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Original article

Cadmium, lead, and mercury exposure assessment among Croatian consumers of free-living game

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Free-living game can be an important source of dietary cadmium and lead; the question is whether exposure to these two elements is such that it might cause adverse health effects in the consumers. The aim of this study was to estimate dietary exposure to cadmium, lead, and mercury from free-living big game (fallow deer, roe deer, red deer, wild boar, and brown bear), and to mercury from small game (pheasant and hare), hunted in Croatia from 1990 to 2012. The exposure assessment was based on available literature data and our own measurements of metal levels in the tissues of the game, by taking into account different consumption frequencies (four times a year, once a month and once a week). Exposure was expressed as percentage of (provisional) tolerable weekly intake [(P)TWI] values set by the European Food Safety Authority (EFSA). Consumption of game meat (0.002-0.5 % PTWI) and liver (0.005-6 % PTWI) assumed for the general population (four times a year) does not pose a health risk to consumers from the general population, nor does monthly (0.02-6 % PTWI) and weekly (0.1-24 % PTWI) consumption of game meat. However, because of the high percentage of free-living game liver and kidney samples exceeding the legislative limits for cadmium (2-99 %) and lead (1-82 %), people should keep the consumption of certain game species' offal as low as possible. Children and pregnant and lactating women should avoid eating game offal altogether. Free-living game liver could be an important source of cadmium if consumed on a monthly basis (3-74 % TWI), and if consumed weekly (11-297 % TWI), it could even give rise to toxicological concern.

KEY WORDS: *brown bear; deer; hare; liver; meat; pheasant; provisional tolerable weekly intake; toxic metal; wild boar*

Hunting in Croatia has a long history (officially since the enactment of the Hunting Bill in 1893) as leisure, economic activity, and source of quality food. Game meat is served in restaurants and households throughout the country. Considered a delicacy, it is far more valued for its exceptional taste than for its nutritive properties; it is rich in proteins, minerals Fe, Mg, P, K, Zn, vitamins riboflavin, niacin, pyridoxine, and cobalamine (1) and has a favourable fatty acid composition (2). However, being part of the terrestrial soil-plant-animal food chain, free-living game have generally been reported having higher Cd, Pb, and Hg levels than farmed animals (3). Food is the main source of non-occupational exposure to Cd, Pb, and Hg in the non-smoking adult population (4-6). Free-living game is dominantly consumed by hunters and their families and friends (2, 7-11). The 65,000 hunters registered in Croatia in 2012 constituted about 1.4 % of the general population (12).

The Scientific Panel on Contaminants in the Food Chain (CONTAM) conducted a risk assessment of various food contaminants in European countries, which was endorsed by the European Food Safety Authority (EFSA). EFSA considers frequent consumers of free-living game meat and offal to be exposed to higher Cd (only from offal) and Pb levels than the general adult population (5, 6). In 2011 and 2012, German, Swedish, Spanish, and British national food authorities advised pregnant women and children to reduce game consumption [reviewed by Green and Pain (13) and Meltzer et al. (11)].

Several recent European studies (7, 10, 14-17) focused on exposure to toxic metals from consumption of free-living animals. Some pointed out that regular intake of free-living game is likely to result in exceeding the provisional tolerable weekly intake (PTWI) for Cd and Pb (14-17) proposed by the EFSA (5, 18). Epidemiological studies reported an association between human blood Pb concentration and consumption of free-living game, small, especially birds (13, 19, 20), and big, like deer, moose, and reindeer (8, 11, 21, 22), but some studies did not report such association for Cd and Hg (21, 22). The prevailing sources of high Pb in free-living game meat appear to be fragments of lead ammunition (16, 23-25). High Cd levels in game are the consequence of accumulation, especially in the liver and kidney (6). The same applies to Hg but at much lower levels. In 2008, the Croatian regulation on maximum allowed levels of metals (26) was harmonised with EU legislation (27) and since then, the monitoring of Hg in animal tissues (other than fish) for human consumption was no longer obligatory. On the national level, at least 100 samples of marketed free-living game are tested for Cd and Pb every year according to the Commission Decision (28). However, the vast majority of game is not controlled for residues because non-marketed food is not subject to these tests. Data about toxic metal levels in free-living game from Croatia are mainly limited to scientific research (29-39). In 2012, the Croatian Food Agency published a heavy metal exposure assessment (40) based on data for wild boar meat, liver, and kidney. Data for the roe and red deer were available only for the liver.

Considering that comprehensive exposure assessments have not yet been conducted in Croatia involving multiple element data from regularly hunted game species, the present study aimed to gather relevant data in one place and contribute to existing knowledge with estimations of Cd, Pb, and Hg exposure of the Croatian population due to consumption of the most common game species, big and small. Our assessment is based on recently published data and available consumption frequency data for the Croatian population. As a novel contribution, this study also provides data on Pb levels in brown bears from Croatia, along with updated Cd and Hg levels in brown bear tissues. The data were discussed according to the current legislative maximum levels (ML) for farmed animals and exposure according to (P)TWI levels of toxic metals by the EFSA.

