

MALLARDS (*Anas platyrhynchos*) - A RISK TO HUMAN HEALTH FROM EXPOSURE TO LEAD SHOTS CONTAMINATING THE ENVIRONMENT

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Summary: The problem around bodies of water used for waterfowl hunting is elevated lead contamination. The aim of the study was to determine which bodily tissues of mallards suffer the most from lead contamination, and whether such contamination can lead to the exceeding of the maximum allowable lead concentrations in meat and giblets set by the EU for poultry. Two groups of hunted mallards were used in the study. One group consisted of ten hunted mallards that spent a part of their life on a pond (experimental group, E). The other group was made up of ten mallards raised without access to a body of water (control group, C). Lead concentrations were determined by high resolution continuum source atomic absorption spectrometry. In experimental group, considerably higher average lead concentrations (mean±SD; mg/kg) were found in breast muscle (E=0.253±0.205; K=0.077±0.031), heart (E=0.272±0.307; K=0.096±0.042), lungs (E=2.721±3.950; K=0.205±0.048), liver (E=7.669±14.048; K=0.287±0.124) and kidneys (E=24.944±30.377; K=0.407±0.106). Significant differences (P<0.05) between E and C group were found in breast and heart muscle, as well as in lung and kidney tissue. A comparison between average lead concentrations in experimental group and maximum lead concentration limits in poultry meat and giblets set forth by the EU showed that the maximum concentration limits were statistically significantly exceeded in the case of breast muscle (P<0.043) and kidneys (P<0.032). It follows from the results that mallards bagged on a pond contaminated with lead from shotgun pellets can pose a risk to human health.

Key words: lead pellets; game; waterfowl; wild duck

Introduction

The problem in the surroundings of bodies of water used for waterfowl hunting is elevated lead contamination (1 - 3). The source of that contamination is the long-term use of lead in the manufacture of shots used in waterfowl hunting (3 - 4). A shotgun cartridge used in waterfowl hunting containing approx. 32 g of lead alloy may be loaded with up to 140 shot pellets (the

weight and quantity differing according to the type of cartridge used). Several cartridges may be fired at a single duck, and only a few pellets will remain in the duck's body. The rest of the pellets will be left unnoticed in the environment for many years but they become a possible source of lead contamination of the environment.

In spite of its relatively high stability in the environment (4), lead may pose a serious risk. The risk materializes when lead enters the food chain (5). Waterfowl may mistake lead pellets left lying around bodies of water for feed (seeds) or small pebbles-gastrolites/grit, which birds ingest to

facilitate food digestion. Ingested pellets will either be soon excreted from the body, or will stay for 18-20 days on average in the proventriculus together with sand and grit (6). Shot pellets retained in the stomach will be fragmented by the action of grit and dissolved by the proventricular acid. Lead salts thus produced are then absorbed into the blood circulation (7). Lead can thus be deposited in various bodily tissues and organs (4). High quantities of ingested lead produce symptoms of acute poisoning, and the birds affected eventually die. Although birds may die of lead poisoning at any time throughout the year, most cases are usually reported at the close of the waterfowl hunting season (8), and in November in particular (9). At lower doses, lead may be deposited for extended periods of time in bodily tissues (10). Under certain circumstances, lead shots may also be the source of environmental contamination. Lead may pass from pellets to sediments at the bottom of bodies of water, and be picked by, and accumulated in, aquatic organisms and plants. Lead from them may enter the organism of waterfowl as part of their diet. The lead ingested as part of contaminated food may be accumulated, with different intensities, in both meat and organs of the birds. Lead-contaminated waterfowl may be a source of lead contamination for bird-hunting predators, scavengers and beast of prey (4, 11). Man can also be exposed to negative effects of lead if he eats lead-contaminated animals. The risk of poisoning is particularly acute in people who are regular game eaters.

One of very common waterfowl raised for hunting in the Czech Republic are mallard ducks. They belong among dabbling ducks, and are often called one of the greatest "gluttons" among waterfowl. Mallard's diet consists mainly of what it finds in water, near the banks and in the close vicinity of bodies of water. Cases of lead poisoning have been known especially amongst waterfowl (5, 7). The mallard, from human nutrition point of view, may be a high-risk lead-contaminated food.

