The Effects of Subacute and Chronic Exposure to 4-Aminopyridine on Reproduction in Coturnix Quail

by E. W. SCHAFER, R. B. BRUNTON, and N. F. LOCKYER
U.S. Fish and Wildlife Service
Wildlife Research Center, Building 16, Federal Center
Denver, Colo. 80225

SUMMARY

When male and female coturnix quail (Coturnix coturnix) were given a single subacute oral dose (5.62 mg/kg) of the avian frightening agent 4-aminopyridine (4AP) and paired with untreated mates, there was no effect on the reproductive performance of males; egg production of females was reduced the third week after treatment but recovered during the fourth week. Chronic exposure to 31.6, 100, and 316 ppm of 4AP in the diet of mated pairs did not affect reproductive performance during or after the 4- or 6-week period the chemical was fed, but no birds fed 1,000 ppm produced live chicks after treatment began and all died within 3 weeks. The 28-day LC_{50} of 4AP was determined to be 447 ppm for male coturnix and 562 ppm for females. F_1 progeny from quail fed 31.6, 100, and 316 ppm 4AP and randomly mated at maturity showed no reproductive effects from their parents' exposure.

INTRODUCTION

The chemical, 4-aminopyridine (4AP), is used as an avian frightening agent and was recently registered (EPA Registration 11649-12) to protect ripening field corn from blackbirds. Earlier studies of the toxicology and pharmacology of 4AP have shown that 4AP is relatively toxic to birds and mammals by acute oral administration, is well tolerated by birds exposed to repeated or continual challenges, and present few hazards to flesh-eating birds and mammals (SCHAFER et al., 1974; SCHAFER and MARKING, 1974). These studies were designed primarily to delineate all-or-none effects (death or survival) when 4AP was used as a bird control agent. The present studies were designed to yield information on the reproductive performance of coturnix quail fed subacute or chronic levels of 4AP.

PROCEDURES

Three studies were conducted: the first, to determine the effects of single, subacute, oral doses on the reproductive function of males and females; the second, to determine the effects of chronic dietary exposure on reproduction in breeding pairs; and the third, to determine the effects of chronic exposure of the parents on reproduction in the F_1 generation.

Bulletin of Environmental Contamination & Toxicology,
Vol. 13, No. 6 © 1975 by Springer-Verlag New York Inc.

All quail used were raised by the Poultry Science Department, University of California at Davis, and were shipped to us when they were 4 weeks old, except for the F_1 generation which we raised. The quail from Davis were randomly divided into pairs and placed under a photoperiod regime of 8 hours light and 16 hours darkness (8L:16D) for 4 weeks. The photoperiod was then advanced to 16L:8D, and records of egg production were kept over the following 7 weeks. Those pairs producing at least 10 fertile eggs during the last 2-week period were used for reproduction studies. Fertile pairs were randomly separated into two groups, one for the single dose subacute study (males and females were separated for 14 days before treatment), the other for the chronic feeding study.

Quail were held in standard quail-breeding racks (GQF Manufacturing Co.[1]) with external waterers, covered internal feeders, and 15-watt light bulbs providing each cage with an average illumination of 107 lux. Eggs were stored in a constant temperature cooler and incubated and hatched in a Jamesway Incubator. Chicks were brooded in a 4-tier, 12-compartment brooder. The room holding the test quail was maintained at 24° ± 3° C and 40% ± 10% relative humidity. Lights were controlled by a 24-hour time clock.

In the oral, single-dose subacute study, males and females were gavaged with a propylene glycol solution containing 0 or 5.62 mg/kg 4AP, then paired with untreated mates. We treated 10 females and 10 males in the event of mortality, but only 6 randomly selected pairs were used in each evaluation.

In the chronic feeding study, breeding pairs were fed ad lib. for 4 weeks on a standard laboratory diet that was treated to contain 0, 31.6, 100, 316, or 1,000 ppm 4AP and thoroughly blended and pelletized. Food consumption, bird weight, egg production, eggshell thinning, hatchability, and chick mortality were measured weekly during four 2-week periods: pretreatment, first treatment period (T1), second treatment period (T2), and posttreatment. Egg production was recorded daily. One group of six pair of quail was used at each treatment level except at the 0-ppm level where two groups were tested. A second test was conducted with 0 and 316 ppm using 12 pairs at each treatment level; 4AP was fed for three continuous 2-week periods, but pretreatment and posttreatment measurements were not made.

