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Blood and Tissue Parameters in Wild Mallards Redosed with Lead Shot

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Mortality of waterfowl from lead poisoning is widely recognized (Sanderson and Bellrose 1986). Sublethal biochemical effects caused by ingestion of a single lead pellet include elevation of protoporphyrin concentrations in blood (Roscoe et al. 1979) and inhibition of delta-aminolevulinic acid dehydratase (ALAD) enzyme activity in blood, liver, and brain (Dieter and Finley 1978), the latter resulting in brain damage (Dieter and Finley 1979). Sublethal lead exposure reduces the numbers of immunological cells (white blood cells and spleen plaque-forming cells) in mallards (*Anas platyrhynchos*) (Rocke and Samuel 1991).

Most waterfowl with ingested shot contain one pellet, which disappears from the gizzard in about 20 days (Sanderson and Bellrose 1986). Waterfowl that ingest one pellet usually survive, but because they can spend about 150 days on migration and wintering areas that are often subject to heavy hunting pressure, some waterfowl ingest shot several times (Sanderson and Bellrose 1986). Many studies have examined the effects of lead poisoning on game-farm mallards, which may be less sensitive than wild mallards to sublethal effects (Rattner et al. 1989). The physiological effects of redosing wild mallards with lead shot have received little attention (Chasko et al. 1984; Rattner et al. 1989), and no studies have investigated the physiological effects of redosing wild mallards at rates typifying ingestion during fall migration. The objectives of this study were to investigate any changes in lead levels in blood, bone, and selected soft tissues; in protoporphyrin IX (PP) concentrations in blood; and in body weight of wild mallards during fall as a result of redosing with a second lead pellet 5 wk after initial dosage.

MATERIALS AND METHODS

Sixty-two flightless mallards (34 females, 28 males) approximately 8 wk old were livetrapped on Horicon Marsh, Wisconsin, in a nonhunted area in August 1984. Exposure to spent lead shot prior to capture should have been minimal. The mallards were placed in an outside holding pen in central Illinois for 1 yr for acclimation and maturation.

On 30 September 1985, the holding pen was divided into three compartments (3x7x1 m) containing tanks (1x1x0.5 m) with running water,

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ad libitum Purina Duck Growena, and washed quartz grit. The mallards were randomly assigned to the three pens to form control (11 females, 9 males), dose (11 females, 9 males), and redose (12 females, 10 males) groups. All birds were weighed (±1 g), and blood samples were taken from the brachial vein for determination of lead and PP concentrations (Anderson and Havera 1985). Ducks in the control group were sham-dosed with a polyethylene esophageal catheter, and ducks in the dose and redose groups were administered one No. 4 commercial lead pellet ($\bar{x}$=210 mg). Every 7 days the ducks were weighed and blood samples taken. After 5 wk, when blood lead and PP concentrations approached predose levels (Roscoe et al. 1979), birds in the redose group were administered a second No. 4 lead pellet. Body weights and blood samples were taken at 7-day intervals from all birds for another 5 wk, after which all birds were sacrificed via cervical dislocation. The brain, liver, kidneys, right supracoracoideus breast muscle, gonads, and right tibia, radius, and ulna were removed. Bones were cleaned of all extraneous material. All tissues were frozen in plastic bags.

Blood samples were analyzed for lead as described in Anderson and Havera (1985) and for PP using an Aviv Hematofluorometer calibrated for waterfowl blood (Roscoe et al. 1979). Lead concentrations in soft tissues were determined from duplicate 1.0-g aliquots of tissue digested with nitric and perchloric acids until the perchloric acid fumed. Bones were broken into 1.0-g pieces. All pieces of a given bone were digested with nitric and perchloric acids to facilitate reporting results as average lead concentration in the bone. Digests were diluted to 50 ml and analyzed by inductively coupled plasma atomic emission spectroscopy using a Jarrell-Ash Model 975 AtomComp interfaced to a Digital PDP8 computer for data acquisition. Representative tissue samples spiked with varying concentrations of lead yielded recoveries of ≥98%. Lead residues are reported as parts per million wet weight for all samples. The lead detection limits were 0.01 ppm for blood and 1.00 ppm for all other samples. A blood lead concentration of 0.20 ppm is above background (Friend 1985), and 0.50 ppm is toxic for mallards (Roscoe 1986). A blood PP concentration of 40 μg/dL is above normal (Roscoe et al. 1979). Threshold concentrations of lead above background are 2.0 ppm in wet liver (Friend 1985) and 20.0 ppm in bones (White and Stendell 1977). Longcore et al. (1974) suggested that lead concentrations of 6 to 20 ppm in kidneys and >3 ppm in brains indicate an advanced state of exposure to lead.

