# Human Health Risk Assessment of Cadmium from Cattle Meat and Offal in Central Bosnia Canton

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## Summary

The aim of this study was to determine the levels of cadmium in cattle meat and offal on the area of Central Bosnia Canton and to estimate the level of population exposure to cadmium through consumption of cattle meat and offal. Fifty samples were analysed using inductively coupled plasma mass spectrometry, out of which twenty samples of kidney tissue, twenty samples of liver tissue and ten samples of muscle tissue. Determined cadmium levels in cattle kidney varied between 0.088 and 4.493 mg/kg, while cadmium levels in liver were determined in the range from 0.016 to 0.206 mg/kg. The mean value of cadmium in kidney was 0.750 mg/kg, while the mean value of cadmium in liver was 0.076 mg/kg. Cadmium levels in muscle tissue were less than 0.008 mg/kg in all analysed samples. In three samples of kidney (15% of the analysed) cadmium levels exceeded maximum permitted level, while no such case was found for liver and muscle tissue. Estimated weekly intake of cadmium due to the consumption of cattle meat is  $1.74 \times 10^{-3}$  g/kg body weight. Weekly intake of cadmium by consuming cattle kidney is 9.08 x  $10^{-3}$  g/kg body weight, whereas weekly intake of cadmium via cattle liver is  $1.23 \times 10^{-3}$  g/kg body weight. The intake of cadmium due to the consumption of cattle meat and offal in the examined population is within the tolerable weekly intake. Exposure to cadmium from cattle meat in the examined population does not pose a risk for health.

## Key words

cadmium, cattle meat, kidney, liver, risk assessment

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## Introduction

Cadmium is a soft, silver-white metal found in the earth's crust, associated with zinc, lead, and copper ores (Agency for Toxic Substances and Disease Registry, 2012). Cadmium is used globally as a metal and as a component in various products: nickel-cadmium (Ni-Cd) batteries 82%, pigments 10%, coatings and plating 6%, stabilizers for plastics 1,5%, non-ferrous alloys, semiconductors, photovoltaic devices, and other 0.5% (United Nations Environment Programme, 2010). The presence of cadmium in the environment has significantly increased over the past few decades, consequently as a result of human activities, which contributed to an increase of cadmium levels in food. The increase in cadmium pollution in the environment and food contamination is caused by various natural sources and human activities, including volcanic activity, forest fires, weathering of rocks, the mining and smelting of ores, metal using industry, fossil fuels combustion, manufacture and application of phosphate fertilisers, waste incineration, and releases from municipal landfills (International Agency for Research on Cancer, 2012).

In the non-smoking general population, food accounts for approximately 90% of human exposure to cadmium, and less than 10% of total exposure occur due to inhalation of the low concentrations of cadmium in ambient air and through drinking water. The main food commodities that contribute to cadmium exposure are cereals and vegetables. Meat and fish normally contain lower cadmium contents, but animal offal such as kidney and liver can contain high cadmium concentrations, because these organs are in animals whose bodies concentrate cadmium. Smokers have on average twice the body burden of a nonsmoker, while occupationally exposed workers may accumulate high cadmium levels, depending on the type of work (European Food Safety Authority, 2009).

Cadmium absorption after dietary exposure in humans is relatively low. Cadmium binds to metallothionein and retains mainly in the liver and kidney in the human body. Approximately 50% of the total cadmium body burden is found in kidneys and 15% in liver. Cadmium is excreted very slowly, with urinary and faecal excretion. According to the World Health Organization (WHO), cadmium has a very long biological half-life, which is estimated between 10 and 33 years (WHO, 2011).

