Environ Sci Pollut Res (2016) 23:4465–4472
DOI 10.1007/s11356-015-5658-1

RESEARCH ARTICLE

Toxic heavy metals in the muscle of roe deer (*Capreolus capreolus*) —food toxicological significance

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Received: 26 June 2015 / Accepted: 20 October 2015 / Published online: 28 October 2015

Abstract The study was performed on 20 (10 males, 10 females) roe deer (*Capreolus capreolus*) to investigate the concentration of cadmium, lead, mercury, and arsenic in the muscle tissue. They reside in forest and meadow, about 50 km distance from industrial activities and traffic. Samples were taken from the *musculus biceps femoris* of each deer without external contamination after shooting during the regular hunting season on a hunting area close to Eger in Hungary. The determination of heavy metal contents was carried out by inductively coupled plasma optical emission spectrometry (ICP-OES). The statistical analysis was performed by statistical package for the social sciences (SPSS) version 11.0. The measured residue concentration of cadmium was below the limit of detection in the roe deer meat indicating no health risk for the consumers. The average lead concentration (0.48± 0.21 mg/kg wet weight) exceeded the regulated maximum limit, but its calculated weekly intake was below the provisional tolerable weekly intake (PTWI). The residue level of mercury is not regulated and the average mercury content of roe deer meat (0.87±0.40 mg/kg wet weight) was about half of PTWI, but the consumption of meat with the highest detected concentrations results in higher PTWI than recommended. The measured concentration of arsenic (0.27± 0.20 mg/kg wet weight) in the roe deer meat may not pose any health risk for the human consumers according to the PTWI set by the World Health Organization.

Keywords Heavy metals · Food safety · Accumulation · Food chain · Roe deer

Responsible editor: Philippe Garrigues

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Introduction

Game meat may be contaminated with metals and metalloids such as cadmium, lead, mercury, and arsenic if animals reside in anthropogenically polluted areas or if ammunition used to kill the game contaminates the meat (Taggart et al. 2011).

Cadmium occurs naturally in the Earth's crust as a part of cadmium-rich geological materials and as a consequence of volcanic eruptions and exfoliation of rocks and minerals (Pacyna and Pacyna 2001). Anthropogenic sources of cadmium include industrial emissions (metal industry, industrial and agricultural wastes, coal combustion, phosphate fertilizers manufactures) and urban pollution (incineration of municipal solid waste, road dust, heating) (EFSA 2009a). Industrial activities are the main sources of cadmium release to the air, and emissions from anthropogenic sources have been found to exceed those of natural origin by an order of magnitude (ATSDR 1999a). Cadmium pollutants present in the air may be transported from a hundred to a few thousand kilometers and have a typical atmospheric residence time of about 1–10 days before deposition occurs by wet or dry processes (Elinder 1985; ATSDR 1999a).

Cadmium concentrates in grasses and food crops, just as in earthworms, domestic animals, and wildlife (ATSDR 1999a).

In food-producing animals, the highest quantity of cadmium can be found in the kidneys and the liver. Cadmium is one of the most toxic heavy metals. It impairs the functioning of several organs, with its nephrotoxic potential being the most significant. Furthermore, cadmium belongs to the carcinogenic compounds; its carcinogenic effect is probably indirect and of epigenetic character (Laczay 2015).

Lead is used in some types of ammunition, which can lead to direct and indirect contamination of the meat and organs of animals when shoot by hunters using this type of ammunition and processed game animal foodstuffs (Taggart et al. 2011). Because of high toxicity of elemental lead as pellet and its all compounds, it is considered as one of the most important metallic environmental pollutants, and the use of lead is strictly regulated.