In the subacute and chronic feeding studies, reproductive parameters were not measured on eggs produced after the death of the male.

The F_1 generation study was conducted by taking chicks hatched during the last 2 weeks of treatment in the 4-week chronic feeding study and measuring their reproductive performance when mature. Nine randomly selected pairs of offspring produced by birds fed 31.6, 100, and 316 ppm 4AP and eight pairs produced by control birds were tested for egg production and hatchability at maturity.

[1] Reference to trade names does not imply Government endorsement of commercial products.

Treatment effects, period effects, and treatment-period interactions were analyzed by a two-way analysis of variance followed by Scheffé's S test of the means.

RESULTS

Subacute study. All birds treated with 5.62 mg/kg 4AP exhibited slight to moderate intoxication symptoms (hyperactivity, tremors, and minor motor seizures) during the 1- to 4-hour period after treatment. Two males died within 24 hours. Egg production by treated females was significantly reduced during the third week of the test (Table 1), although hatchability was not affected. Hatchability in the treated and untreated groups was depressed during the first week, but this was a result of the study design, since test birds were not paired with mates until immediately after treatment.

Table 1

Mean reproductive performance of six male and six female coturnix quail treated with single doses of 5.62 mg/kg 4-aminopyridine and paired with untreated mates

Treatment	Weeks after treatment	Egg production[1] (%)	Hatchability (%)
Control	1	69.0	51.7a[2]
	2	54.8	69.9b
	3	59.5	80.0b
	4	52.4	77.3b
Males treated	1	71.4	60.0a
	2	69.0	93.1b
	3	83.3	97.1b
	4	57.1	79.2b
Females treated	1	57.1a	62.5a
	2	66.7a	92.9b
	3	35.7b	86.7b
	4	66.7a	92.9b

[1] Percent of 42-egg potential each week (6 hens times 7 days).
[2] Numbers followed by different letters are significantly different ($P = 0.10$) within treatments.

Chronic feeding study. Birds fed 31.6 ppm 4AP in their diet for 4 weeks showed no significant effects in any measured parameter. The average daily intake of 4AP was 4.3 mg/kg, and one female died during the second 2-week test period (Table 2).

Table 2

Effects of 4-aminopyridine in the diet on reproductive performance, body weight, and food consumption of mated pairs of coturnix quail

Treatment (ppm)	Pairs tested	2-week period	Egg production (%)[1]	Shell thinning (%)[2]	Hatchability (%)[3]	Live chick production (%)[4]	Average body weight of adults (g) ♂	Average body weight of adults (g) ♀	Average food consumption (g/bird/day)	Average 4AP consumption (mg/kg/day)	Deaths ♂	Deaths ♀
Control (0)	6	Pre	86	7	75	71	120	141	15.6a[5]	0.0	0	0
		T1	96	7	68	64	123	142	17.3ab	0.0	0	0
		T2	88	8	64	55	127	144	17.3ab	0.0	0	0
		Post	77	5	60	55	127	142	19.1b	0.0	0	0
	6	Pre	64	2	61	57	128	138	16.6a	0.0	0	0
		T1	80	8	49	49	132	144	17.4a	0.0	0	0
		T2	79	5	62	62	135	150	17.8a	0.0	0	0
		Post	83	1	57	53	132	147	20.3b	0.0	0	0
31.6	6	Pre	88	0	68	65	125	139	16.3a	0.0	0	0
		T1	83	9	53	53	131	143	18.6ab	4.3	0	0
		T2	85	4	58	55	133	151	19.2ab	4.2	0	1
		Post	81	6	32	27	130	144	19.9b	0.0	0	0
100	6	Pre	80	6	69	69	124	141	14.6a	0.0	0	0
		T1	73	3	61	59	124	145	16.4ab	12.2	0	0
		T2	70	5	69	66	124	154	16.5ab	11.9	1	0
		Post	64	20	52	45	119	156	19.3b	0.0	0	0

