LEAD CONCENTRATIONS IN MOURNING DOVES COLLECTED FROM MIDDLE ATLANTIC GAME MANAGEMENT AREAS.

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Abstract: Bone and liver lead concentrations ($\mu g/gm$, d.w.) were determined by atomic absorption spectrophotometry for 412 hunter-killed mourning doves (Zenaida macroura) collected from 6 Atlantic flyway game management areas located in Maryland, Virginia, North Carolina, and South Carolina, 1977 and 1978 seasons. Juvenile bone lead concentrations ranged from 1.25-763.65 μ g/gm while adults showed levels from 1.02-322.81 μ g/gm. Significant effects in location of collection (P<0.005) and age (P < 0.005) were determined, where adults had higher bone lead concentrations than juveniles. Some juvenile birds had extremely high bone lead concentrations not found in adults. Adult liver lead concentrations ranged from 0.00-74.61 $\mu g/gm$ and from 0.00-76.45 μ g/gm in juveniles. In the 1978 season, liver concentrations were significantly (P<0.025) higher in the early dove season, perhaps from increased lead shot exposure. Neither bone nor liver analyses indicated a differential lead exposure pattern due to sex. Gizzard and crop examination of the 412 birds indicated 11 lead shot in 10 individual doves (2.4%). However, liver analyses indicated that 21 of 412 doves (5.1%) contained elevated lead concentrations that possibly resulted from lead shot exposure. For the combined years, 1977 and 1978, analyses indicated 45 of 412 (10.9%) doves to have bone lead levels > 100.00 μ g/gm, indicating an elevated pattern of lead exposure in this species.

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Lead poisoning has been well documented as a significant mortality factor in eastern and central North American waterfowl populations (Bellrose, 1959, Bagley et al., 1967). However, little is known of the extent of lead exposure in either mourning doves or upland game birds.

As hunting on managed dove fields expands, more birds will become exposed to increasing amounts of spent lead shot. To indicate the exposure potential of lead shot in public dove fields, Lewis and Legler (1968) reported that the concentration of shot per hectare in the top 0.95 cm of soil in prehunt and posthunt samples was 26,898 and 107,593, respectively. The accumulation of spent lead shot on roads of managed dove field areas also is significant in that shot is likely to remain on the surface year-round. This could expose doves to shot when vegetative growth limits their use of fields. Apparently doves are ingesting these shot because investigators in Tennessee (Lewis and Legler 1968), Maryland (Locke and Bagley 1967) and Virginia (Scanlon and Mirarchi, unpublished data) have found lead shot in the gizzards of doves. In a sample (350) of Maryland doves collected on the first 4 days of the 1976 season, Scanlon and Mirarchi (unpublished data) found that 6% of the birds had lead shot in the gizzard. Locke and Bagley (1967) reported that 6.5% of a collection of mourning doves from Maryland had lead shot in the gizzard.

A confirmed case of lead poisoning in a mourning dove was reported by Locke and Bagley (1967). The bird contained 2 lead pellets in the gizzard, was moribund and emaciated, and acid-fast intranuclear inclusions were found in kidney cells. It was suggested by the authors that this could be of a more frequent occurrence in that sickened doves would be extremely difficult to locate thus allowing the problem to go undetected. Another upland game bird, the bobwhite quail (*Colinus virginianus*), has shown similar signs of lead poisoning (Westemeier, 1966). Lead shot were detected in the gizzard and it was noted that this could be of significant consequence to upland game birds.

The exposure of doves to lead may be compounded further by their feeding on roadside grit particles. Goldsmith et al. (1976) have reported up to 126 ppm (d.w.) lead (primarily from automobile exhaust) from roadside soil samples within 6 m from the highway. Considerably higher lead levels in the top 2.5 cm of soil and vegetation were found with higher traffic densities (<1000 ppm, d.w., within 6 m of roads) (Scanlon, 1977). In studies with pigeons in Japan, Ohi et al. (1974) found significant differences in tissue lead levels in birds collected from rural versus urban areas. The data indicated that ingested grit particles, when in the proximity of heavy traffic usage, were more heavily coated with lead and resulted in increased lead intake. Furthermore, Siegfried et al. (1972) reported lead concentrations in bone tissue of laughing doves (Streptopelia senegalensis) in South Africa to be significantly different between rural and urban birds. Doves collected from a city area contained a mean concentration of 84.3 ppm (d.w., range 40-163) lead in bone tissue and rural doves [3.1 ppm (d.w., range 5.5-30.5). Bagley and Locke (1967) reported limited data on tissue lead levels of 17 southeastern bird species. It was noted that concentrations detected in dove livers ($\overline{x} = 3.3$ ppm, w.w.) were high on the list and upper level concentrations (range 0.4-7.0 ppm, w.w.) had previously been reported to be suggestive of lead toxicosis in mallard ducks (Anas platvrhynchos) (Longcore et al. 1974).

