Distribution of mercury in leg muscle and liver of game birds from Serbia

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Abstract. The purpose of this study was to determine the distribution of Hg levels in leg muscle and liver of game birds collected within the Serbian National residue monitoring program from 2013 to 2016. Hg levels in samples (n=464) of: pheasants (n=182), mallard (n=25), Eurasian jay (n=7), partridges (n=5) and woodcocks (n=8) were determined by ICP-MS. The highest mean Hg levels were observed in leg muscle samples of woodcocks (0.071 mg/kg) and mallard (0.059 mg/kg). The lowest mean Hg level in liver was determined in partridges (0.008 mg/kg) while the highest was in pheasants (0.262 mg/kg) and mallard (0.161 mg/kg). Statistical analysis showed significantly differences between Hg levels in liver of woodcocks and mallard, as well as between them and livers of other analysed game birds. During the four years (2013-2016), 87.5% of leg muscle and 50% of woodcock livers had Hg levels that exceeded the MRL, while in mallard muscle and liver those percentages were 36% and 40%, respectively.

1. Introduction
Meat is good source of protein, fat, vitamins and some essential elements and makes up an important part of the food we eat [1]. Beside wide range of nutrients, meat may also carry certain toxic substances [2]. Heavy metals are toxic, and still one of the global environmental problems posing health risk to man and wildlife [3]. Heavy metals enter the environment by natural sources (volcanic activity, erosions and weathering) and by human activities (mining, fossil fuels combustion, industrial emissions, direct application of fertilizers and fungicides). Mercury (Hg) is a persistent global pollutant existing in three forms – elemental, inorganic and organic mercury compounds. The inorganic form of mercury can be transformed to methylated form, methylmercury, by microorganisms and this form is mostly present in fish [4,5,6]. Exposure to mercury causes permanent damage to the brain, kidneys, and developing foetus [7,8]. According to International Agency for Research on Cancer (IARC) methylmercury is classified as possibly carcinogenic for humans (2B).

Game meat is considered and valued for its exceptional taste than for its nutritive properties. However, as game animals are a part of the terrestrial soil-plant-animal food chain, they have been reported containing higher toxic element levels than animals from farms [9]. While toxic element levels in muscle are generally low, liver and kidney accumulate higher concentrations of these elements [10]. This is especially expressed in birds, as their metabolism is more rapid compared to...
other species of animals. So, game birds are considered a wildlife type that is a suitable, representative, potential bioindicator of pollution of the environmental.

Since the meat of bird game is, therefore, a bioindicator of potential pollution in the environment and is consumed by humans, the objective of this study was to examine the levels of Hg in leg muscle and liver of different game bird species and to identify possible differences in Hg levels between species.

2. Materials and Methods
Levels of Hg were measured in leg muscle and liver of game birds \((n=464)\). Pheasants \((n=182)\), mallard \((n=25)\), Eurasian jay \((n=7)\), partridges \((n=5)\) and woodcocks \((n=8)\) were acquired during regular hunting seasons within the Serbian National residue monitoring program from 2013 to 2016.

Frozen samples were thawed at \(+4^\circ C\) for a day before analysis and then homogenized. An amount, approximately 0.5 g of homogenized tissue was transferred into a teflon vessel with 5 mL nitric acid (67% Trace Metal Grade, Fisher Scientific, Bishop, UK) and 1.5 mL hydrogen peroxide (30% analytical grade, Sigma-Aldrich, St. Louis, MA, USA) for microwave digestion. The microwave (Start D, Milestone, Sorisole, Italy) program consisted of three steps: 5 min from room temperature to \(180^\circ C\), 10 min hold at \(180^\circ C\), 20 min ventilation. After cooling, the digested sample solutions were quantitatively transferred into disposable flasks and diluted to 100 mL with deionized water from a water purification system (Purelab DV35, ELGA, Buckinghamshire, UK).

Inductively coupled plasma mass spectrometry (ICP-MS), (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany), was used for analysis of the \(^{209}\text{Hg}\) isotope. Five-point calibration curve (including zero) was constructed for the qualitative analysis for Hg isotope of the samples. Multielement internal standard (\(^6\text{Li}, \, ^{45}\text{Sc}—10 \, \text{ng/mL}; \, ^{71}\text{Ga}, \, ^{89}\text{Y}, \, ^{209}\text{Bi}—2 \, \text{ng/mL}\) was introduced online by an additional line through the peristaltic pump.