Nonparametric statistics were used to compare median values of the measured parameters because distributions were not normal. The Wilcoxon Statistic for rank sums was used to test for sex differences within and between groups for values of blood lead and PP for the same week; for sex differences in the weekly amount and percentage change in body weight within groups; for the percentage change in body weight for all ducks during a period of weeks between groups; and for sex differences in lead concentrations of tissues within and between groups. The Wilcoxon Signed Rank Test for matched pairs was used to test for sex differences in blood lead and PP values at different weeks within groups; for weekly change in body weight for all ducks within groups; and for percentage change in body weight for all ducks during a period of weeks within groups. Spearman's rank correlations were used to compare blood lead-PP and also tissue lead relationships. The level of statistical significance was $P \leq 0.05$.

RESULTS AND DISCUSSION

In the control and dose groups, there were no differences ($P>0.05$) between males and females in weekly concentrations of lead in blood (Fig. 1). In the redose group, females had higher blood lead concentrations than males in week 6 (median 4.35 vs 2.63 ppm; $P=0.01$), week 7 (3.62 vs 1.39 ppm; $P=0.001$), and week 8 (1.69 vs 0.84 ppm; $P=0.06$).

Weekly blood lead concentrations remained elevated ($P<0.0001$) in both males and females in the dose group compared to the control group for weeks 1-8 (Fig. 1). Males in the redose group had higher ($P<0.001$) blood lead concentrations for weeks 1-9 and females for weeks 1-10 than did those in the control group. Blood lead concentrations were also higher in males ($P<0.003$) and females ($P<0.0003$) in the redose group than in the dose group during weeks 6-10. Median lead concentrations remained above the 0.50 ppm toxic level and the 0.20 ppm threshold level in dose group males for 5 and 8 wk; in dose group females for 4 and 7 wk; in redose group males for 9 wk; and in redose group females for 9 and 10 wk, respectively.

Roscoe et al. (1979) reported that wild female mallards dosed with No. 4 lead pellets exceeded 0.40 ppm blood lead within 8 hr after dosing and attained maximum concentrations on the second day. These authors also found that 90% of the mallards dosed with ≤ 8 lead pellets had normal lead concentrations by day 36, and all ducks dosed with one pellet had blood lead concentrations <0.40 ppm by day 29.

Males in the redose group had a lower ($P=0.03$) blood lead concentration in week 10 (0.18 ppm) than in week 5 (0.29 ppm). However, females in the redose group had higher concentrations in week 6 than in week 1 (median 4.35 vs 2.38 ppm; $P=0.01$), in week 7 than in week 2 (3.62 vs 2.13 ppm; $P=0.001$), and in week 8 than in week 3 (1.69 vs 0.91 ppm; $P=0.0002$). These results indicate a greater response in blood lead concentrations in females than in males subjected to a second pellet.

Comparisons of PP concentrations by sex class within groups were similar to those found for blood lead levels (Fig. 1). In the control and dose groups, there were no differences ($P>0.05$) between males and females in weekly PP concentrations. In the redose group, PP concentrations were higher in females than in males for week 6 (median 219.4 vs 151.2 μg/dL; $P=0.03$) and week 7 (207.2 vs 103.8 μg/dL; $P=0.02$).

Concentrations of PP in both the dose and the redose groups were higher than those in the control group for weeks 1-9 for males ($P<0.002$) and weeks 1-10 for females ($P<0.0001$). Males in the redose group had elevated PP concentrations compared with those in the dose group for only 2 wk (week 6, median 151.2 vs 50.0 μg/dL, $P<0.0001$; week 7, 103.8 vs 49.7 μg/dL, $P=0.002$) instead of all 5 wk as with blood lead concentrations. Females in the redose group had higher PP concentrations than those in the dose group for weeks 6-10 ($P<0.02$), the same as with blood lead concentrations. Median PP concentrations remained above the 40 μg/dL threshold in both males and females in the dose group for weeks 1-8 whereas values in the redose group returned to the threshold concentration at 9 wk for males (39.0 μg/dL) and at 10 wk for females (39.5 μg/dL).

