

Health risk assessment of exposure to toxic elements in meat and meat products from Croatia



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Abstract

The aim of this study was to measure the concentrations of arsenic (As), cadmium (Cd), lead (Pb), aluminium (Al), chromium (Cr) and nickel (Ni) in meat (pork and beef neck) and meat products (meat with beans, breakfast meat, chicken pâté, ham, and pork sausage) purchased from supermarkets in several Croatian cities. Element concentrations were analysed using inductively coupled plasma mass spectrometry (ICP-MS). Mean element concentrations in meat and meat products were measured in the ranges ($\mu\text{g}/\text{kg}$): Al 523-19,179, As 2.25-5.63, Cd 2.02-2.86, Cr 20.8-132.6, Ni 4.78-166.9, Pb, 3.53-7.49. The highest mean concentrations of elements were found in: Al in chicken pâté; As and Cd in ham; Cr and Ni in meat with beans; Pb in pork sausage. All measured Cd and Pb levels were below the European Commission limits of 50 and 100 $\mu\text{g}/\text{kg}$, respectively, and there were no significant differences in these elements between products. Significant differences in the content of Al, As, Cr and Ni were determined between meat and meat products. An estimation of the dietary daily

(EDI) and weekly (EWI) intakes of elements associated with the consumption of meat and meat products were calculated. Measured element concentrations in meat and meat products contributed to the provisional tolerable weekly intake level (PTWI) and tolerable weekly intake level (TWI) in the ranges (%): 1.13-43.5 (Al); 0.33-0.87 (As); 0.32-0.68 (Pb); 2-2.4 (Cd), and to the permitted daily exposure (PDE) values in the ranges (%): 0.03-0.17 (Cr); 0.003-0.18 (Ni). Results of comparison with the toxicological reference values suggest no concern with regard to exposure to the analysed elements for consumers who often consume these meats and meat products. The exception is Cr content, which may pose a problem given the values set by the national legislation. Lower concentrations of As, Al, Cd, Cr, Ni and Pb in meat and meat products were determined in this study in comparison with the available literature data from other countries.

Ključne riječi: *meat; meat products; toxic elements; risk assessment; ICP-MS*

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Introduction

Heavy metals are naturally present in the Earth's crust, though human activities such as agriculture, smelting, mining and metal production have an enormous effect on their environmental enhancement (Singh et al., 2011). The environmental contamination by toxic elements in recent decades has given rise to the significant and constant presence of an ecological and global public health concern due to their transfer into the food chain and significant impacts on consumer health (Ikem and Egiebor, 2005).

Accumulation of highly toxic elements, such as arsenic (As), cadmium (Cd) and lead (Pb) in foods such as meat and meat products may cause adverse effects on human health, affecting the nervous system, and accumulating in human adipose tissue and internal organs, thereby increasing the risk for cancer (Turkmen et al., 2008). Those elements pose a risk to human health even at trace levels and therefore are high on the list of public health concerns (Okogwu et al., 2019). Epidemiological studies have shown that As causes cancers of the lung, skin and bladder, as well as skin lesions (EFSA, 2009). Cadmium exposure over a long period of time can cause nephrotoxicity, osteotoxicity and immunotoxicity, and may cause lung, kidney, liver and prostate cancer (WHO, 2011). Chronic exposure to Pb can affect the nervous (lead-associated reduction in IQ in young children), cardiovascular (lead-associated increase in blood pressure), haematological, reproductive and immune systems, and also organs such as liver and kidney (WHO, 2011).

Accumulation of other microelements, such as aluminium (Al), chromium (Cr) and nickel (Ni) may also cause negative effects on health (Turkmen et al., 2008). Aluminium exposure can cause adverse effects on the reproductive and nervous systems and also is associated

with cognitive impairment, dementia and Alzheimer's disease (WHO, 2007; Krewski et al., 2007). Although Cr as Cr(III) plays an important role in the metabolism of sugar and fat, in the form Cr(VI) it can have genotoxic and cytotoxic effects (Okogwu et al., 2019). At higher levels, Ni stimulates adverse health effects such as cardiovascular and kidney diseases, allergies, lung fibrosis, and lung and nasal cancers (Peeters et al., 2017).

