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LEAD TOXICITY IN CAPTIVE AND WILD MALLARDS (*ANAS PLATYRHYNCHOS*) IN SPAIN

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ABSTRACT: Forty captive Mallards (*Anas platyrhynchos*), of both sexes, were separated into five groups and dosed with lead shot via oral intubation; one group was used as a control. Lead dosage differed in terms of shot number and size, as well as administration time. One hundred and thirty-five wild mallards were trapped between 1998 and 2001 in the Boada and Nava lagoons near the Canal de Castilla, in the Spanish province of Palencia. Radiologic techniques (ventrodorsal and lateral views) were used to detect lead shot in the gizzard and to determine degradation in dosed birds over time. Heparinized blood samples were taken from wild and captive mallards and blood lead levels were determined using anodic stripping voltammetry with a dropping mercury electrode. Clinical signs, injuries, and body weight were recorded. In approximately 90% of the experimentally dosed mallards, administered shot stayed in their gizzard until it degraded; this took approximately 30 days. Peak lead levels in blood were observed between days 10 and 20, and 10 days following a repeat dosage; males were more sensitive than females to a repeat dosage. During the experimental phase, 34% of birds died, and those that survived had varying degrees of anorexia, lethargy, and a decreased response to external stimulus. Of 135 tested wild mallards, 41% had a blood lead concentration higher than 0.200 μg/g. Lead shot was found embedded in 3.6% of the wild birds and 1.2% had a lead shot pellet in their gizzard.

Key words: *Anas platyrhynchos*, lead poisoning, lead shot, Mallard.

INTRODUCTION

Lead shot ingestion is the main cause of heavy metal poisoning in waterfowl, and lead has been recognized as a significant cause of death in wild birds for more than 100 yr (Mateo et al., 1998). In some regions of Spain, lead poisoning was considered the second most common cause of mortality (20% of deaths) in waterfowl species including Greylag Goose (*Anser anser*), Northern Shoveler (*Anas clypeata*), Mallard (*Anas platyrhynchos*), Northern Pintail (*Anas acuta*) and others; shooting-related injuries were the number one cause of mortality (>40%; Mateo et al., 1998). Lead intoxication occurs after accidental ingestion of lead shot dispersed by hunters (Mateo et al., 1997) and blood lead levels above 0.200 μg/g indicate a high acute lead exposure (Havera et al., 1992; Mateo et al., 2001). It is most likely that birds confuse shot with small stones, which are routinely ingested to form gastrolites in the gizzard (Pain, 1992), and high lead shot densities in sediment have been reported in numerous Mediterranean wetlands (Mateo et al., 1998, 2007). Soil in these wetland areas lack particles greater than 0.5 mm in diameter, which birds require as grit; this may explain the high levels of shot ingestion by wintering waterfowl (Pain, 1992). Among all waterfowl species, ducks (*Anas* spp.) are the most affected (Pain, 1992; Mateo et al., 1998; Ochiai et al., 1999).

Given the increasing number of birds killed every year because of lead toxicosis, lead shot was banned for use in waterfowl hunting in the USA in 1991, and led to a subsequent decline in the rate of waterfowl poisoning (Brewer et al., 2003). Lead shot also has been banned in the wetlands

of Andalusia (Spain) since 2002 (Mateo et al., 2007). Lead shot has been replaced by alternative nontoxic shot made out of steel or other materials that contain a mixture of tungsten, nickel, and iron, and these types of shot have been well accepted by hunters (Mateo et al., 1998; Brewer et al., 2003).

Waterfowl mortality following ingesting of lead shot is well documented (Sanderson and Bellrose, 1986; Mateo et al., 2007) but less information is available on the rate of lead shot degradation, blood lead levels, and the sublethal effects in birds following acute lead ingestion (Pain and Rattner, 1988; Srebocan and Rattner, 1988; Havera et al., 1992). To the authors' knowledge, there are many wetlands in Spain that are important for migratory and nonmigratory waterbirds where hunting is allowed, but there have been no studies to assess the prevalence of lead shot ingestion and lead poisoning in waterbirds from these areas.

