

## Lead Concentrations in Livers of Maryland Waterfowl with and without Ingested Lead Shot Present in Gizzards

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The significance of lead poisoning in waterfowl caused by ingestion of spent lead shot has long been recognized (WETMORE, 1919) as has the adverse impact on waterfowl populations (BELLROSE, 1959). The majority of observations on lead shot ingestion by waterfowl have been with dabbling duck species. Much less work has been done on diving ducks and geese although the problem of lead toxicity in these species has been studied by such as DIETER et al. (1976) and DIETER (1979) working with canvasback ducks (Aythya valisineria), DANELL et al. (1977) working with pochards (Aythya ferina), tufted ducks (Aythya fuligula) and golden-eye (Bucephala clangula), and COOK & TRAINER (1966) working with Canada geese (Branta canadensis).

Techniques are available for monitoring the presence of ingested lead shot in gizzards of waterfowl such as direct examination of dead birds and fluoroscopic methods for live or dead birds. These are somewhat limited insofar as ingested lead pellets may be eroded relatively rapidly and/or excreted leaving no direct evidence of recent acute lead ingestion regardless of presence of ingested lead shot.

The present report is concerned with comparison of liver lead concentrations in 14 species of waterfowl with and without ingested lead shot present in their gizzards.

### MATERIALS AND METHODS

Waterfowl gizzards of 14 species, details of which are given in Table 1, shot by and collected from Maryland hunters in the counties of Dorchester, Kent, Queen Anne's and Talbot were examined by means of a fluoroscope using procedures similar to those described by PERRY & ARTMANN (1979) for the presence of ingested lead shot. Those with evidence of ingested lead shot had their gizzards

dissected to check for entrance and exit wounds caused by shot and to determine presence and type of ingested shot. Samples of livers were recovered with the corresponding gizzards, placed in individually marked plastic bags and frozen.

Liver specimens were subsampled using stainless steel instruments working on a stainless steel surface. Tissues were placed in acid-washed glass vials for lyophilization which was conducted over 24 h at -50C and 50-100  $\mu$ torr. The dried tissues were stored in dessicators.

The glass vials used for lyophilization were subjected to the following cleansing procedures which were followed for all glassware used in heavy metal determinations; a) soaked in a 5% solution of laboratory soap (Peck's Analytical and Research Laboratory Glass Cleanser, Peck's Products, St. Louis, MO) and deionized water (Barnstead NANO pure 4 system); b) rinsed 10 times with deionized water; c) placed in a 20%  $\text{HNO}_3$  (Fisher Reagent Grade) bath for 24 h; d) placed in a 5%  $\text{HNO}_3$  for 24 h; e) rinsed 6 times with deionized water. Clean glassware was stored, capped or inverted, in aluminum lined containers. Clean glassware was placed in a 5% reagent grade  $\text{HNO}_3$  bath for 24 h and rinsed 10 times with deionized water prior to use.

Lyophilized liver was weighed to the nearest 0.1 mg into preweighed ignition tubes which had been subjected to the cleansing procedures described above. Tissues were ashed in a muffle furnace (Sybron/Thermolyne Furnatrol II) at 525C (i.e. below the volatilization point of lead). Ash was digested in a  $\text{HNO}_3$ :HCl: deionized water (1:1:2) mixture. Acids used were reagent grade (Fisher Scientific). Appropriate dilutions were made subsequently.

The following precision and accuracy procedures were routinely conducted during heavy metal analyses: a) standards were run every 20 samples; b) spiked samples were assayed to determine percent recovery and machine accuracy (recovery averaged 97%); c) deionized water and acids used were routinely checked for heavy metal contamination; d) standard additions were run to correct for possible matrix interferences; e) blank tubes were routinely run through the analytical procedure as a check on possible contamination; f) bovine liver (Standard Reference Material 1577) obtained from the National Bureau of Standards (Washington, D. C.) was used to test for precision;

g) water pollution quality control samples for trace metal analyses (Environmental Monitoring and Support Laboratory, Environmental Protection Agency, Cincinnati, OH) were used to verify analytical procedures.

Data on Canada geese, mallards (Anas platyrhynchos), and black ducks (Anas rubripes), all of which had substantial numbers of individuals with and without ingested lead shot, were analysed by the Wilcoxon rank sum test (CONOVER, 1971).

## RESULTS AND DISCUSSION

Data are presented in Table 1 showing mean concentrations of lead in all species studied. Sufficient data were available for 3 species (Canada goose, mallard, black duck) to allow statistical comparisons between those with ingested lead pellets in gizzards and those without evidence of ingested lead pellets. In all three species highly significant ( $P < 0.0001$  or  $P < 0.0005$ ) differences in lead concentrations between the two categories were apparent. Ingested lead shot were found in gizzards in only 4 individuals of 4 other species (pintail, Anus acuta; shoveler, Spatula clypeata; greater scaup, Aythya marila; bufflehead, Bucephala albeola). Mean concentration ( $\pm$ S.E.) of lead in livers of waterfowl without ingested lead shot present in gizzards varied from  $5.0 \pm 0.5$   $\mu\text{g/g}$  for Canada geese to  $45.9 \pm 40.4$  for pintails.

High concentrations of lead in the liver are diagnostic of recent acute exposure to lead such as ingestion of spent lead shot. FRIEND (1979) considers that concentrations in blood in excess of  $8 \mu\text{g/g}$  are clearly diagnostic of lead poisoning. DIETER (1979) considers that the ratio of blood lead to liver approximates 1:1 and reported that lead concentrations as low as  $0.2 \mu\text{g/g}$  in blood caused substantial, sublethal, harmful effects. Neither worker indicated whether the concentrations were on a wet-weight or dry-weight basis.