MATERIALS AND METHODS

Study area and data collection

The animals included in this study were all legally hunted on Croatian territory (Figure 1) between 1990 and 2012. Of all species, only brown bears were hunted in Gorski kotar and Lika, mountainous regions of Croatia. Fallow deer was hunted on the small north-Adriatic island of Brijuni, and the rest of the species originated from the Pannonian (lowland) region. These three geographical regions vary by climate and geomorphological properties. Considering the here observed metals (Cd, Pb, and Hg) topsoil concentrations in the Pannonian region and Brijuni were generally much lower than in the mountainous regions. A noteworthy exception was the higher Pb levels in the soil of Drava and Mura valley on the very north of Croatia. The highest soil Hg concentrations were reported in the north and north-west part of Gorski kotar, probably due to mining of small cinnabar deposits in Tršće near Čabar (41) and proximity of the now closed second largest Hg-mine in the world (Idrija, Slovenia). Furthermore, elevated levels of Cd and Pb of anthropogenic origin were reported near industrial areas (cities of Zagreb, Sisak, Kutina, Osijek, Šibenik), as well as high Pb deposition in the Drava valley (42).

A part of data on toxic metal levels in the muscle, liver, or kidney tissue used in this study to estimate exposure have been published elsewhere and include pheasant [*Phasianus colchicus* (29)], hare [*Lepus europaeus* P. (30)], fallow deer [*Dama dama* L. (31)], roe deer [*Capreolus capreolus* L. (32, 33)], red deer [*Cervus elaphus* L. (34-36)], wild boar [*Sus scrofa* L. (33, 35, 37, 38)], and brown bear [*Ursus arctos* L. (39)]. Data on pheasant and hare are limited to Hg whereas fallow deer data are limited to Cd. This study also brings our own unpublished data regarding Pb, Cd, and Hg levels in 206 more brown bear tissues,

