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THE TOXICITY FOR DUCKS OF DISINTEGRATED LEAD SHOT IN A SIMULATED-MARSH ENVIRONMENT

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Abstract: Adult mallard drakes were exposed for approximately 14 weeks to distributions of 17.8, 89.0, and 178 g of particulate lead per m^2 in simulted-marsh areas. The ante-mortem and post-mortem findings indicated that exposures of 17.8 g of particulate lead per m^2 had a low toxicity for the experimental birds. The birds exposed to 89.0 g/m^2 experienced a 57% mortality within an average of 72.5 days; all had positive fluorescent erythrocyte tests; and post-mortem examinations showed evidence of chronic lead toxicosis. The birds exposed to 178 g/m^2 showed overt signs of lead poisoning; they experienced 100% mortality within an average of 22.5 days; and post-mortem examinations showed evidence of subacute lead toxicosis. It was concluded that distributions of disintegrated lead shot (i.e. particulate lead) in waterfowl wetlands could probably exceed existing distributions of commercial lead shot in heavily hunted areas by ten fold without presenting a significant toxicity to waterfowl.

INTRODUCTION

The lead poisoning problem in waterfowl has been recognized since the late 1800's[12] and numerous die-offs from this disease have occurred.[2,7] Much effort has been spent in attempts to find a non-toxic shot for waterfowl hunting.[2,10,11,13,14] To date, the most suitable substitute found has been soft iron shot, however, technical problems seemingly still prevent it from being put into production. In recent years the Colloid Chemistry Section of the National Research Council of Canada, Division of Applied Chemistry, has developed a process of spherical agglomeration which provides a method for making rapid and efficient separations of solids from liquid suspensions. The spherical agglomeration technique can be used to make acceptable lead spheres from particulate lead using various water sensitive adhesives. The fabrication of spheres of lead combined with iron or other metals is also possible. The range of possibilities offered by this technique suggested that a suitable disintegrating lead shot might be developed for waterfowl hunting. In anticipation of the development of disintegrating lead shot by this technique, this study was undertaken to test the toxicity of disintegrating lead shot (i.e. particulate lead) for ducks in a simulated-marsh environment.

MATERIALS AND METHODS

Only adult mallard drakes *(Anas platyrhnchos)* were used in this experiment. All birds were penned individually, provided with a simulated-marsh puddling area and given whole corn *ad libutum*. A diet of half corn and half commercial duck pellet was given for a 2 week period midway through the experiment when the supply of corn was low. Groups of six birds were exposed to particulate (325 mesh) lead in the simulated-marsh areas in distributions equivalent in weight to 0, 10, 50 and 100 No. 5 lead shot per square foot for approximately 14 weeks. Prior to exposure, all birds were radiographed to ensure that they were not carrying ingested lead shot.

The simulated-marsh areas consisted of rectangular plastic trays, 57.8 x 70.5 x 23.5 cm deep, containing a 6 cm layer of marsh soil and a 15 cm water overlay. The marsh soil was obtained from the site of a drawn-down marsh near Guelph. Only the upper humic stratum was used

in the experiment. The contents of the experimental trays were renewed every 2 weeks.

Weights and feed consumptions were recorded at 14 day intervals. Blood samples were also taken at 14 day intervals for fluorescent erythrocyte tests.[4] Prior to necropsy, euthanasia was performed by intracardial air injection.

At necropsy, kidney tissues were fixed in buffered formalin, embedded in paraffin, sectioned and stained by the Ziehl-Neelsen acid-fast method.[1] Samples of liver and pectoralis muscle and the complete tibiotarsal bone including the fibula were taken for lead analysis. Dry, fat-free samples of bone were obtained by heating the samples in an oven at 250 C for 5 days. The samples of liver, muscle and bone were ashed in a low temperature Plasma Asher.* The lead residue in liver and muscle samples was then collected with nitric acid and the lead content determined by Atomic Absorption Spectrohphotometry (A.A.) After ashing, the lead content of bone was determined by a modification of the method of Smith et al.[15] for A.A.

RESULTS

The feed consumptions of the lead treated groups were not significantly different ($P < .05$) from those of controls over the experimental period.

The high treatment group had lost significantly more weight on day 15 than the controls but the weights of other lead treated groups were not significantly different from those of the controls.

