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Cadmium, Lead, and Chromium in Large Game: A Local-Scale Exposure Assessment for Hunters Consuming Meat and Liver of Wild Boar

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Abstract Heavy metals are ubiquitous in soil, water, and air. Their entrance into the food chain is an important environmental issue that entails risks to humans. Several reports indicate that game meat can be an important source of heavy metals, particularly because of the increasing consumption of game meat, mainly by hunters. We performed an exposure assessment of hunters and members of their households, both adults and children, who consumed wild boar (WB) meat and offal. We estimated the amount of cadmium, lead, and chromium in the tissues of WB hunted in six areas within Viterbo Province (Italy) and gathered data on WB meat and offal consumption by conducting specific diet surveys in the same areas. The exposure to cadmium, lead, and chromium was simulated with specifically developed Monte Carlo simulation models. Cadmium and lead levels in WB liver and meat harvested in Viterbo Province (Italy) were similar to or lower than the values reported in other studies. However, some samples contained these metals at levels greater then the EU limits set for domestic animals. The chromium content of meat or liver cannot be evaluated against any regulatory limit, but our results suggest that the amounts of this metal found in WB products may reflect a moderate environmental load. Our survey of the hunter population confirmed that their consumption of WB meat and liver was greater than that of the general Italian population. This level of consumption was comparable with other European studies. Consumption of WB products contributes significantly to cadmium and lead exposure of both adults and children. More specifically, consumption of the WB liver contributed significantly to total cadmium and lead exposure of members of the households of WB hunters. As a general rule, liver consumption should be kept to a minimum, especially for children living in these hunter households. The exposure to chromium estimated for this population of hunters may be considered to be safe. However, a specific and complete assessment of chromium speciation in relevant dietary and environmental situations should be conducted.

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Heavy metals are ubiquitous in the environment, and their entrance into the food chain through soil, water, and air circulation is an important environmental issue that includes risks to humans (Nriagu and Pacyna 1988; Wolkers et al. 1994; Falandysz et al. 2005). The literature dealing with heavy-metal contamination of game meat (Hernández et al. 1985; Swiergosz et al. 1993; Falandysz 1994; Wolkers et al. 1994; Kottferová and Koréneková 1998; Guitart et al. 2002; Lord et al. 2002; Falandysz et al. 2005; Lazarus et al. 2008) highlights that heavy metals can reach concentration levels high enough to make some of these products unsafe for human consumption. Wild ungulate populations are increasing throughout Europe (Saez-Royuela and Telleria 1986; Delibes-Mateos et al. 2009; Meriggi et al. 2011). Since the 1980s, the wild boar (WB [*Sus scrofa*]) population has undergone a population explosion, which has resulted in an increased distribution range and population density (Carnevali et al. 2009). Harvesting WB by hunting has also increased (Geisser and Reyer 2004; Bieber and Ruf 2005) in the last decades, thus increasing the amount of WB meat available for human consumption (Ramanzin et al. 2010). Although some countries, such as Scotland, Austria (Winkelmayer and Paulson 2008) and Spain (Morales et al. 2011), have an established game meat market, the majority of this meat is consumed in the home in other European countries. In Italy, game meat is not subjected to the food safety controls outlined in Regulation (EC) 853/2004 (European Parliament 2004) because most of it is consumed in domestic settings or is supplied in small amounts directly and untraceably to the final consumers and local retailers. Moreover, no specific concentration limits for toxic elements (e.g., lead and cadmium) in game meat have been defined; those limits listed in Regulation (EC) 1881/2006 are specific to the meat and offal of farm animals only (European Commission 2006). Nonetheless, it is important to measure the toxic-metal exposure of those populations likely to be exposed whether through work or other settings. Hunters and their families are particularly likely to be at risk (Burger 2002; Vahteristo et al. 2003; Lazarus et al. 2008) because the concentrations of metals in wild animals may vary considerably from location to location (Kålås et al. 1995; Petersson-Grawé et al. 1997; Wlostowski et al. 2006; Bilandzic et al. 2009; Bilandžić et al. 2010). Local-scale exposure studies are thus appropriate for game meat that is consumed mainly within the harvesting area. Risk-assessment studies indicate that health risks related to heavy-metal exposure are greater for hunter populations than for the general populace. For instance, Morales et al. (2011) recently found that Spanish hunters may be exposed to lead at levels of >224 % of the provisional tolerable weekly intake (PTWI) established by the Food and Agriculture Organization/World Heath Organization (Food and Agriculture Organization/World Health Organization 2000). This value is in addition to the amount already taken in by way of other food items, such as drinking water and air (European Food Safety Authority [EFSA] 2010). Lead contamination of food is of major importance to humans, and considerable attention has been focused recently on lead toxicity in children (Hornung et al. 2009; European Food Safety Authority 2010). In fact, there is evidence that cognitive development is affected when children <2 years of age are exposed to lead (Chen et al. 2005; Lanphear et al. 2005). Cadmium has become recognized as a worldwide public health hazard because of its persistence in the environment and its extended biological half-life (Vahter et al. 1996). Similar to other heavy metals, high dietary cadmium intake may cause functional disturbances. These effects are especially serious in children because they adsorb metals more efficiently than adults and are, for biological and developmental reasons, particularly sensitive (de Burbure et al. 2006). A recent study concluded that some European consumers are exposed to cadmium at rates exceeding the PTWI of 7 μg kg^{-1} body weight (bw) $week^{-1}$ (Nasreddine and Parent-Massin 2002). Consequently, there is a clear need for the continued monitoring of cadmium in food and in the environment, particularly for at-risk populations (Coni et al. 1992). Chromium naturally occurs in two stable oxidative states: Cr^{VI} and Cr^{III}. Since the late 1940s, Cr^{VI} has been recognized as highly toxic (Machle and Gregorius 1948), and it was subsequently designated as a Group 1 carcinogenic substance for humans by the International Agency for Research on Cancer (WHO–IARC 1990). Conversely, Cr^{III} is thought to be an essential trace element for mammals because it is apparently involved in glucose and lipid metabolism. However, elucidating its function at a molecular level has proved to be problematic (Vincent 2000). The Food and Nutrition Board of the Institute of Medicine (IOM 1989) tentatively identified the estimated safe and adequate dietary intake (ESSADDI) for adults of chromium to be 50–200 μg Cr/d. However, updated more recent evaluation of the adequate intake (AI) has been performed (Institute of Medicine 2001), and a range from 11 μg Cr/d for children (1–3 years old) to 35 μg Cr/d for men (19–50 years old) has been proposed. Although no specific upper limit (UL) has been established as yet, the potential for adverse effects resulting from high chromium intake cannot be excluded (Institute of Medicine 2001). Furthermore, the classification of hexavalent chromium as a known human carcinogen raises concerns regarding the carcinogenic potential of trivalent chromium (United States Environmental Protection Agency [USEPA] 1998). For these reasons, the study presented here had the following aims: (1) to estimate the amount of cadmium, lead, and chromium in tissues of WBs hunted in six hunting areas within the Viterbo Province (Italy); and (2) to perform an exposure assessment for hunters and their household members, both adults and children, using a specific survey to estimate WB meat consumption.

Materials and Methods

Study Area

The study area encompassed six WB game-management districts within the administrative boundaries of the Viterbo Province (Lazio Region, Central Italy). The landscape is highly fragmented and consists of a large proportion of cultivated fields interspersed with woodland and scrubland. The "A. Volta" electric power plant (southwest area) and some authorized landfills are located within the province, but all are located outside of the six hunting districts. The area is crossed by the Fiora and Paglia rivers, which originate in Monte Amiata, where cinnabar mines and geothermal phenomena are present.

Sampling

The study included 54 WBs shot by hunters during the 2005-to-2006 hunting season (from November 1, 2005, until the end of January 2006). The hunters used the technique of dog-drive hunting. The carcasses of the animals, ranging from 2 to 5 years of age, were randomly selected from each area using a preplanned schedule based on the results of the previous 2 years of hunting. Thus, the sampling may be considered to be representative of WB meat and offal entering the human food chain. Sample selection was performed within sex, and 27 male and 27 were sampled. Meat and offal samples were collected, individually packed in polyethylene bags, and transferred to the laboratory in refrigerated bags. During sampling operations, special care was taken to avoid tissues near the bullet pathway; all tissue samples were taken from >40 cm away from areas of bullet damage. The tissue samples were frozen and stored at −20 °C until analysis.

