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Lead concentration in meat from lead-killed moose and predicted human exposure using Monte Carlo simulation

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Lead-based hunting ammunitions are still common in most countries. On impact such ammunition releases fragments which are widely distributed within the carcass. In Norway, wild game is an important meat source for segments of the population and 95% of hunters use lead-based bullets. In this paper, we have investigated the lead content of ground meat from moose (*Alces alces*) intended for human consumption in Norway, and have predicted human exposure through this source. Fifty-two samples from different batches of ground meat from moose killed with lead-based bullets were randomly collected. The lead content was measured by atomic absorption spectroscopy. The lead intake from exposure to moose meat over time, depending on the frequency of intake and portion size, was predicted using Monte Carlo simulation. In 81% of the batches, lead levels were above the limit of quantification of 0.03 mg kg⁻¹, ranging up to 110 mg kg⁻¹. The mean lead concentration was 5.6 mg kg⁻¹, i.e. 56 times the European Commission limit for lead in meat. For consumers eating a moderate meat serving (2 g kg⁻¹ bw), a single serving would give a lead intake of 11 µg kg⁻¹ bw on average, with maximum of 220 µg kg⁻¹ bw. Using Monte Carlo simulation, the median (and 97.5th percentile) predicted weekly intake of lead from moose meat was 12 µg kg⁻¹ bw (27 µg kg⁻¹ bw) for one serving per week and 25 µg kg⁻¹ bw (45 µg kg⁻¹ bw) for two servings per week. The results indicate that the intake of meat from big game shot with lead-based bullets imposes a significant contribution to the total human lead exposure. The provisional tolerable weekly intake set by the World Health Organization (WHO) of 25 µg kg⁻¹ bw is likely to be exceeded in people eating moose meat on a regular basis. The European Food Safety Authority (EFSA) has recently concluded that adverse effects may be present at even lower exposure doses. Hence, even occasional consumption of big game meat with lead levels as those found in the present study may imply an increased risk for adverse health effects. Children and women of child-bearing age are of special concern due to the neurodevelopmental effects of lead.

Keywords: metals analysis – AAS; exposure modelling; lead; meat

Introduction

Lead is a highly toxic heavy metal, which nowadays is omitted in an increasing number of products, such as gasoline, paint, food cans, pipes and ammunition used over wetlands (Avery and Watson 2009; World Health Organization (WHO) 2011). Nevertheless, lead-based rifle bullets are still common around the world. These lead-based bullets are designed to disintegrate on impact, which leads to significant and widespread fragment dispersion (Hunt et al. 2006; Dobrowolska and Melosik 2008; Hunt et al. 2009; Grund et al. 2010; Knott et al. 2010; Stokke et al. 2010). These findings are based on radiographic measurements of lead in carcasses and meat packages from animals killed with lead-based bullets, chemical analyses of lead in tissues at different distances from the bullet track, or measurement of weight loss of bullets at impact. The bioavailability of the lead fragments has been demonstrated in pigs (Hunt et al. 2009); and elevated

blood concentrations of lead in humans have been associated with consuming gun-killed game meat (Iqbal et al. 2009).

Big game hunting is popular in Scandinavia, and Norway has one of the highest concentrations of hunters in Europe (Rundtom and Steinset 2009). Moose (*Alces alces*) is the largest contributor to the consumption of harvested game meat. Approximately 36,000 moose, with a total slaughter weight of about 4.6 million kg, were harvested yearly in Norway in 2009 and 2010 (Statistics Norway 2011a), for a total human population of 4.8 million (Statistics Norway 2011b). According to Stokke et al. (2010) lead-based ammunition is used by 95% of Norwegian moose hunters. On average, 1.4 bullets are used per killed moose, with an estimated amount of lead deposited in each carcass of 3.77 g. In addition, one extra bullet in the head or neck is used to euthanise 29% of the animals.