Fluorescence of erythrocytes was not found in the blood samples from any of the birds prior to exposure to the particulate lead nor in the blood samples from any of the control birds over the duration of the experiment (Table 1). Fluorescence of erythrocytes was found in all birds in the high treatment group on day 15 and on subsequent testing days, but fluorescence was not as pronounced at the intermediate treatment level, except on day 29. The fluorescence of erythrocytes was seen only in a small percentage of birds in the low treatment group and then only on days 15 and 43.

Overt signs of lead poisoning were not observed in the birds in the low or intermediate treatment groups nor in the controls, but signs of poisoning were seen in the birds of the high treatment group, usually within 15 days of exposure. These signs included green diarrhea and green stained vent, "wing - drop", anorexia, weight loss and lethargy and finally extreme weakness in the legs as depicted by the birds continuously resting on their sternums and having great difficulty when forced to move. Convulsions were noted in two birds for a few hours prior to death.

One hundred percent mortality occurred in the birds in the high treatment group within an average of 22.5 days, while four out of seven birds (57%) in the intermediate and one of the six birds (17%) in the low treatment groups died within average periods of 72.5 and 94.0 days, respectively (Table 2).

Gross lesions of lead poisoning were not observed at necropsy in the controls

TABLE 1. Percentage of mallard drakes exposed to particulate lead which had positive fluorescent erythrocytes.

Lead Treatment (g/m^2)	Day 0	15	29	43	57	71	85
0	0	0	0	0	0	0	0
17.8	0	33	0	17	0	0	0
89.0	0	67	100	84	17	0	50
178	0	100	100	100	—	—	—

* Manufactured by Trapelo Division of LFE Corporation, Walton, Mass.

nor in the birds in the low treatment group (Table 2). Gross lesions were more prevalent in the high treatment group than in the intermediate group, except for gizzard lining erosion. Distended gall bladders and hydropericardiums were not noted in the intermediate group. The lesions observed in this group were mainly found in the birds which died during the course of the experiment.

Although acid-fast intranuclear inclusion bodies were not found in the renal tubules of the kidneys of birds in the low treatment group or in the controls, these inclusion bodies were common in the kidneys of the birds in the intermediate and high treatment groups (Table 2).

The lead content in tissues of the experimental birds is given in Table 3. Lead concentrations in liver tissues increased significantly at each treatment level ($P < .05$). Amounts of lead in muscle tissue were not significantly different between the two lower treatment groups and the controls but the birds in the high treatment group had significantly higher concentrations in muscle than the other groups ($P < .05$). There was no significant difference in the lead content of the bone between the intermediate and high treatment groups nor the lower treatment group and the controls but there was a significant difference between the two lower and two higher treatment groups ($P < .05$). The liver and bone lead concentrations of the bird which died in the low treatment group were 1.6 ppm, wet weight and 18 ppm, dry fat-free weight, respectively.

TABLE 2. Percent occurrence of various lesions in mallard drakes exposed to particulate lead.

Lesions	Lead Treatment (g/m^2) 0	17.8	89.0	178
Emaciation	0	0	29	80
Proventricular impaction	0	0	29	40
Gizzard lining stained green	0	0	14	30
Gizzard lining eroded	0	0	29	10
Atrophied liver	0	0	29	60
Distended gall bladder	0	0	0	50
Hydropericardium	0	0	0	60
Acid-fast intranuclear inclusion in kidney tubules	0	0	57	88

TABLE 3. Mean lead concentrations in tissues of mallard drakes exposed to particulate lead.

Tissue	Lead Treatment (g/m^2) 0	17.8	89.0	178
Liver[a]	0.9 ± 0.19 (6)[c,d]	3.6 ± 0.59 (6)	14.6 ± 3.03 (6)	28.4 ± 3.50 (8)
Muscle[a]	0.6 ± 0.22 (6)	0.4 ± 0.09 (6)	0.7 ± 0.23 (5)	1.9 ± 0.22 (6)
Bone[b]	24 ± 9.2 (5)	38 ± 7.6 (6)	119 ± 34.1 (6)	176 ± 53.7 (6)

a Lead concentrations expressed as parts per million—wet weight
b Lead concentrations expressed as parts per million—dry, fat-free weight
c Sample size
d Lines group data which are not significantly different ($P < .05$)