The objectives of this study were to examine the process of degradation of lead shot in the gastrointestinal tract; to closely monitor lead concentrations in blood; to determine sublethal effects of lead exposure; and to study the prevalence of lead shot ingestion and lead poisoning in mallards from the wetlands in the vicinity of Canal de Castilla in the North of Spain.

MATERIALS AND METHODS

All the animal procedures undertaken in this study were done in compliance with the respective institutional regulations in accordance with Spanish regulations on animal use and care. Experiments comply with European Guideline 86/609/CEE, December 24, 1986, and with the Spanish Royal decree No. 233/1988 from March 14. The capture of wild animals was authorized by the governing entity Consejería de Medio Ambiente de la Junta de Castilla y León.

Captive animals

Forty adult mallards (22 males and 18 females) bred in captivity were distributed into five groups of eight birds, four males and four females, except groups I and IV, which had five males and three females. Mallards were acclimated for 1 wk under routine laboratory conditions before the experiments and were not subject to any treatment during this period. All birds were intubated orally and administered different amounts of lead (Table 1). Animals from groups II, III, and IV also were given a second dose of lead.

Animals were kept in individual cages (40 cm wide×80 cm deep×40 cm high) with a bowl for food and water. Cages were suspended 1 m above a bed of sawdust. Birds were handled only during changing of food or water supply, cage cleaning, oral intubation, blood sampling, weighing, and X-ray examination. The animals were fed a mixture of chicken feed, cereals, and some green vegetables. The food and water were provided to the birds ad libitum. Mallards were supplemented every 7–10 days with a commercial brand of vitamins and amino acids. Small stones and sand (gastrolites) were added each week to the food bowl. The experiment was carried out during the winter, from November to February, to avoid the stress related to breeding season. Cages were placed in closed rooms, with open windows, thus maintaining ambient outdoor temperatures and photoperiod.

Wild ducks

Wild mallards were trapped as described (Bub, 1991) in the Boada (60 ha) and Nava (300 ha) lagoons near the Canal de Castilla in the Spanish province of Palencia (41°50′–42°30′N, 4°11′–5°0′W; Fig. 1). Birds were captured only between August and March (outside of the breeding season) between 1998 and 2001. Birds were held captive for <1 day for blood sample collection, weighing, and clinical and radiographic examination; they were then released in the area where they had been captured. Some dead birds also were examined.

Radiographic examination

Four X-ray examinations were performed on the captive mallards dosed with No. 6 shot (groups II, III, and IV). These were done on the first day of the experiment to determine if shot had been retained or eliminated, and on days 10, 20, and 31 to document shot retention and level of degradation. The level of degradation was estimated by measuring the diameter of shot X-ray density (mean of the largest and smallest diameter, when irregular) using a magnifying glass (5×) and a ruler. Shot size was graded into three size classes based on diameter reductions: <one-third diameter reduction; one-third to two-thirds diameter reduction; and >two-thirds diameter reduc-

TABLE 1. Experimental protocol in captive Mallards (*Anas platyrhynchos*): groups, lead shot administration, X-ray examination, and blood samples.

Groups (mallards)[a]	Lead shot administration by oral intubation		X-ray examinations (days after day 0)	Blood samples (days after day 0)
	No. 4[b]	No. 6[c]		
I (5 M and 3 F)[d]	1 on day 0	—		0, 3, 10, 20, 30, 60, 90, 120, 150, 180, 210, and 240
II (4 M and 4 F)[e]	—	1 on day 0 and 1 on day 70	0, 10, 20, and 31	0, 10, 20, 30, 60, 80, 90, and 100
III (4 M and 4 F)[f]	—	2 on day 0 and 1 on day 70	0, 10, 20, and 31	0, 10, 20, 30, 60, 80, 90, and 100
IV (5 M and 3 F)[g]	—	3 on day 0 and 1 on day 70	0, 10, 20, and 31	0, 10, 20, 30, 60, 80, 90, and 100
Control (4 M and 4 F)	Only oral intubation on day 0 and 70		—	0, 10, 20, 30, 60, 80, 90, and 100

[a] M=males; F=females.