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The present study was undertaken to investigate lead levels in Norwegian moose meat intended for human consumption, and assess lead intake based on these levels. Monte Carlo simulation was used to account for the variability of lead concentration in moose meat.

Materials and methods

Meat samples

Samples of 300–500 g were collected from 52 different batches of ground meat intended for human consumption, and originating from wild moose shot with lead-based ammunition in the fall 2009 and 2010; in most cases the batch was meat from a single animal. Ground meat was selected to obtain a mixed sample from various parts of the moose.

The samples were collected from the most important moose hunting counties according to Statistics Norway (2011a), after a random selection of registered hunters. An additional five consumer packages of ground moose meat were bought at different vendors in the Oslo region.

Sample processing and chemical analysis

The samples of ground meat were chemically analysed for lead content by the accredited laboratory at The Norwegian Veterinary Institute in Oslo. The samples were stored in plastic bags and maintained at -20°C until processing. Each sample was homogenised with nitric acid (ROMIL-SpA super purity nitric acid, Cambridge, UK; 15 v/v %) in a 2:1 weight ratio. The samples were kept in a beaker overnight at 22°C . This slurry was then transferred to a sample container and kept frozen until analysis. In order to prevent contamination from utensils and the homogeniser, every item was washed in 7.5 v/v % of the nitric acid between each processed sample.

For the detection and quantification of lead, digestion of a 0.8 g homogenised subsample was carried out in closed Teflon beakers by microwave heating with a mixture of 4 ml nitric acid (ROMIL-SpA 67%) and 1 ml of hydrogen peroxide solution (Fluka TraceSELECT Ultra, $\geq 30\%$, Sigma-Aldrich, Steinheim, Germany).

Analysis for lead was performed by atomic absorption spectroscopy (Perkin Elmer Analyst 800, Waltham, MA) with graphite furnace, as described by Bernhoft et al. (2002) after dilution to 25 ml, using deionised water (resistivity $> 10 \text{ M}\Omega \text{ cm}^{-1}$). The limit of quantification (LOQ) was $0.03 \mu\text{g lead g}^{-1}$ of meat. All results were reported on a wet weight basis. The quality control sample bovine liver BCR 185 R was utilised as a reference material and the results were in agreement

(87–99%) with the certified value. In addition, an in-house control liver spiked with a known concentration of lead showed results within acceptable limits. All analyses were performed in accordance to an accredited method (Norwegian Accreditation (P110)).

Statistics and prediction of human exposure

The lead content in the 52 different batches was used to model the lead concentration in servings of moose meat originating from animals killed with lead-based ammunition. Unquantifiable levels were modelled with a Uniform (0, 0.03) distribution, which assigns a random value between 0 and 0.03 (= LOQ). Quantifiable levels were modelled by a continuous distribution based on the cumulative distribution of the observed quantifiable values, as described by Vose (2000), with a maximum value of 10% above the maximum observed.

The lead concentration in each serving was randomly drawn from the modelled distribution. The predicted average weekly intake was then calculated from the sampled concentrations and the ratio between serving size and body weight (S/bw), as presented in Table 1. A moderate serving of 2 g meat kg^{-1} body weight (bw) was used in the calculations (50 g meat for a child weighing 25 kg, or 150 g meat for an adult weighing 75 kg). The lead exposure following higher or lower intakes may, however, be easily calculated as the exposure is directly proportional to the intake.

Statistics, modelling and Monte Carlo simulation were done using Microsoft Excel 2002 with @Risk 4.5.5 Professional (Palisade) as add-in. Hypercube sampling with 10,000 iterations was used during simulations to obtain convergence. Inputs and formulas are summarised in Table 1.

Results

Lead at a quantifiable level was found in 42 (81%) of the 52 batches (Figure 1). The mean lead concentration was 5.6 mg kg^{-1} , the standard deviation was 20 mg kg^{-1} and the 95% confidence interval was $0.09\text{--}11 \text{ mg kg}^{-1}$. The median and 95th central inter-percentile range were 0.3 mg kg^{-1} and $0.02\text{--}79 \text{ mg kg}^{-1}$, respectively. The maximum concentration measured was 110 mg kg^{-1} . In the subsample of five packages bought at meat vendors the lead concentration ranged from 0.61 to 3.6 mg kg^{-1} , with a mean of 1.5 mg kg^{-1} .

