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Schafer,E.W.; Marking,L.L.

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9. ABSTRACT

A five year study of five species of birds at the Denver Wildlife Research Center and two species of fish at the Fish Control Laboratory, La Crosse, Wisconsin, was undertaken to gather data to show how granivorous birds and freshwater fishes respond to long-term 4-aminopyridine (4AP) exposure. The birds were fed diets of three percent 4AP treated cracked corn diluted with various ratios of untreated corn for seven to thirty five days. Lethal concentrations were determined for doves (316ppm) and coturnix quail (479ppm). Bobwhites and pheasants showed no adverse affects. Starlings treated by gavage daily for twenty-five days with 1.78 mg/kg 4AP showed no intoxication symptoms. Mortality appeared to be the result of acute poisoning rather than cumulative toxicity. In ninety-six hour static tests, channel catfish and bluegill showed increasing sensitivity to 4AP with time. The lethal concentration for bluegills varied from 2.82 to 7.56 mg/liter, depending on temperature and water quality. The lethal concentration for catfish varied from 2.43 mg/liter to 5.80 mg/liter. However, 4AP appears less toxic than many commonly used pesticides.

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LONG-TERM EFFECTS OF 4-AMINOPYRIDINE EXPOSURE TO BIRDS AND FISH

By E. W. Schafer, Jr. and L. L. Marking

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LONG-TERM EFFECTS OF 4-AMINOPYRIDINE EXPOSURE TO BIRDS AND FISH

E. W. SCHAFER, JR., U.S. Fish and Wildlife Service, Wildlife Research Center, Denver, Colorado 80225

L. L. MARKING, U.S. Fish and Wildlife Service, Fish Control Laboratories, La Crosse, Wisconsin 54601

Abstract: The avian frightening agent, 4-aminopyridine (4AP), was tested on five avian and two fish species to assess the effects of long-term 4AP exposure to nontarget vertebrates. Adult bobwhites (*Colinus virginianus*), mourning doves (*Zenaida macroura*), and ring-necked pheasants (*Phasianus colchicus*) were fed diets containing 3 percent 4AP-treated cracked corn diluted with various ratios of untreated corn for 7 to 35 days. Minimum dilution ratios that produced more than 50 percent mortality were 1:29 for bobwhites and 1:99 for doves. Mortality caused by 4AP could not be determined for pheasants because of their inability to subsist only on corn during the test periods. LC_{50}'s were determined for doves (316 ppm) and coturnix quail (*Coturnix coturnix*) (479 ppm) fed exclusively on uniformly treated laboratory rations for 28 to 40 days. Starlings (*Sturnus vulgaris*) treated by gavage daily for 25 days with 1.78 mg/kg 4AP showed no intoxication symptoms. Mortality in all avian species apeared to be the result of acute poisoning, and no evidence of cumulative toxicity was noted in species fed uniformly-treated rations. In 96-hour static tests, channel catfish (*Ictalurus punctatus*) and bluegill (*Lepomis macrochirus*) showed increasing sensitivity to 4AP with time in test waters ranging from very soft to very hard and from 12 to 22 C. The 96-hour LC_{50}'s for both species ranged from 2.43 to 7.56 mg/liter (ppm). Toxicity appeared to decrease with increasing water temperatures but was generally unaffected by water hardness. On the basis of the data presented, and the currently registered application rate for 4AP in agricultural crops (0.34 g/ha), long-term hazards to nontarget wildlife were judged to be minimal.

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The avian frightening agent, 4-aminopyridine (4AP), is toxic to most avian and mammalian species, with acute LD_{50}'s generally below 10 mg/kg (Schafer et al. 1973: 537). Although acute toxicity is an important measure of the hazard of an animal damage control agent, numerous methods of application (i.e., specific baits, specific placements, dilution rates) exist that can circumvent most acute intoxication of nontarget species. De Grazio et al. (1972) and Stickley et al. (1972) showed that 4AP baits can be used effectively and safely to protect ripening field corn from blackbird damage. Their data led to the registration of 4AP for this use. However, such methods of application are of little value if the control agent is cumulatively toxic. The objective of this study was to gather data to show how granivorous birds and freshwater fishes respond to long-term 4AP exposure.

