Cadmium level trend in liver and kidney of pigs from Serbia during 2014-2018

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Abstract. Concentrations of cadmium (Cd) were measured in samples of liver (n=88) and kidney (n=226) of pigs within the Serbian National Residue Monitoring Program during 2014-2018. Levels of Cd were determined by inductively-coupled plasma mass spectrometry. All of the pigs had measurable amounts of Cd in liver and kidney, but none of the analyzed samples exceeded MRL for Cd – 0.5 and 1.0 mg/kg, respectively. The maximum Cd concentration in kidney amounting to 0.864 mg/kg was established in the first year of the residue monitoring program (2014). No statistically significant differences in Cd levels in liver samples during the examined period were observed, while statistical differences were established only between Cd levels in kidney collected in 2014 and 2015. On the basis of statistical analysis, it can be concluded that Cd levels have not increased in the analyzed organs during the investigated period.

1. Introduction

Cadmium (Cd) is a toxic heavy metal well known as an environmental and food chain contaminant [1,2]. Water and air currents facilitate its transfer over long distances from sources of pollution, until it enters plants through soil deposition, and consequently animals and humans through nutrition. Industrial development and human activities increase the levels of this naturally occurring element in the environment [3,4]. Diet is the primary source of Cd for the nonsmoker population, while tobacco smoke is the major source for smokers [5]. Cd is not degradable and accumulates in various tissue types, specifically in kidney [6]. After exposure, Cd can damage kidney, liver, and nervous system, can cause diabetes, lead to bone disorder, problems with vitamin D metabolism, and cardiovascular disease, etc. [7-10]. Considering the chronic Cd exposure of humans through diet, along with its ability to accumulate and long half-life in the body [11], Cd poses a significant health risk to humans [12]. According to the International Agency for Research on Cancer (IARC) Cd and Cd compounds are carcinogenic to humans [13], as Cd is pronounced as human lung carcinogen [14]. Given that food is one of the most significant sources of Cd exposure, the European Commission [15] and Serbian national regulation [16] has established maximum residue levels for Cd in muscle, liver and kidney intended for human consumption (0.050, 0.5 and 1 mg/kg, respectively). In order to secure the hygiene of food of animal origin and to protect public health, it is necessary to establish control and monitoring programs encompassing adequate numbers of samples, as well as the efficient monitoring of the residue level in tissues and organs of animals [17].
The objective of this study was to review the Cd levels in liver and kidney of pigs collected during the Serbian monitoring program in the period 2014-2018, and to establish whether levels of Cd in pig tissues are increasing.

2. Materials and Methods
The levels of Cd were measured in samples of liver (n=88) and kidney (n=226) of pigs. Analyzed tissues were collected as a part of Serbian monitoring program during 2014-2018. Samples were individually stored in plastic bags at -18 °C prior to analysis.

Amounts of 0.3 g measured with precision of ± 0.001 g were transferred into Teflon vessels and treated with 5 mL of nitric acid (67% Trace Metal Grade, Fisher Scientific, Bishop, UK) and 1.5 mL of hydrogen peroxide (30% analytical grade, Sigma-Aldrich, St. Louis, MA, USA). Sample homogenates were further treated in the microwave (Start D, Milestone, Sorisole, Italy) according to the following temperature program: 5 min from room temperature to 180°C, 10 min hold 180°C, and 20 min vent. Digested homogenates were quantitatively transferred into volumetric flasks and diluted to 100 mL with deionized water (ELGA, Buckinghamshire, UK).

The analysis of $^{111}$Cd isotope was performed by an inductively coupled plasma-mass spectrometry (ICP-MS) instrument iCap Q (Thermo Scientific, Brem men, Germany), equipped with a collision cell, and operating in the kinetic energy discrimination (KED) mode. A five-point calibration curve (including zero) was constructed for quantification. Multielemental internal standard ($^6$Li, $^{45}$Sc – 10 ng/mL; $^{71}$Ga, $^{89}$Y, $^{209}$Bi – 2 ng/mL) was introduced online via another line through the peristaltic pump. Measured concentrations were corrected for the response factors of internal standards. The quality of the analytical process was confirmed by the analysis of the standard reference material SRM 1577c (Gaithersburg, MD, USA), and were within the range of the certified values.