Many individuals in the present report which did not have ingested lead shot present had liver lead concentrations in excess of  $10 \mu\text{g/g}$  (d.w.). In fact, the mean liver lead concentrations of individuals of 4 species (black duck; pintail; shoveler; and lesser scaup, Aythya affinis) without ingested lead shot exceeded  $10 \mu\text{g/g}$  (d.w.) but in all cases the standard errors were high.

Table 1. Mean ( $\pm$ S.E.) lead concentrations ( $\mu\text{g/g}$ , d.w.) in livers of waterfowl shot by Maryland hunters (Data for all species are categorized by positive or negative readings for lead shot with fluoroscopy and direct examination of gizzards).

Species	Positive		Negative	
	N	Mean $\pm$ S.E. ( $\mu\text{g/g}$ d.w.)	N	Mean $\pm$ S.E. ( $\mu\text{g/g}$ d.w.)
Canada Goose <i>Branta canadensis</i>	71	32.1 $\pm$ 9.0 <sup>a</sup>	90	5.0 $\pm$ 0.5
Mallard <i>Anas platyrhynchos</i>	27	43.2 $\pm$ 12.8 <sup>a</sup>	117	8.0 $\pm$ 1.6
Black Duck <i>Anas rubripes</i>	23	36.8 $\pm$ 13.5 <sup>b</sup>	82	11.4 $\pm$ 4.7
Pintail <i>Anas acuta</i>	1	1.4	9 <sup>c</sup>	45.9 $\pm$ 40.4
Gadwall <i>Anas strepera</i>			17	9.1 $\pm$ 1.9
Am. Widgeon <i>Mareca americana</i>			16	6.0 $\pm$ 2.1
Shoveler <i>Spatula clypeata</i>	1	10.8	3	19.4 $\pm$ 17.4
Green-Winged Teal <i>Anas carolinensis</i>			17	5.7 $\pm$ 1.5
Wood Duck <i>Aix sponsa</i>			14	8.7 $\pm$ 4.6
Greater Scaup <i>Aythya marila</i>	1	1.5		
Lesser Scaup <i>Aythya affinis</i>			9	15.5 $\pm$ 6.4
Bufflehead <i>Bucephala albeola</i>	1	10.7	30	5.4 $\pm$ 1.4
Oldsquaw <i>Clangula hyemalis</i>			31	6.4 $\pm$ 2.0
White-Winged Scoter <i>Melanitta deglandi</i>			53	5.7 $\pm$ 1.0

<sup>a</sup>Mean value of lead shot positive significantly ( $P < 0.0001$ ) greater than lead shot negative in species.

<sup>b</sup>Mean value of lead shot positive significantly ( $P < 0.0005$ ) greater than lead shot negative in species.

<sup>c</sup>Mean included one value of 369  $\mu\text{g/g}$  (d.w.). Without this value mean was 5.47 $\pm$ 1.68 (S.E.)

Table 2. Proportion of waterfowl with liver lead concentrations in excess of 10  $\mu\text{g/g}$  d.w. in relation to presence of ingested lead shot.

Species	Shot present in gizzard		No shot in gizzard	
	<10 $\mu\text{g/g}$ d.w. lead in liver	$\geq$ 10 $\mu\text{g/g}$ d.w. lead in liver	<10 $\mu\text{g/g}$ d.w. lead in liver	$\geq$ 10 $\mu\text{g/g}$ d.w. lead in liver
Canada Goose	65	6	82	8
Mallard	11	16	93	24
Black Duck	11	12	74	8
Pintail	1	0	6	3
Gadwall	0	0	10	7
Am. Widgeon	0	0	14	2
Shoveler	0	1	2	1
Green-winged Teal	0	0	13	4
Wood Duck	0	0	12	2
Greater Scaup	1	0	0	0
Lesser Scaup	0	0	6	3
Bufflehead	0	1	27	3
Oldsquaw	0	0	25	6
White-winged Scoter	0	0	45	8
Totals	89	36	409	79
%		(28.8%)		(16.2%)

Data in Table 2 indicate the number of individuals with liver lead concentrations in excess of 10  $\mu\text{g/g}$ . These individuals provide presumptive evidence of lead shot ingestion in the recent past even though lead shot was no longer present in the gizzards. The liver lead concentration exceeded 10  $\mu\text{g/g}$  (d.w.) in 28.8% of individuals with ingested lead shot. While there appears to be a substantially greater frequency of high liver lead concentrations among those with ingested lead shot present it should be considered that those birds with ingested lead shot present did not yet have maximum opportunity to metabolize the lead shot present in gizzards and so both overall concentrations and proportion of individuals with high concentrations may be underestimated. The species with the lowest mean lead concentration was white-winged scoter (*Melanitta deglandi*). None of the 53 specimens had ingested lead shot. In fact a

series of 272 individuals of this species from Maryland waters has been examined without finding ingested lead shot (STOTTS, 1979). The liver lead concentrations in this species may represent background concentrations for a species whose diet is composed of invertebrates.

The data presented in this report indicate that higher concentrations of lead are found in those waterfowl with ingested lead shot and that analysis of liver lead concentrations reveals that substantial numbers of waterfowl have high concentrations, indicative of recent acute dosing, without presence of ingested lead shot in gizzards.

#### ACKNOWLEDGEMENTS

This work was initiated under and partially supported by Federal Aid in Wildlife Restoration funds (Maryland Pittman-Robertson Project W-45-R). Special thanks go to J. E. Schouber, Jr. for his efforts in collecting and examining specimens.

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