DISCUSSION

In order to compare the toxicity of particulate lead in simulated-marsh areas with the toxicity of commercial lead shot which is found in natural marshes, the particulate lead treatments per unit area were chosen to correspond to equivalent weights of No. 5 commercial lead shot per square foot, i.e. the treatments of particulate lead of 17.8, 89.0 and 178 g/m^2 were equivalent in weight to 10, 50, and 100 No. 5 shot per square foot. Bellrose[7] listed 2.71 pellets per square foot as the maximum amount of shot distributed in the bottom sediments of extensively hunted North American marshes. Approximately one pellet per square foot is probably a realistic average (based on areas for which there were one hundred or more bottom sampels taken.[7])

Although the toxicities of the low and intermediate lead exposures were not severe enough to depress feed consumption or body weight, a small percentage of the birds in the low treatment group absorbed a sufficient amount of lead to result in the fluorescence of erythrocytes, apparently as a consequence of a disturbance in heme synthesis. The fluorescence of erythrocytes can probably be attributed to an increase in free erythrocytic protoporphyrin[17] which results from the inhibiting effect of lead on the combination of iron with protoporphyrin in the final step of heme formation.[8] The consistency of positive fluorescent erythrocyte tests for the bird in the high treatment group was in agreement with the findings of Barrett and Karstad[4] for mallards experimentally poisoned with commercial lead shot.

The overt signs of lead poisoning observed in the high treatment group are comparable to those reported for mallards poisoned by lead shot.[5,15] The average of 22.5 days to death calculated for these birds was comparabel with the average of 21 days reported for mallards poisoned by lead shot.[7]

The gross lesions found in the intermediate and high treatment group were similar to those described for metallic lead poisoning by Wetmore[20] and to those described for subacute lead poisoning from soluble lead salts by Coburn et al.[9]

According to Locke et al.[16] the occurrence of acid-fast intranuclear inclusion bodies, such as were found in the birds in the itnermediate and high treatment groups, is strong presumptive evidence of lead poisoning.

The average lead concentrations in the liver of the controls, 0.9 ppm, wet weight, was the same as reported for normal mallards.[3] The lead content of the high treatment group, 28.4 ppm, is comparable with 33 ppm reported in mallards poisoned with lead shot[15] but lower than 43 ppm found in the livers of mallards subacutely poisoned by soluble lead salts.[9]

The lead content of bone tissue of the controls, 24 ppm dry, fat-free weight, was lower than the 67.7 ppm dry weight reported by Coburn et al[9] and the lead content of bone in the group exposed to the highest lead treatment, 176 $\pm$ 53.7 (6) ppm, was also lower than 469 ppm found in mallards dosed with soluble lead.[9] These differences may have partially resulted from the loss of lead from the soft bone tissue when the fat was extracted by heat. Extracting the soft bone tissue may allow more accurate measure of the lead stored in the mineral portion. Blaxter[6] found that increases in the lead content of whole bone was largely accounted for by the high lead content of marrow. Lead has been found to be rapidly deposited in and mobilized relatively easily from the soft bone tissue while it is firmly bound in the mineral portion.[10]

Although the liver lead concentrations of the lower treatment group were significantly greater than those of the controls, the relatively normal lead concentrations in the bone probably indicate that the rate of lead absorption did not greatly exceed the rate of lead excretion.

The liver concentration of the bird which died in the low treatment group (1.6 ppm) was within the range reported for normal mallards (0.3 - 2.0).[8] In addition, the lead content of the bone of this bird (18 ppm) was lower than the average lead content of bone in the control group (24 ppm). In view of these findings it is doubtful that this bird died from lead poisoning.

Thus the ante-mortem and post-mortem

findings of this experiment indicated that exposures of 17.8 g of particulate lead per m^2 had a very low toxicity for adult mallard drakes, exposures of 89.0 g/m^2 resulted in chronic lead toxicosis, and exposures of 178 g/m^2 resulted in subacute lead toxicosis.

On the basis of this experiment, distributions of disintegrated lead shot (i.e. particulate lead) in waterfowl wetlands could probably exceed existing distributions of commercial lead shot in heavily hunted areas by ten fold without presenting a significant toxicity to waterfowl.

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