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LEAD IN GAME MEAT A Study of Bioaccessibility of Lead Metal Fragments

Ulf Qvarfort[1] and Christer Holmgren[2*]

Abstract

The presence of metal lead fragments in the tissue around the wound channel can be observed in animals killed with lead-based bullets. These parts are normally discarded as food but a limited number of fragments may be consumed accidentally. A number of studies are performed to estimate mean lead metal contamination in game meat harvested with rifle and is assumed that all lead metal fragments to its total weight that may accidentally be left in the edible muscle tissue, will be dissolved to an absorbable lead compound which can be taken up by the body severely increasing B-Pb level.

When blood lead level, B-Pb, is an important biomarker for dietary exposure from lead several studies have been conducted comparing blood lead levels in hunters with similar population groups who do not eat wild game. Even taking into account environmental and social conditions, eating habits and lead related occupations or hobbies, few conclusions can be drawn concerning risk assessment. This because lead pathways in general as well as the actual bioaccessibility of any metal lead accidently ingested, and the actual absorbable (bioavailable) fraction of this, are not compared or taken into account.

The aim of this study was, by mean of in-vitro gastrointestinal simulation (IVG), to experimentally examine what percentage of any metal lead fragments present in tissue from a severely contaminated wound channel is converted to water soluble bioaccessible lead salts in the human gastrointestinal tract.

Results obtained show that less than 2 % of all present lead metal fragments in raw meat from wound channel are converted to bioaccessible form in the human GI tract.

Using this knowledge of the percentage of bioaccessible lead released, the impact on human blood lead levels from known or estimated contamination of lead metal in food can be easily calculated with use of Carlisle and Wade (1992) empirical equation for dietary exposure adopted by CONTAM panel 2010 using Slope Factor Models and absorption fractions.

Keywords: Lead metal; Blood lead level; Bioaccessibility; Ammunition; Lead-based bullet; Dietary exposure.

Summary

The presence of metallic lead fragments in meat from game animals shot with lead-based bullets has been well documented. However, little information exists regarding the conversion of metallic lead fragments to bioaccessible lead compounds in the human gastrointestinal tract. Most risk assessments

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which have so far been conducted on lead, especially lead metal in game meat, assume that either all the lead metal fragments by weight from a bullet that may accidentally be left in the edible muscle tissue can be taken up by the body, or that 20 to 50% of it can be taken up depending on age. When blood lead level, B-Pb, is an important biomarker for dietary exposure from lead this study was conducted to investigate the distribution of lead metal fragments around the center of the wound channel and to what extent bioaccessible lead compounds is released from accidentally ingested lead metal fragments in human gastrointestinal tract.

By means of flat X-ray and computed tomography the investigated meat samples was collected from the unginned wound channels, which is the most lead metal contaminated part of the animal. One part of each collected and homogenized sample from the wound channel then was analyzed for total lead content. The second part of same sample was used for analysis of the *in vitro*, digestive-released amount of bioaccessible lead compounds from the lead metal fragments present. The result of *in vitro* gastrointestinal simulation shows that the bioaccessible part of lead metal is less than 2% in most part of the investigated samples (mean 0.5%) regardless size of present fragments. An additional *in vitro* study of US 3 (3.5 millimeter) lead shot only, shows a result of between 0.3 and 0.8%. By means of computed tomography and flat X-ray, an exact distribution of lead particles in the animal could be identified around the wound channel. This shows that the courser lead fragments can only radially penetrate edible muscle tissue a few centimetres around the wound channel, while the finer fragments can appear in the blood, present in the damaged muscle tissue and soft tissue in the chest, due to their low weight and low energy. Risk assessment simulation using the Integrated Exposure Uptake Biokinetic Model (or IEUBK Model), developed by the US Environmental Protection Agency (US EPA) shows that minced moose meat with a total lead metal content of 0.9 mg/kg ww, [The National Food Agency, Sweden 2012] and presuming that all lead metal is bioaccessible, will increase the blood lead level to 7.2 µg/dL.

A re-calculation based on the result from this study, which suggests that the bioaccessible part of metallic lead is only 2%, and assuming an uptake of 50%, (children) will cause a temporary increase of the blood lead level in a child to only 0.3µg/dl.

In a survey by the National Food Agency Sweden (Report 18-2014, Part 1) was conducted an experiment intended to simulate the dissolution of lead in the stomach. As starting material were used metal chips of lead which were placed in 0.1M hydrochloric acid for different time intervals. The samples also underwent some stirring by agitation in test tubes. The results showed, as expected, that both time and rocking increased the release of lead. However, it is doubtful that the relevance of the results. Essentially they only studied leads dissolution in 0.1 M HCl after various time intervals and stirring average result obtained was 8%. Why they don´t use the usually *in vitro* techniques to study the dissolution of lead in the stomach as described in the literature is confusion and unknown.

Previously established PTWI (provisional tolerable weekly intake) of 25 micrograms lead/ week and kg bodyweight (214 µg /day for a 60 kg person corresponding to 0.41 µMol/L) from 2010 is not appropriate anymore. New B-Pb endpoints by CONTAM panel from 2010 is far below PTWI: $BMDL_{01}$ for developmental neurotoxicity = 1.2 µg/dL(B-Pb), effects on kidney (CKD) in adults: $BMDL_{10}$ = 1.5 µg/dL (B-Pb) and effects on systolic blood pressure (SBP) in adults: $BMDL_{01}$ = 3.6 µg/dL (B-Pb) This corresponds with a daily intake of 0.5, 0.63 and 1.5 bioaccessible lead /kg b.w/day respectively for a 60 kg person. For estimation the relationship between B-Pb and dietary lead intake CONTAM panel has from 2010 adopted use of Carlisle and Wade empirical equation (California EPA, Intake slope factor model). Intake (bioaccessibly lead) * 0.04 = B-Pb µg/dL. From a known consumption of game meat with a known or estimated content of lead metal from bullets and the results from this study the realistic daily exposure and impact on B-Pb can easily be calculated.

Presumed an annual dietary intake of 200 grams per day of mentioned mincemeat with a content of 0.9mg lead metal per kilo and from the findings in this study, that 2 % of this lead metal is converted to bioaccessible form in the GI tract. In a steady state B-Pb will increase with 0.144µg/dL. This corresponds, with this unusually high annual consumption of mincemeat (73 kilogram/year), to less than 10 % of the end point B-Pb level for increased risk of CKD. The example used in this study is important because existing theoretical models regarding uptake of lead metal possibly present in game meat, as well as subsequent risk to humans, are assumed to be the same for all lead compounds and are often calculated based on lead acetate.

