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Effects of Atrazine on Penned Pheasants and the Occurrence of Stress Marks on Feathers

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EFFECTS OF ATRAZINE ON PENNED PHEASANTS

AND THE OCCURRENCE OF STRESS MARKS ON FEATHERS

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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TOM

EFFECTS OF ATRAZINE ON PENNED PHEASANTS

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Abstract

THOMAS ORIN MELIUS

Doses of 4 g of atrazine (AAtrex) were not lethal to penned hen pheasants (*Phasianus colchicus*), but levels of 6, 8, 10 and 12 g induced mortality. Pen studies to determine the effects of atrazine on reproduction of hen pheasants were conducted with two replicates of the basic test. Birds were administered treatment levels of 0, 100, 200 or 400 mg of atrazine per hen per week. Egg production and gain in weight of hens were unaffected by the treatments. Replicate A eggs were unaffected in shell thickness and pipping rates, while fertility and hatchability were statistically different. Replicate B eggs were unaffected in fertility, hatchability and shell thickness but indicated a significant difference in pipping rates. Hens receiving 200 and 400 mg of atrazine laid eggs that were significantly lighter in weight than eggs from control birds. Survival and gain in weight of chicks were not different between treatments. Effects of the atrazine on behavior tested with visual cliff performance and susceptibility to hand capture indicated no differences between experimental and control birds. Stress marks were visible in plumage of pheasants subjected to environmental change, limited diet, and caging. Stress marks related to atrazine ingestion were not determinable in the experimental design utilized.

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INTRODUCTION

The herbicide atrazine (2-chloro-4-ethylamine-6-isopropyl-amine-s-triazine) is widely used for selective control of broadleaf and grassy weeds in corn and sorghum in addition to other crops (Geigy Agricultural Chemicals 1971). It is formulated and available as an 80 percent water dispersible powder or a 4 percent granular product. Atrazine acts as an inhibitor of photosynthesis and persists in the soil from 7 to 18 months depending upon soil properties, influence of weather, and the soil environment (Burnside et al. 1971, LeBaron 1970, Mullison 1970, Sheets 1970, Sheets and Shaw 1963).

Herbicides are generally lower in toxicity than many other pesticides. Of 89 pesticides tested by Heath et al. (1972), toxicity of atrazine was not great enough to warrant a toxic rank number. Devany (1967) stated that atrazine when used in accordance with recommended procedure, was not hazardous to fish or wildlife. The LD50 for a single oral dose of atrazine (AAtrex, 80 percent atrazine) for female mallard ducks (Anas platyrhynchos) 6 months of age is > 2000 mg/kg (Tucker and Crabtree 1970). The LC50's of technical grade atrazine in 5-day diets for 2-week-old birds are: (1) bobwhite quail (Colinus virginianus), >5000 ppm, (2) Japanese quail (Coturnix coturnix japonica), >5000 ppm with 7 percent mortality at the 5000 ppm level, (3) ring-necked pheasant, >5000 ppm, and (4) mallard, >5000 ppm with 30 percent mortality at the 5000 ppm level (Heath et al. 1972). Palmer and Radeleff (1969) found that chickens receiving 10 doses of 50 mg/kg showed significant reduction in weight gained and lack or loss of appetite.

Studies have shown that sub-lethal levels of some insecticides are detrimental to birds. Azevedo et al. (1965) found that 10 ppm of DDT in the diet reduced egg production in pheasants. Dahlgren et al. (1974) reported that sub-lethal levels of dieldrin affected egg production in hen pheasants and affected the behavior of chicks hatched from eggs laid by hens receiving the compound. Since the effect of sub-lethal levels of atrazine has received little or no attention, this study was designed to measure sub-lethal effects of the chemical on pheasants. Measurements were made of (1) weight change of hens, (2) egg production, (3) fertility and hatchability of eggs, and (4) weight gain, survival, and behavioral changes of chicks.

The second phase of this study concerned the occurrence of stress marks on the feathers of pheasants. Stress marks are thin, translucent streaks or angular lines found on the vanes of feathers. They have been referred to as "growth bars" by Wood (1950), as "feather bars" by Michener and Michener (1938), and as "fault bars" by Riddle (1908) and Hammerstrom (1967). Stress marks are most distinct in raptors (Hammerstrom 1967). Stress marks have been reported on plumage of purple grackle, (Quiscalus q. quiscula); catbird (Dumetella carolinensis); eastern cardinal (Richmondena c. cardinalis); eastern robin (Turdus m. migratorius); and brown thrasher (Toxostoma rufum) (Wood 1950). Although marks are most commonly seen in the primaries and rectrices, the eastern mourning dove (Zenaidura m. carolinensis) exhibited stress marks on practically all feathers (Wood 1950).

