Exposure and impacts of lead (Pb) in Scandinavian brown bears (*Ursus arctos*)

Helle Bernstorf Hydeskov

A thesis submitted in partial fulfilment of the requirements of Nottingham Trent University for the degree of Doctor of Philosophy

This research programme was carried out in collaboration with the Scandinavian Brown Bear Research Project and the Inland Norway University of Applied Sciences, Evenstad Campus

June 2023

Abstract

Lead (Pb) is a toxic, non-essential element known for its negative effects on human, animal and environmental health. Previous studies have shown that free-ranging Scandinavian brown bears (*Ursus arctos*) have blood Pb at concentrations that would cause concern in humans. However, no studies have been carried out to investigate the health effects of Pb in this population. This thesis determines the gaps of knowledge concerning Pb exposure and its health effects in wild mammals and uses both free-ranging and captive Scandinavian brown bears to investigate 1) Pb exposure and if any commonly used blood variables are correlated with Pb concentration, 2) how Pb is distributed in different tissues and if any potential histopathological changes are present, 3) if Pb concentration is affected by hibernation.

The findings of this thesis show that while many wild mammalian species are exposed to Pb, there are significant knowledge gaps in regard to Pb exposure and its health effects in wild mammals. Two blood variables indicative of chronic kidney disease and two indicative of liver disease were correlated with Pb. Pb was present in all brown bear tissues analysed and the results suggest a body distribution similar to humans (three-compartment model). However, no histopathological changes were identified in liver, kidney or spinal cord tissues, so the definite health effects of Pb in the species are still undetermined. Pb concentration during hibernation is significantly higher than during the active state, potentially posing a higher risk of health effects in hibernating animals.

When monitoring Pb exposure, studying wildlife at high trophic levels is desirable, and the Scandinavian brown bear is a good sentinel species given its varied diet and easy access to samples.

In a One Health context, Scandinavian brown bear products pose a risk to human health if consumed. The author advises against eating or selling products from the Swedish annual licensed hunt and supports the need for regulations of Pb in ammunition and in wild/game products to protect the consumers.

Table of contents

Acknowledgements

When I started my PhD project in October 2019, other NTU PGRs kept telling me *"a PhD project never goes to plan"*. I didn't believe them. But five months later the pandemic was a reality and it turned out they were right…!

I have tons of people to thank as this project has truly been a team effort. Thanks to all my supervisors, Antonio, Jon, Louise and Chris, for their support and always interesting discussions about wildlife health. Thanks to Johan at Zoetis and Else at Triolab for sponsoring my blood analysis equipment. Thanks to Bärbel at Zoetis for helping me understand blood values and graphs. Thanks to Aleksija, Ricardo and Erik at SVA and Inger at SLU for facilitating blood and tissue examinations. Thanks to Ilia at ALS Scandinavia for interesting Pb discussions. Thanks to all involved in the Scandinavian Brown Bear Research Project for letting me use your ongoing project to collect data, and special thanks to all those who helped me get samples when the pandemic prevented me from attending fieldwork. Thanks to Tom, the helicopter pilot, for coming to my rescue when I had two flat tires, no phone service and was 60 km from the nearest town. Thanks to the staff at Länsstyrelsen Gävleborg, especially Sone, Göran, Per and Marielle, for letting me work alongside them to collect samples. Thanks to Yvonne for feeding me delicious food during the long bear hunt sampling days. Thanks to my excellent fieldwork assistants during the bear hunts, Ingrid, Stef and Jennifer. Thanks to Emil for housing me in Sweden during several long periods. Thanks to all the zoos for providing me with samples from captive bears.

Thanks to Becki for initially getting me excited about wildlife health in 2015 and for mentoring me throughout my residency and beyond.

Thanks to my family and friends in Denmark, especially mum, dad, M and Melissa, for keeping my spirit up in times when the pandemic made international fieldwork a living nightmare, and later when three months of sick leave made the beginning of 2022 really difficult. Thanks to Toni for being an amazing friend on the other side of the world. And finally, thanks to my wonderful PGR peers at Brackenhurst, especially Jess, Lori, Katie W., Tizz, Louise, Nourhan, Soojin, Helen B., Alex K., Kat, Tom, Katie P, Helen P. and Alex D. for always being supportive throughout my PhD journey.

I thank you all, without you this PhD project would not have been possible!

Helle Bernstorf Hydeskov

List of figures

List of tables

Chapter 1: Introduction

This chapter will explain the background, aims and objectives of the thesis.

1.1 Exposure to and effects of toxic, non-essential elements in wildlife

A wide range of wildlife taxa is known to be exposed to and negatively affected by toxic, non-essential metals and metalloids due to these elements' ability to trophic transfer, bioaccumulate, and for some even biomagnify (Ali & Khan, 2018). Quite naturally, research in wildlife has often focused on situations where an non-essential toxic element has had the greatest negative health implication (i.e., fatal outcome to the exposure). For this reason, lead (Pb) has received a lot of research attention over the past century, given its ability to severe affect many bird species worldwide (Pain et al., 2019). However, how Pb affects wild mammals, and especially the species at high trophic levels, is less studied (Chapter 2).

1.2 There is no safe level of lead (Pb) exposure

Recently defined by the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (WOAH), the United Nations Environment Programme (UNEP) and the World Health Organization (WHO) as *"One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems."* (FAO et al., 2022). Although it in wildlife disease management has often used when referring to infectious diseases (Buttke et al., 2015), all health issues that involve more than one of these groups (i.e., humans, animals, the environment), are to be considered as One Health issues.

Lead (Pb) is a toxic, non-essential element known for its negative effects (Bergdahl & Skerfving, 2022). Pb exposure continues to be a One Health issue, as it affects human, animal and environmental health (FAO et al., 2022). Pb's negative effects on human health have been documented for millennia (Hernberg, 2000) and include effects on the nervous, renal, cardiovascular, haematological, immune and reproductive systems (Agency for Toxic Substances and Disease Registry (ATSDR), 2020; Bergdahl & Skerfving, 2022). Especially children with developing central nervous systems are at risk of negative health effects from Pb exposure (Bergdahl & Skerfving, 2022; World Health Organization, 2022a).

Data from 2019 show that Pb exposure accounts for over 900,000 human deaths per year (World Health Organization, 2021), and according to the World Health Organization (WHO), there is no known safe level of Pb exposure in humans (World Health Organization, 2022a). However, to identify the worst cases of human Pb exposure, the European Food Safety Authority (EFSA) and the Centers for Disease Control and Prevention (CDC) have set different blood concentration limits as to when actions should be taken (Centers for Disease Control and Prevention, 2022; EFSA Panel on Contaminants in the Food Chain (CONTAM), 2013). The EFSA 95th percentile lower confidence limit of the benchmark dose ($BMDL_{01}$) for developmental neurotoxicity in children is 12 µg/l, and the CDC blood lead reference value (BLRV) is 35 µg/l.

Despite the large amount of evidence documenting Pb's negative health effects, Pb continues to be used in different products across the globe such as ammunition, aviation gasoline, water pipes, paints, glassware and cosmetics (Bergdahl & Skerfving, 2022). Although many developed countries have regulated the use of Pb-based paints, only 45 % of countries globally have any such legally-binding controls (World Health Organization, 2022b). Through anthropogenic activities, the amount of environmental Pb has increased to around 1,000 times the natural levels (Renberg et al., 2001), and potentially increased the environmental exposure of wildlife.

Documentation of the negative effects of Pb in wildlife species started almost 150 years ago when reports on poisoning of common pheasants (*Phasianus colchicus*) after ingestion of Pb shotgun pellets where first published (Calvert, 1876; Holland, 1882). Since then, numerous studies in different wildlife species have been published, often with a focus on avian species (Pain et al., 2019). Birds have been the animal class in focus for Pb research in wildlife because Pb exposure in wild birds often leads to acute multisystemic clinical disease or even death, with waterfowl, scavenging and predatory species being most commonly affected (Pain et al., 2019). Compared to wild avian species, the knowledge of Pb exposure and health effects in wild mammalian species is limited (Pain et al., 2019).

Despite the human blood Pb concentration limits set by the EFSA and the CDC, wildlife health scientists are now suggesting stopping the reporting of "safe levels"

or “threshold levels” of Pb exposure in wildlife (Pain et al., 2019) and instead following the line of the WHO, assuming that no level of Pb exposure is safe.

1.3 Scandinavian brown bears (*Ursus arctos*) and lead (Pb)

The Scandinavian brown bear is a top-predator, omnivore and scavenger with a varied diet (Wilson & Mittermeier, 2009). It has been documented to eat 26 different food and its diet is very dependent on season (Stenset et al, 2016). In the spring and early summer, it primarily eats moose (*Alces alces*) calves and ants. Berries dominate the diet in late summer to autumn. Throughout its active state, it will scavenge on whatever it finds (i.e., gut piles left in the environment by hunters shooting big game). From October to May, the Scandinavian brown bear hibernates for 6-7 months (Manchi & Swenson, 2005). It uses continuous torpor (i.e., no arousals during the hibernation phase), sometimes referred to as “winter sleep”. The species has a long lifespan of approximately 30 years and starts to reproduce at the age of 4-6 years (Chapron et al., 2009; Schwartz et al., 2003).

The Scandinavian Brown Bear Research Project has captured and researched the same population of free-ranging central Swedish brown bears (*Ursus arctos*) since 1984 (Scandinavian Brown Bear Research Project, 2023). A previous study has shown that the entire population is exposed to high Pb concentrations, from suckling cubs to adults (Fuchs et al., 2021). The mean blood concentration in this bear population (96.6 µg/l) is 8 and over 2.5 times higher than the EFSA and CDC limits described in section 1.1, respectively (Centers for Disease Control and Prevention, 2022; EFSA Panel on Contaminants in the Food Chain (CONTAM), 2013; Fuchs et al., 2021). With such a high Pb concentration and the assumption that no Pb exposure is safe, it is expected that it will results in negative health effects in the bears. Yet, no concerns about their health status have been reported by the authority in charge of conducting health surveillance in the species (National Veterinary Institute (SVA), 2022) or others.

Figure 1.1 illustrates the possible exposure route of Pb in Scandinavian brown bears. However, with the Scandinavian brown bear population primarily living far from industrial activities (Scandinavian Brown Bear Research Project, 2023), it is believed that the main exposure route of Pb in this population will be digestion.

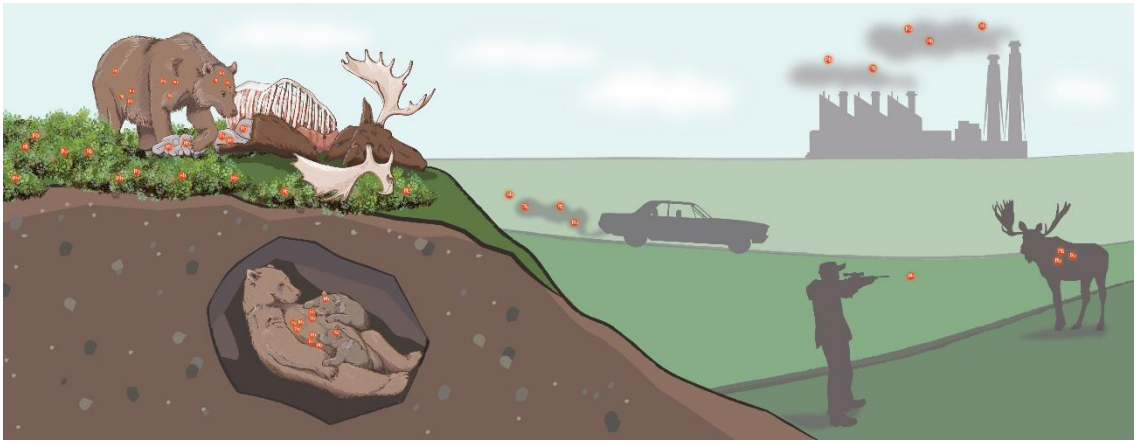

Figure 1.1 Lifecycle of lead (Pb) in Scandinavian brown bears (*Ursus arctos*). The orange spots represents Pb elements. Illustration by Juliana D. Sphar. Reproduced with permission.

With the Scandinavian brown bear being a top predator, scavenger and omnivore (Wilson & Mittermeier, 2009), Pb's ability to bioaccumulate (Clemens, 2006; Radomyski et al., 2018), and the knowledge of the high blood Pb concentrations in the free-ranging population (Boesen et al., 2019; Fuchs et al., 2021), made it an ideal wild mammalian species for studying Pb health effects.

1.4 Thesis aims, objectives and structure

Although relevant for the mitigations of Pb exposure in a One Health context, this thesis will not investigate the sources of Pb exposure.

The thesis aims to investigate the current knowledge of Pb exposure and health effects in wild mammals and whether Pb exposure is affecting the health of Scandinavian brown bears. The free-ranging and captive Scandinavian brown bears included in the thesis are from the same geographical region to allow for comparisons between the two populations.

The thesis chapters 2-5 are written in the style of journal manuscripts, and all will be submitted for publication in peer-reviewed journals.

The thesis has the following objectives:

- To determine the current knowledge of Pb exposure and its health effects in wild mammals.

- To determine if any haematological or biochemical blood variables are correlated with blood Pb concentration in free-ranging Scandinavian brown bears.
- To establish Pb's distribution in the body of free-ranging Scandinavian brown bears.
- To investigate if the presence of Pb is linked to tissue pathologies in free-ranging Scandinavian brown bears.
- To determine if the captive Scandinavian brown bear population is exposed to Pb like the free-ranging population and if any tissue pathologies can be detected.
- To determine if blood Pb concentration is affected by hibernation in free-ranging Scandinavian brown bears.
- To determine if the Scandinavian brown bear is a suitable long-term monitoring sentinel species for environmental Pb exposure in a One Health context.