Reference to trade names does not imply U.S. Government endorsement of commercial products.

METHODS

Studies with birds, conducted at the Denver Wildlife Research Center (DWRC) over a five-year period, involved five species and three methods of oral administration. Groups of 2 to 7 bobwhites, mourning doves, and ring-necked pheasants were fed for 7 to 35 days on 3 percent 4AP-treated cracked corn baits (22 mg average weight) diluted with untreated cracked corn at 1 or more of the following rates: 1:199 (150 ppm), 1:99 (300 ppm), 1:49 (600 ppm), and 1:29 (1,000) ppm). The 1:29 dilution ratio was used by De Grazio et al. (1972: 1317) and the 1:99 ratio by Stickley et al. (1972:1314) for field applications of 4AP. Controls for each species were offered only cracked corn for the test period. Groups of 6 to 12 mourning doves and coturnix were

Table 1. Characteristics of reconstituted test waters.

Water type	Salts added (mg/liter)				pH range	Total as mg/liter $CaCO_3$	
	$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KCl		Hardness	Alkalinity
Very soft	12	7.5	7.5	0.5	6.4–6.8	10–13	10–13
Soft	48	30	30	2.0	7.2–7.6	40–48	30–35
Hard	192	120	120	8.0	7.6–8.0	160–180	110–120
Very hard	384	240	240	16.0	8.0–8.4	280–320	225–245

fed for 28 to 40 days on a diet of Purina Game Bird Breeder Layena into which 0, 31.6, 100, 316, and 1,000 ppm (coturnix only) 4AP had been uniformly incorporated. Ten individually-caged starlings were treated by gavage daily for 25 days with a propylene glycol solution containing a dose of 1.78 mg/kg 4AP; 10 controls were treated by gavage daily with propylene glycol.

Starlings and doves were captured from wild populations and acclimated to test conditions. Quail and pheasants were bred in captivity at DWRC. Food consumption and body weight of all birds were determined every one to seven days, and observations for signs of intoxication were made daily. LC_{50}'s (concentrations calculated to produce 50 percent mortality) were estimated by the method of Thompson (1947).

Studies with fish were conducted at the Fish Control Laboratory, La Crosse, Wisconsin, by the methods of Lennon and Walker (1964). Young catfish and bluegills, obtained from Fairport National Fish Hatchery, Iowa, were fed and maintained according to the procedures outlined by Hunn et al. (1968) and acclimated to the test conditions. Groups of 10 fish each were exposed to concentrations varying from 0 to 50 ppm in 4 different water hardnesses (Table 1) and 3 temperatures. Mortality was observed and recorded at 3, 6, 24, and 96 hours; LC_{50}'s were determined at these time periods by the method of Litchfield and Wilcoxon (1949).

RESULTS

Birds

The minimum dilution ratios of treated baits that produced more than 50 percent mortality were 1:29 for bobwhites and 1:99 for mourning doves (Table 2). Death in both species apparently resulted from acute intoxication caused by the consumption of more than one treated bait at one feeding (single baits are not lethal to bobwhites or doves), and all intoxicated birds showed the typical acute poisoning symptoms described by Schafer et al. (1973).

It was not possible to determine the dilution ratio for 4AP baits that would produce greater than 50 percent mortality in adult ring-necked pheasants because of the erratic mortality observed at every treatment level, including the controls. Deaths apparently were not the result of 4AP poisoning, but of the inability of these birds to subsist for extended periods exclusively on corn. Control and treated birds lost from 15 to 30 percent of their original weight during the test, and the birds that died exhibited the greatest weight loss. Intoxication symptoms typical for birds acutely poisoned with 4AP were not observed. However, in a separate 2-day test, two of four 4- to 6-month-old pheasants died after a single day of feeding on 1:29 diluted bait. Intoxication symptoms typical of acute 4AP poisoning were observed, and mortality was probably the result of two factors—a body weight about

Table 2. Effects of long-term 4AP exposure to birds.