Sample Preparation and Elemental Analysis

Finely ground samples (0.5–0.6 g) were dispersed in HNO_3 (65 %, trace-analysis grade; Sigma, Italy) or HNO_3 plus H_2O_2 (30 %, trace-analysis grade; Sigma, Italy) and submitted to optimized microwave (MW) digestion cycles (Table 1) using a MLS 1200 MW laboratory system (Milestone S.r.l., Italy). At the end of mineralization, the samples were quantitatively recovered in polyethylene tubes and diluted to 10 ml with ultrapure water (specific conductibility 0.055 μS/cm) (Sartorius Stedim, Italy). Samples were diluted to appropriate concentrations with 1.0 % (v/v) ultrapure HNO_3 before analysis. The analyses of cadmium, lead, and chromium were performed using a Varian SpectrAA 30 atomic absorption spectrophotometer equipped with a Zeeman graphite furnace (Varian Inc., USA). Chemical (or matrix) interferences were minimized by the use of platform atomization techniques with the aid of appropriate matrix modifiers. For cadmium 1.0 % (w/v) $NH_4H_2PO_4$ was used, and 1.0 % (v/v) H_3PO_4 was used for lead. No modifier was added for the chromium analysis. Five calibration standards of cadmium (1.56–25.00 μg/ml), lead (3.13–50.00 μg/ml), and chromium (0.79–12.50 μg/ml) were prepared by diluting certified standard solutions (1.000 ± 0.002 g l^{-1}) (Merck, Germany) with 1.0 % (v/v) ultrapure HNO_3 solution. A certified reference material (bovine liver, BCR 185R; Community Bureau of References, Belgium) was treated in the same way as the WB samples as method control. Percent recoveries (Rec %) for cadmium, lead, and chromium were calculated using the values obtained from the certified values as references. Reusable materials, such as glassware and MW liners, were soaked overnight in a mixture of HCl/HNO_3 (3:1 v/v; prepared daily) before each use. The purity of the reagents and the water, as well as the cleanliness of the laboratory materials, was monitored by inclusion of a reagent laboratory blank in each MW mineralization cycle (EPA 1991). The concentrations of all elements analyzed were expressed as wet weights (ww [mg kg^{-1}]) and were calculated as follows:

$$[\mathrm{El}]_{\mathrm{sample}} = \frac{(\mathrm{ABS}_{\lambda} - \mathrm{Int}_{\mathrm{cc}}) \times \mathrm{DF} \times V_{\mathrm{min}}}{\mathrm{Slope}_{\mathrm{cc}} \times W_{\mathrm{sample}}},$$

where $[\mathrm{El}]_{\mathrm{sample}}$ is the element concentration in the tissue sample; ABS_{λ} is the absorbance recorded at 228.8 nm for cadmium 283.3 nm for lead and 357.9 nm for chromium; $\mathrm{Int}_{\mathrm{cc}}$ (in AU) and $\mathrm{Slope}_{\mathrm{cc}}$ (in ml mg^{-1}) are, respectively, the intercept and the slope of the calibration curve developed for each element; DF is the applied dilution factor; V_{min} is the final volume of the mineralized samples (ml); and W_{sample} is the sample weight in grams. Limits of detection (LODs) and quantification (LOQs) were also determined for each element from six separate trials of each metal. These values were determined by following the DIN no. 2345 procedure (DIN 1994). The results were as follows: LOD = 0.003 mg kg^{-1}, LOQ = 0.009 mg kg^{-1}, and Rec % (mean ± SD) = 87.1 ± 0.6 for cadmium; LOD = 0.010 mg kg^{-1}, LOQ = 0.030 mg kg^{-1}, and Rec % = 94.1 ± 1.8 for lead; and LOD = 0.012 mg kg^{-1}, LOQ = 0.037 mg kg^{-1}, and Rec % = 91.1 ± 2.4 for chromium. The data presented in this study were not corrected for recoveries. Because nondetected data constituted far less than 80 % of the whole data set for each metal (see "Results"), the following procedures were adopted for the calculation of dietary exposure (WHO 1995b): The values of all data points falling below than the LOD were set to LOD/2, whereas those data points falling between the LOD and LOQ were numerically reported.

Table 1 MW digestion conditions optimised for WB meat and offal

Matrix	Sample amount (g)[a]	Reagents (ml)	MW cycles (min/W)
Meat	0.600 ± 0.002	HNO_3 (2)	1/600
			5/300
Offal	0.500 ± 0.002	HNO_3 (2)	5/300
		H_2O_2 (1)	0.5/0
			5/600

[a] Tolerance refers to the precision of the analytical balance used

Metal-Exposure Estimation

Weekly exposure estimations for cadmium, lead, and chromium were calculated using the heavy-metal content of meat and offal and meat and/or offal consumption rates. To obtain the latter information, questionnaires were administered to people who hunted in the areas where the WB samples were collected. In total, 118 respondents were assisted by trained personnel in completing the questionnaire, which was designed to obtain the following information: (1) total annual WB meat in the hunter's bag; (2) total amount of WB meat given to people associated with the hunter's family (friends, neighbors, etc.); (3) number of household members, including children (<15 years old) consuming WB meat and offal; and (4) weekly frequency of WB product consumption. No data were collected about the consumption of other types of meat. According to the completed questionnaires, liver was the only WB organ that was consumed. Some questionnaires that included incomplete or inconsistent information were discarded. The percentage of retained questionnaires was 90.7 % of the total number of questionnaires administered. Data were obtained for 262 people, including 107 hunters and 155 household members. Consumption rates were estimated by determining the WB hunter's annual bag minus the amount given to others and taking into account weekly consumption frequency and number of household members. According to Vahteristo et al. (2003), we assumed that consumption by children (<15 years old) was equal to half the amount of WB meat/liver per meal consumed by adults. Among adults, the amounts of WB products consumed per meal were equal for both men and women. The boneless-to-boned meat ratio was estimated to be 0.72, a value calculated from the answers of 40 hunters to questions about boned meat used in food preparation (annual basis) and the amount of meat used per meal. This value is in line with the boneless meat yield reported in the literature (Skewes et al. 2008). Descriptive statistics were used to estimate the amount of WB used in household food preparation. A Monte Carlo simulation model (MCSM) (Metropolis and Ulam 1949) was used to estimate total cadmium, lead, and chromium exposure of hunters and cohabiting relatives using data on meat and liver consumption as in the following equation:

$$\mathrm{WMI} = N_{\mathrm{m}} \times C_{\mathrm{m}} + P_{\mathrm{l}} \times N_{\mathrm{l}} \times C_{\mathrm{l}} \tag{1}$$

where WMI is the weekly metal intake from WB meat and liver consumption; N_{m} is the weekly amount of consumed WB meat; C_{m} is the concentration of each different metal (cadmium, lead, or chromium) found in the WB meat samples; P_{l} is the conditional probability that a person consuming WB meat also consumes liver (the ratio of people eating liver to all of the respondents = 98 of 262); N_{l} is the weekly amount of WB liver consumed; and C_{l} is the trace element concentration found in the liver. This approach takes into account the variability and shape of the input data distributions that influence metal exposure among WB hunters/eaters resulting in exposure profiles rather than mere point estimates (Vahteristo et al. 2003). The MCSM was constructed using the object-oriented programming software Stella 8.0 (HPS, NH) for Microsoft Windows. Simulation of the WMI was performed 96,000 iterations as follows: 32,000 iterations, the highest number allowed by the program, were repeated three times. On the basis of the model described in Eq. 1, two other models were developed to separately estimate the exposure for adults (Eq. 2) and children (<15 years old) (Eq. 3):

$$\mathrm{WMI}_{\mathrm{a}} = N_{\mathrm{am}} \times C_{\mathrm{m}} + P_{\mathrm{al}} \times N_{\mathrm{al}} \times C_{\mathrm{l}} \tag{2}$$

$$\mathrm{WMI}_{\mathrm{c}} = N_{\mathrm{cm}} \times C_{\mathrm{m}} + P_{\mathrm{cl}} \times N_{\mathrm{cl}} \times C_{\mathrm{l}}, \tag{3}$$

where P_{al} (80 of 221) and P_{cl} (18 of 41) represent the conditional probabilities of consumption of both WB liver and meat for adults and children, respectively. For each model, the individual contributions of meat and liver to total heavy-metal ingestion were investigated separately.

