Lead in Tissues of Mallard Ducks Dosed With Two Types of Lead Shot

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Poisoning from ingestion of lead shot is a major cause of death of wild waterfowl. Considerable effort has been devoted to development of commercial shot that is non-toxic, economically feasible, and ballistically satisfactory. Toxicity studies using several types of shot (IRBY et al. 1967; GRANDY et al. 1968; LONGCORE et al. 1974a) have shown that lead-induced mortality is in proportion to the amount of lead present in the shot.

A new lead-iron combination shot has been suggested by the Canadian Wildlife Service as a replacement for the traditional commercial lead shot in preference to steel shot recently recommended by the U. S. Fish and Wildlife Service. In preliminary studies, lead-iron shot containing 50 percent lead caused less mortality of ducks than did commercial lead shot (IRWIN et al. 1974). The present study compares the kinetics and potential toxicity of lead-iron shot and commercial lead shot.

MATERIALS AND METHODS

Test birds were male and female 6-month-old mallard ducks (Anas platyrhynchos) that had been pen-reared at Patuxent Wildlife Research Center. During July, 30 birds were weighed and randomly assigned in pairs to 90 x 90 x 60 cm vinyl coated wire cages with accessible running water. Males weighed an average of 1084 g and females 987 g. Ducks were fed ad libitum diets of one-half yellow corn and one-half commercial breeder pellets ground to a mash. Six males and six females were dosed with one number 4 commercial lead shot; an equivalent group received one number 4 lead-iron combination shot* containing 47.5% lead. The commercial lead shot weighed an average of 193 mg; the lead-iron shot averaged 196 mg. Ducks were dosed through a flexible plastic tube forced down the gullet to the proventriculus. Four males and two females served as controls and received no shot.

All ducks were bled prior to dosage, 24 hours after dosage, and at weekly intervals for 4 weeks. Immediately after each bleeding, birds were weighed and fluoroscoped to ascertain shot retention. Food consumption was measured at weekly intervals. The ducks were killed by cervical dislocation 5 weeks after dosage. Ducks were necropsied and selected tissues were retained for

*Lead-iron shot containing by weight 47.5% Pb, 47.5% Fe, 4% Cu, and 1% Zn manufactured by Arcanum Corporation, Ann Arbor, Michigan 48106.

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chemical analysis and histopathological examination. Tissues selected for microscopic examination were fixed in 10% buffered neutral formalin, embedded in paraffin, sectioned, and stained with Ziehl-Neelsen acid-fast stain. Shot recovered from the digestive tracts were examined and weighed to determine the extent of erosion.

Samples of blood (3 ml) were drawn from alar veins into heparinized disposable syringes. An aliquot of whole blood was frozen and shipped to Environmental Trace Substances Research Center, Columbia, Missouri, for lead determination. A 50 µl blood sample was diluted 1:9 with distilled water and the lead concentration was determined by the method of standard additions followed with the analysis of a standard. Samples were assayed on a Perkin-Elmer Model 403 atomic absorption spectrophotometer at 283.3 nm. [See also Analytical Methods for Atomic Absorption Spectrophotometry, the Perkin-Elmer Corp., Norwalk, Connecticut. (1971)] Limits of sensitivity were 0.02 ppm; residues are reported on a wet weight basis.

Livers, kidneys, and right wings were removed at necropsy and shipped for lead analyses to WARF Institute, Inc., Madison Wisconsin. Whole kidneys and 10-g aliquots of liver were homogenized prior to analysis. Samples were ashed using a nitric and perchloric acid mixture and digested in an acid solution. Samples were assayed on a Perkin-Elmer Model 303 atomic absorption spectrophotometer at 283.3 nm. Residues are reported as ppm wet weight with limits of sensitivity of 0.2 ppm lead in livers and 0.5 ppm in kidneys.

Integument and flesh were dissected from wings; the radii-ulnae bones were removed, and then cleaned by dermestid beetles for 1 to 2 days. Bones were rinsed with deionized water and dried overnight at 100° C. Samples were weighed and ashed for 8 hours in a muffle furnace at 550° C. Samples were digested with 5 ml concentrated nitric acid and analyzed by atomic absorption with limits of sensitivity at 0.5 ppm based on dry weight.