Cadmium is primarily toxic to the kidney. Cadmium causes damage of proximal tubules and failure of tubular reabsorption, which lead to renal dysfunction. Cadmium also has effects on reproduction and development, and the genotoxicity. The International Agency for Research on Cancer (IARC) in 1993 has classified cadmium and cadmium compounds in Group 1 as a human carcinogen (IARC, 1997, 2012). European Food Safety Authority (EFSA) established a tolerable weekly intake (TWI) for cadmium of 2.5  $\mu$ g/kg body weight and estimated that the mean exposure for adults across Europe is close to, or slightly exceeding, the TWI of 2.5  $\mu$ g/kg body weight (EFSA, 2009, 2011). The aim of this study was to determine the levels of cadmium in cattle meat and offal on the area of Central Bosnia Canton and estimate the level of population exposure to cadmium through consumption of cattle meat and offal.

# Material and methods Sample collection

The levels of cadmium were determined in 50 samples of cattle meat and offal from the area of Central Bosnia Canton, out of which 20 samples of kidney tissue (kidney cortex), 20 samples of liver tissue and 10 samples of muscle tissue (hip). Samples were collected in the period from December 2013 to June 2014. The survey included male and female cattle of different age groups, ranged from 1 to 12 year-old, all originated from Bosnia and Herzegovina. Samples were obtained from four cattle breeds: Simmental, Holstein, Montbeliard and Brown Swiss. Samples of 30 cattle were randomly taken at eight slaughterhouses in six municipalities of Central Bosnia Canton: Gornji Vakuf-Uskoplje, Bugojno, Donji Vakuf, Jajce, Travnik and Kiseljak. Samples were packed in sterile polyethylene bags (Whirl-pak®) and transported to the laboratory frozen at -18°C, where they were stored in the freezer until analysis. Analysis of samples was carried out in the laboratory of the Federal Institute for Agriculture Sarajevo.

#### Sample preparation

Samples of homogenized tissue were prepared using pressure digestion technique in the microwave Sineo MDS-8 (Sineo Microwave Chemistry Technology, China), according to standard BAS EN 13805:2005. Approximately 0.5 g was taken from each sample in teflon vessels and added 8 mL of 65% HNO<sub>3</sub> (Merck) for pre-treatment of 20 minutes. After that in vessels was added 3 mL of 65% HNO<sub>3</sub> and 1 mL of 30%  $H_2O_2$  (Merck). Microwave digestion was performed on 900 W for 30 minutes in three steps: heating for 10 minutes from room temperature to 130°C, 5 minutes on 150°C and 15 minutes on the 200°C. After cooling, the clear solutions of the samples were transferred quantitatively in volumetric flasks of 50 mL, and diluted to mark with solution containing 2% (w/w) HNO<sub>3</sub> and 0.5% (w/w) HCl (Fluka). Digested samples were transferred to polypropylene sample tubes for metal determinations.

## Determination of cadmium

Cadmium concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) using Agilent 7700x (Agilent Technologies, Japan), according to standard BAS EN 15763:2011. For calibration of device was used a certified standard for cadmium of 10 mg/L (Agilent Technologies Multielement Calibration Standard-2A). The accuracy and precision of the method were checked by using certified reference material for cadmium (NIM NCS ZC73012). Analytical recoveries were within the reference values. The sample analyses were done in two parallel determinations and all metal concentrations are reported as mg/kg wet weight. The results are shown as the minimum and maximum concentration, mean  $\pm$  standard deviation. The Mann-Whitney U test and Student's t-test were used to determine statistical significance.

#### Food frequency questionnaire

Bosnia and Herzegovina has not done national dietary study of food consumption, so data of cattle meat and offal consumption were obtained on the basis of food frequency questionnaire of an adult population from the area of Central Bosnia Canton. The survey was conducted on a random sample of 150 respondents, out of which 79 women and 71 men. Semiquantitative questionnaire consisted of questions about the frequency (daily, weekly, monthly, yearly) and the amount of consumption of certain types of cattle meat and offal. The results are shown as the mean weekly consumption  $\pm$  standard deviation.

#### Risk assessment

The methodology of the WHO (2010) was used in assessing the risk on human health due to cadmium exposure from cattle meat and offal. Weekly intake of cadmium (expressed as  $\mu$ g/kg body weight/week) was estimated by calculating the average concentration of cadmium determined in the analysed samples, the frequency and duration of exposure and the average weight of respondents, based on the following equation (1):

$$I = \frac{C \times CR \times ED}{BW \times AT}$$
(1)

where: I=Intake; C = Concentration; CR= Contact Rate; ED=Exposure Duration; BW= Body weight; AT= Averaging Time.