The typical concentration of lead in the crust of the Earth is 13 mg/kg, but significantly higher amounts can be found for example along highways where the emissions of lead-based fuel still is present in the soil (ATSDR Agency for Toxic Substances and Disease Registry 1999b; IPCS 1989). Contaminated soil is one of the most important sources of lead exposure for animals because it can serve different dietary components for certain wild vertebrate species (Reglero et al. 2008). Old paint, batteries, and other products that contain lead can contaminate the soil and expose the animals to above maximum tolerable concentrations (Waldner et al. 2002). Lead with the highest concentration is found in the roots of plants that live in acidic soil with low organic matter content. The concentration of lead in edible plants is usually below 0.05 mg/kg wet weight (NRC 2005).

Like cadmium, lead exerts diverse toxic effects on living organisms. Of these, its inhibitory effect on heme synthesis and its neurotoxic effect exerted on the central nervous system are the most important in humans (Laczay 2015).

Mercury is very rare in the crust of the Earth with a mass of only 0.08 mg/kg (Ehrlich and Newman 2008). But where mercury ores are found, the concentration is usually very high, and the lowest concentration in the ores is 0.1 %, which is far greater than the average concentration in the crust. Mercury can be found in higher concentrations around volcanoes or in hot springs, soils, and vegetation (and fungi) in regions with mercuriferous belts (Kojta el al. 2011, 2012). During volcanic eruptions, the concentration of mercury in the atmosphere can increase four to six times. Half of the emissions into the atmosphere are caused by volcanic eruptions, the other half by man-made pollution, particularly comes from power plants that use combustion of coal to produce electricity (Ehrlich and Newman 2008).

Once released into the environment, mercury may undergo a series of complex transformations and cycles between the atmosphere, ocean, and land. The three chemical forms of mercury are elemental or metallic mercury (Hg^0), inorganic mercury (Hg_2^{2+} and Hg^{2+}), and organic mercury (mostly methyl mercury). The predominant form of it in foods other than fish and shellfish is inorganic mercury (JECFA-959 2011).

The maximum tolerable level of inorganic mercury in ruminants is not defined because of a lack of studies and because of its methylation in the rumen, which may create a higher bioavailability. For chronic consumption of methylmercury, the maximum tolerable level is 2 mg/kg diet in ruminants (NRC 2005).

Inorganic mercury is nephrotoxic, causing damage to the renal tubular epithelium and immunological glomerular disease. At higher dosages, it may also cause severe gastrointestinal alteration, just as hematological and hepatic disorders (JECFA-959 2011).

Arsenic is found in the crust of the Earth with an average concentration of 1.5–3 mg/kg (Mandal and Suzuki 2002). It was frequently applied in the past as insecticides and herbicides in the agriculture or as a preservative for wood products, and it is used until now in some countries as anabolic for food-producing animals. It is not considered as an essential part of the diet of animals or humans (Research Council 2005). However, research has shown that arsenic deprivation may cause decreased growth and abnormal reproduction (Nielsen 1998).

The concentration of arsenic in grass species is about 100 μg/kg dry matter (DM), grass that grows close to industrial sites, like a lead smelter, have been measured with concentrations up to 62 mg/kg DM. Most of the contamination of plants not only occurs through their roots, but can also occur, to a lesser extent, through aerial pollution (Woolson 1983; Chilvers and Peterson 1987). Sheep and cattle have been found to not find arsenic contaminated feedstuff repulsive; on the contrary, ruminants have been observed selectively eating contaminated feedstuff (Clarke and Clarke 1975).

The main adverse effects reported to be associated with long-term ingestion of inorganic arsenic in humans are skin lesions, cancer (skin and urinary bladder), developmental toxicity, and neurotoxicity (EFSA 2009b).

Commission of the European Communities (EC) regulated the maximum levels of certain contaminants in foodstuffs, including several heavy metals such as lead, cadmium, mercury, and inorganic tin (Commission Regulation 2006). However, it does not regulate the safe level of important contaminants (e.g., arsenic, mercury) originated from the nature or due to as man-made hazard in edible tissues of food-producing mammals and bird species and game animals (e.g., roe deer or other cervids). The maximum levels for lead and cadmium are listed for bovine meat and offal; however, there are no limit data of meat and edible tissues for mercury and arsenic in mammal and bird species (Table 1).

