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Study on the lead and cadmium contamination in big game meat

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Coordinator: Nadia Waegeneers (Sciensano)
Co-promoter(s): Jim Casaer (INBO)
Researcher(s):

Ann Ruttens (Sciensano)
Cédric Marien (Sciensano)
Anneleen Rutten (INBO)
Jan Vercammen (INBO)
Sander Devisscher (INBO)
Frank Huysentruyt (INBO)

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2. List of abbreviations

BfR	Bundesinstitut für Risikobewertung
BMD	Benchmark dose
BMDL	Lower confidence limit of the BMD
bw	Body weight
Cd	Cadmium
CONTAM	Contaminants in the Food Chain (panel)
DEMNA	Département de l'Etude du Milieu Naturel et Agricole
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GMU	Game management unit
GPC	Game processing center
HNO_3	Nitric acid
ICP-MS	Inductively coupled plasma-mass spectrometry
ID	Identification number
IQ	Intelligence quotient
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD50	50% lethal dose
MOE	Margin-of-exposure
Pb	Lead
PTFE	Polytetrafluoroethene
PTWI	Provisionally tolerable weekly intake
SciCom	Scientific Committee of the Federal Agency of the Food Chain
WHO	World Health Organization

3. Executive summary / Uitgebreide samenvatting

Context - Lead (Pb) is a widely occurring, hazardous contaminant. Humans come into contact with lead through environmental exposure and dietary intake. Although lead has been banned from several consumer products (e.g. gasoline, paint, ...), in order to reduce human exposure, it is still widely used in hunting ammunition. While the use of lead shot has been restricted in several countries over the last decade, the use of lead-containing bullets has not been restricted to the same extend. Nevertheless, several studies have shown that game shot with lead-containing bullets can result in increased lead levels in venison. Hence, the consumption of this meat may result in an increased lead exposure and could be a food safety issue for vulnerable population groups, such as young children, and for consumers of large amounts of game meat.

Objectives - This study aimed to gain insight in the lead (and cadmium) levels of big game meat (and kidneys) shot in Belgium, and to estimate the potential associated risks for high-level consumers. It also aimed to clarify to which extent the results obtained in Belgium fit the observations from similar research in other countries. In a separate investigation, the effect of the distance to the wound channel on meat lead concentrations was explored more in detail, because this information could be helpful when defining measures aiming to reduce lead exposure due to game meat consumption. The results can contribute to the discussion about a potential transition towards non-lead ammunition for hunting ungulate game species. Therefore, other aspects related to a change in ammunition type such as hunting efficiency, animal welfare, cost and availability of alternative ammunition, were analysed as well.

Materials and methods - The intention was to collect edible meat samples of the saddle and haunch of 250 different animals, comprising roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*). Meat samples, representative for one serving (± 200 g), were collected, either directly from hunters or by collaborating with a game processing centre. All efforts were directed towards an optimal geographical distribution of the samples between Flanders and Wallonia, except for red deer, which is only hunted in Wallonia. In addition, portions of saddle, haunch or mixtures (stew meat) of Belgian big game were bought in supermarkets for each of the species. In total, 213 animals have been collected (174 through hunting and 39 via supermarkets), comprising of 99 wild boar, 85 roe deer and 29 red deer. For red deer the number of animals was lower than targeted.

The analysis of lead and cadmium (Cd) concentrations in game meat, potentially affected by fragments of lead bullets, demanded a different sample preparation and homogenisation approach compared to traditional mixing of meat samples. Lead fragments may continue to exist in subsamples after homogenisation by simple mixing, leading to heterogeneous -hence non representative- results in these subsamples. Therefore, ±200 g-meat portions were -after the first homogenization step in a knife mill- blended with diluted nitric acid in a 1:2 solid:liquid weight ratio. The acid blended samples were allowed to shake overnight at room temperature to dissolve the lead fragments, after which they were again mixed thoroughly. Subsamples of the obtained slurries were further mineralised with nitric acid in a microwave and analysed by inductively coupled plasma-mass spectrometry (ICP-MS).

To estimate the influence of age and area in which the animals forage, on the lead and cadmium concentrations in kidney, a joint database of Sciensano and INBO containing (unpublished) data of trace element concentrations in kidneys of 200 roe deer was used. The roe deer were shot in six different Game Management Units (GMU) in Flanders. One GMU, a forest area in central Belgium away from known heavy metal point sources, was used in this study as a control area. The other five GMUs were located in the Noorderkempen, which is known for its historical contamination with cadmium. The age (in months) and sex of the animals was included as well in the database. Within the LECAHUNT project, the database was extended with roe deer kidney from three hunting areas in Wallonia (including the military domain Camp Roi Albert). For these animals it was only known whether the animals were younger or older than one when shot.

To collect more specific information on meat lead concentrations as a function of the distance to the wound channel, a more intensive sample collection took place on a selected number of individuals of roe deer and wild boar (18-19 animals/species). For both species all samples were collected from one hunting area to reduce geographically induced variation in concentrations. Samples were collected in transects going from the shoulder, over the saddle towards the haunch of the animals in 10-cm intervals, at both sides of the animal (shot entry & shot exit). The distance towards the wound channel was thereby measured for each sample.

Dietary lead intake was calculated from the analysis results in edible meat portions following a deterministic and a probabilistic approach, and evaluated against lead exposure levels associated with certain health risks.

To get an overview of international policy recommendations and scientific knowledge related to a transition towards non-lead ammunition for ungulate hunting, an extensive literature review was performed, focussing on existing information about lead bullet composition and behaviour, possible alternatives for lead bullets, national and international regulations, and international policy recommendations.

Results - The majority of the samples (81%) had lead concentrations below 0.10 mg/kg, which is the EU maximum level for lead in meat of bovine animals, sheep, pig and poultry. In 91% of all samples the lead concentration was below 1.0 mg/kg, the current action limit applied by the Federal Agency for the Safety of the Food Chain for big game meat. Within each animal species, differences in median lead concentrations among saddle and haunch (and diverse) were small. None of the samples had cadmium concentrations above 0.050 mg/kg, which is the EU maximum level for cadmium in meat of bovine animals, sheep, pig and poultry.

Statistical modelling revealed no significant differences in lead or cadmium concentrations between species, regions or sample types. The median and 95th percentile lead concentrations of the whole dataset were 0.015 and 4.2 mg/kg respectively. The median concentration was similar to the median concentration in other types of meat in Belgium, but the distribution was more skewed towards higher values. The median and 95th percentile cadmium concentrations of the whole dataset were 0.002 and 0.009 mg/kg respectively, which was similar as in other types of Belgian meat.

The variation in cadmium and lead concentrations in roe deer kidneys was large within each GMU, and the cadmium concentrations were about two orders of magnitude larger than the lead concentrations. The kidney cadmium concentration depended on the age of the animals, and was significantly higher in some, but not all, game management units in the Noorderkempen compared to the control GMU. The lead concentration in kidneys of roe deer did not depend on the age of the animals. The lead concentration in roe deer kidneys shot in military domains, was not larger than in kidneys from other areas.

Lead concentrations detected in the samples at different distances to the wound channel, revealed high to extremely high values in the tissue surrounding the entrance and exit wounds. A large drop in lead concentrations was visible at larger distances from the wound channels at both entry and exit sides, however no clear trend could be observed with increasing distance. As a result, no 'threshold' distance' for the guaranteed 'safe' sampling of meat for consumption could be defined. Meat from the shot wound was not fit for consumption.

Dietary lead intake calculations following a deterministic approach appeared not to provide realistic exposure results because the median lead concentration in large game meat was similar to the median lead concentration in other types of meat (bovine meat, poultry meat) and did not reflect the large variation in lead concentrations found. Following a probabilistic approach, the dietary (and overall) lead exposure depended on the frequency of game meat consumption. There was an increase in lead exposure with increasing frequency of game meat consumption. The probability that persons who eat big game meat a few times a year, consume a portion with elevated lead content, is small. The lead exposure that would occur in such an event does not involve any increased risk. The possibility of lead-related effects cannot be excluded at the population level for adults consuming 2 or more game meat servings per week, for women of childbearing age consuming 2 or more game meat servings per month living in a rural or city environment, and for 5% of women of childbearing age spending a lot of time in an industrial environment who consume 6 or more game meat servings per year. Because of neurodevelopmental effects, foetuses, infants and children are subgroups that are most sensitive to lead. Margins of exposure are below 1 for Belgian children, whether or not they consume game meat.

Regulations that currently exist regarding lead-based ammunition used for hunting, mainly focus on local or national bans on lead shot in wetlands and/or waterfowl. Only in three German states, a total ban has been established on lead-based ammunition. Policy recommendations have been formulated in several countries, mainly concerning the use of lead-free ammunition or concerning the consumption of game meat shot with lead ammunition. Different opinions exist on the mandatory nature of the transition towards non-lead based ammunition for hunting ungulate species. Where some argue that legal enforcing will speed up the availability of high-performance non-lead alternatives at comparable prices, others argue that a smooth transition, sensitization and stimulation allowing the depletion of current stocks and information and training of hunters, will increase the support for this transition.

Conclusions and recommendations - Following conclusions and recommendations concerning the consumption of big game meat and the transition from lead to non-lead bullets for hunting ungulates, in relation to the consumption of the venison of these animals, could be formulated:

- Because of neurodevelopmental effects, foetuses, infants and children are subgroups that are most sensitive to lead. Margins of exposure are below 1 for Belgian children, whether or not they consume game meat. Therefore, small children could be recommended to avoid eating big game meat shot with lead containing ammunition.
- Pregnant women and women of childbearing age who plan on getting pregnant, could be recommended to avoid eating big game meat shot with lead containing ammunition or limit their consumption of this type of meat, depending on the desired margin of exposure. The selection of the desired value of the margin of exposure for women of childbearing age as well as for other adults, is a choice to be made by risk management authorities.

- With exception for some of the smallest calibres that are currently used for roe deer, there are sufficient non-lead alternatives available on the market in Europe to allow a transition of lead-based towards non-lead bullets for hunting ungulate species of which the meat is in general consumed.
- Hunting efficiency, animal welfare, safety risks, barrel fouling, accuracy, impact on venison, toxicity and costs are however all non-negligible concerns that could inhibit the acceptance of non-lead ammunition by the hunting community. Research indicates that bullet material (lead versus non-lead) has no significant effect on the escape distance – being used as a proxy for killing efficiency and thereby animal welfare and hunting efficiency. The concerns over the other aspects mentioned above are justified but are not specific for the transition from lead-based toward non-lead ammunition. They are typical for each change in bullet type or brand hunters make.
- Given the elements above, investing in outreach and stakeholder communication are key elements for a successful transition of lead-based ammunition towards non-lead bullets for hunting ungulate species.

Whether or not the consumption of big game meat shot with lead-containing bullets increases blood lead levels of consumers, and hence increases the possibility of any risks, is still a question of debate. Additional research focusing on the relation between the consumption of big game meat and blood lead levels is recommended before formulating consumption advices for the general adult population.

Uitgebreide samenvatting

Context - Lood (Pb) is een veel voorkomende, gevaarlijke, verontreinigende stof waarmee mensen in aanraking komen via het milieu en via de voeding. Hoewel het gebruik van lood verboden is in verschillende consumentenproducten (bijv. benzine, verf, ...), met het oog op het verlagen van de humane blootstelling, wordt het nog steeds veelvuldig gebruikt in munitie voor de jacht. Terwijl het gebruik van loodhagel in het afgelopen decennium in verschillende landen werd beperkt, is dit niet in dezelfde mate het geval voor loodhoudende kogels. Niettemin hebben verschillende onderzoeken aangetoond dat wanneer grofwild geschoten wordt met loodhoudende kogels, dit kan leiden tot een verhoogd loodgehalte in vlees bestemd voor consumptie. Bijgevolg kan de consumptie van dit vlees leiden tot een verhoogde blootstelling aan lood en kan dit een voedselveiligheidsprobleem vormen voor kwetsbare bevolkingsgroepen, zoals jonge kinderen, en voor personen die vaak wildvlees eten.

Doelstellingen - Deze studie had als doel inzicht te krijgen in het lood- (en cadmium-)gehalte in vlees (en nieren) van grofwild dat in België is geschoten, en de hiermee gepaard gaande potentiële risico’s voor personen die vaak wildvlees eten in te schatten. Deze studie beoogde tevens na te gaan in hoeverre de Belgische onderzoeksresultaten overeenkomen met observaties van soortgelijk onderzoek in andere landen. In een tweede deel werd het effect van de afstand tot het wondkanaal op de loodconcentraties in het vlees nader onderzocht, omdat deze informatie nuttig kan zijn bij het bepalen van maatregelen om de blootstelling aan lood, als gevolg van de consumptie van vlees van wild, te verminderen. De resultaten kunnen bijdragen aan de discussie over een mogelijke overgang naar loodvrije munitie voor de jacht op grofwild. Daarom werden ook andere aspecten bekeken die gerelateerd zijn aan een verandering in het type munitie, zoals jachtefficiëntie, dierenwelzijn, kostprijs en beschikbaarheid van alternatieve munitie.

Materiaal en methoden - De opzet was om eetbaar vlees te verzamelen van de rug en bil van 250 verschillende dieren, met name ree (*Capreolus capreolus*), wild zwijn (*Sus scrofa*) en edelhert (*Cervus elaphus*). Porties vlees, representatief voor een consumeerbare portie (± 200 g), werden hetzij rechtstreeks via jagers, hetzij via samenwerking met een wildverwerkingscentrum, verzameld. Er werd daarbij gestreefd naar een optimale geografische spreiding van de stalen over Vlaanderen en Wallonië, met uitzondering van edelherten waarop alleen in Wallonië gejaagd wordt. Daarnaast werden voor elke diersoort verpakte porties van rug, bil of divers vlees (stoofvlees) van Belgisch grofwild in supermarkten gekocht. In totaal werden 213 dieren bemonsterd (174 via de jacht en 39 via supermarkten), waarvan 99 wilde zwijnen, 85 reeën en 29 edelherten. Voor edelhert werd het vooropgestelde aantal dieren niet behaald.

De analyse van lood- en cadmiumconcentraties in vlees van wild, dat mogelijk fragmenten van loodhoudende kogels bevat, vereiste een andere monstervoorbereidings- en homogenisatieprocedure dan het traditionele mixen van het vleesmonster. Loodfragmenten kunnen immers aanwezig blijven in deelstalen na eenvoudig mixen, wat leidt tot heterogene - en dus niet-representatieve- resultaten in deze deelstalen. Daarom werden de porties vlees van ± 200 g, na de eerste homogenisatiestap met behulp van een snijmolen, gemengd met verdund salpeterzuur in een 1:2 vast:vloeibare gewichtsverhouding. De met-zuur-gemengde monsters werden een nacht geschud bij kamertemperatuur om de loodfragmenten op te lossen, waarna ze opnieuw grondig werden gemixt. Deelstalen van de verkregen suspensies werden verder gemineraliseerd in een microgolfoven en geanalyseerd via inductief gekoppeld plasma-massaspectrometrie (ICP-MS).

Om de invloed van de leeftijd en het gebied waarin de dieren foerageren, op de lood- en cadmiumconcentraties in nieren te schatten, werd een gezamenlijke databank van Sciensano en INBO gebruikt met (niet-gepubliceerde) gegevens van spoorelementconcentraties in nieren van 200 reeën. Deze reeën werden in zes verschillende Wildbeheereenheden (WBE) in Vlaanderen geschoten. Eén WBE, een bosgebied dat ver verwijderd was van gekende puntbronnen voor zware metalen, werd in deze studie gebruikt als controlegebied. De andere vijf WBE’s waren gelegen in de Noorderkempen, dat gekend is voor zijn historische verontreiniging met cadmium. De leeftijd (in maanden) en het geslacht van de dieren werden ook opgenomen in de databank. Tijdens dit LECAHUNT project werd de databank uitgebreid met nieren van reeën uit drie Waalse jachtgebieden (inclusief het militair domein Camp Roi Albert). Van deze dieren was enkel geweten of ze jonger of ouder waren dan 1 jaar bij afschot.

Om meer specifieke informatie te verzamelen over loodconcentraties in vlees van grofwild in functie van de afstand tot het wondkanaal, vond een intensievere monstername plaats op een beperkt aantal reeën en wilde zwijnen (18-19 dieren/soort). De dieren werden per soort in één jachtgebied bemonsterd om geografische variatie in concentraties te beperken. Vleesmonsters werden verzameld over transecten gaande van de schouder, over de rug naar de bil van de dieren in intervallen van 10 cm, aan zowel de inschot- als uitschotzijde van het dier. De afstand tot het wondkanaal werd daarbij voor elk monster gemeten.

Loodinname werd berekend op basis van de analyseresultaten in de porties eetbaar vlees via een deterministische en probabilistische benadering, en vergeleken met blootstellingsniveaus voor lood die geassocieerd worden met bepaalde gezondheidsrisico’s.

Om een overzicht te krijgen van internationale beleidsaanbevelingen en wetenschappelijke kennis gerelateerd aan de overgang naar loodvrije munitie voor de jacht op grofwild, werd een uitgebreide literatuurstudie uitgevoerd, gericht op onderzoek naar de samenstelling en het gedrag van loodhoudende kogels, mogelijke alternatieven voor loodhoudende kogels, nationale en internationale regelgeving en internationale beleidsaanbevelingen.

Resultaten - De meerderheid van de stalen (81%) had een loodgehalte lager dan 0.10 mg/kg, wat het EU-maximumgehalte is voor lood in vlees van runderen, schapen, varkens en pluimvee. In 91% van alle stalen was de loodconcentratie lager dan 1.0 mg/kg, wat het actieniveau is dat momenteel wordt toegepast door het Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV) voor wildvlees. Binnen elke diersoort waren de verschillen in mediane loodconcentraties tussen rug en bil (en divers vlees) klein. Geen van de monsters had cadmiumconcentraties boven 0.050 mg/kg, wat het EU-maximumgehalte is voor cadmium in vlees van runderen, schapen, varkens en pluimvee.

Statistische modelering toonde geen significante verschillen aan in lood- of cadmiumconcentraties tussen de verschillende diersoorten, de verschillende regio's, of de verschillende soorten stalen. De mediane loodconcentratie en het 95^{e} percentiel van de gehele dataset waren respectievelijk 0.015 en 4.2 mg/kg. De mediane concentratie was vergelijkbaar met de mediane concentratie in andere vleessoorten in België, maar de verdeling van de loodconcentraties was meer gespreid richting de hogere waarden. De mediane cadmiumconcentratie en het 95^{e} percentiel van de gehele dataset waren respectievelijk 0.002 en 0.009 mg/kg, wat ook vergelijkbaar is met andere Belgische vleessoorten.

De variatie in cadmium- en loodconcentraties in nieren van reeën was groot binnen verschillende WBEs, en de cadmiumconcentraties waren ongeveer twee grootteordes hoger dan de loodconcentraties. De cadmiumconcentratie in nieren was afhankelijk van de leeftijd van de dieren, en was significant hoger in sommige, maar niet alle, WBE's uit de Noorderkempen in vergelijking met de controle WBE. De loodconcentratie was niet afhankelijk van de leeftijd van de dieren. De loodconcentratie in nieren van reeën geschoten in militaire domeinen, was niet hoger dan in nieren uit andere gebieden.

Loodconcentraties in stalen met een verschillende afstand tot het wondkanaal, vertoonden hoge tot extreem hoge waarden in het weefsel rond de ingangs- en uitgangswonde. Een sterke daling in de loodconcentratie werd waargenomen op grotere afstand van het wondkanaal, dit zowel aan de inschot- als aan de uitschotzijde. Er kon echter geen duidelijke trend worden waargenomen met toenemende afstand tot de schotwonde. Hierdoor kon geen drempelwaarde worden bepaald die de afstand vastlegt waarop vlees gegarandeerd "veilig" is voor consumptie. Schotwondevlees werd niet geschikt bevonden voor consumptie.

Loodinnameberekeningen op basis van een deterministische benadering bleken geen realistische blootstellingsresultaten te genereren, omdat de mediane loodconcentratie in vlees van grofwild nauwelijks verschilde van de mediane loodconcentratie van andere vleessoorten (rundsvlees, gevogelte), en ze de grote variatie aan loodconcentraties die gevonden werd, niet weerspiegelt. Op basis van een probabilistische benadering was de blootstelling aan lood via het dieet (en andere bronnen) afhankelijk van de frequentie van de consumptie van grofwild. De blootstelling aan lood steeg met een toenemende consumptiefrequentie. De kans dat personen die enkele malen per jaar grofwild eten, een portie consumeren met een verhoogde loodconcentratie, is klein. De blootstelling aan lood die in zo'n geval plaatsvindt, houdt geen verhoogd risico in. De kans op loodgerelateerde effecten kan niet uitgesloten worden voor volwassenen die twee of meer porties grofwild eten per week, voor vrouwen die zwanger zijn of zwanger willen worden en die twee of meer porties grofwild eten per maand en in een landelijk of stedelijk gebied leven, en voor 5% van de vrouwen die zwanger zijn of zwanger willen worden en veel tijd doorbrengen in een industriële omgeving wanneer ze zes of meer porties grofwild per jaar verbruiken. Omwille van de effecten op neurologische ontwikkeling vormen foetussen, zuigelingen en kinderen een subgroep die het meest gevoelig is voor lood. Blootstellingsmarges zijn reeds kleiner dan 1 voor Belgische kinderen, ongeacht of zij wild eten of niet.

De reeds bestaande regelgeving met betrekking tot loodhoudende munitie voor de jacht, omvat voornamelijk lokale of nationale verboden op het gebruik van loodhagel in waterrijke gebieden en/of voor watervogels. Enkel in drie Duitse staten werd een totaalverbod ingesteld op loodhoudende munitie. Beleidsaanbevelingen werden in verschillende landen geformuleerd, voornamelijk met betrekking tot het gebruik van loodvrije munitie of de consumptie van vlees van wild dat met loodhoudende munitie werd geschoten. Er bestaan verschillende meningen over het al-dan-niet verplichte karakter van de overgang naar loodvrije munitie voor de jacht op grofwild. Waar sommigen beweren dat een wettelijke verplichting de beschikbaarheid van hoogwaardige, loodvrije alternatieven aan vergelijkbare prijzen zal versnellen, beweren anderen dat de steun voor zo'n overgang zal verhogen bij een soepele overgang waarbij sensibilisatie en stimulering noodzakelijk zijn, waarbij de huidige voorraden kunnen worden uitgeput en waarbij het informeren en trainen van de jagers helpt om een draagvlak te creëren.

Conclusies en aanbevelingen - Volgende conclusies en aanbevelingen met betrekking tot de consumptie van vlees van grofwild en de overgang van loodhoudende naar loodvrije munitie voor de jacht op grofwild, met de intentie om het vlees te consumeren, konden geformuleerd worden:

- Omwille van de effecten op neurologische ontwikkeling, vormen foetussen, zuigelingen en kinderen een subgroep die het meest gevoelig is voor lood. Blootstellingsmarges zijn reeds kleiner dan 1 voor Belgische kinderen, ongeacht of zij wild eten of niet. Daarom zou voor kleine kinderen aanbevolen kunnen worden om het eten van grofwild te vermijden wanneer dit is geschoten met loodhoudende munitie.
- Voor zwangere vrouwen en vrouwen die zwanger willen worden, kan aanbevolen worden om het eten van grofwild te vermijden wanneer dit is geschoten met loodhoudende munitie, of om het eten van dit vlees te beperken, afhankelijk van de gewenste blootstellingsmarge. De selectie van de gewenste waarde van de blootstellingsmarge voor zowel zwangere vrouwen als voor de algemene bevolking, is een keuze die gemaakt moet worden door de autoriteiten die verantwoordelijk zijn voor risicobeheer.
- Met uitzondering van de kleinste kalibers die momenteel gebruikt worden voor reeën, zijn er voldoende loodvrije alternatieven op de markt in Europa om een overgang van loodhoudende naar loodvrije munitie mogelijk te maken voor de jacht op grofwild met de intentie om het vlees te consumeren.
- Jachtefficiëntie, dierenwelzijn, veiligheidsrisico's, vervuiling van de loop, nauwkeurigheid, impact op wild, toxiciteit en kosten zijn allemaal niet te verwaarlozen bekommernissen die het aanvaarden van loodvrije munitie door de jachtgemeenschap zouden kunnen belemmeren. Onderzoek wijst uit dat het materiaal van de kogel (lood versus loodvrij) geen significant effect heeft op de vluchtafstand, dat als maat wordt gebruikt voor de efficiëntie van het doden en daarmee ook voor dierenwelzijn en jachtefficiëntie. De bezorgdheden over de andere bovengenoemde aspecten zijn gerechtvaardigd, maar zijn niet specifiek voor de overgang van loodhoudende naar

loodvrije munitie. Ze zijn typerend voor elke verandering in het type of merk van kogel door jagers.

- Op basis van bovenstaande elementen is duidelijk dat het investeren in voorlichting en communicatie met de belanghebbenden sleutelelementen zijn voor een succesvolle overgang van loodhoudende naar loodvrije munitie voor de jacht op grofwild.

Of de consumptie van grofwild dat geschoten werd met loodhoudende munitie, al dan niet leidt tot een verhoogd loodgehalte in het bloed van de consument, is nog steeds een discussiepunt. Bijkomend onderzoek dat zich focust op de relatie tussen de consumptie van grofwild en het loodgehalte in bloed, wordt aanbevolen vooraleer consumptieadviezen te formuleren voor de algemene volwassen bevolking.

Bijkomend onderzoek dat zich focust op de relatie tussen de consumptie van grofwild en het loodgehalte in bloed, wordt aanbevolen vooraleer consumptieadviezen te formuleren voor de algemene volwassen bevolking.