Based on the high potential for lead exposure in mourning doves, a lead monitoring project was undertaken. The extent of lead exposure in this species, presumably reflecting both lead shot and environmental lead, was investigated. This study represents an analysis of data obtained from mourning doves collected in two hunting seasons from wildlife management areas.

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Methods and Materials

Femur bone and liver tissue were collected from 412 mourning doves killed by hunters during the 1977 and 1978 hunting seasons. For comparison doves collected from September 1 - 20 of each year were considered to be from the early season and were classified as date 1. Doves collected from September 20 to the end of the hunting season in the respective states were noted to be from date 2. Collection points were as follows: James W. Webb Wildlife Center, Hampton County, S.C.; Sandhills Game Lands, Chesterfield County, S.C.; Guilford County Game Lands, Guilford County, N.C.; Sandhills Game Land, Scotland County, N.C.; Elm Hill Wildlife Management Area, Mecklenburg County, Va; and Remington Farms, Kent County, Md. Crop and gizzard contents from all birds were examined for lead shot, (gizzards penetrated by lead shot were noted) and each specimen was sexed, aged (Hanson and Kossack, 1963) and noted as to date and place of collection. Birds were classified and compared as either juveniles or adults.

Before use, all glassware was soaked (>24 hours) and washed in 15% Lakeseal Laboratory Glassware Cleaner (Peck's Products, St. Louis, Mo.) and rinsed 3 times in

distilled-deionized H_2O . Glassware was then leached in 20% HNO₃ (>24 hours) and rinsed 3 times in distilled-deionized H_2O before usage. Utmost care was taken in the laboratory to avoid contamination of glassware and samples.

Frozen tissue samples were lyophilized for 24 hours, added to tared 16 x 125 mm ignition tubes and weighed to the nearest 0.10 mg. Samples were then ashed in a muffle furnace (500°C) and the ash was brought into solution in distilled-deionized H_2O (Barnstead Demineralizer, Fisher Scientific) /HNO₃ (reagent grade, Fisher Scientific) /HC1 (reagent grade, Fisher Scientific). An automatic repipette system (20 ml) was used to maintain precision in acid delivery.

Lead concentrations were determined in each tissue using an Instrumentation Laboratories Model 351 atomic absorption spectrophotometer (flame). A standard curve was established on the instrument utilizing 6.00, 3.00, 0.50, 0.10, 0.05, and 0.00 μ g/ml lead (Pb) concentrations made from Fisher certified atomic absorption standards (Fisher Scientific). Unknown Pb samples were determined from this standard curve and the instrument was checked against a blank between each aspirated sample. Spiked samples yielded 95.26 \overline{x} % Pb recovery.

The limit of detection was 0.10 μ g/ml for lead in bone tissue. This detection limit was adequate for both the 1977 and 1978 bone lead data; therefore, the 2 years were combined for statistical analysis. For 1977 liver tissue, the limit of detection was 0.10 μ g/ml. An improved lead lamp in the spectrophotometer allowed detection limits to be increased to 0.05 μ g/ml for 1978 liver tissue. Often, liver lead levels were near the limit of detection. Since this detection limit was increased (0.05 μ g/ml) for the 1978 data, we separated the 2 years of liver data for statistical analysis. However, liver lead concentrations >0.10 μ g/ml were unaffected by the increase in sensitivity. Lead concentrations were calculated in terms of μ g/g (equivalent to ppm) dry weight of bone or liver.

Nonparametric statistical analysis of the data was accomplished with a modification (Pirie, Dept. of Statistics, VPI & SU, personal communication) of Friedman's Ranked Sum Test with unequal cell sizes (Hollander and Wolfe 1973). Tabular data of means \pm S.E. are provided; however, restricted interferences were drawn from this information.