This regulation has been changed and updated a number of times, and therefore, the Commission wanted to make a new regulation that takes into consideration the new information and research available regarding this topic. It would be important because the anthropogenic pollution and the

Table 1 Maximum levels for lead and cadmium in the European Union (Commission Regulation 2006)

Foodstuffs	Maximum levels (mg/kg wet weight)	
	Lead	Cadmium
Meat (excluding offal) of bovine animals, sheep, pig, and poultry	0.10	0.05
Offal of bovine animals, sheep, pig, and poultry	0.50	–
Liver of bovine animals, sheep, pig, poultry, and horse	–	0.50
Kidney of bovine animals, sheep, pig, poultry, and horse	–	1.0

environmental areas naturally enriched in toxic heavy metals where game animals reside can increase the possibility of risk that the contaminants enter the food chain and induce elevated level of them in game. Furthermore, the lead-containing bullet can remain in processed meat products as a secondary contamination.

According to Ramanzin et al. (2010), the average consumption of ungulate meat in Europe, is 0.1–0.3 kg per inhabitant per year, but in some regions where hunting is popular, the amount can even be 1.0–4.0 kg per inhabitant per year. For the hunters and their families that consume the meat from game, for example, roe deer, heavy metal accumulation in the meat could pose a food safety risk if the concentrations are high and the intake is above the average European intake.

The regulation states that it is crucial to keep the contaminants in the foodstuff at levels that are toxicologically acceptable. This is important for the public health of the European population. The maximum levels of contaminants should be at a level which is possible to achieve by following good standards in regards to the agriculture and manufacturing practice. When the contaminants are considered to be genotoxic carcinogens or when the foodstuff is intended for especially vulnerable groups, for example, infants and young children, the levels should be "as low as reasonably achievable". Products that have too high levels of contaminants should not reach the market, and they should not be mixed or diluted with other products that are intended for the market (Commission Regulation 2006).

As the consumption of game meat may pose a rather poorly regulated risk to human health, the present study was undertaken to determine the concentration of heavy metals and metalloids (cadmium, lead, mercury, arsenic) in the muscle tissue of the roe deer and evaluate the related potential risk to human consumers.

Materials and methods

Animals

The study was performed on 20 (10 males, 10 females) roe deer living in a landscape with forest and land used for agricultural purposes on a hunting area close to Eger in Heves county of Northeast Hungary (Fig. 1). There is industrial activity like lignite, copper, and non-ferrous metal mine, and a busy highway within about 50 km distance from the hunting area.

The roe deer is a member of the Artiodactyla order and of the Cervidae family and Odocoileinae subfamily. They can be found in Asia and widespread in Europe. They feed on grass, leaves, berries, and young shoots. They are adapted to consume easily digestible forages that are rich in cell solubles. The concentration of heavy metals in the tissues of roe deer depends on the dietary intake of these contaminants from different potential sources. Based on the investigation of Tixier et al. (1997), roe deer preferred different species of plants [e.g., bluebells (*Hyacinthiodes non-scripta*), hornbeam (*Caprinus betulus*), ivy (*Hedera helix*), haw thorn (*Crataegus monogyna*)], making it a generalist herbivore. Besides, fungi are seasonally important nutrients for roe deer; thus, they might be an important source of heavy metals due to the high accumulative potential of fungi (Bargagli and Baldi 1984; Demirbaş 2002; Falandysz and Borovička 2013; Falandysz et al. 2012a, 2012b; Kalač et al. 1991; Larsen et al. 1998; Pokorny et al. 2004).

The muscle samples were taken from the *musculus biceps femoris* of each deer after shooting during the regular hunting season. It starts with October 1 till the end of February for female roe deer and from April 15 till September for the male roe deer.

