Effect of Asulam in Wildlife Species Residues and Toxicity in Bobwhite Quail After Prolonged Exposure

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Asulam (methyl 4-aminobenzenesulfonyl carbamate) is a broad-spectrum systemic herbicide active against broadleaf weeds such as dock (Rumex sp.) and bracken fern (Pteridium aquilinum) (SOPER et al., 1948; HOLROYD et al., 1970). These weeds are pests in pasture lands and reforestation areas, respectively. Asulam is registered throughout the world, as Asulox(R) herbicide for use in pastures and other non-crop uses and in the Caribbean for control of johnsongrass (Sorghum halepense) in sugarcane (TUCKETT and BALL, 1968). It is currently under experimentation in the United States for these same purposes.

Acute and chronic studies have indicated that asulam is of a low order of toxicity to all species tested (mammals, birds, and fish) and is not accumulated in fish at exposure levels of 4,000 parts per million (ppm) for 96 hours. (INGHAM and GALLO, 1974)

The work reported in this study was undertaken to ascertain the effects of prolonged feeding of exaggerated doses of asulam on the body weight, feed consumption, reproductive capabilities, and hatchability of bobwhite quail (Colinus virginianus), and to determine the residues of asulam in the meat and eggs of the test birds.

Materials and Methods

Sixteen, 22, and 27-week-old bobwhite quail (240 of each sex) were obtained from a commercial quail farm for use in this study. Four treatments were arranged in a randomized complete block design and consisted of asulam (98.6% technical grade) in an all-mash quail layer ration at levels of 0, 1.5, 6.0, and 25.0 ppm. Sixty male and 60 female quail were assigned to each treatment and were divided into 12 pens of 5 males and 5 females each. The quail were distributed so that each pen contained a similar mixture of the 3 age groups. The quail were housed in 2 Petersime starter batteries each of which contained 6 decks. Each deck was an experimental block containing 1 pen from each treatment group; thus, there were 12 blocks in the study.

The study lasted a total of 7 weeks. Feed and water were supplied ad libitum and artificial lights provided 24 hours daily. Asulam containing feed was discontinued after the first 4 weeks and all quail received control basal ration during the remaining 3 weeks (recovery period).

Bulletin of Environmental Contamination & Toxicology,
Vol. 13, No. 2 ©1975 by Springer-Verlag New York Inc.

Body and feed weights were measured weekly and eggs were collected daily and refrigerated for use in hatchability studies and for analysis of asulam residues.

Three male and three female quail were sacrificed by decapitation and exsanguination at the end of each 7 day period. Breast and leg muscle, skin with fat, liver, kidney, and heparinized blood were quick frozen on dry ice and saved for analysis.

The analysis of total asulam residues, including the possible metabolite, acetylasulam, was carried out using a procedure based on the Bratton-Marshall reaction (1939). In general, 5 grams of tissue, deshelled eggs, or blood were blended with an equal weight of dry ice. After sublimation of the dry ice, 10 volumes (50 ml) of 0.2% acetic acid in ethyl acetate were added and blended at high speed for two minutes. The homogenate was filtered and the blender washed with 15 volumes (75 ml) of acidic ethyl acetate, and the wash was filtered. Five mls of water were added to the clear filtrates and the solvents were evaporated. One ml of 5N NaOH was added to the aqueous residue to hydrolyze the acetylated and/or conjugated asulam and the flask was heated in a 90°C constant temperature bath for 20 minutes. The flask was allowed to cool and its contents acidified with 1:1 HCl (about 1 ml). The hydrolyzed sample was transferred to a separatory funnel with 3 washes of 5 ml each of ethyl acetate and a fourth wash with 2 ml of 1N HCl. After adding 0.5 ml of 0.1N sodium nitrite solution the mixture was shaken vigorously for about five minutes and the phases allowed to separate. The diazotized asulam partitioned into the acidic aqueous phase and the impurities partitioned into the ethyl acetate, which was discarded. The aqueous layer was filtered and the clear filtrate transferred to a 10 ml volumetric flask. The excess nitrite was scavenged by the addition of 1 ml of 1.5% ammonium sulfamate. If necessary, distilled water was added to make up approximately 9 ml, and the solution left to stand for five minutes (pre-coupling reaction). The absorbance of an aliquot of the diazotized solution was measured at 540 mu. This aliquot was returned to the volumetric flask and 1 ml of the chromogenic reagent (N-1-naphthylethylenediamine hydrochloride, 1.0% w/v in 0.1N HCl) was added, mixed thoroughly and the color allowed to develop (post-coupling reaction). After letting the solution stand for 5 minutes, the absorbance was read at 540 mu. The difference between the post and precoupling absorbances is the corrected absorbance.