Eggs laid before and after shot dosage were collected daily, marked, and weighed. A random sample of pre-treatment eggs was selected for lead analysis along with alternate eggs laid after dosage. Eggs were opened at the equator, contents stored in jars, and the shells washed and air-dried. Samples also were analyzed for lead at WARF Institute, Inc. by atomic absorption. Egg contents were homogenized and 12.5-g aliquots were ashed using 5 ml concentrated HNO_3 and digested in an acid solution. Eggshells were washed, dried overnight at 105° C, and placed in a muffle furnace at 500° C for 8 hours. Shells were crushed and digested with 10 ml HNO_3 in an acid solution. Lead in egg contents is reported on a wet weight basis with limits of sensitivity of 0.2 ppm; lead in shells is reported as ppm dry weight with limits of sensitivity of 0.5 ppm.

RESULTS AND DISCUSSION

No mortality occurred during the study and all ducks were considered in excellent condition upon termination of the experiment. Necropsy revealed no muscular or liver atrophy, and all ducks contained heavy fat deposits. During the 5-week test period, ducks gained an average of 66 g and consumed an average of 137 g of feed daily. Weights and feed consumption did not differ between the two dosed groups or in controls. Fluoroscoping revealed that only 1 duck in each dosed group voided shot after 1 week. Gizzards of males given lead-iron shot eroded an average of 71 percent of the original shot compared with 96 percent erosion in all other dosed ducks. Lower rates of erosion for shot containing iron were reported by IRBY et al. (1967) and LONGCORE et al. (1974a). We do not have an explanation for the slower ($P<0.05$) rate of erosion of the lead-iron shot in males compared with females.

Necropsy failed to reveal any of the tissue lesions usually associated with lead poisoning in waterfowl (KARSTAD 1971; LOCKE et al. 1966). Renal tubular cells of kidneys of two females dosed with commercial lead shot exhibited cytolysis and frequently contained swollen nuclei. The kidney of one of these females contained numerous nuclei with small atypical pink bodies (weakly acid-fast) resembling lead inclusion bodies. The kidneys of this duck contained 10.0 ppm lead. We found no inclusion bodies in kidneys of the other ducks. The presence of acid fast intranuclear inclusion bodies in the renal tubular cells has been considered to be presumptive evidence of lead intoxication in mallards (LOCKE et al. 1966 and 1967; BATES et al. 1968). In rats, lead induced intranuclear inclusions in kidneys were related to minimal lead concentrations of 10 to 20 ppm (MAHAFFEY et al. 1973). The major biochemical change in our lead-dosed ducks was a 90 percent inhibition of the erythrocyte enzyme, delta-aminolevulinic acid dehydratase, which will be reported elsewhere.

Overall, lead levels in ducks given commercial lead shot averaged about twice those in ducks given lead-iron shot (Table 1). In both dosed groups, lead levels in wingbones were higher in females than in males that received the same dosage ($P<0.05$). Lead levels in livers and kidneys of females given all-lead shot also were significantly higher than in males. Lead in blood sampled 4 weeks after dosage averaged 0.64 ppm in the 12 ducks given commercial lead shot, and 0.28 ppm in all ducks given lead-iron shot ($P<0.05$). Differences in levels in liver and kidney were of the same magnitude, reflecting the amount of lead in the two shot types (200 mg in the all-lead shot and 100 mg in the lead-iron shot).

Levels of lead in liver and kidney were unexpectedly low. Although the blood contained up to 0.64 ppm lead, residues in livers did not exceed 2.3 ppm and residues in kidneys exceeded 5.0 ppm in only 1 female. Lead poisoning in waterfowl that have ingested shot usually is associated with much higher lead residues

TABLE 1

Lead residues (ppm) in tissues of mallard ducks dosed with one number 4 commercial lead shot or one number 4 lead-iron shot.@

Treatment	Liver	Kidney	Blood	Bone
LEAD				
Females	^{a}1.15 ± 0.29	^{a}3.53 ± 1.43	^{a}0.71 ± 0.25	^{a}112.27 ± 44.27
Males	^{b}0.58 ± 0.19	^{b}1.02 ± 0.22	^{a}0.49 ± 0.10	^{b} 10.22 ± 1.46
LEAD-IRON				
Females	^{b}0.32 ± 0.05	^{ab}1.42 ± 0.65	^{b}0.31 ± 0.06	^{b} 32.65 ± 16.58
Males	^{b}0.25 ± 0.02	^{b}0.75 ± 0.10	^{b}0.23 ± 0.04	^{c} 3.38 ± 0.56
CONTROLS	ND	ND	0.05 ± 0.01	3.97 ± 1.08

@ Means ± S. E., n=6; Controls consist of combined values from 4 males and 2 females. Only blood samples taken 4 weeks after shot dosage are included. Means in a column preceded by a common letter are not significantly different, $P<0.05$, Duncan's Multiple Range Test.