[b] Lead shot pellet with a diameter of 3.25 mm and weight of 0.2 g.

[c] Lead shot pellet with a diameter of 2.75 mm and weight of 0.13 g.

[d] Four mallards died during the experimental study.

[e] Two mallards died during the experimental study.

[f] Four mallards died during the experimental study.

[g] One mallard died during the experimental study.

tion. Ventrodorsal X-ray views of wild ducks were used to detect ingested and embedded lead shot. When shot was detected, a lateral view was also performed to enable a better anatomic localization of the shot.

Blood samples and clinical examination

Heparinized blood samples were collected from the radial vein to determine blood lead levels (Table 1). Blood samples were analyzed by anodic stripping voltammetry with a dropping mercury electrode using the PC-controlled system 757 VA Computrace and version 2.0 software (Metrohm, Herisau, Switzerland), as described (Wilson-Tabor, 1986).

Captive mallards were observed daily to assess general health and to detect clinical signs or injuries. Weight and body condition were also evaluated on days of oral intubation or when birds were sampled (Table 1). Body condition was evaluated by assessing the pectoral muscles and sternal keel; birds were

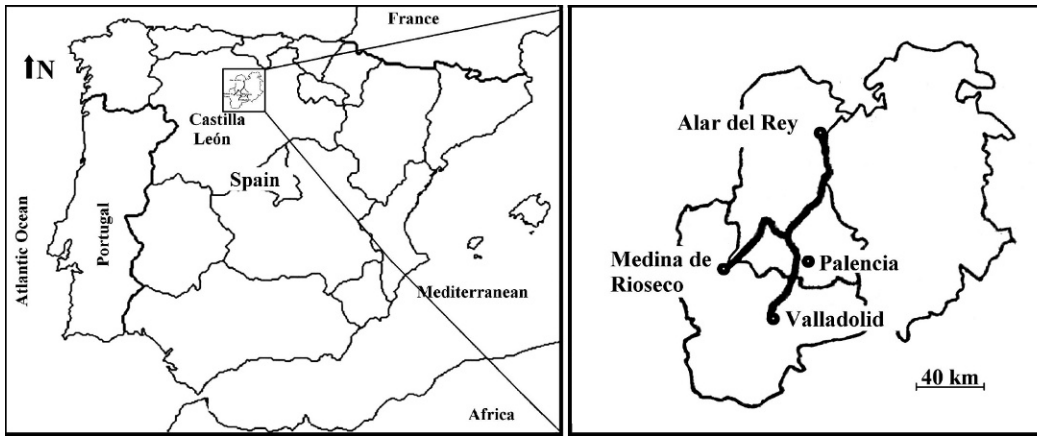

FIGURE 1. Location of the study area in Spain (left) and Canal de Castilla in the Spanish region of Castilla and León (right). (Adapted with permission from Rodríguez, 2004.)

as characterized as very thin (without palpable pectoral muscles), thin, ideal (easy palpation pectoral muscles and sternal keel), or overweight and obese (difficulty on sternal keel palpation; Honour et al., 1995; Hillier, 1997). For wild birds, general health and the presence of clinical signs were assessed at the point of capture. A complete necropsy was performed on all dead birds to detect the presence of lead shot or any lesions characteristic of lead toxicosis (i.e., signs of anemia; compression of the gizzard; hemorrhagic enteritis; bile-stained gizzard; cerebral congestion; congestion of esophagus, crop, and proventriculus; and hepatic congestion).

Data analysis

Statistical analysis was done using the SPSS 12.0 statistical package (SPSS, Chicago, Illinois, USA). Blood lead concentration by treatment and control groups was analyzed for mean differences by time period using a repeated-measures analysis of variance (two-way ANOVA). Comparisons among groups within specific time were done by one-way ANOVA. The post hoc Bonferroni test was used to test comparisons between means on the total number of pairwise comparisons. The level of significance was established at $P<0.05$.

