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Source: Journal of Wildlife Diseases, 25(2) : 175-183

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-25.2.175>

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## COMPARATIVE TOXICITY OF LEAD SHOT IN BLACK DUCKS (*ANAS RUBRIPES*) AND MALLARDS (*ANAS PLATYRHYNCHOS*)

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**ABSTRACT:** In winter, pen-reared and wild black ducks (*Anas rubripes*), and game farm and wild mallards (*Anas platyrhynchos*), maintained on pelleted feed, were sham-dosed or given one number 4 lead shot. After 14 days, dosed birds were redosed with two or four additional lead shot. This dosing regimen also was repeated in summer using pen-reared black ducks and game farm mallards. Based upon mortality, overt intoxication, weight change, delta-aminolevulinic acid dehydratase activity and protoporphyrin concentration, black ducks and mallards were found to be equally tolerant to lead shot. However, captive wild ducks were more sensitive than their domesticated counterparts, as evidenced by greater mortality and weight loss following lead shot administration. This difference may be related to stress associated with captivity and unnatural diet.

**Key words:** Black ducks, *Anas rubripes*, mallards, *Anas platyrhynchos*, lead shot, lead poisoning, experimental study, toxicology.

### INTRODUCTION

Lead poisoning from ingested shot is a major cause of mortality in waterfowl (Sanderson and Bellrose, 1986). Bellrose (1959) estimated that lead poisoning accounts for 2 to 3% of the annual loss of waterfowl in North America. Recent restrictions on the use of lead shot for waterfowl hunting in the United States (Federal Register, 1986) may ameliorate such losses.

In a previous study (Pain and Rattner, 1988), pen-reared black ducks were dosed with one number 4 lead shot to obtain lead-inhibited delta-aminolevulinic acid dehydratase (ALAD) for development of an ALAD reactivation assay (heat reactivation, Tomokuni and Kawanishi, 1975; zinc reactivation, Finelli et al., 1975; Scheuhammer, 1987) for the mass screening of waterfowl. An unprecedented 60% mortality was recorded within 6 days. All 10 dosed birds exhibited classic signs of lead intoxication, including lethargy, bile-stained feces, weight loss, markedly increased blood lead and erythrocyte protoporphyrin (EPP) concentrations, and inhibited blood ALAD activity. These findings suggested that black ducks may be more sensitive to lead intoxication than heretofore believed (Bellrose, 1959; Jor-

dan, 1968; Finley and Dieter, 1978). Alternatively, this apparent sensitivity of pen-reared black ducks to lead shot (Pain and Rattner, 1988) may have been related to the exacerbation of lead toxicity by stressful conditions, including elevated ambient temperature (>35 C), confinement in small pens (1 m<sup>2</sup>), and repeated handling and venipuncture (five 2.5-4-ml samples in 9 days). However, there is some indication that wild black ducks receiving low shot dosages (two number 6 shot, Chasko et al., 1984) are more sensitive than wild mallards, whereas at higher dosages (five number 6 shot, Chasko et al., 1984; eight number 6 shot, Longcore et al., 1974), tolerance of black ducks and mallards (both pen-reared and wild) appears to be comparable.

In view of the above findings (Pain and Rattner, 1988) and the steady decline in black duck populations over the past 20 yr (United States Department of the Interior, 1988), the present investigation was undertaken to compare lead shot toxicity in domesticated and wild black ducks and mallards.

### MATERIALS AND METHODS

Males used in these studies included pen-reared black ducks derived from a colony at the Patuxent Wildlife Research Center (Laurel,

Maryland 20708, USA), commercially obtained game farm mallards (winter trial, adults—Frost Game Farms, Coloma, Wisconsin 54930, USA; summer trial, juveniles—Oak Ridge Game Farm, Gravette, Arkansas 72736, USA), and wild black ducks and mallards collected in baited traps in Maryland at Blackwater (38°27'N, 76°07'W) and Eastern Neck (39°02'N, 76°13'W) National Wildlife Refuges and in Delaware at Bombay Hook National Wildlife Refuge (39°20'N, 75°32'W). Ducks were housed in outdoor pens (4.6 × 9.1 m; 5 birds/pen) and maintained on pelleted feed (Beacon Duck Developer, The Beacon Milling Co., Cayuga, New York 13034, USA) and fresh water ad libitum for a 3-wk conditioning period, and during the studies.