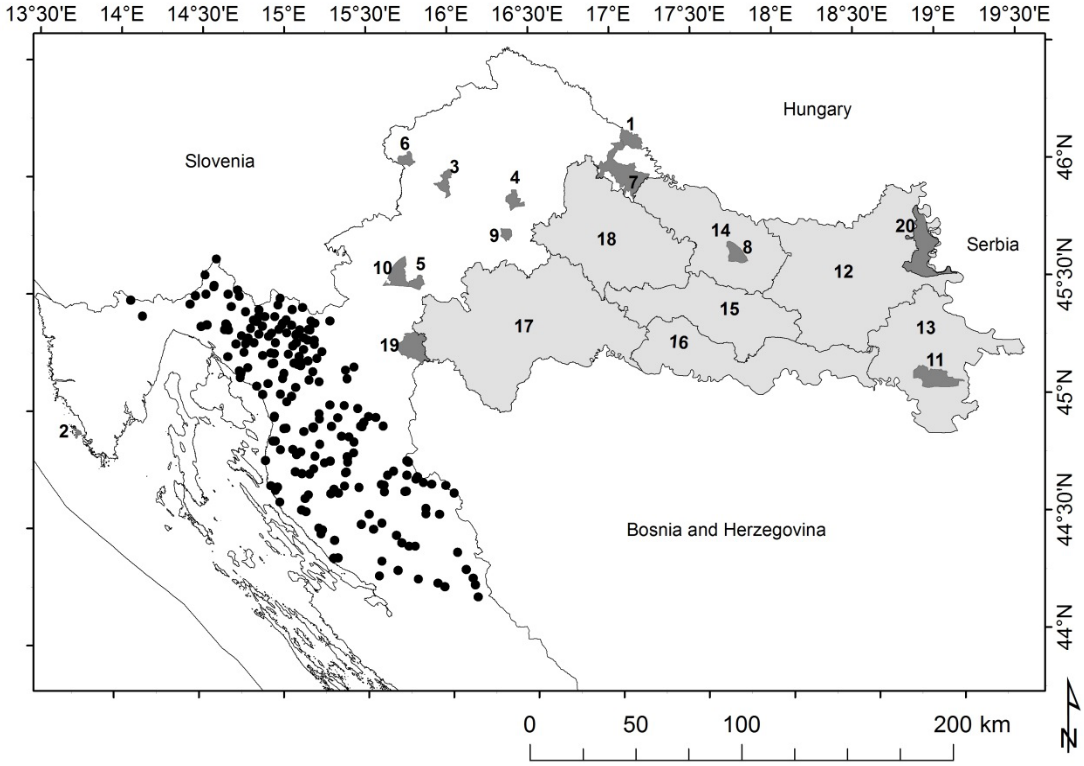

Figure 1 *Map showing the hunting areas where animals were sampled. The hunting estates and areas are marked dark gray (1 - Molve; 2 - Brijuni Islands; 3 - Medvednica; 4 - Vrbovec; 5 - Kupčina; 6 - Tuhelj; 7 - Đurđevac; 8 - Slatina; 9 - Črnovšćak; 10 - Pokupski bazen; 11 - Spačva; 19 - Petrova gora; 20 - Podunavlje-Podravlje). The counties relevant for the study are marked light gray (12 - Osječko-baranjska county; 13 - Vukovarsko-srijemska county; 14 - Virovitičko-podravska county; 15 - Požeško-slavonska county; 16 - Brodsko-posavska county; 17 - Sisačko-moslavačka county; 18 - Bjelovarsko-bilogorska county).*
Black dots represent the exact coordinates of locations where brown bears were hunted (based on data for animals from this study and reference 39). Fallow deer was hunted at Brijuni Islands – 2 (based on reference 32). Roe deer originated from hunting estates 3, 4, 5, 6, 7, 8, 9, 10, 11 (based on references 32, 33). Red deer was hunted at locations 12, 13, 14, 15, 20 (based on references 34-37). Wild boar was hunted at locations 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 (based on references 33-38). Pheasant and hare were hunted at hunting estate Molve – 1 (based on references 29, 30)

which together with previously reported findings (39) represents a total of 317 brown bears tissues analysed in Croatia by 2012.

Details about sampling and metal analysis in tissues of each species, performed by three independent expert analytical laboratories, are available in the respective references. For the purpose of this study, we used raw data (metal mass fractions for each animal) with the approval from the authors. Data on one species from several reports (e.g. red deer, wild boar) were merged and do not include age and sex distribution. All levels are presented in mg kg^{-1} of wet tissue mass (w. m.).

For descriptive statistics of the raw data, which includes arithmetic mean, range, and the 95th percentile, we used Statistica for Windows, version 12.0 (StatSoft, Inc., Tulsa, USA). For this purpose, metal levels below the limit of detection (LOD) were assigned their separate LOD value (upper-bound values) as recommended by the WHO (43). Fallow deer Cd data represent less than 50 samples and less than 25 quantified results, so lower-bound values were reported in the text together with upper-bound values (43), while in the tables and Figure 2 only the upper-bound value was indicated. However, it should be noted that due to the differences in the LODs designated within the analytical methods, the presented results should be taken with reserve.

Exposure assessment

Exposure assessments for the consumers of big game (deer species, wild boar and brown bear) were based on Cd, Pb, and Hg levels in meat and liver. We assumed that kidney consumption is negligible and thus did not include it in the estimation. For small game, only Hg exposure estimation was done. Weekly exposure to Cd, Pb, and Hg from meat and liver was estimated based on mean or 95th percentile metal levels and three consumption scenarios, i.e. consumption once a week (often), once a month (regular), and four times a year (rare). These scenarios were based on free-living game consumption frequency reported by Florijančić (9) in a population group whose 93 % declared to consume game and 51 % were hunters and their household members. Categorisation by consumption frequency was taken over from the study by Meltzer et al. (11). In our calculations we assumed that an average adult person weighs 70 kg and that a meal of raw meat or liver weighed 150 g wet mass [100 g when cooked; (44)], based on average mass of meat and offal eaten a day by adults from other countries (45, 46), since reliable data for Croatia do not exist.

The amount of game eaten by a person in our study scenarios ranged from 2.88 g per person per week (rare consumption) over 37.5 g per person per week (regular consumption) to 150 g per person per week (often consumption). Weekly metal exposure from game consumption was expressed as the percentage of (provisional) tolerable weekly intake [(P)TWI] and termed "added weekly exposure" as existence of other metal intake sources are presumed, apart from game consumption (4-6, 18). For the worst case-scenario we took weekly (often) consumption of meat or liver with the highest metal levels (95th percentile).

RESULTS AND DISCUSSION

Game meat and offal have been recognized by the EFSA as food items with elevated content of Cd and Pb thus raising concern about exposure to these toxic metals in "high consumers" (frequent intake over long time period) (5, 6). By assessing the harmful potential of metals ingested through game consumption, a few important facts should be kept in mind in order to include all possible exposure scenarios, but at the same time not to overestimate health risks thereby discouraging consumption of this nutritionally valuable food item.

In estimating exposure to metals through game meat and offal consumption, one should be aware of (a) heavy metal levels in meat and offal; (b) meat and offal consumption preference; (c) consumption frequency; (d) vulnerable population groups (children, pregnant/lactating women); (e) other sources of metal intake (e. g., other food from the diet, air, smoking, metal-related occupation, hobbies); and (f) individual factors influencing metal toxicity (age, sex, reproductive status, nutritional status).

