

EFFECTS OF DRC-1339 (3-CHLORO-4-METHYLANILINE HYDROCHLORIDE) AVICIDE ON PHEASANT REPRODUCTION

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Abstract: We performed a laboratory experiment to investigate the effects of 2 levels of 3 repeated dosages of compound DRC-1339 (3-chloro-4-methylaniline hydrochloride) on ring-necked pheasants (*Phasianus colchicus*). Twelve females and 4 males were administered 3 doses of 4 mg each (HIGH), a second group received 3 doses of 2 mg each (LOW), and a third group served as controls. Doses were administered over a 5-day period. Three sub-groups of each treatment group of females were randomly mated with each group of males. Reproductive variables (clutch size, fertility, hatchability, 10-day chick survival, and final brood size) were analyzed with ANOVA using a 3 x 3 factorial arrangement of treatments. Birds were necropsied upon death or termination of the experiment. One HIGH group female died of toxicosis. Incidence of egg yolk peritonitis was significantly higher in the females that received DRC-1339. Females showed a trend of decreasing clutch size and brood size with increasing dosage, but these trends were not statistically significant. Males showed decreasing effects on clutch size and fertility, with effect on brood size being significant ($p=0.01$). We conclude that sub-lethal doses of DRC-1339 might affect reproduction in ring-necked pheasants.

Key words: avicide, DRC-1339, *Phasianus colchicus*, reproduction, ring-necked pheasant, sublethal effects.

Red-winged blackbirds (*Agelaius phoeniceus*), yellow-headed blackbirds (*Xanthocephalus xanthocephalus*), and common grackles (*Quiscalus quiscula*) annually damage about \$1.5 million and \$3.5 million of sunflower in South Dakota and North Dakota, respectively (Lilleboe 1996). The U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) is evaluating the reduction of the northern prairie's population of red-winged blackbirds by decoying vernal migrating flocks into plots baited with rice treated with the compound DRC-1339 (3-chloro-4-methylaniline hydrochloride, also known as 3-chloro-4-methyl benzamine HCL, DRC-1339). The bait is diluted to 1 treated and 25 untreated rice kernels. Each treated grain contains about 0.4 mg of toxicant (Avery et al. 1998). Based on the reported ring-necked pheasant (*Phasianus colchicus*) LD₅₀ for DRC-1339 of 10 mg/kg (DeCino et al. 1966), it is possible that wild pheasants could ingest enough rice in a feeding bout to accumulate a lethal dose of the avicide (Avery et al. 1998). However, studies have not reported any mortality of pheasants in areas where experimental blackbird poisoning programs have been conducted (Kenyon 1997, Knutsen 1998, Smith 1999). The timing of DRC-1339 application to fields occurs when vernal migrating blackbirds are present: any time between the third week of March through the third week of April (Lilleboe 1996). Ring-necked pheasants begin their breeding season in South Dakota in early April, with peak nesting occurring in early May (Trautman 1982). Thus, sublethal effects of

DRC-1339 on pheasant reproduction might be possible and warrant investigation.

DRC-1339 causes renal damage and death by uremic poisoning. Once a specific threshold amount of the DRC-1339 is eaten, it takes a susceptible bird a set time to die regardless of total intake of the compound. Schafer et al. (1977) found that feed containing 286 ppm DRC-1339 took an average of 19 days to kill pheasants. In comparison, a concentration of 2860 ppm took an average of 15 days to kill ring-necked pheasants and was not significantly different from the 286 ppm group. In both groups, feed consumption was drastically reduced, but the total amount of DRC-1339 ingested by the groups was 112 mg/kg for the low-concentration group and 848 mg/kg for the high-concentration group. These amounts are equal to an average daily dose of 0.6 times the LD₅₀ per bird per day for the low-concentration group and 5.7 times the LD₅₀ per bird per day for the high-concentration group (assuming an average body weight of about 1000g; Dunning [1993] reported a mean body weight for 759 females of 953 g and a mean of 1317 g for 6378 males). Sub-lethal effects of DRC-1339 on reproduction or behavior have not been reported for ring-necked pheasants, but Schafer et al. (1977) have reported a few experiments on reproduction in Japanese quail (*Coturnix coturnix*) and rock doves (*Columba livia*). Japanese quail experienced increased eggshell breakage and reduced egg and live chick production at daily consumptions of 0.5 times the LD₅₀ or above. Eggshell breakage and egg production returned