To address the objectives, the remaining part of this thesis is divided into the following five chapters:

Chapter 2: A global systematic review of lead (Pb) exposure and its health effects in wild mammals

This chapter reviews the current scientific knowledge of Pb exposure and its health effects in wild mammals, identifies the gaps and provides suggestions for how future studies can be designed to address these gaps. *This chapter is currently under review at the Journal of Wildlife Diseases. For the purpose of the flow of the thesis, formatting has been changed to fit the rest of the thesis and spelling changed from American to British English.*

Chapter 3: Correlations between blood lead (Pb) concentrations and common blood variables in Scandinavian brown bears (*Ursus arctos*)

This chapter, based on data from 86 free-ranging Scandinavian brown bears, investigates if different blood variables are correlated with blood Pb concentration and evaluates if these can be indicative of negative health effects. Additionally, using data from 25 captive Scandinavian brown bears, Pb exposure in this population is examined.

Chapter 4: Lead (Pb) concentrations and associated pathology in tissues of Scandinavian brown bears (*Ursus arctos*)

This chapter, based on data from 56 free-ranging and 26 captive Scandinavian brown bears, investigates tissue Pb concentrations and associated pathologies.

Chapter 5: Hibernation increases blood lead (Pb) concentrations

This chapter, based on data from 15 sub-adult free-ranging Scandinavian brown bears, investigates whether blood Pb concentrations differ during their hibernation and active states.

Chapter 6: Discussion

This chapter summarises and evaluates the findings from Chapters 2-5. Ideas on how the Scandinavian brown bear can be used as a sentinel species of Pb exposure monitoring are discussed in a One Health context.

1.5 Statement of contribution

The author of this thesis singlehandedly conducted the screening of papers, data extraction and analysis of the systematic literature review in Chapter 2. The author organised the collection and analysis of all samples included in the thesis. The pandemic meant that the author herself could not collect all samples as planned and therefore this was delegated to collaborators within the Scandinavian Brown Bear Research Project when needed. The author herself collected the blood samples taken in 2021 used in Chapter 3, four sets of blood samples used in Chapter 5, all tissue samples from the free-ranging bears and samples from 7 captive bears used in Chapter 4. The Pb analysis was coordinated by the author and conducted as an external reference laboratory. The blood variable analyser tests and creation of blood smears were conducted by the author in 2021 and by collaborators within the Scandinavian Brown Bear Research Project in 2020 due to the pandemic. The manual screening of blood smear and histology slides were conducted by the author.

References

Agency for Toxic Substances and Disease Registry (ATSDR). (2020). *Toxicological profile for lead*. https://doi.org/http://dx.doi.org/10.15620/cdc:95222

Ali, H., & Khan, E. (2019). Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs - Concepts and implications for wildlife and human health. *Human and Ecological Risk Assessment: An International Journal, 25*(6), 1353-1376. https://doi.org/10.1080/10807039.2018.1469398

Bergdahl, I. A., & Skerfving, S. (2022). Lead. In G. F. Nordberg & M. Costa (Eds.), *Handbook on the Toxicology of Metals: Fifth Edition: Vol. II: Specific Metals* (Fifth Edition, pp. 427–493). Academic Press. https://doi.org/10.1016/B978-0-12-822946-0.00036-2

Boesen, A. H., Thiel, A., Fuchs, B., Evans, A. L., Bertelsen, M. F., Rodushkin, I., & Arnemo, J. M. (2019). Assessment of the LeadCare® plus for use on Scandinavian brown bears (*Ursus arctos*). *Frontiers in Veterinary Science*, *6*, 285. https://doi.org/10.3389/fvets.2019.00285

Buttke, D. E., Decker, D. J., & Wild, M. A. (2015). The role of one health in wildlife conservation: a challenge and opportunity. *Journal of Wildlife Diseases, 51*(1), 1-8. https://doi.org/10.7589/2014-01-004

Calvert, H. S. (1876). Pheasants poisoned by swallowing shot. *The Field*, *47*.

Centers for Disease Control and Prevention. (2022, December 16). *Blood Lead Reference Value*. Data and Statistics. https://www.cdc.gov/nceh/lead/data/blood-lead-reference-value.htm

Chapron, G., Wielgus, R., Quenette, P. Y., & Camarra, J. J. (2009). Diagnosing mechanisms of decline and planning for recovery of an endangered brown bear (*Ursus arctos*) population. *PLOS ONE*, *4*(10), e7568. https://doi.org/10.1371/JOURNAL.PONE.0007568

Clemens, S. (2006). Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie*, *88*(11), 1707–1719. https://doi.org/10.1016/J.BIOCHI.2006.07.003

EFSA Panel on Contaminants in the Food Chain (CONTAM). (2013). Scientific opinion on lead in food. *EFSA Journal*. https://doi.org/10.2903/j.efsa.2010.1570

FAO, UNEP, WHO, & WOAH. (2022). *One Health Joint Plan of Action (2022-2026). Working together for the health of humans, animals, plants and the environment*. https://doi.org/10.4060/cc2289en

Fuchs, B., Thiel, A., Zedrosser, A., Brown, L., Hydeskov, H. B., Rodushkin, I., Evans, A. L., Boesen, A. H., Græsli, A. R., Kindberg, J., Kindberg, J., & Arnemo, J. M. (2021). High concentrations of lead (Pb) in blood and milk of free-ranging brown bears (*Ursus arctos*) in Scandinavia. *Environmental Pollution*, *287*, 117595. https://doi.org/10.1016/j.envpol.2021.117595

Hernberg, S. (2000). Lead poisoning in a historical perspective. *American Journal of Industrial Medicine*, *38*, 244–254. https://doi.org/10.1002/1097-0274

Holland, G. (1882). Pheasant poisoning by swallowing shot. *The Field*, *59*, 232.

Manchi, S., & Swenson, J. E. (2005). Denning behaviour of Scandinavian brown bears *Ursus arctos*. *Wildlife Biology*, *11*(2), 123–132. https://doi.org/10.2981/0909-6396(2005)11[123:DBOSBB]2.0.CO;2

National Veterinary Institute (SVA). (2022). *Wildlife disease surveillance in Sweden 2021 - SVA report series 76:2022*.

Pain, D. J., Mateo, R., & Green, R. E. (2019). Effects of lead from ammunition on birds and other wildlife: A review and update. *Ambio*, *48*(9), 935–953. https://doi.org/10.1007/s13280-019-01159-0

Radomyski, A., Lei, K., Giubilato, E., Critto, A., Lin, C., & Marcomini, A. (2018). Bioaccumulation of trace metals in aquatic food web. A case study, Liaodong Bay, NE China. *Marine Pollution Bulletin*, *137*, 555–565. https://doi.org/10.1016/J.MARPOLBUL.2018.11.002

Renberg, I., Bindler, R., & Brännvall, M. L. (2001). Using the historical atmospheric lead-deposition record as a chronological marker in sediment deposits in Europe. *Holocene*, *11*(5), 511–516. https://doi.org/10.1191/095968301680223468

Scandinavian Brown Bear Research Project (2023). *Skandinaviska Björnprojektet*. http://brownbearproject.com/

Schwartz, C. C., Keating, K. A., Reynolds III, H. V., Barnes Jr., V. G., Sellers, R. A., Swenson, J. E., Miller, S. D., McLellan, B. N., Keay, J., McCann, R., Smith, R., & Herrero, S. (2003). Reproductive maturation and senescence in the female brown bear. *Ursus*, *14*(2), 109–119.

Stenset, N. E., Lutnæs, P. N., Bjarnadóttir, V., Dahle, B., Fossum, K. H., Jigsved, P, Johansen, T., Neumann, W, Opseth, O., Rønning, O., Steyaert, S. M. J. G., Zedrosser, A., Brunberg, S., & Swenson J. E. (2016). Seasonal and annual variation in the diet of brown bears *Ursus arctos* in the boreal forest of southcentral Sweden. *Wildlife Biology, 22*(3), 107-116.

Wilson, D. E., & Mittermeier, R. A. (Eds.). (2009). *Handbook of the Mammals of the World: Carnivores* (Vol. 1). Lynx Edicions.

World Health Organization. (2021). *The public health impact of chemicals: knowns and unknowns - data addendum for 2019*.

World Health Organization. (2022a). *Lead poisoning*. https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health

World Health Organization. (2022b). *Legally-binding controls on lead paint*. The Global Health Observatory. https://www.who.int/data/gho/data/themes/topics/indicator-groups/legally-binding-controls-on-lead-paint

Chapter 2: A global systematic review of lead (Pb) exposure and its health effects in wild mammals

This chapter is currently under review at the Journal of Wildlife Diseases. For the purpose of the flow of the thesis, formatting has been changed to fit the rest of the thesis and spelling changed from American to British English.

Helle B. Hydeskov,[1,5] **Jon M. Arnemo,**[2,3] **Chris Lloyd Mills,**[4] **Louise K. Gentle,**[1] **and Antonio Uzal**[1]

[1] School of Animal, Rural and Environmental Sciences, Nottingham Trent University, Brackenhurst Campus, Southwell, NG25 0QF, UK
[2] Department of Forestry and Wildlife Management, Faculty of Applied Ecology, Agricultural Sciences and Biotechnology, Inland Norway University of Applied Sciences, Campus Evenstad, 2418 Elverum, Norway
[3] Department of Wildlife, Fish and Environmental Studies, Faculty of Forest Sciences, Swedish University of Agricultural Sciences, 90183 Umeå, Sweden
[4] School of Science and Technology, Nottingham Trent University, Clifton Campus, Nottingham, NG11 8NS, UK

[5] Corresponding author (email: hbhvet@gmail.com)
Helle B. Hydeskov, School of Animal, Rural and Environmental Sciences, Nottingham Trent University, Brackenhurst Campus, Southwell, NG25 0QF, UK, +44 (0)1159418418
Word count = 3,805

Abstract

Lead (Pb) is a toxic non-essential metal, known mainly for causing poisoning of humans and wild birds. However, little is known about Pb exposure and its associated health effects in wild mammals. We conducted a global systematic literature review to identify peer-reviewed studies published on Pb exposure in wild mammalian species and the health effects they identified. In total, 183 studies, conducted in 35 countries and published over 62 years (1961-2022), were included in the review. Only 6 % (11/183) of the studies were conducted in developing countries. Although 153 mammalian species were studied, most studies focused on species that are easy to access (i.e., hunted species and small mammals that are easy to trap). Therefore, carnivores and scavengers were less frequently studied than herbivores and omnivores. Despite all studies reporting Pb concentrations, only 45 (25 %) studies investigated health effects and, of these 45 studies, only 28 (62 %) found any health effect. All health effects were negative, and ranged from subclinical effects to fatality. Methodologies of Pb sampling and quantification, and reporting of results, varied widely across the studies, making both Pb concentrations and health effects difficult to compare and evaluate. Thus, there is a need for more research on Pb exposure and its health effects on wild mammals, especially as carnivores and scavengers could be used as sentinels for ecosystem health.

Key words: bioaccumulation, Mammalia, One Health, sentinel species, toxicology, wildlife population health

2.1 Introduction

2.1.1 Lead (Pb)

Lead (Pb) is a toxic, non-essential metal that has no known biological function for living organisms, contaminates the environment, and causes negative health effects in humans and other animals (European Chemicals Agency, 2018; Lanphear et al., 2018; Ma, 2011; Pain et al., 2019). Pb occurs naturally in the Earth's crust, and has historically been used in many products, including water pipes, paints, gasoline, ammunition, aviation fuel, motor vehicle batteries, glassware and cosmetics (Bergdahl & Skerfving, 2022). Consequently, these anthropogenic activities have increased the amount of environmental Pb to around 1,000 times the natural levels (Renberg et al., 2001), thereby amplifying the potential environmental exposure of wildlife to Pb. Despite legislation banning or reducing the use of Pb, it is still present in many products. For example, very few places (i.e., countries, states or regions) have banned all Pb-based ammunition (Sonne et al., 2022) and, as of June 2022, only 45 % of countries have made legally-binding controls on the production, import, sale and use, of Pb-based paints (World Health Organization, 2022b).

2.1.2 Health effects of lead (Pb) in humans

Children are generally more at risk of health effects from Pb exposure than adults, as children absorb more ingested Pb from their gastrointestinal system (World Health Organization, 2022a). Pb affects multiple organ systems, including the nervous, renal, cardiovascular, haematological, immune and reproductive systems. The health effects of Pb exposure can range from chronic and subclinical, to acute and fatal (Agency for Toxic Substances and Disease Registry (ATSDR), 2020; Bergdahl & Skerfving, 2022). Furthermore, there is no known safe level of Pb exposure in humans (World Health Organization, 2022a). Despite this, concentrations of concern vary between health authorities. For example, the Centers for Disease Control and Prevention (CDC) in the USA currently have a "blood lead reference value (BLRV)" of 35 µg/l, which is used to identify children with high blood Pb concentrations (top 2.5 % with the highest concentration) that require additional actions to be taken by health care professionals (Centers for Disease Control and Prevention, 2022). Whereas, the European Food Safety Authority (EFSA) uses a different system and has set lower confidence limits of 12, 15, and 36 µg/l as benchmark doses for developmental neurotoxicity, effects

on the prevalence of chronic kidney disease, and effects on systolic blood pressure, respectively (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2013). Furthermore, between 2012 and 2021, CDC's BLRV was 50 µg/l; prior to 2012, it was 100 µg/l and called the "level of concern in children" (Centers for Disease Control and Prevention, 2022). With this continuing lowering of the CDC's BLRV, it is clear that both health authorities agree that even very small concentrations of Pb can negatively affect human health, in alignment with the World Health Organization's understanding of Pb exposure (World Health Organization, 2022a).