Treatment of Input Data and Statistical Procedures

Data on weekly meat and liver consumption, as well as metal concentrations, were tested for normality using Shapiro–Wilks W test (Shapiro et al. 1968). The data did not follow the normal distribution. When possible, the variables were log-transformed to achieve a normal distribution (Tables 2, 3), whereas in the remaining cases, the data were merged into frequency classes (Tables 2, 3). For the normalized, log-transformed variables, the probability density function was determined as follows:

$$X = \frac{1}{x\sqrt{2\pi\sigma^2}} \mathrm{e}^{-\frac{(\ln x - \mu)^2}{2\sigma^2}},$$

where x is the untransformed variable; and μ and σ are the mean and the SD of the variable, respectively. Because all of the WB consumers were potentially exposed to each contaminated sample, variable distributions (Tables 2, 3) were used to generate input contamination and consumption data for the estimation of the WB consumers' weekly metal exposure by the MCSMs developed according to the equations described previously (see Eqs. 1 through 3). To determine the accuracy of the simulations, the consumption and concentration data generated by the MCSMs were compared with the original data using Kolmogorov–Smirnov and Chi-square tests (Siegel and Castellan 1988). Autocorrelation for the WMI output data were thoroughly checked using the procedure of Box and Jenkins (Box and Jenkins 1976). Autocorrelation coefficients (r_k) were calculated for *lag values* (k) between 1 and 999 (the limit imposed by the software). The presence of "white noise" was assessed by examining the autocorrelations at various k values using the Ljung–Box Q statistic (Ljung and Box 1978). Descriptive statistics were used to study weekly metal-intake data (WMI, $\mathrm{WMI}_{\mathrm{a}}$, and $\mathrm{WMI}_{\mathrm{c}}$). The data distribution profiles were studied, and differences in WMI values between consumer categories (e.g., meat eaters vs. meat and liver eaters) were assessed for significance by Mann–Whitney U test (Siegel and Castellan 1988) using a significance level of $p < 0.05$. The aforementioned statistics were performed using Statistica 7.0 (StatSoft, Tulsa, OK). The uncertainty of the estimated exposures (European Food Safety Authority 2006) was taken into account by performing bootstrap statistics (Efron and Tibshirani 1986). Nonparametric bootstrapping was performed by randomly drawing 10,000 samples in each of the simulation sets obtained according to Eqs. 1 through 3. The boundaries of the 95 % confidential interval (CI) were then calculated for the mean, SD, median, and 5th and 95th percentiles. The statistical software package SPSS Statistics (release 20.0.0; IBM, NY) was used for these computations.

Results

Concentration of Cadmium, Lead and Chromium in Wild Boar Meat and Liver

Because the only WB offal consumed was liver, data distributions and statistics are presented only for liver and meat (Table 2). Overall, left-censorship in the analytical data set was virtually absent. A low left-censored rate was recorded (<5 %, data not shown) only for chromium in WB liver. With the exception of data regarding chromium levels in WB meat, all metal-concentration data were adequately fitted by log-normal distributions (Kolmogorov–Smirnov and Chi-square tests, $p > 0.1$). For cadmium, the median values found in meat and in liver were similar, with slight differences at the 95th percentiles (0.131 vs. 0.188 mg kg^{-1} ww). Half of the WB meat and liver samples had lead concentrations <0.119 and 0.323 mg kg^{-1} ww, respectively. Median values for chromium in WB liver were slightly greater (0.125 mg kg^{-1} ww) than in meat (0.112 mg kg^{-1} ww), with a proportionally wider scattered distribution, especially to the right (e.g., the 95th percentiles were 0.370 and 0.283 mg kg^{-1} ww for the liver and the meat, respectively).

Consumption of Locally Hunted Wild Boar Products

Virtually all of the respondents (98 %) declared that they regularly consume WB meat and liver throughout the year. The survey results included estimates for WB product consumption for 221 adults and 41 children, of whom 80 and 18 consumed both WB meat and liver, respectively. The data distributions were right-skewed (Table 3), and a log-normal distribution fitted the meat-consumption data well (Kolmogorov–Smirnov and Chi-square tests, $p > 0.1$). The liver-consumption data were not distributed according to any of the most common distributions (normal, log-normal, gamma, Chi-square). Therefore, discrete distribution was adopted for the MCSM simulations (Table 3). Overall, compared with the median values of meat consumption, liver consumption was low, although consumption was high for the 95th percentiles, particularly for adults.

Cadmium-Exposure Estimation

The MCMS for cadmium exposure (Eq. 1) allowed us to estimate the cadmium exposure of hunters and their families resulting from the consumption of WB meat and liver. The distribution of the simulated exposure for all groups of WB consumers (Fig. 1a) was strongly right-tailed with a low median value (7.4 µg Cd week^{-1} person^{-1} [CI = 0.1 µg Cd week^{-1} person^{-1}]) compared with the 95th percentile, which was equal to 23 µg Cd week^{-1} person^{-1} (CI = 0.4 µg Cd

Table 2 Distributions and statistics for cadmium, lead, and chromium concentrations (mg kg^{-1}ww) found in WB meat and offal (liver)

Contamination category	Symbol	Type of distribution	Probability density function	Mean	Geometric mean	Median	SD	5th–95th percentile (range)
Cd in meat	Cd_m	Log-normal	$X = 0.9506 \cdot x^{-1} \cdot e^{-0.0881 \cdot (\ln x + 2.6535)^2}$	0.078	0.070	0.066	0.049	0.040–0.131 (0.031–0.381)
Cd in liver	Cd_l	Log-normal	$X = 0.8129 \cdot x^{-1} \cdot e^{-0.1205 \cdot (\ln x + 2.7005)^2}$	0.084	0.067	0.067	0.061	0.018–0.188 (0.008–0.380)
Pb in meat	Pb_m	Log-normal	$X = 1.9600 \cdot x^{-1} \cdot e^{-0.0207 \cdot (\ln x + 2.1103)^2}$	0.124	0.121	0.119	0.028	0.090–0.173 (0.080–0.226)
Pb in liver	Pb_l	Log-normal	$X = 1.8640 \cdot x^{-1} \cdot e^{-0.0229 \cdot (\ln x + 1.1338)^2}$	0.329	0.322	0.323	0.072	0.231–0.472 (0.179–0.564)
Cr in meat	Cr_m	Discrete	Discrete[a]	0.133	0.123	0.112	0.082	0.089–0.283 (0.069–0.692)
Cr in liver	Cr_l	Log-normal	$X = 0.5068 \cdot x^{-1} \cdot e^{-0.3100 \cdot (\ln x + 2.1859)^2}$	0.146	0.112	0.125	0.110	0.042–0.370 (<LOD = 0.626)

Distributions were used to generate input contamination data of WB products for the estimation of hunters' metal exposure by MCSMs

[a] Fourteen equally spaced frequency classes with upper limits ranging from 0.05 to 0.70 mg kg^{-1} ww

Table 3 Distributions and statistics of consumed amounts (μg week^{-1} person^{-1}) of WB meat and offal (liver)

Consumption category	Symbol	Type of distribution	Probability density function	Mean	Geometric mean	Median	SD	5th–95th percentile (range)
Meat (all)	N_m	Log-normal	$X = 0.7400 \cdot x^{-1} \cdot e^{-0.1454 \cdot (\ln x + 4.4359)^2}$	113	84	92	112	28–278 (14–831)
Meat adults only	N_{am}	Log-normal	$X = 0.8228 \cdot x^{-1} \cdot e^{-0.1176 \cdot (\ln x + 4.4545)^2}$	123	94	92	117	35–323 (28–831)
Meat children only	N_{cm}	Log-normal	$X = 1.2209 \cdot x^{-1} \cdot e^{-0.0534 \cdot (\ln x + 3.7364)^2}$	49	42	35	29	20–92 (14–138)
Liver (all)	N_l	Discrete	Discrete[a]	38	–	13	59	0–192 (0–385)
Liver adults only	N_{al}	Discrete	Discrete[a]	45	–	19	63	0–192 (0–385)
Liver children only	N_{cl}	Discrete	Discrete[b]	10	–	0	15	0–55 (0–55)