RESULTS

Eleven (34%) ducks (eight males and three females) dosed with lead died (Table 1); mortality did not occur within the control group. In the groups subjected to a repeat lead dosage (II, III, and IV), deaths were recorded after the second lead shot administration. The pattern and severity of clinical signs prior to death were similar, regardless of the amount of lead ingested and included weight loss (91%), anorexia (73%), diarrhea (73%), abnormal positions (droop of tail and wings, abduction of one leg, and/or neck "s" lateral shape) associated with motor problems (54%), and reduced or absent reaction to external stimulus (human presence, light, and sound; 54%). At necropsy, macroscopic signs of anemia (18%); compression of the gizzard (36%); hemorrhagic enteritis (36%); bile-stained gizzard (27%); cerebral congestion (27%); congestion of esophagus, crop, and proventriculus (18%); and hepatic congestion (18%) were also observed in these animals.

In the control group, clinical signs of lead intoxication were not observed, body condition was stable or improved (being ideal or overweight at the end of the study), and birds showed an initial increase in weight, which stabilized after day 60 (Fig. 2). Birds that were dosed with lead and did not die had varying degrees of anorexia, signs of dejection, and a decreased response to external stimulus. These clinical signs decreased in intensity during the course of the study. Moderate, intermittent diarrhea was observed in 52% of the ducks, but typical green-stained diarrhea produced by lead intoxication was observed only in one mallard belonging to group III. The body condition of the ducks at the start of the experiment was considered ideal in 38 (95%) and overweight in two birds (5%). This made the deterioration observed in those groups dosed with more lead (groups I, IV), and in groups II and III after the second lead shot administration (day 70), particularly evident. The mean weight of the mallards in group I decreased slightly until day 30, increased considerably between day 30 and 60, and remained constant with little variation after this point (Fig. 2). In groups II and III (lower initial lead administration) weight was steady or increased until the second administration (day 70), then decreased rapidly over the course of 30 days (Fig. 2). Group IV (greater lead dosage) showed a weight decrease over the course of 30 days before beginning to show clear signs of recuperation (i.e., appetite, normal feces or response to external stimulus). Their weight was stable from that point on, even after the second shot administration (day 70; Fig. 2).

During the study 135 mallards (84 males, 41 females, and 10 ducklings) were captured alive and 15 mallards were found dead. Radiographic study was performed in only 83 of these animals. All live animals

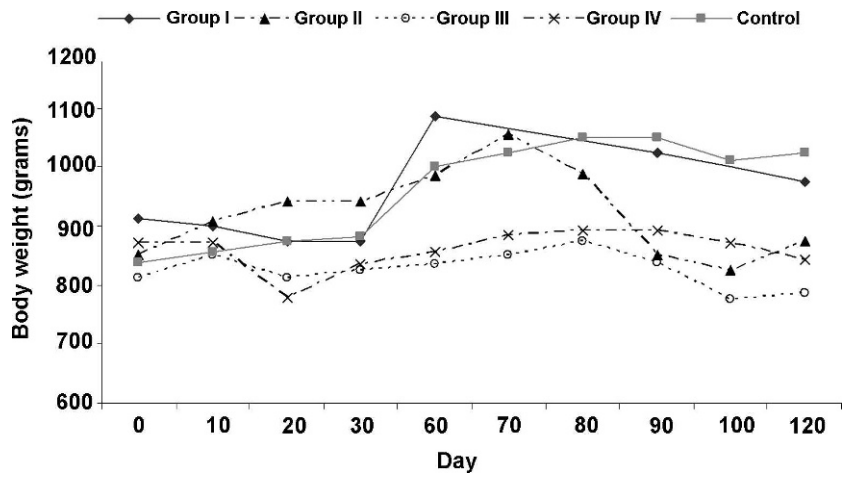

FIGURE 2. Mean body weight variation in Mallards (*Anas platyrhynchos*) intubated with varying amounts of lead shot (group I, II, III, and IV) and control group. Mallards of group I were intubated on day 0 with one No. 4 lead shot pellet, and groups II, III, and IV were intubated on day 0 with one, two, or three No. 6 lead shot pellets, respectively, and on day 70 with a repeat dosage of one No. 6 lead shot pellet. In the control group oral intubation only was performed on days 0 and 70 without lead shot.