2.1.3 Lead (Pb) research in animals

Research on Pb exposure and health effects in wildlife has often been undertaken on avian species, where the first poisonings were recognized nearly 150 years ago (Calvert, 1876). Pb exposure in birds often leads to multisystemic clinical disease or even death, especially in waterfowl, scavenging birds and predatory birds (e.g., Pain et al., 2019). Consequently, Pb exposure has caused population declines, e.g., in the scavenging California condor (Gymnogyps californianus; Green et al., 2008). Although toxicity thresholds in different avian species have previously been proposed (Franson & Pain, 2011), scientists have recently suggested stopping this practice as it gives the impression that some levels of Pb are acceptable (Pain et al., 2019).

Previous reviews on Pb in mammals have focused primarily on animals in laboratory settings or on domestic species. For example, Ma (2011) showed that humans and laboratory mammals have similar susceptibility to Pb exposure (i.e, the same Pb dose-health effects relationship). In domestic mammals, young cattle appear most prone to Pb poisoning, likely due to their tendency to lick different items (Constable et al., 2017). However, young cattle may simply absorb Pb better than adults, as seen in humans (World Health Organization, 2022a).

2.1.4 Importance and impact of a global systematic literature review

To the best of our knowledge, no global systematic literature review has focused on Pb exposure and health effects in wild mammalian species. Synthesizing this scientific evidence is important to understand the breadth of wild mammalian species affected by Pb across the world, as there is no reason to expect wild

mammals to be less at risk from Pb exposure than any other vertebrates, including humans.

This article provides a global systematic review of the literature on environmental Pb exposure and health effects in wild mammals. Gaps and biases in the research are assessed, and suggestions on how to advance knowledge of the effects of Pb are provided, particularly in identifying the types of species that may best indicate environmental Pb exposure. The outcomes of this review are important because they can be used to inform future studies, influence policymakers, and feed into relevant environmental legislation.

2.2 Materials and methods

2.2.1 Systematically identifying peer-review studies to include

This systematic review followed the workflow proposed by the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 statement (Page et al., 2021).

A literature search for peer-reviewed scientific articles on Pb exposure in wild mammals was conducted using the database Scopus (www.scopus.com) on 21 June 2022. Scopus was chosen over other well-known databases and online search engines (e.g., Web of Science [www.webofscience.com], PubMed [www.ncbi.nlm.nih.gov/pubmed], or Google Scholar [www.scholar.google.com]) due to the ability of Scopus to allow the chemical lead (Pb) as a search term, an option not provided by other databases or online search engines. In addition, Scopus permitted the exclusion of articles containing the word “lead” when used as a verb in the absence of any occurrence of lead as a chemical in the source. Furthermore, Scopus only indexed peer-reviewed articles and did not have restrictions on the length of search queries.

In Scopus, different query combinations of the following searches were used: CASREGNUMBER (7439-92-1)/CHEMNAME (lead); wild/wildlife/free-living/"free living"/free-ranging/"free ranging"/semi-wild/"semi wild"/free-roaming/"free roaming"/street/feral; captive/zoo/domestic/domesticated/semi-captive/"semi captive"/semi-domestic/"semi domestic"/semi-domesticated/"semi domesticated"; exposure/expose/exposed/exposing/poisoning/poison/poisoned; keywords for mammals, and scientific nomenclature of all mammalian orders, families and genera taken from the IUCN Red List of Threatened Species (IUCN, 2021) (Appendix A).

The first author conducted the assessment of all records and screening of studies. After identifying records via Scopus, records were checked for duplicates. Unique records were then screened by reading the title and abstract. The selected studies were thoroughly screened by reading the entire article. Articles on Pb exposure in wild mammals that were cited in the reference sections of the Scopus articles passing the full screening were also screened to identify additional publications not found by the original literature searches (i.e., snowball search).
Inclusion criteria included the requirement that studies must be peer-reviewed original research studies reporting Pb concentrations in tissues or body fluids in a non-domestic mammal (called “wild mammals” in this review) species. Studies reporting Pb concentrations in captive wild mammals outside laboratory settings were included to illustrate the variety of wild mammalian species documented to be susceptible to Pb exposure from their environment. Exclusion criteria included articles not reporting Pb concentrations in wild mammalian tissues or blood, studies using purely experimental settings, and studies reporting Pb concentrations in free-ranging domestic mammalian species. Data from studies were excluded if the study reported Pb concentration in tissues or body fluids knowingly contaminated with Pb-based ammunition. Some studies reported non-standard units (i.e., liquid concentrations for tissues or units unknown to the authors of this study). In these cases, or where there was a mismatch between data reported in text and tables, the corresponding author was contacted via the email provided in the publication and given a maximum of 30 days to respond. Without a response, either the data in question or the entire study was excluded.

2.2.2 Data extracted from the studies

For every study included, the following information was extracted where available: taxonomy (order, family, common species name, scientific species name), continent and country where the study was conducted, description of known pollution sources nearby the study site, type of living condition (free-ranging, captive or semi-captive), year of sample collection, season, age, sex, tissue, sample size, Pb analysis method, reporting in wet weight or dry weight concentrations (where both were reported, dry weight concentration was preferred), and Pb concentration arithmetic mean (geometric means and arithmetic weighted means were excluded), standard deviation, standard error, median, minimum value and maximum value. Pb concentrations were converted to

µg/kg or µg/l where applicable, for comparison. Whether the study had investigated and identified any health effects of Pb was also recorded. To investigate in which part of the mammalian food web Pb exposure is most frequently reported, the feeding type of the mammalian species (i.e., herbivore, omnivore or carnivore), and whether or not the species scavenges on animal material, was determined by following species descriptions available in a recently published mammalian textbook (Wilson & Mittermeier, 2009). Articles from Russia were allocated to the European or Asia continent based on the geographical location of the sample collection. To investigate potential economic resource biases in the publications, the Organisation for Economic Co-operation and Development's designation of developed and developing countries was used (Organisation for Economic Co-operation and Development (OECD), 2006).

Different parts of a tissue or body fluid (e.g., whole kidney versus kidney cortex or whole blood versus plasma) were considered separate tissues for the purpose of the review. Additionally, cortical bone and antler were differentiated, as cortical bone is permanent bone, whereas antler is deciduous bone (representing only short-term/annual exposure).

2.3 Results

2.3.1 Characteristics of the studies

A total of 254 records were retrieved from Scopus (Figure 2.1), with no duplicates identified. After the initial screening of the title and abstract, 125 studies remained for full screening, resulting in 69 studies from the Scopus search queries meeting the inclusion criteria. Additionally, 114 studies were identified for inclusion through a snowball search, giving a total of 183 studies for systematic review (Appendix B).

The studies were published over 62 years (1961 to June 2022), with most of the studies (158/183, 86 %) published in the second half of this period (1992 to 2022) (Figure 2.2). The majority of studies (162/183, 89 %) were conducted in Europe (108 studies, 59 %) and North America (54 studies, 30 %), and some studies were conducted in multiple countries (but always within the same continent). The US was the country where most studies were conducted (38 studies), followed by Canada (17 studies), Poland (16 studies), Germany (12 studies) and the UK (11 studies). Only 6 % (11/183) of the studies were conducted in developing countries (Figure 2.3).

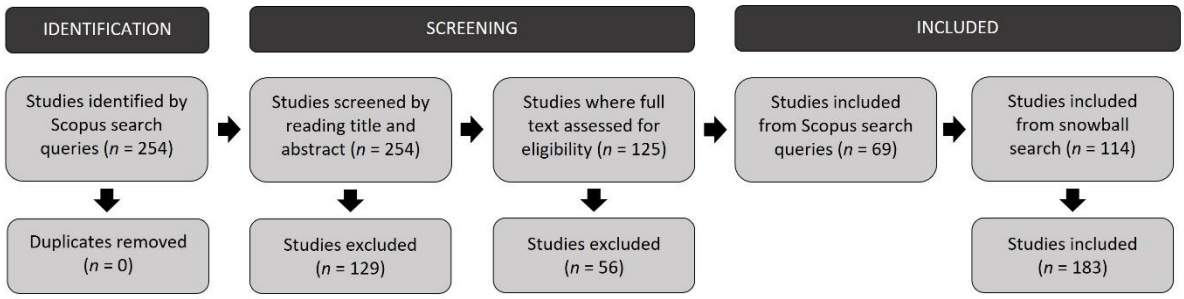

Figure 2.1 Modified PRISMA 2020 flow diagram for systematic reviews indicating the number of studies that were included and excluded in the screening process.

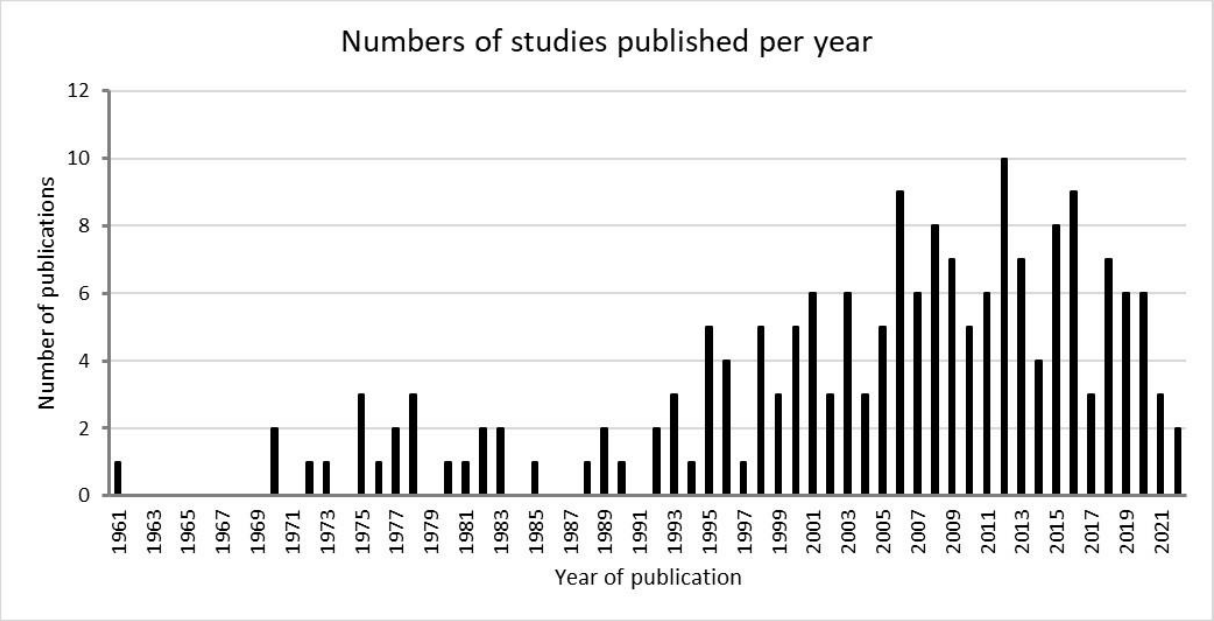

Figure 2.2 The number of studies published per year on lead (Pb) exposure in non-domestic mammals included in this systematic review (*n* = 183). Studies were published in the period 1961-June 2022.

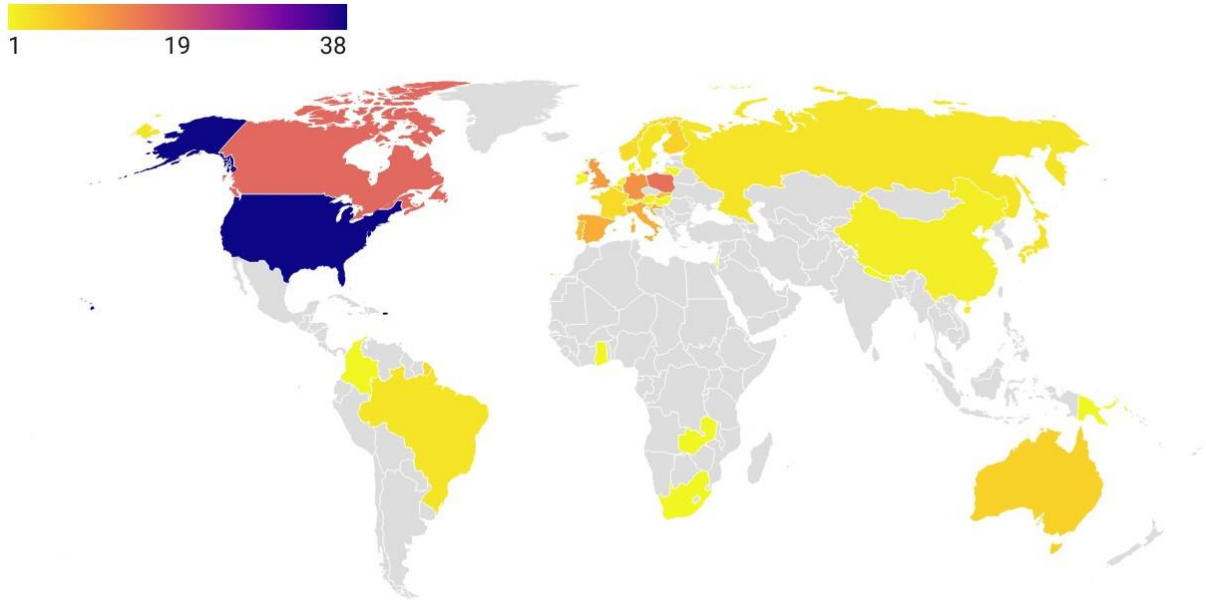

Figure 2.3 The number of studies conducted on lead (Pb) exposure in wild mammals between 1961 and June 2022 per country (*n* = 183). Studies were conducted in 35 countries across 6 continents. Darker colour indicates a higher number of studies conducted. Created using Datawrapper (Datawrapper GmbH 2022).