Cd, Pb, and Hg in game tissues

Figure 2 shows the levels of Hg in small game, and Cd, Pb, and Hg levels in tissues of big game hunted in Croatia. Due to a high level of uncertainty in fallow deer Cd data (low number of samples, high number of quantified samples) we specified upper and lower-bound mean values for muscle: 0.0243 and 0.0193 mg kg $^{-1}$ w. m., and liver: 0.127 and 0.124 mg kg $^{-1}$ w. m.

Keeping in mind the limitations of such presentation (different quantification methods, merged data), toxic metal levels in the game follow a general trend the lowest levels in the muscle tissue and the highest in the kidney. The exception are muscle Pb levels, which may be overestimated due to left-censored data and varying LODs between the methods (that in some cases differed with more than an order of magnitude (0.05 *vs*. 0.0018 mg kg $^{-1}$ w. m.). A high percentage of data below 0.05 mg kg $^{-1}$ w. m. (the LOD of one method) shifted the mean to the right. The other likely reason are outliers, probably Pb ammunition-contaminated samples, which are common in game meat (16, 17, 24, 25). Because of the tiny diameter of Pb particles, their wide radiation from the bullet path, and bioavailability (47, 48), it is almost impossible to absolutely exclude exposure to Pb by consumption of free-living game [especially minced meat, as shown by Meltzer et al. (11)] hunted with Pb-bullets (16), even if meat around the wound is discarded. Switching to lead-free ammunition would definitely lower Pb exposure in humans and wildlife (49) and would allow quantification of exclusively environmental Pb in free-living game.

Left censoring for Cd and Hg in muscle tissue was low because, unlike with Pb, measurements below the method's LOD were far less common (Tables 2-4). Median values, especially for Cd and Pb in muscles, were around one order of magnitude lower than the respective means in all of the analysed animals (data not shown).

Mercury levels in the liver and kidney as organs of metal accumulation were lower in small game species than in the big ones. Furthermore, large herbivores showed lower Cd, Pb, and Hg levels than the omnivores (Figure 2). The liver and kidney of brown bear showed the highest metal accumulation. In contrast, wild boar had the highest metal levels in the muscle, a tissue most relevant for consumption.

Table 1 *Percentage of free-living game samples collected in Croatia between 1990 and 2012 exceeding legislative maximum levels (ML)*

	% of samples exceeding maximum level					
	Kidney		Liver		Muscle	
	Cd	Pb	Cd	Pb	Cd	Pb
ML* (mg kg $^{-1}$)	(1.00)	(0.50)	(0.50)	(0.50)	(0.05)	(0.10)
Brown bear	99	82	76	41	6	4
Wild boar	78	7	24	6	19	11
Red deer	67	2	2	1	23	9
Roe deer	88	0	37	3	4	29
Fallow deer	5	N/A	11	N/A	11	N/A

**Maximum level (27)*
N/A - data not available

Cd, Pb, and Hg game levels in respect to legislative limits (maximum level; ML)

Table 1 shows that the percentage of samples exceeding legislative Cd and Pb ML in meat and offal (27) was highest for brown bear tissues, then roe deer, wild boar and red deer. Cd levels were the most frequently above ML in renal and, in lower portion, in hepatic samples. Pb levels above ML were most frequently found in muscles of wild boars, red deer, and roe deer, unlike brown bears. In brown bears, tissue Pb ML was crossed in the highest number of renal samples, followed by hepatic and very small number of muscle samples. Comparing the number of samples above Cd and Pb ML for muscle, we noticed a similar number that did not comply with legislation. The EU legislation regulates only maximum levels in meat and offal of farmed animals, i.e. cow, sheep, pig, and poultry (27), even though it is an established scientific fact that free-living game animals have higher Cd and Pb levels (3). For example, in the Croatian National Residue Monitoring Program (NRMP) in 2009, Cd and Hg levels in kidney, and Pb in muscle were reported as the most frequent samples above the legislation ML (88 % of all farmed and free-living samples above ML were free-living game

Table 2 *Cadmium exposure estimations for adult consumers of meat and liver of free-living game collected in Croatia between 1990 and 2012, based on mean or 95th percentile levels in tissues*