Results

Standard ANOVA techniques were not appropriate for use with this information because the data were not normally distributed. Figs. I and 2 present plots of liver vs bone concentrations of lead in adults and juveniles, respectively. They are provided to relate the distribution of the data. Many observations are located between 1.25 and 45 μ g/gm bone lead and others are widely dispersed between 200 and 600 μ g/gm. The range was also large within the observations contributing to the nonnormal distribution of the data. Consequently, a nonparametric analog to a two-way ANOVA, Friedman's Ranked Sum test with unequal cell sizes was used for analysis. Tables I and 2 provide descriptive statistics ($\bar{x} \pm S.E.$) of the data and do not indicate the results of Friedman's nonparametric analysis, which in this case, utilizes median values for tests.

In the analysis the most important treatment effects were date, age, and location. A factorial analysis was not possible because no nonparametric tests were available for this type of analysis. Consequently, analyses were set up as a series of blocks in which the variable of interest was tested for treatment effects while the other two factors were used as the blocking factor. To reduce the complexity of the analysis, sex and a sex-age (male-adult, male-juvenile, female-adult, female-juvenile) combination were considered separately with location used as the blocking factor.

In the combined 1977 and 1978 bone lead data, differences between date 1 (early season) and date 2 (late season) as well as the year were not significant. Age affected (P<0.005) the levels of their bone lead. Table 1, although not related to Friedman's test,

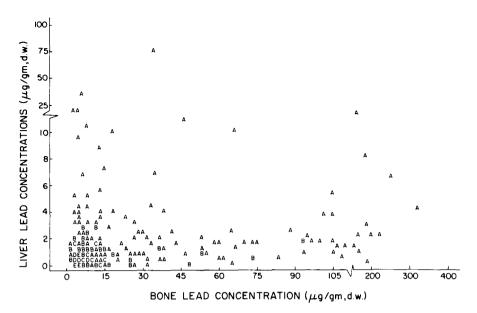


Fig. 1. A plot of liver lead concentrations versus bone lead concentrations (μg , gm, d.w.) for adult mourning doves collected from game management areas, 1977 and 1978 seasons. Legend: A = 1 observation, B = 2 observations, etc.

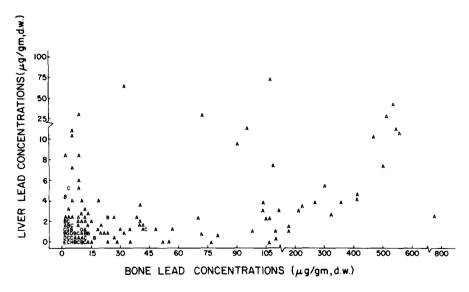


Fig. 2. A plot of liver lead concentrations versus bone lead concentrations (μg gm, d.w.) for juvenile mourning doves collected from game management areas, 1977 and 1978 seasons. Legend: A = 1 observation, B = 2 observations, etc.

TABLE 1. Bone lead levels ($\mu g/gm$, d.w.) of adult and juvenile mourning doves collected from middle Atlantic game management areas, 1977 and 1978 seasons.

	1977 Season					1978 Season				
	N	<u>Adults</u> X±S.E.	N	Juveniles X±S.E.	N	Adults X±S.E.	N	Juveniles X±S.E.		
Kent Co., MD	10	29.42±15.58	-	-	28	40.61± 8.53	40	55.13±22.67		
Meeklenburg Co., VA	15	18.97±_5.44	4	26.00±22.87	11	33.30±13.21	19	16.05± 4.07		
Guilford Co., NC	21	49.09±11.96	26	82.61±26.97	24	42.61±10.46	12	23.49±10.37		
Scotland Co., NC	31	39.76±11.63	27	34.38±13.44	36	27.76± 7.19	23	45.22±22.34		
Chesterfield Co., SC	12	23.36± 8.63	23	123.04±39.81	-	-	-	-		
Hampton Co., SC	6	16.43± 9.09	7	68.07±41.61	26	26.30±11.35	11	6.29± 2.55		

TABLE 2. Liver lead levels (µg/gm, d.w.) of adult and juvenile mourning doves collected from middle Atlantic game management areas, 1977 and 1978 seasons.