Introduction

Humans have used lead for the past 6000 to 9000 years. Indeed it was one of the first metals that humans learned to use. Five thousand years ago, it was discovered that small amounts of silver could be extracted as a by-product from lead, and production increased to a more extensive level. The first gun using lead bullets was introduced in the late 13th century. Bullets made of lead were probably also used as ammunition for crossbows. Lead is a soft metal that has known many applications over the years in metal products, cables and pipelines, but also in paints and pesticides. Lead is one of the four most used metals in the world but is also

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one of the metals which can have the most damaging effects on human health. Lead can enter the human body through uptake from food (65%), water (20%) and air (15%). The use of lead ammunition in various forms has been an issue widely discussed in Sweden in recent years there has been lively political debate on the issue, together with demands for its prohibition. In late 2000 the Swedish government took a decision not to implement a proposed total ban on lead ammunition. The use of lead shot is, however, prohibited for hunting in wetlands and shallow areas of open water. However, it is allowed when hunting mammals outside the wetland areas, and on dry land for hunting birds. For environmental reasons, a ban on shooting clay pigeons on shooting ranges remains. This means that hunting with rifles and shotguns using lead-based ammunition in any context, but with certain exceptions for wetlands, continues to be allowed.

During the last few years a new debate has developed about the risks associated with eating game meat. Game meat may contain variable amounts of lead in the form of fine metallic residues originating from hunting ammunition. The only existing limit for lead levels in meat which currently exists in the EU refers solely to livestock meat (e.g. chicken, lamb, beef etc.), in part because game meat is normally eaten less frequently. In addition, the legislation contains the same health-based reference values of 0.5 to 1.5 µg/kg body weight per day for all types of lead compounds [1]. Several studies have demonstrated radiographically that conventional lead bullets can lead to the presence of lead particles in the tissues of the killed animal, centered upon the bullet path through the body. In 2008, American researchers examined the fragmentation of bullets in white tail deer. The results showed that lead particles could occur at least 45 cm from the bullet path. A Polish study of deer and wild boar shot with lead bullets showed lead fragments about 30 cm from the bullet path. The British Deer Society also concluded that meat in a radius of 30 cm from the bullet path might be affected [2]. Most of the investigations of lead particles or fragments in killed animals are, however, undertaken on slaughtered and eviscerated animals, which may affect the distribution and subsequent interpretation when lead metal fragments easily can be moved around with the blood from the chest cavity. The concentration of lead metal fragments is often highest at the entry and exit wounds and may locally exceed the equivalent of 476 mg/kg in red deer [3], which far exceeds the EU safety level for food of 0.1 mg/kg. Lead scatter and contamination at exit wounds was higher in wild boar, where 1955 mg/kg meat was found, reflecting the tougher skin and greater bullet fragmentation in this species. Some researchers used another approach. They calculated the mean of metallic lead emitted per projectile from four of the most common types of cartridge to be 2.69 grams per bullet. They also calculated that the average number of hits per moose was 1.4, and that therefore 3.77 g (1.40 x 2.69 g) of metallic lead was deposited on average in a killed moose. However, the investigation does not answer the crucial question: to what extent is meat from different parts of the moose affected by lead particles in its bioavailable form [4]. The published reports of high lead levels bear further scrutiny [5]. Many of the studies are based on randomly selected packs of mincemeat. It should be noted that at least two factors may have increased the likelihood of encountering lead in these packs. First, the minced meat may have been processed from poor cuts, trims and possibly the tissue damaged by bullets. Secondly, in one of the studies the packs analyzed were donated to the Community Action Food Centre. One cannot help but ponder whether those households donating gave away their best game meat? There have been a number of investigations of lead in game meat from different countries. Table 1 below is a summary from some of those investigations. Sampling method is unknown.

Table 1: Lead in game meat from different countries

Investigator	Type of animal	Ammunition	Samples	Median mg/kg	Mean mg/kg	Max mg/kg
a	Game meat	---	2521	0.02	3.15	867
b	Wild boar	Bullet	---	0.02	4.7	684(1998)
c	Moose meat*	Bullet	52	0.3	5.6	110
d	Bird	Lead shot	128	---	2.55	---
e	Moose meat*	Bullet	54	0.027	0.9	31

*Minced meat

a) EFSA. EFSA panel on contaminants in the food chain (CONTAM); Scientific opinion on lead in food. EFSA J 8 (2010): 1570.

b) Bundesinstitut fur Risikobewertung, "Bleibelastung von Wildbret durch Verwendu", Stellungnahme Nr 040/2011

c) Lindboe. M., et al. Lead. Concentration in meat from lead-killed elk and predicted human expo- sure using Monte Carlo simulation. Food Additives and Contaminants (2012): 1-6, I First.

d) Mateo.R, et al. Bioaccessibility of Pb from ammunition in game meat is affected by cooking treatment. PLoS One 6 (2010): e15892.

e) Report from Swedish National Food Agency.

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Bioavailability

The term bioavailability has different meanings across various disciplines of toxicology and pharmacology. In this investigation the following definition has been chosen:

The bioavailable fraction or "oral absorption fraction" is defined as, and equal to, the fraction of an ingested bioaccessible dose that actually crosses the gastrointestinal epithelium and becomes available for distribution to internal target tissues and organs. This in turn varies with the water solubility of the present lead salts released [6].

Absorption of ingested inorganic lead in the gastrointestinal tract depends on physiological factors (such as age, pregnancy, etc.) As well as the physicochemical characteristics of the ingested particles (size, solubility, etc.). The presence of food in stomach also decreases the absorption of water-soluble lead compounds. What is more, absorption of lead compounds is higher in children than in adults. Absorption of inhaled sub-micron sized particles occurs in the respiratory tissues whereas larger-sized particles are transferred into the pharynx and are then swallowed [7]. From studies of the uptake of stable isotopes of lead in adults, an average absorption of 15 to 20% has been estimated, and for children 50% has been estimated [8].

Health risks

It is commonly understood that human consumers of wildlife killed with lead ammunition may be exposed to health risks associated with lead metal ingestion. This hypothesis is based on published studies showing elevated blood lead concentrations in hunter populations, related to ammunition residues in the tissues of the animals. A study from North Dakota showed that those who regularly ate wild game had a blood lead concentration of 1.27µg/dL. People who do not eat wild game meat regularly have a concentration of 0.84µg/dL - a difference of 0.43µg/dL. However, although these levels are significantly different, both are well below levels detected previously in subsistence hunting communities as well as the currently established level of concern (10µg/dL) [9]. Moreover, the frequency of game meat consumption in North Dakota was not associated with increased blood lead levels and only consumers eating more than 56.7 grams of game meat per meal showed higher blood lead levels [10]. In a Swiss study, blood lead in hunters varied between 2–17.1 µg/dL. Likewise, there was no association with the frequency of game meat consumption [10].