ND - Not detected.

than are reported in this study. LONGCORE et al. (1974b) reported that 6 to 20 ppm lead in the liver of mallards was indicative of acute lead exposure and should be diagnostic of active lead intoxication. These investigators also concluded that 20 ppm lead in the kidney caused serious lead effects. These values presumptive of toxic lead levels are within the range reported to be lethal to several species of waterfowl (IRWIN and KARSTAD 1972; BATES et al. 1968; CHUPP and DALKE 1964). Our results indicate that liver and kidney tissues may not accumulate extremely high lead levels in mallards 1 month after ingesting a single lead shot. The fact that our ducks were maintained on a nutritionally adequate mash diet would be a contributing factor to such low residues and the good physical condition of the dosed birds. JORDAN and BELLROSE (1951) concluded that diet was more important than dosage level in determining whether ducks would be poisoned by lead. Mallards that were fed whole corn suffered much higher mortality than those fed commercial duck pellets or corn ground to a meal. The single shot dosage, type of diet fed, and age of the test birds should all be considered when interpreting these residues.

Lead levels in wingbones of females were about 10 times higher than those in males (Table 1). Levels ranged from 2.3-270.0 ppm in females given all-lead shot and 3.9-108.0 ppm in those given lead-iron shot. Levels in wingbones of males dosed with the all-lead shot ranged from 5.9-14.9 ppm and from 1.5-5.1 ppm in those given the lead-iron shot. Accumulation of lead in bone is a commonly used criterion for diagnosing chronic lead exposure but is not necessarily a useful criterion for determining acute lead poisoning. High concentrations of lead in bone may result from either acute high-level exposure or chronic low-level exposure (LONGCORE et al. 1974b).

The hens that laid the greatest number of eggs after dosage contained the highest residues of lead in bone. The level of lead in the wingbone was correlated ($P<0.01$) with the number of eggs that were laid by dosed hens (Fig. 1). Of the 6 hens given all-lead shot, 4 laid an average of 8.8 eggs each (range, 3-18). All 6 of the hens given lead-iron shot laid eggs, averaging 5.5 eggs each (range, 1-11). Lead levels in contents and shells of eggs laid by hens dosed with all-lead shot were about twice those in eggs laid by hens dosed with lead-iron shot, again reflecting the amount of lead in the two types of shot (Table 2). Eggshells from both shot treatments contained about 5 times the lead levels in the egg contents and best reflect levels in the blood. Residues in individual eggs were not related to the number of days after dosage and there were no significant correlations between lead levels in eggs and those in tissues.

We have not yet determined the biological significance of the lead levels found in eggs but currently are investigating the possible transfer of harmful amounts of lead from the eggshell to the developing embryo. HAEGELE et al. (1974) reported 2.50 and 1.34 ppm lead in eggshells and contents from a pooled aliquot of