	1977 Season				1978 Season				
	N	Adults X±S.E.	N	Juveniles X±S.E.	N	Adults X±S.E.	N	Juveniles X±S.E.	
Kem Co., Md.	10	2.81±1.10	~	-	28	1.47±0.59	40	1.19±0.19	
Meeklenburg Co., VA	15	1.50±0.50	4	7.59± 4.39	H	1.38±0.29	19	1.31±0.23	
Guilford Co., NC	21	1.51±0.30	26	3.99± 1.73	24	5.77±3.18	12	1.28±0.68	
Scotland Co., NC	31	3.55±0.77	27	3.58± 1.26	36	2.05±1.00	23	3.37±1.81	
Chesterfield Co., SC	12	2.21±0.65	23	4.00± 1.00	-	-	-	-	
Hampton Co., SC	6	1.58 ± 0.57	7	12.53±10.67	26	1.95±0.69	11	7.87±5.58	

does appear to show a trend of differences in bone lead levels between adults and juveniles. However, mean values are misleading in that Fig. 2 shows outlying data points which enlarge the mean values. This further substantiates the use of a nonparametric test which is testing median values instead of means. Indeed, these data points (Fig. 2) were important in relating the detected high levels of bone lead in some individuals; however, the Friedman's Ranked Sum test relates trends in the data using ranked values. The results of the Friedman's Ranked Sum test demonstrated that adult birds had higher bone lead concentrations than juveniles.

Bone lead was affected (P<0.005) by location. It was not possible to determine which areas were significantly different as there are no separation techniques in nonparametric analysis available for such test (e.g., Duncan's Multiple Range Procedure). However, the corrected ranked sums (CRS) for bone lead used in Friedman's test do provide useful information. The Guilford Co., N.C. area CRS was larger than any of the other collection points (CRS = Kent Co., Md. = 19, Mecklenburg Co., Va. = -190.5, Chesterfield Co., S.C. = -182.5, Hampton Co., S.C. = -1045.0, Guilford Co., N.C. = 1167.0, Scotland Co., N.C. = 232.0). The mean values in Table 1 also illustrated that this area had birds with higher lead levels, but they must be considered while understanding that means can be misleading due to the range in the data. Bone lead was not determined to be influenced by the sex of the individual doves.

The 1977 liver lead data were not significantly affected by date, age and location of collection. Table 2 illustrated potential differences between adults and juveniles, but, disregarding mean values, the CRS revealed no consistent differences (CRS: adult = -

40.5, juvenile = 40.5). There were no differences attributed to sex in either the 1977 or 1978 liver lead data.

The 1978 liver lead data revealed no differences as related to age and location. However, date of collection was significantly different ($P \le 0.025$). Liver lead levels, as indicated by the CRS, showed higher levels in the early season (CRS: date 1 = 114.5, date 2 = -114.5).

Gizzard and crop examination indicated 11 lead shot were detected in 10 individuals of the 412 birds examined. Ten doves (2.4%) of those examined contained a detectable shot; however, liver lead levels showed that 21 of 412 (5.1%) had concentrations greater than 10 μ g/gm, d.w., that being a level which has been determined to be indicative of lead exposure possibly resulting from lead shot ingestion (Kendall, unpublished data).

Of interest was the relatively high degree of lead exposure that these birds have in the environment. Figs. 1 and 2 indicate that 45 of 412 (10.9%) of the birds had bone lead levels $>100 \ \mu g/gm$, a level which is associated with an acute dosage of lead or chronic exposure.

Discussion

Our data confirm earlier observations (Bagley and Locke, 1967) of the relatively high lead exposure in mourning doves. These birds are exposed to spent lead shot in dove fields as well as other forms of environmental lead.

Although no differences were detected in liver lead concentrations in the 1977 birds, the 1978 doves did show an effect due to the date of collection in the hunting season. Birds collected in the early season possibly had a greater exposure potential presumably from lead shot because these lead concentrations in the liver as corroborated by laboratory studies (Kendall, unpublished data) could have resulted from chronic lowlevel environmental lead but more probably from lead shot exposure. Lewis and Legler (1968) reported an increase of lead shot in dove fields during the hunting season. Possibly, more shot are available in the earlier phase of the dove season in that most hunting occurs at this time. The shot, not having been on the ground long enough to be deposited under a layer of soil, may be more accessible to mourning doves.