Therefore, risk evaluations of dietary exposure by elements from all types of food, including meat and meat products are necessary. A previous study showed that concentrations of essential and toxic trace elements vary widely, even in different cuts of beef, which also indicates large variations in the element content in meat products (López-Alonso et al., 2016; Gálvez et al., 2019). Toxic and trace element contents in meat and meat products have been reported in different countries: Chile (Munoz et al., 2005), Turkey (Demirezen and Uruc, 2006), Lebanon (Nasreddine et al., 2010), France (Arnich et al., 2012; Millour et al., 2012), United Kingdom (Rose et al., 2010), Sweden (Becker et al., 2011; Sand and Becker, 2012), Spain (González-Muñoz et al., 2008; González-Weller et al., 2013; Perelló et al., 2015), Serbia (Mitić et al., 2012), Iran (Abedi et al., 2011), Saudi Arabia (Alturiqi and Albedair, 2012), Brazil (Avegliano et al., 2011) and China (Liang et al., 2018).

The aim of this study was to determine the concentrations of toxic elements Al, As, Cd, Cr, Ni and Pb in meat and meat products and to verify whether the measured concentrations exceed the maximum prescribed levels set by the EU legislation. An additional aim was to compare the obtained concentrations with the literature data and with the defined recommended toxicological

limits, to determine a potential consumer health risk.

Materials and methods

Sample collection

During 2018, meat (pork and beef neck) and meat products (beef meat with beans, pork breakfast meat, chicken pâté, ham, and pork sausage) were purchased from supermarkets located in different Croatian cities. Samples were homogenized and kept stored and frozen at -18 °C until analysis.

Sample preparation

Meat and meat products (0.5 g) were digested with 2.5 mL HNO₃ (65% v/v), 2 mL H₂O and 1 mL H₂O₂ (30% v/v) using a high-pressure laboratory microwave oven Multiwave 3000 (Anton Paar, Ostfildern, Germany). The digestion programme consisted of three steps with potency: first step 500 W for 4 min, second step 1000 W for 5 min, third step 1200 W for 10 min. Digested samples were diluted to a final volume of 50 mL with ultra pure water.

Quantitative analysis was performed via the calibration curve method. Calibration curves were built with a minimum of five concentrations of standards per element. The limits of detection (LODs) were calculated as three times the standard deviation of 10 consecutive measurements of the reagent blank, multiplied by the dilution factor used for sample preparation. LOD values determined were (µg/kg): Al 10, As 4, Cd 3, Cr 2, Ni 10, Pb 2.

Element analysis

Inductively coupled plasma instrument with mass detector Agilent ICP-MS system Model 7900 (Agilent, Palo Alto, CA, USA) was used for element concentration measurement. The working parameters and experimental conditions for ICP-MS and mercury analyser are shown in Table 1.

Table 1. ICP-MS operating conditions and measurement parameters.

Torch injector	Quartz
Spray chamber	Peltier Cooled Cyclonic
Sample uptake	0.4 rps (rounds per second)
Nebulizer Type	MicroMist
Interface	Pt-cones
RF power	1550 W
Ar gas flow rate (L/min)	plasma 15; auxiliary 0.9
Nebulizer pump	0.1 rps
He gas flow rate	0.03 mL/min
Ion lenses model	x-lens
Lens voltage	10.7 V
Omega bias	- 90 V
Omega lens	10.2 V
Acquisition mode	Spectrum
Peak Pattern	1 point
Integration time	2000 ms
Replicate	3
Sweeps/replicate	100
Tune mode (Stabilization time; Integ.Time/mass)	No gas: 0 s; 0.1 s He: 5 s; 0.5 s HEHe: 5 s; 1 s
ICP-MS (standard mode)	No gas: Al ²⁹ , Pb ²⁰⁸ He mode: Cr ⁵² , Ni ⁶⁰ , Cd ¹¹¹ , As ⁷⁵
Internal standards	²⁰⁹ Bi, ¹¹⁵ In, ⁴⁵ Sc

Estimation of daily and weekly intake and comparison with toxicological values

The estimated daily intake (EDI) of the toxic metals Cr and Ni were calculated by the equation: $EDI = (C \times MS) / BW$, where C is the element content (µg/kg w.w.), MS is meal size (g per portion of meat and meat products), and BW is body weight (adult BW of 70 kg). The acute

food consumption survey conducted on adults in Croatia in 2011 showed a daily consumption of 226.96 g of meat and meat products (EFSA, 2011a).