Method and species	Duration (days)	Treatment[a]	No. tested	Mortality (%)	LC_{50} in ppm (95% confidence interval)	Acute oral LD_{50} (mg/kg)[b]
Bait						
Bobwhite quail	32	1:29	4	100	NC[c]	15.0
		1:99	4			
		0:1	4			
Mourning dove	7	1:99	7	72	NC	8.1
		1:99[d]	7	43		
		0:1	43			
Ring-necked pheasant	35	1:29	2	50	NC	7.5♀
		1:49	2	50		
		1:99	3	67		
		1:199	3			
		0:1	3	33		
Feed						
Coturnix quail	28	1,000	12	100	479	7.65♂
		316	12	8	(354–645)	8.05♀
		100	12	8		
		31.6	12	8		
		0	24			
	40	316	24	8	>316	
		0	24	4		
Mourning dove	30	316	6	50	316	
		100	6		(100–1,000)	
		31.6	6			
		0	6			
Gavage						
Starling	25	1.78	10		>1.78[e]	4.9
		0	10			

[a] Expressed as dilution ratio for baits (parts 3% 4AP baits:parts untreated corn); ppm for feed and mg/kg/day for gavage.
[b] From Schafer et al. (1973:533).
[c] NC = not calculable.
[d] 2% 4AP baits.
[e] Mg/kg/day.

half that of adults and a correspondingly higher food intake per unit of body weight.

LC_{50}'s for birds offered feed uniformly treated with 4AP were 479 ppm for coturnix (28 days) and 316 ppm for mourning doves (30 days). Treatment at 31.6 and 100 ppm had no observable effect on either species. Coturnix consumed 1.5 LD_{50} and doves 1.3 LD_{50} doses per day without effect at the 100-ppm level. At 316 ppm, 3 of the 38 coturnix died after showing symptoms typical of acute 4AP poisoning. Survivors exhibited occasional minor symptoms such as hyperactivity and mild body tremors; however, food consumption and body weight were not affected. Average daily intake of 4AP was 5.3 LD_{50} doses. At 316 ppm, 3 of the 6 doves died after exhibiting acute 4AP poisoning symptoms, whereas survivors exhibited light to moderate symptoms of acute 4AP intoxication throughout the test. Average daily ingestion of 4AP was 4.3 LD_{50} doses, and food consumption was depressed.

Table 3. Toxicity of 4AP to channel catfish and bluegill.

Temp. (C)	Water hardness	LC_{50} in mg/liter (95% confidence interval) at 3 hours	6 hours	24 hours	96 hours
			Channel catfish		
12	Very soft		9.40 (8.0–11.0)	6.10 (5.5– 6.8)	4.00 (3.2–5.0)
	Soft			8.74 (7.9– 9.7)	4.00 (3.4–4.7)
	Hard			7.26 (6.2– 8.6)	4.00 (3.6–4.5)
	Very hard			4.36 (3.9– 4.9)	2.43 (2.2–2.8)
17	Soft		16.4 (13.8–19.4)	9.80 (8.6–11.2)	4.36 (3.9–4.8)
22	Soft	13.8 (12.3–15.5)	13.8 (12.3–15.5)	9.35 (8.3–10.6)	5.80 (5.2–6.4)
			Bluegill		
12	Very soft	38.1 (26.2–55.3)	26.0 (18.7–36.2)	10.4 (8.6–12.6)	3.40 (3.0–3.9)
	Soft	38.1 (26.2–55.3)	23.2 (18.2–29.5)	11.2 (10.2–12.3)	4.41 (3.8–5.1)
	Hard		38.1 (26.2–55.3)	12.3 (10.0–15.2)	2.82 (2.3–3.5)
	Very hard			8.60 (7.3–10.2)	3.20 (2.7–3.8)
17	Soft	18.1 (15.3–21.4)	16.2 (14.1–18.6)	11.8 (9.7–14.3)	5.60 (4.8–6.5)
22	Soft	18.1 (15.3–21.4)	15.0 (13.0–17.3)	12.3 (10.7–14.1)	7.56 (6.3–9.1)