A small piece of muscle, around 1–3 g, was immediately removed from each deer without external contamination. Collected samples were cooled in a portable cooler and transported to the analytical laboratory and stored at −80 °C freezer until analysis.

Analytical method

Chemicals used

Deionized water was produced by a Vitro-Tech Lab vdb-3A two-step water purification system. The salt-free water obtained at the end of the second step had 1.4 µs electronic conductivity. Concentrated nitric acid (HNO_3) and hydrochloric acid (HCl) were obtained from Scharlau. For sample digestion, aqua regia (nitro-hydrochloric acid) solution was prepared freshly from concentrated nitric acid (65 m/m%) and hydrochloric acid (37 m/m%) in a volume ratio of 1:3. All the

Fig. 1 Location of the study

laboratory glassware and plastic tools were cleaned by 0.15 M nitric acid (HNO_3) solution, then rinsed with deionized water.

Analytical standards, ICP measurements

ICP multi- and mono-element standards used for quantitative ICP measurement were obtained from MERCK. Argon gas of 4.6 purity was from Linde. QC standards were prepared from standard bovine liver (NIST-1577C).

Sample preparation

One gram from each roes was weighted into a closed vessel after homogenization, and 12 mL aqua regia was added to each sample then digested for 60 min at 150 °C in a Gerhardt Kjeldatherm KB40 block digestion system. The samples were left to cool down and then made up to 50 mL with deionized water. These diluted samples were filtered into 100-mL Erlenmeyer flasks on Macherey Nagel MN619Gs phosphate-free filter paper. The filtered samples were stored in 25-mL plastic bottles until the analysis. Blank samples were also prepared by the same method, without adding roe flesh. QC samples were prepared from NIST-1577C standard by the same method.

Analytical measurements

Determination of heavy metals was carried out by a Perkin Elmer Optima 3300 DV inductively coupled plasma optical emission spectrometry (ICP-OES) instrument among the following measurement parameters: RF generator, 40 MHz; RF power, 1500 W; nebulizer type, concentric (Meinhard Type A); nebulizer gas flow rate, 0.9 dm^3/min; cooling water flow rate, 1 dm^3/min; sheath gas flow rate, 0.9 dm^3/min; sample feeding flow rate, 0.9 cm^3/min; and observation height, 15 mm.

Calibration range was between 0.5 and 250 mg/kg covered by 9 calibration points (0.5, 1, 2.5, 5, 10, 25, 50, 100, and 250 mg/kg standard solutions). The calibration curve was accepted if at least by 7 points the calculated and the nominal concentrations did not deviate more than 15 %. Limit of detection (LOD) was 0.001 mg/kg for cadmium and mercury, 0.01 mg/kg for lead, and 0.02 mg/kg for arsenic.

Internal quality control of the measurements was carried out via measuring QC samples of known heavy metal concentration at least ten times. After discarding the extremes, the standard deviation of data (s) was established, which must have remained within the ±15 % of the nominal concentration value in order to accept the QC measurement.

Exposure calculation

The dietary weekly exposure was calculated based on the detected concentration of heavy metals in the samples and a standard portion of 200 g meat consumed by a human consumer that is sometimes used for assessment. This amount was then divided with an average body weight of man (60 kg) and multiplied with 7 (days/week).

Statistical analysis

t test was used to compare the heavy metal concentration between males and females. It was calculated using the TTEST equation with two-tailed distribution and two-sample unequal variance (heteroscedastic). Those samples in which the concentration was below the limit of detection were excluded. The statistical analysis was performed by statistical package for the social sciences (SPSS) version 11.0.

Results and discussion

The heavy metal concentrations (mg/kg wet weight, w.w.) of muscle tissue of males, females and of both sexes, just as their statistical differences, are presented in Table 2.