2.3.2 Lead (Pb) exposure

Pb was present in all mammalian species studied, with concentrations reported in a total of 153 wild mammalian species belonging to 45 families and 11 orders. The most studied order, family and species, were *Cetartiodactyla*, *Cervidae* and red deer (*Cervus elaphus*), with 67, 46 and 20 studies, respectively (Table 2.1). In terms of feeding type, herbivores, omnivores and carnivores were researched in 92, 79 and 49 studies, respectively, and 49 studies researched scavenging species.

Thirty-three different tissues and body fluids were tested. The five most studied tissues were the liver (123 studies), kidney (92 studies), skeletal muscle (54 studies), bone (32 studies) and blood (25 studies). At 1,506,000 µg/kg dry weight, the highest Pb mean concentration reported across all 11 mammalian orders studied was recorded in the kidney of a Northern short-tailed shrew (*Blarina brevicauda*) (Table 2.2).

Table 2.1 Top 5 most studied mammalian orders, families and species by the number of studies published.

Order	**No. of studies**
Cetartiodactyla	67
Carnivora	55
Rodentia	42
Lagomorpha	15
Eulipotyphla	14
Family	**No. of studies**
Cervidae	46
Muridae	23
Ursidae	19
Suidae	17
Cricetidae	16
Species	**No. of studies**
Red deer (*Cervus elaphus*)	20
European roe deer (*Capreolus capreolus*)	19
Wild boar (*Sus scrofa*)	17
Long-tailed field mouse (*Apodemus sylvaticus*)	12
Brown bear (*Ursus arctos*)	11

Table 2.2 Highest mean lead (Pb) concentration (µg/kg or µg/l) reported in each mammalian order studied. Solid tissue concentrations are reported in either wet weight (w.w.) or dry weight (d.w.).

Order	Concentration	Species	Sample size	Tissue	Location[a]	Country[b]	Reference
Carnivora	861,900 µg/kg w.w.	Ringed seal (*Pusa hispida*)	1	Brain	F-R	Canada	(Chan et al., 1995)
Cetartiodactyla	97,300 µg/kg w.w.	Beluga whale (*Delphinapterus leucas*)	1	Blubber	F-R	Canada	(Chan et al., 1995)
Chiroptera	500,000 µg/kg w.w.	Grey-headed flying fox (*Pteropus poliocephalus*)	2	Liver	Captive	USA	(Zook et al., 1972a)
Dasyuromorphia	62 µg/l	Tasmanian devil (*Sarcophilus harrisii*)	26	Blood	Captive	Australia	(Hivert et al., 2018)
Didelphimorphia	1,300 µg/kg w.w.	Virginia opossum (*Didelphis virginiana*)	4	Liver	F-R	USA	(Lewis et al., 2001)
Diprotodontia	2,950 µg/kg[c]	Common wombat (*Vombatus ursinus*)	4	Hair	F-R	Australia	(Penrose et al., 2022)
Eulipotyphla	1,506,000 µg/kg d.w.	Northern short-tailed shrew (*Blarina brevicauda*)	1	Kidney	F-R	USA	(Stansley and Roscoe, 1996)
Lagomorpha	9,000 µg/kg w.w.	European hare (*Lepus europaeus*)	9	Muscle	F-R	Austria	(Ertl et al., 2016)
Primates	212,500 µg/kg w.w.	Stump-tailed macaque (*Macaca arctoides*)	2	Liver	Captive	USA	(Zook et al., 1972a)
Rodentia	672,000 µg/kg d.w.	Long-tailed field mouse (*Apodemus sylvaticus*)	5	Bone	F-R	UK	(Johnson et al., 1978)
Sirenia	100 µg/l	American manatee (*Trichechus manatus*)	4	Blood	Captive	Brazil	(Anzolin et al., 2012)

[a] F-R: The wild mammal was from a free-ranging setting. Captive: The wild mammal was from a captive setting.
[b] Country in which the study was conducted.
[c] No indication of whether the concentration is reported in w.w. or d.w.

2.3.3 Health effects of lead (Pb)

Forty-five (25 %) studies investigated the health effects of Pb and, of these, 28 (62 %) studies identified a negative health effect. No studies identified a positive health effect. Negative health effects were reported in 57 species (32 herbivores, 21 omnivores and 4 carnivores) and ranged in severity from ‘interference with developing permanent teeth’ to ‘fatality’ (Table 2.3).

Table 2.3 Negative health effects associated with lead (Pb) exposure in wild mammals by organ system affected. ALAD: Aminolevulinic acid dehydratase; INIBs: Eosinophilic acid-fast Intranuclear inclusion bodies; MNEs: Micronucleated erythrocytes.

Organ/organ system affected	Effects found	References
Whole body	Fatality, DNA damage, oxidative stress	Brumbaugh et al., 2010; Burco et al., 2012; da Silva et al., 2000; Lazarus et al., 2020; North et al., 2015; Sauer et al., 1970; Shlosberg et al., 1997; Skerratt et al., 1998
Nervous system	Neurological clinical signs; demyelination of spinal cord and subcortical white matter of brain and optic tracts	Diters & Nielsen, 1978; Sauer et al., 1970; Skerratt et al., 1998; Zook et al., 1973
Circulatory system	Decreased ALAD activity; decreased haemoglobin; decreased haematocrit; increased MNEs; increased total bilirubin; decreased calcium; decreased lactate dehydrogenase	Chen et al., 2018; Ieradi et al., 1996; Mouw et al., 1975; Rogival et al., 2006; Sánchez-Chardi et al., 2009; Stansley & Roscoe, 1996
Reproductive system	Testicular structural and functional disruption; abnormal sperm cells; decreased proportion of live sperm	Chen et al., 2018; Ieradi et al., 1996; Parelho et al., 2016
Kidney	Decreased ALAD activity; necrosis and cell degeneration; INIBs in proximal tubular epithelium; oedema; mitochondrial abnormalities; increased weight of kidneys; hyperplasia of tubules, atrophy of glomeruli, interstitial fibrosis; degenerate cells in the lumen of seminiferous tubules; mineralisation and degeneration of proximal tubules; karyocytomegaly; lymphocyte infiltrations; cell debris and change in glomerular aspect and size	Brumbaugh et al., 2010; Ceruti et al., 2002; Damek-Poprawa & Sawicka-Kapusta, 2003; Diters & Nielsen, 1978; Mouw et al., 1975; Roberts et al., 1978; Sauer et al., 1970; Skerratt et al., 1998; Stansley & Roscoe, 1996; Tête et al., 2014; Zook et al., 1970, 1972
Liver	INIBs; necrosis; apoptosis; increased number of pyknotic nuclei; interstitial fibrosis, decreased glycogen content; increased liver-body ratio; steatosis; lymphocyte infiltrations	Damek-Poprawa & Sawicka-Kapusta, 2003; Diters & Nielsen, 1978; Sánchez-Chardi et al., 2009; Sauer et al., 1970; Tête et al., 2014; Zook et al., 1970, 1972
Teeth	Interference with developing permanent teeth	North et al., 2015; Rappaport et al., 1975

2.3.4 Variation in methodology

The 183 studies used a large variety of sampling approaches and Pb analysis techniques, and reported the results in different ways, making it hard to compare findings. For example, some studies only reported mean values, while others reported mean, standard deviation/standard error, median and range of Pb concentration. Full details on sex and age of the animals tested, and on whether the sampling was undertaken in an area with known Pb pollution, were not provided in 158 (86 %), 134 (73 %), and 101 (55 %) studies, respectively.

2.4 Discussion

2.4.1 Coverage of lead (Pb) exposure in wild mammals in the literature

The number of publications of Pb studies on wild mammalian species has increased over the last three decades. Nevertheless, the frequency of Pb publications on wild avian species still appears to be much higher, indicated by the large number of avian reviews published in recent years (e.g., Ives et al., 2022; Monclús et al., 2020; Pain et al., 2019).

Pb concentrations were reported in 153 wild mammalian species, highlighting that this Class of animals is exposed to Pb on a large scale. Despite this clear evidence of Pb exposure across many taxa, based on IUCN taxonomic classification, only 41 % (11/27) of orders, 28 % (45/162) of families and 3 % (153/5,968) of species were studied, which evidences a large group of mammals with no information on Pb exposure (IUCN, 2021). Understandably, there has been a tendency to study species that are easiest to acquire (i.e., hunted species and small species that are easy to trap without chemical immobilization). Consequently, the studies identified in this review are skewed towards herbivores and omnivores. It is not possible to determine whether tissues were selected due to easy access, or because the researcher hypothesised that the selected tissue would provide the most information in their study species. However, liver, kidney, skeletal muscle, bone and blood are commonly collected and stored for further investigations during wildlife necropsies (McAloose et al., 2018).

2.4.2 Health effects of lead (Pb) in wild mammals

Only 28 studies identified negative health effects of Pb in wild mammals. As expected, no positive effects were found. From the breadth of health effects identified and organ systems affected, it is clear that Pb affects many parts of the body in wild mammals, just like in other species (Bergdahl & Skerfving, 2022; Pain et al., 2019). Many of the identified health effects would most likely be subclinical, so it is not possible to determine how exactly these impact wild mammalian populations.

It is expected that, due to their feeding behaviours, mammals are more likely than birds to be exposed to smaller amounts of Pb, relative to body mass, but over extended periods of time. For example, waterfowl are known to pick up large numbers of Pb-based shotgun pellets or fishing weights from the bottom of wetland areas when foraging (Pain et al., 2019), yet wild mammals would not be exposed to such accumulations of Pb, as they forage using different methods. This difference in feeding behaviour would reduce the risk of acute poisonings, seen in many waterfowl, scavenging and predatory bird species, but cause more chronic and often subclinical health effects (e.g., on the nervous and immune systems). Chronic effects tend to be much less apparent than acute effects in free-ranging wildlife, so may be overlooked unless investigated explicitly. Ecke et al. (2017) concluded that sublethal Pb exposure in golden eagles (*Aquila chrysaetos*) might impair flight performance and thus increase the risk of mortality from other causes. Yet, other eagle studies could not find evidence that subclinical Pb concentrations are linked to “increased risk of trauma” (Isomursu et al., 2018) or “other causes of mortality” (Franson & Russell, 2014). These examples demonstrate the difficulty in investigating subclinical and chronic health effects in wildlife, especially when studies do not classify effects similarly. Furthermore, ethical permits to conduct Pb toxicological effect studies on wild mammals and other wildlife are, rightly so, unlikely to be granted. Therefore, subclinical effects are difficult to confirm in wildlife.

Although the impacts of Pb exposure on human, laboratory mammalian, and wild avian health are well-documented, there is still much to learn about the impacts on wild mammal health. In contrast to important infectious diseases in animals, the World Organisation for Animal Health does not have any reference laboratories for

any non-infectious diseases such as Pb poisoning, which could guide or help standardize how the diagnostic steps should be conducted (World Organisation for Animal Health, 2022). Potential strategies to research the health effects of Pb on wild mammals include behavioural studies, reproductive success analyses and immune system function assessments, which all come with challenges in free-ranging animals. Research which would be easier to achieve in free-ranging settings includes correlations between blood Pb concentrations and haematological and biochemical parameters, investigations of blood and tissue Pb concentrations to understand the distribution of Pb in the body, and investigations of tissue pathologies in Pb-exposed populations. Ideally, studies should use the most sensitive techniques available for measuring Pb (i.e., ICP-MS) (Apostoli, 2002) and report the results in dry weight for easier comparisons. Regardless of the approach taken by future studies to investigate Pb exposure and health effects in wild mammals, studies should report details of the study area, sex and age of animals, analytical methods, and also provide an appropriate range of descriptive statistics (e.g. sample sizes, standard deviations/errors), which would facilitate future evidence-based policies.

2.4.3 Methodologies, kinetics and thresholds

This systematic review has found a lack of consistency in estimating Pb concentrations, health effects and how this is reported. The different Pb concentration analytical methods used in the literature reviewed (e.g., atomic absorption spectroscopy and inductively coupled plasma mass spectrometry) vary in sensitivity and differ in susceptibility to matrix effects (Apostoli, 2002). Therefore, some studies could have failed to detect Pb if present in low concentrations. In addition, some studies reported tissue concentrations by dry weight and others by wet weight, impeding direct comparison of such concentrations. Furthermore, Pb uptake and storage distribution could potentially differ between species. As there are no studies available on Pb kinetics in wild mammals, this remains unknown. Variations in methodologies and target species make it difficult to compare Pb concentrations between studies and between species. Consequently, it is not possible to establish toxicity thresholds for wild mammals based on the current body of literature. However, although thresholds could be a valuable tool for some professions (e.g., veterinarians in clinical practice), a pragmatic reason for not attempting to establish threshold values for

different wild mammalian tissues and/or species is the risk of creating the false impression of acceptable or safe levels of Pb exposure in wild mammals. Therefore, we recommend stopping the use of threshold values for Pb concentrations in wildlife research, as already suggested by Pain et al. (2019).

2.4.4 Designing future studies on lead (Pb) exposure and health effects

From a One Health perspective, human, animal and ecosystem health are interlinked. Whilst it would be beneficial to study Pb concentrations in species at differing trophic levels in the wild, the cost of periodic screening programs is likely to be restricted to only a few selected species. Since Pb bioaccumulates in both plants and animals (Clemens, 2006; Radomyski et al., 2018), testing for Pb in carnivorous and scavenging mammalian species (i.e., species at higher trophic levels) could aid monitoring of environmental Pb exposure, which may also indicate the potential exposure of humans to Pb (O'Brien et al., 1993). Using higher trophic-level species as sentinels is already being utilized in avian Pb research (Monclús et al., 2020), and in mammalian research on infectious diseases and antimicrobial resistance (Millán et al., 2014; Sacristán et al., 2020). Another benefit of studying top predatory and/or scavenging mammals, which are often iconic and charismatic (Albert et al., 2018), is that these mammals might attract more attention from stakeholders and policymakers involved in the regulation of Pb, a phenomenon already seen with charismatic species receiving most conservation attention (Sitas et al., 2009).