The lead intake following one serving of the sampled meat can easily be calculated from the measured values, knowing the serving size. A single moderate serving ($2 \text{ g kg}^{-1} bw$) of such meat would result in a lead intake of $11 \mu\text{g kg}^{-1} bw$ on average

Table 1. Input data for the prediction of human exposure of lead from lead-killed moose.

Variable	Unit	Symbol	Value, distribution or formula
Observed quantifiable fraction			42/52
Measured lead concentrations	mg kg ⁻¹	X_i	0.03, 0.04, 0.04, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.07, 0.09, 0.13, 0.14, 0.16, 0.26, 0.26, 0.28, 0.29, 0.35, 0.44, 0.48, 0.58, 0.61, 0.88, 0.89, 0.93, 0.95, 0.99, 1.1, 1.6, 2, 2.3, 2.4, 2.7, 3.6, 4.3, 4.6, 7.9, 9.4, 35, 95, 110
<i>Modelled lead concentrations</i>			
Fraction quantifiable		f_q	= Beta (43, 11)
Quantifiable?		q	= Binomial (1, f_q)
If nonquantifiable ($q=0$)	mg kg ⁻¹	C_i	= Uniform (0, 0.03)
If quantifiable ($q=1$)	mg kg ⁻¹	C_i	= Cumulative (0.03, 110*1.1, X_i , rank(X_i)/(42 + 1))
Number of servings per year		n	1, 2, ..., 260
Average lead concentration	mg kg ⁻¹	$C_{y,n}$	= Average (C_1, C_2, \dots, C_n)
Serving size/body weight	g kg ⁻¹	S/bw	2
Average weekly intake	µg kg ⁻¹	WI	= (S/bw)* $C_{y,n}$ * n /52

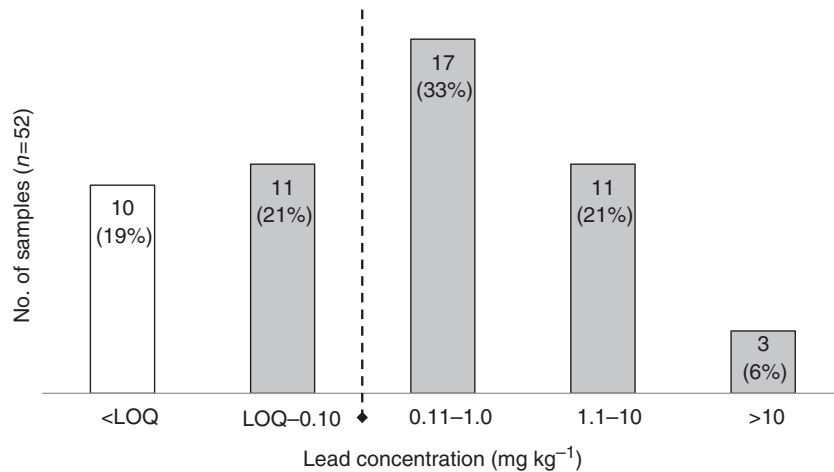


Figure 1. Lead concentration (mg kg⁻¹) in ground meat from moose shot with lead bullets ($n = 52$). The dotted line indicates the maximum level in meat, laid down by the European Commission (0.10 mg kg⁻¹). Limit of quantification (LOQ) = 0.03 mg kg⁻¹.

(5.6 µg g⁻¹ meat × 2 g meat kg⁻¹ bw), with a maximum 220 µg kg⁻¹ bw (110 µg g⁻¹ meat × 2 g meat kg⁻¹ bw).