Distributions were used to generate input consumption data for the estimation of hunters' metal exposure by MCSMs

[a] Eight equally spaced frequency classes with upper limits ranging from 50 to 400 g week^{-1} person^{-1}

[b] Three equally spaced frequency classes with upper limits ranging from 20 to 60 g week^{-1} person^{-1}

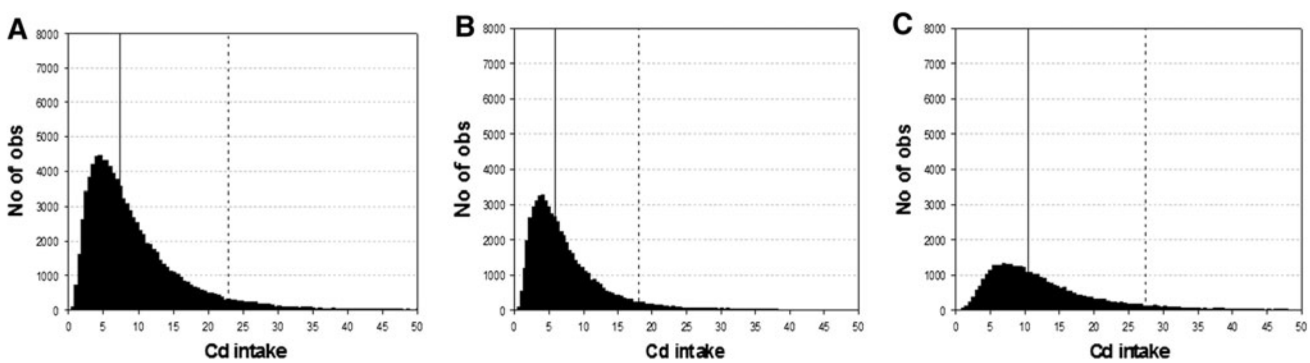

Fig. 1 Cadmium exposure estimation profile (μg week^{-1} person^{-1}) for hunters and cohabiting relatives due to meat and liver consumption (**a**) and separately due to only meat (**b**) and only liver consumption (**c**). Straight and dotted vertical lines represent the median and the 95th percentile, respectively

week^{-1} person^{-1}). The 5th to 95th percentile range (20.8 μg Cd week^{-1} person^{-1}) was a function of the variability of exposure due to meat consumption (5th to 95th range of 16.3 μg Cd week^{-1} person^{-1}) (Fig. 1b), for which the maximum of the simulated metal intake has been recorded to be 113.1 μg Cd week^{-1} person^{-1}. The median value of cadmium exposure from liver consumption was 10.4 μg week^{-1} person^{-1} (CI = 0.2 μg Cd week^{-1} person^{-1}), with the 95th percentile consuming >27 μg week^{-1} person^{-1} (Fig. 1c). Cadmium exposures of adults and children eating the meat and liver of hunted WB were estimated according to Eqs. 2 and 3, respectively (CIs are reported in brackets) (Table 4). The median value for adult cadmium exposure was 8.2 μg week^{-1} person^{-1}, whereas the 95th percentile was >24 μg week^{-1} person^{-1}. Liver consumption had a rather high impact on cadmium exposure in the subgroup of adults that regularly ate this product. The contribution of liver consumption to total cadmium intake represents a nonnegligible additional exposure in addition to the exposure resulting from WB meat consumption: The median exposure of liver-eating adults was 11.5 μg week^{-1} person^{-1} (+41 %), whereas the 95th percentile increased from 24.3 to 29.4 μg week^{-1} person^{-1}. Estimations of cadmium exposure due to WB-product consumption by children (Table 4) showed a similar pattern when WB liver-eaters were compared with the entire consumer pool. However, the median levels (and the 95th percentiles) were lower than for adults: 3.6 (9.2) and 4.8 (10.8) μg week^{-1} person^{-1} for liver-eaters and the entire consumer pool, respectively.

Lead-Exposure Estimation

Figure 3a shows the simulated lead exposure distribution for the entire population studied. As was the case for cadmium, the lead exposure profile is skewed widely to the right, with median and mean values equal to 10.3 μg week^{-1} person^{-1} (CI = 0.2 μg Pb week^{-1} person^{-1}) and 12.1 μg week^{-1} person^{-1} (CI = 0.2 μg Pb week^{-1} person^{-1}), respectively

Table 4 Cadmium exposure estimations (μg week^{-1} person^{-1}) for adults and children[a]

Consumption category	Mean	SD	Median	5th percentile	95th percentile	Minimum–maximum
Adults						
All	10.1 [10.1; 10.2]	7.4 [7.3; 7.5]	8.2 [8.1; 8.2]	2.6 [2.6; 2.7]	24.3 [24.1; 25.5]	0.6–107.2
Meat	8.2 [8.1; 8.2]	5.9 [5.8; 6.0]	6.6 [6.6; 6.7]	2.3 [2.3; 2.3]	19.0 [18.9; 19.2]	0.6–107.2
Meat + Liver	13.5 [13.4; 13.6]	8.4 [8.2; 8.5]	11.5 [11.4; 11.6]	4.3 [4.2; 4.3]	29.4 [29.0; 29.8]	0.8–101.9
Children						
All	4.2 [4.2; 4.3]	2.9 [2.5; 2.6]	3.6 [3.6; 3.7]	1.4 [1.4; 1.4]	9.2 [9.1; 9.3]	0.3–42.5
Meat	3.4 [3.4; 3.4]	1.9 [1.9; 2.0]	3.0 [2.9; 2.9]	1.2 [1.2; 1.3]	7.1 [7.0; 7.2]	0.3–31.1
Meat + Liver	5.4 [5.3; 5.4]	2.9 [2.8; 2.9]	4.8 [4.7; 4.8]	2.0 [1.9; 2.0]	10.8 [10.7; 10.9]	0.5–42.5

[a] For both the age categories, cadmium intake was simulated for all consumers (All) and those who consumed only WB meat (Meat) or WB meat and liver (Meat + Liver). Nonparametric bootstrap CI values are presented in brackets

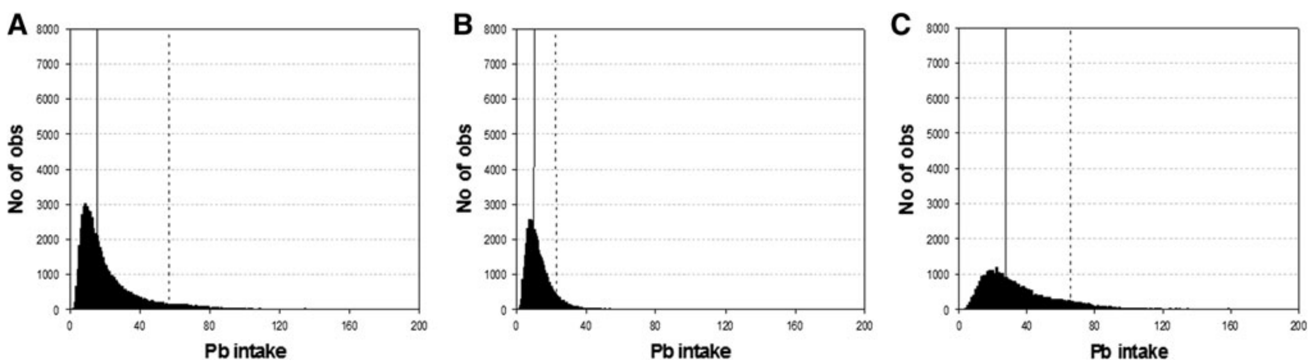

Fig. 2 Lead exposure estimation profile (μg week^{-1} person^{-1}) for hunters and cohabiting relatives due to meat and liver consumption (**a**) end separately due to only meat (**b**) and only liver consumption (**c**). Straight and dotted vertical lines represent the median and the 95th percentile, respectively