Cd	Level in game (mg kg $^{-1}$ w. m.)		Added weekly exposure to Cd as % TWI by[2]:		
	Mean (P95)[1]	% <LOD	Often consumption	Regular consumption	Rare consumption
Muscle					
Fallow deer	0.0243 (0.129)	47	2 (11)	1 (3)	0.04 (0.2)
Roe deer	0.0107 (0.046)	6	1 (4)	0.2 (1)	0.02 (0.1)
Red deer	0.0584 (0.278)	28	5 (24)	1 (6)	0.1 (0.5)
Wild boar	0.0519 (0.200)	17	4 (17)	1 (4)	0.09 (0.3)
Brown bear	0.0252 (0.0521)	-	2 (4)	1 (1)	0.04 (0.1)
Liver					
Fallow deer	0.127 (0.714)	37	11 (61)	3 (15)	0.2 (1)
Roe deer	0.511 (1.24)	-	44 (106)	11 (27)	0.8 (2)
Red deer	0.152 (0.449)	3	13 (38)	3 (10)	0.3 (1)
Wild boar	0.393 (1.42)	4	34 (122)	8 (30)	1 (2)
Brown bear	1.25 (3.46)	-	107 (297)	27 (74)	2 (6)

[1]P95 - 95th percentile; % <LOD - percent of samples whose metal level was below the limit of detection
[2]Calculations were made for mean and P95 (in parenthesis) Cd levels in game tissue, a meal size of 150 g wet mass (raw), a 70 kg body mass person, and Cd tolerable weekly intake (TWI) of 2.5 μg kg $^{-1}$ body mass (18).
Often, regular, and rare consumption refers to once a week, once a month, and four times a year, respectively

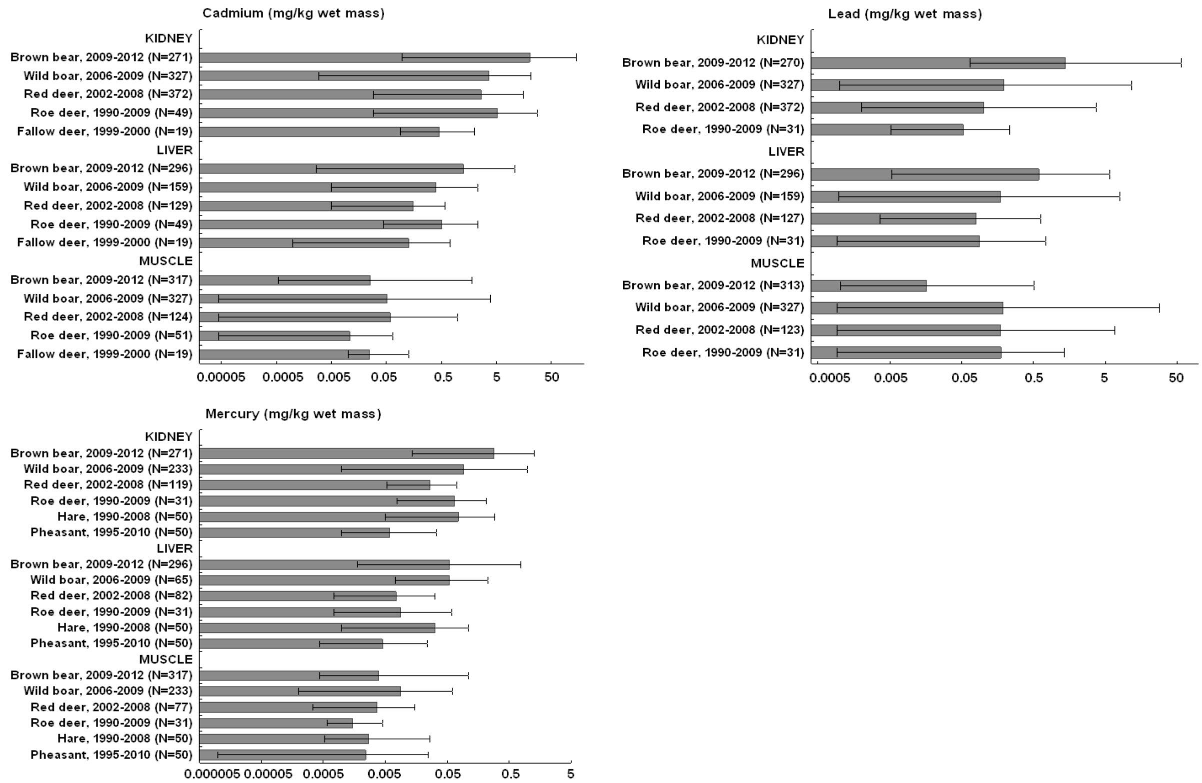

Figure 2 *Cadmium, lead, and mercury in tissues of game collected in Croatia between 1990 and 2012. Bars represent mean levels and whisker range of levels (min-max) on a log scale (base 10)*

samples) (50) and in 2008, Cd, Pb, and Hg levels were above the ML in 76 %, 26 %, and 18 % of free-living game liver and kidney samples, respectively (51). Current practice requires that foodstuffs exceeding these limits are safely disposed of, which incurs financial loss to the product owner. Clearly, current regulations need to include separate limit values for free-living game and farmed animals, as the former reflect environmental conditions better, contain higher metal level but also are consumed much less frequent.