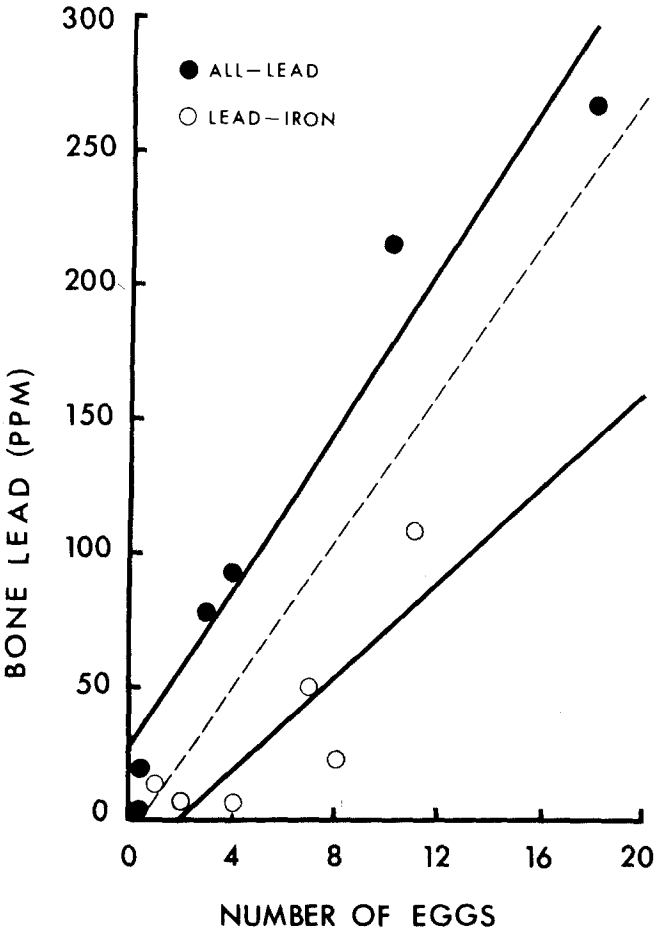

Figure 1. Correlation between wingbone lead concentration and number of eggs laid by hens dosed with two types of lead shot. The broken line represents combined residues from both shot treatments ($n = 12$, $r = 0.85$, $P<0.01$). All-Lead: $r = 0.97$, $P<0.01$. Lead-Iron: $r = 0.84$, $P<0.05$.

eggs laid by mallards fed diets containing 100 ppm metallic lead for about 3 months; there was no eggshell thinning.

The higher levels of lead in bone of laying hens may be related to the competitive effect between calcium and lead during active bone metabolism (SIX and GOYER 1970). In laying birds, medullary bone undergoes sequences of bone formation and destruction connected with storage and liberation of calcium (SIMKISS 1961; BLOOM et al. 1958; TAYLOR and MOORE 1954). In pigeons, dietary dosage of DDE altered calcium mobilization as a result of induced hepatic hydroxylation of circulating steroids (OESTREICHER et al. 1971). Mallards fed DDE in combination with metallic lead had higher levels of lead in bone than those fed lead alone (HAEGELE et al. 1974). In rats, low dietary calcium significantly enhanced the deposition of lead in bone (MAHAFFEY et al. 1973). Also in rats, a single injection of lead acetate mobilized the calcium in bone (YAMAMOTO et al. 1974). Our results indicate that lead deposition in bone of mallard hens dosed with sublethal levels of lead may be greatly increased as a result of mobilization of calcium from the bone during eggshell formation.

TABLE 2

Lead residues in contents and shells of eggs laid by mallard ducks dosed with either one number four commercial lead shot or one number four lead-iron shot.@

Treatment	N	Lead (ppm)	
		Contents	Shell
Lead Shot	20	0.50 ± 0.06^{a} (0.20-1.10)	2.80 ± 0.25^{a} (0.90-5.30)
Lead-Iron Shot	20	$0.30 \pm 0.10^{b*}$ (0.20-1.2)	1.42 ± 0.26^{b} (0.50-4.70)
Controls [d/]	10	$<0.20^{b}$	0.69 ± 0.12^{c} (0.50-1.30)

@ Means ± S. E.; Range in parenthesis; Means within a column followed by different letters are significantly different, $P<0.05$. Student's t test.

* Only 10 egg contents analyzed.

[d/] Control eggs were randomly selected from eggs laid by all hens before shot dosage.

SUMMARY

Mallard ducks (Anas platyrhynchos) were sacrificed one month after ingesting one number 4 all-lead shot or one number 4 lead-iron shot. Livers, kidneys, blood, wingbones, and eggs were analyzed for lead by atomic absorption.

Necropsy of sacrificed ducks failed to reveal any of the tissue lesions usually associated with lead poisoning in waterfowl. Lead levels in ducks given all-lead shot averaged about twice those in ducks given lead-iron shot, reflecting the amount of lead in the two types of shot. Lead in the blood of ducks dosed with all-lead shot averaged 0.64 ppm, and 0.28 ppm in ducks given lead-iron shot. Lead residues in livers and kidneys of females given all-lead shot were significantly higher than in males. In both dosed groups, lead levels in wingbones of females were about 10 times those in males, and were significantly correlated with the number of eggs laid after dosage. Lead levels in contents and shells of eggs laid by hens dosed with all-lead shot were about twice those in eggs laid by hens dosed with lead-iron shot. Eggshells were found to best reflect levels of lead in the blood.

Our results indicate that mallards maintained on a balanced diet and dosed with one lead shot may not accumulate extremely high lead levels in the liver and kidney. However, extremely high lead deposition may result in the bone of laying hens after ingesting sublethal amounts of lead shot as a result of mobilization of calcium from the bone during eggshell formation.

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