Prior to experimental use, ducks were weighed, fluoroscoped (Dinex 150 X-ray Inspection System, TFI Corp., New Haven, Connecticut 06506, USA) and bled by jugular venipuncture. All birds were in good flesh (>1 kg). Birds were excluded from study upon detection of shot or if ALAD activity was depressed (generally 2 standard deviations below the group mean).

One, two or four number 4 lead shot (196–264 mg/shot) were administered ( $n = 10$  or 20 ducks/group) through a flexible plastic tube inserted to the level of the proventriculus. Control birds ( $n = 10$ /group) were sham-dosed (i.e., intubated without giving shot). Birds were observed daily, and, at various intervals, they were weighed and bled (3- to 4-ml sample collected into a heparinized syringe).

Hematocrits were determined on blood samples. An aliquot of whole blood was stored at –70°C for quantification of red blood cell ALAD activity (Burch and Siegel, 1971; optimized for black ducks and mallards with pH 6.4 buffer). The remainder of the sample was stored at 4°C for 48 hr before determining EPP concentration with a hematofluorometer (AVIV Biomedical, Inc., Lakewood, New Jersey 08701, USA) modified according to Roscoe et al. (1979).

Differences in body weight (absolute and percent weight change), hematocrit, ALAD activity and EPP concentration among groups of ducks and lead shot dosage were evaluated by analysis of variance (ANOVA) (SAS Institute, Inc., 1985). Initial analyses revealed that pen variability did not differ significantly from bird variability (i.e., no pen effect); and, because ducks were individually dosed, bird-to-bird variability was used as the error term in subsequent analyses. Groups of ducks (pen-reared black ducks, wild black ducks, game farm mallards and wild mallards) and the number of shot administered were crossed factors in the ANOVA; day was a within-bird factor to model

sequential measurements. Separate analyses were used to evaluate the response to one shot and additional shot administered after 14 days. When significant higher order interactions were detected, appropriate subsets of data were analyzed by ANOVA to quantify the interaction. Mortality in each dosage group at specific times was compared to that of sham-dosed controls by Pearson's chi-square test (Larntz, 1978).

## RESULTS

### Toxicity of lead shot in black ducks and mallards during winter

In March 1986, four groups of males (adult), including pen-reared black ducks, wild black ducks, game farm mallards (Frost Game Farm), and wild mallards, were either sham-dosed ( $n = 10$ /group) or dosed with one number 4 lead shot ( $n = 20$ /group) on day 0. During the ensuing 14-day period, transient signs of intoxication, including lethargy, droop of tail and wings and green watery feces, were noted in a few lead-dosed birds in each test group; however, there was no mortality. Body weight of dosed birds decreased slightly ( $P < 0.01$ ) during the first week of lead exposure (mean reduction of <1 to 3% of day 0 weights) (Fig. 1). Predosing weight was restored by day 14, with the exception of wild mallards (mean reduction of 4% of day 0 weight). Hematocrit was not affected by exposure to one lead shot. However, log transformed ALAD activity was inhibited ( $P < 0.01$ ) by shot exposure compared to sham-dosed controls (Fig. 2). Depression of ALAD activity was most pronounced 3 days following shot intubation, and recovered slightly thereafter. Log transformed EPP concentration was markedly elevated ( $P < 0.01$ ) in birds receiving shot (Fig. 3). Erythrocyte protoporphyrin concentration increased steadily in wild mallards through day 14, whereas EPP concentration in the other dosed groups appeared to reach a plateau by day 7. By day 14, the deviation of EPP concentration decreased in pen-reared birds receiving shot, whereas this deviation remained constant or increased in wild birds.