4. Problem

Lead (Pb) is an ubiquitous environmental pollutant. It occurs in the environment both naturally and due to anthropogenic activities such as mining and smelting, manufacturing and the (former) use of leaded gasoline. Lead is a hazardous substance and human exposure to it is associated with a wide range of effects, including various neurodevelopmental effects, nephrotoxicity and mortality due to cardiovascular diseases (Lanphear et al. 2005; Muntner et al. 2005; Nawrot and Staessen 2006). Generally, no adverse health effects have been observed after a single exposure. An oral LD50 (50% lethal dose) greater than 2000 mg/kg_{bw} has been established for Pb salts (AESAN 2012). In 2010 the EFSA panel on Contaminants in the Food Chain (CONTAM panel) concluded that the -at that time available- provisionally tolerable weekly intake (PTWI) of 25 µg Pb/kg bodyweight was no longer appropriate as there was no evidence of a threshold for critical Pb induced effects (EFSA 2010). In its evaluation at the 73rd meeting, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) reached similar conclusions (JECFA 2011). Since then, a dose-response method is applied, calculating the amount of Pb in the diet (the Benchmark Dose or BMD) that would result in a blood Pb concentration associated with defined health responses in people. EFSA and JECFA defined significant health responses at a population level for developmental neurotoxicity and cardiovascular effects, while EFSA also included health responses for chronic kidney effects (EFSA 2010; JECFA 2011). These health responses were based on dose-response tests on chronic effects in humans, with the lowest 95% confidence limit of the benchmark dose (BMDL) as a point of references for critical effect characterisation. The risk assessment is then performed by using the Margin of Exposure (MOE) approach, where the MOE is calculated by dividing the BMDL for a considered effect by an estimated daily intake.

Due to its hazardous effects, Pb has been banned from gasoline, paint[1] and various household items in several countries over the past decades, resulting in declining human exposure (Strömberg et al. 2003). Lead is, however, still widely used in hunting activities. Lead has a low melting point, is ductile, easy to form into shot and bullets and has excellent ballistic properties, which are the reasons it has been used as ammunition for hundreds of years (Lead Ammunition Group 2015). Recently, the use of lead shot has been regulated in several countries. The most common restriction is the ban of lead shot for hunting of waterfowl over wetlands (Avery and Watson 2009). In Flanders the use of lead shot is prohibited for each form of hunting while in Wallonia it is prohibited to use lead shot while hunting waterfowl in or within 50 m distance of marshes, lakes, ponds, water reservoirs, streams, rivers and canals. Nickel-plated lead shot is, however, still authorized in Wallonia. Though lead has been banned in gunshot, it is still widely used in bullets to hunt big game. Lead is the major constituent of traditional rifle bullets although it may be cased (jacketed) in a metal such as copper or copper alloys.

In the period 2013-2015, 12% of the game meat samples, collected in the framework of official controls in Belgium, exceeded the action level for Pb (1 mg/kg; personal communication Federal Agency for the Safety of the Food Chain). In Germany a dedicated study on the presence of metals in game meat, resulting from the use of hunting ammunition, was performed

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[1] http://www.who.int/gho/phe/chemical_safety/lead_paint_regulations/en/

(BfR 2014). It was shown that the use of Pb ammunition leads to significantly higher mean Pb contents in meat of roe deer and wild boar in comparison to non-Pb ammunition, irrespective of the distance to the wound channel. These findings were also significant when taking into account the effect of regions with different soil Pb concentrations. A similar trend was observed for red deer. A Swedish survey on game meat from different hunted animals also showed that lead fragments from bullets and shot occurred in game meat intended for consumption (Kollander et al. 2014): more than 40% of the cuts from roe deer, fallow deer and wild boar contained Pb levels above the legal limit for beef, pork and poultry (0.10 mg/kg; EU 1881 (2006)). There was a significant decrease in Pb level with increasing distance from the wound channel. In an American study, Hunt et al. (2009) fluoroscopically revealed metal fragments in 32% of ground meat samples, intended for consumption, from white-tailed deer. ICP-MS analysis and isotope ratios of Pb revealed that the metal fragments consisted of Pb in the majority of selected samples and that the Pb in meat originated from bullets and not from background exposure.

Most studies agree that the consumption of game meat shot with Pb-containing bullets poses no significant risk towards the general population (BfR 2014; Lead Ammunition Group 2015). High level/extreme consumption of game meat by e.g. hunters may, however, considerably contribute to Pb exposure (BfR 2014; Bjermo et al. 2013; Lead Ammunition Group 2015). Due to the high vulnerability of young children and the developing foetus towards the effects of Pb, risks for these groups can generally not be excluded and advices against the consumption of game meat or game offal are often formulated for children and women of childbearing age.

5. Research objectives

In the context of a potential health risk due to the presence of Pb in big game meat shot with Pb containing ammunition, the aim of this project is to collect representative data on the presence of Pb in edible meat of big game (roe deer, wild boar and red deer) shot in Belgium. The samples are collected in collaboration with hunters and game processing centres spread throughout the main hunting areas in Belgium, and the data are analysed in relation to the geographical area where the animals were shot (in order the estimate the influence of environmental exposure), their age, the location of the wound channel and potential bone hit. Attention is also paid to data on Pb and Cd concentrations in kidney of roe deer of an existing database (owned by INBO and Sciensano), which is delivered to the project, complemented with additional data of roe deer kidneys sampled in the Walloon region within this project. The data are processed in relation to geographical origin and animal age. In a separate investigation the effect of the distance to the wound channel on meat Pb concentrations is explored more in detail, because this information may be helpful when defining measures that can help to reduce Pb exposure due to game meat consumption. Based on various consumption scenarios, the exposure to Pb via the consumption of game meat is assessed for high-level consumers of big game meat, i.e. hunters and their relatives, and a risk evaluation is performed. To complement the Belgian data, a detailed overview of international studies related to the use and risks of Pb containing bullets is provided. Also policy recommendations are formulated concerning the consumption of big game meat and the option of using non-lead ammunition.

Research questions to be answered are:

- What is the lead (Pb) and cadmium (Cd) content in edible meat and in kidneys of big game shot in Belgium?
- What is the influence of environmental exposure on the Pb and Cd content in Belgian big game meat (and kidneys)
- What is the influence of the distance from the wound channel on the Pb content in Belgian big game meat?
- What is the dietary intake of Pb through the consumption of Belgian big game meat by hunters and their relatives?
- Are there risks related to the intake of Pb through the consumption of Belgian big game meat for these consumers?
- Which recommendations concerning the consumption of big game meat and the use of non-lead ammunition can be formulated based on the results of this study and experiences in the matter in other countries.

6. Materials & methods

6.1. WP 1 – Belgian data concerning the concentration of lead and cadmium in big game meat and kidney

6.1.1. Sample collection

6.1.1.1. Meat samples

The aim was to collect meat samples of at least 250 different animals, comprising roe deer (*Capreolus capreolus*; ± 84 animals), wild boar (*Sus scrofa*; ± 84 animals) and red deer (*Cervus elaphus*; ± 84 animals).

Meat samples were collected either directly from hunters or by collaborating with a game processing centre (GPC). All efforts were directed towards an optimal geographical distribution of the samples between Flanders and Wallonia, except for red deer that is only hunted in Wallonia.

The hunters/GPC were instructed to provide a 200-g sample of both the saddle and the haunch. In order to guarantee the necessary data collection, a fill-in formulary was supplied to the collaborating hunters/GPC. This formulary had to be filled out for each animal and included requests to provide:

- The animal species
- The identification number of the animal shot (ID)
- An indication whether the bullet was still present in the animal
- Bone hit (yes or no)
- Information on the bullet type when known
- A graphical indication of the wound channel and sample location on a drawing of the species

For animals shot in Wallonia, following information was requested as well:

- Estimated age of the animal (<12 months; 12-24 months; >24 months)
- Sex of the animal
- The body weight of the animal (+/- 0.5 kg)
- Hunting date
- Hunting location

An example of a fill-in formulary is provided in Appendix A. The provided animal ID was used by INBO to determine the geographic location of the samples collected in Flanders, as well as the age class and sex of each animal shot based on the existing information in the Game Management Database Flanders ("Wildbeheerdatabank Vlaanderen"). For Wallonia, this information was collected in the fill-in formularies. All gathered information was stored in a database for further data treatment.

In addition, consumer packages of saddle, haunch or mixtures (stew meat) were bought in supermarkets for each of the species. It was verified whether the packages contained following labels: "*Dit product van de jacht kan projectielresten bevatten*"/"*Ce produit issu de la chasse peut contenir de restes de projectilles*" as well as "*origine: België*/*origine: Belgique*" to be sure that the meat was game meat of Belgian origin. The batch number and GPC that packed the meat were stored as well in the database.

6.1.1.2. Kidney samples

To estimate the influence of age and area in which the animals forage, on the Pb and Cd concentrations in kidney, a joint database of Sciensano and INBO containing (unpublished) data of trace element concentrations in kidneys of 200 roe deer was used (REE database). Within the LECAHUNT project, the REE database was extended with roe deer kidneys from three hunting areas in Wallonia.

Roe deer kidney samples, original REE database – The roe deer originated from six different game management units (GMU) in Belgium: (1) Meerdaalwoud, a forest area in central Belgium away from known heavy metal point sources, which is considered as a control group (2) valley of the Zwarte beek, a nature reserve at the southern border of the Noorderkempen (the GMU where roe deer were collected from in WP2), (3) Netebroek Balen, a nature reserve about 5 km southeast of an area historically known for its metallurgical activities in the Noorderkempen, (4) De Vart, a wildlife area in the northern part of the Noorderkempen, (5) valley of the Bosbeek, a wildlife area in the eastern part of the Noorderkempen, (6) Molenbeersel, a wildlife area in the north-eastern part of the Noorderkempen, about 20 km east of an area historically known for its metallurgical activities. The animals (99 males, 101 females) were shot between January 15 and September 15, 2006. For each animal, age (age class + age expressed in months), sex, shooting date and location were recorded.

Roe deer kidney samples, samples of the present project – the GPC was asked to provide us with a kidney whenever there was one available in the roe deer from which saddle and haunch samples were collected. Hence, information about the age class of the animals, sex, shooting date and location were provided via the fill-in formularies. In addition, kidney samples from roe deer hunted in Camp Roi Albert (Marche-en-Famenne; the GMU where wild boar were collected from in WP2) and Saint-Michel Freÿr (Saint Hubert) were provided by DEMNA (Département de l’Etude du Milieu Naturel et Agricole). For these samples information about the age class of the animals (“animals up to one year old” or “animals older than one”), sex and shooting date was provided as well.

All meat and kidney samples were stored at -18°C until further processing.

6.1.2. Chemical analysis

6.1.2.1. Meat samples

The samples were handled according to a slightly modified version of the method described by Lindboe et al. (2012). In short, samples of 200 g (or less if the collected samples were smaller) were cut into pieces and grinded to a homogenous paste in a Büchi B-400 mixer (equipped with ceramic knives; Büchi Labortechnik AG, Flawil, Switzerland) with diluted nitric acid (HNO_3 15% SpA grade, Romil, Cambridge, UK) in a 2:1 (acid:meat) weight ratio. The samples were constantly shaken in a beaker for 24 hours at 22°C, after which the slurry was again mixed in the Büchi B-400 mixer. The homogenized slurries were either directly processed or frozen at -18°C until further processing. The analytical procedure is visualized in Figure 1.

Total Pb and Cd concentrations were determined by ICP-MS (Varian 820; Varian, Melbourne, Australia) after acid digestion in teflon vessels (PTFE, polytetrafluoroethene) in a microwave oven (Anton Paar, Multiwave 3000, Graz, Austria). Aliquots of 1.5 g of the homogenized slurry, which corresponds to 0.5 g fresh meat, were weighed in triplicate in the microwave vessels. After addition of 4 mL nitric acid (HNO_3 65-67% SpA grade, Romil, Cambridge, UK) and 4 mL double distilled water, the vessels were closed and placed in the microwave system. The samples were heated to 180°C in 20 min and maintained at that temperature during 30 min, followed by 20 min at 50°C. After cooling, the samples were diluted by adding 9 mL double distilled water to 0.8 mL of the digest. Quantification of Pb and Cd in the digests was performed by external calibration (calibration range 1-10 µg/L) using acidified dilutions (4% nitric acid) of a multi-element stock solution (Analytika, Prague, Czech Republic). The certified reference material BCR-185R (bovine liver, JRC-IRMM, Geel, Belgium) was added to each sample batch. A schematic overview of the sample analysis procedure is given in Figure 1. The limit of quantification for total Pb and Cd determination, calculated as 10 times the standard deviation of 20 blank samples, corresponds to 0.003 and 0.0015 mg/kg respectively after taking into account the applied dilution factor. All results, except for the certified reference material, are expressed on a fresh weight basis.

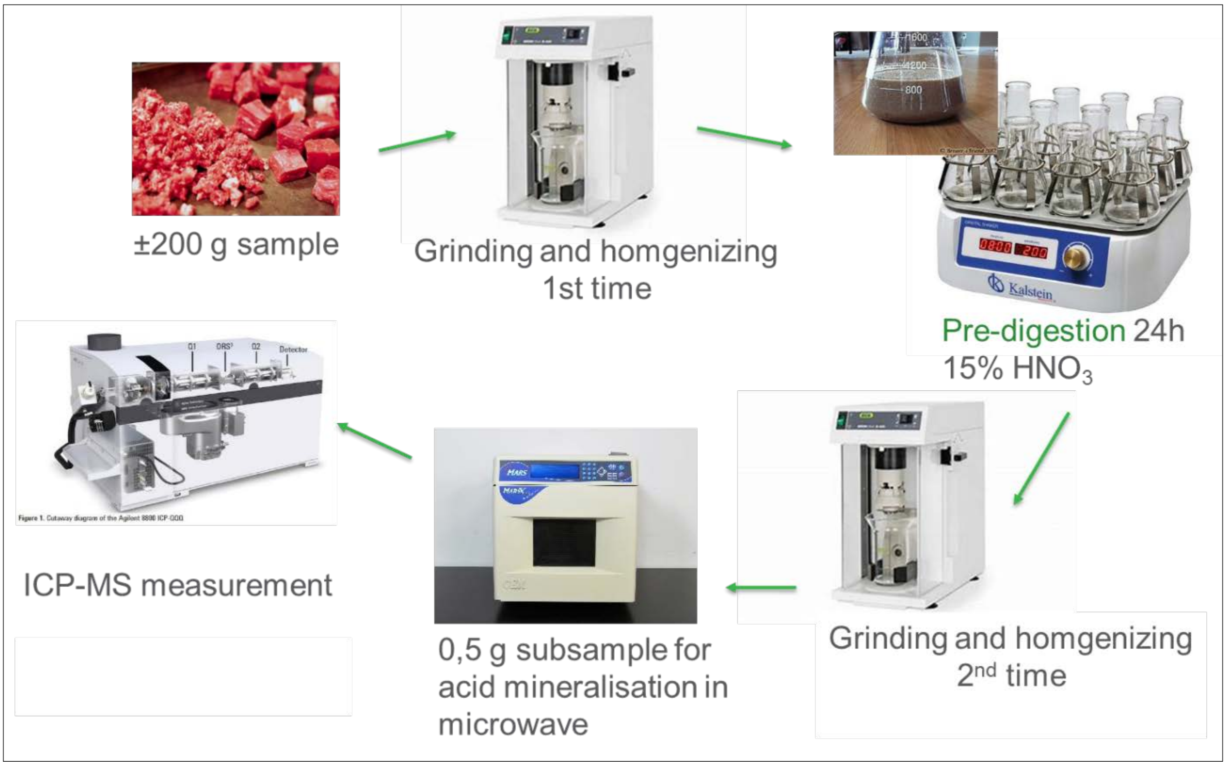


Figure 1. Schematic overview of the sample analysis procedure

6.1.2.2. Kidney samples

Samples of the present project - At the time of analysis, the tissues were partially thawed and mixed in a kitchen mixer (Braun- HB701AI) to homogenize the samples. Consequently, the kidney cortex and medulla, which contain unequal trace element concentrations, were homogeneously mixed and metal concentrations are expressed on the whole organ. For each kidney, three homogenized subsamples were mineralised by acid digestion (nitric acid) in pressure vessels of an X-PRESS microwave digestion system (CEM Corporation, Matthews, NC, USA). Aliquots of 0.5 g of the homogenized kidney was weighed in triplicate in the microwave vessels. After addition of 4 mL nitric acid (HNO_3 65-67% SpA grade, Romil, Cambridge, UK) and 4 mL double distilled water, the vessels were closed and placed in the microwave system. The samples were heated to 180°C in 15 min and maintained at that temperature during 30 min. After cooling, samples were diluted on a weight basis (1g sample solution + 9 g double distilled water). Lead and Cd concentrations in all digests were quantified by ICP-MS (Agilent 8800, Agilent Technologies, Santa Clare, CA, USA). The certified reference material BCR-185R (bovine liver, JRC-IRMM, Geel, Belgium) was added to each sample batch. The limit of quantification for total Pb and Cd determination, calculated as 10 times the standard deviation of 20 blank samples, corresponds to 0.008 and 0.0014 mg/kg respectively after taking into account the applied dilution factor.

Samples of the REE database – The samples were homogenized and digested as described above, except for the number of replicates. Fifteen kidney samples, randomly chosen, were mineralised both as a single replicate and in duplicate to verify whether a single replicate analysis was sufficient. Figure 2 shows that the difference in trace element concentrations between the single replicate and duplicate analyses was negligible and the analysis of single replicates was considered sufficient. Therefore, the other kidneys were mineralised in a single replicate. The concentrations in the extracts were measured by ICP-MS (VG PQ-ExCell Thermofisher Scientific, Winsford, UK). Each batch additionally included four procedure blanks and one duplicate reference material sample (IAEA-407, International Atomic Energy Agency, Vienna, Austria). The limit of quantification was calculated as 10 times the standard deviation of ten procedure blanks, multiplied with the dilution factor and equalled to 0.006 and 0.002 mg/kg on a fresh weight basis for Pb and Cd respectively.

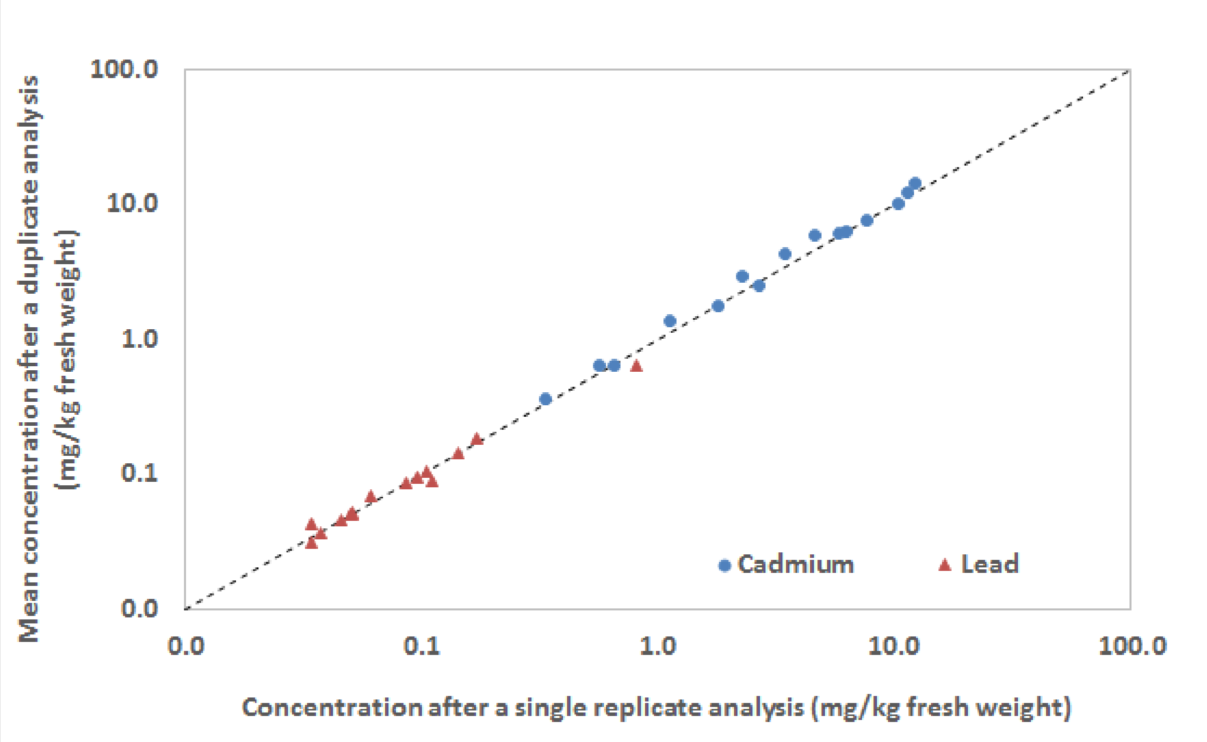


Figure 2. Mean Cd (blue dots) and Pb (red triangles) concentrations in kidneys after duplicate sample preparation and analysis versus a single replicate sample preparation and analysis. The dotted line is the 1:1 line.

6.1.3. Data treatment

6.1.3.1. Meat samples

All information collected via the animal ID or fill-in questionnaires was assembled in a database, together with the Pb and Cd analyses results. Data analysis was performed in the software environment of R (R Studio, Boston, USA). Maps were made to visualize the distribution of the samples for each species. Boxplots in overlay with violin plots were made to compare lead and cadmium contents between different animals and sample types.

In addition to the samples collected specifically for WP1, some samples collected for WP2 (two to three per animal) were added to the database. Because the samples of WP2 were in general smaller compared to those of WP1, the weighted average Pb and Cd concentrations were calculated for either 2-3 consecutive transect samples or two portions of the same sample (e.g. the haunch) to obtain the metal concentrations in 200-g portions.

Handling of data below the LOQ – Measurement results below the LOQ are associated with a large relative standard deviation between replicate samples and with large measurement uncertainties. Nevertheless, the means of the actual measured values of triplicate samples were used for statistical and model calculations as these represent the best estimate of the true Pb or Cd concentration in the sample. Using zero or the LOQ value for measurement results below the LOQ would respectively lead to an underestimation or an overestimation of the actual concentration. Values below the limit of detection (LOD; 0.001 mg/kg for Pb and 0.00015 mg/kg for Cd) were set equal to the LOD in the statistical and model calculations.

Statistical analysis - To assess if there are significant differences of lead and cadmium contents between species (wild boar, roe deer, red deer), regions (Flanders, Wallonia, Belgium (for supermarket samples)) or sample types (saddle, haunch, diverse), linear mixed-effect models were used.

To conduct a model with sufficient data of each group, some samples were excluded (red deer samples, samples with regions Belgium and diverse sample type). Moreover, samples with a Pb value larger than 1 mg/kg were also excluded from dataset for statistical analysis of Pb samples (34 out of 317 samples).

A full model including all variables (species, region and sample type) as well as the interaction between species and sample type (in case sample types affect the Pb or Cd concentration differently across species). The individual animal was added as an offset variable because multiple samples per animal were analysed.

Starting from the full model, a backwards model selection approach based on AIC-values (Akaike information criterion) was used: in each step, the variable with the lowest variable importance was excluded. The model with the lowest AIC value was selected as the best explaining model for the assessment of significant differences of lead or cadmium contents between species, or sample types.

6.1.3.2. Kidney samples

REE database - Statistical analysis was performed with the statistical software RStudio version 1.1.463 (© 2009-2018 RStudio, Inc). The normal distribution of data was verified by the Shapiro-Wilk test. Trace element concentrations and age were log-transformed to fulfil the requirement of normality before further statistical analysis.

To assess whether there are significant differences in the kidney Cd or Pb concentrations between the two age classes and/or between the GMUs, data in the extended REE database (Flanders + Wallonia) were analysed by two-way type III ANOVA because of unequal sample sizes in each level of the independent variables (GMU and age class). The assumptions behind ANOVA were verified with the Shapiro-Wilk test (to check the normal distribution of residuals), the Durbin-Watson test (to check independence) and Levene's test (to check for homoscedasticity). Some outlying values were detected (Grubbs outlier test), but there was no good reason to remove them from the dataset.

Data in the original database (Flanders) were analysed by linear regression modelling to assess whether the kidney Cd or Pb concentrations depended on the age of the animals or the GMUs. The models included the variables log(age in months) (centered around the mean log(age)) and GMU, as well as the interaction term between log(age) and GMU. Type III ANOVA was applied to determine the significance of the variables.

6.2. WP 2 – Lead content as a function of the distance to the wound channel

6.2.1. Sample collection

Whole wild boar animals (n = 18) and one red deer were collected after two hunting events at "Camp Roi Albert", Marche-en-Famenne (17/11/2018 and 08/12/2018). The animals were transferred to the animal testing facilities of Sciensano (Machelen, Belgium) where they were skinned and cut into pieces. Samples were cut in transects going from the shoulder, over the saddle towards the haunch of the animals (shoulder, loin, leg, ham) in 10-cm intervals, at both sites of the animal (shot in & shot out). The distance towards the wound channel was thereby measured for each sample. Additional samples were taken from the front legs (arm shoulder), the inside of the back leg (ham) and the tenderloin. Some of these latter samples were used for WP1 as "diverse" samples.

Whole roe deer animals (n = 19) were collected in the GMU "Vallei van de Zwarte Beek" over the period 15/01/2019-15/06/2019 to obtain both males and females. Similar to wild boar, samples were cut from the shoulder, over the saddle towards the haunch of the animals (shoulder, loin, haunch) in 10-cm intervals, at both sites of the animal (shot in & shot out). An example is given in Figure 3. The distance towards the wound channel was thereby measured for each sample. Additional samples were taken from the front legs (shoulder, shank), the inside of the back leg and the tenderloin.

All samples were stored at -18°C until further processing. The same formulary as used in WP1 was filled out for each whole animal collected in WP2.

Figure 3. Example of a roe deer meat transect from shoulder (left) to haunch (right).

6.2.2. Chemical analysis

All samples were analysed as described in WP1, except that the sample weight was ~100 g instead of 200 g.

6.2.3. Data treatment

To assess the relation between lead contents in relation to the distance from the wound channel, data were grouped into different distance classes being: class 1: 0 cm (= in the wound channel); Class 2: > 0-25 cm, class 3: > 25-50 cm, class 4: > 50 cm. Because the criteria for performing parametric statistics were not fulfilled (normality of data distribution, equality of variance) a non-parametric test (Kruskall-Wallis, followed by a multiple comparison procedure in GraphPad Prism 8) was used to reveal potential differences in Pb concentrations between the classes. In addition, scatterplots were constructed to visualize the presence/absence of potential trends, and the percentiles of Pb concentrations (P25, P50, P75, P95) were calculated for each class.