Table 2 Heavy metal content of the muscle tissue of roe deer (mg/kg wet weight)

Metal	Male Mean±SD Range	Female Mean±SD Range	Both sexes Mean±SD Range	Statistical difference
Cadmium	<LOD	<LOD	<LOD	–
Lead	0.42±0.22a 0.04–0.74	0.55±0.20a 0.30–0.82	0.48±0.21 0.04–0.82	0.26
Mercury	1.07±0.30a 0.70–1.46	0.61±0.38b 0.24–1.43	0.87±0.40 0.24–1.46	0.02
Arsenic	0.33±0.18a 0.12–0.45	0.23±0.23a 0.03–0.51	0.27±0.20 0.03–0.51	0.57

a-a in pairs, not significant; a-b in pairs, statistical difference between males and females ($p<0.05$)

Concentration of cadmium was below the LOD in each sample. In 2003, due to the possible atmospheric deposition, the level of cadmium was 4.2 mg/kg dry weight (d.w.) in mosses, which was used as bioindicator of heavy metal burden of an area, in Hungary around Miskolc which is close to our investigated site because of industrial activities (Ötvös et al. 2003). The very small concentration of cadmium in roe deer meat measured in our study is probably due to the reduced industrial emission during the past 10–15 years.

Similarly, low residue level of cadmium (0.006± 0.004 mg/kg w.w.) was detected in roe deer in central region of Poland where the game reside in forest and meadow far away from heavy industry (Długaszek and Kopczyński 2013). Residue of cadmium of roe deer meat was <5–64 μg/kg w.w. in the northern part of Poland in 1987–1991 (Falandysz 1994).

However, cadmium concentration may be elevated due to pollution in nearby area or emission that can be transported by the air from locations far away. The detected level of cadmium was higher (0.22±0.13 mg/kg d.w.) in red deer (*Cervus elaphus*) in the northeastern part of Poland (Jarzyńska and Falandysz 2011). Highly elevated residue of cadmium ranged from 0.007 to 1211 mg/kg d.w. was detected in Spain at an area historically used for mining, smelting, and refining various metals and metalloids (Taggart et al. 2011).

The provisional tolerable monthly intake (PTMI) value of cadmium set by World Health Organization (WHO) is 25 μg/kg (JECFA-960 2011).

In spite of the fact that the international scientific literature present data about the high level of cadmium in the tissues of different game animals, the existing European regulation (Commission Regulation 2006) does not contain the maximum level for it in game meat (and offal) only for meat of bovine species.

The residue of lead was detected in the muscle of 18 roe deer (9 males, 9 females). The concentration of lead was found to be 0.48±0.21 mg/kg w.w. on average with a range of 0.04–0.82 mg/kg w.w. The average level of it was about 30 % higher in females (0.55±0.20 mg/kg w.w.) than that in males (0.42±0.22 mg/kg w.w.) without statistical difference.

The recommended provisional tolerable weekly intake (PTWI) value established by WHO is 25 μg/kg for lead (JECFA-960 2011), and the maximum residue level of it is 0.10 mg/kg w.w. for bovine species (Commission Regulation 2006). Based on our measured concentrations, if a consumer consumes a 200-g steak of roe deer meat daily, the total weekly intake of lead will be 0.89–19.2 μg/kg (on average, 11.2 μg/kg) that is below the PTWI. However, the measured average concentration in the muscle was above the permitted residue level for bovine animals that may pose a risk for the consumers during a long-term consumption.

Długaszek and Kopczyński (2013) measured a similar level of lead (0.58±0.39 mg/kg w.w.) in the meat of roe deer at the central region of Poland. However, Medvedev (1999) detected much higher lead concentration in the muscle of moose (*Alces alces*) (1.43±0.17 mg/kg w.w.) and reindeer (*Rangifer tarandus fennica*) (2.14±0.47 mg/kg w.w.) in Karelia, Russia that exceeds the maximum level, i.e., 0.10 mg/kg, established for meat of bovine animals (Commission Regulation 2006). Similarly, the average level of it was above the regulated limit in the muscle of roe deer (0.18 mg/kg d.w.) at Warmia and Mazury region of Poland (Jarzyńska and Falandysz 2011). The range of lead level in the meat of roe deer that was <10–2600 μg/kg w.w. in the northern part of Poland in 1987–1991 (Falandysz 1994) exceeded the maximum level by the highest detected concentrations.