Results

Asulam when fed in the diet of bobwhite quail at 25 ppm or less for 28 days had no effect on food consumption, body weight or cumulative mortality (Table 1). No signs of toxic response to asulam were observed during or after the 28 day feeding period. The depression of body weight and food intake in all groups during the 4th week of the study was attributable to an outbreak of ulcerative enteritis which was treated with bacitracin, 0.5 gms/gallon in the drinking water, beginning the 28th day of the study. The success of the treatment is evidenced by the decrease in mortality and the subsequent weight gain of the survivors.

No effect of treatment was observed on egg production, fertility, or hatchability of fertile eggs (Tables 2 & 3). No abnormalities were observed in the F_1 generation chicks.

TABLE 1
Summary of Body Weight - Feed Intake
Asulam Intake and Mortality

Group	Asulam (ppm)	Weeks 1	2	3	4*	5	6	7	Cum
				Average Body Weight (gms)					
1	0	200	207	209	193	191	195	199	
2	1.5	196	205	201	189	185	194	200	
3	6.0	197	206	204	197	201	206	204	
4	25.0	197	205	206	192	200	200	203	
				Average Feed Consumption (gms)					
1	0	98	113	111	76	100	118	113	732
2	1.5	95	108	102	69	95	107	116	708
3	6.0	90	106	104	75	104	107	108	697
4	25.0	95	108	110	76	97	115	112	717
				Average Asulam Intake (mg/kg Body Weight)					
1	0	-	-	-	-	-	-	-	
2	1.5	0.72	0.79	0.76	0.53	-	-	-	2.83
3	6.0	2.75	3.10	3.05	2.27	-	-	-	11.15
4	25.0	12.01	13.20	13.36	9.69	-	-	-	48.16
				Mortality No. of Birds					
1	0	1	0	4	14	10	1	2	32
2	1.5	4	1	3	13	11	2	0	34
3	6.0	2	1	5	10	8	3	1	30
4	25.0	0	0	2	16	15	0	0	33

* Body weight and feed consumption decreased and mortality increased due to an outbreak of ulcerative enteritis in all groups. Disease treated with bacitracin beginning on day 28.

Normal quail tissue spiked with varying amounts of asulam and acetylasulam (one of the main metabolites) was analyzed and the results indicated an average recovery of approximately 88% (Table 4).

Total asulam, as asulam equivalents, recovered in tissue during the 28 day feeding of 25 ppm of asulam ranged from 0.10 - 0.14 ppm, depending on the tissue analyzed. The kidney, leg muscle, and liver contained the highest amounts: 0.13, 0.14, and 0.11 ppm, respectively (Table 4). During the withdrawal period the liver contained 0.12 ppm of total asulam equivalents, but residues were not found in any other tissue (Table 4).

Discussion

Asulam is effective in the control of many broadleaf pasture weeds and bracken fern in reforestation areas (SOPER *et al.*, 1968; HOLROYD *et al.*, 1970). The application of

TABLE 2

Fertility and Hatchability of Eggs From Bobwhite Quail Fed Asulam

Group	No. of Eggs Set	% Fertile[1]	Hatched % of Fertile	Hatched % of Total
		Week 1		
0.0	17	29.4	40.0	11.8
1.5	26	23.1	16.7	3.8
6.0	32	21.9	14.3	3.1
25.0	20	20.0	0.0	0.0
		Week 2		
0.0	58	34.5	30.0	10.3
1.5	40	62.5	64.0	40.0
6.0	58	32.8	10.5	3.4
25.0	38	39.5	46.7	18.4
		Week 3		
0.0	76	51.3	38.5	19.7
1.5	66	59.1	61.5	36.4
6.0	104	48.1	44.0	21.2
25.0	69	46.4	56.3	26.1
		Week 4		
0.0	84	54.8	32.6	17.9
1.5	51	62.7	21.9	13.7
6.0	93	49.5	23.9	11.8
25.0	56	51.8	31.0	16.1
		Week 5[2]		
0.0	31	38.7	33.3	12.9
1.5	23	56.5	53.8	30.4
6.0	74	51.4	57.9	29.7
25.0	31	67.7	66.7	45.2
		Week 6		
0.0	32	62.5	45.0	28.1
1.5	36	75.0	55.6	41.7
6.0	67	58.2	69.2	40.3
25.0	56	78.6	68.2	53.6
		Week 7		
0.0	32	68.8	59.1	40.6
1.5	37	56.8	71.4	40.5
6.0	63	65.1	63.4	41.3
25.0	30	70.0	52.4	36.7

1. Fertility determined by candling 14 or 15 days of incubation.
2. No asulam fed during 5th through 7th weeks.

Total for 7 Weeks

Group	No. of Eggs Set	Hatched	Hatched/set x 100%
0.0	330	64	19.4
1.5	279	85	29.3
6.0	491	111	22.6
25.0	300	89	29.7

1. Fertility determined by candling at 14 or 15 days of incubation.

this compound in these areas necessitated the study of this herbicide in bobwhite quail (Colinus virginianus). It has been shown that asulam is degraded rapidly in plants and soil (RHODIA, 1972). Therefore, the doses fed the quail are very exaggerated and are unlikely to be encountered in the field.