to normal after treatment was withdrawn, but live chick production remained suppressed. Pigeons experienced an increase in the proportion of infertile eggs. These authors also reported that "Two other reproductive studies indicated that no reproductive dysfunction occurred following a single sublethal dose of 1.8 mg/kg of DRC-1339 to adults or in F₁ generation *Coturnix* raised from adults fed 10 ppm of DRC-1339 for 14 days" (Schafer et al. 1977:245). The general objectives of this study were to investigate the sublethal effects of DRC-1339 on reproduction in ring-necked pheasants. Specifically, the objectives were to: (1) compare egg production, hatchability, fertility, eggshell thickness, 10-day chick survival, and brood size of females and males and (2) compare pathological changes of females and males fed repeated, but limited, sublethal doses of DRC-1339 with that of a control group.

METHODS

Adult female ($n=36$) and male ($n=12$) pheasants were purchased from Countryside Wild Bird Farm and Hatchery, Lennox, South Dakota, and brought to the lab on 16 April 1997. All birds received a commercially prepared complete ration for breeding pheasants ("COOP Game Bird Breeder PC" [16% crude protein], Farmland Industries, Inc., Kansas City, MO) and water *ad libitum*. The birds were housed at the Animal Disease Research and Diagnostic Laboratory at South Dakota State University (SDSU) and were cared for in accordance with the guidelines of the SDSU Institutional Animal Care and Use Committee. Each female was housed in an individual layer-type wire cage measuring 46 X 46 X 46 cm; males were housed in larger cages (46 X 92 X 46 cm) suitable for hand-breeding pairs.

Photoperiod in the lab at arrival was that of seasonal conditions (ca. 12-13 hrs) via the laboratory windows. After 5 days of adjustment to lab conditions, photoperiod was increased to 16 hrs by supplemental lighting. On the 7th day after arrival, 2 groups each of 12 randomly selected females and 2 groups each of 4 randomly selected males began receiving doses of DRC-1339. Three doses of DRC-1339 were administered in aqueous solution via a tube into the cervical esophagus; each dose administered at approximately 48-hr intervals. One group of each sex (12 hens and 4 roosters) received three 2-mg doses (LOW groups) and another group of each sex received three 4-mg doses (HIGH groups). The remaining 12 females and 4 males received 3 placebos of deionized water (CONTROL groups).

Four randomly selected females from each of the 3 groups were randomly mated to males from the CONTROL group. Likewise, 4 females from each of the groups were randomly mated with males from the LOW group and 4 females per group were mated to males from the HIGH group. This design resulted in 9 treat-

ments with 4 females per treatment. Matings began 8 days after the last dose of toxicant was administered and occurred approximately every 72 hrs thereafter. The 8-day time period between dosing and mating was selected as a possible "worst-case-scenario" should DRC-1339 field application occur in late April and, hence, closer to peak nesting, rather than an early spring application. For each mating, females and males were re-randomized so that each female was not always mated with the same male within their respective groups. However, 1 male each from the LOW and HIGH groups were observed to have a poor libido and were left out of the mating schedule after the second mating. Females that were to be mated to those 2 individuals were re-mated to another male from their appropriate group. Thus, only 3 males were used for mating from the LOW and HIGH groups.

Eggs were collected daily, stored at room temperature, and set every 4 days in an incubator. Temperature in the incubators was maintained between 37.5 C and 37.6 C. Wet bulb temperature was maintained at 28.9 C. Chicks were held in commercially obtained brooders until about 4 weeks old, at which time they were relocated to outdoor pens.

A 3X3 factorial ANOVA was used to detect differences between treatment groups for clutch size, fertility, hatchability, 10-day chick mortality, brood size, and eggshell thickness. Egg collections for clutch size and other reproductive variables began on the second day after the third mating; observations of the matings indicated that this was the appropriate time to begin collections in order to ensure that all eggs had the potential to be fertilized (based on observations of confirmed copulations), due to poor libido of 2 of the males (see above). Egg collections terminated on the day that the running average of the control clutches (clutches of the CONTROL females mated with CONTROL males) reached or exceeded 12, a reasonably large clutch size for wild pheasants (Trautman 1982). Fertility was determined by candling on the 10th day of incubation and was expressed as the percent of a clutch that was fertile. Hatchability was calculated as the percent of the clutch with eggs producing a free-standing chick. A piped, but not emerged, chick was not counted as hatched. The 10-day chick mortality was expressed as the percentage of hatched chicks alive at the end of the 10th day post-hatch. Brood size was the total number of live chicks from a clutch at the end of the 10th day post-hatching. Egg-shell thickness was measured to the nearest 0.0001 mm with a micrometer at 3 locations around the transverse equator of the shell and were considered "samples" of an egg; the mean of the eggs in a clutch represented a clutch's value.