6.3. WP 3 – Dietary intake assessment and risk evaluation

6.3.1. Consumption of big game meat by specific consumers

It was considered from the outset of the research that hunters and their relatives are likely to eat higher quantities of game meat than the general population (FSA, 2012). Therefore, this project focussed on the consumption of game meat, and its associated risks, by high-level consumers, i.e. hunters and their relatives (including children). A literature survey was performed to estimate consumption frequencies and serving sizes of game meat by this target population, and these were further translated in consumption scenario's.

6.3.2. Lead exposure through the consumption of big game meat by specific consumers

In 2011, the Scientific Committee of the FASFC calculated the total exposure to Pb for different groups in the Belgian population (SciCom 2011). The dietary intake was based on the evaluation performed in 2009 (SciCom 2009a), to which additional sources of Pb exposure were added (exposure through air, soil and dust, and cigarette smoke or consumption). The calculations were performed for people living or working in different environments (rural, city, industrial) to take into account environmental exposure. An overview of these exposure calculations is given in Table 1.

These total exposure data were considered as the background Pb to which people in Belgium are exposed and to which the dietary intake of Pb through the consumption of game meat were added. The consumption scenarios (cfr. Section 6.3.1) were combined with the Pb concentrations measured in edible meat (WP1), to calculate the additional dietary Pb intake and total Pb exposure through the consumption of game meat per person.

Two strategies were followed to select appropriate Pb concentrations in meat for the intake calculations and calculate the Pb exposure: a deterministic approach and a probabilistic approach.

Table 1. Total background exposure to Pb, calculated by the Scientific Committee of the FASFC in 2011, for different populations in Belgium, taking into account the relative bioavailability of Pb from different environmental sources compared to Pb in food (SciCom 2011).

	Pb exposure through the diet	Pb exposure through intake of soil & dust	Pb exposure through outdoor air	Pb exposure through inhalation of cigarette smoke	Pb exposure through cigarette consumption	Total Pb exposure
Rural area						
Adult	0.13	0.01	0.012	0.0425	0/0.083	0.19/0.28
Women of childbearing age	0.13	0.01	0.012	0.0425	0	0.19
Children (2.5-6.5 y)	0.42	0.21	0.0034	0.0120	0	0.65
City area						
Adult	0.13	0.04	0.0255	0.0425	0/0.083	0.24/0.32
Women of childbearing age	0.13	0.04	0.0255	0.0425	0	0.24
Children (2.5-6.5 y)	0.42	0.74	0.0072	0.0120	0	1.25
Industrial area						
Adult	0.13		0.088	0.0425	0/0.083	0.37/0.46

Deterministic approach

The median Pb concentration of the meat samples analysed in WP1 was multiplied with the game meat serving size per person and consumption frequency to calculate the Pb exposure due to the consumption of big game meat. The background total Pb exposure (dietary exposure + environmental exposure; SciCom, 2011) was added to the exposure through the consumption of big game meat. The exposure to Pb through the consumption of other meat types was thereby reduced in accordance with the frequency of game meat consumption.

$$Pb\ exposure\ \left({}^{\mu g}/_{kg_{bw} \cdot d}\right) = \frac{[Pb]_{median}\ \left({}^{\mu g\ Pb}/_{g\ meat}\right) \cdot serving\ size\ \left({}^{g\ meat}/_{kg_{bw} \cdot serving}\right) \cdot {}^{\#\ servings}/_{year}}{365\ d/year} + Background\ Pb\ exposure\ \left({}^{\mu g}/_{kg_{bw} \cdot d}\right)$$

Probabilistic approach

The Pb concentration of the meat samples analysed in WP1 were used to model the Pb concentration in big game meat servings. The data were, therefore, logarithmically transformed, after which a lognormal distribution was fitted over the data. This approach resulted in a better fit of the higher percentile Pb concentrations compared to fitting a normal distribution over the log-transformed data, as shown in Figure 4. The maximum concentration of Pb in meat was set at 200 mg/kg, as calculations demonstrated that Pb concentrations > 200 mg/kg are caused by lead fragments that are larger than tactile sensibility thresholds (thickness 70 µm & minimal longest side 7.1 mm – thickness 700 µm & minimal longest side 2.25 mm) and will probably not be swallowed (Hinton et al., 2004 cited in (Engelen et al. 2005), Enkling et al. (2007)). The Pb concentration in each serving was randomly drawn from the modelled distribution and multiplied with the game meat serving size per person. This was repeated for the number of servings per year (corresponding to the respective consumption frequency scenario) over a lifetime period (age 18-64 years). The background total Pb exposure (dietary exposure + environmental exposure; SciCom, 2011) was added to the exposure through the consumption of big game meat. The exposure to Pb through the consumption of other meat types was thereby reduced in accordance with the frequency of game meat consumption. The total lifetime Pb exposure was finally recalculated on a per-day basis (µg/kg_{bw}/d). A Monte Carlo simulation was executed in Excel 2016. An iteration size of 10000 was used.

As the calculations by SciCom (2011) demonstrated that children in the general population already exceed health based guidance values (HBGV) for Pb exposure, no probabilistic Pb exposure estimates were performed for children.

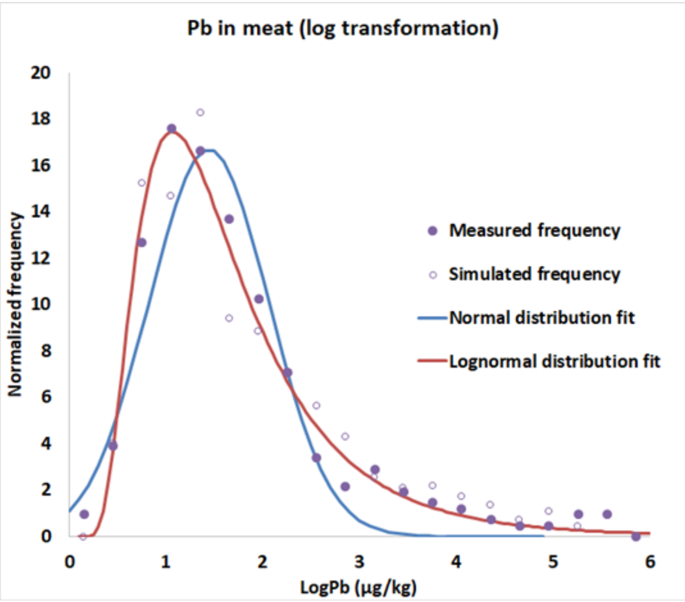


Figure 4. Frequency distribution of the measured Pb concentrations in big game meat (log transformed), normal and log-normal distribution fit over these data, and frequency of the simulated log-transformed Pb concentrations in big game meat after applying the log-normal distribution model.

6.3.3. Risk evaluation of lead intake through the consumption of big game meat

Neither for acute exposure nor for chronic exposure to Pb it is currently possible to identify a tolerable intake level or minimal risk level since there is no threshold for critical Pb-induced effects (ATSDR, 2007; EFSA, 2010; JECFA, 2011).

EFSA defined significant health responses at a population level as being a 1% decrease in IQ (i.e. a 1-point reduction in IQ), a 1% increase in systolic blood pressure, and a 10% increase in the incidence of chronic kidney disease. The lower one-sided 95% confidence limit of the Benchmark Dose Level (i.e. the BMDL), was taken as the reference point for margin-of-exposure estimations (MOE). The use of a BMDL as a reference point is a conservative approach and considered to be health protective. The BMDLs determined by EFSA expressed in µg/L blood, and the corresponding dietary intake values in µg/kgbw/day, are given in Table 2. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) used the review of EFSA (EFSA, 2010) as the starting point for its evaluation of Pb in 2011, together with newer studies that were considered to be informative. JECFA agreed with EFSA that the neurodevelopmental effects should be the pivotal data in a risk assessment for children, and considered the increase in systolic blood pressure as the pivotal data for adults. JECFA calculated a range of IQ impacts in children associated with a range of dietary exposures. The differences compared to the estimates by EFSA can be attributed to different choices made in dose-response modelling of blood Pb levels and the extrapolation to dietary exposure.

The potential health risks associated with the intake of Pb through the consumption of edible big game meat shot in Belgium was evaluated against the Pb exposure levels and associated risks derived by EFSA and JECFA presented in Table 1. The BMDL values were used as health based guidance values and margin of exposure (MOE) levels were calculated as

$$MOE = \frac{BMDL\ (^{\mu g}/_{kg_{bw}} \cdot day)}{Estimated\ Pb\ exposure\ (^{\mu g}/_{kg_{bw}} \cdot day)}$$

Table 2. Lower one-sided 95% confidence limit of the Benchmark Dose Levels (BMDL) for blood lead concentration and dietary lead exposure, corresponding to a certain level of effect

Effect on	Effect level (at population level)	BMDL Blood Pb level (µg L^{-1})	BMDL Dietary Pb exposure (µg kg_{bw}^{-1} d^{-1})	Reference
Developmental neurotoxicity	1% decrease in IQ	12	0.50	(EFSA, 2010)
	0.5% decrease in IQ	-	0.3	(JECFA, 2011)
	1% decrease in IQ	21	0.6	(JECFA, 2011)
	3% decrease in IQ	-	1.9	(JECFA, 2011)
Systolic blood pressure	1% increase	36	1.50	(EFSA, 2010)
	increase with 1 mmHg		1.3	(JECFA, 2011)
Chronic kidney disease	10% increased prevalence	15	0.63	(EFSA, 2010)

6.4. WP 4 – Data comparison with international literature and policy recommendations

For the purpose of comparison between the data collected in this research project and international research data, and in order to get an overview of international policy recommendations, an extensive literature review is performed. Scientific papers and reports were collected through a thorough search on key words such as 'lead', 'wildlife', 'lead bullets', 'game meat', 'lead ammunition' 'non-lead ammunition', 'lead consumption', etc. During the literature review, we focussed on four subjects: background information about lead bullet composition and behaviour, possible alternatives for lead bullets, national and international regulations, international policy recommendations. These four topics will be extensively discussed in WP4, providing a broad overview of the available information.

7. Results achieved

7.1. WP 1 – Belgian data concerning the concentration of lead and cadmium in big game meat and kidney

7.1.1. Lead and cadmium concentrations in Belgian big game meat

7.1.1.1. Sample collection

Sampling of the animals proved to be more difficult than expected. Despite fruitful contacts with the GPC prior to the start of the project, all but one of the GPC located in Wallonia refused to participate in the project, while the Flemish GPC claimed to either not handle Belgian game meat or to buy game meat from one of the GPC located in Wallonia. Especially the collection of red deer was thereby hampered. For hunters the major issue was to convince them to deliver the best parts of the animals, despite the compensation they received for it. As an alternative, some game meat samples were bought in supermarkets. Table 3 gives an overview of all collected animals.

Information collected from the questionnaires revealed that 23% of the animals were males, 32% were females, and for 45% of the animals the sex was unknown. The animals were shot in the thorax (29%), followed by the shoulder (14%), head (4%) and vertebra (4%). For 30% of the animals the shot location was not available, the other animals were hit elsewhere (e.g. legs, neck, gastrointestinal tract, …) or more than once.

Figures 5-8 give an overview of the location where the different animals were hunted. The areas where the respective species are hunted are indicated as well in Figures 6-8. During the course of the project, hunting activities were restricted in the most southern parts of Belgium due to the outbreak of African Swine Fever.

The target was to collect two samples per animal, one sample from the saddle and one sample from the haunch. This was not possible for supermarket samples, where only one sample per animal could be collected. To compensate for the supermarket samples, three samples per animal were used from the animals that were collected in the framework of Work Package 2. Not all samples that were collected, originated from the saddle or the haunch. These samples originated from the front legs, the neck, etc. and were labelled as “diverse”. Every sample was inspected visually and some samples were excluded from the database because the meat did not appear to be edible (e.g. full of tendons). In total, 411 samples were analysed in the framework of Work Package 1: 197 wild boar samples (93 saddle, 94 haunch, 10 diverse), 168 roe deer samples (83 saddle, 69 haunch, 16 diverse) and 46 red deer samples (22 saddle, 22 haunch and 2 diverse).

It was the intention that all meat samples would originate from animals shot with Pb-containing bullets. This was, however, not entirely successful. Information provided through the questionnaires revealed that 20% of the animals were indeed shot with lead-based bullets, three animals (1.5%) were, however, shot with lead-free bullets. For 78% of the animals, no information on the bullet type was provided in the questionnaires or could be retrieved (e.g. for supermarket samples). Because of the large fraction of samples without information about the bullet type, it was decided to keep the meat samples originating from the three animals shot with lead-free bullets, in the database as well.

Table 3. Overview of collected numbers of animals.

	Wallonia			Flanders			Total
	Wild boar	Roe deer	Red deer	Wild boar	Roe deer	Red deer	
Hunters	10	11	-	45	19	-	85
Processing Centers	14	29	8	-	-	-	51
Hunting for WP2	18	-	1	-	19	-	38
Supermarket	12	7	20	-	-	-	39
Total	54	47	29	45	38	-	213
Target	44	44	44	40	40	-	212
%	123	107	66	113	95	-	100

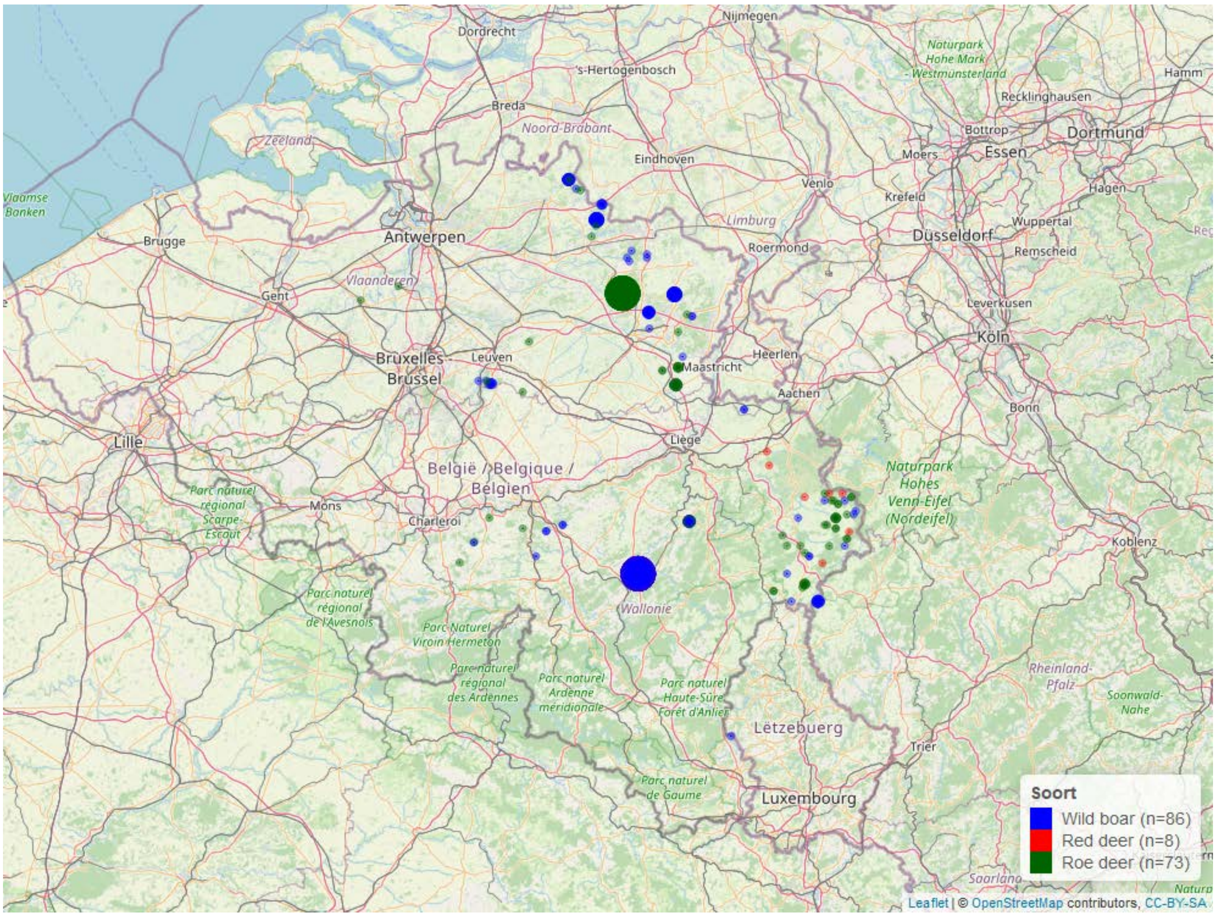


Figure 5. Overview of sample collection of all species in Flanders and Wallonia.

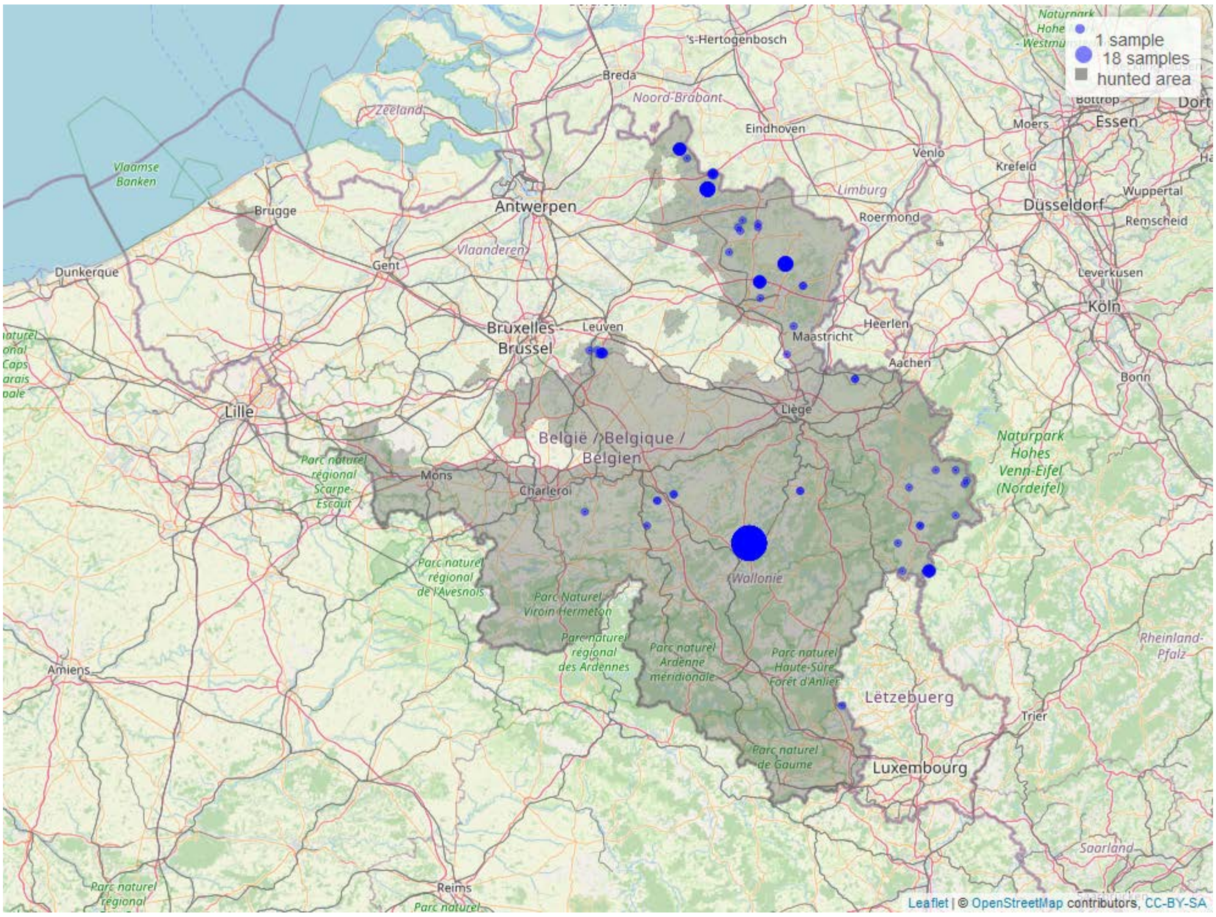


Figure 6. Overview of sample collection of wild boar (blue dots) and areas where wild boar are hunted in Flanders and Wallonia (shaded area).

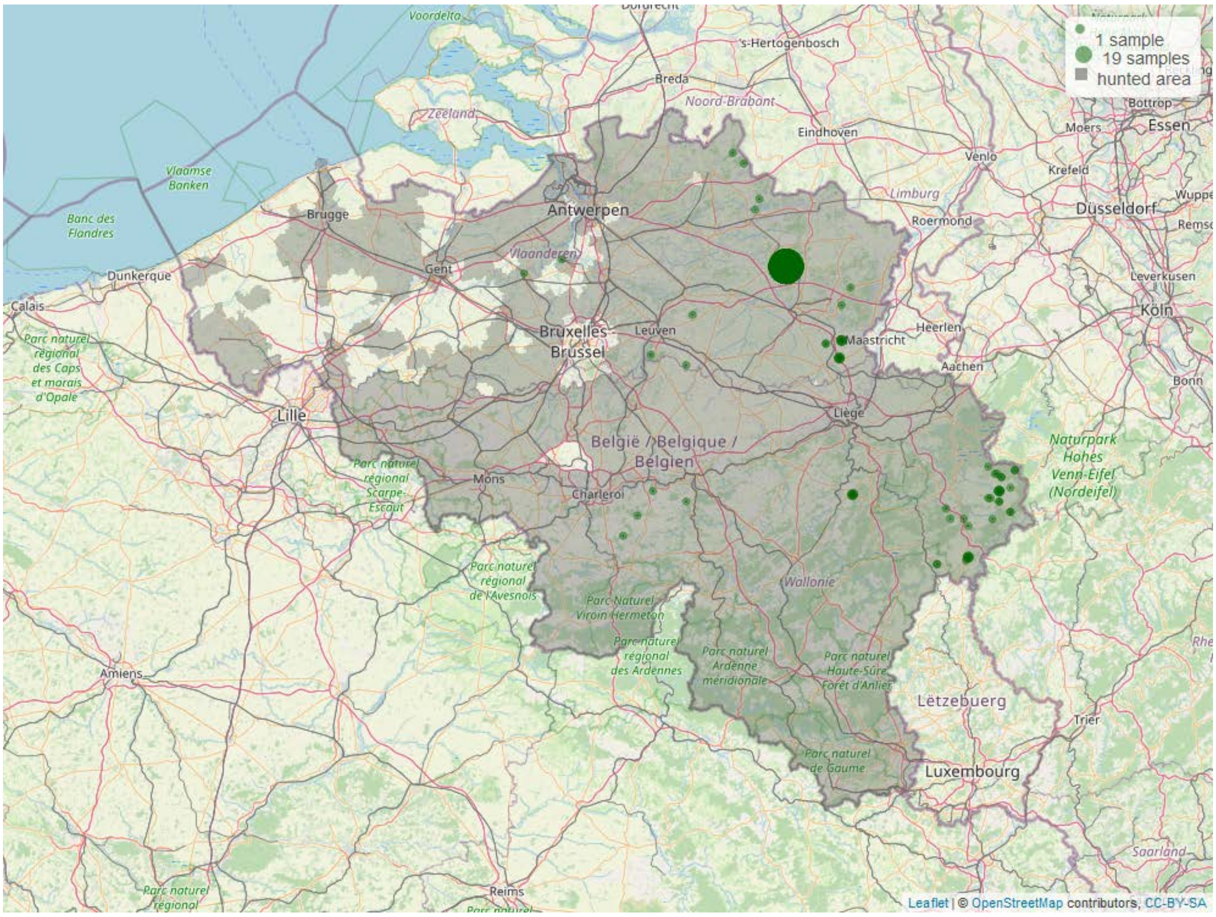


Figure 7. Overview of sample collection of roe deer (green dots) and areas where roe deer are hunted in Flanders and Wallonia (shaded area).

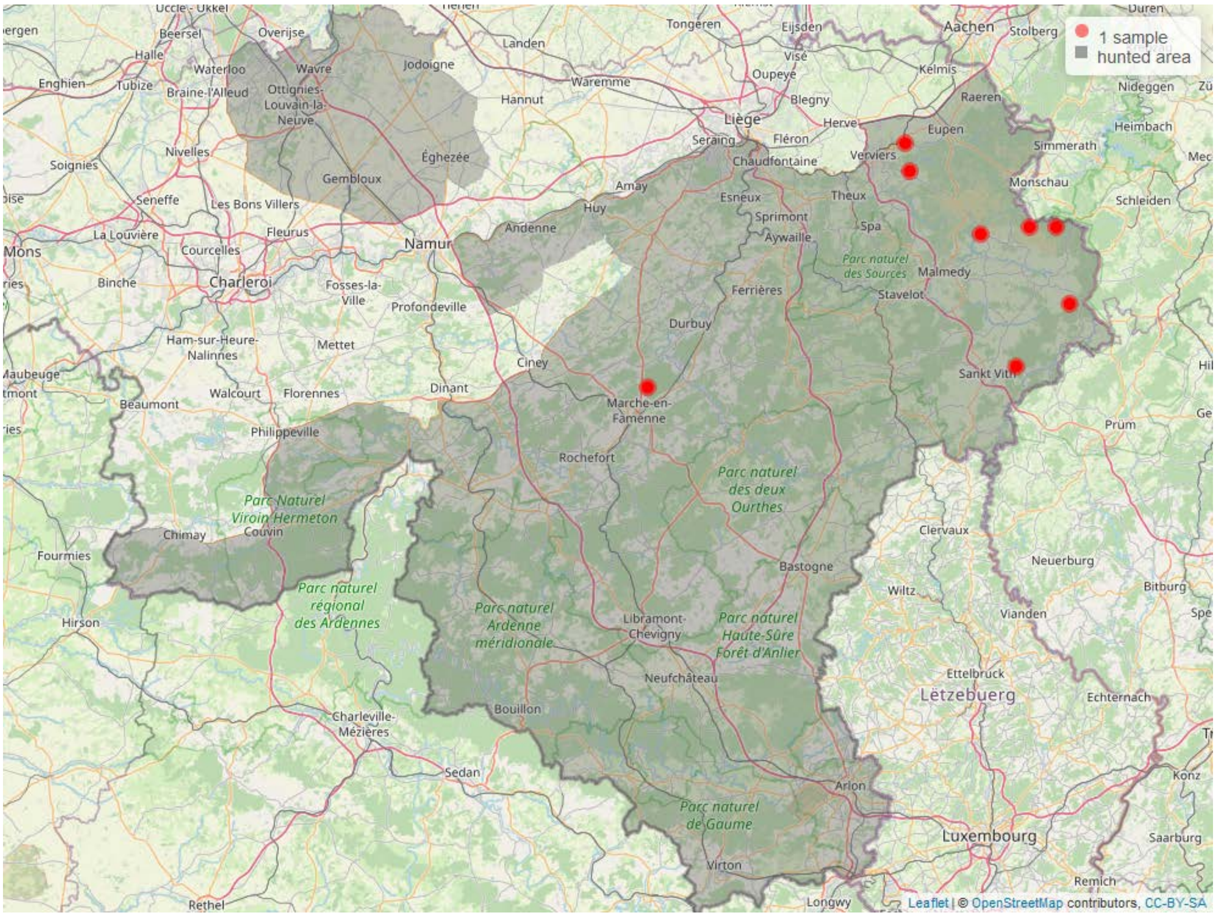


Figure 8. Overview of sample collection of red deer (red dots) and areas where red deer are hunted in Wallonia (shaded area).