The estimated weekly intake (EWI) of the toxic metals Al, As, Cd, and Pb were expressed as ($\mu\text{g}/\text{week}$) and was calculated by the equation: $\text{EWI} = \text{EDI} \times 7$.

The calculated EDI and EWI values were used to calculate the contributions of each element to the toxicological values (PTWI, provisional tolerable weekly intake; TWI, tolerable weekly intake; PDE, permitted daily exposure).

Statistical analysis

Statistical analyses were performed using STATA[®] 13.1 (StataCorp LP[®], USA). The element concentrations were expressed as the minimum and maximum, mean \pm standard deviation (SD). For concentrations below the LOD, a value of 50% of the LOD was assigned and these values were included in statistical testing (Clarke, 1998). Where all obtained results were below the LOD, the results are presented as <LOD. Statistically significant differences in element concentrations between meat and meat products were determined using the Student *t*-test. Statistically significant differences were expressed at a level of probability of 0.05.

Results and discussion

The mean concentrations and ranges of Al, As, Cd, Cr, Ni and Pb in meat and meat products are shown in Tables 2 and 3. Mean element concentrations in meat and meat products were measured in the ranges ($\mu\text{g}/\text{kg}$): Al 523-19,179, As 2.25-5.63, Cd 2.02-2.86, Cr 20.8-132.6, Ni 4.78-166.9, Pb, 3.53-7.49. Element concentrations were measured in the ranges ($\mu\text{g}/\text{kg}$): Al 10-32,180; As 1.50-6.92; Cd 1.30-5.63; Cr 2.32-263.2; Ni 1.22-290; Pb 1-28.9.

The highest mean element concentrations were found in: Al 19,179 $\mu\text{g}/\text{kg}$ in chicken pâté; As 5.63 $\mu\text{g}/\text{kg}$ and Cd 2.86 $\mu\text{g}/\text{kg}$ in ham; Cr 132.6 $\mu\text{g}/\text{kg}$ and Ni 166.9 $\mu\text{g}/\text{kg}$ in beef meat with beans; Pb 6.69 $\mu\text{g}/\text{kg}$ in pork sausage. Significant differences in the content of Al, As, Cr and Ni were determined between meat and meat products. Statistically higher Al concentrations were determined in: beef meat with beans compared to pork breakfast meat, beef and pork neck and ham; pork breakfast meat than in pork neck; chicken pâté than in pork neck and ham; pork neck than in ham ($P < 0.05$, all). Also, a statistically higher As content was found in ham than in other meat and meat products, with the exception of pork sausage ($P < 0.05$, all). Statistically lower Cr concentrations were determined in pork neck than in beef meat with beans and beef neck ($P < 0.05$, all). Statistically higher Ni levels were measured in beef meat with beans compared to pork neck and ham ($P < 0.05$, all). Also, a statistically higher Ni content was found in pork sausage than in beef neck ($P < 0.05$). There were no significant differences in the concentrations of Cd and Pb. All measured Cd and Pb levels were below the European Commission limits of 50 and 100 $\mu\text{g}/\text{kg}$ (EC, 2006).

Within the Total Diet Study (TDS) in France, levels of As, Al, Cd, Cr, Ni and Pb were determined in meat and meat products (Arnich et al., 2012; Millour et al., 2012; Noel et al., 2012). The French study reported lower Al concentrations (600 $\mu\text{g}/\text{kg}$) in meat than in the present study (Arnich et al., 2012; Millour et al., 2012). However, As, Ni and Pb levels found in meat and meat products in the ranges 20-36 $\mu\text{g}/\text{kg}$, 63-93 $\mu\text{g}/\text{kg}$ and 9-14 $\mu\text{g}/\text{kg}$ were higher than those in this study (Millour et al., 2012). Also, significantly higher Cr levels in the range 296-337 $\mu\text{g}/\text{kg}$ were determined in France than in this study (Noel et al., 2012). Cadmium contents found in this study were similar to those from France.