The possibility of an adverse health effects was estimated based on the relationship of estimated weekly intake of cadmium by beef and offal, and the reference values of TWI for cadmium of  $2.5 \mu g/kg$  body weight per week.

### **Results and discussion**

## Cadmium concentrations

Determined cadmium levels in cattle kidney were in the range from 0.088 - 4.493 mg/kg, while the cadmium levels in liver were determined in the range from 0.016 - 0.206 mg/kg. The mean value of cadmium in kidney was  $0.750 \pm 0.99$  mg/ kg, while the mean value of cadmium in liver was 0.076  $\pm$  0.05 mg/kg. The presence of cadmium was detected in all samples of kidney and liver. The lowest concentrations of cadmium were detected in muscle tissue and were less than 0.008 mg/kg in all analysed samples. The maximum permitted level for cadmium in meat and offal of bovine animals in Bosnia and Herzegovina is prescribed by national Regulation of maximum permitted quantities of certain contaminants in food ("Official Gazette of Bosnia and Herzegovina", no. 68/14). The regulatory maximum level for cadmium in bovine muscle, liver and kidney are 0.05, 0.5 and 1.0 mg/kg wet weight, respectively. In three samples of kidney (15% of the analysed) cadmium concentrations exceeded maximum permitted level, while no such case was found for liver and muscle tissue (Table 1).

These results confirm the accumulation of cadmium in the tissues of cattle in the following order: muscle < liver < kidney. The results of this study showed that levels of cadmium in cattle tissues were associated by several factors as the age and sex of cattle. Statistical analysis showed a significant correlation of cadmium concentrations in kidney and age of cattle (r=0.98, p<0.0001) (Figure 1) and of cadmium concentrations in liver and age of cattle (r=0.77, p<0.0001) (Figure 2), which is consistent with chronic exposure to this heavy metal and its bioaccumulation with time.

In females, the cadmium levels in kidney were higher than those recorded in males, and the difference was statistically significant (Mann-Whitney U test, p=0.0064) (Figure 3).

#### Table 1. Concentration of Cd in cattle tissue

	Cd in kidney	Cd in liver	Cd in muscle
Mean ± standard	$0.75 \pm 0.99$	$0.076 \pm 0.05$	-
deviation (mg/kg)			
Range (mg/kg)	0.088 - 4.493	0.016 - 0.206	< 0.008
Maximum permitted	1	0.5	0.05
level (mg/kg)			
Analysed samples	20	20	10
Samples exceeding	3	0	0
permitted level			

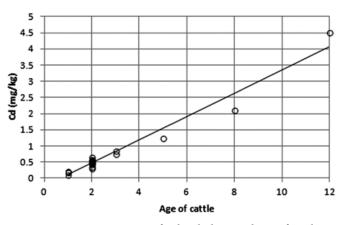


Figure 1. Concentration of Cd in kidney and age of cattle

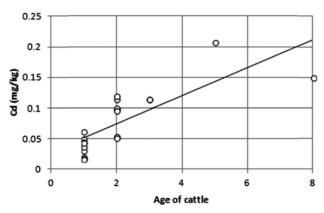


Figure 2. Concentration of Cd in liver and age of cattle

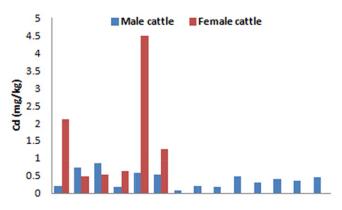
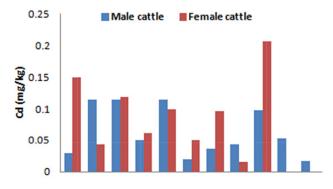
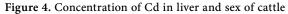


Figure 3. Concentration of Cd in kidney and sex of cattle





This research has not established statistically significant differences between cadmium concentrations in liver and sex of cattle (t=1.39, p=0.179) (Figure 4).