7.1.1.2. Analytical results for lead

To demonstrate that Pb fragments, and hence Pb concentrations, in the meat samples may be not homogeneously distributed among an edible portion of meat, three 200-g samples were each divided in three 70-g subportions, which were homogenized independently. As can be seen in Figure 9, the variation in Pb concentration among the three subportions was large. This is in contrast to the Cd concentrations, which vary only marginally among the subportions (Figure 10). It is, therefore, important that the Pb concentration in game meat is determined on a portion of meat that is representative for a serving.

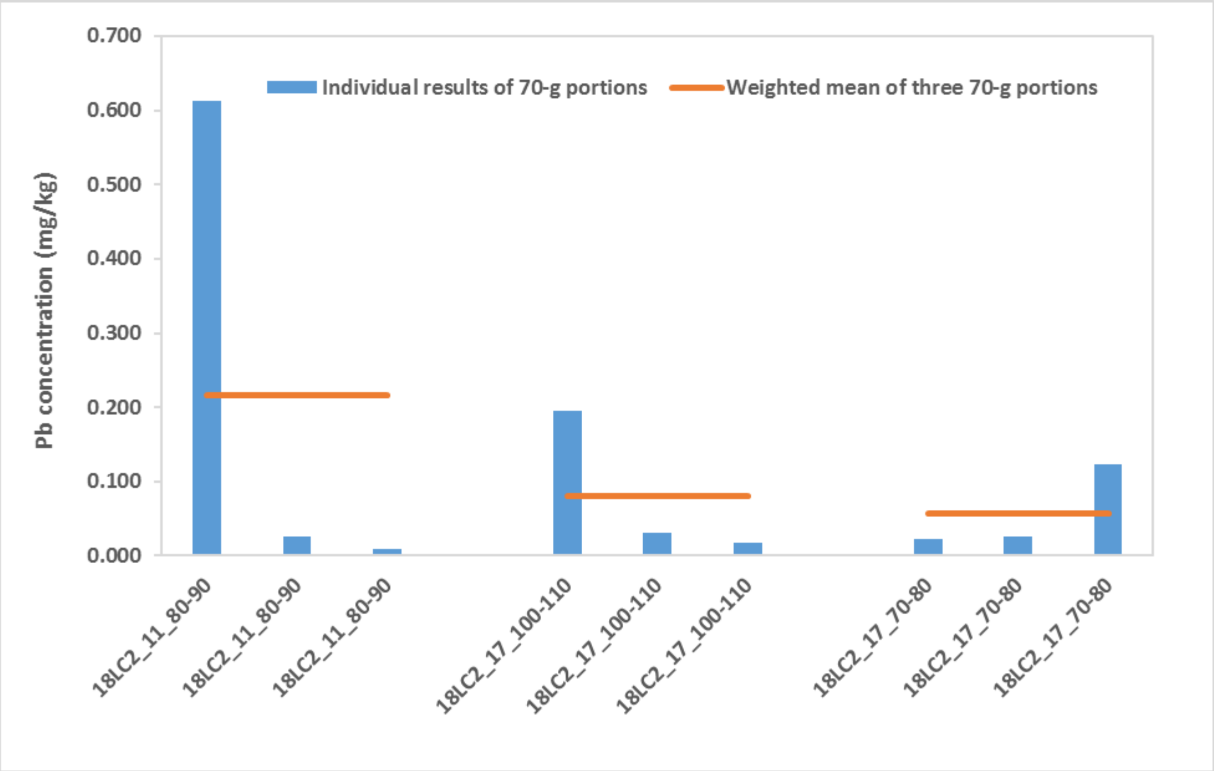


Figure 9. Individual Pb concentrations (blue bars) and weighted mean concentration (orange line) in three 200-g samples that were homogenized and analysed in three independent 70-g portions. The x-axis represents the sample numbers.

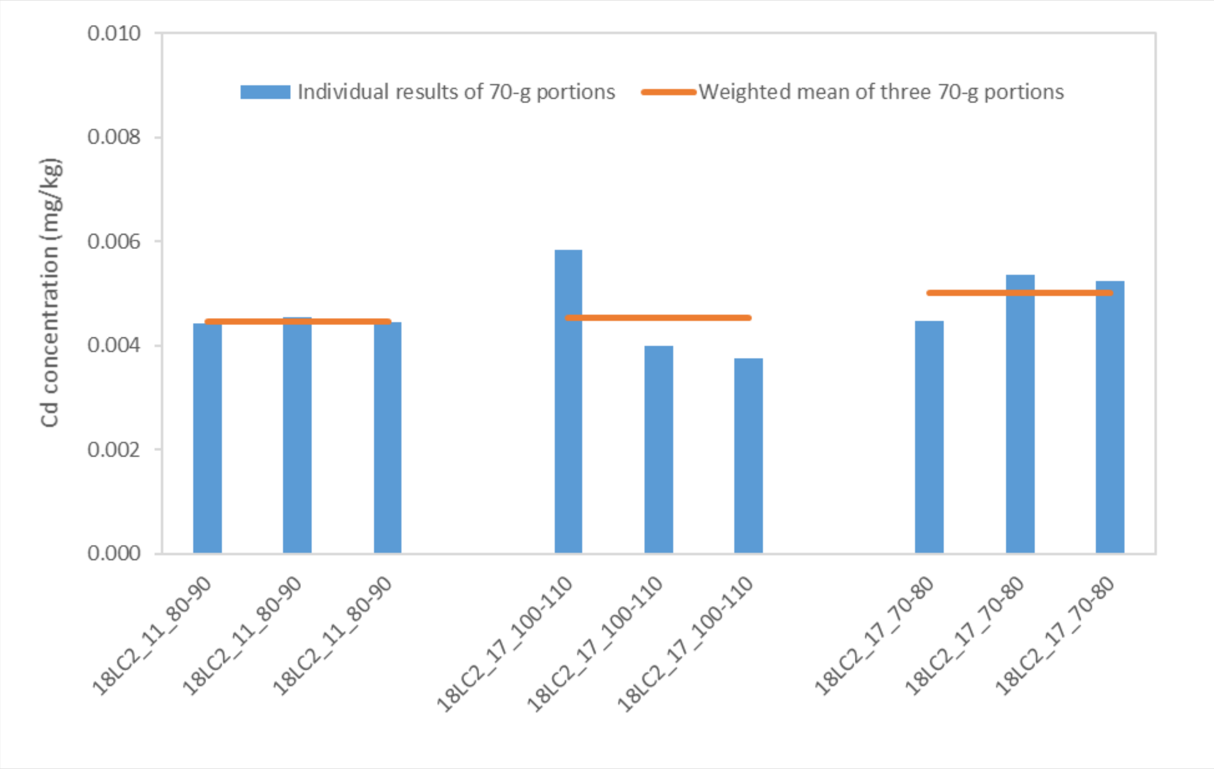


Figure 10. Individual Cd concentrations (blue bars) and weighted mean concentration (orange line) in three 200-g samples that were homogenized and analysed in three independent 70-g portions. The x-axis represents the sample numbers.

Out of the 409 analysis results for Pb, 43 results were below the LOQ of 0.003 mg/kg , and 4 results were below the LOD of 0.001 mg/kg. For 81% of the samples, the Pb concentration was below 0.10 mg/kg, which is the EU maximum level for Pb in meat of bovine animals, sheep, pig and poultry (EU 1881 2006). For wild boar, 78% of the Pb concentrations were below 0.10 mg/kg, while for roe deer 82% of the Pb concentrations were below this value. In 91% of all samples the Pb concentration was below 1.0 mg/kg, the action limit applied by the Federal Agency for the Safety of the Food Chain. Again, less roe deer samples (4.5%) exceeded this action limit compared to wild boar samples (12.5%).

Figure 11 shows the comparison of Pb concentrations in samples of the three animal species. The shape of the plot represents the estimated density of the data, the boxplot shows the median and the interquartile spacing. Only concentrations below 1.0 mg/kg are included in the plot to better visualize the data. Figure 11 illustrates once more that the majority of the samples have a low Pb concentration. Wild boar tended to have the highest Pb concentrations in all selected meat parts, followed by roe deer. In red deer the observed Pb concentrations were the lowest. Within each animal species differences in median Pb concentrations among saddle and haunch (and diverse) are small. In all species the median Pb concentration in saddle was similar to slightly larger than in haunch: 0.026 versus 0.022 mg/kg in wild boar, 0.011 versus 0.009 mg/kg in roe deer, and 0.013 versus 0.004 mg/kg in red deer. The median Pb concentration in the “diverse” samples was 0.035, 0.019 and 0.006 mg/kg in wild boar, roe deer and red deer respectively. Lead concentrations in saddle and haunch of animals collected in “Camp Roi Albert” (wild boar) or “Vallei van de Zwarte Beek” (roe deer) are similar to those collected in other Belgian regions (Figure 12).

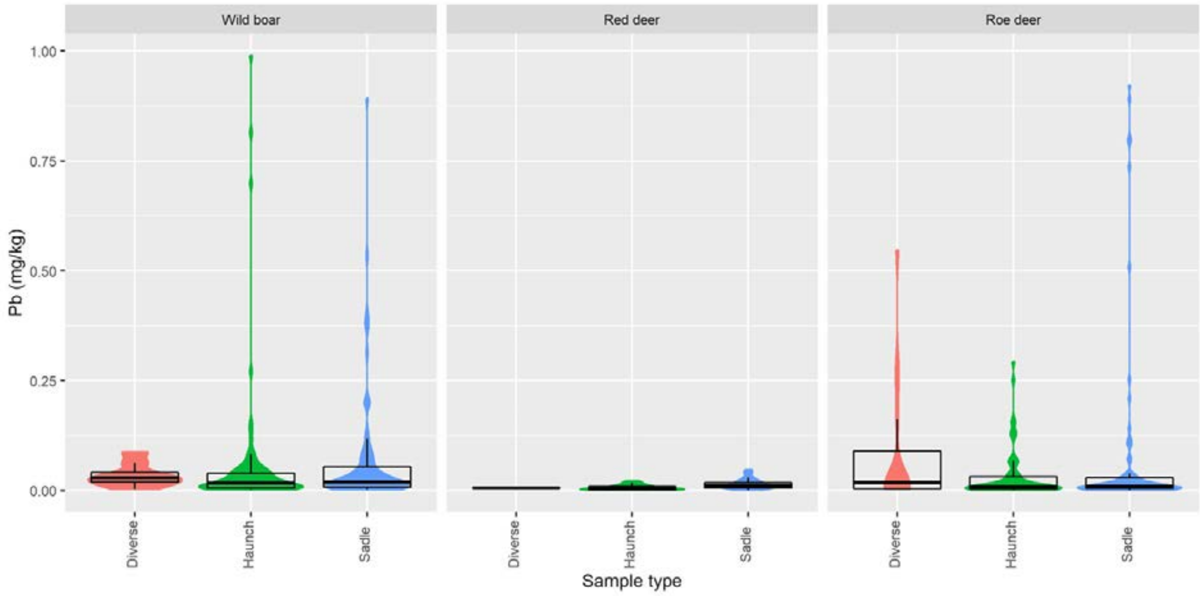


Figure 11. Comparison of lead concentrations below 1.0 mg/kg in edible parts of wild boar, red deer and roe deer. The shape of the plot represents the estimated density of the data, which is combined with the box plot. The boxplot shows the median and the interquartile spacing.

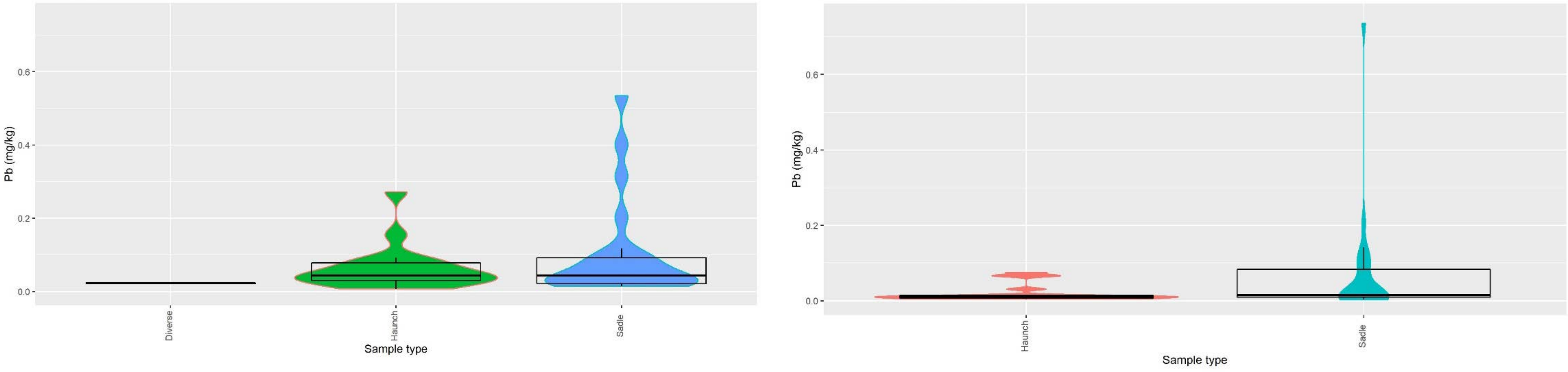


Figure 12. Comparison of lead concentrations below 1.0 mg/kg in edible parts (200-g portions) of wild boar collected in Camp Roi Albert (left), and roe deer collected in GMU Vallei van de Zwarte Beek (right). The shape of the plot represents the estimated density of the data, which is combined with the box plot. The boxplot shows the median and the interquartile spacing.

Table 4 and Table 5 show lead concentrations in meat from animals, obtained through respectively the game processing centre and hunters, for which the Pb concentration in at least one sample exceeds 1.0 mg/kg, taking into account an extended measurement uncertainty of 23.8%. The Pb concentrations above 1.0 mg/kg vary widely, from 1.3 mg/kg to 1185 mg/kg. In addition, five wild boar samples and 1 red deer samples obtained from the animals collected in the framework of WP2, contained Pb levels exceeding 1.0 mg/kg, taking into account the extended measurement uncertainty. The Pb concentrations in these samples ranged from 1.4 to 77 mg/kg.

Table 4. Lead concentration in meat from animals, obtained via the game processing center, for which the lead concentration in at least one sample exceeded 1.0 mg/kg, taking into account an extended measurement uncertainty of 23.8%.

Species	**Pb concentration (mg/kg)**		**Remark**
	Saddle	**Haunch**	
Roe deer	0.016 ± 0.004	215 ± 51	Animal hit in shoulder
Roe deer	0.007 ± 0.002	10 ± 2	Animal hit in shoulder
Roe deer	215 ± 51	0.024 ± 0.006	Animal hit in shoulder
Wild boar	1185 ± 282	0.050 ± 0.012	Animal hit in the vertebra, meat sample cut close to the shot wound
Wild boar	3.8 ± 0.9	0.004 ± 0.001	Animal hit in shoulder
Red deer	4.4 ± 1.0	0.007 ± 0.002	Animal hit in the saddle and thorax, bullet fragments were still present in the animal during cutting in the GPC.

Table 5. Lead concentration in meat from animals, obtained via hunters, for which the lead concentration in at least one sample exceeded 1.0 mg/kg, taking into account an extended measurement uncertainty of 23.8%.

Species	**Pb concentration (mg/kg)**	
	Saddle	**Haunch**
Roe deer	1.3 ± 0.3	-
Roe deer	2.8 ± 0.7	0.25 ± 0.06
Roe deer	43 ± 10	0.067 ± 0.016
Wild boar	26 ± 6	6.6 ± 1.6
Wild boar	0.20 ± 0.05	3.5 ± 0.8
Wild boar	1.33 ± 0.32	0.032 ± 0.008
Wild boar	16 ± 4	3.0 ± 0.7
Wild boar	10 ± 02	0.005 ± 0.001
Wild boar	118 ± 028	0.14 ± 0.03
Wild boar	104 ± 025	35 ± 8.3
Wild boar	0.045 ± 0.011	159 ± 38
Wild boar	155 ± 037	0.036 ± 0.008
Wild boar	4.0 ± 01.0	1.1 ± 0.3
Wild boar	3.2 ± 0.8	3.7 ± 0.9

A literature review with regard to Pb levels in wild boar, roe deer or red deer revealed that often mean Pb concentrations are reported, despite the fact that Pb in big game meat hunted with Pb containing bullets is rarely normally distributed (Bilandžić et al. 2009; Falandysz et al. 2005; Florijancic et al. 2015; Lazarus et al. 2014; Pokorny and Ribarič-Lasnik 2002; Rudy 2010). The median or geometric mean values would be better descriptors of such data. In the German

LEMISI project, Pb levels were measured in saddle and haunch meat of wild boar, roe deer and red deer shot with either Pb-containing bullets or Pb-free bullets (BfR 2014). The median Pb concentration in wild boar saddle and haunch meat were respectively 0.021 and 0.014 mg/kg for wild boar shot with Pb-containing bullets. Concentrations up to 650 mg/kg were found in the saddle. These results are similar as in the current study. In roe deer, median Pb concentrations in saddle and haunch were respectively 0.009 and 0.006 mg/kg, with a maximum of 189 mg/kg. In red deer, median Pb concentrations in saddle and haunch were 0.015 and 0.010 mg/kg, with a maximum of 1.1 mg/kg, The results for all three species found in the current study are similar to those in the LEMISI project.

In Viterbo, Italy, minimal and median Pb concentrations in muscle meat of 58 wild boar were 0.080 and 0.12 mg/kg respectively (Amici et al. 2012; Danieli et al. 2012). These values are much larger than in the current study or in the LEMISI project while the maximum concentration, 0.227 mg/kg, was much lower. Similarly, in Slovakia, the minimal and median Pb concentrations of 40 wild boar meat samples were 0.039 and 0.441 mg/kg respectively, although a maximum value of 61 mg/kg was observed (Gašparík et al. 2017). In other studies, such as those of Bilandžić et al. (2009), Florijancic et al. (2015) and Rudy (2010), no median concentration data are presented. The reported ranges in these studies were 0.002-4.7 mg/kg in Croatia (Bilandžić et al. 2009; Florijancic et al. 2015) and 0.039-0.087 mg/kg in Poland (Rudy 2010). Lazarus et al. (2014) reported a 95th percentile Pb concentration in wild boar muscle meat of 0.23 mg/kg in Croatia. It is unclear which factors could explain the observed differences among studies (e.g. differences in environmental contamination levels, differences in hunting practices, differences in analytical practices, etc.).

Lead concentrations in roe deer meat ranged from <0.05 to 0.76 mg/kg in a Slovenian study (Pokorny and Ribarič-Lasnik 2002) and from 0.04 to 0.82 mg/kg in Northeast Hungary (Lehel et al. 2016).

Lead concentrations in red deer tend to be lower than those found in wild boar or roe deer. Jarzyńska and Falandysz (2011) reported a median concentration of 0.011 mg/kg (recalculated from 0.076 mg/kg_{dw}) for red deer hunted in Poland, and a range from 0.002 to 0.19 mg/kg. In an earlier study Falandysz et al. (2005) reported a range from 0.010 to 1.5 mg/kg, while Skibnieuwski et al. (2015) reported a range from 0.04 to 0.48 mg/kg, with a median of 0.08 mg/kg. In Croatia, Pb concentrations ranging from 0.001 to 0.80 mg/kg are reported (Bilandžić et al. 2009).

7.1.1.3. Analytical results for cadmium

Out of the 394 analysis results for Cd, 16 results were below the LOQ of 0.0005 mg/kg. None of the results were below the LOD of 0.00015 mg/kg. In all samples, the Cd concentration was below 0.050 mg/kg, which is the EU maximum level for Cd in meat of bovine animals, sheep, pig and poultry (EU 1881 2006). Figure 13 shows the comparison of Cd concentrations in samples of the three animal species. The Cd concentrations in all three species and in the different samples types are all very similar, with median values ranging from 0.001 in red deer haunch to 0.0045 in the diverse samples of wild boar. Cadmium concentrations in saddle and haunch of animals collected in “Camp Roi Albert” (wild boar) or “Vallei van de Zwarte Beek” (roe deer) are similar to those collected in other Belgian regions (Figure 14).

In Italy, the median Cd concentrations in wild boar muscle meat was 0.07 mg/kg, which is above the EU maximum level for Cd in meat of domestic animals (Amici et al. 2012; Danieli et al. 2012; EU 1881 2006). The minimal Cd concentration these authors found was already 0.04 mg/kg, and the maximum 0.38 mg/kg. Gašparik et al. (2017) found even higher median Cd concentrations in Slovakian wild boar meat, 0.155 mg/g, although the maximum concentration was comparable to that of the Italian studies. In contrast, Rudy (2010) found maximally 0.018 mg/kg in wild boar shot in Poland. In Croatia, large regional differences were observed in wild boar muscle meat Cd concentrations (Bilandžić et al. 2009).

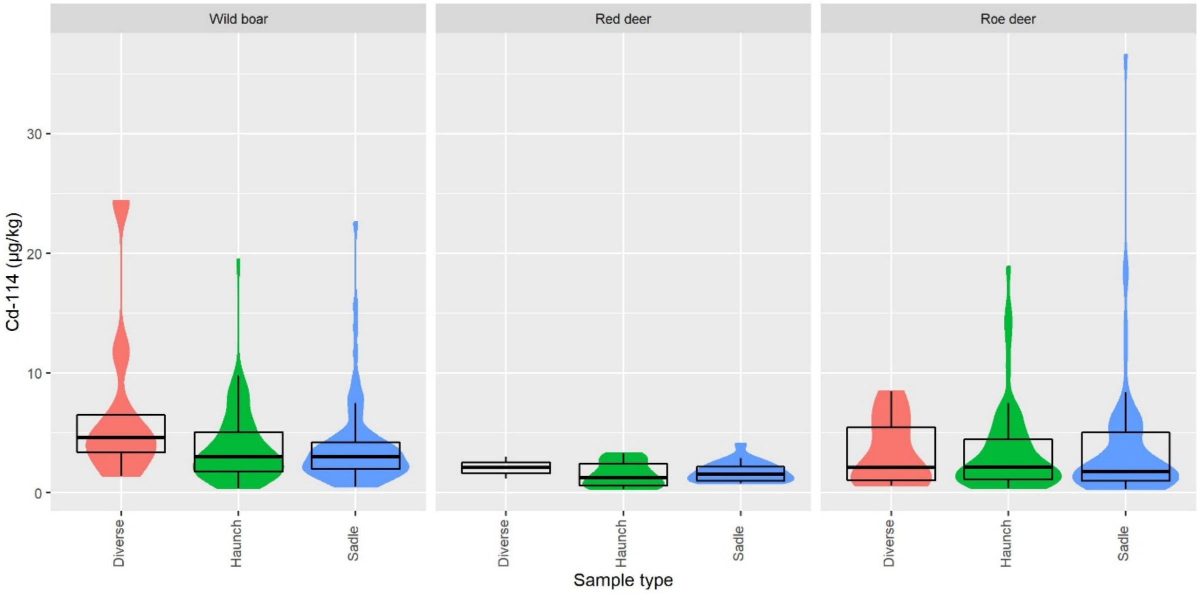


Figure 13. Comparison of cadmium concentrations in edible parts of wild boar, red deer and roe deer. The shape of the plot represents the estimated density of the data, which is combined with the box plot. The boxplot shows the median and the interquartile spacing.

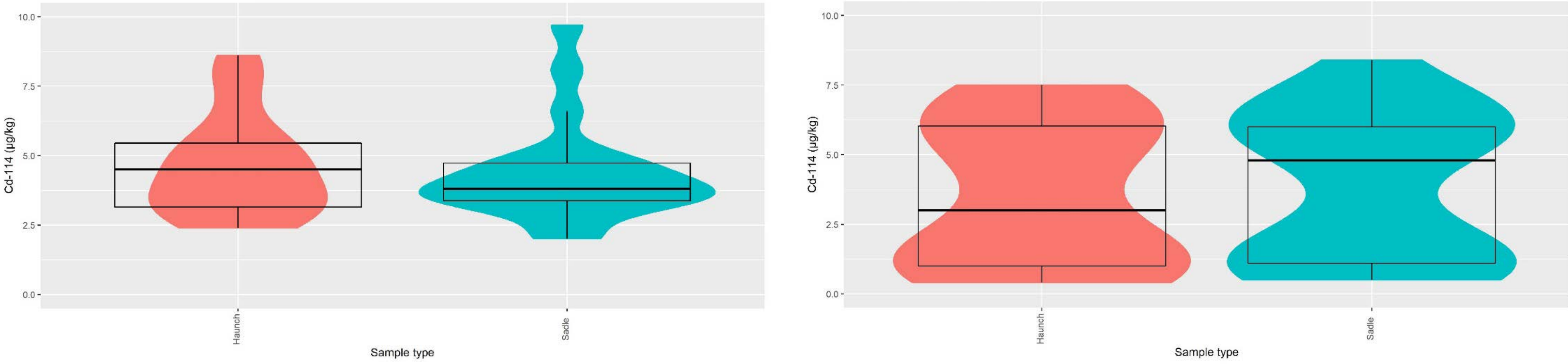


Figure 14. Comparison of cadmium concentrations in edible parts (200-g portions) of wild boar collected in Camp Roi Albert (left), and roe deer collected in GMU Vallei van de Zwarte Beek (right). The shape of the plot represents the estimated density of the data, which is combined with the box plot. The boxplot shows the median and the interquartile spacing.