According to Wood (1950) stress marks develop as the growing barbs emerge from the calamus sheath of the feather; the undeveloped barbules within the sheath show no signs or indications of a mark. Riddle (1908) stated that poor nutritive conditions during the night slowed the formation of barbule-forming cells.

The objectives of this phase of the study were to determine if (1) environmental change, (2) limited diet, or (3) ingestion of atrazine would induce stress marks on pheasant feathers.

MATERIALS AND METHODS

Toxicity Test

A basic toxicity test was conducted in order to form a base for comparison of lethal dosages and doses used in the experiments of sub-lethal effects. The procedure was similar to the protocol described by Tucker (1969, unpublished report of U.S. Fish Wildl. Ser., Denver, Colorado) for determining a single-dose or acute oral toxicity-level of a pesticide to juvenile pheasants under laboratory conditions.

Pen-reared pheasants, approximately 6 months of age, were weighed and held indoors for 15 days under controlled temperature of 60 F and 12 hour photoperiod. Food and water were withheld for 24 hours before oral administration of atrazine. Birds had free access to water, corn, oats, and commercial pheasant feed at other times.

Experimental and control groups contained 5 birds of each sex or 10 individuals. Birds in the experimental groups were given No. 00 gelatin capsules containing weekly dosages of 4, 6, 8, 10, and 12 g of atrazine (AAtrex 80 W; 80 percent atrazine). Control birds received empty capsules.

Birds were observed until death or for 14 days. Date of death, reaction to the chemical, and food consumption were noted and compared to data from the control group.

Sub-lethal Experiments

Adult pheasants were obtained from the South Dakota Pheasant Company, Canton, South Dakota, or were male offspring of the control group from a study conducted the previous year. Two groups of hens

(Replicate A with 32 and Replicate B with 40) and 13 cocks were caged individually in late January and subjected to controlled photoperiod. Individual hens were mated to different cocks each week starting the first of March. Cages and feeding procedures used were as described by Dahlgren et al. (1972).

Three treatment groups of eight birds each in Replicate A and ten birds each in Replicate B were fed capsules of atrazine weekly in No. 00 capsules at dosage levels of 100 mg, 200 mg, and 400 mg, respectively. Birds in the control groups were fed empty capsules. All hens were weighed each week.

Eggs were collected, individually marked, and placed in a force-draft incubator at the Poultry Research Center, South Dakota State University, at weekly intervals for 15 weeks. Individual daily records were kept of the number of eggs laid, weight of each egg, and fertility (eggs with observable embryo development) and hatchability (number of fertile eggs hatched) of eggs.

Thickness of the eggshells was measured with an Ames thickness micrometer to 1/100 mm at three points around the widest part (transverse section) of the egg. Unhatched eggs were rinsed with water to remove excess yolk and albumin. Shells of hatched eggs were measured without membranes.

Chicks were banded and pinioned at hatching and held in battery brooders until 6 weeks of age. All chicks were tested within 36 hours of hatching on the visual cliff as described by Baxter et al. (1969).

Chicks were moved to outside pens (4.9 m x 4.9 m) when 6 weeks old.

Susceptibility of the birds to hand-capture as described by Dahlgren et al. (1970) was conducted in the outside pens when the birds reached 8 weeks of age.

Stress Mark Experiments

To test environmental change, primaries were removed from 25, 5-week-old pheasant chicks. They were held in brooders for 1 week after which they were placed in outside pens. The move from the brooders to the outside pens was the stress applied. The primaries were examined for stress marks when the chicks were 12 weeks of age and the primaries had regrown.

To measure effects of limited diet, primaries were removed from 26 pheasants 22 weeks of age. Food was withheld from 20 birds for 4 days each week. Six control birds had access to food at all times. The primaries were removed and examined for stress marks after regrowth occurred.

The stress of ingestion of atrazine on pheasants was measured by giving birds 3 g of atrazine weekly for 4 weeks. Twenty-four birds 22 weeks of age were held in cages in a heated building and received the chemical. Twelve control birds were given empty capsules. Primaries were removed before the first treatment and removed again after regrowth had occurred.