Cd, Pb, and Hg exposure assessment

Number of registered hunters and hunted large game in Croatia increased in the period 2009-2012 (12, 52). These facts, together with the enhanced availability of game meat to the average consumer via restaurants and supermarkets, lead to a possibility that game consumption is also increasing. Generally, game consumption frequency data with average amount eaten per year/week are very scarce in the literature (7, 10, 53) for both hunters and general population, with no data for Croatia. Each year the Central Bureau of Statistics of the Republic of Croatia (CBS) publishes rough estimates of annual average personal consumption of particular food items. In that report, game and rabbit meat belong to one food category, game offal and farmed animal offal to another, which means that data on the consumption of game tissue alone are not available. According to CBS reports, 0.43 kg of game and rabbit meat per year per person (8 g per person per week) and 1.10 kg game and farmed animal offal per year per person (21 g per person per week) was consumed in Croatian households on average in the period 1999-2011 (12). Italy is the only neighbouring country with available data about consumed game meat and liver amounts for hunters and their household members (mean: 113 g per person per week for meat, 38 g per person per week for liver) (10), but only for wild boar. Vahteristo et al. (7) reported mean consumption of moose meat among moose hunters in Finland to be 392 g per person per week and moose liver 18 g per person per week. In its dietary exposure model for especially high exposure to Cd and Pb EFSA has assumed that a person eats 200 g of meat and 100 g of offal per week (5, 6).

It is important to differentiate exposure estimation for general population, and hunters, their household members and friends who in Croatia, like in other countries, are the vast majority of free-living game consumers (7-11). Consumption frequency in this subpopulation is independent of the availability of

Table 3 *Lead exposure estimations for adult consumers of meat and liver of free-living game collected in Croatia between 1990 and 2012, based on mean or 95th percentile levels in tissues*

Pb	Level in game (mg kg $^{-1}$ w.m.)		Added weekly exposure to Pb as % PTWI by[2]		
	Mean (P95)[1]	% <LOD	Often consumption	Regular consumption	Rare consumption
Muscle					
Roe deer	0.176 (1.11)	35	2 (10)	0.4 (2)	0.03 (0.2)
Red deer	0.171 (0.354)	43	1 (3)	0.4 (0.8)	0.03 (0.06)
Wild boar	0.188 (0.230)	31	2 (2)	0.4 (0.5)	0.03 (0.04)
Brown bear	0.016 (0.091)	32	0.1 (1)	0.03 (0.2)	0.003 (0.01)
Liver					
Roe deer	0.087 (0.484)	10	0.7 (4)	0.2 (1)	0.01 (0.08)
Red deer	0.080 (0.191)	20	0.7 (2)	0.2 (0.4)	0.01 (0.03)
Wild boar	0.174 (0.606)	24	1 (5)	0.4 (1)	0.03 (0.1)
Brown bear	0.594 (1.65)	-	5 (14)	1 (4)	0.1 (0.3)

[1]P95 - 95th percentile; % <LOD - percent of samples whose metal level was below the limit of detection
[2]Calculations were made for mean and P95 (in parenthesis) Pb levels in game tissue, a meal size of 150 g wet mass (raw), a 70 kg body mass person, and Pb provisional tolerable weekly intake (PTWI) of 25 µg kg $^{-1}$ body mass (5).
Often, regular, and rare consumption refers to once a week, once a month, and four times a year, respectively

Table 4 *Mercury exposure estimations for adult consumers of meat and liver of free-living game collected in Croatia between 1990 and 2012, based on mean or 95th percentile levels in tissues*

Hg	Level in game (µg kg $^{-1}$ w.m.)		Added weekly exposure to Hg as % TWI by[2]		
	Mean (P95)[1]	% <LOD	Often consumption	Regular consumption	Rare consumption
Muscle					
Pheasant	2.43 (6.00)	62	0.1 (0.3)	0.03 (0.08)	0.002 (0.01)
Hare	2.71 (9.00)	57	0.1 (0.5)	0.04 (0.1)	0.003 (0.01)
Roe deer	1.48 (3.53)	-	0.1 (0.2)	0.02 (0.05)	0.002 (0.004)
Red deer	3.71 (12.7)	-	0.2 (0.7)	0.05 (0.2)	0.004 (0.01)
Wild boar	8.87 (26.0)	1	0.5 (1)	0.1 (0.3)	0.009 (0.03)
Brown bear	3.92 (10.3)	11	0.2 (0.6)	0.05 (0.1)	0.004 (0.01)
Liver					
Pheasant	4.52 (18.0)	40	0.2 (1)	0.06 (0.2)	0.005 (0.02)
Hare	31.7 (79.0)	-	2 (4)	0.4 (1)	0.03 (0.08)
Roe deer	8.82 (21.3)	-	0.5 (1)	0.1 (0.3)	0.01 (0.02)
Red deer	7.42 (16.0)	-	0.4 (1)	0.1 (0.2)	0.01 (0.02)
Wild boar	53.4 (142)	-	3 (8)	0.7 (2)	0.05 (0.1)
Brown bear	53.9 (143)	-	3 (8)	0.7 (2)	0.06 (0.1)