were in a healthy condition. In dead mallards, lead shot was not detected in the gastrointestinal system of any bird, nor were there macroscopic lesions characteristic of lead poisoning.

X-ray study

At the beginning of the study, 100% of shot was found in the gizzard of experimental ducks. On day 10, 91% of shot remained in the gizzard and there was a reduction in shot size: 11% of the shot was within two thirds of initial size, 74% was between one and two thirds of initial size, and 16% was smaller than one third of initial size. At the third X-ray examination performed on day 20, all lead shot found on day 10 was still present in the gizzard. However, it was substantially worn down and less than one third of its original size. By day 31, all lead shot were virtually gone (only detected via an indistinct image in 50% of animals) from gizzards. Lead shot found in gizzards of birds that died during the study showed a pattern of erosion similar to the results obtained by X-ray.

In the wild birds, embedded lead shot was detected in three (3.6%) mallards; only one (1.2%) had lead shot in the gizzard.

Lead in blood

There were no detectable differences between mean blood lead levels in the different groups on day 0 ($P>0.05$). Blood lead levels the control group did not vary and remained below 0.200 μg/g. Blood lead concentration in group I increased rapidly (about 10 times greater than normal values) 72 hr after administering the No. 4 lead shot pellet. Values peaked around day 20 (mean 3.551 ± 0.08 μg/g) and decreased progressively until initial concentrations were reached between 120 and 150 days (Fig. 3). In group II, after administering only one No. 6 lead shot pellet, blood lead levels peaked (about 10 times greater than normal values) after 10 days and then progressively decreased. The ingestion of the second lead shot pellet of the same size caused a similar increase in blood lead concentration; however, a higher peak (about 50%) in blood lead concentration was observed (Fig. 4). Blood lead concentration in group III was similar to group II (Fig. 4). In the fourth group, after the first three shots were administered, a sudden increase in blood lead concentration was observed that peaked on day 10

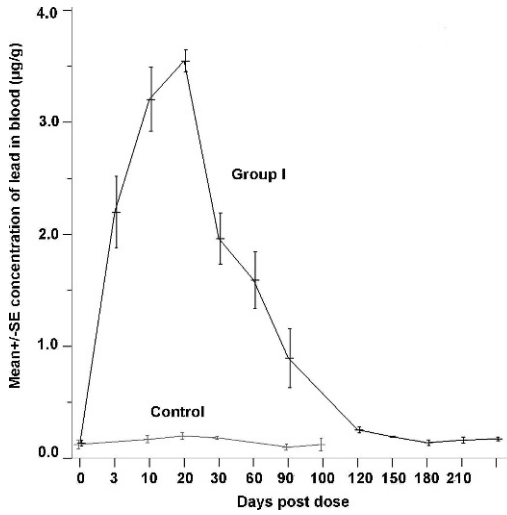

FIGURE 3. Mean±SE concentrations (µg/g) of lead in blood collected from Mallards (*Anas platyrhynchos*) in different days, intubated with one No. 4 lead shot pellet on day 0 (Group I), and control group. The control group was only intubated, without lead shot.