RESULTS

Toxicity Experiment

Pheasants appeared unaffected by the 4 g dosage of atrazine (Fig. 1 and Table 1). A male pheasant died following an injury and a resulting weakened condition. Mortality increased with dosages of 6 to 12 grams. Six died in the 6 g group while 8 were lost in the 10 g group and 9 died following administration of 12 grams. An accidental death resulted among the control birds fed empty gelatin capsules.

Response of the birds to atrazine ranged from normal appearance at lower dosages to severe tremors, atoxia, and death at higher dosages. Several days after administration of the atrazine, all birds were excitable. Birds on higher dosages developed a droopy appearance with ruffled feathers and death soon followed. Birds on all dosage levels lost weight (Table 2) and 18 females and 12 males died (Table 1).

Sub-lethal Experiments

Weight of hens from Replicate A and Replicate B were combined to test differences in weight change among treatment groups (Table 3). No significant difference ($P > 0.05$) in weight change among treatment groups was detected using analysis of variance. There was a significant difference ($P < 0.05$) for the change in weights among weeks. This change in weight among weeks could not be attributed to atrazine.

Of the 18 control hens and 54 experimental hens in the 2 replicates, 8 hens died that received atrazine and 3 control hens died. Cause of death was attributed to severe pecking by cocks during breeding, prolapsed cloaca, and accidental injury. Necropsy reports by the