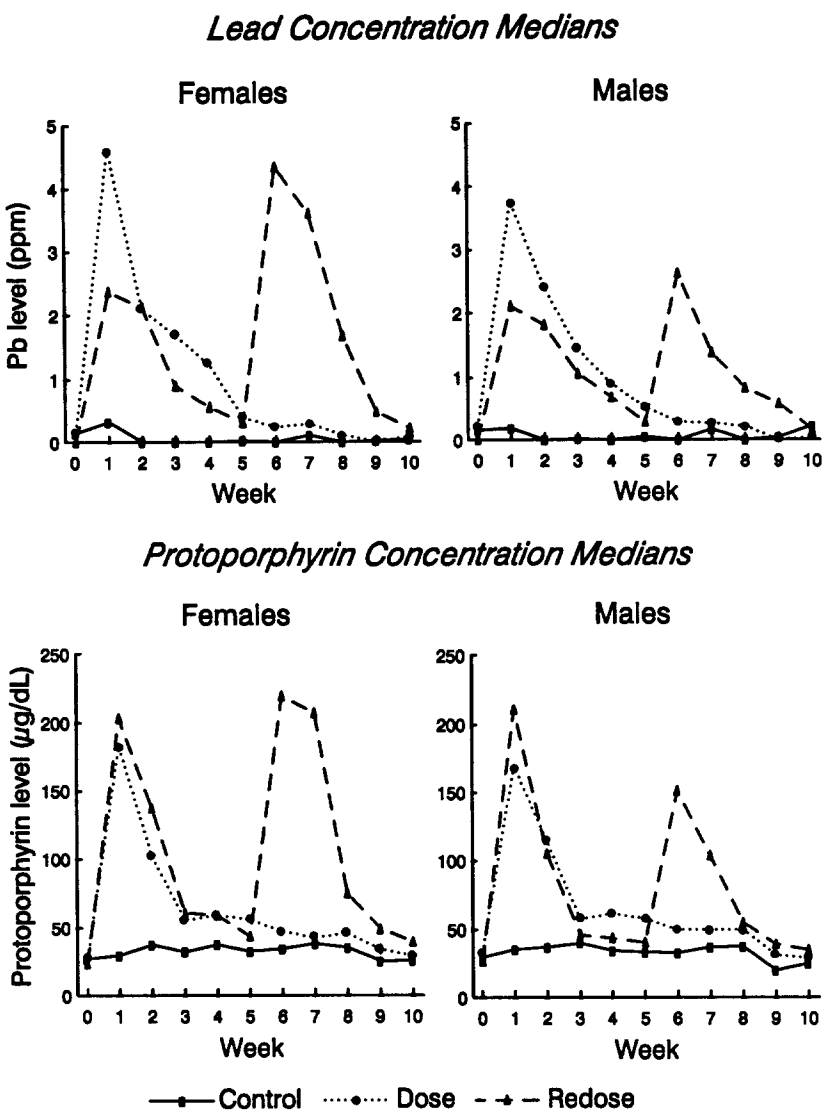

Figure 1. Weekly median values of blood lead and protoporphyrin concentrations in wild male and female mallards. Control group birds were sham-dosed. Dose group birds received one No. 4 lead pellet at week 0. Redose group mallards received one No. 4 lead pellet at week 0 and a second similar pellet at week 5.

Roscoe et al. (1979) found that blood PP levels in wild female mallards dosed with ≤8 lead pellets exceeded 40 µg/dL 2 days after shot ingestion, maximum PP concentrations were attained by day 8, and PP concentrations were below 40 µg/dL on day 29 for all birds receiving one pellet and for 90% of all lead-treated birds by day 36. In the only other study monitoring PP in wild mallards redosed with lead pellets, Rattner et al. (1989) noted a rise in PP concentrations when birds dosed with one No. 4 lead pellet were administered two or four pellets 14 days later.

In the redose group, males had lower PP concentrations in week 6 than in week 1 (median 151.2 vs 211.0 μg/dL; P=0.004) and in week 10 than in week 5 (35.0 vs 40.6 μg/dL; P=0.003). Females had lower concentrations of PP in week 9 than in week 4 (49.0 vs 59.0 μg/dL; P=0.01) and in week 10 than in week 5 (39.5 vs 43.7 μg/dL; P=0.03). Unlike blood lead concentrations, PP levels were no higher (P>0.05) in females after redosing than during the corresponding weeks after initial dosage.