Dietary exposure of lead is ranged from 0.36 to 2.43 μg/kg body weight (b.w.)/day in adult high consumers, from 0.21 to 0.94 μg/kg b.w./day in infants and from 0.80 to 3.10 μg/kg b.w./day in children (up to 5.51 μg/kg b.w./day in high consumers) in Europe, respectively. European Food Safety Agency (EFSA) (2010) concluded that the valid PTWI is no longer acceptable as there is no safe threshold for critical lead-induced effects, particularly nephrotoxicity in adult people and developmental neurotoxic alterations in the fetus and mainly in young children from 1–7 years of age (Jedrychowski et al. 2009; Jusko et al. 2008).

The muscle is not the dominant site of lead accumulation, the tissue with the highest concentrations of it is the kidney, the brain, the spleen, and, certainly, the skeleton and other bone tissues, such as the teeth and antlers. However, lead-containing bullets are fragmented in the muscle after shooting, and the very small fragments can be scattered radiately into the tissues (Cornatzer et al. 2009). Their complete removal is almost impossible, and they can remain as a secondary contamination in the processed game meat resulted in higher level of lead in the muscle. Cornatzer et al. (2009) detected 4200–55,000 μg/g d.w. lead levels in packaged ground venison, and Tsuji et al. (2009) measured 2948 μg/g d.w. in white-tailed deer (*Odocoileus virginianus clavium*) and 19, 468 μg/g d.w. in caribou (*Rangifer tarandus caribou*) muscle. If the hunter does not take suitable precautions during the handling of hunted game, the parts of the animal that are contaminated by a lead-based bullet may be ingested which might be a health risk for the human consumers (Bernhoft 2013; Lindboe et al. 2012; Stokke et al. 2010).

Despite the results of scientific research, there is no regulated maximum level of lead in force for game meat in the European Union. However, for example, the Norwegian Food Safety Authority published a dietary recommendation for the intake of meat from cervids in August 2013. It stated that pregnant women, women in fertile age, and children should not eat the meat of cervids more than once a month.

Mercury was detected in 17 roe deer (9 males, 8 females). The mean concentration of mercury determined in the roe deer was 0.87±0.40 mg/kg w.w. There was a statistical difference ($p<0.05$) in the average level between the males (1.07±0.30 mg/kg w.w.) and the females (0.61±0.38 mg/kg w.w.). The highest concentration found was 1.46 mg/kg w.w., and the lowest concentration was 0.24 mg/kg w.w. These variations may be due to a difference in age, sex, and nutrition habitat. The absorption of mercury in young animals is far greater than that in old animals, and this may lead to differences in tissue concentration even if the roe deer ingest the same feedstuff (Kostial et al. 1979). The research by Pokorny et al. (2004) showed that mercury in fungi may significantly affect the tissue concentrations of mercury in the roe deer. The mercury levels measured in the present study was much higher than those reported in the muscle of roe deer in Poland between 1985 and 1991 (Falandysz 1994; Falandysz and Gajda 1988). The reason of it is unclear, but it might be due to their difference in fungal ingestion.

According to the WHO, the PTWI value is 1.6 μg/kg for methylmercury and 4 μg/kg for total mercury (JECFA-776 1989; JECFA-959 2011). With a daily intake of 200 g meat, a consumer would ingest 0.173 mg mercury/day on average corresponding to an average intake of 2.88 μg/kg with individual values between 0.8 and 4.9 μg/kg. While the calculated average intake is below the PTWI, the individual values may exceed it.

Previously, the concentration of mercury was regulated in the raw game meat and game meat product as a maximum of 0.05 mg/kg wet weight in many countries in national regulations (e.g., 17/1999 EüM regulation in Hungary), but the existing European regulation does not contain any related tolerance limit (Commission Regulation 2006).