At termination of the experiment, all adult birds underwent a gross necropsy and gross lesions noted. All birds, excepting those terminated early, were

ethanized at the conclusion of egg collection; 30 days post-dosing. When findings at necropsy suggested the possibility of infection, diseased tissues were taken and appropriate bacterial and mycotic examinations were performed (Carter and Cole 1990). Nine birds from each dose group, 5 randomly selected females and all 4 males, had a comprehensive examination including gross and microscopic pathological examinations and bacterial culture of liver and any grossly suspicious organs. Microscopic examinations were conducted on sections of spleen, gonad, liver, muscle, lung, kidney, heart, intestine, uterus, cloaca, and any other tissue with a gross lesion. Listed tissues were placed in 10% neutral-buffered formalin, processed by standard paraffin techniques, sectioned at 5 micrometers, stained with hematoxylin and eosin, and examined by light microscopy (Prophet et al. 1992).

RESULTS

Reproductive Experiment

One female from the HIGH dose group died on the second day after her last dose of DRC-1339, but prior to hand mating. Necropsy indicated she died from DRC-1339 poisoning (see below). The clutch produced from the bird that died of DRC-1339 toxicosis (0 eggs) was left in the analysis of clutch size, but was not used in the analyses of the other reproductive variables since its values for the other variables are meaningless. This female was scheduled to be mated with a HIGH male. To maximize the sample sizes in the CONTROL/CONTROL and HIGH/HIGH clutches, a female was randomly selected from those HIGH group females that were to be mated to LOW group males to replace her. Clutches from 3 other females that died from causes unrelated to DRC-1339 were not included in the analyses of reproductive variables.

Three of the CONTROL, 3 of the LOW, and 6 of the HIGH group females produced uncalcified eggs. Most of these produced uncalcified eggs sporadically. However 1 HIGH dose female produced only uncalci-

fied eggs beginning on the 5th day post-dosing and produced no eggs from the 15th day post-dosing to termination, although she produced 12 normal eggs in the 15 days previous to the 5th day post-dosing. Uncalcified eggs were included in clutch size analysis, but since they are nonviable were considered not fertile and not hatchable in those analyses. Occasionally, eggs were collected that were either crushed or cracked. All cracked and crushed eggs were included in analysis of clutch size, but were not included in analyses of fertility or hatchability as we assumed that these effects were artifacts of experimental conditions. Cracking was probably caused by eggs being laid on the slightly inclined wire cage floor and rolling forward to the egg collection receptacle on the front of the cage. Crushed eggs appeared to be caused by eggs failing to roll forward to the collection receptacle and consequently trampled by the bird. Eggshell measurements were obtained from cracked eggs and included in analysis, but crushed eggs were not measured as it was not possible using our methods.

Results of the 3x3 factorial analyses of variance on the reproductive variables are listed in Table 1. All analyses were nonsignificant except for analysis of the male effect on brood size. Even though most comparisons were nonsignificant, there was a tendency for several reproductive variables to decrease with dosage. For the male effect on brood size, least squares mean separation procedures showed that the mean brood size produced from HIGH dose males was significantly lower than the mean brood size produced from LOW dose males ($p=0.04$) and broods produced from CONTROL males ($p=0.002$). Means of broods produced from LOW and CONTROL males were not different ($p=0.24$). There were no differences between groups for eggshell thickness (Table 2).

Pathology

One female from the HIGH dose group died on the second day after her last dose of DRC-1339, but prior to hand mating. During the necropsy, we discovered

Table 1. Effects of 2 levels of repeated sublethal doses of DRC-1339 on selected reproductive variables in ring-necked pheasants.