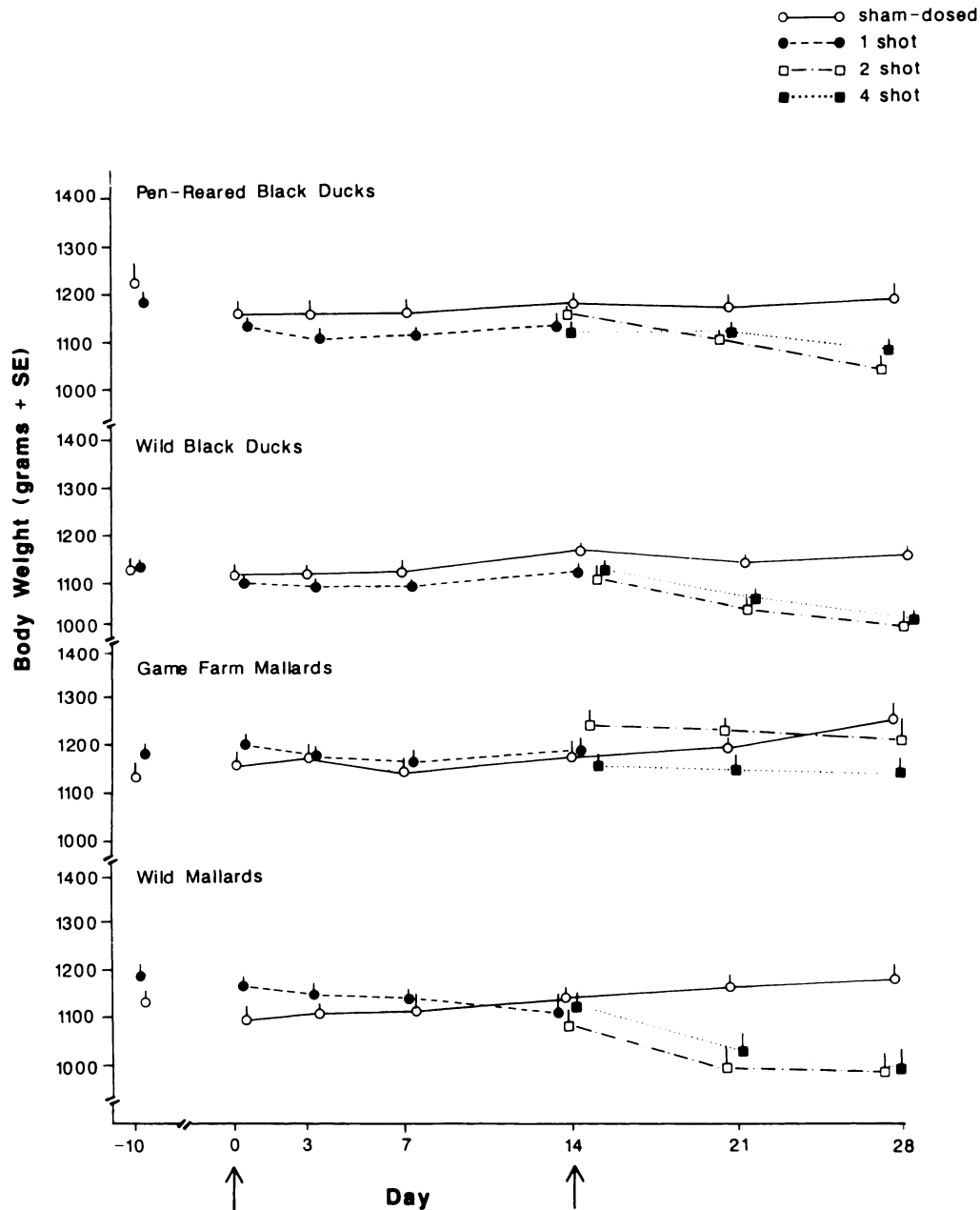


FIGURE 1. Mean body weight of ducks dosed with lead shot in the winter. On day 0, ducks were sham-dosed ( $n = 10$ ) or dosed with one number 4 shot ( $n = 20$ ). On day 14, controls were again sham-dosed and ducks that had received one shot were redosed with either two ( $n = 10$ ) or four ( $n = 10$ ) number 4 shot. Arrows indicate days of shot administration.