Coturnix fed 1,000 ppm exhibited acute 4AP poisoning symptoms within 5 days. Food consumption decreased 70 percent, and body weight declined rapidly. Severe intoxication symptoms lasted from a few hours to three days, and all test birds died within three weeks. Two males and one female were completely paralyzed after severe and prolonged tonic convulsions. The average daily intake of 4AP was 6.6 LD_{50} doses before death.

The treated starlings showed no visible effects when treated by gavage daily for 25 days with 1.78 mg/kg of 4AP. The daily dose represented 36 percent of the acute oral LD_{50} of 4AP for starlings or a total of 9 LD_{50} doses over the test period.

Fish

The 96-hour LC_{50} of 4AP for channel catfish ranged from 2.43 mg/liter in very hard water to 5.80 mg/liter in standard (soft) test water at 22 C (Table 3). In standard test water 4AP was significantly less toxic ($P \leqslant 0.05$) at 22 C than at 12 C and 17 C, even though mortality occurred more rapidly at 22 C. At 12 C, 4AP showed the same toxicity in very soft, soft, and hard water but significantly greater toxicity in very hard water ($P \leqslant 0.05$).

The 96-hour LC_{50}'s for bluegills varied from 2.82 to 7.56 mg/liter, depending upon temperature and water quality (Table 2). As with catfish, 4AP was significantly less toxic ($P \leqslant 0.05$) in standard test water at 22 C than at 12 C.

DISCUSSION

The results with birds indicate that 4AP is not cumulative in doves or coturnix according to the criteria used in the "Index of Chronicity" (Kenega 1973). Repeated sublethal doses had no greater effects than single sublethal doses. All acute toxicity apparently resulted from the ingestion of more 4AP in a period than could be excreted or detoxified. In the feeding studies where surviving mourning doves and coturnix showed symptoms of intoxication, the symptoms disappeared as soon as treated food was removed.

The data for bobwhites (Table 2) are of particular importance to the use of 4AP in the field situations for which it is registered and for which registrations are pending. Bobwhites fed at the 1:99 dilution rate for

a 32-day period showed no adverse effects. Although the sample size was small (four), the data indicated relatively little hazard to bobwhites under field-use conditions. The data for adult pheasants suggested a similar conclusion. The data for doves and young pheasants indicated some hazard to individuals of these species or age classes. However, because the registered use directions for protecting field corn from bird damage (4.5 g of 3 percent 4AP-treated corn/0.405 ha) results in an application rate of about 500 treated corn particles (0.34 g of 4AP) per ha (about 200 particles/acre), and since the bait is diluted 1:99 (99 untreated particles are supplied for every 1 treated), it would seem unlikely that even the more susceptible species and individuals would find and eat enough 4AP-treated particles to adversely affect a population during a baiting program.

Fish apparently are less able to cope with 4AP in their environment and become increasingly sensitive to 4AP with increasing exposure. However, even after 96 hours of exposure to 4AP, the LC_{50}'s for channel catfish and bluegill indicated that 4AP is less toxic than many commonly used pesticides. At recommended use levels (0.34 g/ha or 0.14 g/acre), the possibilities of ponds and streams containing detectable levels of 4AP are remote, particularly in view of the recent finding by Starr and Cunningham (1974) that 4AP is readily adsorbed onto soil colloids.

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