Furthermore, Lewis and Legler (1968) reported that only 1% of the doves had ingested 1 - 24 lead shot from a Tennessee dove field. Possibly the determination of lead shot exposure by counting shot in the crop or gizzard of mourning doves is not adequate. Experience has revealed that partially eroded lead pellets are difficult to detect even in the laboratory. Also, studies in our laboratory have demonstrated the rapid erosion (Kendall, unpublished data) patterns of lead pellets in doves. This decreases the odds of detecting shot in a dove gizzard. We detected only 11 shot in 10 of the 412 doves examined. Liver analyses indicated that >5% were potentially exposed to lead pellets.

Although there is the possibility that penetration of lead shot through soft tissue, such as the liver, could produce artifacts in some individual birds, studies in our laboratory have revealed that this is probably the exception rather than the rule. Ringed turtle doves (*Streptopelia risoria*) from our captive colony have been shot (penetrating the liver) in flight with lead pellets and have not indicated elevated liver concentrations but a trend towards background levels. However, bone lead concentrations can be greatly increased at the point of impact with lead shot. For this reason, we were particularly careful in selecting femurs for analysis which had not been hit by a pellet (Kendall, unpublished data). In light of the data that we have to date, birds collected by shooting can provide a useful source of information for lead analyses. However, it should be realized that the most ideal situation would have probably been live-trapping and sacrificing by compression but hunter-killed animals provide a valuable alternative. Highway lead studies (Goldsmith et al. 1976) have indicated high concentrations in roadside areas. These birds, in frequenting the road for grit particles, can become exposed to lead in some places. Siegfriend et al. (1972) reported lead concentrations in the bones of South African city and country laughing doves to be much higher in the urban area (40.0-163.0 μ g/gm bone lead, d.w.). In other words, the high lead levels in the South African environment were reflected by this species. Furthermore, Tansey and Roth (1970) demonstrated that city pigeons had bone lead concentrations often greater than 100 μ g/gm, reflecting a high level of lead exposure. Bagley and Locke (1967) reported bone lead concentrations in 13 species of birds (mostly waterfowl). Of the 82 reported bone lead analyses, the highest level reported was 26.0 μ g/gm, (w.w.) but most levels were well below this concentration.

Our data indicate that bone lead varied with location. Doves from Guilford County, N.C. were associated with the Interstate 85 highway. Other areas were generally more rural than Guilford County. Probably these birds reflected an increased amount of environmental lead in this area.

We have as well performed lead analyses on hunter-killed ruffed grouse (*Bonasa umbellus*) collected from southwest Virginia. These data indicated low tarsometatarsus bone lead concentrations ($\overline{x}\pm S.E. = 2.78\pm 0.57$, N = 16) as compared to mourning doves (Kendall, unpublished data).

Lead has an affinity for bone tissue; therefore, chronically exposed adults should demonstrate higher concentrations than juveniles (Vallee and Ulmer, 1972). Our statistical analysis revealed that adult doves had higher bone lead concentrations than juveniles. However, the juveniles tended to have a greater number of high outlying values which were reflected in enlarged means. In adult birds, these high values were consistently of a lower magnitude suggesting that juveniles with elevated bone lead concentrations could have been lost to the population before becoming adults. Finley and Dieter (1978) have reported that high lead concentrations in labile medullary bone would be subject to mobilization during bone metabolism (e.g., egg-laying). Their study corroborates our findings relating that the significance of high skeletal lead concentrations is poorly understood in birds.

We found no differences between male and female liver or femur bone lead concentrations. However, laboratory studies (Finley and Dieter, 1978) have indicated that when each sex of mallards is exposed to 1 No. 4 lead shot during the egg-laying cycle, hens will deposit significantly more lead in bones than drakes. Perhaps we did not detect such differences due to the chronic exposure patterns of lead in doves as well as acute exposure due to lead shot ingestion. Furthermore, our results agreed with data obtained from a national survey of lead concentrations in mallard wingbones which also indicated no sex differences (White and Stendell, 1977).

Our results indicate that the mourning dove, a major game species, has a high degree of exposure to lead in the environment, apparently from lead shot and environmental lead. Further work is needed to characterize the patterns of lead exposure and to determine its biological significance.

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