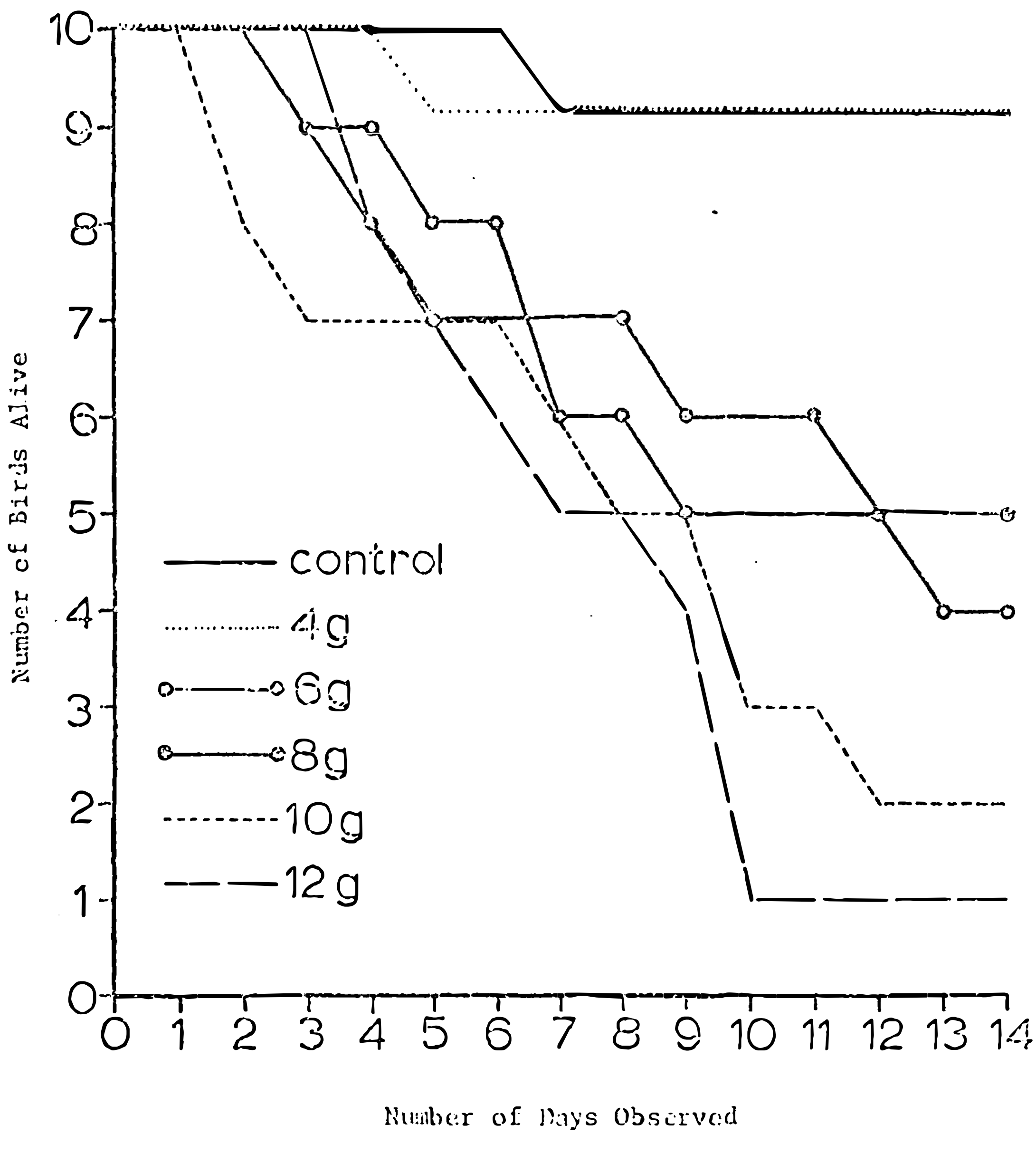

Figure 1. Number of birds alive at each dosage level for days of toxicity test.

Table 1. Response of pheasants administered atrazine and number of pheasants alive at the completion of the toxicity test.

Dosage Level (g Atrazine)	Number Of Birds		Condition of Birds Following Treatment				Number Of Birds Alive At End of Test	
	Male	Female	1 day	7 days	14 days	Extended Period	Male	Female
0	5	5	Appeared normal	One dead Appeared normal	Appeared normal	Appeared normal	5	4
4	5	5	Appeared normal	One dead Excitable	Appeared normal	Appeared normal	4	5
6	5	5	Appeared normal	Four dead Excitable	Two dead Appeared normal	Appeared normal	3	1
8	5	5	Appeared normal	Three dead Reduced food intake Droopy appearance	Two dead Droopy appearance	One dead Recovery 19 days	3	1
10	5	5	Reduced food intake	Eight dead Reduced food intake Droopy appearance	Reduced food intake	Recovery 19 days	2	0
12	5	5	Reduced food intake	Nine dead Tremors Atoxia	Normal appearance	Recovery 19 days	1	0

Table 2. Average deviation from initial weight of pheasants for each dosage level in toxicity test.

Dosage Level (g)	Initial Weight (g)		Weight Change of Birds Alive (g)			
			Day 7		Day 14	
	Male	Female	Male	Female	Male	Female
0	726	675	+21	+28	+26	+26
4	737	680	-27	-53	-6	-14
6	717	759	-45	-14	-2	-4
8	729	653	-45	-106	-46	-103
10	730	644	-47	-141	-23	
12	740	657	-83	-129	-54	

Table 3. Average weights (g) of control hens and hens receiving atrazine for Replicates A and B combined in sub-lethal experiments.

Week of Treatment	Average Weight of Hens in Groups 0 mg	100 mg	200 mg	400 mg
1	705	701	688	693
2	712	703	692	695
3	703	701	688	698
4	704	708	696	700
5	714	707	698	695
6	708	707	696	697
7	716	711	702	701
8	715	703	696	698
9	714	703	700	698
10	716	710	706	706
11	715	699	698	695
12	707	698	697	690
13	704	696	702	695
14	710	702	697	694
15	704	696	693	688
16	721	710	704	697

Veterinary Diagnostic Laboratory at South Dakota State University indicated that death was not from atrazine ingestion.

No significant difference ($P > 0.05$) was detected among treatment groups in eggs laid per hen per day for Replicates A or B (Table 4) when data were examined using analysis of variance. Chi-square analysis showed fertility and hatchability of the fertile eggs differed significantly among treatment groups in Replicate A but not in Replicate B (Table 4). This test also detected a significant difference ($P < 0.05$) among treatment groups in pipping rates for fertile eggs not hatched in Replicate B but not in Replicate A (Table 4).

An analysis of variance test detected a significant difference ($P < 0.01$) in egg weights among treatment groups when combining data from Replicates A and B. Dunnet's T test showed that eggs from 200 mg group and 400 mg group were significantly lighter than eggs of the control hens (Table 5). Shell thickness did not vary among treatment groups in Replicates A or B by the use of an analysis of variance test (Table 6).