To assess or monitor short-term and recent Pb exposure using reduced sampling effort and non-invasive sampling, antlers or hairs might be suitable tissues to consider. Antlers represent around six months' worth of Pb exposure, spring to autumn, and have previously been used to assess Pb contamination (Ludolphy et al., 2022). Antlers do, however, introduce potential biases into a Pb monitoring program. For most wild cervid species, detecting differences between age classes and sexes would not be possible using this tissue. Hair is often easily available even from museum specimens (i.e., on pelts), and it can be collected from free-ranging mammals non-invasively using hair traps. The period of Pb exposure can be estimated using laser ablation techniques if the particular species' hair growth cycle is known and the sampling is carried out in a consistent manner (Sela et al., 2007).

To gain information on Pb kinetics in a particular wild mammalian species, researchers would have to analyse multiple tissues and blood all obtained from the same individual at the same time (Bergdahl & Skerfving, 2022; Krone, 2018). However, obtaining this amount of samples would likely only be possible in freshly dead or mildly autolyzed individuals (McAloose et al., 2018). Additionally, extra expenses from analysing multiple samples from each individual could be cost-prohibited. Therefore, more controlled studies on the kinetics and clinical pathology of Pb in captive individuals could help to understand the health effects of Pb in wild mammals.

This review highlights that few studies on Pb exposure in wild mammals have been conducted in developing countries. However, it is important to consider that this does not mean the problem is less prevalent in these countries. Indeed, the lack of studies could be explained by lower access to research funding (Kpokiri et al., 2022). On the contrary, environmental exposure to Pb might be greater in developing countries due to fewer legislative controls on Pb use in products, as seen with Pb-based paints (World Health Organization, 2022b). Indeed, children in developing countries are more exposed to Pb than children in developed countries (Bergdahl & Skerfving, 2022; Hwang et al., 2019). Therefore, more research on Pb exposure in wild mammals must be conducted and published in these countries to understand the levels of environmental exposure to Pb, the risk to human and animal health, and to study whether legislation on Pb in products affects the Pb present in the environment.

As all organisms are exposed to Pb, studies helping to determine the impacts of Pb on different species will add to current knowledge of this toxic metal. Collectively, this increase in knowledge will create stronger evidence to influence policymakers involved in the regulation of Pb in products which continue to act as sources of environmental contamination and be a major One Health issue. Overall, Pb exposure in wild mammalian species must be recognised as an issue, as this systematic review demonstrates. Control and regulation of Pb need to take a One Health approach and not focus on single taxa groups when developing policies.

Acknowledgements

We thank Victoria Boskett (Research Librarian at Nottingham Trent University) for her help with journal indexing and database search engines.

References

Agency for Toxic Substances and Disease Registry (ATSDR). (2020). *Toxicological profile for lead*. https://doi.org/http://dx.doi.org/10.15620/cdc:95222

Albert, C., Luque, G. M., & Courchamp, F. (2018). The twenty most charismatic species. *PLOS ONE*, *13*(7), e0199149. https://doi.org/10.1371/JOURNAL.PONE.0199149

Apostoli, P. (2002). Elements in environmental and occupational medicine. *Journal of Chromatography B*, *778*(1–2), 63–97. https://doi.org/10.1016/S0378-4347(01)00442-X

Bergdahl, I. A., & Skerfving, S. (2022). Lead. In G. F. Nordberg & M. Costa (Eds.), *Handbook on the Toxicology of Metals: Vol. II: Specific Metals* (Fifth Edition, pp. 427–493). Academic Press. https://doi.org/10.1016/B978-0-12-822946-0.00036-2

Brumbaugh, W. G., Mora, M. A., May, T. W., & Phalen, D. N. (2010). Metal exposure and effects in voles and small birds near a mining haul road in Cape Krusenstern National Monument, Alaska. *Environmental Monitoring and Assessment*, *170*(1–4), 73–86. https://doi.org/10.1007/s10661-009-1216-y

Burco, J., Myers, A. M., Schuler, K., & Gillin, C. (2012). Acute lead toxicosis via ingestion of spent ammunition in a free-ranging cougar (*Puma concolor*). *Journal of Wildlife Diseases*, *48*(1), 216–219. https://doi.org/10.7589/0090-3558-48.1.216

Calvert, H. S. (1876). Pheasants poisoned by swallowing shot. *The Field*, *47*.

Centers for Disease Control and Prevention. (2022). *Blood Lead Reference Value*. Childhood Lead Poisoning Prevention. https://www.cdc.gov/nceh/lead/data/blood-lead-reference-value.htm

Ceruti, R., Ghisleni, G., Ferretti, E., Cammarata, S., Sonzogni, O., & Scanziani, E. (2002). Wild rats as monitors of environmental lead contamination in the urban area of Milan, Italy. *Environmental Pollution*, *117*(2), 255–259. https://doi.org/10.1016/S0269-7491(01)00273-1

Chen, Y.-P., Liu, Q., Ma, Q.-Y., Maltby, L., Ellison, A. M., & Zhao, Y. (2018). Environmental toxicants impair liver and kidney function and sperm quality of captive pandas. *Ecotoxicology and Environmental Safety*, *162*, 218–224. https://doi.org/10.1016/j.ecoenv.2018.07.008

Clemens, S. (2006). Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie*, *88*(11), 1707–1719. https://doi.org/10.1016/J.BIOCHI.2006.07.003

Constable, P. D., Hinchcliff, K. W., Done, S. H., & Grünberg, W. (2017). Diseases of the Nervous System. In *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats* (11th ed., pp. 1155–1370). Elsevier Ltd. https://doi.org/10.1016/B978-0-7020-5246-0.00014-0

da Silva, J., de Freitas, T. R. O., Heuser, V., Marinho, J. R., Bittencourt, F., Cerski, C. T. S., Kliemann, L. M., & Erdtmann, B. (2000). Effects of chronic exposure to coal in wild rodents (Ctenomys torquatus) evaluated by multiple methods and tissues. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *470*(1), 39–51. https://doi.org/10.1016/S1383-5718(00)00094-2

Damek-Poprawa, M., & Sawicka-Kapusta, K. (2003). Damage to the liver, kidney, and testis with reference to burden of heavy metals in yellow-necked mice from areas around steelworks and zinc smelters in Poland. *Toxicology*, *186*(1–2), 1–10. https://doi.org/10.1016/S0300-483X(02)00595-4

Datawrapper GmbH. (2022). Datawrapper. https://www.datawrapper.de/

Diters, R. W., & Nielsen, S. W. (1978). Lead poisoning of raccoons in Connecticut. *Journal of Wildlife Diseases*, *14*(2), 187–192. https://doi.org/10.7589/0090-3558-14.2.187

Ecke, F., Singh, N. J., Arnemo, J. M., Bignert, A., Helander, B., Berglund, Å. M. M., Borg, H., Bröjer, C., Holm, K., Lanzone, M., Miller, T., Nordström, Å., Räikkönen, J., Rodushkin, I., Ågren, E., & Hörnfeldt, B. (2017). Sublethal Lead Exposure Alters Movement Behavior in Free-Ranging Golden Eagles. *Environmental Science and Technology*, *51*(10), 5729–5736. https://doi.org/10.1021/acs.est.6b06024

EFSA Panel on Contaminants in the Food Chain (CONTAM). (2013). Scientific opinion on lead in food. *EFSA Journal*. https://doi.org/10.2903/j.efsa.2010.1570

European Chemicals Agency. (2018). *Annex XV investigation report: A review of the available information on lead in shot used in terrestrial environments, in ammunition and in fishing tackle*.

Franson, J. C., & Pain, D. J. (2011). Lead in Birds. In W. N. Beyer & J. P. Meador (Eds.), *Environmental contaminants in biota: Interpreting tissue concentrations* (2nd ed., pp. 563–593). CRC Press.

Franson, J. C., & Russell, R. E. (2014). Lead and eagles: demographic and pathological characteristics of poisoning, and exposure levels associated with other causes of mortality. *Ecotoxicology*, *23*(9), 1722–1731. https://doi.org/10.1007/S10646-014-1337-0/FIGURES/3

Green, R. E., Hunt, W. G., Parish, C. N., & Newton, I. (2008). Effectiveness of action to reduce exposure of free-ranging California condors in Arizona and Utah to lead from spent ammunition. *PLoS ONE*, *3*(12), e4022. https://doi.org/10.1371/journal.pone.0004022

Hwang, Y. H., Hsiao, C. K., & Lin, P. W. (2019). Globally temporal transitions of blood lead levels of preschool children across countries of different categories of Human Development Index. *Science of The Total Environment*, *659*, 1395–1402. https://doi.org/10.1016/J.SCITOTENV.2018.12.436

Ieradi, L. A., Cristaldi, M., Mascanzoni, D., Cardarelli, E., Grossi, R., & Campanella, L. (1996). Genetic damage in urban mice exposed to traffic pollution. *Environmental Pollution*, *92*(3), 323–328. https://doi.org/10.1016/0269-7491(95)00109-3

Isomursu, M., Koivusaari, J., Stjernberg, T., Hirvelä-Koski, V., & Venäläinen, E. R. (2018). Lead poisoning and other human-related factors cause significant mortality in white-tailed eagles. *Ambio*, *47*(8), 858–868. https://doi.org/10.1007/s13280-018-1052-9

IUCN. (2021). *The IUCN Red List of Threatened Species. Version 2021-3*. https://www.iucnredlist.org

Ives, A. M., Brenn-White, M., Buckley, J. Y., Kendall, C. J., Wilton, S., & Deem, S. L. (2022). A Global Review of Causes of Morbidity and Mortality in Free-Living Vultures. *EcoHealth*, *19*(1), 40–54. https://doi.org/10.1007/S10393-021-01573-5/TABLES/5

Kpokiri, E. E., Sri-Pathmanathan, C., Shrestha, P., Navaid, S., Wiwatpanit, T., Wijegunawardana, A., Umar, M. A., Jackson, D., Alger, J., Bohren, M. A., Hoole, M., Labarda, M., Juban, N., Launois, P., Tang, W., Halpaap, B., & Tucker, J. D. (2022). Crowdfunding for health research: a qualitative evidence synthesis and a pilot programme. *BMJ Global Health*, *7*(7), e009110. https://doi.org/10.1136/BMJGH-2022-009110

Krone, O. (2018). Lead poisoning in birds of prey. In J. H. Sarasola, J. M. Grande, & J. J. Negro (Eds.), *Birds of Prey: Biology and conservation in the XXI century* (pp. 251–272). Springer International Publishing AG. https://doi.org/10.1007/978-3-319-73745-4_11/FIGURES/6

Lanphear, B. P., Rauch, S., Auinger, P., Allen, R. W., & Hornung, R. W. (2018). Low-level lead exposure and mortality in US adults: a population-based cohort study. *The Lancet Public Health*, *3*(4), e177–e184. https://doi.org/10.1016/S2468-2667(18)30025-2

Lazarus, M., Orct, T., Sergiel, A., Vranković, L., Marijić, V. F., Rašić, D., Reljić, S., Aladrović, J., Zwijacz-Kozica, T., Zięba, F., Selva, N., & Huber. (2020). Metal(loid) exposure assessment and biomarker responses in captive and free-ranging European brown bear (*Ursus arctos*). *Environmental Research*, *183*, 109166. https://doi.org/10.1016/j.envres.2020.109166

Ludolphy, C., Kierdorf, U., & Kierdorf, H. (2022). Antlers of European roe deer (*Capreolus capreolus*) as monitoring units to assess lead pollution in a floodplain contaminated by historical metal ore mining, processing, and smelting in the Harz Mountains, Germany. *Environmental Pollution*, *302*. https://doi.org/10.1016/j.envpol.2022.119080

Ma, W.-C. (2011). Lead in mammals. In W. N. Beyer & J. P. Meador (Eds.), *Environmental Contaminants in Biota - Interpreting Tissue Concentrations* (Second Edition, pp. 595–608). CRC Press.