Although a lag of about 6 days occurred in a previous study between the peaks in blood lead and PP concentrations in wild mallards (Roscoe et al. 1979), the concentrations of blood lead and PP have been related in mallards (r=0.70, P<0.01, Scheuhammer 1989; r=0.84, P<0.01, Mautino and Bell 1987) and black ducks (*A. rubripes*) (r=0.68, P<0.001, Pain 1989). In this study, Spearman's rank correlations indicated that lead and PP concentrations for birds receiving one pellet were positively related in weeks 1-3 and 7 for males (r=0.49-0.69; P=0.04-0.001) and in weeks 1, 5-7, and 9 for females (r=0.35-0.71; P=0.05-0.007). Lead-PP concentrations for all birds receiving one pellet were related (r=0.31-0.70; P=0.02-0.0001) in each of the first 7 wk following dosage except in week 4 (P=0.39). After redosing at week 5, lead-PP relationships for males approached significance in weeks 6-8 (r=0.48-0.54; P=0.06-0.08) and reached significance in week 9 (r=0.67; P=0.02). Stronger blood lead-PP relationships were found in redosed females in week 6 (r=0.75; P=0.003) and week 8 (r=0.52; P=0.04).

Anderson and Havera (1985) found that lead in blood was a more sensitive indicator of lead poisoning in waterfowl than PP. Among 399 blood samples from all ducks in the dose and redose groups, 10.5% with blood lead concentrations ≥0.20 ppm and 3.5% with concentrations ≥0.50 did not have elevated PP values (≥40 μg/dL). Less discrepancy in elevated blood lead and PP concentrations occurred in females than in males (≥0.20 ppm but <40 μg/dL, females: 7.8%, males: 13.8%; ≥0.50 ppm but <40 μg/dL, females: 1.4%, males: 6.1%). Scheuhammer (1989) reported that 9.5% of mallards with blood lead concentrations >0.80 ppm and 21% with concentrations >0.15 ppm did not have elevated PP concentrations (≥40 μg/dL). Pain (1989) found false negative results of only 1.5% for both the ALAD activity and activity ratio methods in black ducks when compared with blood lead concentrations >25μg/dL. ALAD activity ratios in mallards (Scheuhammer 1989) and both ALAD activities and activity ratios in black ducks (Pain 1989) were better correlated with blood lead concentrations than were PP concentrations.

Lead concentrations in tissues did not differ between sexes in the dose group (Table 1). In the redose group, however, females had higher tissue lead concentrations than males in tibia (P=0.032), radius (P=0.002), and ulna (P=0.002). These results differ from those of Stendell et al. (1979), who reported no differences in lead concentrations of bones between sexes of mallards outside the breeding season. Finley and Dieter (1978) reported that female mallards dosed with one No. 4 lead pellet in spring had higher (P<0.05) lead concentrations in bones than males similarly dosed; however, the lead concentration in males increased threefold from dosage with a second pellet whereas concentrations in females did not increase.

Males and females in the dose group had higher lead concentrations than their respective sex in the control group for kidney (males, P=0.015;

Table 1. Median (ppm wet weight) and first and third quartiles of lead in selected tissues and bones of wild mallards in the control group; the dose group, which received one No. 4 lead pellet; and the redose group, which received a second similar pellet 5 wk after initial dosage. Sample sizes are in parentheses; values denoted by the same letter within a column differ significantly (P<0.05).

Group	Tissues					Bones		
	Breast	Liver	Kidney	Brain	Gonads	Tibia	Radius	Ulna
Control								
Male	<1.0	<1.0	<1.0^{A}	<1.0	<1.0	2.3^{B}	2.1^{B}	2.0^{B}
						1.0-4.6	1.0-4.7	1.4-4.2
	(9)	(8)	(9)	(9)	(9)	(9)	(9)	(9)
Female	<1.0	<1.0	<1.0^{B}	<1.0	<1.0	1.7^{A}	1.1^{A}	1.3^{A}
						<1.0-2.5	<1.0-1.7	<1.0-1.9
	(11)	(11)	(11)	(11)	(11)	(9)	(9)	(9)
Dose								
Male	<1.0	<1.0	<1.0^{AC}	<1.0	<1.0	17.9^{BD}	9.5^{BC}	11.5^{BD}
		<1.0-1.0	<1.0-1.0			9.6-22.0	6.9-12.6	6.6-12.8
	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)
Female	<1.0	<1.0^{A}	<1.0^{BD}	<1.0^{A}	<1.0	13.0^{AC}	13.6^{AD}	10.5^{AC}
			<1.0-1.2			9.3-20.1	7.7-16.1	8.0-14.3
	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)
Redose								
Male	<1.0	<1.0	1.9^{C}	<1.0	<1.0	26.5^{DE}	17.7^{CE}	16.3^{DE}
		<1.0-2.3	1.1-4.6	<1.0-1.4		18.7-34.1	12.5-24.9	10.2-19.8
	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Female	<1.0	1.2^{A}	3.1^{D}	1.0^{A}	<1.0	42.6^{CE}	39.5^{DE}	32.8^{CE}
		<1.0-1.5	2.1-4.6	<1.0-1.8		27.0-67.1	22.7-63.3	20.1-43.3
	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)