In 1988 lead contamination of seabirds from the use of lead shot in Greenland was studied in thick-billed murex which were hunted. In each bird pellets were located and counted using X-ray. The birds were skinned and viscera, head, wings and legs removed, after which the carcass was cooked. The soup and breast meat were then analyzed for lead after removal of visible shot pellets. The lead concentration in the soup was quite low, with a mean of 0.63µg/dL, whereas breast meat lead values revealed a mean of 0.22µg/g ww. This was more than 10 times higher than in birds not killed with lead shot. The study also indicated that lead exists as small lead fragments or lead oxides, left during the passage of pellets through the breast. Because of inhomogeneous lead distribution in samples, the uncertainty of estimated lead concentration in breast meat was high. Based on this study, it was concluded that birds killed with lead shot are a significant source of lead, (probably the single most important source), in the diet of many people in Greenland [11]. Compared to the *in vivo* digestive uptake of lead in soil and the *in vitro* digestive uptake of lead from lead ammunition, the latter has only been studied a few times. In a Spanish study the bioaccessible part of lead from ammunition (lead shots) was found to be between 0.7 and 6.75%. The effect of cooking using recipes which contained vinegar was linked to the higher value [12].

Objectives of the study

One purpose of the present investigation was to study the distribution of metallic lead fragments around the wound channel and in cavities created upon impact by means of flat X-ray and computed tomography (CT). In addition to the total content of lead in wild boar meat, *in vitro* digestive release from lead metal fragments in the meat was also investigated. The method used is a simulation of human digestion as material passes from the gastric to intestinal portions of the gastro- intestinal tract. To optimize the lead content in the meat, the study focused on the wound channel and the area around it. This gave a realistic possibility of showing the extent to which metallic lead can be converted into a bioaccessible form in the human digestive system. This is one of the key questions when dealing with theoretical models simulating uptake of lead in humans. It must be noted that all results and conclusions in this report are based solely on the metal lead fragments that may be present in muscle tissue.

Materials and Methods

Most of the coarser lead fragments are usually found on the exit side of the animal if the projectile penetrates the thick muscle tissue on the shoulder. For example, when a projectile hits the heart or large blood vessels in the thoracic cavity, small lead fragments which stop in the heart and lung tissue because of their low kinetic energy are rinsed with the blood sequenced out of the cavity, formed due to impact when the lungs collapse. This gives the opportunity for lead fragments to follow the blood to other parts of the body cavity at evisceration of the carcass. This is likely to be one of the reasons that lead particles in small quantities can be found by conventional X-ray far from the actual wound channel.

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Computed Tomography[1] (CT) X-ray and sample collection:

The unconeviscerated animals, deep-frozen in natural posture, were subjected to compute tomography. The apparatus used limited the living weight of the object to be analyzed to approximately 70 kg. As far as we know, this method has not been used anywhere before. And yet this method is vastly superior compared to flat X-ray in order to establish the distribution of the lead fragments around a wound channel and in the carcass. The "patient" can be studied from all directions down to a thickness of 1 millimeter in transverse and dorsal plane. Lead fragments smaller than 1/10 millimeter is easily observed in a high magnification image. The diameter of the distribution of actual lead fragments can then be determined with great accuracy. When the radio density of the individual fragments can be measured in Hounsfield Units (HU) it is also possible to distinguish lead fragments from splinters of bone

In addition it is also possible to view in 3D the distribution of lead fragments along the wound channel. This is quite unique, even if somewhat expensive. So far we have established that the lead fragments, in practice; lodge exclusively very close to the wound channel and in those cavities between membranes created upon impact, as well as in those blood clots that are created in the cavities between the lungs and the breast cage walls. Courser lead fragments can penetrate edible parts only a few centimeters around the wound channel, while the finer fragments can appear in the blood due to their low weight and low energy. From the study it was impossible to verify the penetration of lead particles tens of centimeters from the center of the wound channel.

Examples below show the advantages of using CT in the analysis of the distribution pattern of lead fragments in game tissue.

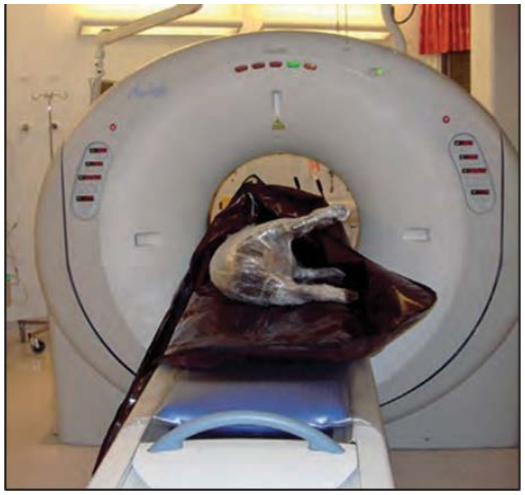

Figure 1: Computed Tomograph, Toshiba Aquilion 16 used in the study

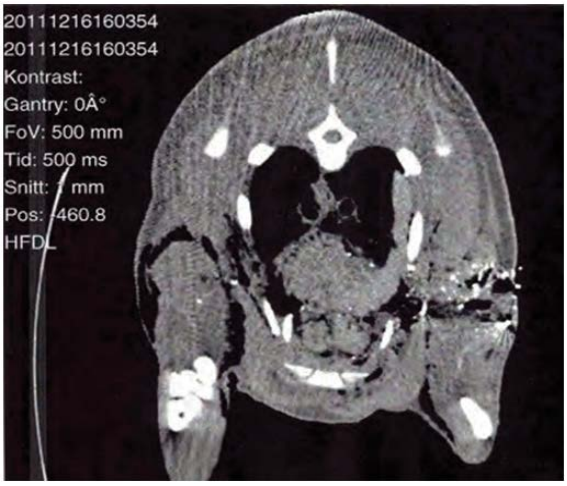

Figure 2: Transverse, CT picture from centre of the exit wound. (Object 1.)

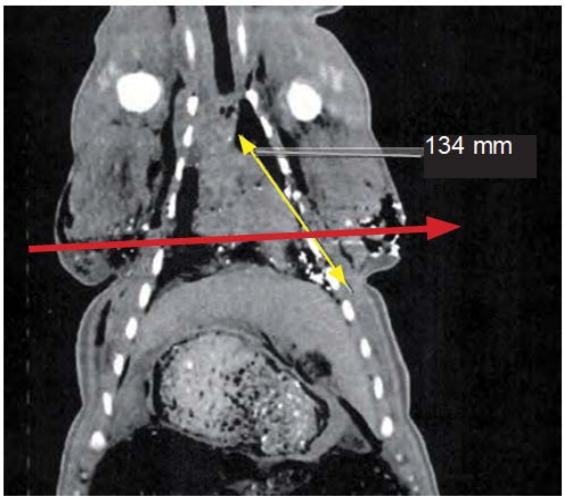

Figure 3: Dorsal CT picture centre of wound channel. Maximum distance between the lead fragments followed by the bloodstreams in the chest cavity is 134 mm. (Object 1.)