7.1.1.4. Statistical analysis

Due to the exclusion criteria to conduct the model selection with sufficient data for each group within variables, as a result, a dataset of 283 samples for lead and 306 samples for cadmium could be used.

Using the backward model selection process, the null model was obtained as the best model, both for the lead as well as for the cadmium dataset (Table 6).

Table 6. Model selection process with corresponding AIC-values for cadmium and lead models.

Model	AIC - Cd	AIC - Pb
Pb/Cd ~Species + Region + Sample type + Sample type:Species + (1|Individual animal)	-2767.430	-230.2665
Pb/Cd ~Species + Region + Sample type + (1|Individual animal)	-2784.087	-235.8139
Pb/Cd ~ Region + Sample type + (1|Individual animal)	-2799.090	-243.8298
Pb/Cd ~ Region + (1|Individual animal)	-2816.496	-247.9149
Pb/Cd ~ 1 + (1|Individual animal)	-2829.835	-250.6150

With the null model being the best explanatory model for this dataset, no significant differences in lead or cadmium concentration between species, regions or sample types could be found.

Hence, the whole dataset of Pb concentrations is used in WP3 to estimate the Pb exposure due to the consumption of big game meat in Belgium. A description of the full dataset is given in Figure 15 for Pb and Figure 16 for Cd. The median Pb concentration is similar to the median concentration in other types of meat in Belgium, such as bovine or poultry meat, but the distribution was more skewed towards higher values (SciCom 2009a). The median and range in Cd concentrations are similar to those in other types of meat in Belgium (SciCom 2009b; Waegeneers et al. 2009).

	Pb (mg/kg) All samples
N	409
P25	0.006
P50	0.015
P75	0.057
P81	0.10
P91	1.0
P95	4.2
Max	1185

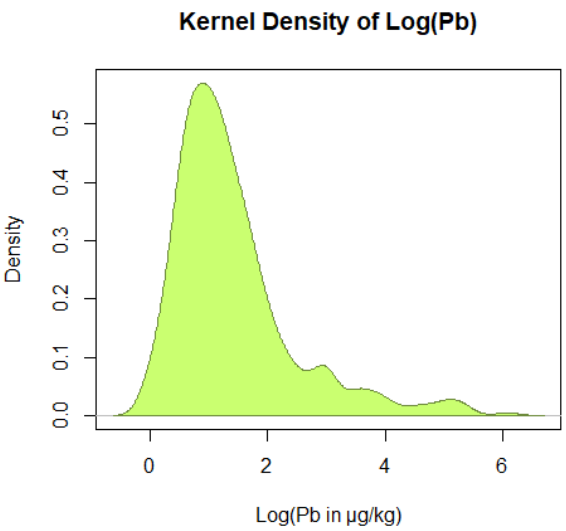


Figure 15. Percentiles and Kernel density plot of the lead concentrations measured in meat of big game hunted in Belgium.

	Cd (mg/kg) All samples
n	394
P25	0.001
P50	0.002
P75	0.004
P95	0.009
Max	0.037

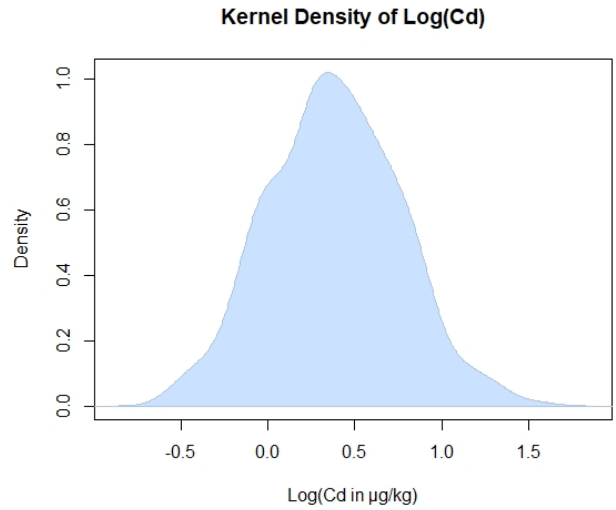


Figure 16. Percentiles and Kernel density plot of the cadmium concentrations measured in meat of big game hunted in Belgium.

7.1.2. Lead and cadmium concentrations in Belgian roe deer kidney

The south-eastern part of The Netherlands and the north-eastern part of Belgium are areas which are mainly enriched by zinc (Zn), Cd and Pb due to the historical presence of several zinc smelters. Until the 1970's a pyrolytic procedure was used for zinc refining, with temperatures in the blast furnaces up to 1300°C. Due to the lower boiling point of Cd, it was released by vaporization, which resulted in a diffuse contamination of the environment. Increased Cd levels have been found in garden soils (Nawrot et al. 2006), agricultural soils (Waegeneers et al. 2011) and forest litter (De Vos et al. 2006) in the Belgian part of this area, also known as the Noorderkempen. Furthermore, increased Cd levels have also been found in human blood and urine (Staessen et al. 1991) and bovine tissues (Waegeneers et al. 2009). Up till now it has not been investigated yet whether wildlife from the Noorderkempen also has an increased Cd burden. Higher kidney Cd levels have been found in voles, shrews and moles in the Dutch part of the Kempen area compared to a site 100 km away (Ma 1987; Ma et al. 1991). Information on Cd loads in big game mammals such as wild ungulates is, however, still missing from the Noorderkempen.

In a study on forest litter in different areas of the Noorderkempen, exceedance of critical concentrations for Cd and Zn were found in the region Balen-Overpelt (De Vos et al. 2006). To determine whether this increased Cd (and Pb) burden is reflected in big game mammals, roe deer kidneys were collected in different GMU in the Noorderkempen, including the GMU "Netebroek/Balen". The GMU "Meerdaalwoud", a forest area in central Belgium away from heavy metal point sources, was chosen a reference are. In WP2, roe deer and wild boar are collected from two GMU, "Vallei van de Zwarte Beek" and "Camp Roi Albert". As both areas are located in military domain, there could be an increased Pb burden. Therefore, roe deer kidneys from these areas were included as well.

Table 7 shows Cd and Pb concentrations that have been measured in the roe deer kidneys collected from the different wildlife areas in Flanders and Wallonia. A large variation in concentrations was observed within each GMU. The Cd concentrations in kidneys varied by a factor 20 to 50 (and even a factor 160 in the "Vallei van de Zwarte beek") and Pb concentrations varied by a factor 5 to 70 within the GMUs. The Cd concentrations were about two orders of magnitude larger than the Pb concentrations. It is long known that Cd accumulates in kidneys and that renal Cd concentrations are age-related (Andersen and Hovgård Hansen 1982; Neathery and Miller 1975).

In the original REE database (only GMUs of Flanders), where the age of the animals was available as a continuous variable, analysis of covariance showed that both the age of the animals and the GMU had a significant effect on the kidney Cd concentration ($P < 0.001$, after log-transformation of age and Cd concentration; Figure 17). The renal Cd concentration was thereby significantly higher in GMUs “De Vart” ($P < 0.001$), “Molenbeersel” ($P < 0.001$) and “Bosbeekvallei” ($P <0.05$) compared to GMU “Meerdaalwoud” ($P < 0.05$). GMU “Netebroek Balen” did not differ significantly compared to “Meerdaalwoud”. With regard to Pb, the variation in Pb concentrations was not due to differences in the age of the animals ($P > 0.05$; Figure 17), neither was there a significant difference in renal Pb concentrations between the different GMUs ($P > 0.05$).

In the extended REE database, including kidneys originating from Wallonia, the age of the animals was only known as two discrete classes, animals up to one year old and animals older than one. Two-way type III ANOVA revealed a significant difference between both age categories and between some GMUs for Cd (after log-transformation). There was no interaction effect between both variables. The mean log-transformed Cd concentration in kidneys was significantly lower for roe deer up to the age of one compared to older roe deer (Figure 18, left), and it was significantly higher in GMU “De Vart” compared to “Meerdaalwoud” and “Netebroek Balen” (Figure 19, top). The mean log-transformed Pb concentration in kidneys did not differ significantly between both age categories (Figure 18, right), but it was significantly lower in “Camp Roi Albert” compared to the other GMUs (Figure 19, bottom). Apparently, the Cd concentrations in roe deer kidneys shot in Netebroek Balen, and the Pb concentrations in roe deer kidneys shot in military domains, were not larger than in kidneys from other areas.

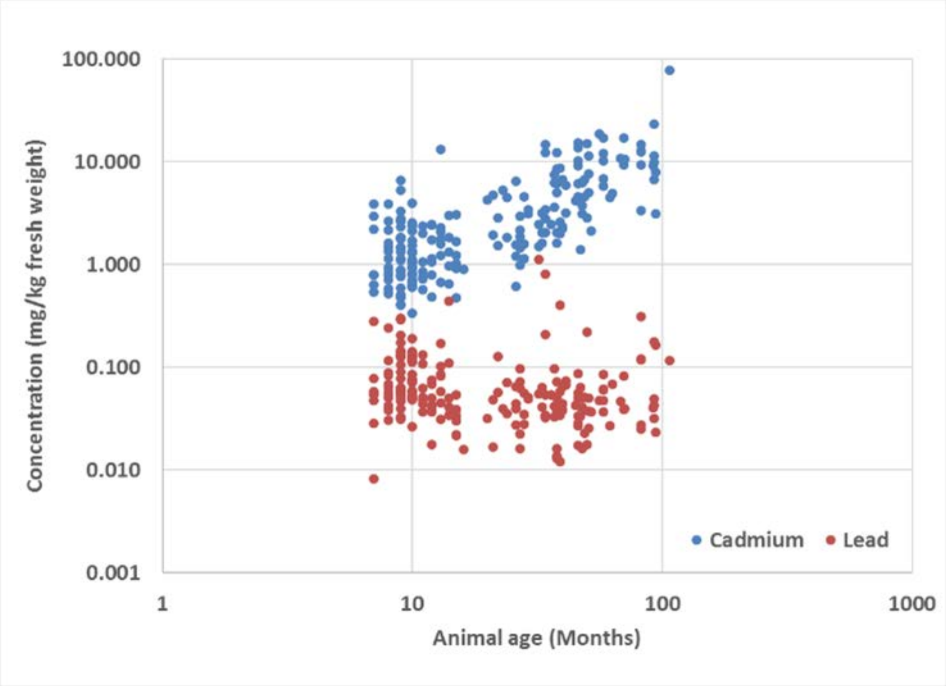


Figure 17. Cd (blue dots) and Pb (red dots) concentrations (mg/kg fw) in roe deer kidney as a function of the age of the animals (expressed in months). The kidneys are sampled in six game management units in Flanders (REE database).

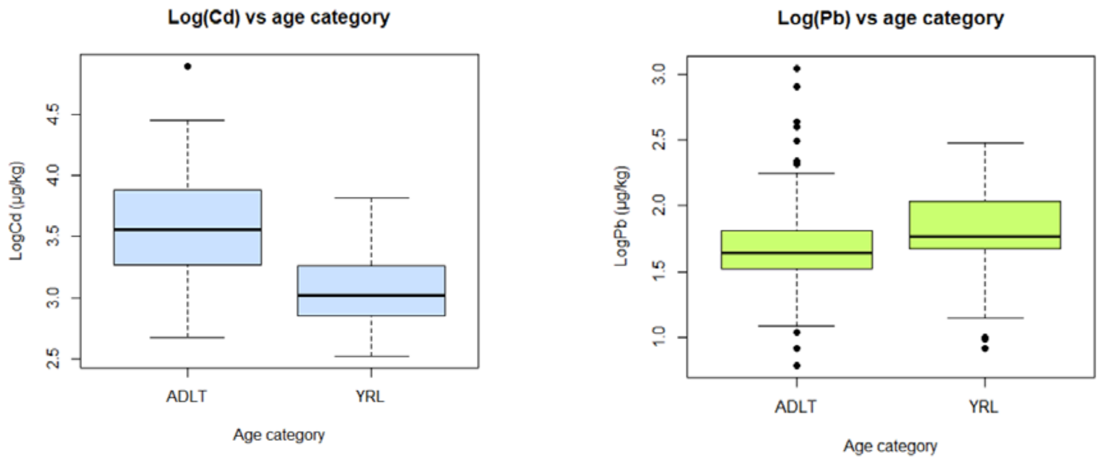


Figure 18. Box plots of log-transformed cadmium (left) and lead (right) concentrations as a function of the age categories "up to one year old" (YRL) and "older than one year" (ADLT). The boxes indicate the interquartile range, with the median as the thick black line, the top and bottom whiskers indicate the minimum and maximum values, while outliers are indicated by circles.

Table 7. Descriptive statistics of trace element concentrations in roe deer kidneys collected from different wildlife areas in Belgium. Concentrations are expressed on a wet weight basis.

		Cadmium			Lead		
GMU	**N**	**Median**	**Geometric mean**	**Range**	**Median**	**Geometric mean**	**Range**
Meerdaal Bos	44	2.0	2.0	0.34-13	0.056	0.054	0.025-0.18
Netebroek (Balen)	44	1.6	1.5	0.47-15	0.051	0.061	0.016-1.1
Vallei van de Zwarte Beek	44	2.4	2.7	0.50-78	0.050	0.055	0.008-0.31
Bosbeekvallei	28	2.7	2.7	0.65-17	0.051	0.056	0.016-0.81
Molenbeersel	20	2.3	3.2	0.71-18	0.049	0.050	0.012-0.40
De Vart	20	5.4	4.3	0.79-13	0.062	0.074	0.037-0.44
Hertogenwald	22	2.3	2.8	0.58-28	0.038	0.040	0.015-0.097
Camp Roi Albert	12	3.6	2.7	0.59-13	0.013	0.015	0.006-0.043
Saint-Michel Freÿr	8	3.0	2.4	0.36-14	0.064	0.055	0.023-0.12
All	242	2.2	2.4	0.34-78	0.050	0.052	0.006-1.1

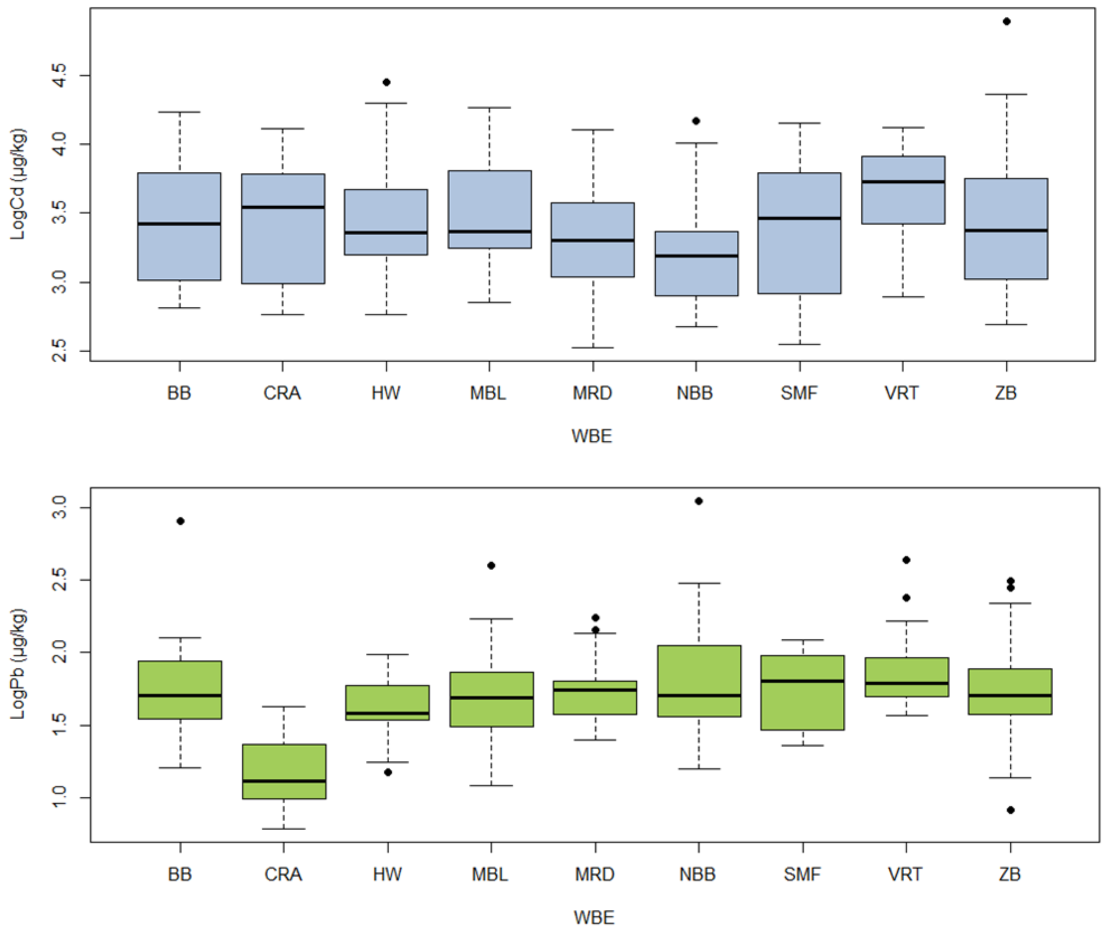


Figure 19. Box plots of log-transformed cadmium (top) and lead (bottom) concentrations as a function of the game management unit. BB = Bosbeekvallei, CRA = Camp Roi Albert, HW = Hertogenwald, MBL = Molenbeersel, MRD = Meerdaalwoud, NBB = Netebroek Balen, SMF = Saint-Michel Freÿr, VRT = De Vart, ZB = Vallei van de Zwarte Beek. The boxes indicate the interquartile range, with the median as the thick black line, the top and bottom whiskers indicate the minimum and maximum values, while outliers are indicated by circles.

7.1.3. Conclusions

Game meat

The median Pb concentration in big game meat was similar to the median Pb concentration in bovine and poultry meat. The distribution of the Pb concentrations was, however, more skewed towards higher values and some extreme high concentrations were observed. Still, in 81% of the samples, the Pb concentration was below 0.10 mg/kg, the EU maximum level for Pb in meat of bovine animals, sheep, pig and poultry. In 9% of the samples, the Pb concentration exceeded 1.0 mg/kg. None of the samples had Cd concentrations above 0.050 mg/kg, which is the EU maximum level for Cd in meat of bovine animals, sheep, pig and poultry No significant influence of animal species, meat sample type, or environmental exposure on the Pb and Cd concentration in meat could be demonstrated.

Roe deer kidney

The mean kidney Cd concentration depended on the age of the animals, and differed between some of the game management units. The mean Pb concentration in kidneys of roe deer did not depend on the age of the animals, but was significantly lower in Camp Roi Albert compared to the other game management units. Apparently, the Cd concentrations in roe deer kidneys shot in Netebroek Balen, and the Pb concentrations in roe deer kidneys shot in military domains, were not larger than in kidneys from other areas.

7.2. WP 2 – Lead content as a function of the distance to the wound channel

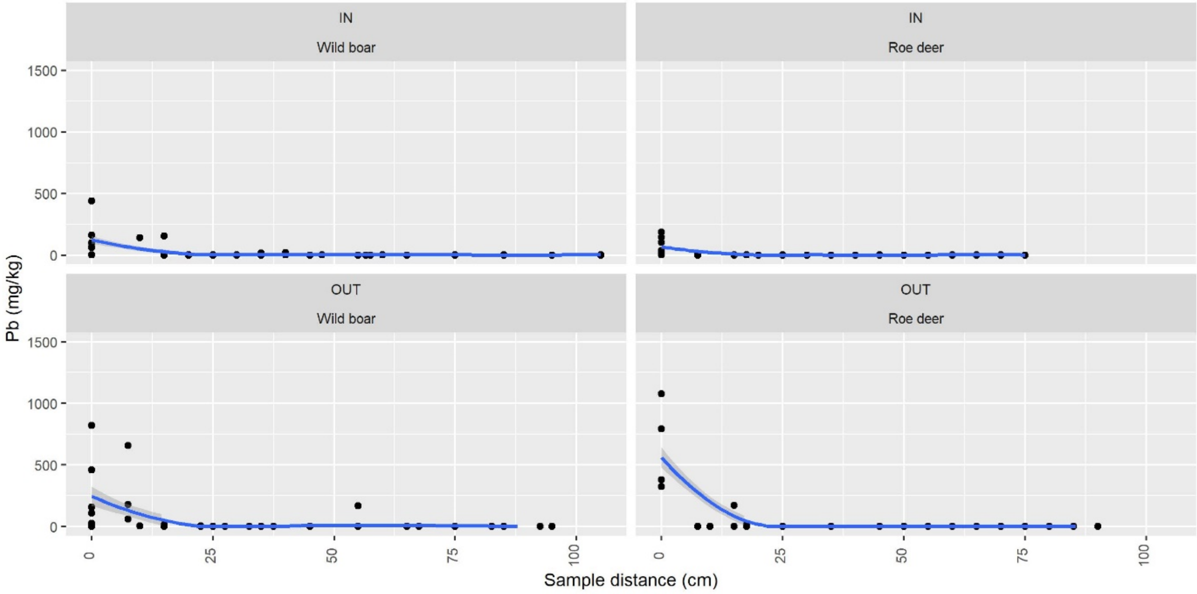
7.2.1. Results

Results of Pb concentrations detected in the samples at different distances to the wound channel, revealed high to extremely high values in the tissue surrounding the entrance and exit wounds. Concentrations ranged from 0.7 mg kg^{-1} to 13600 mg kg^{-1} in wild boar and from 1.4 to 3400 mg kg^{-1} in roe deer (Table 8). Maximum concentrations tended to be higher in the exit wounds compared to the entrance wounds (Figure 20). Differences in Pb concentration between the entrance and exit sides of the bullet have been reported earlier and have been linked to the place of maximal mushrooming of the bullet (higher concentrations close to location where maximum mushrooming occurs). Depending on tissue resistance and bullet construction this can be either close to the entrance or the exit wound (Dobrowolska and Melosik 2008).

A large drop in Pb concentrations was visible at larger distances from the wound channels at both entry and exit sides, however no clear trend could be observed with increasing distance (Figure 20). Since the tissue collected in the wound channel could not be considered as edible meat, these samples were discarded from the further statistical data analysis, which aimed to verify if such analysis could help to define a 'threshold' distance or 'cut off' distance for the 'safe' sampling of meat.

After division of the edible meat in the distinct distance classes (>0-25 cm, >25-50 cm, >50 cm) no significant difference in Pb concentration between the classes could be observed in wild boar (Kruskall Wallis test, $p=0.76$). In roe deer Pb-concentrations were significantly lower in the 25-50 cm class compared to the <0-25 cm class (Kruskall Wallis test $p= 0.0029$; adjusted $P = 0.0021$), but no significant differences were observed for the largest distance class compared to the other classes.

A.



B.

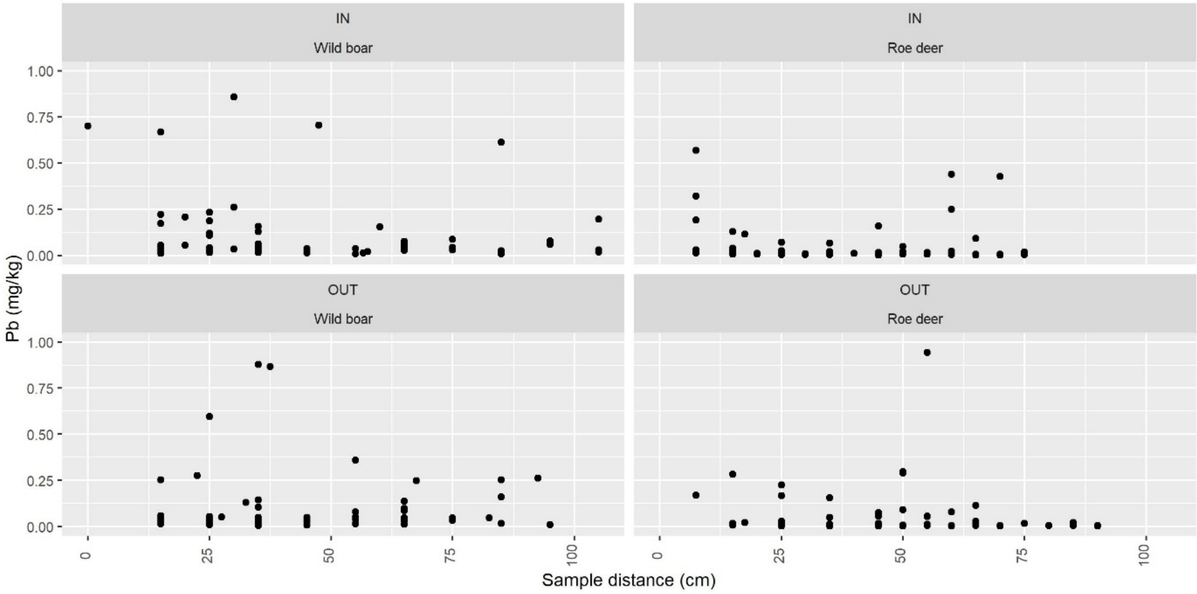


Figure 20. Figure x: Scatter plots of Pb concentrations in samples in the wound channel (0cm) and at increasing distance from the channel at two sides of the animal i.e. at the entrance side of the bullet (IN) and at the exit side (OUT). Only values < P75 are displayed. Partim B of the figure is zooming in on the lower Pb-concentrations (< 1 mg/kg).