females, P=0.035), tibia (P=0.0001), radius (males, P=0.001; females, P=0.0001), and ulna (P=0.0001) (Table 1). Males in the redose group had higher lead concentrations than those in the dose group for kidney (P=0.002), tibia (P=0.025), radius (P=0.007), and ulna (P=0.036). Females in the redose group had higher lead concentrations than those in the dose group for brain (P=0.019), liver (P=0.032), kidney (P=0.0001), tibia (P=0.0001), radius (P=0.0001), and ulna (P=0.0001). Thus, dosage with the second pellet increased lead concentrations in the brain and liver of females more than in males. Nonetheless, median lead concentrations were <2.0 ppm in both liver and brain. No lead was detected in breast and gonadal tissue (Table 1).

In this and other dosing studies involving mallards, lead concentrations in tissues typically examined were highest in bone, intermediate in kidney, and lowest in liver (Finley et al. 1976; Mautino and Bell 1987). Lead concentrations increased with dosage in soft tissues and bone (Table 1). Chasko et al. (1984) reported higher wing bone and liver lead concentrations in mallards and black ducks receiving five No. 6 lead pellets over 14 days than in birds receiving the pellets in one dosage.

Spearman's rank correlations of lead concentrations in kidney, liver, and bones were conducted for 14 birds in the redose group with detectable amounts in all of these tissues. Lead concentrations among bones were highly correlated ($r=0.87$-0.96; $P=0.0001$). Lead concentrations in kidney were related to amounts in liver ($r=0.51$; $P=0.03$), tibia ($r=0.49$; $P=0.04$), radius ($r=0.56$; $P=0.02$), and ulna ($r=0.53$; $P=0.03$). Other studies found positive relationships ($P<0.05$) in lead concentrations in birds between liver and kidneys (Szymczak and Adrian 1978; Beyer et al. 1988), liver and bones (Anderson 1975), and bones (Finley and Dieter 1978).

Both the amount of change and the percentage change in body weight by week did not differ ($P>0.05$) between sexes in the control, dose, and redose groups. Mallards in the redose group had a greater percentage increase in body weight for the 5 wk after redosing than did birds in the control group (median change of 5.7% vs 2.5%; $P=0.02$). In addition, birds in the redose group had a greater percentage increase in body weight during the 5 wk after redosing than during the 5 wk following initial dosage (median change of 4.8% vs 0.3%; $P=0.001$). These results indicate no negative effects on body weight in birds dosed with a second lead pellet.

In this initial study of wild adult mallards during fall, redosage with a second No. 4 lead pellet resulted in higher ($P<0.05$) lead concentrations for males in kidneys and bones and for females in liver, kidney, brain, and bones when compared with concentrations for those receiving only the initial pellet 5 wk earlier. After redosage, females (1) had higher ($P<0.05$) blood lead concentrations than values at a corresponding time after initial dosage, (2) had higher ($P<0.05$) concentrations than males of lead and PP in blood and lead in bones, and (3) had more lead than males in most tissues examined.

If parameters from the 22 mallards 5 wk after redosage were used for a lead poisoning monitoring program, results indicate that 18.2% had lead concentrations in liver ≥ 2.0 ppm, 40.9% had blood PP concentrations ≥ 40 μg/dL, 45.5% contained blood lead concentrations ≥ 0.20 ppm, and 86.4% had at least one bone with lead concentrations ≥ 20.0 ppm. Thus, lead poisoning monitoring programs for waterfowl, which sometimes employ only examination of gizzards, should incorporate samples of at least one soft tissue (including blood) for studies of short-term exposure to lead and also bone for studies of both short- and long-term exposure to lead.

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