Variable	Effect		High dose group			Low dose group			Control group		
	Sex	<i>P</i>	\bar{x}	sd	<i>n</i>	\bar{x}	sd	<i>n</i>	\bar{x}	sd	<i>n</i>
Clutch size	F	0.16	8.1	5.0	12	9.5	5.4	10	12.6	1.3	11
	M	0.46	8.9	4.5	11	10.7	4.1	10	11.5	4.3	11
Fertility (%)	F	0.87	72.4	18.9	10	69.4	39.9	9	78.5	16.5	11
	M	0.18	63.3	31.5	11	73.5	21.2	9	85.5	18.1	10
Hatchability (%)	F	0.17	52.1	21.7	10	39.6	24.7	9	63.7	21.1	11
	M	0.11	41.0	26.0	11	58.5	10.5	9	60.1	26.8	10
10-day chick survival (%)	F	0.53	98.1	5.6	9	97.9	5.9	8	95.3	7.2	11
	M	0.98	97.5	5.6	10	96.2	7.7	9	97.1	6.0	9
Brood size	F	0.24	5.9	2.9	9	5.4	3.0	8	7.8	3.0	11
	M	0.01	4.4	3.4	10	6.8	1.9	9	8.6	2.2	9

Table 2. Effects of 2 levels of repeated sublethal doses of DRC-1339 on eggshell thickness (mm) in ring-necked pheasants.

Variable	Effect		High dose group			Low dose group			Control group		
	Sex	P	\bar{x}	sd	n	\bar{x}	sd	n	\bar{x}	sd	n
Eggshell thickness	F	0.56	0.2463	0.0130	10	0.2393	0.0312	8	0.2462	0.0186	11
	M	0.43	0.2445	0.0186	10	0.2397	0.0187	9	0.2484	0.0254	10

a dry, white material over the surface of the heart and liver. Microscopically, there were extensive deposits of eosinophilic homogenous material with streaking. Some of the deposits had nonsuppurative inflammation around them. These deposits were found in the epicardium, pericardium, myocardium, spleen, liver, and kidney, and were consistent with urate trophi. There was severe nephrosis characterized by renal tubular epithelium with cytoplasmic vacuolation, degeneration, and necrosis. Much of the epithelium was sloughing into tubular lumens. There were also large trophi in the kidney. These findings confirmed that the cause of death was from renal toxicosis, consistent with DRC-1339 poisoning (DeCino et al. 1966).

Males did not have gross lesions, however 7 females had egg yolk peritonitis severe enough to be grossly noted: 5 females from the HIGH group and 2 from the LOW group. Chi-square analysis of the number of females with peritonitis showed that the incidence of peritonitis was significantly higher in the dosed groups (Table 3). One of the 5 females with peritonitis from the HIGH group was the one that died from DRC-1339 toxicosis. CONTROL females did not exhibit gross lesions. The peritonitis was characterized by off-white to yellow multifocal to diffuse discoloration of the serosal surfaces of the peritoneal organs. Histopathological examination was conducted on 3 of these females. Microscopically, the peritonitis varied in severity and was characterized by multifocal accumulation of non-suppurative inflammatory cells. Homogeneous eosinophilic material in low to moderate amounts was present in the cytoplasm of macrophages or was free. Small amounts of a golden brown pigment were also present in the cytoplasm of some macrophages.

Table 3. Effects of DRC-1339 on incidence of peritonitis in ring-necked pheasant females receiving repeated sublethal doses of DRC-1339.

	Experimental groups (n)		
	HIGH (12)	LOW (11)	CONTROL (11)
Peritonitis present ^a	5	2	0
Normal	7	9	11

^aLikelihood ratio chi-square = 7.843, 2 df, p = 0.02.

At necropsy, 2 of HIGH dose females that had peritonitis also had egg abnormalities. One of these females had 2 small misshapen mineralized eggs in the distal uterus and some coagulated off-white material (egg white) in the mid-uterine lumen. This was the same female that had produced only uncalcified or no eggs at all commencing on the 5th day post-dosing. The other female had a well formed, but uncalcified egg free in the peritoneal cavity. This latter female is the same female that died from DRC-1339 toxicosis. Both of these females had grossly discernible peritonitis. In the former female, tissue samples from the liver and uterus, and a peritoneal swab were cultured for bacteria. In the latter female, bacterial cultures were obtained from liver, intestine, heart, and kidney tissues as well as a peritoneal swab. All cultures were negative. A mycotic examination of a peritoneal swab of 1 of the females and the heart of the other female also yielded no significant organisms.