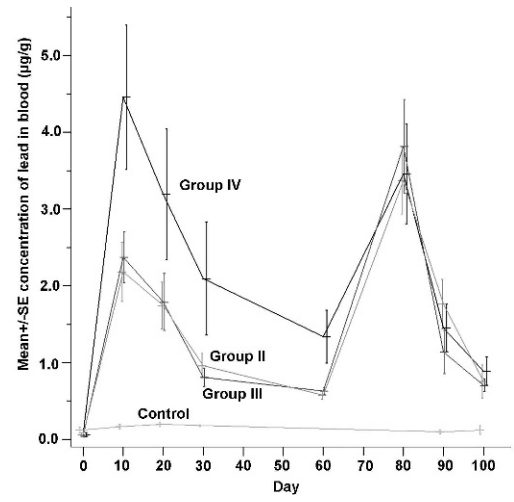

FIGURE 4. Mean±SE concentrations (µg/g) of lead in blood collected from Mallards (*Anas platyrhynchos*) intubated with varying amounts of lead shot (groups II, III, and IV) and control group. Mallards of groups II, III, and IV were intubated on day 0 with one, two or three No. 6 lead shot pellets, respectively, and on day 70 with a repeat dosage of one No. 6 lead shot pellet. The control group was only intubated on days 0 and 70, without lead shot.

(about 22 times greater than normal values). This concentration decreased, and after administration of the final shot blood lead concentration was similar to that observed in groups II and III (Fig. 4). Mean lead blood concentrations in groups II and III, which were supplied different doses of lead shot on day 0 and 70, developed similar blood lead levels over the course of the experiment ($P>0.05$). Differences in blood lead levels were apparent in group IV (Fig. 4); mean lead blood concentrations exceeded those observed in groups II and III ($P<0.05$). Significant differences between treatment groups were not observed during the second intubation on day 70 ($P>0.05$). Given that blood lead concentrations in groups II and III were similar over the course of the study, these data were pooled and used to compare lead blood levels by gender (eight males and eight females). Differences were only detected 10 days after the second lead shot administration, when males had a higher lead concentration than females ($P<0.05$; Fig. 5).

In wild mallards, mean lead concentration in blood was above 0.200 µg/g and no significant differences ($P>0.05$) were detected among males (0.201±0.161), fe-

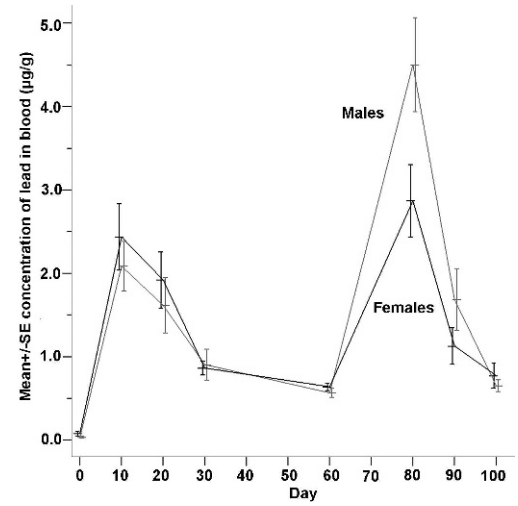

FIGURE 5. Comparison of mean±standard error (SE) concentration (µg/g) of lead in blood collected from male and female Mallards (*Anas platyrhynchos*) intubated on days 0 and 70 with varying amounts of No. 6 lead shot (groups II and III).

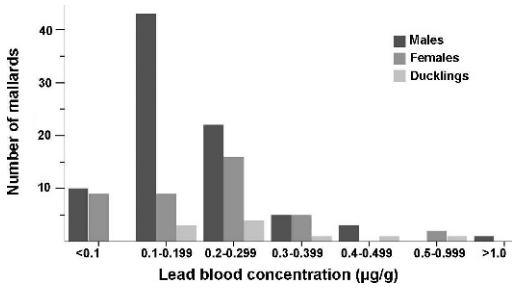

FIGURE 6. Frequency distribution of lead concentrations in blood collected from male, female, and duckling Mallards (*Anas platyrhynchos*) trapped alive from 1998 to 2001 (between August and March) in the region of Castilla and León of Spain.

males (0.224±0.141), and ducklings (0.288±0.133). However, blood lead level was higher than 0.200 µg/g in only 51 (41%) of the mallards (Fig. 6). The maximum level detected was 1.428, in the single mallard that had shot in its gizzard.