[1]Computed tomography, often abbreviated to DT or CT and in Swedish also abbreviated to tomography, is a further development of the standard X-ray machine (plain X-ray). Computed tomography is used in medical diagnostics to image the patient in three dimensions. By comparison a plain X-rays obtains a 2-dimensional image where the density differences in the projection direction is interleaved and thus cannot be distinguished. CT provides an anatomical image in three dimensions, so it also helps to visualize organs and tissues with otherwise low density for plain X-rays.

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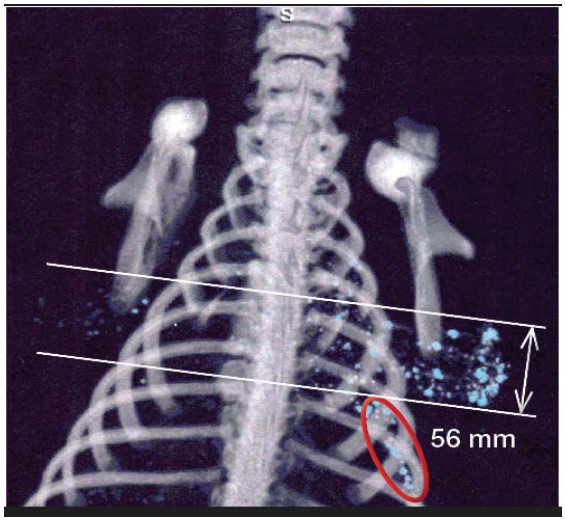

Figure 4: 3D picture of wound channel. In the red ring, lead fragment in clot between lung and chest wall, outside diaphragm. (Object 1.)

Sample Collection and Preparation

The animals used in this study were free living and shot during normal hunting conditions. Wild boars were chosen as the subject for this study for two reasons: they are a very common prey over a large part of the world and their compact physique in relation to their weight provides a greater mechanical impact on the projectiles used. Samples were collected from muscle tissue around the wound channel from the parts of the animal that are normally used as food. Samples were also collected from the chest wall, inside the shoulder area, and parts of the lungs and heart where, from experience, the presence of lead fragments is suspected or confirmed by radiographs. The treatment and preparation of the samples are described in the sections below. The samples are then marked and deep-frozen in an insulated cassette and X-rayed. On a computer-enlarged picture, the dispersion of lead fragments can easily be observed and so it was possible to divide the sample into two parts, manually by sight, to ensure the content of lead fragments in each part is was as equal as possible. Samples were taken around the center of the wound channel (9 centimeters diameter) and from a radius of 6.5 centimeters from the center of the wound channel, both from the exit and entrance side. In the chest cavity, samples were collected from the entrance and exit side of the lung and heart tissue to the vertical line of the chest to a diameter of approximately 10 centimeters. Contaminated meat was subjected both to analysis of the total content of lead fragments and to analysis based on *in vitro* gastrointestinal simulation. Since one of the objectives of the study was to determine the actual relationship between the total content of lead metal and the amount of lead which converted to bioaccessible form in the human digestive system, meat samples with single and small lead fragments were excluded when it not is practically possible to divide them into two equal parts.

Data for the animal, ammunition used and shooting distance

OBJECT 1.

Species	Wild boar (Sus scrofa)
Sex	Male
Live Weight	45.5 kg
Distance when shot	75 metres
Calibre used	6.5 -284 Norma
Muzzle velocity	890 m/sec
Calculated impact velocity	840 m/sec
Bullet weight Nosler Partion	9.1 grams (140grs)

OBJECT 2.

Species	Wild boar (Sus scrofa)
Sex	Female
Live Weight	72 kg
Distance when shot	25 metres
Calibre used	6,5x55
Muzzle velocity	870 m/sec
Calculated impact velocity	845 m/sec
Bullet weight	10.1 grams (156 grs)
Bullet type	NORMA Oryx, bonded core
Bullet entrance	Left side, 3th rib
Bullet exit	Right side, 4th rib

Sampling points

The image below shows the location of the main sampling areas in the outer muscle tis- sue of object 1, around the wound channel. In addition to sampling the wound channel (blue circle - 9 cm diameter), three samples were collected symmetrically around the wound channel center (in a 6.5 cm radius) to assess the potential for radial spread of lead fragments. Additional samples were taken of outer tissue in the membranes between the shoulder muscles and the chest wall. This area was suspected to contain microscopic lead particles that cannot be observed in a computed tomography image. Samples were also collected from the chest wall (1st to 7th rib) after the shoulder had been removed, and from lungs and heart tissue, both from the entrance and exit side. Control samples were taken from unaffected muscle tissue in the neck vertically above the wound channel.

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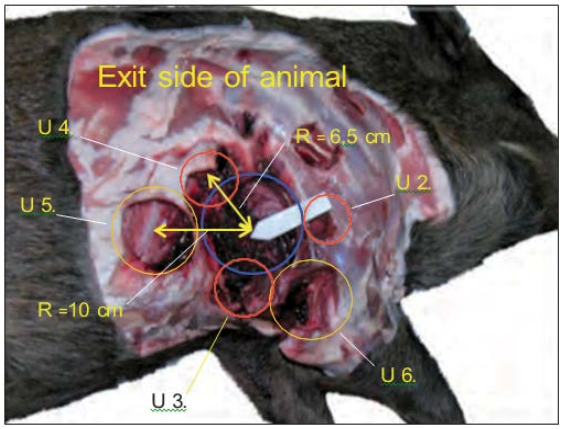

Figure 5: Image shows sampling model used in this study. Samples were collected when object was half thawed to avoid movement of lead particles.

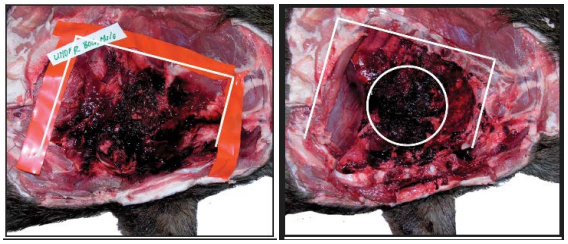

Figure 6: Left image shows object with the shoulder removed. All parts of chest wall, tissue and ribs, inside the marking is cut away from the body and subject to analysis for total lead and *in vitro* simulation. (*Sample U 7 object 1*).