McAloose, D., Colegrove, K. M., & Newton, A. L. (2018). Wildlife Necropsy. In K. A. Terio, D. McAloose, & J. st. Leger (Eds.), *Pathology of Wildlife and Zoo Animals* (p. 19). Academic Press. https://doi.org/10.1016/B978-0-12-805306-5.00001-8

Millán, J., García, E. J., Oleaga, Á., López-Bao, J. V., Llaneza, L., Palacios, V., Candela, M. G., Cevidanes, A., Rodríguez, A., & León-Vizcaíno, L. (2014). Using a top predator as a sentinel for environmental contamination with pathogenic bacteria: The Iberian wolf and leptospires. *Memorias Do Instituto Oswaldo Cruz*, *109*(8), 1041–1044. https://doi.org/10.1590/0074-0276140258

Monclús, L., Shore, R. F., & Krone, O. (2020). Lead contamination in raptors in Europe: A systematic review and meta-analysis. *Science of the Total Environment*, *748*, 141437. https://doi.org/10.1016/j.scitotenv.2020.141437

Mouw, D., Kalitis, K., Anver, M., Schwartz, J., Constan, A., Hartung, R., Cohen, B., & Ringler, D. (1975). Lead. Possible toxicity in urban vs rural rats. *Archives of Environmental Health*, *30*(6), 276–280. https://doi.org/10.1080/00039896.1975.10666699

North, M. A., Lane, E. P., Marnewick, K., Caldwell, P., Carlisle, G., & Hoffman, L. C. (2015). Suspected lead poisoning in two captive cheetahs (*Acinonyx jubatus jubatus*) in South Africa, in 2008 and 2013. *Journal of the South African Veterinary Association*, *86*(1). https://doi.org/10.4102/jsava.v86i1.1286

O'Brien, D. J., Kaneene, J. B., & Poppenga, R. H. (1993). The use of mammals as sentinels for human exposure to toxic contaminants in the environment. In *Environmental Health Perspectives* (Vol. 99, pp. 351–368). National Institute of Environmental Health Sciences. https://doi.org/10.1289/ehp.9399351

Organisation for Economic Co-operation and Development (OECD). (2006). *Developed, developing countries*. Glossary of Statistical Terms. https://stats.oecd.org/glossary/detail.asp?ID=6326

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., … Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*, *372*, n71. https://doi.org/10.1136/BMJ.N71

Pain, D. J., Mateo, R., & Green, R. E. (2019). Effects of lead from ammunition on birds and other wildlife: A review and update. *Ambio*, *48*(9), 935–953. https://doi.org/10.1007/s13280-019-01159-0

Parelho, C., Bernardo, F., Camarinho, R., Rodrigues, A. S., & Garcia, P. (2016). Testicular damage and farming environments - An integrative ecotoxicological link. *Chemosphere*, *155*, 135–141. https://doi.org/10.1016/j.chemosphere.2016.04.043

Radomyski, A., Lei, K., Giubilato, E., Critto, A., Lin, C., & Marcomini, A. (2018). Bioaccumulation of trace metals in aquatic food web. A case study, Liaodong Bay, NE China. *Marine Pollution Bulletin*, *137*, 555–565. https://doi.org/10.1016/J.MARPOLBUL.2018.11.002

Rappaport, S. C., Strebel, R. F., Garner, E., & Craston, D. (1975). Lead insult to the dentition in the leopard. *Pharmacology and Therapeutics in Dentistry*, *2*(3–4), 201–207.

Renberg, I., Bindler, R., & Brännvall, M. L. (2001). Using the historical atmospheric lead-deposition record as a chronological marker in sediment deposits in Europe. *Holocene*, *11*(5), 511–516. https://doi.org/10.1191/095968301680223468

Roberts, R. D., Johnson, M. S., & Hutton, M. (1978). Lead contamination of small mammals from abandoned metalliferous mines. *Environmental Pollution (1970)*, *15*(1), 61–69. https://doi.org/10.1016/0013-9327(78)90061-7

Rogival, D., Scheirs, J., De Coen, W., Verhagen, R., & Blust, R. (2006). Metal blood levels and hematological characteristics in wood mice (*Apodemus sylvaticus* L.) along a metal pollution gradient. *Environmental Toxicology and Chemistry; Oxford*, *25*(1), 149–157. https://doi.org/10.1897/04-659R1.1

Sacristán, I., Esperón, F., Acuña, F., Aguilar, E., García, S., López, M. J., Cevidanes, A., Neves, E., Cabello, J., Hidalgo-Hermoso, E., Poulin, E., Millán, J., & Napolitano, C. (2020). Antibiotic resistance genes as landscape anthropization indicators: Using a wild felid as sentinel in Chile. *Science of the Total Environment*, *703*, 134900. https://doi.org/10.1016/j.scitotenv.2019.134900

Sánchez-Chardi, A., Ribeiro, C. A. O., & Nadal, J. (2009). Metals in liver and kidneys and the effects of chronic exposure to pyrite mine pollution in the shrew Crocidura russula inhabiting the protected wetland of Doñana. *Chemosphere*, *76*(3), 387–394. https://doi.org/10.1016/j.chemosphere.2009.03.036

Sauer, R. M., Zook, B. C., & Garner, F. M. (1970). Demyelinating encephalomyelopathy associated with lead poisoning in nonhuman primates. *Science*, *169*(3950), 1091–1093. https://doi.org/10.1126/science.169.3950.1091

Sela, H., Karpas, Z., Zoriy, M., Pickhardt, C., & Becker, J. S. (2007). Biomonitoring of hair samples by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). *International Journal of Mass Spectrometry*, *261*(2–3), 199–207. https://doi.org/10.1016/J.IJMS.2006.09.018

Shlosberg, A., Bellaiche, M., Regev, S., Gal, R., Brizzi, M., Hanji, V., Zaidel, L., & Nyska, A. (1997). Lead toxicosis in a captive bottlenose dolphin (*Tursiops truncatus*) consequent to ingestion of air gun pellets. *Journal of Wildlife Diseases*, *33*(1), 135–139. https://doi.org/10.7589/0090-3558-33.1.135

Sitas, N., Baillie, J. E. M., & Isaac, N. J. B. (2009). What are we saving? Developing a standardized approach for conservation action. *Animal Conservation*, *12*(3), 231–237. https://doi.org/10.1111/J.1469-1795.2009.00244.X

Skerratt, L. F., Speare, R., Berger, L., & Winsor, H. (1998). Lyssaviral infection and lead poisoning in black flying foxes from Queensland. *Journal of Wildlife Diseases*, *34*(2), 355–361. https://doi.org/10.7589/0090-3558-34.2.355

Sonne, C., Adams, D. H., Alstrup, A. K. O., Lam, S. S., Dietz, R., & Kanstrup, N. (2022). Denmark passes total ban of leaded ammunition. *Science*, *377*(6610), 1054–1055. https://doi.org/10.1126/SCIENCE.ADE3150/ASSET/9C28339E-1FF5-4662-B2F0-E5B14D9A2C4F/ASSETS/SCIENCE.ADE3150.FP.PNG

Stansley, W., & Roscoe, D. E. (1996). The uptake and effects of lead in small mammals and frogs at a trap and skeet range. *Archives of Environmental Contamination and Toxicology*, *30*(2), 220–226. https://doi.org/10.1007/BF00215801

Tête, N., Durfort, M., Rieffel, D., Scheifler, R., & Sánchez-Chardi, A. (2014). Histopathology related to cadmium and lead bioaccumulation in chronically exposed wood mice, *Apodemus sylvaticus*, around a former smelter. *Science of the Total Environment*, *481*(1), 167–177. https://doi.org/10.1016/j.scitotenv.2014.02.029

Wilson, D. E., & Mittermeier, R. A. (Eds.). (2009). *Handbook of the Mammals of the World: Vol. 9 volumes*. Lynx Edicions.

World Health Organization. (2022a). *Lead poisoning*. https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health

World Health Organization. (2022b). *Legally-binding controls on lead paint*. The Global Health Observatory. https://www.who.int/data/gho/data/themes/topics/indicator-groups/legally-binding-controls-on-lead-paint

World Organisation for Animal Health. (2022). *Reference Laboratories*. https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3

Zook, B. C., Eisenberg, J. F., & McLanahan, E. (1973). Some factors affecting the occurrence of lead poisoning in captive primates. *Journal of Medical Primatology*, *2*(3–4), 206–217. https://doi.org/10.1159/000460323

Zook, B. C., Sauer, R. M., & Garner, F. M. (1970). Lead poisoning in Australian fruit bats (*Pteropus poliocephalus*). *Journal of the American Veterinary Medical Association*, *157*(5), 691–694.

Zook, B. C., Sauer, R. M., & Garner, F. M. (1972). Lead poisoning in captive wild animals. *Journal of Wildlife Diseases*, *8*(3), 264–272. https://doi.org/10.7589/0090-3558-8.3.264

Chapter 3: Correlations between blood lead (Pb) concentrations and common blood variables in Scandinavian brown bears (*Ursus arctos*)

Abstract

Lead (Pb) is a toxic non-essential element affecting human, animal and environmental health. The free-ranging Scandinavian brown bear (*Ursus arctos*) population is highly exposed to Pb as evidenced by high blood Pb concentrations. To investigate whether these high blood Pb concentrations affect the health of the bears, correlations between blood Pb concentration and 24 haematological and 16 biochemical variables commonly used in-house were analysed (118 samples from 86 free-ranging bears). Haemoglobin, alanine aminotransferase, total bilirubin and creatinine showed significant positive correlations with blood Pb concentration. These are indicative of liver and chronic kidney disease. However, no variable had a strong correlation (i.e., $| r_s | > 0.5$) with blood Pb concentration, so a cheap in-house screening test to monitor Pb exposure is yet to be identified. Captive brown bears in Scandinavia are also exposed to Pb, and the different age and sex groups show similar exposure patterns to free-ranging bears. Monitoring Pb exposure in top predatory and scavenging wildlife continues to be important from a One Health perspective. Further investigations into other health variables are warranted to determine if brown bear health is affected by Pb exposure.

Key words: biochemistry, haematology, health effects, renal disease, toxicology, wildlife population health

3.1 Introduction

Lead (Pb) is a non-essential toxic element known to cause negative health effects in humans and other vertebrates, affecting a large range of organ systems, including the neurological, hepatic, renal, haematopoietic and immune systems (Bergdahl & Skerfving, 2022; Flora, 2014).

It has previously been shown that the free-ranging Scandinavian brown bear (*Ursus arctos*) population is highly exposed to environmental Pb (Boesen et al., 2019; Fuchs et al., 2021). For example, the mean whole blood Pb concentration of 96.6 µg/l (range: 38.7-220.5 µg/l; 153 samples from 110 bears) is 8 times higher than the European Food Safety Authority's (EFSA) 95th percentile lower confidence limit of the benchmark dose ($BMDL_{01}$) for developmental neurotoxicity in children (12 µg/l), and over 2.5 times higher than the Centers for Disease Control and Prevention's (CDC) "blood lead reference value" (35 µg/l) used to identify children exposed to Pb, which require additional actions to be taken by health care professionals (Centers for Disease Control and Prevention, 2022; EFSA Panel on Contaminants in the Food Chain (CONTAM), 2013; Fuchs et al., 2021). Whilst it is unrealistic to expect zero presence of Pb in living organisms, such high whole blood Pb concentrations are assumed to have a negative health effect on the bears, especially given that no Pb exposure is considered safe in humans (World Health Organization, 2022). Additionally, a systematic literature review on Pb exposure and health effects in wild mammals (Chapter 2) showed that Pb exposure has been confirmed in a wide variety of species and that health effects in this taxon are similar to the health effects seen in humans and other mammals (Bergdahl & Skerfving, 2022; Ma, 2011). However, negative health effects might be subclinical or difficult to detect because the bears are free-ranging wild animals (Ryser-Degiorgis, 2013).

The World Health Organization (WHO) uses the following definition of health: "*Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity"* (World Health Organization, 2020). Therefore, it is clear that "health" might be difficult to measure. The World Organisation for Animal Health (WOAH) does not have a definition of animal health, and when it comes to the term "wildlife health" there is also no single definition. Despite this, the analysis of haematological and biochemical variables is

widely used in human and veterinary clinical medicine and research settings, including wildlife medicine. Such analyses can inform on the severity of disease in a patient with clinical signs but also detect disease in the subclinical patient (Evans, 2009; Stockham & Scott, 2008). When used as diagnostic screening tools in subclinical patients, haematological and biochemical variables play a crucial part in preventive medicine.

As disease is difficult to ascertain in free-ranging animals (Ryser-Degiorgis, 2013), this chapter aims to determine 1) if any common in-house haematological or biochemical blood variables are correlated with whole blood Pb concentrations in brown bears, which could indicate disease processes occurring in specific organ systems; 2) whether a cheap blood variable could be used as a screening test over the expensive Pb analysis in the monitoring of Pb exposure in Scandinavian brown bears; 3) if captive Scandinavian brown bears from the same geographical region as the free-ranging bears are also exposed to Pb.

3.2 Material and methods

3.2.1 Free-ranging brown bears

A total of 86 free-ranging brown bears were captured in Dalarna and Gävleborg Counties in central Sweden (~61°N, 15°E; Figure 3.1. The study area consists of intensively managed coniferous forest (80 %) with trees of different ages up to 100 years old. Lakes and bogs are the other two main land cover types (Martin et al., 2010). There are no industrial activities close to where the brown bears live. The captures were conducted by the Scandinavian Brown Bear Research Project (SBBRP) between mid-April and mid-June (ordinal dates 101-163) of 2020 (*n* = 62) and 2021 (*n* = 56). Thirty-two bears were captured in both 2020 and 2021, resulting in a total of 118 sampling events. Not all analyses were carried out at all sampling events. For bears captured in both 2020 and 2021, only the 2020 sample was used for population Pb concentration calculations. The bears were darted from a helicopter with a mix of medetomidine and tiletamine-zolazepam in the darts for general anaesthesia, as described by Arnemo & Evans (2017).