(Fig. 2a). The 95th percentile of the distribution decreases at 26.5 μg week^{-1} person^{-1} (CI 0.6 μg Pb week^{-1} person^{-1}), and the highest estimated value is 287.9 μg week^{-1} person^{-1}. When comparing the relative contributions of meat and liver with the total lead exposure for the entire consumer population, it should be noted that liver consumption has a major effect because of its highly skewed distribution compared with the distribution of weekly lead exposure for respondents consuming only meat (Fig. 2b). For the distribution containing liver-eaters, the median, 95th percentile, and maximum are equal to 0, 44.13, and 269.64 μg week^{-1} person^{-1}, respectively (Fig. 2c). If the people consuming only meat are compared with those who eat both meat and liver, the 50th and the 95th percentile of the WB meat consumers were exposed to >10.2 (CI 0.2 μg Pb week^{-1} person^{-1}) and 26.6 μg week^{-1} person^{-1}(CI 0.4 μg Pb week^{-1} person^{-1}), respectively. Conversely, the people consuming both WB meat and liver experienced a considerably greater level of exposure, with the median and the 95th percentile values equal to 27.6 μg week^{-1} person^{-1} (+269 %) (CI = 0.8 μg Pb week^{-1} person^{-1}) and 75.6 μg week^{-1} person^{-1} (+284 %) (CI = 0.9 μg Pb week^{-1} person^{-1}), respectively. For adults, the median and the 95th percentile values were 15.4 and 61.3 μg week^{-1} person^{-1}, respectively (Table 5). Adults who ate both WB meat and liver had a significantly greater lead exposure (median and 95th percentile values of 30.2 and 78.9 μg week^{-1} person^{-1}, respectively) (Mann–Whitney U test $p < 0.01$) than those who consumed only WB meat (Table 5). Children had a median lead exposure of 6.6 μg week^{-1} person^{-1} with the 95th percentile being equal to 20.8 μg week^{-1} person^{-1} (Table 5). As with the adults, liver consumption had a great effect (Mann–Whitney U test, $p < 0.001$) on lead exposure in children, increasing the median value for this consumer category (95th percentile in parentheses) from 5.1 (9.6) μg week^{-1} per child eating only WB meat (data not shown) to 11.5 (24.5) μg week^{-1} per child in the case of families using both WB meat and liver in food preparation.

Chromium-Exposure Estimation

The simulated chromium exposure distributions are summarized in Fig. 3. As observed from the distribution that

Table 5 Lead-exposure estimations (μg week^{-1} person^{-1}) for adults and children[a]

Consumption category	Mean	SD	Median	5th percentile	95th percentile	Minimum–maximum
Adults						
All	21.6 [21.5; 21.7]	18.8 [18.5; 19.0]	15.4 [15.3; 15.5]	5.4 [5.4; 5.4]	61.3 [60.7; 61.8]	1.1–239.7
Meat	13.1 [8.1; 8.2]	7.4 [7.3; 7.5]	11.4 [6.6; 6.7]	4.8 [2.3; 2.3]	27.1 [18.9; 19.2]	1.1–93.7
Meat + Liver	36.1 [13.4; 13.6]	22.9 [22.5; 23.2]	30.2 [11.4; 11.6]	10.9 [4.2; 4.3]	78.9 [29.0; 29.8]	2.4–239.7
Children						
All	8.5 [8.5; 8.5]	5.6 [5.6; 5.6]	6.6 [6.6; 6.7]	3.0 [3.0; 3.0]	20.8 [20.7; 21.0]	0.8–50.7
Meat	5.5 [5.5; 5.5]	2.2 [2.2; 2.2]	5.1 [5.1; 5.1]	2.7 [2.7; 2.7]	9.6 [9.5; 9.7]	0.8–26.0
Meat + Liver	12.6 [12.6; 12.7]	6.2 [6.1; 6.2]	11.3 [11.2; 11.3]	4.9 [4.8; 4.9]	24.5 [24.3; 24.6]	1.7–50.7

[a] For both the age categories, cadmium intake was simulated for all consumers (All) and those who consumed only WB meat (Meat) or WB meat and liver (Meat + Liver). Nonparametric bootstrap CI values are presented in brackets

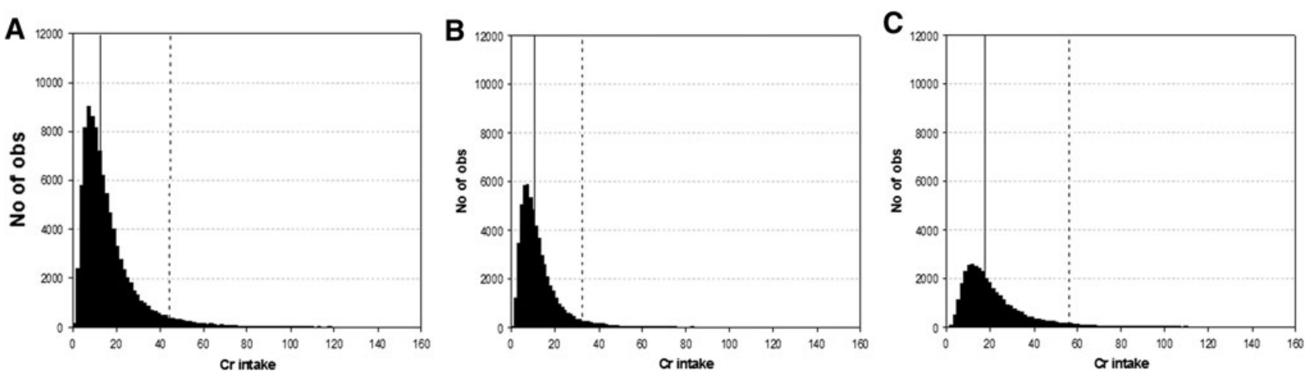

Fig. 3 Chromium exposure estimation profile (μg week^{-1} person^{-1}) for hunters and cohabiting relatives due to meat and liver consumption (**a**) and separately due to only meat (**b**) and only liver consumption (**c**). Straight and dotted vertical lines represent the median and the 95th percentile, respectively

includes the entire consumer population (Fig. 3a), the estimated exposure distributions indicate that half of the population is exposed to levels greater than 12.5 μg week^{-1} person^{-1} (CI for the median = 0.5 μg Cr week^{-1} person^{-1}). In fact, the top 5 % is exposed to 44.1 μg week^{-1} person^{-1} (CI for the 95th percentile = 0.7 μg Cr week^{-1} person^{-1}). A comparison of chromium exposure levels between those consumers eating only meat and those eating meat plus liver (Fig. 3b, c) suggests that consuming WB liver is associated with a significant increase in the overall chromium exposure: The median values for these two consumer categories were 10.0 (CI = 0.4 μg Cr week^{-1} person^{-1}) and 17.6 μg week^{-1} person^{-1} (+76 % increase associated with liver consumption) (CI = 0.5 μg Cr week^{-1} person^{-1}), respectively. In addition, the 95th percentile for the respondents who ate meat and liver (56.4 μg week^{-1} person^{-1} [CI = 0.9 μg Cr week^{-1} person^{-1}]) was 1.7-fold greater than for the 95th percentile of people who ate only WB meat. Analysis of the right extremes of the chromium exposure distribution indicates that regardless of their consumer categorization, a small, nonnegligible portion of the population might have been exposed to high weekly chromium doses, which range from approximately 30–60 to 330–400 μg week^{-1} person^{-1}. The outcomes obtained by running the MCSM according to Eqs. 2 and 3 for adults and children exposed to chromium by way of WB product consumption are listed in Table 6 (CIs are reported in brackets). Liver consumption had a significant (Mann–Whitney U test, $p < 0.001$) effect on the weekly chromium exposure for adults eating liver plus meat compared with the exposure of adults eating only WB meat. The simulated chromium exposure for children (Table 6) is lower than that for adults (Mann–Whitney U test, $p < 0.001$). Median values (95th percentile in parentheses) for the total child population (including both meat-only and meat-plus-liver eaters) were 5.9 (17.25), 5 (12.4), and 7.7 (20.9) μg week^{-1} person^{-1}, respectively. Compared with the respondents eating only WB meat, those eating liver also were exposed to significantly greater chromium levels (median = +154 %; 95th percentile = +169 %; Mann–Whitney U test, $p < 0.001$).