Right image shows the chest cavity with collapsed lungs. Tissue inside the white ring (≈10 cm) was subject for sampling of total lead content and in vitro simulation.

Sample preparation

The metallic lead contained in meat samples is not usually evenly distributed since it occurs as isolated solid particles of varying size. Consequently a special method of analysis is required compared with when lead occurs in ionic form. When semi-thawed a full part of the sample, from 20 to 120 gram, was very finely homogenized to a smooth meat paste using mechanical processing. Tools and equipment were washed between each homogenization. It can be assumed that some larger lead metal fragments and flakes were to some extent cut into smaller pieces, changing the surface/ weight ratio and that; possibly, invisible fine-grained lead metal dust was evenly distributed throughout the whole sample. After homogenization the samples were wrapped in plastic film to avoid leakage, then packaged in round paper molds with a diameter of 50 or 90 and a height of 25 millimeters, depending on the weight of the sample. The samples were flattened to a uniform thickness in the mold, labeled, placed separately and indexed in an insulated cassette before being deep-frozen again. After freezing all samples was subject to high resolution radiography. On these highly magnified images of the samples, where even relatively small fragments were easily visible (< 1/10 of a millimeter) it was possible to visually assess how the samples would be assigned to allow the same test materials to determine both total lead content and dissolution of bioaccessible lead compounds through in-vitro gastrointestinal simulation, giving a percentage figure with reasonable accuracy.

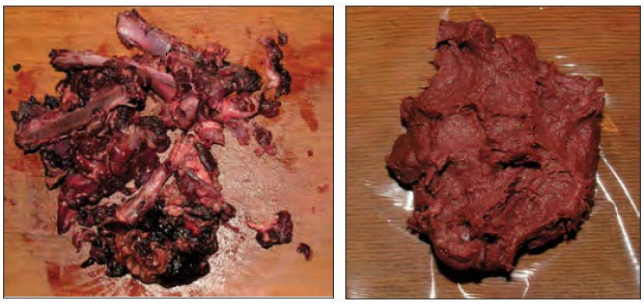

Figure 7: OBJECT 1. Sample U 7 Left picture shows the part of the chest wall, subject to analysis, in the first step of perparations. To the right all soft tissues from 1th to 7th rib has been mechanically homogenised.

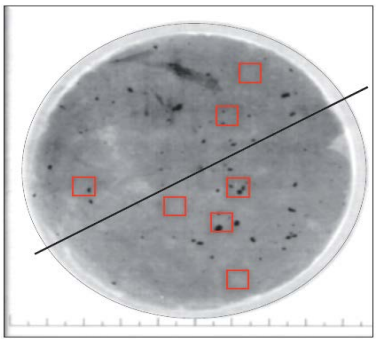

Figure 8: Samples packed in the the paper moulds were subjected to X-ray. This sample weight is 118 grams and total lead is 589 mg/kg ww. The black line indicates the instruction to the laboratory for how the sample should be divided. Red squares illustrate uncertainty when using small meat samples.

Lead analysis

Total lead content

Contaminated meat was subjected both to analysis for the total content of lead and to *in vitro* gastrointestinal simulation in order to analyze the bioaccessible lead content. One part of each sample was heated at 450^0 C for 12 hours in platinum pots with lids to obtain an accurate measurement of total lead in the sample. Tests on meat with known lead content show that heating results in a lead loss of < 0.1%. After heating the remaining ash were leached for lead with 7M HNO3. The leaching was performed in an autoclave at 200 kPa (120°C) for 30 minutes. The sample was diluted with high purity water prior to analysis by Method DS 259th. Analysis has

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been undertaken according to SS 028150-2/ ICP-MS and SS 028150-2/ ICP-AES.

Lead concentration in procedural blanks < 0.0005 mg/L.

Figure 9: By "ashing" a greater amount of tissues sample could be used to get more accurate results for analysis of the total content. A very limited amount of nitric acid is used for digestion.

In vitro gastrointestinal simulation

In vitro gastrointestinal simulation was performed on the second part of each meat sample using methods from [12] modified from [13]. A summary of the method is given below. The meat was mixed with the simulated gastric juice, put in a polypropylene tube and adjusted to pH 3, purged with argon, heated to 37°C and placed in an orbit shaker for 2.5 hours at 37° C. The "slurry" in the tubes was then divided into two parts and to one part intestinal juice was then added. The intestinal part pH was adjusted to 6.3 and the solution was homogenates, purged with argon and, again, placed in the orbit shaker for an additional 2 hours at 37°C. The final homogenates were then centrifuged at 14,000 rpm. The liquid and the meat were then analyzed for the lead released, both gastric and intestinal. The bioaccessible lead released in the *in vitro* gastrointestinal test was calculated as a percentage of the total content of metallic lead in the sample. The results for object 1 can be seen in table 3 and for object 2 in table 4.

Gastrointestinal lead

To become bioaccessible, lead metal fragments that may be present in game meat first needs to be mobilized from the game meat during digestion. In the *in vitro* human digestion model used, it was possible to assess the bioaccessibility of lead and obtain results for both the gastrointestinal and intestinal part of the digestive system. Some results of the study can be seen in table 2 below which shows the dissolutions in the gastrointestinal and intestinal part of the digestive system for object 1. (The results for object 2 are similar).

As can be seen from the table above dissolution happens mainly in the gastrointestinal part of the digestive system and very little occurs in the intestinal part of the system. It would be expected that the lead concentration in the intestinal sample would be equal or higher than the result from the gastrointestinal sample. However, most of the contents show

Table 2: Object 1 Gastrointestinal dissolution

Name	Total lead	In vitro	Intestinal	Differences
	mg/kg ww	Gastrointestinal mg/kg ww	mg/kg ww	mg/kg ww
I 1	131	0.54	0.58	0.04
I 2	72.39	1.12	1.02	-0.1
I 3	341	1.09	1.01	-0.08
I 4	221	2.03	1.21	-0.82
U 1	1955	1.25	0.89	-0.36
U 3	84.8	0.88	0.62	-0.26
U 4	22.3	0.34	0.23	-0.11
U 6	13.8	0.06	0.04	-0.04
U 7	589	1.25	1.17	-0.08

Samples with a total lead content less than 1 mg/kg w.w were excluded.

negative values, which means that no further dissolution happens in the intestinal tract. The small differences in the results are probably a result of the fact that lead forms complex ions in the solution, especially as the pH value is higher. (Cf. Method chapter). To further check this irregularity an *in vitro* test was conducted on lead shot. This test showed that no further dissolution happened in the intestinal part of the digestive system. See Table 5. The conclusion is therefore that most dissolution of lead occurs in the stomach where after metallic lead fragments are more or less unaffected as they pass through the intestinal tract.