The quality of the analytical process was verified by analysis of the certified reference material NIST 1577c (Gaithersburg, MD, USA). Reference material was prepared as samples using microwave digestion. Replicate analyses were in the range of certified values.

Statistical analysis of experimental data was performed using software Minitab 16 Statistical Software. One-way analysis of variance – ANOVA and Tukey’s HSD test were applied for comparison of Hg levels between leg muscles as well as between livers from different game birds.

3. Results and Discussion
The results of Hg levels in leg muscle and liver of the analysed samples are presented in Table 1 and Table 2, respectively. For calculation, when the levels of Hg were below the limit of detection (LOD, LOD=0.001 mg/kg), that value was assumed to be equal to one half of the LOD (1/2 LOD).

| Table 1. Hg levels in leg muscle of game birds from Serbia |
|----------------------------------------------------------|
|               | n1 | min-max | Mean ± SD    | n2 |
| Pheasants     | 182| < LOD-0.030 | 0.001±0.002\(^a\) |     |
| Mallard       | 25 | 0.005-0.220 | 0.059±0.058\(^a\) | 9  |
| Eurasian jay  | 7  | < LOD-0.012 | 0.008±0.004\(^a\) |     |
| Partridges    | 5  | < LOD-0.013 | 0.003±0.006\(^a\) |     |
| Woodcocks     | 8  | 0.021-0.130 | 0.071±0.036\(^a\) | 7  |

n1 – number of samples
n2 – number of non-compliant samples
a-b Different superscripts within the same column indicate significant differences of means according to Tukey’s HSD test \((p < 0.05)\)

| Table 2. Hg levels in liver of game birds from Serbia |
|-----------------------------------------------------|
|           | n1 | min-max | Mean ± SD    | n2 |
| Pheasants | 182| < LOD-0.039 | 0.004±0.007\(^c\) |     |
| Mallard   | 25 | 0.015-0.484 | 0.175±0.161\(^a\) | 10 |
Eurasian jay  7  < LOD-0.035  0.046±0.014c
Partridges  5  < LOD-0.036  0.008±0.016c
Woodcocks  8  0.071-0.212  0.132±0.060b  4

n1 – number of samples
n2 – number of non-compliant samples
a-b-c Different superscripts within the same column indicate significant differences of means according to Tukey’s HSD test (p < 0.05)

Figure 1. Percentage of non-compliant leg muscle or liver of mallard and woodcocks with regard to Hg levels.

The levels of Hg in leg muscle were within the range < 0.001-0.220 mg/kg. The highest mean Hg levels were established in leg muscle samples of woodcocks (0.071 mg/kg) and mallard (0.059 mg/kg), while liver samples of other game birds contained very low Hg levels, often close to the LOD (Table 1). These could be a consequence of the characteristic feed of mallard and woodcocks, which is rich in crustaceans, worms and fish, food with high levels of Hg. Statistical analysis did not show any significant differences in Hg levels between leg muscle samples of game birds. National legislation [11] does not prescribe maximum residue levels (MRL) for Hg in game tissue. Considering that, in this study, the MRL for Hg in poultry tissue (muscle, liver) is used as the MRL for game. MRL for muscle is 0.030 mg/kg [11]. Nine leg muscle samples of woodcocks and seven of mallard exceeded this level. So, during 2013-2016, 87.5 and 36% of analysed leg samples of woodcocks and mallard, respectively, were non-compliant (Figure 1).

While Hg levels in leg muscle were generally low, liver accumulates higher levels [12, 13, 14]. The lowest mean Hg levels established in liver samples were from partridges (0.008 mg/kg) while the highest was in the liver from pheasants (0.262 mg/kg) and mallard (0.161 mg/kg). Significant differences were established between Hg levels in liver of mallard and woodcocks. Also, Hg levels in liver of mallard and woodcocks significantly differ from the other analysed livers of game birds. National legislation [11] established 0.100 mg/kg as MRL for Hg in game liver. Some liver samples of woodcocks (n=4) and mallard (n=10) exceeded this MRL level (Figure 1). The highest established Hg levels were in liver of mallard (0.484 mg/kg) and woodcocks (0.212 mg/kg).

4. Conclusion
Game meat, including meat of game birds, is commonly consumed by hunters and their families. Meat and offal of game have been recognized as food items with elevated content of toxic elements thus raising concern about exposure to these elements even in a small part of the population. So, periodic control and investigation of Hg and other toxic elements in game meat and offal are needed to assess
the safety of these meats with respect to human health and to widen the knowledge base for this type of meat.

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