#### Cadmium exposure and risk assessment

The results of food frequency questionnaire, which was used for the collection of cattle meat, kidney and liver consumption data, showed that the average age of respondents was  $34.08 \pm 14.23$  years, and body weight amounted to  $76.05 \pm 11.96$  kg. The average weekly consumption of beef per capita was estimated to be  $125.95 \pm 107.32$  g (about 18 g/day). The average weekly consumption of kidney and liver were estimated to be  $26.71 \pm 34.87$  g and  $30.91 \pm 43.42$  g (approximately 3 g/day and 4 g/day).

Based on the Equation 1, estimated weekly intake of cadmium due to the consumption of cattle meat is 1.74 x 10<sup>-3</sup>  $\mu$ g/kg body weight. Weekly intake of cadmium by consuming cattle kidney is 9.08 x  $10^{-3}$  µg/kg body weight, whereas weekly intake of cadmium via cattle liver is  $1.23 \times 10^{-3} \mu g/kg$  body weight. The intake of cadmium due to the consumption of cattle meat and offal in the examined population is below the tolerable weekly intake of 2.5  $\mu$ g/kg body weight. The amount of consumed muscle tissue greatly exceeds the amount of consumed offal where were detected higher concentrations of cadmium, and thus the risk of cadmium intake via cattle tissues in the amount exceeding the recommended value is small. Quantitative risk assessment showed that the average consumption of beef and offal in the examined population from Central Bosnia Canton negligible increase the intake of cadmium. Average weekly intake of cadmium from cattle meat and offal is low with contribution less than 1% of tolerable weekly intake (Table 2).

The mean cadmium concentrations found in kidney are similar to values reported by various researches from Poland, Croatia, Czech Republic and Ireland (Falandysz, 1993; Bilandžić et al., 2010; Drápal et al., 2012; Canty et al., 2014). The mean cadmium concentrations in liver obtained in this study are consistent with the results of other studies on cattle in Sweden, Finland, Slovenia and Spain (Jorhem et al., 1991; Niemi et al., 1991; Tahvonen and Kumpulainen, 1994; Doganoc, 1996; López Alonso et al., 2000, 2004). Research in Belgium has shown that 75% of kidney samples from cattle raised in contaminated industrial areas, and 47% from cattle raised in uncontaminated rural areas had concentrations of cadmium in excess of the maximum permitted level (Waegeneers et al., 2009a). These authors concluded that the

Table 2. Weekly intake of Cd				
Type of tissue	Average consumption ± standard deviation (g/week)	Level of Cd exposure (µg/kg b.w./week )	% TWI	
Muscle	$125.95 \pm 107.32$	1.74 x 10 <sup>-3</sup>	0.0696	
Liver	$30.91 \pm 43.42$	1.23 x 10 <sup>-3</sup>	0.0492	
Kidney	$26.71 \pm 34.87$	9.08 x 10 <sup>-3</sup>	0.3632	