Arsenic was detected in 8 of the investigated 20 roe deer (3 males, 5 females). Its average concentration was found to be 0.27±0.20 mg/kg w.w. with a range of 0.03–0.51 mg/kg w.w. The mean arsenic content was more than 40 % higher in males (0.33±0.18 mg/kg w.w.) than that in females (0.23±0.23 mg/kg w.w.), but the difference was not statistically significant. Some ruminants will prefer feedstuff with arsenic (Clarke and Clarke 1975); thus, it might be possible that an individual can ingest higher amounts than the usual of arsenic and thereby had a higher concentration in its tissues. Fungi may be a source of arsenic for the roe deer, and especially during the summer and autumn, the concentration of fungi found in the feces of roe deer can increase (Pokorny et al. 2004). Arsenic level was found to be 0.041 to 0.649 mg/kg d.w. in the muscle of red deer in Spain at a mined area (Taggart et al. 2011). The concentration of arsenic in the muscle tissue of cattle is usually below 20 μg/kg w.w. (Michels 1986). The maximum tolerable level of arsenic in the diet of domestic animals is 30 mg/kg diet.

The PTWI value of arsenic set by the WHO was previously 15 μg/kg b.w. (JECFA-776, 1989; JECFA-959, 2011). The WHO withdraws this PTWI value and has not yet created a new one. Based on the previous PTWI value (15 μg/kg b.w.) and the highest level of arsenic (0.51 mg/kg) in the roe deer meat measured in our study, a person weighing 60 kg would ingest 11.8 μg/kg arsenic if 200 g meat/day is consumed. It shows that the measured level of arsenic in the meat would not cause a health risk for the consumer.

Based on a survey across 19 European countries performed by the EFSA, the inorganic arsenic exposure from food and water is between 0.13 and 0.56 μg/kg b.w./day for average consumers and is ranged from 0.37 to 1.22 μg/kg b.w./day for 95th percentile consumers. Dietary exposure of children under 3 years of age is two-to threefold higher than that in adults. Based on the available scientific data, EFSA concluded that the present PTWI is no longer appropriate because data had shown that inorganic arsenic induces cancer in the lung, urinary bladder, and skin at lower exposures than those reviewed by the JECFA (EFSA 2009b).

Conclusions

The measured residue concentration of cadmium in the roe deer meat was below the LOD indicating no health risk for the consumers. The residue of lead (0.48±0.21 mg/kg w.w.) exceeded the maximum limit established for domesticated ruminants, but its calculated weekly intake was below the PTWI. The average mercury content of roe deer meat (0.87 ±0.40 mg/kg w.w.) was about half of PTWI; however, the consumption of meat with highest measured concentration results in higher PTWI than recommended. The measured amount of arsenic (0.27±0.20 mg/kg w.w.) in the roe deer meat may not pose any health risk for the consumers according to the previous PTWI set by World Health Organization.

Due to the doubt in allowable intake rate assessment and the relatively rare consumption of game for majority of population, the risk of mercury and lead in roe deer meat is not inevitably high. However, the lead from the lead-containing bullets can often cause food hygienic problem if it is not properly recognized during sanitary inspection. Furthermore, because of man-made hazards (e.g., industrial pollution), the environment in which the roe deer reside can be contaminated and this could lead to accumulation of heavy metals in their tissue. Present regulations of European Community in force lay down maximum levels for limited number of metals and metalloids, and the range of regulated foods of animal origin is also narrower than that in the past, e.g., game animals are not included. Since game is now being made increasingly available to the consumers via supermarkets, restaurants, and mass catering promoted as a healthy "wild" alternative to intensively reared meat products, the food safety regulation of game meat (including offal) would have particularly sense to be reconsidered.

Acknowledgement This research was supported by the 8525-5/2014/TUDPOL grant of the Hungarian Ministry of Human Resources.

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