed ≤ 235.0 $\mu\text{g/g}$ strychnine showed roughly equal initial intakes of the diets, with increased consumption across weeks of the study.

As for quail, complex increases and decreases in the body weights of ducks occurred as a function of diets, genders, and weeks, with all main and interaction effects significant: diets: $F = 29.98$, $df = 5/46.2$, $p < 0.0001$; genders: $F = 31.02$, $df = 1/46.4$, $p < 0.0001$; weeks: $F = 52.43$, $df = 4/163$, $p < 0.0001$; diets \times genders: $F = 3.82$, $df = 5/46.2$, $p < 0.0056$; diets \times weeks: $F = 22.31$, $df = 20/163$, $p < 0.0001$; genders \times weeks: $F = 9.86$, $df = 4/163$, $p < 0.0001$; and diets \times genders \times weeks: $F = 4.83$, $df = 20/163$, $p < 0.0001$. Here again, a plot of the cell means for the triple interaction effect revealed key patterns of weight loss/recovery by the ducks. Three points gleaned from the plots were: (1) as for quail, the random assignment of ducks to groups in the absence of a matching procedure led to sizable differences at the start of the study; (2) ducks fed ≥ 235.0 $\mu\text{g/g}$ diets showed dramatic weight losses (16–26%) throughout the initial 3 weeks of the study; and (3) for ducks fed ≤ 91.1 $\mu\text{g/g}$ mean strychnine, females actually gained weight, while males in these groups remained unchanged or lost weight, during the study.

NOEC/LC₅₀: Based on the mortality/pathology/sign data, the NOEC for mallards was 91.1 $\mu\text{g/g}$. Probit analysis yielded an LC₅₀ of 679.8 $\mu\text{g/g}$ strychnine ($\pm 95\%$ CL between 194.3 and 442.6 $\mu\text{g/g}$) for the ducks.

Discussion

Subchronic dietary toxicity of strychnine was much less pronounced in the gallinaceous than waterfowl species. Compared to mallards, relatively few clinical signs occurred in bobwhite quail, with NOECs and LC₅₀s $\approx 10\times$ and $\approx 7\times$ greater for quail than ducks, respectively. These findings concur with other literature for galliformes and anseriformes (Hudson *et al.* 1984; USSD 1994); acute oral toxicity (LD₅₀) values of strychnine are 27 mg/kg for ring-necked pheasant (*Phasianus colchicus*), 35–50 mg/kg for sage grouse (*Centrocercus urophasianus*), 50 mg/kg for turkey (*Meleagris gallopavo*), and 112–161 mg/kg for California quail (*Callipepla californica*) versus 2.27–5.88 mg/kg LD₅₀ for mallard ducks. Interestingly, passeriformes are also sensitive (*e.g.*, 4.18 mg/kg for sparrows [*Passer domesticus*] and 3.98–5.0 mg/kg in blackbirds/starlings [*Agelaius phoeniceus*/*Sturnus vulgaris*]); whereas, columbiformes show intermediate sensitivity (*e.g.*, 7.7–21.3 mg/kg for pigeons/doves [*Columba livia*/*Zenaida macroura*]).

The differential toxicity of quail and ducks to strychnine is difficult to explain. It has long been known that the poison blocks inhibitory motor pathways controlled by Renshaw cells in the spinal cord—the cause of tetanic convulsions (Murphy 1986). Still, we found no information on neurological/biochemical function to account for the species differences. Of course, the gross anatomy of gallinaceous and waterfowl species differs, with a spindle-shaped crop structure in the alimentary canal of ducks and a unilateral sac in quail (Ziswiler and Farner 1972). This temporary food store and thick epithelium sac in the esophagus of quail could act to slow strychnine deposition into the gizzard, thereby titrating dose delivery and accounting for the reduced toxicity observed for bobwhites.

Regarding the uniformity/stability of diets, statistically significant variation does not always infer biological/toxicological significance. Guidelines for diet preparation and test substance variation in wildlife hazard studies do not specify limits of acceptability for test diets; these only mandate that chemical determinations be performed (US EPA 1982, 1986). Although mean $\mu\text{g/g}$ strychnine differed significantly among batches (weeks), roughly two-thirds of the batch mixes were within 10% of proposed concentrations. Granted, the week 3 Batch (136.0 $\mu\text{g/g}$) fed to the mallards in the mean 91.1 $\mu\text{g/g}$ strychnine group was an outlier sufficient to affect study results, yet no ducks in this group died or displayed clinical signs. Additionally, while diets lost potency across days when exposed to quail or duck room conditions, even freezer-stored diets varied across days; mean $\mu\text{g/g}$ strychnine determinations varied between -12.7% and $+1.2\%$ of the day 21 values. Observed statistical effects in diet uniformity/stability were of minor importance to biological/toxicological interpretations due to the wide separation among nominal diet concentrations.