The TDS in the United Kingdom showed similar As, Cd and Pb levels in meat and meat products to those found here (Rose et al., 2010). However, the present study found higher Al levels in meat and Ni and Cr in meat products than in the UK (240, 70 and 37 µg/kg), while Al in meat products and Ni and Cr in meat were similar. Significantly higher Al (4640 µg/kg), Cr (870 µg/kg) and Ni (445 µg/kg) in meat and meat products were determined in the TDS in Catalonia, Spain in comparison with this study (Perelló et al., 2015).

In relation to the As and Pb concentrations determined in this study, the values presented in Chile were significantly higher in meat (34 and 112 µg/kg) and meat products (24 and 98

µg/kg) (Munoz et al., 2005). Very high concentrations of As (43 and 77 µg/kg), Cd (3 and 15 µg/kg), Cr (483 and 504 µg/kg) and Pb (29 and 201 µg/kg) were measured in pork and beef meat from China, a highly industrialized country with a dense population (Liang et al., 2018). Also, higher Cd (8.6 and 7.9 µg/kg), Cr (88 and 540 µg/kg), Ni (130.2 and 121 µg/kg), and Pb (125 and 115 µg/kg) concentrations were measured in meat and minced meat from Turkey (Demirezen and Uruc, 2006).

Significantly lower levels of Cr, Cd, Ni and Pb in ham were found in the present study than in smoked pork meat from Serbia, with concentrations (µg/kg): Cd 50, Ni 510 and Pb 52 (Mitić et al., 2012). In different sausage types from Iran, Cd

Table 2. Concentrations of Al, As, Cd, Cr, Ni and Pb in meat and meat products from the Croatian market.

Meat and meat products	N	Mean ± SD (µg/kg)					
		Al	As	Cd	Cr	Ni	Pb
Beef meat with beans	5	2703 ± 347.6 ^a	3.04 ± 1.66 ^e	2.39 ± 1.03	132.6 ± 90.5 ^e	166.9 ± 115.5 ^g	3.53 ± 3.22
Pork breakfast meat	4	1542 ± 942.9 ^{ab}	2.69 ± 1.15 ^e	2.24 ± 1.48	38.5 ± 39.8	80.4 ± 72.1	6.69 ± 10.1
Pork neck	5	523.0 ± 114.2 ^{bc}	2.25 ± 0.46 ^e	< 3	20.8 ± 5.55 ^{ef}	29.5 ± 29.3 ^g	3.25 ± 3.60
Beef neck	4	497.2 ± 132.5 ^a	2.72 ± 0.99 ^e	< 3	44.5 ± 20.5 ^f	4.78 ± 0.38 ^h	< 2
Ham	5	1636 ± 966.3 ^{acd}	5.63 ± 1.39 ^e	2.86 ± 1.27	32.5 ± 15.5	22.1 ± 22.4 ^g	5.16 ± 5.68
Chicken pâté	5	19179 ± 16020 ^{cd}	2.52 ± 0.97 ^e	2.56 ± 1.80	45.0 ± 40.9	89.7 ± 67.1	5.75 ± 8.84
Pork sausage	5	2460 ± 2704	3.71 ± 2.29	2.02 ± 1.16	67.4 ± 56.4	51.6 ± 27.1 ^h	7.49 ± 12.0

Statistically significant differences $P < 0.05$: Al^{abcd}; As^e; Cr^{ef}; Ni^{gh}

Table 3. Minimum and maximum concentrations of Al, As, Cd, Cr, Ni and Pb determined in meat and meat products from the Croatian market.

Meat and meat products	N	Minimum – Maximum (µg/kg)					
		Al	As	Cd	Cr	Ni	Pb
Beef meat with beans	5	2176 - 3104	2.0 - 5.95	1.50 - 3.96	43.1 - 263.2	5.0 - 290.0	1.0 - 8.84
Pork breakfast meat	4	209.0 - 2430	2.0 - 4.39	1.50 - 4.47	2.32 - 92.3	24.4 - 183.6	1.0 - 21.8
Pork neck	5	330.4 - 628.2	2.0 - 3.08	< 3	14.8 - 28.8	1.22 - 72.5	1.0 - 9.27
Beef neck	4	365.1 - 630.0	2.0 - 3.85	< 3	22.5 - 63.2	4.35 - 5.00	< 2
Ham	5	850.3 - 2983	4.0 - 6.86	1.50 - 4.30	16.8 - 56.8	6.58 - 60.8	1.0 - 14.5
Chicken pâté	5	1322 - 32,180	1.50 - 3.59	1.45 - 5.63	2.91 - 106.3	5.0 - 147.6	1.0 - 21.5
Pork sausage	5	10.0 - 6695	2.0 - 6.92	1.50 - 4.09	3.00 - 156.8	7.0 - 73.1	1.0 - 28.9