[1]P95 - 95th percentile; % <LOD - percent of samples whose metal level was below the limit of detection
[2]Calculations were made for mean and P95 (in parenthesis) Hg levels in game tissue, a meal size of 150 g wet mass (raw), a 70 kg body mass person, and tolerable weekly intake (TWI) for inorganic Hg of 4 µg kg $^{-1}$ body mass (4).
Total Hg level in game from second column was regarded as inorganic mercury for the purpose of calculation % TWI, as done by EFSA (4).
Often, regular, and rare consumption refers to once a week, once a month, and four times a year, respectively

game in food stores and its price - two reasons most often indicated in the general population as responsible for the under-representation of free-living game consumption (9). It will more likely depend on the hunting score. Johansen et al. (20) reported the highest consumption of game upon hunting season closure in ethnic Greenlanders. On the other hand, freezing game meat allowed extended consumption throughout the year (7, 53) but frequency of game consumption was shown to also depend on the consumers' preferences to eat particular game species.

Tables 2-4 show that free-living game meat is a low source of Cd, Pb, and Hg, regardless of the consumption scenario, given the respective (P)TWI and mean metal concentrations (0.002-5 %). However, weekly consumption of big game meat (relevant for hunters) with high Cd levels (95^{th} percentile) could significantly contribute to exposure to Cd (4-24 % TWI). Meat with high Pb and Hg levels is a low source of metal intake even if often consumed.

Game meat preferences can influence the intake of metals due to differences in metal content between the species. One Croatian study showed that roe deer meat is the first on the list of preferences, followed by red deer, wild boar, and small game meat (9). Brown bear consumption has not been investigated. To the authors' knowledge, bear meat is less frequently consumed in the general population compared to other investigated game, but among bear hunters there is a possibility of higher consumption preference and frequency due to availability of their own hunted bear meat. In 2012 wild boar was the most hunted large game species in Croatia according to number of hunted individuals, followed by roe deer, red deer (12), and brown bear (52).

Mean weekly dietary intake of Pb and Cd from food in the Croatian general population was estimated in 1996 to be 701 and 121.4 μg per person per week [40 and 69 % of the last proposed (P)TWI], respectively (54). Weekly consumption of big game meat from our study adds 1-7 % and 0.3-4 % (if calculated with mean Cd and Pb concentrations in meat) or 6-34 % and 2-24 % (if calculated with P95 concentrations in meat) to the estimated mean weekly dietary intake of Cd and Pb, respectively, in the general population, depending on the species consumed.

Same as for domestic animals, game meat consumption largely prevails over consumption of offal (7, 10). There are no official data towards game offal consumption preferences in Croatia. Results of the Italian specific diet survey conducted on 262 hunters and their household members revealed that, all 37 % of examinees who stated wild boar offal consumption ate only liver, none consumed kidney (10). On the other hand, among Finish moose hunters and moose meat eaters (N=711), 69 % consumed liver and 23 % kidneys (7). Our assumption was thereby that free-living game consumers in Croatia avoid eating kidneys or consume it in a negligible number of occasions. Although there is a general recommendation for consumers to avoid eating liver and kidneys of free-living game animals in Croatia because of their high Cd content (40, 55), it is assumed that offal is still being consumed in some households, preferentially that of young animals (40). Our findings (Tables 2-4) show that game liver is a low source of Pb and Hg, regardless of the consumption scenario. Regarding the consumed species, consumers of bear liver had the highest intake of Cd, Pb, and Hg, although wild boar liver also had similar Hg content. If consumed on a regular basis (once a month), game liver can be a low to significant (3-74 % TWI) source of Cd, depending on the concentration. Depending on species and Cd concentration range, frequent consumption (weekly) can result in significant to even very high exposure to Cd (11-297 % TWI) in the worst case scenario. Even a single meal of bear liver with average Cd levels, or roe deer/wild boar/brown bear liver with high Cd levels can exceed the EFSA weekly intake considered safe for health. Often consumption of big game liver from this study adds 16-155 % and 2-13 % (if calculated with mean Cd and Pb concentrations in meat) or 55-428 % and 4-35 % (if calculated with P95 concentrations in meat) to the estimated mean weekly dietary intake of Cd and Pb, respectively, in the general population, depending on the species consumed.