maximum level for cadmium in the EU is realistic in Belgium only for cattle up to 2 years of age (Waegeneers et al., 2009b). Similar results has shown the recent report from Ireland, where the concentration of cadmium in kidney of most cattle under 3 years of age conform EU requirements (Canty et al., 2014). The content of cadmium was determined in bovine kidney tissue from rural regions of Croatia. The mean concentration of cadmium in bovine kidney was 0.5 mg/kg and ranged from 0.001 to 4.55 mg/kg. Cadmium levels exceeding the maximum permitted quantity were found in 13% of bovine kidney samples. The results have confirmed the need to control cadmium levels in bovine kidney tissue on slaughter line (Bilandžić et al., 2010). In Bosnia and Herzegovina, Grujić et al. (2000) determined the content of cadmium in meat products from the Banja Luka market. The highest average content of cadmium was found in brined chopped meat 0.60 mg/kg, then in cooked sausages 0.34 mg/kg and in dry sausages 0.24 mg/kg. The authors concluded that special attention should be paid to the use of edible offal for the preparation of some types of sausages and other meat products, because many authors have found that the content of cadmium in kidney, liver and other internal organs often could be critical. Sapunar-Postružnik et al. (2001), based on the research results, pointed to the importance of regular and systematic control, not only of animals and their tissues and organs, but also of animal feed and its ingredients. The lowest concentrations of cadmium were detected in muscle tissue of cattle and were less than 0.008 mg/kg in all analysed samples, which is consistent with the results of previous studies in Poland, Slovenia, Spain and Iran (Falandysz, 1993; Doganoc, 1996; Miranda et al., 2001, 2005; González-Weller et al., 2006; Rahimi and Rokni, 2008). Many studies in the world have established that cadmium is accumulated in large quantities in liver and kidney, and its accumulation is associated with age and type of animal and cadmium content in soil and plants used for animal feeding (Zasadowski et al., 1999; Farmer and Farmer, 2000; Miranda et al., 2000; Sedki et al., 2003; Wlostowski et al., 2006; Nriagu et al., 2009; Cai et al., 2009; Yatmark et al., 2010; Saipan et al., 2014).

According to the results of this survey, the amount of beef and offal consumption is similar to the data of the Food and Agriculture Organization where in Bosnia and Herzegovina in 2013 consumption of beef per capita was 24.99 g/day, while consumption of edible offal was 6.38 g/day (FAOSTAT, 2013). Estimated intake of cadmium through cattle meat and offal consumption in Central Bosnia Canton is similar to those reported in the UK Total Diet Study where it was found that meat and offal have a small contribution (<1%) in the total dietary exposure to cadmium (Food Standards Agency, 2009). EFSA (2009) had developed high cadmium exposure cases to assess the potential contribution of extreme diets, which include consumption of offal at a level of 100 g/week. Frequent consumption of kidney from older animals would increase the total weekly intake of cadmium to 0.96 µg/kg body weight, so total dietary exposure to cadmium would be 3.23 µg/kg body weight per week. According to the results of the European Commission (2004), weekly intake of cadmium via meat was estimated about 10.81 µg/week, while weekly intake of cadmium via offal amounted average 1.11 µg/week. In Serbia, daily intake of cadmium through meat and meat products consumption was estimated at 0.382 µg/day (Janković et al., 2013), and in Netherlands the average daily intake of cadmium via beef was estimated at 0.209 µg/day, with contribution of 4% in dietary exposure to cadmium (Winter-Sorkina et al., 2003). The average dietary cadmium intake due to the consumption of meat in Spain is 0.246 µg/day (González-Weller et al., 2006). In China, daily intake of cadmium through offal consumption was estimated at 0.15  $\mu$ g/day, with contribution of 1.19% of the total dietary exposure to cadmium (He et al., 2013).

The research results indicate that liver and kidney of cattle are rarely consumed in relation to muscle tissue, and in the case of their consumption offal are often from young animals. For kidney samples average age of cattle was  $2.8 \pm 2.7$  years, for liver  $2.1 \pm 1.71$  years and for muscle  $2.4 \pm 2.11$  years-old. This means that the average consumption of cattle meat, kidney and liver with established levels of cadmium does not pose a risk for health of the examined population.

## Conclusion

Current dietary exposure to cadmium through cattle meat and offal consumption is not of toxicological concern for the health of consumers. However, because of the limited sample size in the conducted study, further health risk assessments are required in order to protect consumers. Due to the fact that eating habits and cadmium contamination of the environment and food are changing, it is necessary to continuously monitor intake of cadmium through diet and its impact on human health. On the basis of the obtained results, it can be concluded that Total Diet Study in Bosnia and Herzegovina is essential to determine dietary habits of the population and food consumption data. Studies of presence and content of cadmium in meat and other foodstuffs are important to assess dietary intake and probability of occurrence and severity of an adverse health effects in the population living on the territory of Bosnia and Herzegovina.

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