Material and methods

In the study, we used a group of ten mallards raised on a pond in southern Moravia (Czech Republic), which had been used for duck hunting with lead shot for several years. The ten ducks were nagged during the autumn 2010 hunting

season (experimental group, E). To rule out lead contamination from lead shot pellets embedded in the body, only ducks killed with steel shot were used. The control group was made up of ten mallard's carcasses coming from the same breeding facility but raised in an enclosure without access to a body of water (control group, C). Samples of the right breast muscle, heart, lung, liver and kidneys were collected from all of the birds. All samples were packed individually in polypropylene bags and frozen (-18°C). Lead concentrations in collected tissue and feedstuff samples were determined by high-resolution continuum source atomic absorption spectrometry (HR-CS AAS) using ContrAA 700 spectrometer (Analytik Jena AG, Germany). Soft tissues were determined by electrothermal atomization. Prior to the determination of lead concentrations, samples were decomposed. Weight of the tissue 1g was fumed with 6 ml nitric acid and 1 ml hydrogen peroxide in a microwave-heated laboratory autoclave (ETHOS SEL, Milestone, Italy). The sample solution was made up to volume 10 ml by water. The detection limit for lead (3σ) was $0.021 \text{ mg}\cdot\text{kg}^{-1}$, the reproducibility was expressed from five measurements as RSD 3.7 %. Standard reference materials, DORM-2 (NRC, dogfish muscle), 1577b (NBS, bovine liver) and H-5 (IAEA, animal bone), were used to verify the validity of the method. Statistical analyses were done using Statistica 8.0 for Windows software. After testing for normality, data were subjected to one-way ANOVA and subsequently to Tukey-HSD test or nonparametric ANOVA and Kruskal-Wallis test.

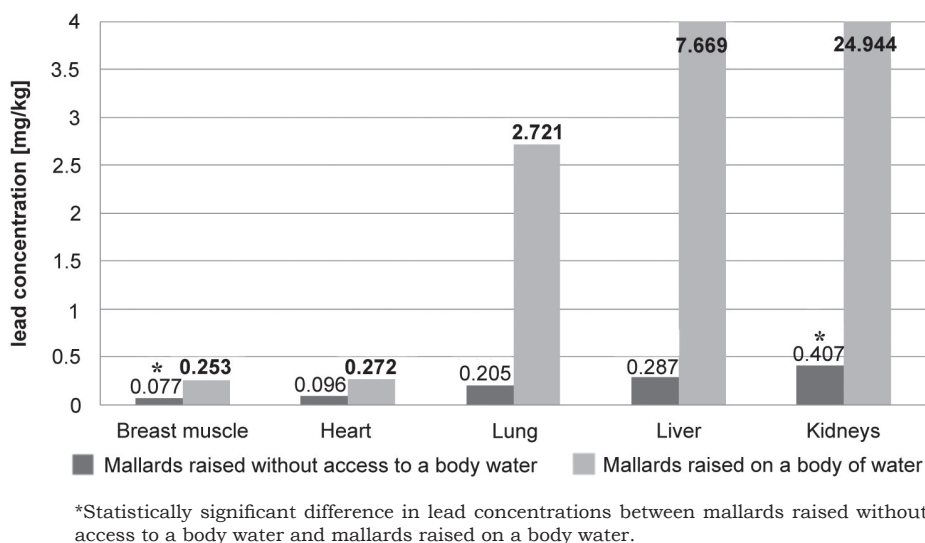
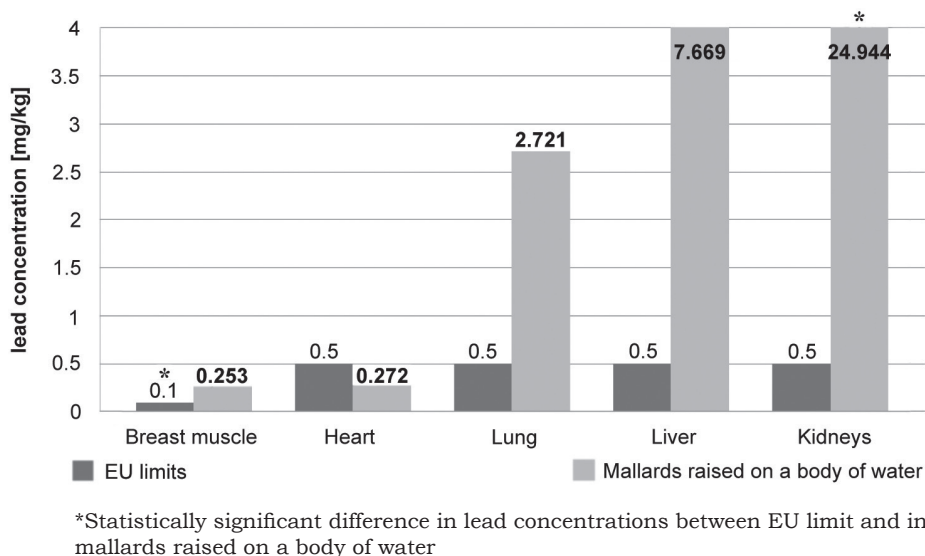
Results