TABLE 3

The Effect of Asulam Feeding
On Egg Production in Bobwhite Quail

	Weeks on Study[1]						
Group	1	2	3	4	5	6	7
	% Hen-Day Production						
0.0	6.2	14.5	20.0	28.1	25.4	40.8	44.6
1.5	8.8	15.1	22.8	19.9	20.5	33.6	60.7
6.0	9.7	17.9	31.6	33.5	46.5	42.9	61.3
25.0	6.2	11.8	21.8	19.4	15.4	36.8	35.3

1. Asulam fed during weeks 1-4.

TABLE 4

Asulam Equivalents

		Found (Avg 28 day feeding)[1,3]		Recovery		
Tissue	Dose Fed	ug	ppm	ug	ppm	% in spiked samples
Skin with fat	0	<0.5	<0.10	<0.5	<0.10	(106)
	25.0	0.5	0.10	<0.5	<0.10	
Breast muscle	0	<0.5	<0.10	<0.5	<0.10	(88)
	25.0	<0.5	<0.10	<0.5	<0.10	
Leg muscle	0	<0.5	<0.10	<0.5	<0.10	(82)
	25.0	0.7	0.14	<0.5	<0.10	
Liver	0	<0.5	<0.10	<0.5	<0.10	(73)
	25.0	0.56	0.11	0.6	0.12	
Kidney	0	<0.5	<0.10	<0.5	<0.10	(85)
	25.0	0.64	0.13	<0.5	<0.10	
Blood	0	<0.5	<0.10	<0.5	<0.10	(88)
	25.0	0.55	0.11	<0.5	<0.10	
Eggs	0	<0.5	<0.10	<0.5	<0.10	(88)
	25.0	0.5	0.10	<0.5	<0.10	

1. Average of the residues sampled at weekly intervals throughout the feeding period. Very little weekly variability was observed.
2. Average of the residues sampled at 2 and 14 days post treatment. Little or no variation was observed between sampling days.
3. No residues were detected in tissues from quail receiving 6.0 ppm asulam in the diet.

When fed in the diet at 25 ppm to bobwhite quail (Colinus virginianus), asulam caused no adverse effects as measured by food consumption, growth rate or mortality. This herbicide did not alter the reproductive capabilities of the quail or result in the formation of any terata.

The birds fed lower concentrations of asulam in their diet (6.0 ppm and 1.5 ppm) were normal throughout the experimental and recovery periods, and no residues were detected in tissues from quail receiving 6.0 ppm asulam in the diet.

Asulam residues in the kidney, liver, and leg muscle of the sacrificed quail are a function of the metabolic activity of the tissues. It appears that asulam, like other sulphonamides, is actively excreted and reabsorbed by the kidney (WEINSTEIN, 1970). Sulphonamides are metabolized in the mammalian liver to the more polar acetylated compound and this appears to be the site and mechanism of metabolism of asulam in the quail. The observation of asulam in the leg muscles, but not in the breast muscles, leads one to suspect that the higher rate of oxidative metabolism in the flight muscles is responsible for a more rapid catabolism and excretion of the compound; hence, the difference in tissue residues.

The residues found are very close to the limit of detection (0.1 ppm) and may be considered negligible.

However, since the bobwhite quail were not affected by the feeding of these exaggerated doses, and the herbicide is rapidly degraded to innocuous compounds, it is concluded that no imminent or long term hazards exist to quail from the application of asulam in the field.

References

BRATTON, A.C. and E.K. MARSHALL: J. Biol. Chem. 128:537 (1939).

HOLROYD, J., C. PARKER, and A. ROWLANDS: Proc. 10th Brit. Weed Control Conference 1:371 (1970)1

INGHAM, B. and M.A. GALLO: Bull. Env. Cont. & Tox., In Press. 1974.

Rhodia Technical Bulletin: No. N.B. 11-72, p.7, Rhodia Inc., New Brunswick, N.J. (1972).

SOPER, D., H.J. TERRY, and B.M. SAVORY: Proc. 9th Brit. Weed Control Conference: p. 508 (1968).

TUCKETT, M.J. and R.W.E. BALL: ibid, p. 744.

WEINSTEIN, L: The Sulphonamides, p. 1178-1203 in L.S. GOODMAN and A. GILMAN(ed.) The Pharmacological Basis of Therapeutics, 4th ed. MacMillan Co., London (1970).