Statistical analysis was performed using the Minitab 16.0 software. One-way (unstacked) ANOVA analysis of variance and Tukey’s test were used in order to compare the differences in Cd concentrations between liver and in kidney from different years.

3. Results and Discussion
The Cd levels measured in liver and kidney samples are presented in Tables 1 and 2, respectively. As expected, all analyzed samples were above the limit of detection (LOD = 0.001 mg/kg) since Cd primarily accumulates in liver and kidney, bonding to metallothionein in these tissues [18,19]. Liver had lower mean Cd concentrations than kidney. Also, none of the analyzed samples exceeded the MRLs for Cd in pig liver and kidney (0.5 and 1.0 mg/kg, respectively).

Table 1. Levels of Cd in pig liver by year

| Year | Mean Cd, mg/kg | min-max     |
|------|----------------|-------------|
| 2014 | 0.022          | 0.007-0.085 |
| 2015 | 0.025          | 0.013-0.046 |
| 2016 | 0.024          | 0.008-0.042 |
| 2017 | 0.025          | 0.005-0.049 |
| 2018 | 0.030          | 0.009-0.080 |
Table 2. Levels of Cd in pig kidney by year

| Year | Mean Cd, mg/kg | min-max |
|------|---------------|---------|
| 2014 | 0.182<sup>a</sup> | 0.014-0.864 |
| 2015 | 0.126<sup>b</sup> | 0.007-0.261 |
| 2016 | 0.135<sup>ab</sup> | 0.038-0.306 |
| 2017 | 0.131<sup>ab</sup> | 0.024-0.351 |
| 2018 | 0.161<sup>ab</sup> | 0.046-0.469 |

**Different superscripts indicate significant differences of means according to Tukey’s HSD test (p < 0.05)**

Measured Cd levels of all analyzed liver samples were similar to data reported by Jankovic et al. (2012) for pig liver (0.033 mg/kg) [1]. They [1] examined Cd levels in offal of cattle, pigs, lambs and horses collected during the Serbian monitoring program from 2011 to 2012. Cd levels determined in kidney samples during 2014 were higher while data obtained in other years (2015, 2016, 2017 and 2018) were similar to Cd levels reported in the previously mentioned study [1]. The mean Cd levels in pig liver of this study were in line with levels reported by Nikolic et al. (2013) [20], who analyzed samples of liver and kidney in swine from the Serbian market. Comparing mean Cd levels of kidney from the current study with concentrations determined by [20], the same situation was observed as in data sets established by Jankovic et al. (2012) [1], except for Cd levels from 2014. Another study of Nikolic et al. (2017) [21] analyzed mineral composition of tissues (muscle, liver and kidney) of intensively and extensively reared pigs. Cd levels from our current study can be compared with data for Cd levels in liver and kidney of swine reared in intensive systems if it is assumed that pigs were kept in comparable conditions and were fed with similar feed. Our results for Cd in liver were lower than those reported by Tomović et al. (2011) (0.412 mg/kg) [22] and López-Alonso et al. (2007) (0.073 mg/kg) [23]. Also, the mean Cd levels in kidney analyzed during the period 2014-2018 were lower compared to some other previously reported studies [21,23-26].

According to some studies [27-29], Cd levels in feedstuffs strongly influence the Cd levels in liver and kidney of animals, and this could be one of the reasons why the Cd levels measured in the current study were different to data reported in these previously mentioned studies.

4. Conclusion

In summary, all samples of liver and kidney taken from the pigs tested within the Serbian National Residue Monitoring Program during 2014-2018 contained Cd. Liver had lower mean Cd concentrations than kidney. The highest Cd level was measured in kidney (0.864 mg/kg) collected during 2014. No increasing trend of Cd concentrations in the analyzed organs during five years (2014–2018) was observed.

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