For each of the distance classes, percentiles of the Pb concentrations are presented in Table 8. Despite the results of the statistical analysis, the table illustrates that for both animal species, in particular in the higher percentiles, the Pb concentrations tended to be the highest in the zone closest to the wound channel, compared to the values at larger distance. This implies that high Pb concentrations were found with a higher frequency close to the wound channel compared to at a larger distance. In both species about 15% of the samples in de 0-25 cm zone had Pb concentrations above 1 mg kg^{-1}, while this percentage was considerably lower at larger distance (8-9% in wild boar versus 1% in roe deer). The difference was the most pronounced in roe deer, which is in agreement with the significant difference detected for this species. Similarly, Kollander at al. (2014) found significant differences in lead content of samples taken from the wound channel versus samples taken further away in wild boar, but there was only a tendency for difference in lead content between the samples from 5-10 and 10-15 cm from the wound channel.

Nevertheless it can be concluded that it was not possible to define a ‘threshold’ distance or ‘cut off’ distance for the ‘safe’ sampling of meat. Even at small distance about 85% of samples are below the intervention value, and also at higher distance high concentrations can be observed. A similar conclusion was drawn by Dobrowolska and Melosik (2008), who stated that it is difficult to unambiguously determine how much tissue should be removed from around the bullet pathway because the animals differed in their lead contents found at various sites along the bullet pathway and with distance away from it.

Table 8. Percentiles of the Pb-concentrations in various ‘distance classes’ depending on the distance to the wound channel (=0 cm).

Wild boar	0 cm	>0-25 cm	>25-50 cm	>50 cm
N	15	37	36	45
Min	0.7	0.011	0.008	0.009
P25	14	0.028	0.027	0.032
P50	98	0.054	0.039	0.047
P75	300	0.234	0.169	0.155
P95	4700	40	4.8	1.6
Max	13600	176	18	166
≤1.0 mg/kg	(3%)	84%	92%	91%

Roe deer	0 cm	>0-25 cm	>25-50 cm	>50 cm
N	12	43	46	34
Min	1.4	0.003	<0.003	<0.003
P25	87	0.009	0.005	0.005
P50	283	0.022	0.009	0.011
P75	1250	0.217	0.015	0.027
P95	2900	1.9	0.258	0.491
Max	3400	172	1.6	1.1
≤1.0 mg/kg	-	85%	99%	99%

7.2.2. Conclusions

The results of WP2 illustrate that Pb concentrations vary at various distances from the wound channel in the animal but do not show a clear trend in function of the distance. Therefore no ‘threshold’ distance’ for the guaranteed ‘safe’ sampling of meat for consumption could be defined. A ‘random’ variability in Pb concentrations was also illustrated in WP1 by analysing three subsamples of one 200 g-sample, resulting in very different results.

Both observations suggest that establishing specific Pb limits would not be an adequate option regarding managing measures aiming to limit Pb intake from big game meat, as Pb concentrations within the same animal may vary widely. Moreover official controls would not be very effective since a large amount of big game meat is consumed directly by hunters and their families.

Meat from the shot wound is not fit for consumption.

7.3. WP 3 – Dietary intake assessment and risk evaluation

7.2.3. Consumption of big game meat by specific consumers

Literature data demonstrate that game meat consumption among consumers of this type of meat, varies by a factor of four approximately. In a report by the Scottish Food Safety Authority, a consumption of 11.7 g per day is reported for those in the general population who reported eating game, based on data from the National Diet and Nutrition Survey (NDNS) (FSA 2012). It was estimated that high level consumers (hunters) would eat an average of 47 g daily. According to Morales et al. (2011), Andalusian hunters (Spain), consuming both red deer and wild boar, consumed on average 23 g/day of this meat. The 95th percentile consumption was 97 g/day. According to the National Survey of Spanish Dietary Intake conducted by the Spanish Agency for Food Safety and Nutrition (AESAN), the average game meat consumption by consumers of large game was 46 g/day (AESAN 2012). Haldimann et al. (2002) estimated a daily game meat intake of approximately 50 g for hunters, based on a consumption of on average 2.2 servings per week (range 0.3-6 servings/week) during the hunting season. In its Scientific Opinion on Lead in Food, EFSA assumed a daily consumption of 28 g game meat to test the impact of game meat consumption on the dietary Pb exposure (EFSA 2010). This portion size corresponded to a weekly serving of 200 g. The same serving size of 200 g was used in the German research project “Safety of game meat obtained through hunting” and combined with a consumption frequency of 1-5 servings/year (normal to high female consumers) or 2-10 servings/year (normal to high male consumers) (BfR 2014). For consumers of hunters’ households, up to 90 servings/year were assumed. In an exposure assessment for the consumers of big game meat in Croatia, servings of 150 g were combined with consumption frequencies of once a week, once a month or four times a year (Lazarus et al. 2014). Lindboe et al. (2012) used moderate servings of 2 g meat/kg bodyweight, corresponding to a serving of 150 g meat for a 75-kg weighing adult, in their Pb exposure calculations for Norwegian moose eaters. During qualitative interviews in the Scottish FSA study mentioned above, 200 g serving sizes were deemed appropriate, although from a commercial perspective, serving sizes larger than 125 g per person were considered too expensive for supermarket customers (FSA 2012). During the quantitative phase of the research, the majority of respondents (which were persons consuming game meat at least once a week) thought that the serving size of wild game meat was the same as the serving size of other meats. According to the Belgian Food Consumption Survey of 2014-2015, the average usual intake of meat (all type of meats) and meat products is 140 g/day for adult males and 89-93 g/day for adult females (Lebacq 2016). The 95th percentiles go up to 273 g/day for males and 155 g/day for females.

The most practical approach to estimate the impact of eating big game meat on the Pb exposure of hunters and their relatives, is using different consumption scenarios. In the current study it was chosen to work with different consumption frequencies (ranging from 2 servings/year to 3 servings/week) and a fixed serving size of 2.3 g meat/kg bodyweight. The latter corresponds to a serving size of 193 g for adult males (mean weight Belgian adult males = 83.8 kg), 162 g for adult females (mean weight Belgian adult females = 70.4 kg), and 69 g for a 20-kg child. At a consumption frequency of 2 servings per year (as a measure for the general population consuming large game meat), the average daily game meat consumption corresponds to 0.9 to 1.1 g/day for adults females and males respectively. At a consumption frequency of 3 servings per week, the average daily game meat consumption corresponds to 69 to 82 g/day for adults females and males respectively. An overview of daily game meat consumption corresponding to different consumption frequency scenarios, is given in Table 9. The scenarios are focussed on adults aged 18-64 years and children aged 6-17 years. This corresponds to the age categories used in the Belgian Food Consumption Survey (Cuypers et al. 2015). In a study of the Scottish Food Safety Authority on the habits and behaviours of high level consumers of wild game meat in Scotland, a drop in game meat consumption levels was observed for children under 5 years old (-23%) and for adults aged 65-74 years (-18%) (FSA 2012).

Table 9. Applied daily big game meat consumption by adults and children for different consumption frequency scenarios.

Consumption frequency	Servings/year	Game meat consumption/serving g/kg$_{bw}$/d	Average daily game meat consumption Males g/day	Females g/day	20-kg child g/day
2/year	2	2.3	1.1	0.9	0.3
6/year	6	2.3	1.8	1.5	0.4
1/month	12	2.3	6	5	1.5
1/two weeks	26	2.3	14	12	3.3
1/week	52	2.3	27	23	6.6
2/week	104	2.3	55	46	13
3/week	156	2.3	82	69	20

7.2.4. Lead exposure through the consumption of big game meat by specific consumers

The median Pb concentration of the meat samples analysed in WP1 was 0.015 mg/kg (see section 7.1.1.4). This value was used in the deterministic approach to calculate the Pb exposure in relation to the game meat consumption frequency.

Due to the small difference between the median Pb concentration in large game meat and the Pb concentration in other types of meat (sheep, pig, poultry and bovine meat: median Pb concentration = 0.012 mg/kg), differences in total Pb exposure (including game meat consumption) were small compared to the background Pb exposure, even at an extreme high consumption rate of one game meat serving per day (Table 10). The median Pb concentration in big game meat, does however, not reflect the large variability observed in Pb concentrations in big game meat, and although the occurrence of (extreme) large Pb concentrations in big game meat is not abundant (88% of Pb concentrations ≤ 1.0 mg/kg), the more big game meat is consumed, the larger the probability of consuming game meat with (extreme) large Pb concentrations. A probabilistic approach seems therefore more reliable to estimate the Pb exposure for those consuming big game meat on a regular basis.

The probabilistic approach allowed the calculation of different percentiles of Pb exposure (median, 95th percentile) in a population of persons consuming game meat at a certain frequency (Table 11). In contrast to the calculations performed following a deterministic approach, the probabilistic approach demonstrates an increasing exposure to Pb with increasing consumption frequency. The results given in Table 11 are those for non-smoking adults, but at a consumption frequency of two game meat serving per week (or higher), the total Pb exposure for smoking adults is the same as for non-smoking adults (median total Pb exposure = 1.5 µg/kgbw/day; 95th percentile total Pb exposure = 1.7 µg/kgbw/day).

To express the results as “added Pb exposure”, the Pb exposure calculated for the general population (cfr paragraph 6.3.2.) was subtracted again. The median added exposure, based on the probabilistic approach in the current study, is in the range from 0.03 to 2 µg/kgbw/day, depending on the consumption frequency, while according to the deterministic approach, it is in the range from 0.01 to 0.03 µg/kgbw/day. Unfortunately, 95th percentile total exposure data were not available for the general population.

Table 10. Total exposure to lead (in µg/kg$_{bw}$/day for different consumption frequencies of big game meat, for people living or working in different environments. The exposure to lead was calculated following a deterministic approach.

	Rural environment			City environment			Industrial environment	
Consumption frequency	**Adult (non-smoker/smoker)**	**Women of childbearing age**	**Children**	**Adult (non-smoker/smoker)**	**Women of childbearing age**	**Children**	**Adult (non-smoker/smoker)**	**Women of childbearing age**
General population*	0.19/0.28	0.19	0.65	0.24/0.32	0.24	1.18	0.37/0.45	0.37
2/year	0.19/0.28	0.19	0.65	0.24/0.32	0.24	1.18	0.37/0.45	0.37
6/year	0.19/0.28	0.19	0.65	0.24/0.32	0.24	1.18	0.37/0.45	0.37
1/month	0.20/0.28	0.20	0.65	0.24/0.32	0.24	1.18	0.37/0.45	0.37
1/two weeks	0.20/0.28	0.20	0.65	0.24/0.32	0.24	1.18	0.37/0.45	0.37
1/week	0.20/0.28	0.20	0.65	0.24/0.32	0.24	1.18	0.37/0.46	0.37
2/week	0.20/0.28	0.20	0.65	0.24/0.33	0.24	1.19	0.38/0.46	0.38
3/week	0.20/0.29	0.20	0.66	0.25/0.33	0.25	1.19	0.38/0.46	0.38
7/week	0.22/0.30	0.22	0.67	0.26/0.34	0.26	1.20	0.39/0.48	0.39

* Calculated by (SciCom 2011)

Table 11. Total exposure to lead (in µg/kg$_{bw}$/day) for different consumption frequencies of big game meat, for non-smoking adults living or working in different environments. The exposure to lead was calculated following a probabilistic approach.

	Median total Pb exposure (µg/kg$_{bw}$/day)			95th percentile total Pb exposure (µg/kg$_{bw}$/day)		
Consumption frequency	**Rural environment**	**City environment**	**Industrial environment**	**Rural environment**	**City environment**	**Industrial environment**
General population*	0.19	0.24	0.37	-	-	-
2/year	0.22	0.26	0.39	0.25	0.29	0.43
6/year	0.26	0.31	0.44	0.32	0.37	0.50
1/month	0.34	0.39	0.52	0.42	0.47	0.60
1/two weeks	0.53	0.56	0.70	0.63	0.66	0.81
1/week	0.90	0.90	1.0	1.0	1.0	1.2
2/week	1.5	1.6	1.7	1.7	1.8	1.9
3/week	2.2	2.2	2.4	2.4	2.5	2.6

* Calculated by (SciCom 2011) following a deterministic approach.

The number of studies in which Pb exposure due to the consumption of (big) game meat is calculated, is limited. The Spanish Agency for Food Safety and Nutrition estimated the daily Pb intake due to game meat consumption at 0.8-1.12 µg/kg_{bw}/day (AESAN 2012). This estimation was based on weighted mean Pb occurrence data in deer, wild boar and partridge, a daily serving of 50 g game meat and a 60-kg adult (deterministic approach). This consumption rate corresponds to almost 3 servings/week for an adult male in the current study.

In Germany, Pb exposure was calculated as well by a deterministic approach for different consumption scenarios (BfR 2010). For adult males consuming two 200-g servings of game meat per year, the total Pb exposure was estimated at 0.52 or 0.59 µg/kg_{bw}/day when respectively the median and mean Pb concentrations in game meat were used (calculated as Pb exposure from the general diet, 0.52 µg/kg_{bw}/day, plus additional Pb exposure through consumption of game meat). For male hunters, a consumption of 91 200-g servings of game meat per year were assumed, resulting in a total Pb exposure of 0.53 or 3.9 µg/kg_{bw}/day based on respectively the median and mean Pb concentrations in game meat. The assumed consumption frequency for hunters corresponds to approximately 2 serving/week for an adult male in the current study.

In Croatia, Pb exposure through the consumption of game meat was calculated for three consumption scenarios: rare consumption (2.88 g game meat/week, corresponding to approximately 1 game meat serving per year for an adult male in the current study), regular consumption (37.5 g game meat/week, corresponding to approximately 1 game meat serving per month for an adult male in the current study), and frequent consumption (150 g game meat/week, corresponding to almost 1 game meat serving per week for an adult male in the current study). The Pb exposure was expressed as “added” exposure and calculated according to a deterministic approach based on mean Pb concentrations in game meat. Based on these assumptions, the added Pb exposures were 0.001, 0.014 and 0.071 µg/kg_{bw}/day respectively.

Morales et al. (2011) estimated added Pb exposure through the consumption of red deer and wild boar meat both in a deterministic (geometric mean consumption x geometric mean concentration) and a probabilistic (modelled consumption & concentration) way(Morales et al. 2011). The mean and median additional exposure in the probabilistic assessment was estimated at respectively 0.127 and 0.027 µg/kg_{bw}/day (recalculated from a per week basis), while the mean additional Pb exposure in the deterministic assessment was estimated at 0.043 µg/kg_{bw}/day based on geometric mean data.

Lindboe et al. (2012) estimated the added Pb exposure related to the consumption of moose meat for Norwegian hunters by means of a probabilistic approach(Lindboe et al. 2012). Estimations were based on a meat consumption of 2 g/kg_{bw}/day and calculated for one to five servings per week. Using Monte Carlo simulation, the median predicted daily intake was 1.7 µg/kg_{bw}/day for one serving/week and 3.6 µg/kg_{bw}/day for a consumption of two servings/week

The added Pb exposure due to the consumption of big game meat and calculated by deterministic approaches in several international studies, correspond to the deterministic approach applied in the current study, which is in the range 0.01-0.03 µg/kg_{bw}/day when expressed as added Pb exposure, except for the AESAN study (AESAN 2012). However, in the latter study the Pb concentration in meat of partridge (hunted with Pb shot) was included as well. In the German study, the background dietary Pb exposure (0.52 µg/kg_{bw}/day) is larger than in the current study (0.19-0.45 µg/kg_{bw}/day), but when expressed as added Pb exposure, the deterministic calculation based on the median Pb concentration in large game meat, gives similar results as the current study (BfR 2010). Several studies or organisations significantly overestimate, however, the additional Pb exposure due to game meat consumption, when applying the deterministic approach, as pointed out by (Fustinoni et al. 2017). EFSA, for example, estimated the additional Pb intake from game meat consumption in European consumers at 0.54 µg/kg$_{bw}$/day, based on an average Pb concentration of 3.15 mg/kg in game meat (EFSA 2010). The latter value was largely influenced by a few samples with very high concentrations. The median Pb concentration was only 0.02 mg/kg, which is similar as in the current study.

The deterministic approach does not take into account the large variability in Pb concentrations that is often observed in big game meat. While the probability of the consumption of big game meat with very high Pb concentrations is low, it may happen multiple times in a lifetime, depending on the consumption frequency of big game meat. The probabilistic approach takes this probability into account. There is a large difference in the probabilistic Pb exposure estimations between the studies of Morales et al. (2011), Lindboe et al. (2012) and the current study. In the study of Morales et al. (2011) the consumption data were modelled as well, hence is not possible to allocate a certain exposure scenario that corresponds to the estimated intake values. The exposure data calculated by Lindboe et al. are about twice as high as in the current study, but are estimated for moose meat, in which the Pb concentrations appear to be higher than in game meat shot in Belgium.

7.2.5. Risk evaluation of lead intake through the consumption of big game meat

Margin of exposure values (MOE) were calculated both for the deterministic and probabilistic exposure calculations and for the different BMDL values (Table 2 of section 6.3.3).

MOE’s calculated based on the deterministic approach are given in Table 12 to Table 16. Given the small differences in Pb exposure compared to the general population, no additional risks due to the consumption of big game meat can be demonstrated by means of the deterministic approach, even at an extreme consumption frequency of one serving per day.

The MOE values for children are all below 1, whether or not they consume game meat, which is in accordance with other international studies (e.g. (BfR 2014, 2010; EFSA 2010)). As a result, it has been recommended in some studies that children under 6 or 7 years of age should avoid eating the meat of big game that has been shot with Pb ammunition (AESAN 2012; BfR 2011; Bjerselius et al. 2014). Due to the fact that the MOE for children is already below 1 in the general population, the probabilistic approach was not applied on children in families of hunters as the conclusions will remain the same.

Table 12. Margin of exposure values(MOE) based on developmental neurotoxicity effect endpoints for children living in rural or city environments and consuming large game meat at different consumption frequencies. Total Pb exposure values are calculated according to a deterministic approach.

			MOE for Developmental neurotoxicity (decrease in IQ)			
			EFSA, 2010		JECFA, 2011	
Environment	**Consumption frequency**	**Total Pb exposure (µg/kg_{bw}/day)**	**1%**	**0.5%**	**1%**	**3%**
Rural	**General population***	0.65	0.8	0.5	0.9	2.9
Rural	**1 portion/year**	0.65	0.8	0.5	0.9	2.9
Rural	**1 portion/day**	0.67	0.7	0.4	0.9	2.8
City	**General population***	1.18	0.4	0.3	0.5	1.6
City	**1 portion/year**	1.18	0.4	0.3	0.5	1.6
City	**1 portion/day**	1.20	0.4	0.2	0.5	1.6

* Total Pb exposure calculated by (SciCom 2011) following a deterministic approach.

Table 13. Margin of exposure values based on cardiovascular effect endpoints for non-smoking adults living or working in rural, city or industrial environments and consuming large game meat at different consumption frequencies. Total Pb exposure values are calculated according to a deterministic approach.

			MOE for Cardiovascular effect (increase in systolic blood pressure)	
			EFSA, 2010	JECFA, 2011
Environment	**Consumption frequency**	**Total Pb exposure (µg/kg_{bw}/day)**	**1% change**	**1 mm Hg increase**
Rural	**General population***	0.19	8	7
Rural	**1 portion/year**	0.19	8	7
Rural	**1 portion/day**	0.22	7	6
City	**General population***	0.24	6	5
City	**1 portion/year**	0.24	6	5
City	**1 portion/day**	0.26	6	5
Industrial	**General population***	0.37	4	4
Industrial	**1 portion/year**	0.37	4	4
Industrial	**1 portion/day**	0.39	4	3

* Total Pb exposure calculated by (SciCom 2011) following a deterministic approach.

Table 14. Margin of exposure values based on cardiovascular effect endpoints for smoking adults living or working in rural, city or industrial environments and consuming large game meat at different consumption frequencies. Total Pb exposure values are calculated according to a deterministic approach.

			MOE for Cardiovascular effect (increase in systolic blood pressure)	
			EFSA, 2010	JECFA, 2011
Environment	**Consumption frequency**	**Total Pb exposure (µg/kg$_{bw}$/day)**	**1% change**	**1 mm Hg increase**
Rural	**General population***	0.28	5	5
Rural	**1 portion/year**	0.28	5	5
Rural	**1 portion/day**	0.30	5	4
City	**General population***	0.32	5	4
City	**1 portion/year**	0.32	5	4
City	**1 portion/day**	0.34	4	4
Industrial	**General population***	0.46	3	3
Industrial	**1 portion/year**	0.45	3	3
Industrial	**1 portion/day**	0.48	3	3

* Total Pb exposure calculated by (SciCom 2011) following a deterministic approach.

Table 15. Margin of exposure values based on developmental neurotoxicity effect endpoints for women of childbearing age living or working in rural, city or industrial environments and consuming large game meat at different consumption frequencies. Total Pb exposure values are calculated according to a deterministic approach.

			MOE for Developmental neurotoxicity (decrease in IQ)			
			EFSA, 2010		JECFA, 2011	
Environment	**Consumption frequency**	**Total Pb exposure (µg/kg_{bw}/day)**	**1%**	**0.5%**	**1%**	**3%**
Rural	**General population***	0.19	2.6	1.6	3.2	10
Rural	**1 portion/year**	0.19	2.6	1.5	3.1	9.8
Rural	**1 portion/day**	0.22	2.3	1.4	2.8	8.8
City	**General population***	0.24	2.1	1.3	2.5	7.9
City	**1 portion/year**	0.24	2.1	1.3	2.5	7.9
City	**1 portion/day**	0.26	1.9	1.2	2.3	7.3
Industrial	**General population***	0.37	1.4	0.8	1.6	5.1
Industrial	**1 portion/year**	0.37	1.3	0.8	1.6	5.1
Industrial	**1 portion/day**	0.39	1.3	0.8	1.5	4.8

* Total Pb exposure calculated by (SciCom 2011) following a deterministic approach.

Table 16. Margin of exposure values based on cardiovascular effect endpoints for smoking adults living or working in rural, city or industrial environments and consuming large game meat at different consumption frequencies. Total Pb exposure values are calculated according to a deterministic approach.

Environment	Consumption frequency	Total Pb exposure ($\mu g/kg_{bw}/day$)		MOE for Nephrotoxicity effect (increase in systolic blood pressure) EFSA, 2010 10% increased prevalence	
		Non-smokers	Smokers	Non-smokers	Smokers
Rural	**General population***	0.19	0.28	3.3	2.3
Rural	**1 portion/year**	0.19	0.28	3.2	2.3
Rural	**1 portion/day**	0.22	0.30	2.9	2.1
City	**General population***	0.24	0.32	2.6	2.0
City	**1 portion/year**	0.24	0.32	2.6	2.0
City	**1 portion/day**	0.26	0.34	2.4	1.8
Industrial	**General population***	0.37	0.46	1.7	1.4
Industrial	**1 portion/year**	0.37	0.45	1.7	1.4
Industrial	**1 portion/day**	0.39	0.48	1.6	1.3

* Total Pb exposure calculated by (SciCom 2011) following a deterministic approach.

Figure 21 - Figure 24 represent the median and 95th percentile total Pb exposure of non-smoking adults (including women of childbearing age) living or working in rural, city or industrial environments together with the different BMDL values derived by EFSA and JECFA (EFSA 2010; JECFA 2011). JECFA and EFSA agreed that the neurodevelopmental effects were pivotal data in the assessment for children (including foetuses). For adults, JECFA concluded that the pivotal data were those of the Pb-associated increase in systolic blood pressure, as this was associated with the lowest blood Pb concentrations and the greatest and most consistent weight of evidence. They did therefore not derive BMDL values for nephrotoxicity as EFSA did (JECFA 2011).

For adults, the median total Pb exposure estimates range from 0.22 to 2.6 µg/kg_{bw}/day, depending on the consumption frequency and environmental background exposure. At the higher end of the range, corresponding to 2 or more servings per week, the MOE for cardiovascular effects drops below 1, and the possibility of cardiovascular effects at the population level cannot be excluded. For the more controversial nephrotoxicity effects, the MOE drops below 1 at two or more servings per month. JECFA and EFSA affirmed that because of the neurodevelopmental effects, foetuses, infants and children are subgroups that are most sensitive to Pb. For women of childbearing age, the median total Pb exposure exceeds the BMDL value corresponding to a 1% population decrease in IQ that was derived for children, at two or more servings per month. In areas with a high Pb burden (industrial areas), this BMDL is exceeded at a consumption rate of more than 6 servings/year for 5% of the population (Figure 23). At three or more servings per week, the estimated median exposure is higher than the level associated with a population decrease of 3% in IQ, which is deemed to be a concern by JECFA (Figure 24). For similar reasons, it is recommended in some other studies, that small children, pregnant women or women planning to have children should avoid consumption of big game meat shot with Pb-containing bullets (AESAN 2012; BfR 2011; Bjerselius et al. 2014). It should be noted that a lifetime consumption frequency of more than two game meat servings per week is elevated and could be considered as a worst case scenario (see also Table 9 in section 7.2.3).

Whether or not the consumption of big game meat shot with Pb-containing bullets increases blood Pb levels, and hence increases the possibility of any risks, is still a question of debate. Haldimann et al. (2002) studied the effect of frequent game meat consumption on the blood Pb levels of active hunters (and their household members) in Switzerland and of a control group. They found no correlation between the individual game meat frequency data and the corresponding blood Pb levels, and concluded that the frequent consumption of wild game meat has no significant effect on blood Pb levels. Fustinoni et al. (2017) corroborated this conclusion in their study of Italian consumers of game meat. They found that blood Pb was not influenced by consumption of game meat (among other variables), but that there was an association with hunting, with blood Pb almost double in hunters, and with wine drinking. It was, thereby, not clear if the higher blood Pb levels in hunters were due to inhalation of Pb fumes during shooting, handling of Pb ammunition, or both. In a study among the general Swedish adult population, higher blood-Pb levels were observed among those adults consuming more game according to a food frequency questionnaire (Bjermo et al. 2013). Meltzer et al. (2013) investigated whether high consumption of cervid meat, shot with Pb containing ammunition, was associated with increased blood Pb levels. The study showed that regular consumption of cervid meat (once a month or more) was associated with approximately 31% higher blood Pb concentrations than never or rare consumption. The increase seemed to be mostly associated with the consumption of minced cervid meat. Hunters who assembled their own ammunition had 52% higher blood Pb concentrations than persons not making ammunition. Tsuji et al. (2008) investigated the source of Pb exposure in First Nations people of Canada, and identified Pb ammunition (both lead shotshell pellets and bullets as no distinction between both could be made) as a source of Pb exposure.