Additionally, 4 females from each dosage group were randomly selected for aerobic and microaerophilic bacterial culture of uteri. No significant bacteria were isolated from any of the 12 females with either technique. Five females (randomly selected) from each group and all 4 males from each group were selected for gross and microscopic pathological examination and bacterial culture of liver and any grossly suspicious organs. Tissues examined microscopically were spleen, gonad, liver, kidney, heart, intestine, cloaca, brain, and uterus. Microscopically there were no differences between groups and no significant bacteria were isolated from the livers. Trauma caused by the wire floors of the cages was indicated in 1 female from each group and 1 male from the HIGH dose group by subcutaneous abscesses over the sternum.

Three other females either died or were terminated during the course of the experiment due to injuries or other causes unrelated to the avicide.

DISCUSSION

The 3 doses of DRC-1339 administered to the birds was designed to simulate a pheasant eating 3 meals of rice bait over a 3-day period. The LOW dose groups received a total of 6 mg of DRC-1339, equivalent

to 60% of an LD₅₀ accumulated over 3 days for a 1-kg bird. The HIGH dose groups received a total of 12 mg of DRC-1339, equivalent to 120% of an LD₅₀ accumulated over 3 days for a 1-kg bird. The effective doses of our experimental birds, however, were variable as initial weights varied from 867 g to 1752 g.

The dilution ratio, used by USDA, of plain rice bait to DRC-1339-laced rice bait is about 25:1 (G. M. Linz, personal communication). At this dilution ratio, the amount of rice bait that would contain the amount of toxicant administered to the HIGH dose group would average about 250 kernels at each of 3 feedings over a 3-day period. This amount of rice weighs about 5 g. The LOW groups received half of that amount; or the equivalent in rice of about 2.5 g of rice at each of 3 feedings. These amounts of rice are probably realistic of the amounts that a pheasant might eat in typical feeding bouts. Trautman (1982:57) reported that captive pheasants eat about 98 g per bird per day, with 2 major feeding periods per day. Avery et al. (1998) concluded that consumption of up to 325 kernels of rice in a day by a pheasant seemed reasonable. Thus, these amounts of rice are probably realistic of the amounts that a pheasant might eat in typical feeding bouts.

Under the conditions of this experiment, 1 female fed the HIGH dosage died and 4 additional HIGH and 2 LOW dosage group females demonstrated a pathological insult (egg yolk peritonitis) that could not be attributable to infection. This would indicate that both the HIGH and LOW dosed female groups were exhibiting effects of DRC-1339. In addition, 1 male each from the HIGH and LOW dose groups exhibited poor libido, but we do not know if it was caused by DRC-1339.

Several of the reproductive variables studied in this experiment showed decreasing trends with increasing DRC-1339 dosage, although they were not statistically significant. The male effect on brood size, however, was significant. This variable is a cumulative expression of most of the other variables studied.

We speculate that 1 possible cause of the lack of statistical significance of treatment effects may be due to variable responses of the birds to DRC-1339. Mull (1971) found that for 1 strain of chicken (white leghorn, males, 6 weeks old) the LD₅₀ was about 10 mg/kg, while for another strain ("Foster Farms," males, 6 weeks old) the LD₅₀ was 32 mg/kg. This is about a 300% difference between strains within a single species. DeCino et al. (1966) reported the LD₅₀ for ring-necked pheasants as 10 mg/kg but did not report either mean time to death nor any other measure of the variability among individuals. Another possible cause of lack of statistical significance may be due to differences in effective dosages due to variability in the mass of the birds. The effective doses for the LOW group hens ranged from 3.8 to 6.1 mg/kg and the HIGH dose hens from 8.1 to 12.8 mg/kg.

For males, the LOW group ranged from 4.6 to 5.4 mg/kg and the HIGH group from 8.0 to 9.4 mg/kg.

CONCLUSIONS

The DRC-1339 caused the death of 1 female from the HIGH group and the occurrence of egg yolk peritonitis in 4 additional HIGH and 2 LOW group females. No egg yolk peritonitis was seen in the controls. This, combined with the decreasing trend of several of the reproductive variables with increasing dosage, the incidence of egg abnormalities in 2 HIGH dose females, and the significantly lower brood sizes with increasing dosage of males suggests that reproductive suppression by DRC-1339 may be dose-dependent. These results suggest that further research using exact dosing may be warranted.

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