DISCUSSION

Ten percent of the ingested lead shot was eliminated without complete degradation in the first 10 days following intubation; remaining shot was retained in gizzards until it was totally degraded. The nonretention of some lead shot in the gizzard is normal. Brewer et al. (2003) reported elimination of 8.8% of shot between days 4 and 22 after ingestion. The gradual reduction of shot size observed in our study, until its complete degradation by around day 30, also is normal and in agreement with previous studies (Srebocan and Rattner, 1988). Sanderson and Bellrose (1986) report periods of 20 days for the total degradation of lead shot, which is only slightly less than our results. Shot pellets retained in the gizzard are ground by the abrasive action of grit and dissolved by the acid secretions of the proventriculus, with lead salts absorbed into the bloodstream (Mateo et al., 1997). The degree of metal erosion of individual shot in the mallard gizzard is directly correlated to the amount of metal that is available for uptake and circulation (Brewer et al., 2003). Differences in degradation can be related to varied diets used in experiments or other factors and should be considered when estimating prevalence of lead shot exposure based on gizzard examinations.

In all the groups, the highest blood lead concentrations were reached between 10 and 20 days after administration of lead shot, which was not consistent with a previous study by Roscoe et al. (1979). These authors reported peak values of lead in blood 48 hr after the administration of one No. 4 lead shot pellet. In our study, samples taken 72 hr after administering a No. 4 lead shot pellet had mean values of 2.199 µg/g, with the maximal value of 3.551 µg/g obtained on day 20. These differences could be related to the diets used in the experiments, which are considered to be the most important factor affecting lead-shot toxicity in waterfowl (Rattner et al., 1989).

Mean lead blood level at day 0 and in the control group throughout the study was normal, and below a threshold blood concentration of 0.200 µg/g (Roscoe et al., 1989; Havera et al., 1992; Mateo et al., 2001). In the groups with lower initial amounts of lead (II and III), the increase in blood lead concentration after the second lead dosage was greater than after the first. A similar trend was noted with the birds' general condition, which worsened after intubation of the second shot. However, in group IV, in which the initial lead dose was greater (3 No. 6 shots), these responses were not observed after the second administration; physical deterioration was less pronounced and the increase in blood lead concentration was not as pronounced. This suggests that mallards may develop a tolerance to lead intoxication after successfully surviving high doses of this compound. This fact could be associated with the secondary anemia, which can reduce the extent of lead attachment to erythrocytes and transport to soft tissues (Casteel, 2001).

Lead blood concentrations were higher in males than in females after the second administration, and male deaths were more frequent over the experimental period (eight males and three females). These differences could be related to males' greater sensitivity to lead intoxication (Rocke and Samuel, 1991). However, Havera et al. (1992) observed higher blood lead levels in females after the second lead shot pellet was administrated (day 35). In both studies, adult animals were used outside of the breeding season, and therefore factors such as age and reproductive condition do not explain this difference. Rocke and Samuel (1991) proved experimentally that male mallards are more sensitive to lead intoxication in terms of their immune response, which is probably related to lead's differing absorption and accumulation patterns in viscera and bones. Finley and Dieter (1978) showed that female mallards could accumulate more lead in their bones than males. Janiga and Zemberyova (1998) also found higher concentrations of lead in the bones of female than male Rock Pigeons (*Columba livia*). The different capacity of both sexes to accumulate lead in their bones could explain the higher blood lead concentrations found in males, and their greater propensity for acute toxicity, as a smaller percentage of absorbed lead can be excreted (Casteel, 2001).