Figure 10: Image show polypropylene tube with the "slurry" after in vitro gastric treatment. After shaking, sample is divided and one part is analysed for dissolved lead. The second part is subjected to chemicals that simulate the environment in the intestinal tract. After an additional 2 hour treatment of the second part, bioavailable lead is analysed

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Results and Discussion

The total amount of lead in the samples listed in mg / kg ww, and the bioaccessible portion is listed as a percentage of the total amount of lead. The result of the investigation for object 1 is shown in table 3 below.

The total lead content varies in object 1, between 23.8 and 1955 mg/kg ww and for object 2 (table 4) between 14 and 590 mg/kg ww. There are tendencies for the meat from the exit side to have a higher content than the meat from the entrance side. The differences between the two objects can be explained by the differences in shooting distance, type of bullet and the animal's weight. The total mean lead content for lead in all samples (n=17) was 273.6 mg/kg ww. The reason for the overall high lead content in the meat is associated with the sampling methodology where the aim was to obtain sufficiently high levels of lead in order to obtain relevant data to conduct an *in vitro* gastrointestinal study. It must, however, be noted that the meat which was investigated came from the wound channel which is the most contaminated part of the animal. This sampled section of the animal is normally cleared away during normal butchering practices and the meat would not be used for consumption.

Table 3: Object 1. Used bullet nosler partition.

Name	Total lead	*In vitro*	Bioaccessible part [14].		Samples nr.
	mg/kg ww	Gastrointestinal and intestinal mg/kg ww	% of total lead		Figure 11.
I 1	131	0.54	0.41	Centre wound channel. Entr.	1
I 2	72.39	1.12	1.55	" r= 3 cm **I**	2
I 3	341	1.09	0.32	" r= 3 cm **II**	3
I 4	221	2.03	0.92	Chest wall. Entr.	**4**
U 1	1955	1.25	0.06	Centre wound channel. Exit	5
U 3	84.8	0.88	1.04	" r= 6.5 cm **I**	6
U 4	22.3	0.34	1.52	" r= 6.5 cm **II**	7
U 6	13.8	0.06	0.43	" r= 10 cm **III**	8
U 7	589	1.25	0.21	Chest wall. Exit	9

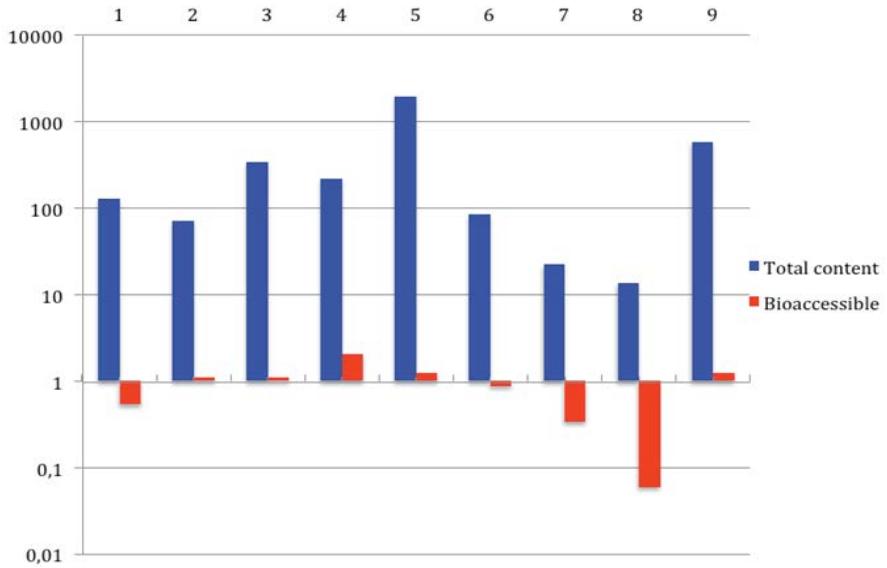

Figure 11: Diagram showing the distribution of the total lead con- tent in the meat vs. the bioaccessible part in object 1. The sampling numbers can be seen in table 3. *Note that the scale is logarithmic*. All lead content is in mg/kg ww.

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Table 4: Object 2. Used bullet Norma Oryx, bonded core.

Name	Total lead	*In vitro*	Bioaccessible part [14].		Samples nr.
	mg/kg ww	Gastrointestinal and intestinal mg/kg ww	% of total lead		Figure 12.
In 1	58	0.83	1.43	Centre wound channel. Entr.	1
In 3	12	0.24	2	" r= 6.5 cm **II**	2
In 4	13	0,19	1.46	" r= 6.5 cm **III**	3
In 5	3.7	0.09	2.38	Chest wall. Entr.	**4**
In 6	590	0.74	0.13	Lung and heart tissue. Entr.	5
U 1	390	1.6	0.41	Centre wound channel. Exit.	6
U 3	100	0.73	0.73	" r= 6.5 cm **II**	7
U 5	14	0.23	1.64	Chest wall. Exit.	8
U 8	0.097	–	–	Control sample. Neck tissue	

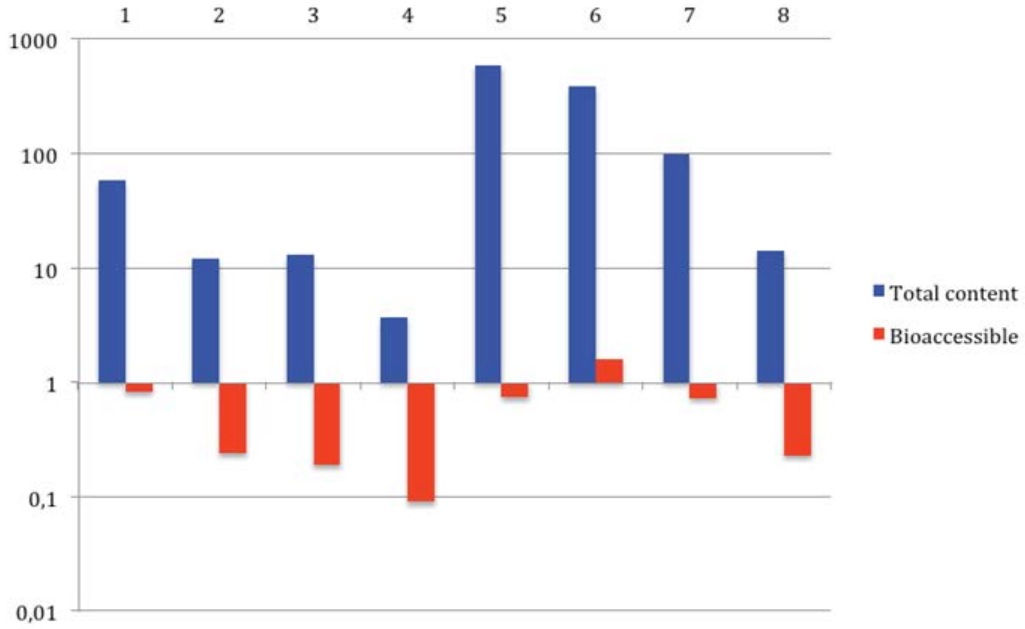

Figure 12: Diagram showing the distribution of the total lead content in the meat vs. the bioaccessible part in object 2. The sampling numbers can be seen in Table 4. *Note that the scale is logarithmic*. All lead content is in mg/kg ww.