Table 6 Chromium-exposure estimations (μg week^{-1} person^{-1}) for adults and children[a]

Consumption category	Mean	SD	Median	5th percentile	95th percentile	Minimum–maximum
Adults						
All	18.2 [18.1; 18.3]	16.6 [16.3; 16.9]	13.6 [13.5; 13.7]	4.7 [4.7; 4.7]	47.0 [46.4; 47.5]	0.0–404.4
Meat	14.2 [14.1; 14.3]	12.4 [12.1; 12.7]	11.1 [11.1; 11.2]	4.2 [4.1; 4.2]	33.3 [32.9; 33.8]	0.0–285.9
Meat + Liver	24.9 [24.7; 25.1]	20.2 [19.7; 20.8]	19.4 [19.2; 19.5]	7.2 [7.1; 7.3]	60.8 [59.6; 61.9]	1.5–404.4
Children						
All	7.4 [7.4; 7.4]	5.6 [5.5; 5.7]	5.9 [5.9; 5.9]	2.5 [2.5; 2.5]	17.3 [17.1; 17.5]	0.7–101.9
Meat	6.0 [5.9; 6.0]	4.4 [4.3; 4.5]	5.0 [5.0; 5.0]	2.2 [2.2; 2.2]	12.4 [12.2; 12.6]	0.7–76.1
Meat + Liver	9.3 [9.3; 9.4]	6.5 [6.3; 6.6]	7.7 [7.6; 7.7]	3.4 [3.4;3.4]	20.9 [20.6; 21.2]	0.8–101.9

[a] For both the age categories, cadmium intake was simulated for all consumers (All) and those who consumed only WB meat (Meat) or WB meat and liver (Meat + Liver). Nonparametric bootstrap CI values are presented in squared brackets

Discussion

Concentrations of Cadmium, Lead and Chromium in Wild Boar Meat and Liver

To date, no specific limits have been established in the European Union (EU) for toxic metals in game meat and offal, and the regulations regarding lead and cadmium are limited to the minimum risk levels (MRLs) listed in Reg. 1881/2006 for pigs and other farmed species (European Commission 2006). In our study, only 13 of 54 WB meat samples were found to contain cadmium levels lower than the EU MRL for pig meat: 0.05 mg kg^{-1} ww. However, all of the liver samples analyzed had cadmium levels lower than the MRL set for pig offal: 0.5 mg kg^{-1} ww. Our data on cadmium occurrence in WB meat fell within the range of published data, i.e., between 0.001 and 0.355 mg kg^{-1} ww (Medvedev 1999; Rudy 2010; Taggart et al. 2011). Compared with the data presented by Hernández et al. (1985) regarding wild Spanish fauna, we observed lower levels of cadmium contamination in WB meat (geometric means of 0.07 vs. 0.12 mg kg^{-1} ww) as well as WB liver (geometric means of 0.067 vs. 0.45 mg kg^{-1} ww). Our data regarding cadmium in WB liver also differs from data published by Wolkers et al. (1994), who reported a median cadmium concentration as high as 2.05 mg kg^{-1} dry weight (dw) in liver samples of WBs age 1.5–5 years. By adopting a conversion factor of 3.4, as per Holben (2002), the cadmium concentration reported by Wolkers et al. (1994) corresponds to 0.60 mg kg^{-1} ww, which is approximately 10 times greater than the median (0.067 mg kg^{-1} ww) observed in our study. Previous work indicates that such variations in cadmium levels are possible among WBs hunted in different areas, even within the same country (Swiergosz et al. 1993).

Regarding the occurrence of lead in WB meat, only 6 of the 54 samples included here contained lead levels that were lower than the MRL of 0.1 mg kg^{-1} ww, whereas 85 % of the samples had levels between 0.1 and 0.2 mg kg^{-1} ww. Conversely, only one WB liver sample was found to exceed the EU MRL for pig liver of 0.5 mg Pb kg^{-1} ww, even though >50 % of the liver samples had lead levels between 0.3 mg kg^{-1} ww and the MRL. However, if the 0.02 mg Pb kg^{-1} ww MRL set in Reg. 1881/2006 (European Commission 2006) to protect children is used as a safety threshold, than all of the meat and liver samples had lead values greater than this limit. Several studies reported highly variable lead concentrations in WB meat and liver (Swiergosz et al. 1993; Kottferová and Koréneková 1998; Medvedev 1999). Wolkers et al. (1994) reported a median concentration of 0.917 mg Pb kg^{-1} dw (0.270 mg Pb kg^{-1} ww) in livers of WBs that belonged to an age group comparable with those in this study that were shot in The Netherlands. These investigators also found that farmed WB meat products exhibited a significantly lower lead concentration (median 0.082 mg kg^{-1} ww) than free-living animals. Finally, a recent survey performed in Croatia (Bilandžić et al. 2010) noted that lead levels in WB meat may vary considerably depending on the region where the animals live and are hunted. For instance, the mean meat contamination data from seven Croatian hunting areas ranged from 0.028 up to 0.150 mg Pb kg^{-1} ww, with pooled data ranging from 0.001 to 1.01 mg Pb kg^{-1} ww (Bilandžić et al. 2010). Although comparisons are sometimes difficult because of differences in data manipulation, our results for lead fall within the range of values found in the surveyed literature, with the exception of the highest values reported by some investigators (Morales et al. 2011; Taggart et al. 2011). In those studies, lead levels in WB were most likely caused by lead dispersion into the animal body mass after bullet fragmentation (Hunt et al. 2009).

Although chromium may be a contaminant introduced to humans by way of diet (Gunderson 1995), there is currently no established EU limit for this element in animal food products.

The absence of relevant regulations is the most likely reason that only a limited number of studies have been performed recently regarding chromium occurrence in either small or large game animals. Swiergosz et al. (1993) reported a mean chromium level in WB liver of 0.705 mg kg^{-1} ww. That result is nearly five times greater than the level we observed (0.143 mg kg^{-1} ww) and is most likely caused by the high degree of environmental pollution where the animals were hunted. However, we cannot make a conclusion regarding whether our data might reflect a low to moderate environmental pollution because, to date, no reference values in WBs hunted in unpolluted areas in the Central Italy are available. In an Italian study performed in the Urbino-Pesaro Province, Alleva et al. (2006) reported fairly low chromium levels in the liver of five wild mammals. These values ranged from <0.01 mg kg^{-1} ww for badger liver (*Meles meles*) to a maximum of 0.22 mg kg^{-1} ww for red fox liver (*Vulpes vulpes*). For red fox, an omnivorous species, the mean value reported by Alleva et al. (2006) in liver was 0.03 mg Cr kg^{-1}. Chromium accumulation in the livers of WB and red fox was studied by Piskorová et al. (2003), and the average concentration in WB liver was approximately 60 % less than in red fox liver. Taking this finding into account, it is reasonable to assume that liver chromium levels of WBs living in the same environment as the red fox should be low as well even though WB was not specifically addressed by Alleva et al. (2006). Senczuk (1990), as cited by Swiergosz et al. (1993), indicated that physiological levels of chromium in WB liver and kidney should be within the range of 0.015–0.220 mg kg^{-1} dw (0.004–0.064 mg kg^{-1} ww). In contrast, the chromium content of pork meat reported by Bratakos et al. (2002) ranged from 0.05 to 0.14 mg kg^{-1} ww. Chromium is not authorized as a feed additive in the EU because of the lack of a documented UL of human tolerance (Mantovani et al. 2009), hence these values may be considered as initial reference levels for chromium in farmed pigs. Although wild animals living in an unpolluted habitat tend to have greater amounts of trace elements in their tissues and organs compared with farmed animals (Wlostowski et al. 2006), in our study the chromium levels in WB meat and liver ranged from undetectable to 0.626 mg kg^{-1} ww. This range is more than 4 times broader than that reported for farmed pigs by Bratakos et al. (2002). Furthermore, this range encompassed the data reported by Piskorová et al. (2003) (0.02–0.49 mg kg^{-1} ww) for WB hunted in a region stressed by polluted air in the Slovak Republic.

Taken together, the data reported in the literature suggest that the values observed in our study can be considered reasonably high and that chromium accumulation due to aging and/or to a moderate environmental chromium load may be hypothesized. However, the relationship between accumulation and aging for chromium in game is unclear (Gamberg et al. 2005). Thus, there is no unequivocal explanation of our data on chromium accumulation in WB tissues. Further investigations are needed to ascertain if our data on chromium accumulation in WB unambiguously reflect a moderate environmental load of this element.