Because exposure to one shot in this trial failed to evoke the severe intoxication or mortality that had previously been observed in black ducks (Pain and Rattner, 1988), additional shot were administered to further compare tolerance of black ducks and mallards. Controls were sham-dosed ( $n = 10$ /dosage) again, and ducks that had

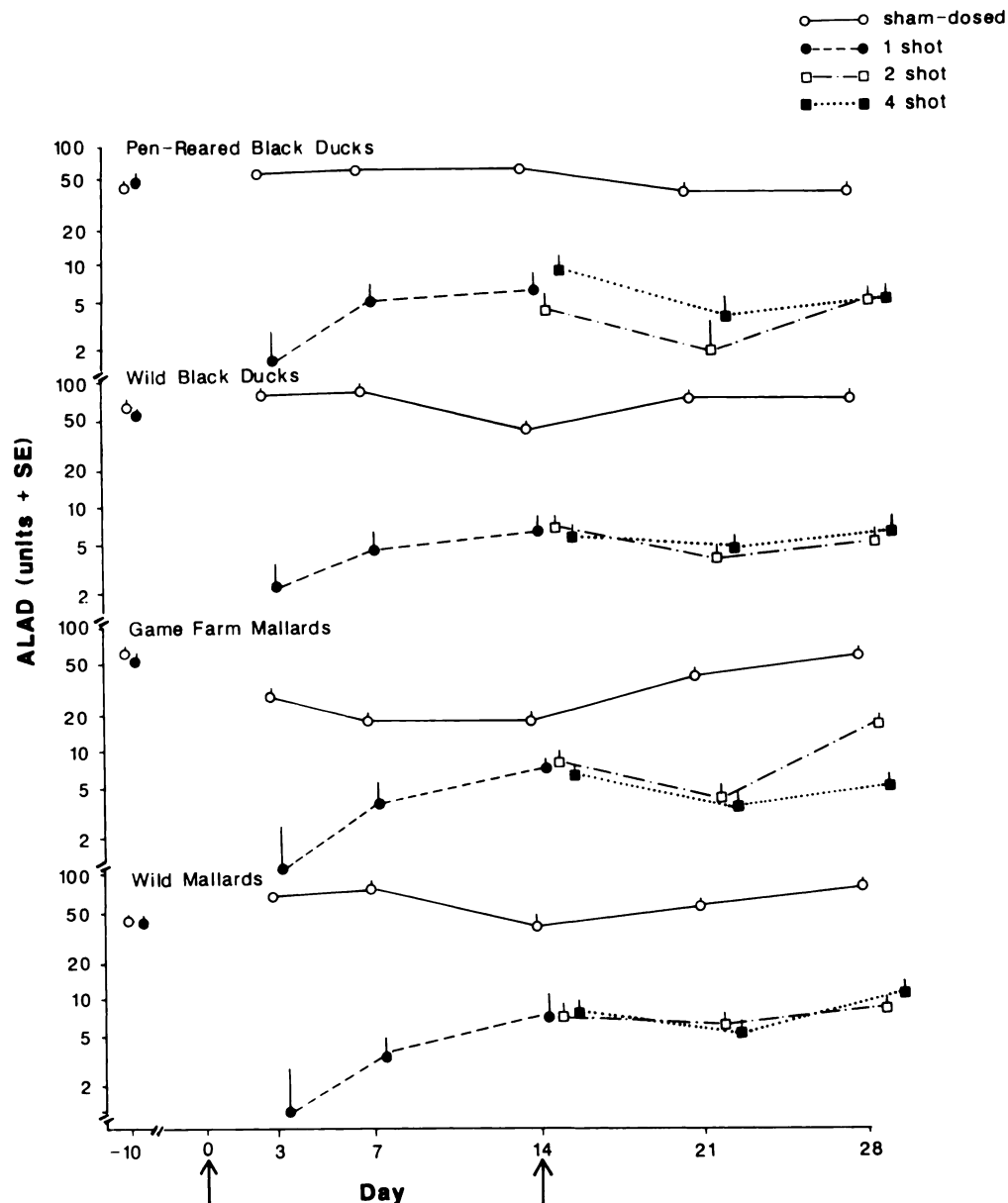


FIGURE 2. Mean red blood cell delta-aminolevulinic acid dehydratase activity of ducks dosed with lead shot in the winter.

received one shot were redosed with either two or four number 4 lead shot ( $n = 10/\text{dosage}$ ). The incidence and severity of intoxication (e.g., weight loss and elevation of EPP) were more pronounced in redosed ducks, particularly in wild ducks (Figs. 1, 3). After a 28-day period, none of the wild

black ducks and 25% of wild mallards that received shot had succumbed, however, after 49 days, mortality was about equal for wild black ducks (40%) and wild mallards (45%) treated with shot (Table 1). The average daily minimum and maximum temperatures during the 4-wk test