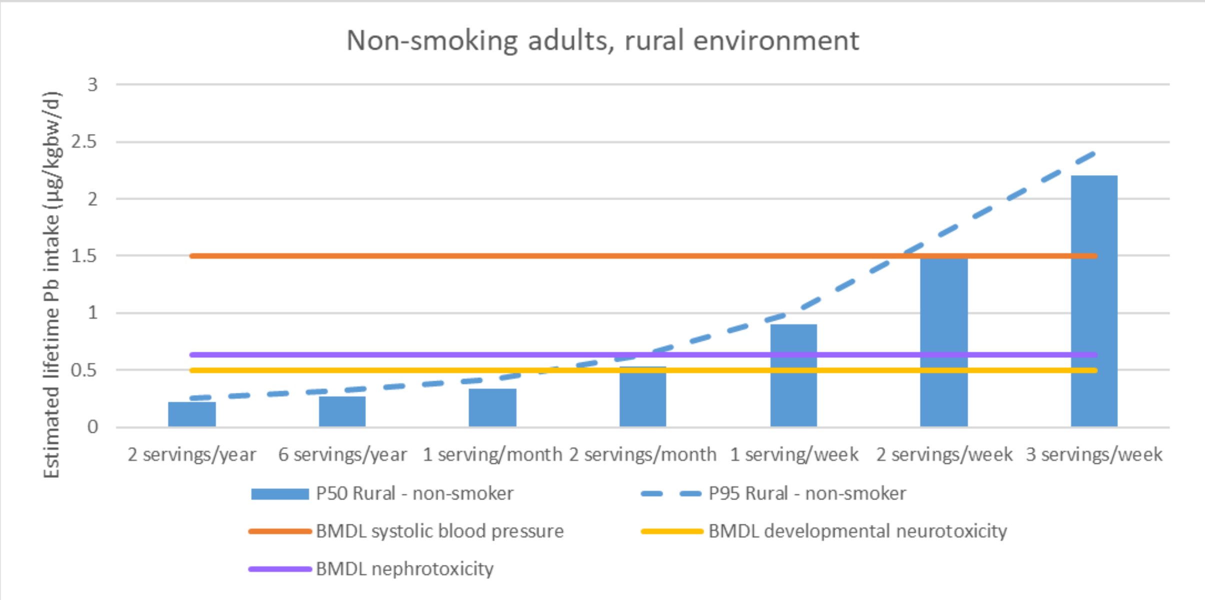


Figure 21. Estimated exposure to lead for non-smoking adults, living or working in a rural environment, as a function of big game meat consumption frequency.

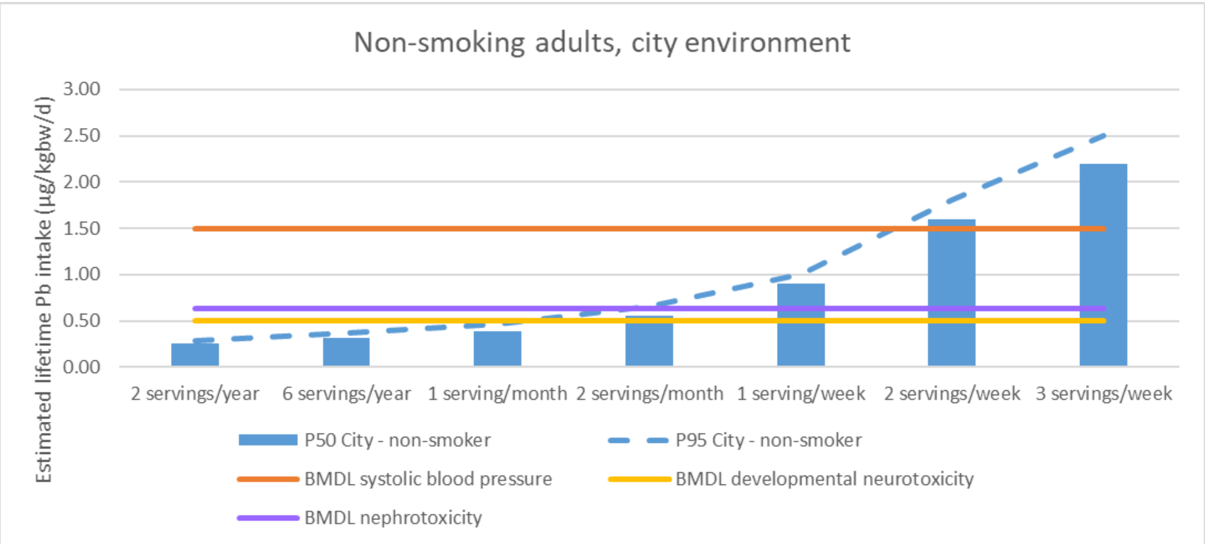


Figure 22. Estimated exposure to lead for non-smoking adults, living or working in a city environment, as a function of big game meat consumption frequency.

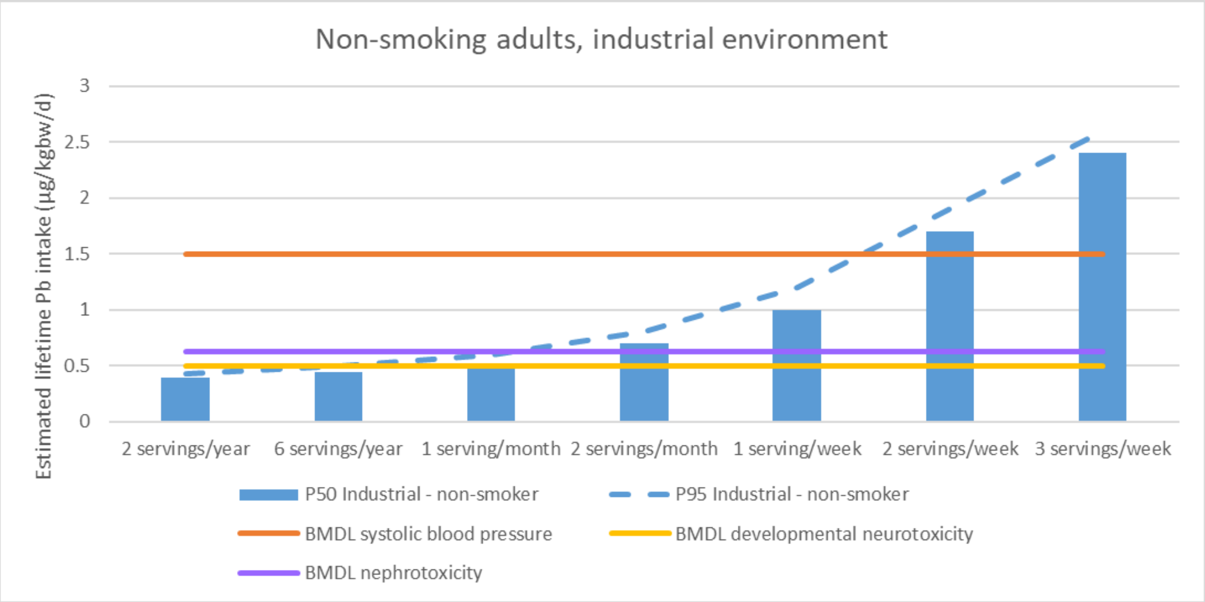


Figure 23. Estimated exposure to lead for non-smoking adults, living or working in an industrial environment, as a function of big game meat consumption frequency.

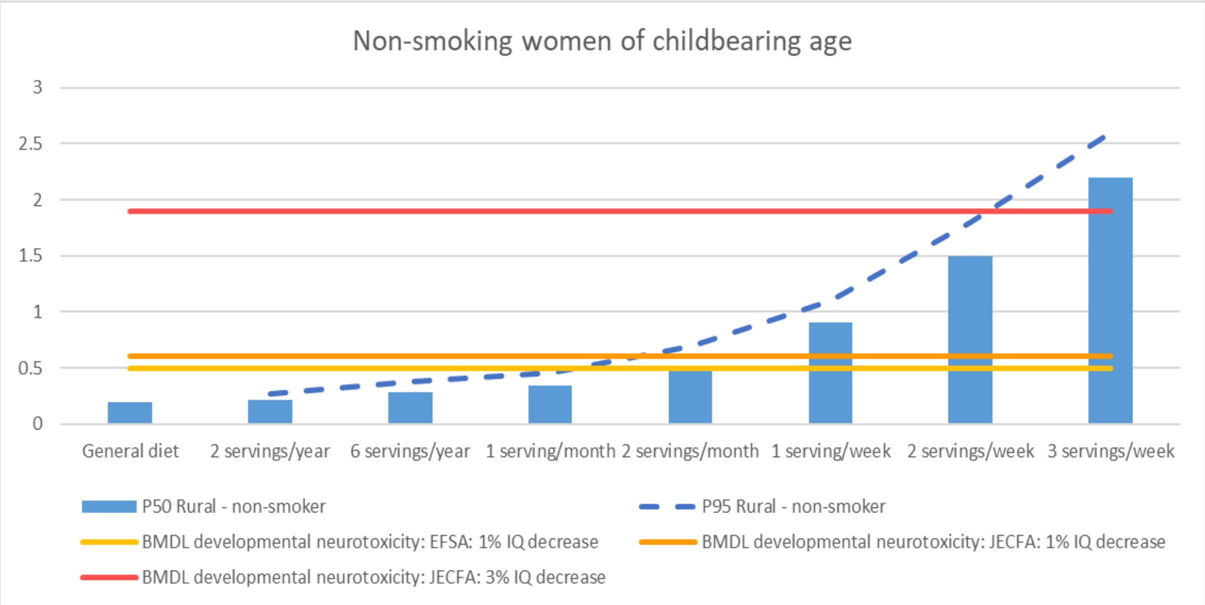


Figure 24. Estimated exposure to lead for non-smoking women of childbearing age, living or working in a rural environment, as a function of big game meat consumption frequency.

7.2.6. Conclusions

Dietary Pb intake calculations following the deterministic approach appeared not to provide realistic exposure results because the median Pb concentration in large game meat is similar to the median Pb concentration in other types of meat (bovine meat, poultry meat) and does not reflect the large variation in Pb concentrations found.

The probability that persons who eat big game meat a few times a year, consume a portion with elevated Pb content, is small. The Pb exposure that would occur in such an event does not involve any increased risk.

Because of neurodevelopmental effects, foetuses, infants and children are subgroups that are most sensitive to Pb. Margins of exposure are below 1 for children, whether or not they consume game meat. Therefore, small children could be recommended to avoid eating big game meat shot with Pb containing ammunition.

Pregnant women and women of childbearing age who plan on getting pregnant, could be recommended to avoid eating big game meat shot with Pb containing ammunition or limit their consumption of this type of meat, depending on the desired MOE.

The selection of the desired value of the MOE for women of childbearing age as well as for other adults, is a choice to be made by risk management authorities.

Whether or not the consumption of big game meat shot with Pb-containing bullets increases blood Pb levels, and hence increases the possibility of any risks, is still a question of debate. Additional research focusing on the relation between the consumption of big game meat and blood Pb levels is recommended before formulating consumption advices for the general adult population.

7.4. WP 4 – Data comparison with international literature and policy recommendations

7.3.1. Regulations

7.3.1.1. International

Overwhelming scientific evidence has shown that lead from ammunition poses a health hazard for wildlife, ecosystems and humans (Bellinger et al. 2013; Gremse et al. 2014; Kanstrup et al. 2016b). The toxicity of lead as a heavy metal has been shown to poison scavenger birds that eat animals' remains after being shot with lead ammunition (Church et al. 2006; Nadjafzadeh et al. 2015, 2013; Tavernier et al. 2004). Moreover, there is a growing concern about the health risk to people who frequently eat game meat shot with lead-containing bullets (Pain et al. 2010).

The ECHA (European Chemicals Agency) published a report in which they recommend regulations towards the use of lead ammunition in terrestrial environments due to the health risk for hunters and people who frequently consume game meat (ECHA 2018). However, there are no European countries which have currently banned the use of lead-based ammunition for all hunting purposes on a national level (Thomas et al. 2016).

In Germany, lead-based hunting ammunition affecting the mortality of raptors attracted considerable political attention and questioned the use of lead-based bullets. Moreover, the result of the scientific evidence on the risk to human and wildlife health increased the pressure to search for alternatives. Although the German federal hunting act does not specify material composition of bullets, 14 of 16 states do not allow the use of lead shot in wetlands and water bodies for hunting water birds. Moreover, in three German states, a total ban is established on lead-based ammunition (Gremse and Rieger 2015). In the UK, in 1999, regulations on lead shot were introduced to protect waterfowl from lead poisoning (Cromie et al. 2010). Local (in certain protected areas) or national bans on lead shot in wetlands and/or waterfowl also exists in the Netherlands, Denmark, Norway, Cyprus, Hungary, Latvia, Finland, Spain, Switzerland, France and Sweden (Cromie et al. 2010). In California, USA, a partial ban on lead-based ammunition has been implemented to protect predatory and scavenging birds such as the critically endangered California condor (*Gymnogyps californianus*) (Finkelstein et al. 2012; Kelly et al. 2011).

7.3.1.2. National

Since 2005, a partial ban on use of lead shot is implemented in Belgium. In Flanders a total ban was implemented in 2008, in the Walloon region only restrictions on the use of lead shot for hunting in wetlands was enforced in 2005 (Cromie et al. 2010; Jachtvoorwaardenbesluit 2014). At the moment there are no restrictions on the use of lead-based bullets in Flanders nor in the Walloon region (Arrêté du Gouvernement wallon 2005; Jachtvoorwaardenbesluit 2014).

7.3.2. Bullet characteristics

7.3.2.1. General

The ballistic behaviour of a bullet can be divided in four parts: internal ballistics (study of bullet inside the barrel), intermediate ballistics (behaviour of bullet as it leaves the barrel), exterior ballistics (behaviour between barrel and target) and terminal ballistics (penetration of bullet in an animal) (Caudell 2013). These ballistics are determined by the characteristics of a bullet as well as the match between the bullet and the riffle, all influencing the different ballistics parts.

Internal ballistics focuses on the twist inside a barrel. A barrel is grooved in the inner wall to guide a particular twist designed to stabilize a bullet in flight (Caudell et al. 2012). The accuracy of a rifle bullet (defined by Thomas et al. (2016) as the technical ability of the rifle in combination with the actual cartridge to achieve a consistent hitting point independently of the shooters' skills) depends on bullet length, state of the rifle barrel, the pressure and speed of burning powder, the bullet velocity and how the bullet is led through the barrel. Longer bullets demand a faster twist in the barrel of a rifle to ensure sufficient stabilization to reach a high accuracy (Thomas et al. 2016). A mismatch between bullet length and twist rate of the barrel can lead to reduced precision (Caudell et al. 2012).

Exterior ballistics are defined by the weight of the bullet and the distance to the animal. For example: heavier bullets are applied to shoot at long range as they retain more speed and energy and are thus less affected by wind (Caudell et al. 2012).

Terminal ballistics are important in determining the killing ability of a bullet. The lethality of a bullet is determined by the location where the bullets hits an animal, the strike-angle, the size of the animal and the amount of energy transferred from the bullet to the animal hit, which is determined by the bullet weight and construction, its velocity and the shooting distance (Gremse and Rieger 2015; Martin et al. 2017). The ability to deposit a bullets' energy and to increase cavity volume are important in relation to the killing efficiency of a bullet: an increased energy deposition of bullets upon hitting the animal reduces escape distances (Gremse et al. 2014). Permanent damage caused by a (fragmenting) bullet within the animal occurs when the tissue or organ which is hit, moves further than the elastic properties of the tissue causing strains, tears or fractures (Caudell 2013). Most energy of a bullet is being released upon impact (Gremse et al. 2014). The cavity volume upon impacting an animal cannot be predicted only from the deposited energy but depends also on the bullet type and organs hit. A bullet can tumble, expand or fragment causing different shapes of cavities (Gremse et al. 2014). Extreme forces on a bullet can result in fragmentation, creating numerous small fragments (Cruz-Martinez et al. 2015; Hunt et al. 2006). Expansion, deformation and/or fragmentation is regarded to be the determining characteristic of a bullet. The number of fragments increases significantly with increasing deposited energy and are mostly located close to the cavity due to the small size of fragments (Gremse et al. 2014). However, lead particles were found up to a maximum of 45 cm from the primary wound tract (Grund et al. 2010).

7.3.2.2. The use of lead for hunting ammunition

Lead is relatively cheap, easy to extract and to be shaped into bullets. Moreover, the high density of lead enables bullets to retain their kinetic energy when shooting. In addition, the softness of lead results in a deformation, expansion and/or fragmentation when hitting an animal (Kanstrup et al. 2016a). These characteristics make lead a highly suitable material taking into account preferred internal, external and terminal ballistics for a bullet.

However, due to the negative effect of lead poisoning on humans, wildlife and the environment, non-lead bullets are more frequently proposed as alternative. There are numerous types of non-lead bullets available on the market with copper-based bullets as the most widely used alternative (Caudell et al. 2012; Thomas 2013). Also brass (an alloy of copper and zinc) composition is possible (Gremse et al. 2014).

7.3.3. Comparing lead and non-lead bullets for hunting ungulate game species

7.3.3.1. Introduction

When considering enforcing regulations on the use of non-lead ammunition as an alternative because of human health or environmental lead contamination, aspects such as game management effectiveness, animal welfare, possible safety risks and the willingness to use alternative ammunition by the hunters should be taken into account (Arnemo et al. 2016; Friend et al. 2009; Schlichting et al. 2017; Thomas et al. 2016). Restrictions for lead-based ammunition are affected by complicated political processes and reflect the challenge of harmonizing conservational and political goals (Finkelstein et al. 2012). European and US hunters' concerns about non-lead bullets involve perceptions of availability, costs, efficacy, accuracy, toxicity, and barrel fouling (Kanstrup et al. 2016a; Thomas et al. 2016). These concerns are politically powerful and, if not addressed, could thwart greater use of non-lead ammunition (Cromie et al. 2010; Thomas et al. 2016). Effective mitigation of lead-based ammunition will require a clear understanding of technical, economic and social dimensions of the problems (Epps 2014).

Many studies have been undertaken to evaluate whether non-lead rifle ammunition fulfils the demands of ethical and humane hunting of ungulate species by causing a rapid kill of hunted animals equivalent to lead rifle ammunition (Kanstrup et al. 2016b). A survey among German hunters revealed that almost 70% are willing to change towards non-lead ammunition when the appropriateness of these bullets for their use in hunting activities in the field is clearly proven (Zielschank und Schuck-Weris in Hackländer et al. (2015). Epps (2014) states that "efforts to change hunter behaviour must recognize the true costs and challenges of changing to non-lead ammunition. Likewise, hunters should recognize and accept their important role in wildlife conservation and work to embrace effective alternatives to lead as they become available". Moreover, Seltenhammer et al. (2011) argue that the implementation of lead-free ammunition should be supported by the hunters community as part of their objective of reducing all possible negative impacts of hunting as guarantee for an ethical hunting that will be accepted by society. This argument was already used in 1992 in the USA by Sparrowe (in Friend et. al. (2009)): "Deposition of lead into the environment is still being used by major anti-hunting groups in the USA to argue against hunting. Removal of that argument is a big plus for retaining the social, economic, cultural and recreational values of hunting.

Different opinions exist on the mandatory nature of the transition towards non-lead based ammunition. Some argue that legal enforcing will speed up the availability of high-performance non-lead alternatives for all calibres at comparable prices to current lead-based bullets (Thomas 2015). Others argue that sensitization and stimulation allowing the depletion of current stocks, the adaptation of riffles as well as time for practice and outreach are the better way to phase-out lead-based ammunition. This would also allow further research to improve non-lead based alternatives for all possible situations (Hackländer et al. 2015).

Many of the above mentioned issues also played a role in the transition from lead shot to non-lead alternatives for hunting waterfowl and other small game species, so many lessons can be learned from the past. It became evident that societal aspects of such a transition are as important as the biological components and will have to be adequately addressed before non-lead bullets will be accepted as an investment in wildlife conservation (Friend et al. 2009). As in many other decision making processes related to nature management there is the need to clearly and unambiguously stipulate all objectives, the desired outcomes, the possible alternatives and to study the consequences of each of these alternatives in order to make defensible decisions (Gregory et al. 2012; Hammond et al. 2002; Knutson et al. 2010; Runge et al. 2013). To ensure that objectives are clear and unambiguous they should always be formulated using terms such as maximizing and minimizing, or other formulations clearly indicating the desired direction (Casaer and Huysentruyt 2017).

Within the decision framework of the transition towards non-lead bullets for ungulate hunting, the final objective of the transition is to minimize the amount of lead contamination in game meat for human consumption as well as lead contamination of the environment, due to bullets used. However at the same time other objectives should be taken into account when making this decision. The total list of objectives involved in the choice of ammunition is – regardless of their importance and consequently the attributed weight in the decision:

i) Minimizing health risk due consumption of venison shot
ii) Minimizing environmental lead contamination
iii) Minimizing the risk of environmental contamination by alternative ammunition
iv) Minimizing human safety risks
v) Maximizing hunting efficiency
   (a) Maximizing shot accuracy
   (b) Minimizing the search time for wounded animals
      1. Maximizing clear marks when an animal is hit
      2. Minimizing escape distances
vi) Minimizing costs for hunters
vii) Minimizing animal suffering: Minimizing time between hit and death of the animal

Gremse and Rieger (2014) mention 7 very similar criteria that can be used to evaluate and compare the appropriateness of different types of bullets for ungulate hunting:

i) What is the price of the bullet ?
ii) Is the bullet suitable to be used in a certain rifle type ?
iii) Is the bullet commonly available on the market ?
iv) What is the impact of the bullet on the possibility to use/sell the game meat ?
v) Is the bullet resulting in a quick death of the animal shot ?
vi) Does the bullet results in short escape distances distance once the animal is hit ?
vii) Does the bullet results in clear marks such as blood, flesh, etc. when the animal is hit ?

The first 4 points mentioned by Gremse and Rieger (2014) are not related to the killing effect of the bullet but do relate to the ease of use and the acceptance of the bullet by hunters. The last 3 are related both the appropriateness of the bullet for the use in ungulate hunting and to animal welfare. An element not mentioned in this list by Gremse and Rieger (2014), but mentioned often in connection to the use of non-lead ammunition is the safety of the use – mainly in relation to the risk of ricochet (rebounding of a bullet). Several of the above mentioned criteria can be analysed based on available data (points 1 & 3) or experimental lab research (points 2 & 4). However, points 5 till 7 require field data, sometimes combined with test data. The time between the shot and the death of the animal can in many cases not be measured in the field by the hunters themselves. However the escape distance (point 6) is a variable that can easily be measured and reported by the hunters in the field during hunting activities. The escape distance also can be used as a proxy for the time between hit and death of the animal shot (point 5). For this reason, this variable is often used to compare the killing effectiveness of different bullet types under different circumstances in the field and in relation to other variables such as shooting distance, shot placement, species, age class and body mass of the animal (Bahr 2014; Hackländer et al. 2015; Kanstrup et al. 2016a; Martin et al. 2017). Other studies use the wound diameters as a proxy for the bullet's killing potential (Trinogga et al. 2013).

An overview of these issues, scientific results of tests regarding these aspects and considerations taken into account in other countries are discussed in this chapter.

7.3.3.2. Price, availability, certain rifle compatibility and impact on venison of bullet types

In comparison to lead bullets which are regarded to be cheap, concerns on availability and prices of lead-free bullets have been raised. However Knott et al. (2009) and Thomas (2013) have shown that copper bullets are 6.3% less expensive than lead bullets in the UK and they report these prices will probably decline further with increasing economic importance and an increase in the availability of copper-based lead-free ammunition. Regulation has been pointed out to be the most important factor establishing availability which in term affects prices (Thomas 2015). Epps (2014) draw the attention on the large variation in bullet types that can be used for hunting activity and the need to recognize this variation in outreach programs towards non-lead ammunition. Epps (2014) suggests to conduct a survey to get a better view on variation in local use of bullet types. An assessment of current market prices in Belgium for some calibres currently used for ungulate hunting, revealed that, when comparing lead-containing and non-lead bullets from the same manufacturer and calibre, the non-lead ammunition are still more expensive (Figure 25). However, there are lead-free bullets for most bullet calibres frequently used for ungulate hunting at a price per bullet that does not differ from lead-based bullets. Moreover, the difference per bullet is limited and, contrary to shotgun-ammunition off which hunters use large amounts, the number of bullets used per hunter is limited. E.g. in the German field study (Gremse and Rieger 2012) the average large game bag per person per year ranged between 3.2 and 11.2 animals.