Table 2. - Continued

Treatment (ppm)	Pairs tested	2-week period	Egg production (%)[1]	Shell thinning (%)[2]	Hatchability (%)[3]	Live chick production (%)[4]	Average body weight of adults (g) ♂	Average body weight of adults (g) ♀	Average food consumption (g/bird/day)	Average 4AP consumption (mg/kg/day)	Deaths ♂	Deaths ♀
316	6	Pre	81	6	54	54	118	146	16.8ab	0.0	0	0
		T1	70	9	67	61	117	152	14.7a	34.5	1	0
		T2	81	4	64	57	119	158	19.0ab	43.4	0	0
		Post	62	12	54	45	117	153	20.1b	0.0	0	0
1,000	6	Pre	80a	9a	42a	42a	115a	138a	19.6a	0.0	0	0
		T1	20b	29b	0b	0b	106b	108b	5.6b	52.4	4	3
		T2	0c	-	-	-	-	-	7.9b	-	2	3
		Post	-	-	-	-	-	-	-	-	-	-
Control (0)	12	T1	65	10	61	53	125	145	15.7	0.0	0	0
		T2	75	18	54	44	126	145	15.8	0.0	0	0
		T3	71	27	44	36	130	148	15.6	0.0	0	1
316	12	T1	64	12	45	41	117	140	14.3	35.2	0	1
		T2	59	10	35	29	111	139	14.6	36.9	1	0
		T3	68	15	30	28	112	142	14.9	37.1	0	0

[1] Percent of 84-egg potential (6 hens times 14 days).
[2] Percent of eggs produced with shells that cracked during handling.
[3] Percent of eggs produced that hatched.
[4] Percent of eggs produced from which chicks survived 10 days after hatching.
[5] Numbers followed by different letters are significantly different ($P \leq 0.10$) within treatments.

Birds fed 100 ppm 4AP in their diet for 4 weeks showed no significant reproductive effects; however, males did not gain weight as they did in the control and 31.6-ppm groups. The average daily intake of 4AP was 12.1 mg/kg, and one male died during the second 2-week test period.

Two tests were conducted with birds fed 316 ppm 4AP; one group wasted for 4 weeks, the other for 6 weeks. Neither treatment group showed significant reproductive effects of 4AP, but again males did not gain weight. Food consumption during the first 2 weeks of feeding (T1) in the group fed 4 weeks was significantly reduced from that in the controls during the same period. The average daily intake of 4AP in the two treatment groups ranged from 34.5 to 43.4 mg/kg, and although many birds showed slight acute intoxication symptoms (hyperactivity and tremors), only two males and one female died during the test periods.

All birds fed a diet containing 1,000 ppm of 4AP died within 3 weeks. They began showing severe intoxication symptoms (clonic and tonic convulsions) within 3 to 5 days after treatment began. During the T1 period all measured parameters were significantly reduced from pretreatment levels and from control values during the same period. The average amount of 4AP ingested per day was 51.9 mg/kg.

The data from the feeding study were used to calculate a median lethal concentration (LC_{50}) by the moving-point interpolation method of THOMPSON (1948) and THOMPSON and WEIL (1952). The 28-day LC_{50} of 4AP for coturnix quail was 447 ppm for males (confidence limits at P = 0.05, 289-714 ppm) and 562 ppm for females (limits at P = 0.05 not calculable).

F_1 fertility. When the F_1 progeny of birds used in the feeding study were mated at maturity, there were no significant differences in egg production or hatchability between the offspring of treated birds and the offspring of control birds.

DISCUSSION

The results of these studies indicate that exposure of coturnix quail to 4AP does not adversely affect reproduction as long as single subacute doses do not exceed 5.62 mg/kg and chronic dietary exposure does not exceed 316 ppm daily for 6 weeks. In addition, chronic exposure of parent birds to 4AP at levels up to 316 ppm does not adversely affect the reproductive ability of the F_1 generation. The data from the chronic feeding study indicate that 4AP may have differential effects in males and females during breeding, since weight gain in males was reduced by 4AP at levels of 100 ppm or more; however, reproduction was not impaired. Perhaps a larger sample size would have shown weight gain effects with some meaningful level of significance.

In the 4-week feeding studies, live chick production at all treatment levels of 4AP were consistently but not significantly less during the posttreatment periods (Table 2). This contrasted with the pattern established by the control and treated birds during both the pretreatment and the treatment periods. Although the posttreatment reduction may have been due to some unknown environmental factor, it may have been a response to the removal of 4AP from the diet.

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