Bioaccessible lead varies between 0.13 to 2% (mean 0.98 %) of the total content for the samples. The results also show that only a marginal dissolution happens in the intestine (cf table 2). The total bioaccessible lead represents less than 1 to 2 % of the total lead content in meat. There is no close correlation between the total content and the bioaccessible part (r = 0.635). This means that it is impossible to estimate the bioaccessible part from the total content. This fact can probably be explained by differences in particle form and size and the uneven distribution of lead particles in the meat samples. It also means that samples with many small particles with a higher specific surface area have a higher dissolution than courser particles independent of the total metal lead content in the samples. Furthermore, it is technically difficult to divide a meat sample of 20 to 120 grams with very small numbers of lead particles which are hardly visible on X-ray, into exactly equal parts for analysis of the total content and *in vitro* simulation. The results from the study of lead shot, with

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a relatively equal weight and size, confirm this hypothesis (cf. table 5). Independent of this fact, the main conclusion of this part of the study is that the bioaccessibility of lead in game meat varies between 1 to 2% of the total content also taking into account normal variations in gastric pH level. This result is important as many conclusions presented in the wider literature are based on the assumption that metallic lead is 100% bioaccessible.

Table 5: Weight loss for lead shot in gastrointestinal simulation

Sample	Gastric juice %	Intestinal juice %
1	0.64	<0.05
2	0.29	<0.05
3	0.74	<0.05
4	0.35	<0.05
5	0.66	<0.05
6	0.57	<0.05
Mean value	***0.61***	–
Median	***0.54***	–

Bioaccessible Lead From Lead Shot

To complete the meat study a control simulation was also performed for lead pellets according to the method below.

Method

A weighted lead shot US 3, nominal diameter 3.5 mm, was placed into a polypropylene tube with 30 ml of solution (gastric juice: 1% pepsin solution with 0.15 M NaCl adjust to pH 3 with 37% HCl.) First Argon gas was flushed over the surface of the solution before the tube was sealed. The tube was placed in a shaking water bath for 2.5 hours at 37°C. After treatment the lead shots were picked out and washed with de-ionized water before drying at 105°C. After drying and cooling, the weight loss was recorded. The lead pellet was then transferred to intestinal solution (0.14 g/ml of porcine bile extract and 0.014 g/ml of pancreatic juice). Argon gas was flushed over the surface of the solution before the tube was sealed. The mixture is shaken for 4 hours at 37°C. After that the lead pellet was re-weighed. The results are given as weight lost in gastric juice and weight lost in intestinal juice. The test was undertaken only on lead shots without meat. The shots weighed 18 -22mg (scale used Mettle AT 20). All leads shot were collected from the same cartridge. The results are given in table 5 below.

The results in the table above show a similar result compared with the results from meat even if the content is somewhat lower, at between 0.29 and 0.74%. The preparation of ashing and the *in vitro* simulation of the samples were undertaken by *MoRe Resesrch Örnsköldsvik*. The lead analysis was undertaken by *Eurofins Environment Sweden AB Lidköping*.

Calculation of lead ingestion through food

Recent scientific studies on lead show that adverse health effects can occur at lower levels of exposure than previously thought. At low levels of exposure the main health effect observed is on the nervous system: specifically, exposure to lead may have subtle effects on the intellectual development of infants and children. Infants and toddlers are particularly vulnerable to the harmful effects of lead because they are undergoing a period of rapid development. Furthermore, their growing bodies absorb lead more easily and excrete lead less efficiently than adults. In addition, infants and young children are more likely to ingest lead because of their natural habit of putting objects into their mouths [15]. The limits for maximum content of Bioaccessible lead in foodstuffs are laid down by the European Commission and vary between different food types, but is generally in the range 0.02 to 0.3 milligrams per kilogram. For example, the maximum allowable lead content of organ meats and seafood varies from 0.5 to 1.5 milligrams per kilogram, for dietary supplements 3.0 milligrams per kilogram and for tap water 0.010 milligrams per liter [16].

In addition, from European Food Safety Authority's scientific opinion (EFSA 2010) risk assessment has established three reference points (RP) for lead exposure [18].

1st Reference point for developmental effects - blood lead level of 1.2 µg /dL corresponding to a lead ingestion from food of 0.5 µg/kg bw / day.

This exposure has been associated with a lowering of IQ on population level

(4 to 10 year old children). In addition to the RP for children this is also considered to be applicable to infants and fetuses.

2nd Reference point for chronic kidney disease in adults - blood lead level of 1.5 µg /dL corresponding to a lead ingestion from food of 0.63 µg/kg bw / day.

3rd Reference point for effects on systolic blood pressure in adults -blood lead level at 3.6 µg/dL corresponding to a lead ingestion from food of 1.5 µg/kg bw / day.

There are approximately half a million U.S. children between the ages of 1 and 5 with blood lead levels above 5 µg/dL, the reference level at which the Centers for Disease Control and Prevention (CDC) recommends public health actions to be initiated. Lead exposure can affect nearly every system in the body. Because lead exposure often occurs with no obvious symptoms, it frequently goes un-recognized [18].

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Use of the IEUBK Model

The lead content in the blood provides an indicator of lead exposure in humans. As the use of leaded gasoline has declined, the lead content in blood has fallen in Sweden and in other countries. In 1978, the average blood lead level in a group of southern Swedish school children was 5-7 µg/dL. Sixteen years later, in 1994, the blood lead level was on average 2 -3µg/dL in children of the same age, and blood lead levels today are on average about 1µg/dL. In order to predict the potential risk presented by consuming elk mincemeat the results presented here have not been analyzed using a suitable model to predict blood level in children. The US Environmental Protection Agency, (EPA). [23] Has developed an *Integrated Exposure Uptake Bio-kinetic model* for lead in children (IEUBK). The model utilizes four integrated modules (exposure, uptake, bio-kinetic, and probability distribution) to estimate blood lead levels in children exposed to lead contaminated media. The default daily dietary lead intake is based on a typical child in the United States. These estimates are derived based upon data from the US Food and Drug Administration (FDA) [24]. The model assumes a default uptake of 50 % and presumes that lead is in the form of lead acetate [23]. The model also permits the inclusion of alternative food and also allows the lead bioavailability to be modified.