Several authors have estimated exposure to metals (mostly Pb and Cd) from game consumption (5-7, 15-17, 25) using different methods, scenarios, and variables (e.g. meat portion) which makes any comparison unreliable. Nevertheless, metal content in game tissues have the strongest impact on exposure estimation so it is pretty safe to say that exposure to Cd and Pb from Croatian big game is similar or lower than the expected exposure in Finland (7), Italy (10), Poland (14), Spain (15, 16), or Norway (17).

Implications of our exposure assessment for vulnerable population groups

Foetuses and children, breastfed infants in particular, are considered vulnerable population group because of the transfer through the placenta (foetuses) or milk (breastfed infants) (4-6) or because of the hand-to-mouth behaviour, greater intestinal absorption, and not fully developed blood-brain barrier (infants and children), which all increase metal bioavailability to young organisms in respect to adults. Greater bioavailability increases the risk of adverse health effects because of the higher sensitivity of developing organisms.

Another vulnerable group are child-bearing and lactating women, who also absorb more toxic metals from food. Health risks are associated with higher bone turnover and therefore higher Pb blood content (Pb released from bone).

Because of the new evidence that there are no safe threshold levels below which Pb does not induce developmental neurotoxicity in children, and cardiovascular effects and nephrotoxicity in adults, EFSA has withdrawn the current PTWI for Pb, except for the calculations of the margin of exposure (5). With this in mind, children and pregnant/lactating women should be advised to avoid free-living game altogether, as its meat may be contaminated by lead ammunition (13) and its offal is likely to have high Cd and Hg content.

CONCLUSIONS

Metal content monitoring of free-living big game in Croatia could expand to include wild boar and deer species from the mountainous parts of Croatia and small game from the whole country. Muscle contamination with Pb in the most frequently consumed species (wild boar, roe deer, red deer) might be avoided by using non-lead ammunition. Because of the high percentage of free-living game offal samples exceeding the limits for Cd and Pb, adults should keep its consumption as low as possible, while children and pregnant and lactating women should avoid it.

Our study estimates suggests that rare consumption of free-living game meat and liver (which is a likely scenario for the general population) does not pose a health risk to consumers. However, free-living game liver could become an important additional source of Cd if consumed on a monthly basis, and weekly consumption may give rise to toxicological concern.

Future estimates should focus on food (free-living game and other items of toxicological concern) preference and frequency surveys among the Croatian population to enable more reliable exposure and risk assessments.

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Sažetak

Procjena izloženosti kadmiju, olovu i živi pri konzumaciji slobodne divljači u Hrvatskoj

Procjena izloženosti kadmiju, olovu i živi pri konzumaciji slobodne divljači u Hrvatskoj Slobodna divljač značajan je izvor kadmija i olova u prehrani ljudi te se nameće pitanje je li izloženost ovim metalima tolika da može štetno utjecati na zdravlje potrošača. Procijenili smo izloženost kadmiju, olovu i živi pri konzumaciji velike slobodne divljači (jelen lopatar, srna, obični jelen, divlja svinja i smeđi medvjed) te živi pri konzumaciji male divljači (fazan, zec) koja je ulovljena u Hrvatskoj između 1990. i 2012. godine. Procjena izloženosti temelji se na dostupnim literaturnim podacima i našim rezultatima mjerenja metala u tkivima divljači te različitoj učestalosti konzumacije (četiri puta godišnje, jedanput mjesečno, jedanput tjedno). Izloženost je prikazana kao postotak vrijednosti (privremeno) prihvatljivog tjednog unosa [(P)TWI] koju je postavila Europska agencija za sigurnost hrane (EFSA). Izloženost toksičnim metalima pri rijetkoj konzumaciji (pretpostavljena za opću populaciju) mesa (0,002-0,5 % PTWI) i jetre divljači (0,005-6 % PTWI) ne predstavlja zdravstveni rizik za potrošače, kao ni redovita (0,02-6 % PTWI) i česta (0,1-24 % PTWI) konzumacija mesa divljači. Preporuka je što više smanjiti konzumaciju iznutrica nekih vrsta divljači zbog visokog postotka uzoraka jetre, a posebno bubrega, koji prelaze zakonom propisane maksimalne razine kadmija (2-99 %) i olova (1-82 %). Djeca, trudnice i dojilje trebale bi izbjegavati konzumaciju iznutrica divljači. Jetra divljači može biti značajan dodatni izvor kadmija ako se redovito konzumira (3-74 % PTWI), a čestom konzumacijom jetre (11-297 % PTWI) može se povećati rizik od štetnih učinaka na zdravlje.

KLJUČNE RIJEČI: *divlja svinja; fazan; jelen; jetra; meso; prihvatljivi tjedni unos; smeđi medvjed; toksični metali; zec*

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