Chicks that hatched from eggs laid by hens in Replicates A and B were tested on the visual cliff. No significant difference ($P > 0.05$) was found among treatment groups in either Replicates A or B using analysis of variance (Table 7). This test also showed no significant difference among treatment groups for weights of 6-week-old chicks (Table 8). Survival of chicks to 6 weeks of age was tested by using Chi-square analysis. There was no significant difference ($P > 0.05$) among treatment groups for either Replicates A or B chicks (Table 9).

Table 4. Reproductive performance of hen pheasants in Replicates A and B that were given weekly treatments of atrazine in sub-lethal experiments.

Treatment Level	No. Eggs Per Day Per Hen	No. Eggs Set In Incubator	Fertile Eggs		Fertile Eggs Hatched		Fertile Eggs Pipped But Not Hatched	
			No.	Percent	No.	Percent	No.	Percent
Replicate A								
0	0.65	362	191	53	40	21	30	16
100	0.54	256	210	82	108	51	30	14
200	0.52	349	219	63	83	38	24	11
400	0.50	264	176	67	78	44	15	9
Replicate B								
0	0.64	454	281	62	74	26	33	12
100	0.47	210	139	66	35	25	26	19
200	0.59	405	244	60	72	30	17	7
400	0.45	221	138	62	38	28	18	13

Table 5. Average weights (g) of eggs laid by control hens and hens receiving weekly treatments of atrazine in sub-lethal experiment.

Treatment Level	Replicate A Egg Weights	Replicate B Egg Weights	Replicates A and B Egg Weights Combined
0 mg	32.7(362)[a]	33.5(454)	33.1(816)
100 mg	32.4(256)	32.7(210)	32.6(466)
200 mg	32.3(349)	31.1(405)	31.7*(754)
400 mg	30.5(264)	30.4(221)	30.4*(485)

*Significant difference (0.05) from control mean by Dunnet's T test.

[a]Number of eggs weighed shown in parenthesis.

Table 6. Mean thickness of eggshells in mm of eggs laid by hens receiving varying dosages of atrazine in two replications as compared with eggshell thickness of eggs laid by control hens.

Treatment Level (mg Atrazine weekly)	Hatched Eggs		Unhatched Eggs	
	No. of Eggs	Eggshell Thickness for 15 weeks	No. of Eggs	Eggshell Thickness and Membrane for 15 weeks
0	109	0.25	491	0.35
100	134	0.26	221	0.35
200	142	0.23	442	0.34
400	116	0.23	281	0.33

Table 7. Behavior on the visual cliff of chicks hatched from eggs laid by control hens and hens receiving weekly treatment of atrazine for the two replicates.

Treatment Levels (mg)	No. that Jumped to Visually Deep Side		No. that Jumped to Visually Shallow Side		No. Not Jumping Within 5 Minutes	
	A	B	A	B	A	B
0	3	9	7	27	30	38
100	18	5	40	17	50	13
200	16	9	25	28	42	35
400	8	7	27	11	43	20

Table 8. Average weight (g) of 6-week-old chicks for Replicates A and B in sub-lethal experiment.

Treatment Levels	Replicate A: No. Alive at 6 Weeks	Replicate A: Average Weight	Replicate B: No. Alive at 6 Weeks	Replicate B: Average Weight
0 mg	18	327.6±13.9	38	407.4±9.6
100 mg	72	327.3±6.9	24	428.1±12.0
200 mg	52	347.9±8.2	44	399.3±8.9
400 mg	41	330.2±9.2	23	376.2±12.3

Table 9. Survival of offspring hatched from eggs laid by control pheasants and pheasants given atrazine in Replicates A and B in sub-lethal experiment.

Treatment Levels (mg)	No. of Chicks to Brooders		No. of Chicks Alive After 6 Weeks		Percent Survival	
	A	B	A	B	A	B
0	40	74	18	38	45	51
100	108	35	72	24	67	69
200	83	72	52	44	63	61
400	78	38	41	23	53	61

The susceptibility of individual pheasants to capturing by hand while contained in outdoor pens was analyzed. Order of sequence of capture was recorded and the null hypothesis that experimental and control birds would be evenly divided within the first and second halves of the captured birds was tested by Chi-square analysis (Table 10). The test was applied to the total captures of birds from each level of treatment and the control over the period from 21 June to 20 September. A significant difference in susceptibility to capture was detected in the control group and the group receiving the 100 mg dosage. Fewer of the control birds than was expected ($P < 0.01$) were captured and more of the birds receiving 100 mg dosage than expected ($P < 0.01$) were captured. The groups receiving 200 mg and 400 mg dosages exhibited no differential susceptibility to capture.