Lead concentrations in breast muscle and individual organs in ducks raised on a pond (experimental group, E) and ducks raised away from the pond (control group, C) are given in Table 1. A comparison of average lead concentrations in breast muscle and individual organs between ducks raised on a pond (experimental group, E) and ducks raised away from the pond (control group, C) is given in Figure 1.

Table 1: Lead concentrations in mallard

	Breast muscle [mg/kg]		Heart [mg/kg]		Lung [mg/kg]		Liver [mg/kg]		Kidneys [mg/kg]	
	K1	E2	K1	E2	K1	E2	K1	E2	K1	E2
min	0.015	0.118	0.015	0.064	0.134	0.129	0.185	0.193	0.247	0.370
max	0.114	0.647	0.158	0.894	0.287	11.77	0.618	35.53	0.595	76.91
SD3	0.031	0.205	0.042	0.307	0.048	3.950	0.124	14.048	0.106	30.377
A4	0.077	0.253	0.096	0.272	0.205	2.721	0.287	7.669	0.407	24.944
P5	0.015		0.090		0.0593		0.114		0.020	

¹ control group raised away from a body of water; ² a group raised on a pond; ³ standard deviation; ⁴ arithmetic mean; ⁵ probability level.

Figure 1: Comparison of lead concentrations between mallards raised without access to a body of water and mallards raised on a body of water**Figure 2:** Comparison of lead concentrations in mallards raised on a body of water with EU limits

From Table 1 and Figure 1 it follows that higher concentration both in the breast muscle, heart, lungs, liver and kidneys were on average found in the group raised on a pond (experimental group) than in the group raised without access to it (control group), and the difference between the experimental and the control groups was statistically significant in the case of breast muscle ($P < 0.015$) and kidneys ($P < 0.020$).

Next, distribution of lead in ducks raised on a pond and exposed to elevated lead contaminations (experimental group) was studied. It follows from Table 1 and Figure 1 that the highest lead concentrations are in the kidneys, followed by the liver, lungs and the heart. The lowest lead concentrations were found in breast muscle. A statistically significant difference was found between mean lead concentrations in the kidneys and lead concentrations in breast muscle ($P < 0.005$), heart ($P < 0.005$) and lungs ($P < 0.016$).

Mean lead concentrations in breast muscle, heart, lungs, liver and kidneys in mallards raised on a body of water contaminated with lead as a result of the site having been used for duck hunting with lead shot for many years (experimental group) were then compared with the maximum allowable limits of lead in meat and giblets set forth by the Commission Regulation (EC) No. 1881/2006 (Figure 2).

From results showed in the Figure 2 it follows that mean lead concentrations found in the mallards raised on a body of water exceed the maximum allowable EU limit set for breast muscle, lungs, liver and kidneys. The amounts in excess of allowable lead limits in foods set forth by the EU were statistically significant in the case of breast muscle ($P < 0.043$) and kidneys ($P < 0.032$). The statistically significant lowest concentrations compared with EU limits were, on the other hand, found in the heart ($P < 0.043$).

Discussion

The environment is contaminated with lead from various sources, with human activities being the most important. Bodies of water used for duck or other waterfowl hunting are among the sites with the highest lead contamination levels. The probability of an elevated lead contamination of bodies of water is the result of the use of cartridges loaded with lead shot in duck or other waterfowl

hunting (11). Such pellets left behind around bodies of water after the hunt may be ingested by waterfowl and cause lead accumulation in bodily organs with negative effects on the health of the birds affected, or even causing their death (1, 3 - 4). Under certain circumstances, lead from shot pellets may be released directly to the environment. Lead particles are more readily degradable if the contaminated soil or water is acid in reaction, or if they contain higher concentrations of dissolved oxygen. Lead particles may then be dissolved in soil water, and subsequently assimilated by plants. Contaminated plants may then be the source of lead for the animals that eat those plants (4). In our study, higher lead concentrations in breast muscles and bodily organs were found in ducks raised on a pond (experimental group) compared with ducks raised away from the pond (control group). That confirms the hypothesis that the surroundings of bodies of water where waterfowl hunting takes place belong among environments with elevated lead contamination.