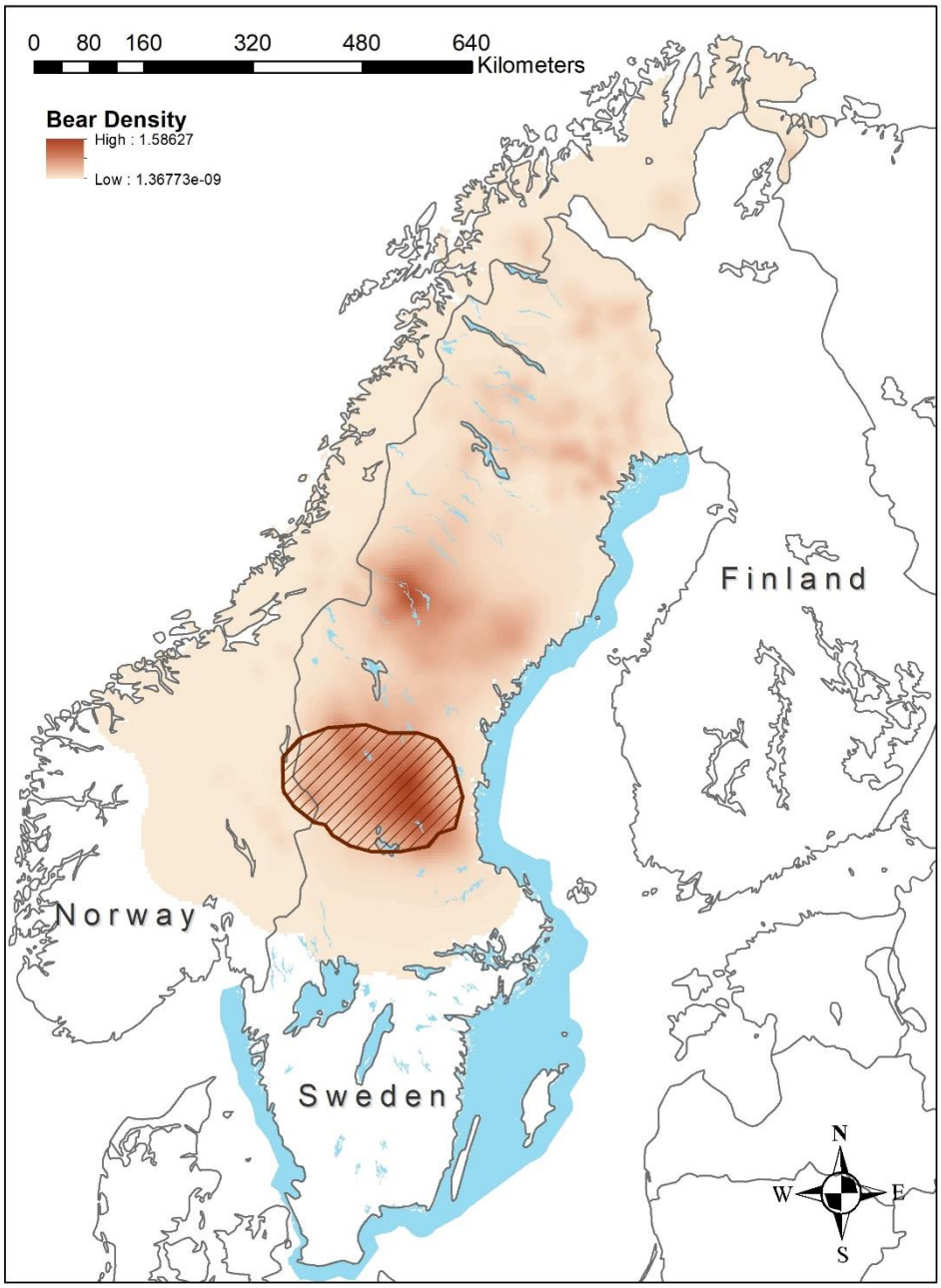

Figure 3.1 Scandinavian brown bear (*Ursus arctos*) density in Sweden and Norway. The study area of the Scandinavian Brown Bear Research Project is indicated by the dark red hashed area (Scandinavian Brown Bear Research Project, 2023).

Blood samples were collected from the jugular vein using a vacutainer system while the bear was fully anaesthetised and in lateral or dorsal recumbency. Blood samples were collected in VACUETTE® 8 ml CAT Serum Separator Clot Activator tubes, 4 ml K3E K3EDTA tubes (EDTA), 6 ml NH Trace Elements Sodium Heparin tubes (TE) and 8 ml LH Lithium Heparin Separator tubes (LH, Greiner Bio-One International GmbH, Kremsmünster, Austria). The blood samples were stored with

ice packs in the field for optimising storage temperature (i.e., to prevent haemolysis).

Ninety-five EDTA samples were haematologically analysed immediately upon return to the SBBRP field station or stored at 5 °C prior to analysis the same day as the sample collection. Twenty-four haematological variables (Table 3.1) were analysed using a desktop veterinary analyser for in-house haematology (ABAXIS VETSCAN® HM5 Hematology Analyzer, Union City, CA, USA), using the species setting "dog". This setting was chosen following advice from the manufacturer, as brown bear blood cells were expected to be most similar to dog blood cells out of the domestic species for which the analyser was calibrated.

Table 3.1 Haematological variables analysed using a VETSCAN® HM5 Hematology Analyzer in Swedish brown bears (*Ursus arctos*).

Blood cell type	Abbreviation	Name of variable	Unit
Leucocytes (white blood cells)	WBC	White blood cell count	10^9/l
	LYM	Lymphocyte count	10^9/l
	MON	Monocyte count	10^9/l
	NEU	Neutrophil count	10^9/l
	EOS	Eosinophil count	10^9/l
	BAS	Basophil count	10^9/l
	LYMp	Lymphocyte percentage	%
	MONp	Monocyte percentage	%
	NEUp	Neutrophil percentage	%
	EOSp	Eosinophil percentage	%
	BASp	Basophil percentage	%
Erythrocytes (red blood cells)	RBC	Red blood cell count	10^{12}/l
	HGB	Haemoglobin	g/l
	HCT	Haematocrit	%
	MCV	Mean corpuscular volume	fl
	MCH	Mean corpuscular haemoglobin	pg
	MCHC	Mean corpuscular haemoglobin concentration	g/l
	RDWs	Red cell distribution width, standard deviation	fl
	RDWc	Red cell distribution width, coefficient of variation	%
Thrombocytes (platelets)	PLT	Platelet count	10^9/l
	PCT	Platelet crit	%
	MPV	Mean platelet volume	fl
	PDWs	Platelet distribution width, standard deviation	fl
	PDWc	Platelet distribution width, coefficient of variation	%

Blood smears were created manually using EDTA whole blood from the 95 samples which haematological analyses were carried out on. Each blood smear was manually stained with Pappenheim's stain (May-Grünwald-Giemsa stain) following the manufacturer's instructions (Merck KGaA, Darmstadt, Germany). Blood smears were stored in glass slide boxes at room temperature until analysis.

The blood smears were screened by the author using a Nikon ECLIPSE Ci microscope (Nikon Europe B.V., Amstelveen, the Netherlands) for pathological evidence of Pb poisoning, including anisocytosis, poikilocytosis, polychromasia, basophilic stippling and increased (>5/100 leucocytes) presence of nucleated red blood cells (Cornell University College of Veterinary Medicine, 2020; Stockham & Scott, 2008). Additionally, a manual white blood cell differential count was conducted by counting 100 leucocytes (band neutrophils, segmented neutrophils, eosinophils, basophils, lymphocytes and monocytes), as a quality control of the VETSCAN® HM5 Hematology Analyzer results.

Concentrations and histograms produced by the VETSCAN® HM5 Hematology Analyzer were evaluated visually by the author and an ABAXIS Senior Specialist in Clinical Diagnostics, to determine if there was evidence of sample mixing error or platelet clumping issues. If such problems were identified, all variable results regarding the particular cell type (i.e., leucocytes, erythrocytes, thrombocytes) were removed from the analysis (4, 7 and 7 from leucocytes, erythrocytes and thrombocytes, respectively). Therefore, 91 leucocytic, 88 erythrocytic and 88 thrombocytic sample results were used in the analysis.

Serum blood collection tubes were centrifuged on the day of collection (30,000 RPM, 15 minutes, room temperature), and serum stored at room temperature prior to biochemical analysis, which occurred within 1 hour of the tube being centrifuged. Sixteen biochemical variables of the serum (Table 3.2) were analysed at the SBBRP field station on serum using a desktop veterinary analyser for in-house biochemistry (VETSCAN® VS2 Chemistry Analyzer, ABAXIS). To get the broadest selection of variables indicating liver and kidney disease, the VS2 Comprehensive Diagnostic Profile test rotor (ABAXIS) was originally chosen for bears captured in 2020; measuring 14 variables. With additional funding available for VS2 test rotors in 2021, the VS2 T4/Cholesterol Profile test rotor (ABAXIS) was added for bears captured in 2021 to add variables indicative of endocrine disorders. Therefore, the Comprehensive Diagnostic Profile test rotor was used on 117 serum samples in 2020-2021, and the T4/Cholesterol Profile test rotor was used on 56 serum samples in 2021. Thirteen measurements across all biochemical variables were beyond the limits of the VETSCAN® VS2 Chemistry Analyzer. For each of these measurements, the measurement was edited to the

highest or lowest possible value for measurements below and above the analyser limits, respectively.

Table 3.2 Biochemical variables analysed using an VETSCAN® VS2 Chemistry Analyzer in Swedish brown bears (*Ursus arctos*).

Test profile name	Abbreviation	Name of variable	Unit
Comprehensive Diagnostic	ALB	Albumin, bromocresol green	g/dl
	ALP	Alkaline phosphatase	U/l
	ALT	Alanine aminotransferase	U/l
	AMY	Amylase	U/l
	TBIL	Total bilirubin	µmol/l
	BUN	Blood urea nitrogen	mmol/l
	CALC	Calcium	mmol/l
	PHOS	Phosphorus	mmol/l
	CRE	Creatinine	µmol/l
	GLU	Glucose	mmol/l
	SODI	Sodium	mmol/l
	POTA	Potassium	mmol/l
	TP	Total protein	g/l
	GLOB	Globulin, calculated value	g/l
T4/Cholesterol	T4	Thyroxine	nmol/l
	CHOL	Cholesterol	mmol/l

Although the selection of variables was focused on variables indicating disease in the haematopoietic, renal and hepatic systems due to Pb's known health effects (Bergdahl & Skerfving, 2022), other variables were included in the analysis when possible (i.e., as part of the haematological test or Comprehensive Diagnostic Profile test rotor, or when funds for additional test profile rotors were available). This was done to widen the search for a screening test that could potentially replace the costly Pb analysis for future exposure monitoring in the Scandinavian brown bear population, should a strong correlation (i.e., $| r_s | > 0.5$) be detected between Pb concentration and one of the variables.

3.2.2 Captive brown bears

Whole blood samples were obtained from 25 captive brown bears from 7 zoological institutions in Sweden and Denmark (Appendix C). The samples were collected by either the attending zoo veterinarian (n = 18) between 2019 and 2021, or by the author (n = 7) in 2021. All blood samples were collected when the animal was anaesthetised for another reason (e.g., general health check, translocation or surplus animal euthanasia). If multiple blood samples were taken from the same

bear on different dates, the one taken closest to spring (mid-April to mid-June) was used to get the most similar sampling period possible as for the free-ranging brown bears. Samples were collected between early March and mid-October (ordinal dates 66-294). These blood samples were collected using three different types of whole blood tubes of different brands depending on availability at the different zoological institutions: 9 TE, 9 LH and 7 EDTA tubes.

3.2.3 Lead (Pb) analysis and conversion factors

Whole blood samples collected from free-ranging (TE tubes) and captive brown bears (TE, EDTA and LH tubes) were stored at -20 °C and shipped frozen to an accredited laboratory (ALS Scandinavia AB, Luleå, Sweden) for whole blood Pb concentration analysis using sector field inductively coupled plasma mass spectrometry (ICP-SFMS, Söderberg et al., 2023).

TE tubes were preferred for Pb concentration analysis as these tubes are considered the gold standard blood collection test tube to use when measuring trace elements as per ALS Scandinavia. Therefore, conversions of LH and EDTA Pb concentrations were needed for the captive bears, but not needed for the free-ranging bears where TE tubes were collected from each individual.

EDTA concentrations from 6 captive bears were converted into TE equivalent concentrations as described by Fuchs et al. (2021):

Trace element concentration = 1.013 x EDTA concentration

LH concentrations from 9 captive bears were converted into TE equivalent concentrations using a conversion factor created from the Pb results from 28 TE and LH sample paired sets from 21 free-ranging and 7 captive brown bears collected in 2021 (R^2 = 0.9774; Appendix D):

Trace element concentration = (0.9743 x lithium heparin concentration) - 0.4658

For internal laboratory quality control, ALS Scandinavia analysed each whole blood sample between 1 and 3 times. Using a conservative approach, the lowest concentration for each sample was used.

3.2.4 Statistical analysis

A Shapiro-Wilk test for normality showed that the whole blood Pb concentration data in the free-ranging bears was not normally distributed ($p < 0.001$). Correlations between whole blood Pb concentrations and 24 haematological and 16 biochemical variables were calculated using Spearman's rank-order correlations. The Bonferroni correction was applied to avoid type I errors for both the haematological and biochemical variables (Van der Weele & Mathur, 2019). A Mann-A Whitney U test was used to determine if there was a difference between whole blood Pb concentration in free-ranging and captive bears. The analyses were carried out in IBM SPSS Statistics 28 (IBM Corp, 2021), with α set to 0.05.

3.2.5 Ethical approval

This study was approved by the Swedish Ethical Committee on Animal Research, Uppsala, Sweden (Dnr. 5.8.18-03376/2020), the Swedish Environmental Protection Agency, Stockholm, Sweden (NV-00741-18) and the School of Animal, Rural and Environmental Sciences Research Committee, Nottingham Trent University, Southwell, United Kingdom (ARE192009). This study only used a portion of samples already collected by the SBBRP or samples from captive brown bears anaesthetised or euthanised for reasons other than this study.

3.3 Results

3.3.1 Free-ranging brown bears

Analysis of the blood Pb concentrations from free-ranging brown bears showed that all age groups were exposed to Pb, with adult lactating females having the highest blood Pb concentration (Table 3.3).

Table 3.3 Whole blood concentrations (µg/l) of lead (Pb) in 86 free-ranging Scandinavian brown bears (*Ursus arctos*) captured in 2020 (*n* = 62) and 2021 (*n* = 24).