In retrospect, the use of a blocking procedure for weights of birds would have aided assessment of dietary effects. Guide-

Table 5. Gross pathology of adult mallard ducks fed respective diets (nominal) containing strychnine

Duck	Fate	Test Day	Anatomy	Pathology
0 µg/g (Control) group				
158 ♂	Sacrificed—Final	PE	Intestines	Slightly hemorrhagic
287 ♂	Sacrificed—Final	PE	NA	NA
457 ♀	Sacrificed—Final	PE	NA	NA
458 ♀	Sacrificed—Final	PE	NA	NA
20 µg/g group				
19 ♂	Sacrificed—Final	PE	Testes Intestines	Slightly hemorrhagic Slightly hemorrhagic
291 ♂	Sacrificed—Final	PE	Testes Intestines	Slightly hemorrhagic Slightly hemorrhagic
230 ♀	Sacrificed—Final	PE	Intestines	Hemorrhagic and gaseous
472 ♀	Sacrificed—Final	PE	NA	NA
75 µg/g group				
72 ♂	Sacrificed—Final	PE	Testes Intestines	Hemorrhagic Extremely gaseous
157 ♂	Sacrificed—Final	PE	NA	NA
390 ♀	Sacrificed—Final	PE	Intestines	Extremely hemorrhagic and gaseous
467 ♀	Sacrificed—Final	PE	NA	NA
250 µg/g group				
166 ♂	Sacrificed—Final	PE	Skin Testes	Slightly hemorrhagic Hemorrhagic
171 ♂	Sacrificed—Final	PE	Testes Lungs	Hemorrhagic Blood pooled at right side of right lower lung
446 ♀	Sacrificed—Final	PE	Intestines	Hemorrhagic and gaseous
474 ♀	Sacrificed—Final	PE	Intestines	Slightly hemorrhagic and gaseous
500 µg/g group				
161 ♂	Found Dead	4	Right Leg Vent	Stretched behind body Protruding
443 ♀	Found Dead	20	Body Backbone	Slightly emaciated Blood along right side
32 ♂	Found Dead	25	Body Intestines	Emaciated Gaseous
147 ♂	Sacrificed—Final	PE	Testes Intestines	Slightly hemorrhagic Slightly hemorrhagic
176 ♂	Sacrificed—Final	PE	Intestines	Slightly hemorrhagic
460 ♀	Sacrificed—Final	PE	Intestines	Gaseous
469 ♀	Sacrificed—Final	PE	NA	NA
1,000 µg/g group				
165 ♂	Found Dead	1	Legs	Stretched behind body
170 ♂	Found Dead	3	Legs	Stretched behind body
456 ♀	Found Dead	5	Legs Intestines Gizzard	Stretched behind body Void of feed Void of feed
204 ♀	Found Dead	19	Intestines	Dark green-colored conte
145 ♂	Found Dead	22	Intestines Lungs	Slightly hemorrhagic Blood pooled on right side
152 ♂	Found Dead	23	Skin Testes Intestines	Hemorrhagic Hemorrhagic Hemorrhagic
468 ♀	Found Dead	25	Body Intestines	Emaciated Gaseous
150 ♂	Sacrificed—Final	PE	Intestines	Gaseous
402 ♀	Sacrificed—Final	PE	Body Skin Intestines	Slightly emaciated Hemorrhagic Hemorrhagic
454 ♀	Sacrificed—Final	PE	Skin Intestines	Slightly hemorrhagic Gaseous

PE = Postexposure; NA = not applicable, no pathology

lines for these types of subchronic and chronic dietary tests omit specifications for measurement of individual food consumptions (US EPA 1982, 1986). Although individual food intake measurements would add to the costs of chemical registration

studies, these data are critical to statistical quantification of effects. Our food consumption and body weight data reflect initial aversion of quail and ducks fed diets containing >3,500 and >480 µg/g strychnine, respectively—effects obscured in 2

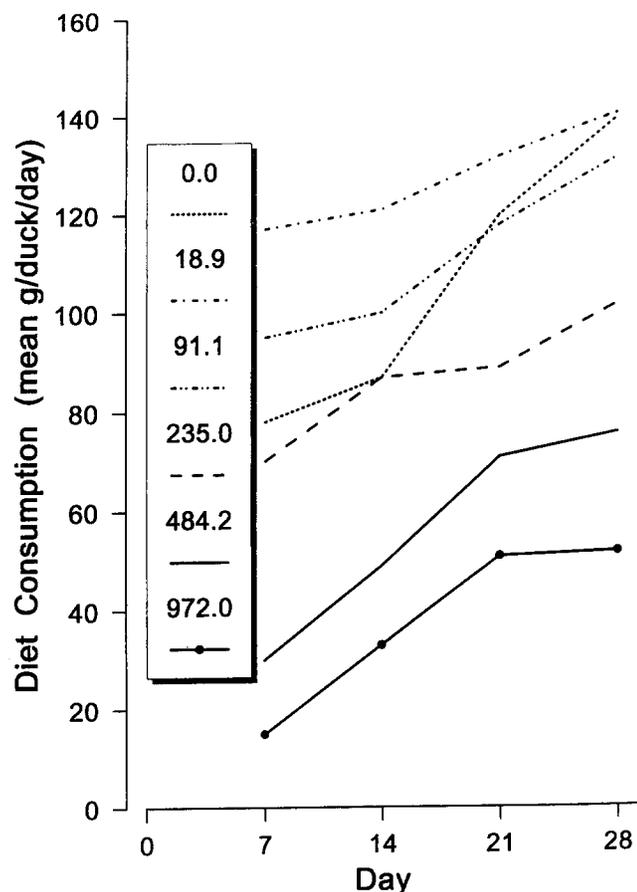


Fig. 2. Mean estimated diet consumption (g/duck/day) for mallard ducks (note—The estimates reflect total weekly consumption based upon the number of ducks surviving in each group of five birds)

weeks due to the single-choice paradigm and mortality of strychnine-susceptible birds in these groups.

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