Lead from ingested shot pellets or contaminated food is easily absorbed in the digestive tract. Blood then distributes the lead to body tissues (10). Lead accumulates in vitally important organs (4, 10). The highest lead concentrations are registered several days or even months after exposure in the kidneys and liver. High Pb in bones indicates a long term exposure to lead, and this deposited lead can be mobilized in situations when Ca would be mobilized from bones (12). In our study, we found the highest lead concentrations in the kidneys and liver. That confirms the hypothesis of high lead accumulation in parenchymatous organs, such as liver and kidneys. Our results are in agreement with the previously reported results (13 - 14), where the highest lead concentrations in ducks were found in the liver; high lead concentrations in the kidneys were also mentioned in previously presented studies (4, 10). On the other hand, other authors, who investigated lead concentrations in adult individuals, found the highest levels in bones and breast muscles, kidneys and the brain, and the lowest lead concentrations in the liver (2). The study investigated the use of mallards for the biomonitoring of heavy metal contamination in the environment.

Mallards are game birds whose meat serves as food for people. For that reason it is important to monitor lead contamination in the birds shot from also the human health point of view. Commission

Regulation (EC) No. 1881/2006 (629/2008), which sets maximum limits of certain contaminants in foodstuffs, does not mention any specific maximum allowable limit for lead concentrations in game meat. A comparison of our results with maximum limits for lead in the above regulation set for poultry meat showed that the allowable lead concentrations (0.1 mg/kg) would have been exceeded in eight cases. The maximum allowable limit laid down in the regulation for lead in offal, liver and giblets is 0.5 mg/kg. In our study, lead concentrations in the liver exceeded several times the maximum allowable limit for lead in foodstuffs in six cases. The limit was exceeded in four liver tissue samples, seven pulmonary tissue samples, and heart tissue samples failed to meet the limit in only two cases. From the results reported here it follows that both meat and organs of ducks raised on ponds contaminated with lead from lead shot pellets may pose a health threat to humans once the EU limits for lead contamination have been exceeded.

Cartridges loaded with lead pellets used to be used in the hunting for wild ducks on and around ponds. Lead shot left in and around ponds may be the source of environmental contamination, and a possible source of contamination and even poisoning of waterfowl as well as other animals and even man. Lead can be ingested by waterfowl directly, or lead dissolved in soil water is assimilated by plants and from there, as part of their diet, the lead gets into the organism of waterfowl.

In our study, we looked at the levels of possible lead contamination of the aquatic environment caused by lead shot pellets, and the subsequent impact on the safety of meat and internal organs of waterfowl (mallards). We found that mallards raised on a pond contaminated with shotgun pellets can pose a risk to human health.

Heart is made up of muscle tissue and the results of our study indicate that trends of lead accumulation in it are different from those in parenchymatous organs. It follows from the results that the maximum lead concentration limit for the heart set by the EU should not be the same as that for parenchymatous organs (0.5 mg/kg).

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(representative for all authors) confirm that the authors have no protected, financial, occupational or other personal interests in a product, service and/or a company which could influence the content or opinions presented in the manuscript.