Stress Mark Experiments

Distinctive stress marks developed on the primaries in six of the 25 pheasants moved from the brooders to outside pens (Fig. 2). Six hens and 20 cocks deprived of food developed stress marks of varying degree, while the three female and three males in the control group did not. Rectices and contour feathers also exhibited stress marks. The number of marks per primary varied from one to six though most primaries showed two to three stress marks. Rectrices of male pheasants showed more stress marks than any other feathers examined. Primaries from all birds receiving weekly doses of atrazine showed stress marks, however, marks were also present on the primaries of the 12 control pheasants.

Table 10. Susceptibility to hand capture of young pheasants hatched from eggs laid by control hens and hens receiving atrazine. (Numbers represent pheasants caught in the first half of all birds caught; numbers in parentheses represent one-half the number of that category in the pen. Chi-square analysis was used to compare numbers actually caught with half of the number in each category.)

Dates of Capture	Milligram of Atrazine Given Weekly to Hens				
	0	100	200	400	Total
June 21 and 28	10(15)	17(20)	18(11)	11(8)	110
July 5 and 12	14(19)	35(32)	28(27)	16(15)	186
July 19 and 26	14(19)	40(30)	27(31)	18(15)	194
August 2 and 9	18(22)	45(26)	28(36)	17(18)	210
August 16 and 23	13(28)	48(27)	34(36)	22(21)	229
August 30 and September 6	19(22)	45(30)	30(44)	25(20)	235
September 13 and 20	18(22)	38(37)	31(41)	32(16)	235
Total	106(147)	268(202)	196(226)	141(113)	1399
Percent Caught 1st Half	42	57	46	56	