Food safety agencies way of calculation

The National Food Agency in Sweden presented an investigation of lead in elk mincemeat in 2012 [20]. They used the mean lead metal content of a preliminary study on moose mincemeat (0.9 mg lead metal/kg, see Table 1) which results in an additional lead intake of 0.44 µg/kg bw/ day for a person weighing 60 kg. This provided of a weekly intake of 200 grams of mince and that 100 % of the lead metal in the mince was absorbed by the body. When it is added to the average intake of 0.2µg/lead/kg body weight per day from other foodstuffs the total exposure will be 0.64 µg/kg/bw/day (compared with the RP for foetuses that is 0.5 µg of lead/kg bw/day). It is notable that lack of knowledge concerning bioaccessibility of the lead metal fragments in the mince also was identified as a key uncertainty in the risk assessment in the same study but not taken in consideration. The same approach was taken in a Norwegian study [21]. This study also assumed 100 % uptake of lead to the body from metallic lead in game meat in their calculations, which gives a value far over the RP value of 0.5 µg of lead/kg bw/day. The calculation referred to above presumes, however, that all the metallic lead in the meat is bioaccessible, which is not the case. It is difficult from literature to determine the available lead compounds from metallic lead in meat. The EFSA state that around 3-21 %, average ≈ 8 %, of bioaccessible lead compounds can be taken up by the body in adults depending on the water solubility of the compound, fed state and type of food ingested. [18].

Exposure from a given amount of lead metal

In any case, to be bioaccessible, lead metal must to some extent be dissolved in the acidic stomach environment. To what extent was the main goal of this study to clarify. As an example we can use the values from the study conducted by the National Food Agency in Sweden that moose mincemeat in mean was contaminated with 0.9 mg lead metal/ kg and from the result from this study presume that 1% of the lead metal is converted to a bioaccessible lead compound we get a value of 0.0043 µg/kg body weight/day instead of 0.44 µg/kg b.w/day. This based on a consumption of 200 grams of mince per week. This can be expressed: 900 µg lead metal/kg /5 (0.2 kg week) = 180 µg/7 days = 25.7 µg intake of lead metal/day/100 (1%) = 0.257µg/60 kg body weight = 0.0043 µg/kg b.w/day If we presume that 2% bioaccessible lead compound is dissolved from 0.9 mg lead metal we end up with an additional intake of 0.0086 µg/kg bw/day from this mincemeat. Still this value is low especially compared with a normal 2 liters daily consumption of tap water on the new EU limit for lead of 10 µg/L. (Lead present in water is in bioaccessible form). Only this normal consumption of tap water will give a lead intake of ≈ 0.33 µg/kg b.w/day (20µg /day/ 60 kg body weight) and increase B-Pb with 0.80µg/dL. To estimate the blood lead level in children from eating elk mincemeat of 0.9 mg/kg and a presumed consumption of 120 g/day and using the IEUBK Model we get a blood lead level rise for 6-7 year old children of 0.3 µg/dL using the accessibility for lead according to the present study of 2% and an uptake of 50%. Children who do not eat elk mincemeat will have a blood lead level of 1.1µg/dL (default value) and those who do, will have a blood lead level of 1.4µg/dL. It should be noted that all the default values follow conditions in the USA where the blood lead level is on average higher than in Sweden.

Relationship between B-Pb and dietary lead intake for adults

To estimate the relationship between bioaccessible, dietary lead intake and B-Pb level for adults CONTAM panel have applied Carlisle and Wade (1992) empirical equation, (California EPA slope factor model). For adults the slope factor (a constant) is 0.04. Same factor is applicable for drink water. To calculate B-Pb from a given intake of dietary lead expressed in µg day, just multiply with the slope factor. Answer is given in µg/dL (micrograms per deciliter). If B-Pb level have to be expressed in micrograms per litre (µg/L), multiply the daily intake in µg with slope factor 0.4 to get the result in for B-Pb in µg/L [7].

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Summing up

To sum up the present study it has been shown that the main part of lead metal fragments emitted from bullets appear fairly close upon the bullet path through the game animal. The highest lead metal level is found close to the center of the wound channel in an approximate diameter of ≈10 centimeter around entrance and exit hole. If this parts is cut away with a good marginal and discarded as food the risk to accidently ingest lead metal fragments is negligible. The most important result from this study is that if some lead metal particles are ingested only 1-2 % of it is converted to a bioaccessible lead compound that can give impact on B-Pb. Some studies claim that game meats have high lead levels with a mean of 3-5 mg/kg depending on use of lead based bullets. In some reports claimed levels from 850 to 19300 mg/kg. Such values is obvious obtained from random sampling directly in a wound channel and the latter is obvious a result from a totally inaccurate and inadequate measurement method. It can be presumed that estimating lead metal content in wild animals of practical an economic reasons is based on normal methods as random sampling of small biopsies 0.5 to 1 gram from different parts of the carcass. If a sample of 1 gram contains 1 mg of lead metal the statistical result will be 1000 mg/kg. From a number of samples a mean result can be calculated but the reliability of such investigations can be questioned when information of methodology used for such investigations always is missing. And it has to be remembered that one can never be exposed to a higher level than the physical weight of present fragments. Another important matter to take in consideration is to keep apart lead metal, bioaccessible lead and bioavailable lead which is the fraction of the bioaccessible lead that can be absorbed by the human body. Lead present in cereals, potatoes, beef, vegetables, water and whatever food stuff is not lead metal. It is bioaccessible lead in ionic form from which a certain fraction can be directly absorbed by the body. EU limit for livestock is 0.1 mg/kg to be allowed to sell to the public. An annual consumption of 30 kg will give a steady state daily exposure of 8.3µg. In turn this gives by use of EPA intake slope factor model, an increase on B-Pb of 0.33µg/dL. Compared with annual consumption of 30 kg game meat with a claimed content of 3-5 mg lead metal per kg and that 1-2 % of it is converted to a bioaccessible form the result will be a daily steady state exposure of 4.9µg. This corresponding to B-Pb 0.2µg/dL. To get a better understanding of health perspective connected with use of lead based ammunition it is important that future research provides a better understanding of the bioaccessibility and bioavailability of lead metal that may be present in meat. Also that risk assessments and exposure a level is calculated from known science and adopted methods.

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