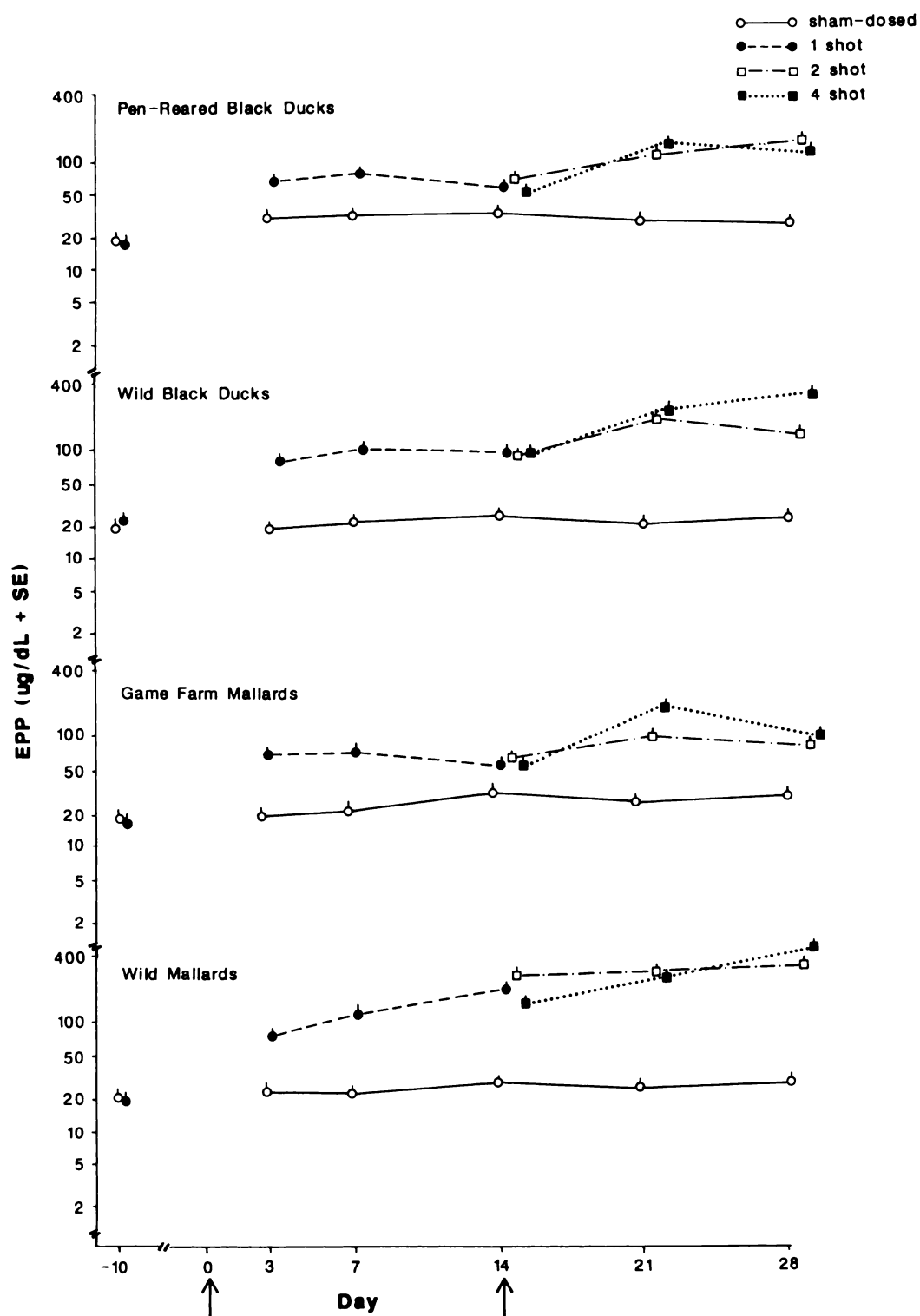


FIGURE 3. Mean erythrocyte protoporphyrin concentration of ducks dosed with lead shot in the winter.