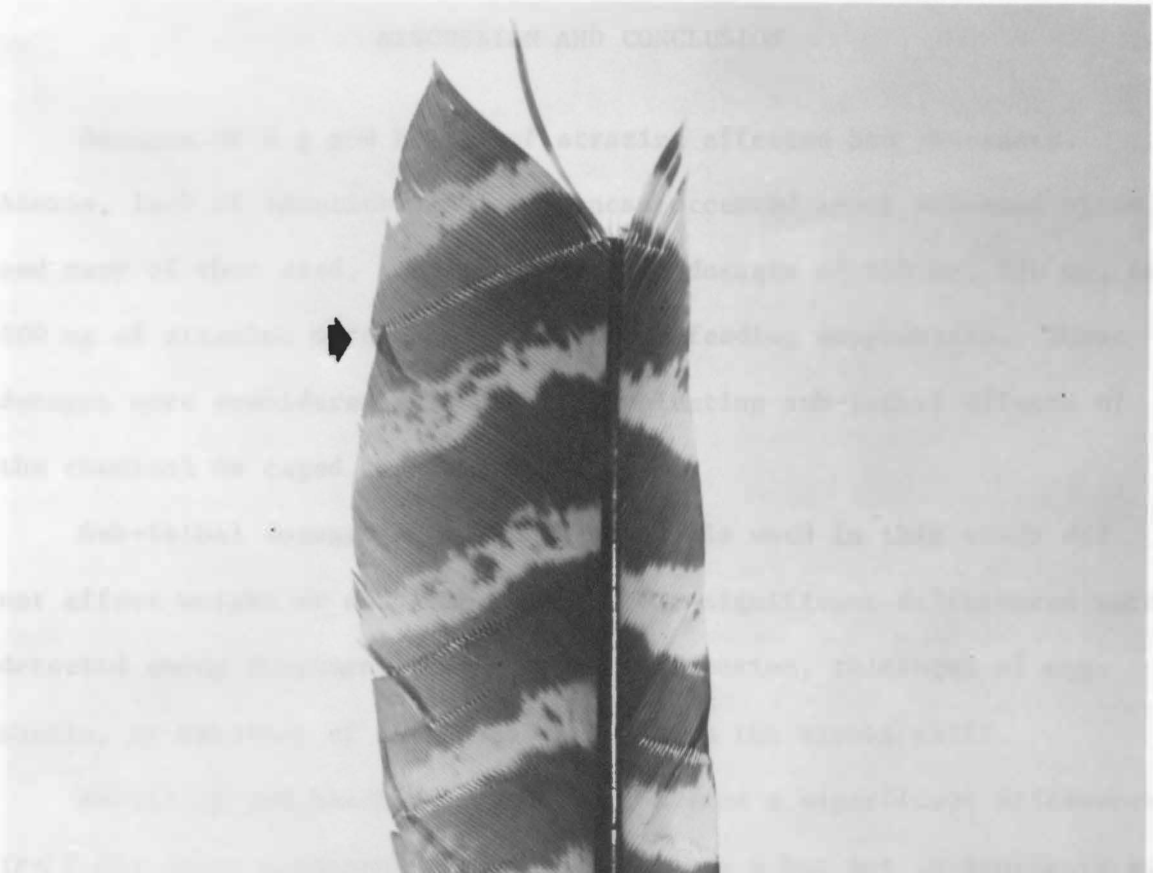

Figure 2. Stress marks on a primary feather from a young hen pheasant subjected to environmental change.

DISCUSSION AND CONCLUSION

Dosages of 6 g and higher of atrazine affected hen pheasants. Atoxia, lack of appetite, and droopyness occurred among affected birds and many of them died. Weekly sub-lethal dosages of 100 mg, 200 mg, and 400 mg of atrazine were used in the force-feeding experiments. These dosages were considered adequate for evaluating sub-lethal effects of the chemical on caged hen pheasants.

Sub-lethal dosages of atrazine at levels used in this study did not affect weight or survival of hens. No significant differences were detected among treatment groups in egg production, thickness of egg-shells, or behavior of chicks as measured on the visual cliff.

Fertility and hatchability of eggs showed a significant difference ($P < 0.05$) among treatment groups in Replicate B but not in Replicate A. Fertility and hatchability were highest at the 100 mg level in both Replicates A and B although differences were not significant. Atrazine may have a stimulatory effect on fertility and hatchability at low dosage levels. Atkins (1966) reported that dosages of 2 mg weekly of dieldrin might have had a slight stimulatory effect on hatchability of eggs in his experiments with caged hen pheasants. Alzevedo et al. (1965) also reported that eggs from hen pheasants fed diets containing 10 ppm DDT and 500 ppm DDT during egg laying had slightly higher hatchability than eggs from hens in the control group. The effect of a chemical acting as a stimulus at low doses while acting as a depressant or even toxic at higher levels has been reported in other animals. Paton and Perry (1953) and Lundberg and Thesleff (1953) administered nicotine and TMA to cats and reported that nicotine possesses a mixed action, by stimulating the ganglion at low levels (50 µg) and acting as a depressant at higher levels (300 µg).

Chicks hatched from eggs laid by hens receiving 0 mg and 100 mg showed a significant difference ($P < 0.05$) in susceptibility to hand capture. There appears to be no biological explanation why the chicks in the 100 mg group were caught more readily than the expected 50 percent distribution and the chicks in the 200 and 400 mg groups were not.

The significant difference ($P < 0.05$) in egg weights among treatment groups for both Replicates A and B appeared to be related to treatment levels; as dosage levels increased weight of eggs decreased. Changes in egg weights are usually related to the condition of the birds. Breitenbach et al. (1963) found that egg weight was generally related to the condition of the hen, with limited intake diets causing a reduction in egg size. I did not measure food consumption but weight change of hens was not significantly different ($P > 0.05$) among treatment groups which indicates that food consumption was not affected to a great degree.

Pheasants will develop stress marks on their feathers if they are subjected to stressful conditions. Moving the chicks from a heated building to outside pens evidently placed chicks 6 weeks of age under sufficient stress to show the marks. Limiting the diet of older birds also produced stress marks. The birds on restricted diets showed stress marks on many of the feathers, while the control birds showed none. Stress resulting from confinement or other factors prevented using stress marks as indication of effect of atrazine ingestion in my experiment. Kabat et al. (1956) reported that caging acts as a stress comparable in effect to reproduction. To be able to use stress marks as a measure for stress conditions it would be necessary to have holding facilities that did not affect the birds enough for marking to develop. Under those conditions the measurement of stress associated with intake of chemicals might be measured.

Stickel (1974) reported that field application of organic herbicides, which include all herbicides in common use today, are essentially non-toxic to birds. The findings of my study indicated that atrazine administered at levels of 100, 200 or 400 mg has no sub-lethal effect on penned hen pheasants.

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