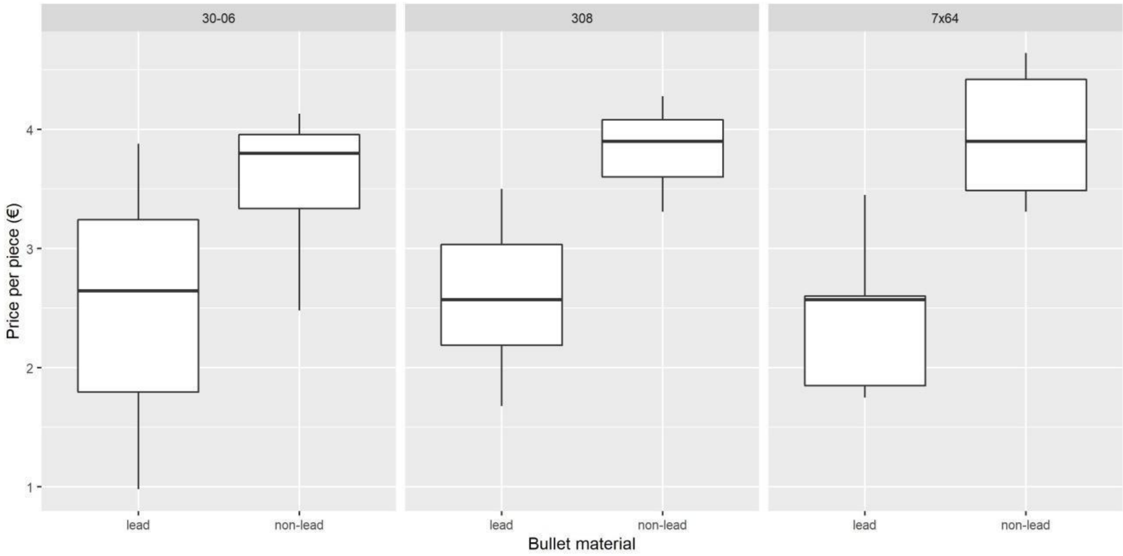


Figure 25. Bullet prices (Belgium – based on internet search) per calibre (30-06, 308, 7x64) for lead-based and lead-free bullets.

The availability of non-lead bullets for ungulate hunting is in general no problem in Belgium/Europe. One of the exceptions is however the small number of available non-lead alternatives for the smallest calibres that can be used for roe deer hunting. In non-lead bullets both fragmenting, partial fragmenting as well as deforming bullets exists. Given that the impact of the bullet on the venison and consequently the possible use or marketing of the venison depends largely on the fact whether a fragmenting or deforming bullet is used, the hunter can make this choice equally for non-lead as for lead-bullets. This allows the hunter to make a choice that has the desired effect on the venison.

Non-lead bullets typically do not fragment as much as lead-contained bullets (Grund et al. 2010). Non-lead bullets are now made in both non-fragmenting and fragmenting types (Thomas et al. 2016). Non-fragmenting lead-free bullets are designed to expand, typically double in diameter and form a mushroom shape (Kanstrup et al. 2016a). Fragmenting lead-free bullets are designed to fragment creating adjacent tissue damage to the wound channel behaving more like lead-core bullets (Kanstrup et al. 2016a). In a study comparing the number of fragments of both copper expanding bullets and lead expanding bullets up to more than 200 fragments were found in animals killed with lead bullets and a maximum of 6 fragments in animals killed with the copper bullets (Hunt et al. 2006). Copper fragments are however generally larger in size compared to lead fragments and penetrate deeper into the tissue (Gremse et al. 2014). Fragments that are released upon hitting an animal, spread throughout the body and cause an increased amount of wounded tissue (Caudell 2013; Gremse et al. 2014). This affects the marketing potential of game meat. Moreover, due to the high amount and potentially very small size of bullet fragments, not all fragments can be removed from the shot animal thereby posing a risk of ingestion. It is therefore suggested that deforming non-fragmenting bullets can be used to prevent ingestion of bullet fragments (Nadjafzadeh et al. 2015) and a reduction of damage to game meat.

The choice to use a certain bullet in a given rifle influences the accuracy and thereby killing efficacy in hunting praxis. Copper has a density of 8.96 g/cm3 while the density of lead is 11.0 g/cm3. This results in lead-core bullets having a density that is 20% larger than copper bullets

(Thomas et al. 2016). For a particular rifle calibre bullet mass, non-lead bullets are thus longer. The consequences of a longer bullet can be counteracted by reducing bullet weight. However, this results in the need for a higher velocity to achieve the same ballistic effect as a similar lead-core bullet and to reach the needed striking power (Thomas 2013). These deficiencies can be bypassed with the development of new specific equipment or decreased shooting ranges (Caudell et al. 2012). Most of the factors (weight and length of the bullet, twist in the rifle barrel) are equally impacting the accuracy of lead-core bullets. Given the large availability of both lead-core and non-lead bullets, professional advice on bullet-type, rifle barrel and testing the grouping of the rifle-bullet combination are necessary – but easy and logic – steps to take when changing from lead-core to non-lead bullets (Hackländer et al. 2015; Thomas et al. 2016).

Repeated firing of copper-jacketed bullets, leaves more residue, also called barrel fouling, in the barrel compared to lead-bullet resulting in the need for a more frequent barrel cleaning (Thomas et al. 2016). However, given the low number of bullets used on a typical hunting day in Europe and the classical maintenance of hunting rifles, extensive barrel fouling is an avoidable issue.

The non-toxicity of ingested copper originating from copper-based bullets for humans is scientifically well supported (Caudell et al. 2012; Thomas et al. 2016). Copper is an essential trace element for humans and is tolerated in low amounts. Also for wildlife, copper does not pose a health risk as illustrated by Franson et al. (2012) who showed that copper levels in blood of raptors did not rise when orally administered in the form of pellets, and no clinical signs could be observed. Although copper fragments were found to increase copper levels in the muscle of a shot animal, the physical hazard of detected fragments is considered to be low because of the low number of fragments in comparison to lead bullets (Irschik et al. 2013). Moreover, the amount of copper ingested per portion of meat shot with fragmenting copper-based bullets is unlikely to exceed the daily recommended intake when fragments are removed before preparation of the meat (Irschik et al. 2013). However, different preparation/heating methods can affect the amount of copper released from the bullet or bullet fragments and therefore attention should be given to methods used to prepare game meat (Schuhmann-Irschik et al. 2015).

7.3.3.3. Killing effectiveness

The killing effectiveness of bullets plays a key role in ungulate hunting. It influences both the animal welfare dimension (short suffering of animals shot) and the possibility to market the venison (all animals hit are found in time to allow the consumption of the meat). Moreover it also influences the efficiency of hunting given that more time is required for searching animals that do not die on the spot or at close distance.

Energy deposition
As mentioned above, the impact of a bullet on the animal – and therefore its killing effect – depends on the amount of energy transferred from to bullet to the body of the animal when it is hit. This depends partly on the weight and velocity of the bullet. Velocity can be inferred from tables describing muzzle velocity/distance relationships. The amount of energy that transferred to the animal’s body and the killing effect however also differs by other parameters such as calibre and bullet type (fragmenting/deforming) and could be influenced by the material used (lead-core bullets versus non-lead bullets). This impact at a certain point in the body of the animal can be simulated and measured using ballistic soap, in which the permanent cavity

formed by the bullet as well as the number and distance of fragments can be measured under controlled, standardized and repeatable circumstances (Gremse et al. 2014). The research of Gremse et al. (2014) reports comparable ballistic behaviour of lead-free bullets compared to lead-core bullets except for the lower fragmentation. Gremse et al. (2014) conclude that "deforming lead-free bullets closely resemble deforming lead-based bullets considering energy conversion, deflection angle, cavity shape and reproducibility", thereby showing that similar terminal ballistic behaviour can be achieved. Given that the ability of a bullet to transfer sufficient energy upon impacting the animal is the key factor determining killing efficiency, Gremse et al. (2014) suggest that this bullet specific characteristic in relation to distance and muzzle velocity should be made available on ammunition packaging.

Escape distance

The information derived from tests using ballistic soap however only gives information on the energy transfer and fragmentation of the bullet under controlled circumstances without the ability to include parameters such as bullet placement (where was the animal hit), animal characteristics (species, sex, age, body weight), shooting distance and type of hunting (stalking, drive hunt, …). All these parameters, in combination with the bullet type used, are believed to have an impact during hunting activities on whether the animals dies quickly, the escape distance and the marks found in the field that guide searching for wounded animals.

The escape distance is a variable that easily can be measured and documented in the field by the hunter (Gremse and Rieger 2012). As mentioned above this measure can also be used as a proxy for the time between the moment that the animal is hit and the moment of death (Kanstrup et al. 2016a). Rephrased to the issue of non-lead bullets the scientific research question therefore becomes: is the escape distance significantly greater when non-lead bullets are used compared to lead-core bullets. To answer this question field data during hunting have to be collected.

Gremse and Rieger (2012) collected data from almost 12.000 ungulates shot in Germany and combined this information with results of lab tests of the projectiles using ballistic soap . Their results showed no difference in escape distances between lead-core and non-lead ammunition when the minimum terminal ballistic requirements were met. They found that in those field conditions that equal an energy transfer from a bullet greater than 1500 Joule in the first 15 centimetres of a ballistic soap under lab conditions, the average escape distance is less than 30 meters. This study established for the first time a link between lab tests concerning impact of bullets and field observations determining the killing effectiveness.

The research of Knott et al. (2009) during red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) control operations in Scotland did not find a difference in copper bullet accuracy and killing power compared to traditional lead bullets under field conditions.

Similar conclusions were drawn by Hackländer et al. (2015). They found that when analysing escape distances from 1231 hunted animals of various species in Austria taking 55 potential variables in account the bullet material did not appear to influence escape distance significantly.

Research in Denmark comparing the escape distance of roe deer and red deer using both lead and non-lead bullets, showed no difference in performance in terms of lethality and animal welfare (Kanstrup et al. 2016a). This result was consistent over a range of different bullet brands, calibers and bullet types. Only at shooting distances above 100 m, escape distances for roe deer increased for lead-free bullets.

Trinogga et al. (2013), who conducted a structural analysis of wound channels of shot animals in Germany, showed that wound diameter did not differ between lead-core and lead-free bullets.

Moreover, similar injury patterns demonstrated that lead-free bullets are equal to lead-core bullets in determining killing effectiveness and thus meet animal welfare requirements for hunting.

The study of Martin et al. (2017) on 1254 (745 shot with lead bullets, 509 with non-lead bullets) roe deer and 845 (514 shot with lead bullets, 340 with non-lead bullets) wild boar in Germany found no difference in escape distances based on bullet materials. The escape distance was found to depend on shot placement, shooting distance, hunting method or age of animals. Bullet composition was not found to have a significant impact.

7.3.4. Conclusions

Following conclusions and recommendations can be drawn regarding the transition from lead to non-lead bullets for hunting ungulates, and this in relation to the consumption of the venison of these animals:

- There are **sufficient non-lead alternatives** available on the market in Europe to allow a transition of lead-based towards non-lead bullets for hunting ungulate species in Europe. Nevertheless, for some exceptional cases, the current non-lead alternatives are not yet satisfactory. For the smallest calibres that can be used for roe deer hunting, the number of non-lead alternatives is, however, limited.
- Research indicates that key factors **influencing escape distance** (as a proxy for killing efficiency and thereby animal welfare and hunting efficiency) are shot placement, shooting distance and animal characteristics. **Bullet material showed no significant effect.**
- The **ability of a bullet to transfer sufficient energy upon impacting the animal** is the key factor determining killing efficiency. This energy is not only related to the bullet weight, distance and muzzle velocity , but depends also on the bullet construction. This information is currently missing on ammunition packages and should, given its importance, be made available to the hunters.
- **Barrel fouling, accuracy, impact on venison, toxicity and costs** are all non-negligible concerns that could inhibit the acceptance of non-lead ammunition by the hunting community. Hunters' concerns over these aspects are justified but are not specific for the transition from lead-based toward non-lead ammunition. They are typical for each change in bullet type or brand hunters make.
- Given the elements above, investing **in outreach and stakeholder communication** are key elements for a successful transition of lead-based ammunition towards non-lead bullets for hunting ungulate species.
- Different opinions exist on the **mandatory nature of the transition** towards non-lead based ammunition for hunting ungulate species. Where some argue that legal enforcing will speed up the availability of high-performance non-lead alternatives at comparable prices others argue that a smooth transition, sensitization and stimulation allowing the depletion of current stocks and information and training of hunters, will increase the support for this transition.

8. Conclusions and recommendations

In the context of a potential health risk due to the presence of Pb in big game meat shot with Pb containing ammunition, the research questions can be answered as follows:

What is the lead (Pb) and cadmium (Cd) content in edible meat and in kidneys of big game shot in Belgium?

The median lead and cadmium concentrations in edible big game meat shot in Belgium are similar as the median lead and cadmium concentrations in other types of meat. The distribution of the lead concentrations is, however, more skewed towards higher values, with 9% of the samples having a lead concentration above 1.0 mg/kg. No significant influence of animal species or meat sample type on the lead and cadmium concentration in meat could be demonstrated.

The cadmium concentration in kidneys of roe deer shot in Belgium are related to the age of the animals and can reach high values. The lead concentrations are not age-dependent and much lower than the cadmium concentrations.

What is the influence of environmental exposure on the Pb and Cd content in Belgian big game meat and kidneys?

No significant influence of environmental exposure on the lead and cadmium concentration in edible big game meat shot in Belgium could be demonstrated.

The cadmium concentration in kidneys of roe deer shot in Belgium was significantly higher in some, but not all, game management units in the Noorderkempen compared to a reference area away from known heavy metal point sources. The lead concentration in roe deer kidneys shot in military domains, was not larger than in kidneys from other areas.

What is the influence of the distance from the wound channel on the Pb content in Belgian big game meat?

Lead concentrations vary at various distances from the wound channel in the animal but do not show a clear trend in function of the distance. Therefore no ‘threshold’ distance’ for the guaranteed ‘safe’ sampling of meat for consumption could be defined. Meat from the wound channel is not fit for consumption.

What is the dietary intake of Pb through the consumption of Belgian big game meat by hunters and their relatives?

The dietary (and overall) lead exposure depends on the frequency of game meat consumption. There is an increase in lead exposure with increasing frequency of game meat consumption.

Are there risks related to the intake of Pb through the consumption of Belgian big game meat for these consumers?

The probability that persons who eat big game meat a few times a year, consume a portion with elevated Pb content is small. The Pb exposure that would occur in such an event does not involve any increased risk.

Margins of exposure are below 1 for children, whether or not they consume game meat.

The possibility of an effect cannot be excluded at the population level for adults consuming **2 or more** game meat servings **per week**, for women of childbearing age consuming **2 or more** game meat servings **per month** living in a rural or city environment, and for 5% of women of childbearing age spending a lot of time in an industrial environment who consume **6 or more** game meat servings **per year**.

Which recommendations concerning the consumption of big game meat and the use of non-lead ammunition can be formulated based on the results of this study and experiences in the matter in other countries.

Because of neurodevelopmental effects, foetuses, infants and children are subgroups that are most sensitive to Pb. Margins of exposure are below 1 for children, whether or not they consume game meat. Therefore, **small children** could be recommended to avoid eating big game meat shot with Pb containing ammunition.

Pregnant women and women of childbearing age who plan on getting pregnant, could be recommended to avoid eating big game meat shot with Pb containing ammunition or limit their consumption of this type of meat, depending on the desired MOE. The selection of the desired value of the margin of exposure for women of childbearing age as well as for other adults, is a choice to be made by risk management authorities.

Different opinions exist on the **mandatory nature of the transition** towards non-lead based ammunition for hunting ungulate species, in relation to the consumption of venison of the animals shot. Where some argue that legal enforcing will speed up the availability of high-performance non-lead alternatives at comparable prices others argue that a smooth transition, sensitization and stimulation allowing the depletion of current stocks and information and training of hunters, will increase the support for this transition.

With exception for some of the smallest calibres that are currently used for roe deer, there are **sufficient non-lead alternatives** available on the market in Europe to allow a transition of lead-based towards non-lead bullets for hunting ungulate species.

Hunting efficiency, animal welfare, safety risks, barrel fouling, accuracy, impact on venison, toxicity and costs are however all non-negligible concerns that could inhibit the acceptance of non-lead ammunition by the hunting community. Research indicates that bullet material (lead versus non-lead) has no significant effect on the escape distance – being used as a proxy for killing efficiency and thereby animal welfare and hunting efficiency. The concerns over the other aspects mentioned above are justified but are not specific for the transition from lead-based toward non-lead ammunition. They are typical for each change in bullet type or brand hunters make.

Given the elements above, investing **in outreach and stakeholder communication** are key elements for a successful transition of lead-based ammunition towards non-lead bullets for hunting ungulate species.

Recommendations for further research

Whether or not the consumption of big game meat shot with lead-containing bullets increases blood lead levels of consumers, and hence increases the possibility of any risks, is still a question of debate. Additional research focusing on the relation between the consumption of big game meat and blood lead levels is recommended before formulating consumption advices for the general adult population.

9. State of affairs compared to schedule

		2018			2019												2020				
		O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M
Task 1.1 Sample collection	anticipated	x	x	x	x	x			x	x	x	x		x	x						
	realized		■	■	■	■	■		■	■	■	■	■	■	■	■					
Task 1.2 Chemical analysis	anticipated		x	x	x	x	x	x	x	x	x			x	x	x	x				
	realized							■	■	■	■	■	■			■	■	■			
Task 1.3 Data treatment	anticipated				x	x	x	x	x	x	x	x	x			x	x	x			
	realized						■	■	■	■	■	■	■	■	■	■	■	■	■		
Task 2.1 sample collection	anticipated				x	x	x	x	x	x											
	realized		■	■	■	■	■			■											
Task 2.2 chemical analysis	anticipated				x	x	x	x	x	x	x	x									
	realized					■	■	■	■	■	■	■	■				■	■	■		
Task 2.3 data treatment	anticipated							x	x	x	x	x	x	x	x	x					
	realized									■	■	■	■					■	■		
Task 3.1 Consumption scenario selection	anticipated			x	x	x	x	x	x												
	realized															■	■				
Task 3.2 Dietary intake calculations	anticipated										x	x	x								
	realized																	■	■		
Task 3.3 Risk evaluations	anticipated										x	x	x								
	realized																	■	■		
Task 4. Results & recommendations foreign studies	anticipated	x	x	x	x	x	x	x	x	x	x	x	x								
	realized							■	■	■	■	■	■	■	■	■	■	■	■		

realized ■ (yellow)
not realized (blue)

10.Travel (conferences, symposia, etc.)

Poster presentation:
Ruttens A., Casaer J., Marien C., Rutten A., Waegeneers N. (2019). Is there too much lead in Belgian big game meat? 4th IMEKOFOODS, September 16-18, 2019, Tervuren, Belgium.

- Abstract: see Appendix B

11.List of publications

Poster presentation:
Ruttens A., Casaer J., Marien C., Rutten A., Waegeneers N. (2019). Is there too much lead in Belgian big game meat? 4th IMEKOFOODS, September 16-18, 2019, Tervuren, Belgium.

- Abstract: see Appendix B

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13. Appendices

Appendix A. Example of a fill-in formulary

sciensano
Dienst Spoorelementen en Nanomaterialen
Leuvensesteenweg 17, 3080 Tervuren
T 02 769 22 29 – nadia.waegeneers@sciensano.be

INSTITUUT NATUUR- EN BOSONDERZOEK
Dienst Faunabeheer en Exoten
Havenlaan 88 bus 73, 1000 Brussel
T 0478 281 701 - jim.casaer@inbo.be

FORMULAIRE LECAHUNT

Pourquoi ce formulaire?

Son but est de collecter des données concernant l'animal à partir duquel les échantillons de viande sont prélevés. Ces données sont nécessaires pour le traitement ultérieur des échantillons dans le cadre du projet LECAHUNT. Il faut remplir 1 formulaire par animal abattu et échantillonné.

Contexte?

Cet échantillonnage fait partie du projet LECAHUNT, financé par le SPF Santé Publique, Sécurité de la chaîne alimentaire et Environnement. Le projet est mené par le consortium Sciensano – Instituut voor Natuur- en Bosonderzoek. L'objectif du projet est de déterminer le niveau de plomb dans la viande comestible du gros gibier abattu en Belgique.

Données de l'animal et de la chasse

1. **L'échantillon provient de quelle espèce animale:**
   - ☐ chevreuil
   - ☐ sanglier
2. **Cochez l'âge estimé de l'animal:**
   - ☐ < 12 mois
   - ☐ 12-24 mois
   - ☐ > 24 mois
3. **Cochez le sexe de l'animal:**
   - ☐ femelle
   - ☐ masculin
4. **Donnez une estimation du poids de l'animal éviscéré (précision à 0.5 kg):** .... kg
5. **Notez le numéro de bracelet de l'animal:**

   ....

   Si vous n'avez pas de numéro de bracelet, utilisez le code d'identification suivant :
   Code : ....

   > Écrivez ce numéro de bracelet ou ce code clairement et à l'encre indélébile sur l'emballage de l'échantillon suivi par « **SELLE** » ou « **CUISSE** ».

6. **Y a-t-il un rein dans la carcasse (seulement dans le cas des chevreuils)?**
   - ☐ Non
   - ☐ Oui => Si le chevreuil est abattu dans la Région wallonne, emballez le rein.

   > Écrivez le numéro de bracelet ou le code d'identification unique clairement et à l'encre indélébile sur l'emballage de l'échantillon suivi par « **REIN** ».

7. **La balle est-elle toujours présente dans l'animal:**
   - ☐ Oui
   - ☐ Non
8. **Est-ce qu'un os a été touché par la balle?**
   - ☐ Oui Quel os? ....
   - ☐ Non

federal public service
HEALTH, FOOD CHAIN SAFETY AND ENVIRONMENT

sciensano

Dienst Spoorelementen en Nanomaterialen
Leuvensesteenweg 17, 3080 Tervuren
T 02 769 22 29 – nadia.waegeneers@sciensano.be

INSTITUUT NATUUR- EN BOSONDERZOEK

Dienst Faunabeheer en Exoten
Havenlaan 88 bus 73, 1000 Brussel
T 0478 281 701 - jim.casaer@inbo.be

9. Savez-vous avec quel type de balle a été tiré?

- ☐ Oui Quel type? ……………………………………………………
- ☐ Non

10. Date de la chasse : ……………………………………………………

11. Localité de la chasse?

- ☐ Région flamande
- ☐ Région wallonne

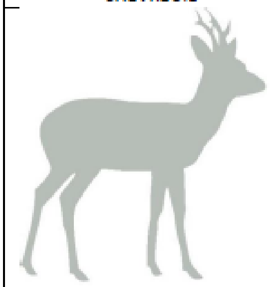
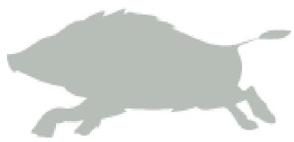
Lieu-dit: ………………………

Veuillez indiquer l'endroit approximatif avec un croix sur le dessin suivant :

Données de l'échantillon de viande

12. Indiquez sur le dessin:

- ☐ Avec un cercle où se trouve la blessure par balle
- ☐ Avec une croix où les 2 échantillons de viande (selle et cuisse) ont été découpés

CHEVREUIL	SANGLIER
	

Indentification du preneur de l'échantillon

Nom : ……………………………… **Prénom:** ………………………………

Numéro de téléphone: ……………………………………………………

Conservez ce formulaire avec soin et remettez-le à l'employé du projet lorsqu'il viendra chercher les échantillons.

Merci pour votre coopération!

Appendix B. Abstract poster presentation, 4th IMEKOFOODS

IS THERE TOO MUCH LEAD IN BELGIAN BIG GAME MEAT ?

Ruttens A.[1], Casaer J.[2], Marien C.[1], Rutten A.[2] and Waegeneers N.[1]

[1]*Sciensano, Service Trace Elements and Nanomaterials, B-3080 Tervuren, Belgium (ann.ruttens@sciensano.be)*

[2]*Research Institute for Nature and Forest (INBO), Research group on wildlife management, B-1000 Brussels, Belgium*

Lead (Pb) is a widely occurring, hazardous contaminant. Human exposure to Pb is associated with a wide range of effects, including neurotoxicity, nephrotoxicity and increased systolic blood pressure (Nawrot & Staessen, 2006). Although Pb has been banned from several applications (e.g. gasoline, plumbing,…), it is still widely used in hunting ammunition. Several studies have shown that hunting big game with Pb-containing bullets can result in increased Pb levels in meat for consumption (Knott et al; 2010; Lindboe et al., 2012). The presence of small bullet fragments in venison was demonstrated by radiographic studies even at large distance of obviously injured tissue (Hunt et al., 2009).

The present study aims to collect data of Pb in edible meat of 250 different animals hunted in Belgium (work package WP1), comprising roe deer, wild boar and red deer, and to evaluate human dietary exposure and risk. Work package 2 focuses on potential differences in Pb concentration in meat as a function of the distance to the wound channel. Information about bullet type, age class and sex of the animal, and geographic origin of all samples, is collected based on the official ID numbers of the animal complemented with questionnaire data, and will be included during data processing. Samples for WP1 are bought from hunters, game processing centres or supermarkets, while for WP2 entire animals are bought from the hunters.

Because Pb fragments may continue to exist in traditionally blended meat samples, leading to heterogeneous -and hence non representative- sample results, an alternative sample treatment and homogenisation step is applied as described by Lindboe et al. (2012). According to this method each meat sample (±200 g in WP1; ±100 g in WP2) is -after the first grinding step- blended with nitric acid (15% v/v) and shaken ±20h at room temperature. This allows potential Pb fragments to dissolve (Kollander et al., 2014). The obtained slurry is further digested in a microwave and analysed by ICP-MS.

The first results of WP1 (currently ±65 samples of roe deer and wild boar) indicate that maximum Pb concentrations were similar in both species (min: <0.003 mg kg^{-1}; max: ±0.800 mg kg^{-1}), while P50 and P90 concentrations were higher in wild boar (P50 = 0.029 µg kg^{-1}; P90 = 0.190 µg kg^{-1}) compared to roe deer (P50 = 0.006 mg kg^{-1}; P90 = 0.134 mg kg^{-1}). In both species respectively 12% and 13% of the samples exceeded the European maximum limit of 0.1 mg kg^{-1} Pb for meat of various farm animals (EU 1881/2006). None of the samples exceeded the Belgian action limit of 1 mg kg^{-1} for game meat as applied by the Federal Agency for the Safety of the Food Chain. In WP2, roe deer samples taken close to the wound channel (5-20 cm) showed higher P50 and P90 Pb concentrations compared to samples taken at larger distance (40-70 cm), while this trend was not observed in wild boar. Data treatment will be finalized in the coming months and the results obtained will be discussed in relation to literature data.

Keywords: Lead – big game - meat – risk - hunting – bullets

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