Consumption of Locally Hunted Wild Boar Products

To our knowledge, the present study is currently the only Italian investigation providing data on the consumption of WB meat and offal. Because there are no official data on WB consumption by hunters' families in Italy, we compared our data on WB meat and liver consumption with the results of earlier total diet studies. In a 1994–1996 description of the Italian market basket (Turrini and Lombardi-Boccia 2002), unspecified game meat consumption for the Italian general population (excluding individuals age <1 year old) was reported to be, on average, approximately 6.4 g $week^{-1}$ $person^{-1}$. Although that study did not report data on game offal consumption, the average total liver and offal consumption in that period was reported to be 17.4 g $week^{-1}$ $person^{-1}$ (Turrini and Lombardi-Boccia 2002). Compared with those data, the mean WB meat and liver consumption rates documented in our study (113 and 38 g $week^{-1}$ $person^{-1}$, respectively) were high, and these results may be explained by the ready availability of WB products to hunters and their families. Similar observations have been made in comparable studies performed in other countries (Burger 2002; Lazarus et al. 2008). Overall, the rates of consumption of WB meat found in our study were similar to the levels reported for hunters and their families in a recent Spanish study (Morales et al. 2011), with the exception of the right-end values (e.g., from the 95th percentile to the maximum), which were greater in our study.

Cadmium, Lead, and Chromium Exposure Assessments for Hunters and Their Families

Uncertainty analysis is a requirement for the reliable estimation of chemical exposure and thus for performing realistic risk assessments (Kroes et al. 2002; European Food Safety Authority 2006). In this study, sources of uncertainty in the food-consumption data (e.g., weekly WB meat consumption/person) (Kroes et al. 2002), chemical data (e.g., left-censored data) (WHO 1995b; Antweiler and Taylor 2008), and simulated data input for MCSMs (e.g., accordance between the distributions of simulated and raw data) were taken into account and managed appropriately. In addition, uncertainties relating to the correctness of the applied model may be as important as the aforementioned sources of uncertainty (van der Voet et al. 2009). Our MCSMs focused exclusively on exposure to heavy metals resulting from WB meat and liver consumption and were designed to cover a spatially and temporally well-defined "harvesting-consumption household system." For these reasons, the correlation between local consumption data and the occurrence of heavy metals is guaranteed. In addition, bootstrapping was used to test the stability and reliability of the developed MCSMs, thus enhancing the reliability of the exposure estimates for cadmium, lead, and chromium. The bootstrap confidence intervals presented (see Tables 4, 5, 6) are quite narrow for all of the simulation estimates, particularly for the medians and upper 95th percentiles, and are in line with those reported in previous exposure studies regarding heavy metals (Sand and Becker 2012) and other food contaminants (Cano-Sancho et al. 2012). Overall, the approach adopted in this study permits the adequate separation of uncertainty and variability as recommended by the European Food Safety Authority (2006).

Meat and other products of animal origin (fish, seafood, and eggs) accounted for approximately 15 % of the estimated cadmium exposure for a sample of the general Italian population (Turconi et al. 2009) or approximately 14.3 μg Cd $week^{-1}$ $person^{-1}$ (range 1.5–34.3 μg $week^{-1}$ $person^{-1}$). Compared with these estimates, the simulated cadmium exposure for our hunter population (range 0.6–107.2 μg $week^{-1}$ $person^{-1}$) was nonnegligible, especially for hunters consuming both WB meat and liver. Recently, the European Food Safety Authority (2009) established a tolerable weekly intake (TWI) level for cadmium of 2.5 μg $week^{-1}$ kg^{-1} bw. This TWI was based on the amount of dietary cadmium exposure that corresponds to the critical urinary cadmium concentration of 1.0 μg g^{-1} creatinine. Thus, for an adult weighing 60 kg, cadmium intake should not exceed 150 μg $week^{-1}$. The mean and 95th percentile for cadmium exposure in our adult hunter population consuming meat and liver accounted for 9.0 and 19.6 % of this safety limit, respectively. For hunters who did not consume WB liver, these estimated rates were >40 % lower.

Thus, the WB consumption in hunters' families should be considered as the manner in which this specific population group could be exposed to cadmium at levels greater than those reported in the general Italian population from the consumption of all animal products. Because products obtained by way of hunting WB most likely represent only a portion of the meat and meat-related products in each hunter family's market basket, these figures suggest that the risks of cadmium exposure by consumption of game meat and offal must be carefully evaluated for this specific population. The weekly cadmium exposure for a 5-year-old child weighing 18 kg (WHO 2000) living in a hunter's family and consuming both hunted WB meat and liver may be estimated to be 0.3 and 0.6 μg $week^{-1}$ kg^{-1} bw in the 50th and 95th percentiles, respectively. These estimates represent 8.7 and 10.9 %, respectively, of the total exposure assessed for European children age 0.5–12 years (Turrini et al. 2001; Turrini and Lombardi-Boccia 2002; Wilhelm et al. 2005; European Food Safety Authority 2009). Similar results may be obtained by comparing our exposure estimates for children with those reported by de Winter-Sorkina et al. (2003) for children in the general Dutch population. Compared with the United States Food and Drug Administration (USFDA) data (Gunderson 1995), our mean cadmium exposure estimate for children consuming both WB meat and liver (0.3 μg $week^{-1}$ kg^{-1} bw) is 13 % of the total cadmium exposure estimated for Americans age 2–16 years. Because approximately 80 % of the cadmium exposure in the above-reported studies comes from cereals, tubers, fruits, and vegetables, it becomes obvious that the exposure of our child population to cadmium by WB-product consumption is dramatically high if only the contributions of meat and meat products are considered. However, these estimates are approximately 35 % lower when WB liver is excluded from the diets of children. Because the total exposure of children to cadmium is normally greater than that of adults (European Food Safety Authority 2009; Food Standards Agency 2009), and because children may be highly susceptible to the effects of cadmium exposure (de Burbure et al. 2006), our estimates suggest that children's exposure to cadmium by way of WB product consumption may represent a health concern that requires more thorough assessment. In addition, such an assessment should be performed because some essential elements (e.g., zinc and copper) may interact with cadmium. In fact, it is known that the intake of zinc exceeding normal nutritional requirements may decrease the toxicological potential of ingested cadmium (Brzóska and Moniuszko-Jakoniuk 2001). However, exposure to cadmium may impair zinc absorption and distribution in organs and tissues, particularly in conditions of nutritional deficiency (Lönnerdal 2000; Schrey et al. 2000).

Lead exposure in hunters' households may be particularly high if the animals are shot using lead bullets and contaminated tissues not carefully discarded (Hunt et al. 2009; Tsuji et al. 2009). In our study, this issue was not included in MCM exposure simulations because the bullet pathway area was carefully excluded during sampling. The estimated lead exposure due to consumption of WB meat and liver was 2.4 and 5.4 % of the 25 μg $week^{-1}$ kg^{-1} bw provisional TWI (PTWI) established by the WHO (1995a) for the mean and 95th percentile, respectively. These percentages are >60 % lower for the group that does not consume WB liver. Although the PTWI has not been updated, the established PTWI for lead is no longer considered appropriate by the European Food Safety Authority (2010) because a threshold for developmental neurotoxicity and nephrotoxicity in adults cannot be set.