and Pb levels were determined in the range 2.2–13.5 µg/kg and 24–158.7 µg/kg, which is similar to the Cd values but higher than the Pb values obtained for pork sausage in this study (Abedi et al., 2011). Similar concentrations of Cd (2 µg/kg) and Pb (4 µg/kg), but lower Ni (< 17 µg/kg) and Cr (19 µg/kg) were reported in meat products from Sweden than in the present study (Becker et al., 2011). Significantly higher Cr concentrations of 708 and 402 µg/kg were reported in ham and sausage from Spain than those in this study (González-Weller et al., 2013).

Given the fact that certain food additives and contaminants in foods have adverse effects and increase the incidence of cancer, the World Health Organization (WHO) has defined critical levels such as the provisional tolerable weekly intake level (PTWI) or tolerable weekly intake level (TWI) dietary as the guidelines for health authorities and institutes (WHO, 2005). The PTWI values defined for toxic metals are (µg/kg/BW): Al 2000 (WHO, 2012), As 15 (WHO, 1989); Pb 25 (WHO, 2000). The TWI value established for Cd is 2.5 µg/kg/BW (EFSA, 2011b). The European

Medicines Agency (EMA) defined the permitted daily exposure (PDE) for the elements: Cr 250 µg/day; Ni 300 µg/day (EMA, 2008).

An estimation of the dietary daily (EDI) and weekly (EWI) intakes of elements associated with the consumption of meat and products were calculated and are presented in Table 4. All obtained EWI and EDI values were below the defined PTWI, TWI and PDE values. The measured Al concentrations in meat and meat products obtained in this study contributed to the PTWI from lowest 11.3 µg/week/BW obtained in beef neck to the highest 435.3 µg/week/BW found in chicken pâté. Measured As, Pb and Cd contents in meat and meat products contributed to PTWI and TWI values between (%): 0.33-0.87; 0.32-0.68; 2-2.4.

The measured Cr and Ni concentrations in meat and meat products contributed between 0.03-0.17% and 0.003-0.18% to the PDE values.

According to the national legislation in Croatia, the permitted Cr content is 40 µg, which refers to the concentration intended for consumption as a single

Table 4. Estimation of daily and weekly intakes (EDI, EWI) of elements in meat and meat products and toxicological values.

Meat and meat products	EWI (µg/week/BW)				EDI (µg/day)	
	Al	As	Cd	Pb	Cr	Ni
Beef meat with beans	61.3	0.07	0.05	0.08	0.43	0.54
Pork breakfast meat	35.0	0.06	0.05	0.15	0.12	0.26
Pork neck	11.9	0.05	-	0.07	0.07	0.10
Beef neck	11.3	0.06	-	-	0.14	0.01
Ham	37.1	0.13	0.06	0.12	0.11	0.07
Chicken pâté	435.3	0.06	0.06	0.13	0.14	0.29
Pork sausage	55.8	0.08	0.05	0.17	0.22	0.17
PTWI ^a / TWI ^b / PDE ^c	1000	15	2.5	25	250	300

^a PTWI (provisional tolerable weekly intake; µg/kg/BW): Al (WHO, 2012), As (WHO, 1989); Pb (WHO, 2000).

^b TWI (tolerable weekly intake; µg/kg/BW): Cd (EFSA, 2011b),

^c PDE (permitted daily exposure; µg/day): Cr, Ni (EMA, 2008).

meal (Anonymous, 2013). If this prescribed quantity is applied to the determined quantities of Cr in meat and meat products in this study, it can be concluded that the Cr contents in beef meat with beans, beef neck, chicken pâté and pork sausage were above the prescribed value. This indicates the need for continuous monitoring of Cr content in meat and meat products. For example, by comparing measured concentrations of Cr in meat and offal between the first TDS (Leblanc et al., 2005) and the second TDS in France (Noel et al., 2012), the second TDS reported Cr levels was 3.3 times higher than in the first survey.