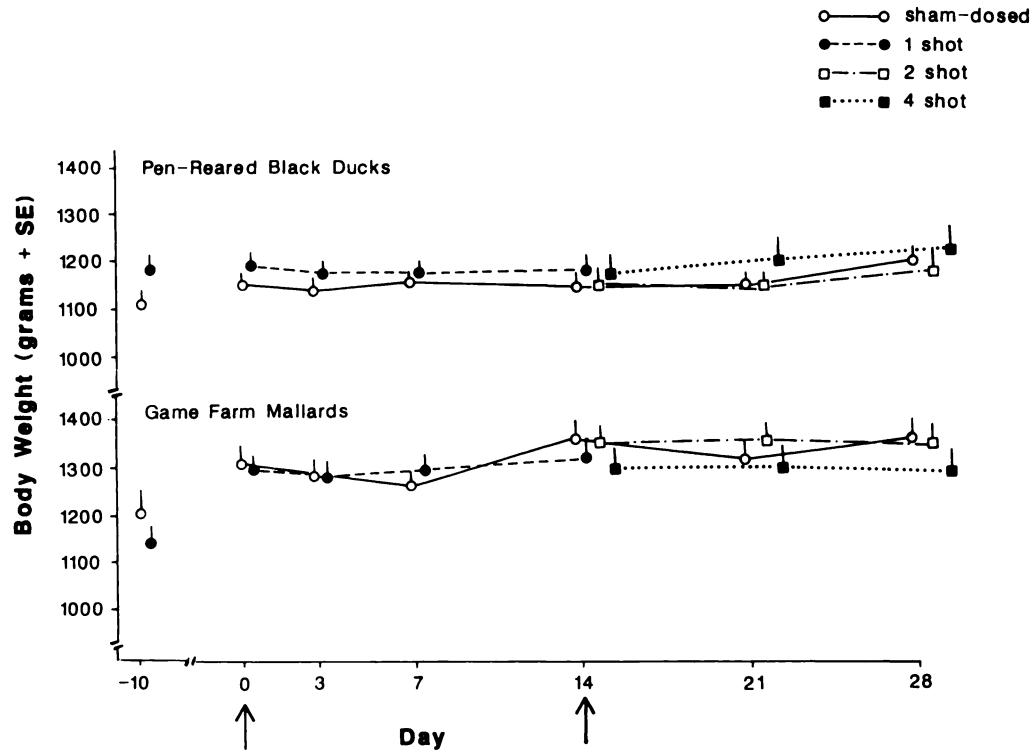


FIGURE 4. Mean body weight of ducks dosed with lead shot in the summer.

period were 10.8 and 15.7 C (National Oceanic and Atmospheric Administration, 1986).

#### Toxicity of lead shot in black ducks and mallards during summer

In August 1987, male (4- to 5-mo-old juveniles) pen-reared black ducks and game farm mallards (Oak Ridge Game Farm) were either sham-dosed ( $n = 10/\text{group}$ ) or dosed with one number 4 lead shot ( $n = 20/\text{group}$ ). Overt signs of intoxication in birds exposed to lead shot were less pronounced in summer than in winter. Neither body weight (Fig. 4) nor hematocrit were significantly reduced in dosed birds. The patterns of inhibition of log transformed ALAD activity and elevation of log transformed EPP concentration were similar to those observed in the winter trial. On day 14, control ducks were again sham-dosed and birds that received shot were

redosed with either two or four number 4 shot ( $n = 10/\text{dosage}$ ). In contrast to the winter trial, redosing with additional shot had little effect on body weight and hematocrit, but did evoke a further increase in EPP concentration. Mortality was limited to a single black duck that died on day 21 after being dosed with one shot and redosed with four shot. The average daily minimum and maximum ambient temperatures during the 4-wk test period were 17.6 and 30.9 C (National Oceanic and Atmospheric Administration, 1987).

#### DISCUSSION

Based upon overt signs of intoxication, body weight change, heme-biosynthetic parameters (i.e., ALAD and EPP) and mortality, pen-reared black ducks and game farm mallards seem to be equally sensitive to low dosages (i.e., one to four shot) of number 4 lead shot. Previous re-

TABLE 1. Cumulative mortality following administration of number 4 lead shot to black ducks and mallards.