Over time, however, consumers are exposed to different batches with different concentrations. We simulated this situation by drawing random values for the lead concentrations from a distribution based on the samples of moose meat, and calculated the predicted average weekly intake. Results for a varying number of servings of moderate size are shown in Figure 2. The model predicted a weekly lead intake (median and 95th central inter-percentile range) following the regular consumption of moderate portions of lead-killed moose meat of 12 µg kg⁻¹ bw (2.6–27 µg kg⁻¹ bw) when eating one serving per week, and 25 µg kg⁻¹ bw (10–45 µg kg⁻¹ bw) when eating two servings per week. The intake is directly proportional to the portion size. For example, for portions twice as big (4 g kg⁻¹ bw, for example 100 g for a child of 25 kg) the exposure would be twice as high.

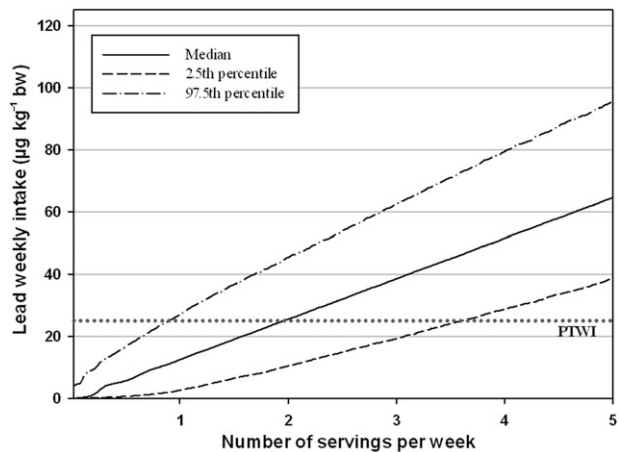


Figure 2. Predicted weekly lead intake (µg kg⁻¹ bw) through moose meat shot with lead bullets. Serving sizes of 2 g kg⁻¹ bw. The 2.5th, 50th and 97.5th percentiles are shown, as well as the provisional tolerable weekly intake (PTWI) set by the FAO/WHO.

Discussion

Lead content in game meat killed with lead-based ammunition as a source for human exposure

Detectable levels of lead were found in the majority of samples of ground meat intended for human consumption, ranging up to 110 mg kg^{-1} . Levels were considerably higher than what could be expected from environmental exposure alone. The background level of lead in the liver and kidneys of Norwegian moose is below 0.03 mg kg^{-1} (median; unpublished data from the Norwegian Veterinary Institute). As lead is poorly biodistributed into mammalian muscle tissue, the background concentration is even lower in muscle samples.

All moose were killed with lead-based ammunition, which is the most likely source of the lead measured. Previous studies have demonstrated a massive and differential distribution of lead from bullet ammunitions (Hunt et al. 2006; Dobrowolska and Melosik 2008; Hunt et al. 2009; Grund et al. 2010; Knott et al. 2010; Stokke et al. 2010). Grund et al. (2010) collected muscle tissue samples 5, 25 and 45 cm from the wound channel in white-tailed deer and sheep and found lead at all distances, although the highest concentrations were found closest to the wound. Similar results were found by Dobrowolska and Melosik (2008) in samples from wild boar and red deer collected up to 30 cm from the bullet pathway. The high variability in lead levels observed supports this acute source of contamination, since ground meat may be produced from variable parts of the animal. In addition, the lead distribution in meat from a hunted moose may vary with the type and number of bullets, the velocity at impact, distance and angle of the shot, and the possible encountering of bones (Grund et al. 2010; Stokke et al. 2010).

Maximum levels of lead in foodstuff are laid down by the European Commission (2006) in Regulation (EC) No. 1881 (2006). Neither moose meat nor any other big game meat is listed in this regulation. However, since meat from moose and other big game is such an important meat source for people in these population groups, it is reasonable to assign moose meat into the foodstuff category 'Meat (excluding offal) of bovine animals, sheep, pig and poultry'. The maximum level for this category is $0.10 \text{ mg lead kg}^{-1}$ wet weight. In 31 (60%) of the batches sampled in our study, lead concentrations exceeded this maximum level.