Normal blood lead levels (less than 0.200 μg/g) were reached around day 120, clearly later than in other studies (Finley et al., 1976; Roscoe et al., 1979; Havera et al., 1992). There is little correlation between lead concentrations in blood (recent exposure) and in bone (cumulative exposure; Casteel, 2001). Roscoe et al. (1979) reported blood lead concentrations below 0.400 μg/g after 29 days and suggested that after 36 days mallards attained normal levels, as long as eight or fewer No. 4 lead shot pellets were administered. Havera et al. (1992) determined periods of 35 days for females and 42 days for males as the time necessary to reach values below 0.200 μg/g after a dosage of one No. 4 shot, and Finley et al. (1976) obtained mean values of 0.64 μg/g 30 days after having supplied one No. 4 shot. We believe that these differences could also be related with the diets used in the experiments and the rates of lead corrosion.

The clinical signs and macroscopic injuries that we observed are in agreement with those described by other researchers (Harrison, 1986; Srebocan and Rattner, 1988; Beyer et al., 1998; Burger and Gochfeld, 2000). All birds that died following lead exposure had symptoms and injuries consistent with lead intoxication, whereas the control group did not and gained weight. However, previous studies indicate that small amounts of lead are not sufficient to cause death in mallards, but may increase sensitivity of birds to concurrent situations such as parasitism, stress, or greater susceptibility to some infectious agents due to altered immunologic function (Rattner et al., 1989; Rocke and Samuel, 1991). In the present study, four mallards died within 15 days of the administration of a No. 4 lead shot pellet (group I); these birds received an additional blood sample collection on day 3. Three animals died two days after being moved to another location (laboratory rearrangement, day 75), and two birds showed intestinal parasitism. Pain and Rattner (1988) and Rattner et al. (1989) believed stress was the cause of the high mortality rate observed during each of their experimental phases after the administration of variable amounts of lead shot to mallards. Finley et al. (1976) and Srebocan and Rattner (1988) did not observe any deaths after the administration of one No. 4 lead shot pellet to mallards. Hatch (1987) suggested that nonlethal parasitism could aggravate lead toxicity.

The prevalence of lead-shot ingestion observed here (1.2%) for wild mallards trapped alive in the Boada and Nava lagoons is lower than in other previously

studied Spanish wetlands, where prevalence in ducks was 6.3% (Mateo et al., 2007) and 20.6% (Mateo et al., 1998). The absence of lead shot in the gizzards of dead birds was also in contrast with a previous study that reported shot in 27% of gizzards (Mateo et al., 1998). These differences could be explained by the relatively low intensity of hunting activity in these wetlands. However, lead levels of higher than 0.200 μg/g in 41% of the samples suggest a chronic subclinical lead poisoning that is probably associated with ingestion of lead shot. Given that some of these ducks migrate through other regions (Rodriguez, 2004), lead-shot ingestion could have occurred elsewhere and the shot could have degraded prior to sampling in Spain.

Our results clearly demonstrate the significant health and environmental problems caused by lead-contaminated waterfowl (live or dead), even in areas under low hunting pressure. The residence time of lead in blood is about 4 mo, but other studies indicate that lead is incorporated into the bone mineral matrix with a residence time spanning decades (Casteel, 2001). Humans, raptors and other predatory animals are subject to secondary poisoning by preying on lead-laden birds and mammals, or by scavenging contaminated carcasses. A mean lead concentration of 15 μg/g in bone ash of contemporary Americans is 1000 times greater than the natural level of lead in the bones of prehistoric native North Americans (Casteel, 2001). Chronic exposure to lead is toxic for a multitude of organ systems, tissues, cell types, and enzymes (Casteel, 2001). The health effects of lead exposure include developmental neurotoxicity, reproductive dysfunction, and toxicity to the kidneys, blood and endocrine systems (Sanborn et al., 2002). Meta-analysis and reviews suggest that any level of lead exposure is potentially detrimental and the relationship between blood lead levels and cognitive outcomes is robust even at low levels of lead exposure (Miranda et al., 2007). This evidence of general lead toxicity in mallards supports the adoption of immediate action to stop waterfowl's being exposed to lead, such as a ban on lead-shot shots in waterfowl hunting.

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