References

1. Mateo R, Belliure J, Dolz JC, et al. High prevalences of lead poisoning in wintering waterfowl in Spain. *Arch Environ Contam Toxicol* 1998; 35: 342–7.
2. Kalisińska E, Salicki W, Myslek P, et al. Using the Mallard to biomonitor heavy metal contamination of wetlands in north-western Poland. *Sci Total Environ* 2004; 320: 145–61.
3. Rodríguez JJ, Oliveira PA, Fidalgo LE, et al. Lead toxicity in captive and wild mallards. *J Wildl Dis* 2010; 46: 854–63.
4. Francisco ND, Troya JDR, Agüera EI. Lead and lead toxicity in domestic and free living birds. *Avian Pathol* 2003; 32: 3–13.
5. Guillemain M, Devineau O, Lebreton J, et al. Lead shot and teal (*Anas crecca*) in the Camargue, Southern France: effects of embedded and ingested pellets on survival. *Biol Conserv* 2007; 137: 567–76.
6. Ferreyra H, Romano M, Uhart M. Recent and chronic exposure to wild ducks to lead in human-modified wetlands in Santa Fe Province, Argentina. *J Wildl Dis* 2009; 45: 823–7.
7. Mateo R, Martínez-Vilalta A, Guitart R. Lead shot pellets in the Ebro delta, Spain: densities in sediments and prevalence of exposure in waterfowl. *Environ Pollut* 1997; 96: 335–41.
8. Friend M. Lead poisoning: the invisible disease. In: Cross D, Vohs P, eds. *Waterfowl management handbook*. Fort Collins: Fish and Wildlife Service, 1989: 1–5. http://www.nwrc.usgs.gov/wdb/pub/wmh/13_2_6.pdf (April 2014)
9. Marjánková K. Otravy kachen způsobené broky. *Československé rybníkářství* 1975; 1: 32–4.
10. Gad SC. Lead. In: Wexler P, ed. *Encyclopedia of toxicology*. Oxford: Academic Press, 2005: 705–9.
11. Scheuhammer AM, Norris SL. A review of the environmental impacts of lead shotshell ammunition and lead fishing weights in Canada. Ottawa: Canadian Wildlife Service, 1995: 56 p. (Occasional paper, no. 88) <http://publications.gc.ca/collections/Collection/CW69-1-88E.pdf> (April 2014)

12. Fisher IJ, Pain DJ, Thomas VG. A review of lead poisoning from ammunition sources in terrestrial birds. *Biol Conserv* 2006; 131: 421–32.

13. Bojar H, Bojar I. Assessment of the lead and cadmium contamination levels in the Lublin Region wetlands using mallards (*Anas platyrhynchos*) as a contamination vector. *Environ Toxicol*

II 2008; 110: 139–47.

14. Walkuska G, Bojar H, Chalabis-Mazurek A, et al. Mallard (*Anas platyrhynchos* L.) as a bioindicator of pollution with selected heavy metals of some water reservoirs in the Lublin district. *Fresen Environ Bull* 2010; 19: 383–9.

RACE MLAKARICE (*Anas platyrhynchos*) - TVEGANJE ZA ZDRAVJE LJUDI ZARADI IZPOSTAVLJENOSTI SVINČENIM IZSTRELKOM, KI ONESNAŽUJEJO OKOLJE

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Povzetek: Okrog vodnih teles, ki se uporabljajo za lov vodnih ptic, se pojavlja problem povišane onesnaženosti s svincem. Namen raziskave je bil ugotoviti, katera tkiva mlakaric so najbolj prizadeta zaradi onesnaženja s svincem in ali lahko tako onesnaženje privede do prekoračitev največje dovoljene koncentracije svinca v mesu in drobovini, določene v okviru EU za perutnino. V študiji sta bili uporabljeni dve skupini uplenjenih mlakaric. Ena je bila sestavljena iz desetih ulovljenih mlakaric, ki so del svojega življenja preživele na ribniku (eksperimentalna skupina E), druga pa je bila sestavljena iz desetih mlakaric, ki niso imele dostopa do ribnika (kontrolna skupina C). Koncentracije svinca so bile določene z atomsko absorpcijsko spektrometrijo visoke ločljivosti. V poskusni skupini smo izmerili znatno višje povprečne koncentracije svinca (povprečje \pm SD mg / kg) v prsni mišici ($E = 0,253 \pm 0,205$; $K = 0,077 \pm 0,031$), srcu ($E = 0,272 \pm 0,307$; $K = 0,096 \pm 0,042$), pljučih ($E = 2,721 \pm 3,950$; $K = 0,205 \pm 0,048$), jetrih ($E = 7,669 \pm 14,048$; $K = 0,287 \pm 0,124$) in ledvicah ($E = 24,944 \pm 30,377$; $K = 0,407 \pm 0,106$). Razlike ($p < 0,05$) so bile statistično značilne v prsni in srčni mišici, pljučih in tkivu ledvic. Primerjava s povprečnimi koncentracijami svinca v eksperimentalni skupini in mejnih vrednosti koncentracije svinca perutninskega mesa in drobovine, ki jih določa EU, je pokazala, da so bile mejne vrednosti koncentracij statistično značilno presežene v prsni mišici ($P < 0,043$) in ledvicah ($P < 0,032$). Naši rezultati kažejo, da vodni lov mlakaric vodi v onesnaženost ribnikov s svincem in lahko predstavlja tveganje za zdravje ljudi.

Ključne besede: igre; vodne ptice; divja raca