The human exposure to lead through the consumption of ground meat from moose killed with lead-based ammunition was predicted according to the number of servings. The findings indicate that occasional consumers, who eat such meat at most every second week on average, are unlikely to ingest levels above the provisional tolerable weekly intake (PTWI) set by the WHO (2011) at $25 \mu\text{g kg}^{-1}$ bw through this

source only. However, frequent consumers who eat such meat twice or more a week, are likely to ingest such levels through this source, particularly if the portion size increases.

According to the European Food Safety Authority's (EFSA) Panel on Contaminants in the Food Chain (CONTAM) (2010), the estimated national dietary exposure to lead in Europe ranges from 2.5 to $8.7 \mu\text{g kg}^{-1}$ bw per week in average adults, up to $17 \mu\text{g kg}^{-1}$ bw per week in high consumers. Estimated exposure in children ranges from 5.6 to $22 \mu\text{g kg}^{-1}$ bw per week (average consumers) up to $39 \mu\text{g kg}^{-1}$ bw per week (high consumers). Cereals, vegetables and tap water were identified as the most important contributors to lead exposure in the general European population. High consumers of game meat were estimated to be exposed to 14 – $17 \mu\text{g kg}^{-1}$ bw per week.

The results suggest that the frequent consumption of ground big game meat killed with lead ammunition may greatly increase the lead intake in this sub-group.

To what extent the lead fragments in the meat, in comparison with lead in other foods, are absorbed in the human body is nevertheless an uncertain factor. The laboratory processing of the meat samples with nitric acid and hydrogen peroxide may possibly resolve more lead than the gastrointestinal process. Most of the hundreds of fragments radiographically counted in deer shot with lead bullets were reported to weigh only 0.1 – 1.0 mg , and a considerable number are supposed to be missed due to their even smaller size (Knott et al. 2010). Therefore, a large surface area of lead fragments is exposed to gastrointestinal absorption processes. The demonstration of elevated blood levels of lead in pigs fed with lead-hunted venison (Hunt et al. 2009) as well as the association between human blood lead levels and the consumption of gun-killed game (Iqbal et al. 2009) indicate a significant bioavailability. Physical removing of the lead fragments before the ground meat is ingested is assumed to be almost impossible.

Recent research indicates that the bioavailability of lead in game meat is increased after cooking and even more so if using acidic ingredients (Mateo et al. 2011). This way of cooking includes modern and increasingly popular methods such as marinating and the use of wine, fruit juices or vinegar in the food. Additional studies on bioavailability are required to assess how large the fraction of the observed lead is absorbed.

The consumption pattern of moose meat in Norway has never been adequately investigated. However, one can expect that hunters and their families in general eat more meat than the national average, with a larger proportion of this being big game meat. The authors' knowledge of this subpopulation suggests that they most likely eat moose 1.5 times a week, ranging from 0.5 to four times. If the

moose have been killed with lead-based ammunition, this alone may lead to intake levels exceeding the PTWI in a large part of this subpopulation. However, hunters and their families know whether or not lead-based ammunition is used, and may be able to sort out meat from the area affected by the bullet. Therefore, to some extent they have the opportunity to decrease their lead intake from game, and particularly consider women of child-bearing age and children, compared with the general population buying meat from vendors.

Implications for human health

In a human toxicological perspective, lead is mainly a chronic poison, which accumulates in the body and may cause severe intoxications, even at low doses (EFSA CONTAM 2010). Health effects are hardly observed after a single oral exposure, although high-level acute exposure may cause colic and encephalopathy in children. Food is the main source of chronic lead exposure. During pregnancy, stored lead is mobilised due to bone catabolism to produce the foetal skeleton (Gulson et al. 1997). Maternal lead is also transferred to infants during breastfeeding with a maternal blood/milk ratio of up to 0.9 (Ettinger et al. 2006).