Group	n	Number of shot administered		Cumulative mortality % (probability) <sup>a</sup>	
		Day 0	Day 15	Day 28	Day 49
Pen-reared black ducks	10	0	0	0	0
	10	1	2	10 ( <i>P</i> = 0.305)	10 ( <i>P</i> = 0.305)
	10	1	4	0	10 ( <i>P</i> = 0.305)
Wild black ducks	10	0	0	0	0
	10	1	2	0	50 ( <i>P</i> = 0.010)
	10	1	4	0	30 ( <i>P</i> = 0.060)
Game farm mallards	10	0	0	0	0
	10	1	2	0	0
	10	1	4	0	0
Wild mallards	10	0	0	0	0
	10	1	2	30 ( <i>P</i> = 0.060)	60 ( <i>P</i> = 0.003)
	10	1	4	20 ( <i>P</i> = 0.136)	30 ( <i>P</i> = 0.060)

<sup>a</sup> Mortality in groups receiving shot was compared to that of sham-dosed controls by Pearson's chi-square test. *P* values are in parentheses.

ports (Chasko et al., 1984; Pain and Rattner, 1988) indicated that black ducks may be more sensitive to low dosages of ingested lead shot than mallards. The unprecedented mortality of pen-reared black ducks dosed with one number 4 shot (Pain and Rattner, 1988) was probably related to exacerbation of lead toxicity by stressful conditions (e.g., elevated ambient temperature, confinement in small pens, repeated handling and venipuncture). Since elevated ambient temperature failed to enhance lead shot toxicity in game farm mallards (Srebocan and Rattner, 1988), reduced food intake, or possibly a combination of stressors, now seem to be likely factors that contributed to the demise of lead-dosed black ducks in the earlier report (Pain and Rattner, 1988).

The onset and severity of intoxication in recently captured ducks, maintained on pelleted feed and dosed with one to four lead shot, suggest that wild mallards are slightly more sensitive than wild black ducks. This contention is substantiated by continued weight loss (Fig. 1) and mortality (Table 1; day 28) observed in wild mallards. In contrast, lead-dosed wild black ducks were found to be more severely affected than wild mallards when natural

foods were provided (Chasko et al., 1984). These seemingly conflicting findings are probably related to differences in diet (pelleted feed versus natural foods), the single most important factor affecting lead shot toxicity in waterfowl (Jordan, 1968; Sanderson and Bellrose, 1986).

Body weight change and mortality in the winter trial provide evidence that wild waterfowl are more sensitive to lead shot than their domesticated counterparts. In a more extensive investigation, Jordan (1968) reported that wild mallards are more sensitive than game farm mallards, regardless of diet. The apparent tolerance of domesticated ducks to lead shot was attributed to their greater food intake.

Cold and harsh winter weather is generally thought to exacerbate lead toxicity in birds (e.g., Kendall and Scanlon, 1984). Comparison of lead toxicity between the winter and summer trials seemingly indicates that the toxicity of lead shot is greater in winter than in summer. However, this finding may be compromised by differences in age (winter trial adults versus summer trial juveniles). Experiments comparing weight loss and survival in immature (7-mo-old) and adult mallards dosed with shot indicate that immature



birds appear to be less sensitive than adults, possibly due to the removal of lead from the circulation and its deposition in ossifying bone (Sanderson and Bellrose, 1986).

The present study did not attempt to differentiate responses due to genetic strain (e.g., game farm versus wild) or the stress associated with captivity and unnatural diet. Future investigations assessing responses of wild and domestic waterfowl exposed to environmental contaminants should attempt to separate strain-related responses from those due to captive conditions. This could be accomplished by hatching and rearing wild strain birds in captivity prior to conduct of toxicity trials (e.g., Heinz, 1980).

In conclusion, the present study indicates that there is little difference in the toxicity of lead shot to pen-reared black ducks and game farm mallards. Likewise, no pronounced differences in sensitivity to lead shot were detected in wild black ducks and mallards maintained in captivity. Extrapolation of these data to free-ranging birds suggest that black ducks ingesting shot are no more likely to succumb to lead poisoning than mallards.

#### ACKNOWLEDGMENTS

We thank Lynette D. Aiken, Velora P. Anders, Margret A. Fitzgerald, Leonard J. Le-Captain, Maria A. Lum, Jeffery J. Momot and Caroline J. Sanderson for their assistance in the conduct of this study. We also thank W. Nelson Beyer, Jerry R. Longcore and Deborah J. Pain for their comments during the preparation of this paper.

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- Received for publication 7 September 1988.*