Conclusions

Meat and meat products represent an important and representative food ingredient in the human diet. It is therefore important to know the content of toxic elements that pose a serious threat to health due to toxicity, bioaccumulation and biomagnification in the food chain. This study determined the differences in the concentrations of As, Al, Cr and Ni between the analysed meat and meat products. Similarly, Cd and Pb contents were measured with no significant differences between products. The highest mean concentrations of elements were found in: Al in chicken pâté; As and Cd in ham; Cr and Ni in meat with beans; Pb in pork sausage.

The concentrations of As, Al, Cd, Cr, Ni and Pb determined in meat and meat products in this study were mostly lower than reports in the available literature for meat and meat products from other countries.

Comparison of the measured As, Al, Cd, Cr, Ni and Pb concentrations with the defined toxicological values suggests no concern with regard to exposure to the analysed elements for consumers who often consume these tested meat and meat products. The exception is Cr

content, which may pose a problem given the values set by national legislation.

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Procjena zdravstvenog rizika izloženosti toksičnim elementima u mesu i mesnim proizvodima iz Hrvatske

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Cilj ovog istraživanja bio je određivanje koncentracija toksičnih metala arsena (As), kadmija (Cd) i olova (Pb), kao i aluminijska (Al), kroma (Cr) i nikla (Ni) u mesu (svinjska i goveđa vratina) i mesnim proizvodima (meso s grahom, mesni doručak, pileća pašteta, šunka i svinjska kobasica) nabavljenih u trgovačkim lancima različitih hrvatskih gradova. Koncentracije elemenata analizirane su primjenom masene spektrometrije induktivno spregnute plazme (ICP-MS). Srednje koncentracije elemenata u mesu i mesnim proizvodima mjerene su u rasponu ($\mu\text{g}/\text{kg}$): Al 523-19179, As 2,25-5,63, Cd 2,02-2,86, Cr 20,8-132,6, Ni 4,78-166,9, Pb 3,53-7,49. Najveće srednje koncentracije elemenata određene su u: Al u pilećoj pašteti, As i Cd u šunki, Cr i Ni u mesu s grahom, Pb u svinjskoj kobasici. Sve izmjerene koncentracije Cd i Pb bile su ispod granica Europske komisije od 50 i 100 $\mu\text{g}/\text{kg}$. Ustvrdene su statistički značajne razlike u sadržaju Al, As, Cr i Ni između mesa i mesnih proizvoda. Nije bilo značajnih razlika u koncentracijama Cd i Pb. Izračunata je dnevna (EDI) i tjedna (EWI) količina unesenih elemenata povezanih s

potrošnjom mesa i proizvoda. Određene koncentracije Al, As, Pb i Cd u mesu i mesnim proizvodima pridonijele su privremenim podnošljivim tjednim nivoima unosa (PTWI) i podnošljivom tjednom unosu (TWI) u rasponima (%): 1,13-43,5; 0,33-0,87; 0,32-0,68; 2-2,4. Također, koncentracije Cr i Ni izmjerene u mesu i mesnim proizvodima pridonijele su dopuštenim dnevnim vrijednostima izlaganja (PDE) u rasponima (%): 0,03-0,17 i 0,003-0,18. Rezultati usporedbe s toksikološkim referentnim vrijednostima ne ukazuju na zabrinutost s obzirom na izloženost analiziranim elementima za potrošače koji često konzumiraju istražene vrste mesa i mesnih proizvoda. Izuzetak je sadržaj Cr, što može predstavljati problem s obzirom na vrijednosti ustvrđene nacionalnim zakonodavstvom. U ovom istraživanju ustvrđene su uglavnom niže koncentracije As, Al, Cd, Cr, Ni i Pb u mesu i mesnim proizvodima u usporedbi s dostupnim literaturnim podacima u mesu i proizvodima iz drugih zemalja.

Ključne riječi: meso, mesni proizvodi, toksični elementi, procjena rizika, ICP-MS