Lead exposure related to game meat consumption was recently studied in Spain by Morales et al. (2011): Their mean and 95th percentile estimates for hunters were greater than ours because of the greater reported lead concentrations and greater rate of WB meat consumption (169 g week^{-1} person^{-1}, on average). For the Italian population, the estimated daily exposure to lead is high relative to the PTWI (Kumpulainen 1996). Because approximately half of the lead exposure in EU populations is thought to be the result of consuming lead-contaminated food (Nasreddine and Parent-Massin 2002), it is imperative to put practices in place to support a general strategy for the decrease of alimentary lead exposure. Compared with the FDA estimate of the average exposure of an adult American (41 μg Pb week^{-1} person^{-1}) (Gunderson 1995), the lead exposure estimates for adults in hunters' families in this study are high: 31.9 and 73.5 % for those who consumed only meat and those who consumed meat and liver, respectively. These same comparisons yield different results (10.0 and 27.5 % for hunters consuming WB meat and meat plus liver, respectively) if the study by Seifert and Anke (2000), in which the lead exposure of citizens of four towns in Turingia (Germany) was examined, is used as a reference. The lead exposure linked to WB-product consumption for our population groups, which is 129 % of the estimated mean lead exposure due to consumption of meat and other animal food products for the general Italian population (>1 year old) as estimated by Turrini et al. (2001), is a matter of concern. A similar study was performed by Alberti-Fidanza et al. (2003) on a sample of the general population living in the Gubbio area (Umbria, Italy). That study recorded a median of 44.7 μg week^{-1} for adult men; in our study, the median lead exposures were 25 and 67.6 % of that amount for adults who ate only WB meat and those who ate WB meat and liver, respectively. No details were reported by Alberti-Fidanza et al. (2003) about the specific contributions by meat to total lead exposure. For children, the mean and the 95th percentile are 3 and 5.3 %, respectively, of the PTWI calculated for an 18-kg child living in a hunter's family consuming only WB meat, whereas the concurrent consumption of WB liver significantly increases these percentages to 7.0 and 13.6 % for the mean and 95th percentile, respectively. The estimated medians for lead exposure levels in children due to consumption of WB meat and WB meat plus liver (5.1 and 11.3 μg week^{-1} per child) are between 40 and 90 % of the total dietary lead exposure for children in the general Dutch population (de Winter-Sorkina et al. 2003), and our estimated mean exposures (5.5 and 12.7 μg week^{-1} per child) are between 21 and 49 % of the mean lead exposure estimated by the FDA for children in the United States (Gunderson 1995). However, Gunderson (1995) did not provide details about the contributions of meat and meat-related products to the total exposure of American children to lead.

Taken together, these figures on children's lead exposure suggest a low but nonnegligible risk from WB product consumption, especially when the fact that exposure to this metal might occur by way of consumption of other foods is taken into account. Children are highly susceptible to lead exposure for several reasons, such as efficient absorption from the diet (approximately 50 %) (de Winter-Sorkina et al. 2003) and the potential for younger children to be exposed to doses greater than the PTWI (Boon et al. 2010b). Thus, minimizing lead in the diets of children should be regarded as an important aspect of an overall strategy to decrease neurodevelopmental problems in this population group (European Food Safety Authority 2010). Furthermore, high exposure to lead should be avoided because it impairs synthesis of heme, which in turn decreases the insertion of iron into porphyrin (ATSDR 1999a). When a human is coexposed to both cadmium and lead, an additive effect may be expected due to the decrease in iron absorption caused by cadmium (ATSDR 1999b).

Chromium is considered to be an essential nutrient for several animal species, but it can be toxic at high levels (Anderson 1997; Shrivastava et al. 2002; Bielicka et al. 2005). An AI level (35 μg day^{-1} for a 60-kg adult) has been recommended by the Institute of Medicine (2001). A greater range of intake levels (from 50 to 200 μg day^{-1} person^{-1}) was previously set as the ESSADDI) (Anderson 1997). Several international health organizations (United States-FNB 2001; United Kingdom-Expert Group on Vitamins and Minerals 2003; Scientific Committee on Food 2003) have attempted to evaluate the toxicity of chromium and its compounds, but no limits have been established because of the scarcity of toxicological data. Thus, the current upper limits are limited to the precautionary maximum dose of 250 μg Cr day^{-1} person^{-1} established by the WHO (1996) and the oral MRLs established by the Agency for Toxic Substances and Disease Registry (2008) (i.e., 5 μg Cr^{VI} day^{-1} kg^{-1} bw for intermediate exposure) [15–365 days] and 1 μg Cr^{VI} day^{-1} kg^{-1} bw for chronic [>1 year] exposure. However, the limits for CR^{VI} have poor, if any, applicative significance for dietary exposure to the metal. In fact, even although no data on chromium speciation in food are available (Boon et al. 2010a), Cr^{III} is the metal's most stable form and is presumably the predominant form occurring in food (EPA 1998; Institute of Medicine 2001; Levina and Lay 2008). In addition, Cr^{III} is the most bioavailable form that occurs in food. Approximately 0.4–2.5 % of Cr^{III} is gastrointestinally absorbed (Institute of Medicine 2001), and it must be regarded as the ultimate toxic form of chromium available for metabolism by animal cells (Léonard and Lauwerys 1980; Levina and Lay 2008). Because no chromium deficiency has been consistently reported amongst healthy human populations (Stallings and Vincent 2006; Stearns 2007), the fact of its "essentiality" remains a controversial matter (Stearns 2000; Levina et al. 2003; Stallings and Vincent 2006). Furthermore, because chromium may accumulate and is possibly genotoxic to animals and humans (Smith 1970; Debetto and Luciani 1988; Snow 1991; Bridgewater et al. 1994; Stearns et al. 1995; Błasiak and Kowalik 2000), excessive chromium intake (or exposure) should be avoided. For the two categories of adults in our study, the median and 95th percentile values are, respectively, 0.6 and 1.9 % (consumers of WB meat only) and 1.1 and 3.5 % (consumers of WB meat plus liver) of the safe limit established by the WHO (1996). In our study, there were no groups for which intake levels were greater than the WHO safe limit. If the highest acceptable intake of 35 $\mu g\ day^{-1}\ person^{-1}$ (Institute of Medicine 2001) is used as a reference, the median exposure and the 95th percentile values for people consuming WB meat were 4.6 and 13.6 %, respectively, and these percentages increased to 7.9 and 24.8 % for people who also consumed liver.

Overall, it was difficult to compare levels of chromium-related WB consumption because of the lack of available data gathered by other researchers. Compared with the exposure values reported by Cigna-Rossi et al. (1978), the mean chromium exposure values recorded for our adult population ranged between 3.4 % (WB meat consumers) and 12.7 % (WB meat and liver consumers). For children, Boon et al. (2010a) estimated a median and 95th percentile for long-term dietary chromium exposure of 5-year-old Italian children (18 kg weigh) to be 491 and 793.8 $\mu g\ week^{-1}$. We estimated a weekly chromium exposure of <3 % of other populations of children in the EU (Boon et al. 2010a). Based on these comparisons, the contribution of WB products to total chromium exposure in both adults and children could be considered to be safe, even though the existing gap in chromium speciation and exposure knowledge should be addressed. Finally, in light of the chromium-accumulation data for WBs hunted in the areas where our studied population lives, further studies are needed to estimate the total chromium exposure to which hunters and their families are subjected.

Conclusion

Cadmium, lead, and chromium contents of WB liver and meat harvested from six hunting areas in the Viterbo Province (Italy), as well as the WB product consumption and heavy-metal exposure of a specific hunter population, were investigated in light of data reported in previous studies. The highest values for lead contamination in WB products that were reported in some studies are most likely artifacts caused by bullet lead dispersion. This is not a factor in our research because tissue samples were carefully collected at appropriate distances from the bullet paths. In most cases, however, lead and cadmium levels in WB meat were greater than the reference EU MRLs set for pork meat. Levels of these metals in liver samples did not exceed the EU MRLs. There are no EU standards against which the safety of chromium in WB meat or liver can be evaluated, but comparisons with data reported in the literature regarding farm-raised pigs and research on WB physiology allow us to hypothesize a moderate environmental load for this metal. To date, there are no official data, either for WB hunters or the general population, on the consumption of WB meat and liver in Italy. Thus, our WB consumption data are novel.

As outlined in other international studies, our survey of hunters confirmed that they consumed greater amounts of WB meat and liver than the general population, undoubtedly because WB meat is more readily available to hunters. To the best of our knowledge, this is the first study of exposure to cadmium, lead, and chromium as a result of consuming hunted WB meat and liver for an Italian hunter population. When compared with total diet studies and, wherever possible, studies of the "meat and offal" food category, our study showed that the consumption of WB products contributed significantly to the cadmium exposure of both adults and children. Overall, despite the fact that rates of WB liver consumption are lower than for WB meat and that cadmium and lead concentrations in WB liver did not exceed the reference EU MRLs set for farm-raised pigs, liver consumption contributes significantly to total cadmium and lead exposure of members of WB-hunter households. Thus, as long as there is no specific risk assessment for Italian and EU hunter populations, and for children of hunters in particular, WB liver-consumption rates should be kept as low as possible. As outlined in other studies, exposure assessments for heavy metals in hunter populations are needed for reliable risk-assessment studies. Such studies are justified by the increasing quantity of game that is being harvested and consumed throughout Europe and by the need to establish a specific regulatory framework for contaminants in game.

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