The main target organ for lead toxicity is the central nervous system with effects on cognitive functions – particularly in the developing brain (EFSA CONTAM 2010). In children, elevated blood lead concentrations are associated with reduced IQ scores. The EFSA CONTAM Panel identified neurotoxicity in young children, and kidney and cardiovascular effects in adults as potential critical adverse effects of lead exposure. The risk assessment used benchmark modelling of effects and intake estimation modelling based primarily on blood lead levels from seven human studies. Risk of neurodevelopmental effects associated with lead intake in children was estimated at $3.5 \mu\text{g kg}^{-1}$ bw per week. This dose level was estimated to correspond to a 1% decrease in full scale IQ score. In adults, the risk of nephrotoxicity and increased systolic blood pressure were derived at 4.4 and $11 \mu\text{g kg}^{-1}$ bw per week, respectively. These dose levels were estimated to correspond to a 10% change in the prevalence of chronic kidney disease and a 1% change of systolic blood pressure, respectively. Thus, the CONTAM Panel concluded that the PTWI of $25 \mu\text{g kg}^{-1}$ bw is no longer appropriate and that there is no evidence for a threshold for the critical endpoints (EFSA CONTAM 2010). Based on these risk estimates, there seems to be a strong need to avoid unnecessary lead exposure. Although additional studies are needed to assess the magnitude of the effect, it is likely that even the occasional consumption of big

game meat with elevated lead concentrations may imply a risk for adverse effects.

Several other species of big game hunted for food are shot with lead-based ammunition. In Norway, this includes other deer species, mouflon, muskox and wild boar. Similar lead levels can therefore be expected to be found in meat from these species, and the total consumption of big game meat should be considered.

Although the lead content in big game meat primarily is of human concern, and probably in particular for hunters and their families, the companion animals should not be forgotten. In veterinary toxicology, acute and sub-acute lead intoxications are well known, primarily in cattle and waterfowl, but also in companion animals. In dogs, the range of acute toxic dose is approximately $190\text{--}1000 \text{ mg kg}^{-1}$ bw, whereas a chronic cumulative toxic dose is $1.8\text{--}2.6 \text{ mg kg}^{-1}$ bw per day (Osweiler et al. 2011). This indicates that repeated feeding with big game meat from the area close to the bullet pathway may constitute a significant toxic risk also for dogs.

Study design

Monte Carlo simulation is a method widely used in risk assessment to account for the variability of biological systems, as well as the uncertainty related to our knowledge. This study used this approach to account for the variability of lead concentration in different servings. The lead levels measured in 52 random batches of ground meat were used to model a continuous distribution, reducing the impact of having relatively few samples. The uncertainty related to the proportion of samples with quantifiable levels of lead was also considered when modelling the variability of lead concentration in ground meat.

Ground meat was chosen as the grinding process mixes and homogenises the meat. Moreover, it is produced from various parts of the animal, and may contain both the highest and the lowest lead levels. The heterogeneous distribution of lead through the carcass should be acknowledged when estimating the risk imposed by certain parts of the carcasses from animals killed with lead-based ammunition. More information on the variation of lead levels in different parts of the animal and between animals, as well as the consumption patterns, would increase the precision of the predictions.

Conclusion

Population groups with an elevated intake of meat from moose and other big game harvested with lead-based ammunition are likely to have an exposure to lead that exceeds the exposure of concern. Particularly at risk are children, either directly through

consumption of big game meat or indirectly through a placental or breast-milk exposure. Based on EFSA's recent risk assessment, even occasional consumption of big game meat with lead levels as determined in the present study may imply a risk for adverse effects. In light of these findings, it may be warranted for health and food safety authorities to investigate further consumption patterns and public health implications, and apply appropriate measures. The use of non-toxic substitutes to lead-based ammunition, which are available on the market, would easily and significantly reduce lead exposure.

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