Age/sex group[a]		*n*	Mean	SD	Median	Minimum	Maximum
Unknown		5	77.0	38.7	64.3	42.2	135.1
Yearlings		18	79.1	39.0	70.9	40.1	216.0
Sub-adults		22	77.9	42.7	58.2	38.1	199.3
Adults	Males[b]	8	56.0	29.3	51.9	22.6	102.4
	Non-L females[c]	20	75.5	20.9	73.3	24.6	109.1
	L females[d]	13	100.3	24.9	93.1	69.6	154.7
Total		86	78.9	34.7	71.7	22.6	216.0

[a] Yearlings: 1 year old, sub-adults: 2-3 years old, adults: 4 or more years old. [b] Adult male age range: 4-19 years. [c] Non-lactating (Non-L) female age range: 19 females aged 4-9 years + 1 female aged minimum 4 years. [d] Lactating (L) female age range: 11 females aged 5-23 years + 2 females aged minimum 4 years.

There were significant positive correlations between whole blood Pb concentration of free-ranging bears and one erythrocyte variables (Table 3.4). There were no significant correlations with any leukocyte or thrombocyte variables. No pathologies were identified through screening of the blood smears.

Table 3.4 Pearson's product-moment correlations between lead (Pb) concentration and 24 haematological variables in free-ranging Scandinavian brown bears (*Ursus arctos*). Bonferroni-adjusted *p*-value was applied. Correlations were significant at the 0.05 level (two-tailed) and significant correlations are highlighted in bold.

Blood cell type	Variable[a]	r_s	*n*	*p*-value	Bonferroni[b]
Leucocytes (white blood cells)	WBC	-0.121	91	0.252	BL
	LYM	-0.230	91	0.028	0,672
	MON	-0.174	91	0.099	BL
	NEU	-0.075	91	0.479	BL
	EOS	-0.251	91	0.017	0.408
	BAS	-0.244	91	0.020	0.480
	LYMp	-0.113	91	0.288	BL
	MONp	-0.066	91	0.531	BL
	NEUp	0.175	91	0.097	BL
	EOSp	-0.185	91	0.080	BL
	BASp	-0.226	91	0.031	BL
Erythrocytes (red blood cells)	RBC	0.255	88	0.017	0.408
	HGB	**0.325**	**88**	**0.002**	**0.048**
	HCT	0.317	88	0,003	0.072
	MCV	0.177	88	0.100	BL
	MCH	0.254	88	0.017	0.408
	MCHC	0.138	88	0.201	BL
	RDWs	0.143	88	0.184	BL
	RDWc	0.013	88	0.902	BL
Thrombocytes (platelets)	PLT	-0.147	88	0.171	BL
	PCT	-0.148	88	0.170	BL
	MPV	-0.073	88	0.501	BL
	PDWs	-0.014	88	0.897	BL
	PDWc	0.001	88	0.993	BL

[a] White blood cell count (WBC); lymphocyte count (LYM); monocyte count (MON); neutrophil count (NEU); eosinophil count (EOS); basophil count (BAS); lymphocyte percentage (LYMp); monocyte percentage (MONp); neutrophil percentage (NEUp); eosinophil percentage (EOSp); basophil percentage (BASp); red blood cell count (RBC); haemoglobin (HGB); haematocrit (HCT); mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); red cell distribution width, standard deviation (RDWs); red cell distribution width, coefficient of variation (RDWc); platelet count (PLT); platelet crit (PCT); mean platelet volume (MPV); platelet distribution width, standard deviation (PDWs); platelet distribution width, coefficient of variation (PDWc).
[b] Bonferroni-adjusted *p*-value. BL: adjusted *p*-value exceeded 1 (beyond limit).

There was a significant positive correlation between whole blood Pb concentration of free-ranging bears and three biochemical variable (Table 3.5). There were no significant correlations with any variable of the T4/Cholesterol Profile.

Table 3.5 Pearson's product-moment correlations between lead (Pb) concentration and 16 biochemical variables in free-ranging Scandinavian brown bears (*Ursus arctos*). Bonferroni-adjusted *p*-value applied. Correlations were significant at the 0.05 level (two-tailed) and significant correlation is highlighted in bold.

Test profile name	Variable[a]	r_s	*n*	*p*-value	Bonferroni[b]
Comprehensive Diagnostic	ALB	-0.143	117	0.123	BL
	ALP	-0.217	117	0.019	0.304
	ALT	**-0.280**	**117**	**0.002**	**0.032**
	AMY	-0.100	117	0.282	BL
	TBIL	**0.291**	**117**	**0.001**	**0.016**
	BUN	-0.118	117	0.204	BL
	CALC	-0.245	117	0.008	0.128
	PHOS	-0.215	117	0.020	0.320
	CRE	**0.307**	**117**	**<0.001**	**0.016**
	GLU	-0.150	117	0.107	BL
	SODI	-0.252	117	0.006	0.096
	POTA	-0.245	117	0.008	0.128
	TP	-0.119	117	0.201	BL
	GLOB	-0.023	117	0.807	BL
T4/Cholesterol	T4	0.179	56	0.186	BL
	CHOL	0.066	56	0.631	BL

[a] Albumin, bromocresol green (ALB); alkaline phosphatase (ALP); alanine aminotransferase (ALT); amylase (AMY); total bilirubin (TBIL); blood urea nitrogen (BUN); calcium (CALC); phosphorus (PHOS); creatinine (CRE); glucose (GLU); sodium (SODI); potassium (POTA); total protein (TP); globulin, calculated value (GLOB); thyroxine (T4); cholesterol (CHOL).
[b] Bonferroni-adjusted *p*-value exceeded 1 (beyond limit, BL).

3.3.2 Captive brown bears

Analysis of the blood Pb concentrations from captive brown bears showed that all age groups were exposed to Pb, with adult females having the highest blood Pb concentration (Table 3.6).

Table 3.6 Whole blood concentrations (µg/l) of lead (Pb) in 25 captive brown bears (*Ursus arctos*) from Sweden (*n* = 24) and Denmark (*n* = 1) sampled between 2019 and 2021.

Age/sex group[a]		*n*	Mean	SD	Median	Minimum	Maximum
Cubs of the year		5	64.9	14.7	76.8	43.1	78.2
Yearlings		5	49.8	16.7	39.9	34.9	71.8
Sub-adults		4	32.1	12.3	34.1	15.6	44.9
Adults[b]	Males	4	31.1	12.7	30.0	19.3	45.1
	Females	7	96.9	48.8	84.8	38.2	154.7
Total		25	60.2	37.5	44.9	15.6	154.7

[a] Cubs of the year: Less than 1 year old, yearlings: 1 year old, sub-adults: 2-3 years old, adults: 4 or more years old. [b] Adult male age range: 11-19 years, adult female age range: 9-21 years.

A Mann-Whitney U test was run to determine if there was a difference in blood Pb concentration between free-ranging and captive bears. Distributions of the blood Pb concentrations for free-ranging and captive bears were not similar, as assessed by visual inspection. There was a statistically significantly difference in blood Pb concentration between free-ranging (mean rank = 60.90) and captive (mean rank = 39.16) bears, U = 654, z = -2.972, asymptotic p-value = 0.003.

3.4 Discussion

3.4.1 Free-ranging brown bears

Although no statistical testing was performed due to low group sample sizes, the Pb exposure results showed a similar age and sex group pattern as that documented by Fuchs et al. (2021). It appears that yearlings are exposed through the milk, and as they grow older and drink less milk (i.e., sub-adults) they become less exposed. Likewise, it seems like lactating females have high blood Pb concentrations, as Pb is being released from bones along with calcium (Bergdahl & Skerfving, 2022; Fuchs et al., 2021). Contrary, non-lactating females could potentially experience higher blood Pb concentration than males if they stopped lactating shortly before being captured. On the other hand, males cannot as easily remove Pb from their bones (i.e., only faecal and urinary excretion routes), so their whole-body burden of Pb through high bone Pb concentrations might be higher than that of adult females. Statistical testing on bigger group sample sizes is needed in order to explore blood Pb concentration differences between the different age and sex groups.

One variable concerning erythrocytes (haemoglobin) and three biochemical variables (alanine aminotransferase, total bilirubin and creatinine) showed significant positive correlations with whole blood Pb concentrations. Haemoglobin and creatinine can be indicative of chronic kidney disease, whereas alanine aminotransferase and total bilirubin can be indicative of liver disease- As Pb is a surrogate for calcium, it is stored in kidneys to a large extent, especially in the proximal tubules in the cortex (Lentini et al., 2017). Renal function is therefore likely to be affected by chronic Pb exposure (Bergdahl & Skerfving, 2022; Evans, 2009; Lentini et al., 2017; Stockham & Scott, 2008).

Polycythaemia (increased haemoglobin and/or haematocrit) can be linked to chronic kidney disease due to increased secretion of erythropoietin (EPO, Stark et al., 2007). EPO is mainly secreted from the kidneys and released as needed to regulate erythrocyte production (i.e., an increase in secreted EPO leads to an increase in erythrocyte production) (Evans, 2009). Chronic kidney disease can result in inappropriate secondary erythrocytic disorder from increased secretion of EPO, which leads to an increase in erythrocytes, and in turn causing polycythaemia (Evans, 2009; Stockham & Scott, 2008). Conversely, a recent study by Powolny et al. (2023) investigated kidney Pb concentrations and found a negative correlation between kidney Pb concentration and erythrocyte concentration in bank voles (*Clethrionomys glareolus*). In addition, Pb has been known to cause anaemia (i.e., decreased haematocrit or haemoglobin) in humans (Bergdahl & Skerfving, 2022). Therefore, whilst the effects of Pb exposure on the haematopoietic system could be different in different species and at different concentrations, for the brown bear it seems likely that it results in polycythaemia due to chronic kidney disease. It is important to keep in mind that polycythaemia could simply be a result of the sampling method. The free-ranging bears are being chased by a helicopter prior to being darted (Arnemo & Evans, 2017). In flight mode, stress will cause the spleen to contract, which results in increased erythrocytes as well as polycythaemia (Stewart & McKenzie, 2002). To rule this out, stress-free animals would need to be sampled. Unfortunately, the sampling setup within the SSBRP does not allow for this currently. In the future, the use of a minimally invasive capture system (MICS) could be an option to reduce the stress animals incur during captures, as seen used in other free-ranging mammalian species (Ryser et al., 2005).

Creatinine is produced during normal muscle metabolism when creatine degrades (Evans, 2009; Stockham & Scott, 2008). Therefore, in a healthy animal, the production of creatinine is relatively constant. Creatinine is filtered in the renal glomeruli and does not get reabsorbed in the renal tubules, hence why it is a good indicator of glomerular filtration rate (GFR). If GFR is decreased, more creatinine will be held back (i.e., not filtering through the glomeruli) and thereby the serum creatinine concentration will increase. Increased serum creatinine concentration is therefore an indicator of kidney disease (Evans, 2009; Stockham & Scott, 2008).

Pb also accumulates in the liver (Bergdahl & Skerfving, 2022) and two biochemical variables indicative of liver disease (alanine aminotransferase and total bilirubin; Evans, 2009; Stockham & Scott, 2008) showed a significant correlation with blood Pb concentration.

Alanine aminotransferase is an enzyme located in the cytoplasm of liver, muscle, and kidneys. In dogs and cats, it is liver specific where increased alanine aminotransferase indicates injury to hepatocytes. It can indicate both primary and secondary hepatic disease and is not specific to the cause of the hepatic disease (Evans, 2009; Stockham & Scott, 2008).

Total bilirubin consists of unconjugated (bound to albumin) and conjugated bilirubin (water soluble). Increased total bilirubin indicates liver disease (with or without cholestasis) but does not indicate liver failure on its own without increases of other hepatic indicators (e.g., alkaline phosphatase; Evans, 2009; Stockham & Scott, 2008).

It is still unclear how exactly Pb exposure is affecting the health of this brown bear population, if the bears have mechanisms protecting them against some ofPb's negative health effects, or if the another variable yet to be studied would clarify the health impact. Recommendations for future research is discussed in Chapter 6.

It is crucial to note that there are currently no expected normal reference ranges for the blood variables measured in this free-ranging brown bear population, and no EPO concentration measurements are available. Although two blood reference ranges for brown bears exist (Græsli et al., 2014; Species360, 2021), there are issues with both, so they have not been used to compare with the results from this study. Firstly, the Zoological Information Management System (Species360, 2021) holds a database of blood reference ranges from captive bears only and is based on results from different methodologies (i.e., it does not consider which machine/technique was used to analyse each variable, nor does it always consider if serum or plasma was used in the analysis of biochemical variables) which could affect the result. Secondly, Græsli et al. (2014) calculated reference ranges for free-ranging Scandinavian brown bears in the same study population as this study, based on bears captured between 2006 and 2013. However, the study was

published before the population was found to be highly exposed to Pb (Boesen et al., 2019), and the bears captured within the time period of the Græsli et al. (2014) study were later shown to have been exposed to Pb. Consequently, these established reference ranges do not necessarily represent the normal blood variables expected in a healthy free-ranging Scandinavian brown bear. Additionally, that study was conducted using other analysers than the ones used in this study. For example, this study used the volumetric impedance method to analyse haematological variables, whereas Græsli et al. (2014) used either the volumetric impedance or peroxidase staining (as the reference laboratory used changed equipment during the study). Direct comparison of variables would therefore also be challenging.
Unfortunately, we do not have access to a population of brown bears in Scandinavia that is not exposed to Pb, so it is impossible to determine normal blood variable reference ranges in healthy individuals. For example, we do not know if the bears' reproductive rate would be higher had they not been exposed to Pb.

The results showed that none of the 40 analysed variables were strongly correlated with whole blood Pb concentration. Although it would have been useful to have identified a blood variable that could function as a cheap screening test instead of reference laboratory Pb analysis to monitor Pb exposure, the results from this study showed that such an in-house blood variable has yet to be identified for this purpose in brown bears. However, it is still valuable to study haematopoietic and renal health as part of the ongoing monitoring of brown bear health, to assess changes over time in relation to Pb exposure.

3.4.2 Captive brown bears

Although no statistical testing was performed due to low group sample sizes, it appears that adult captive females have high blood Pb concentrations, presumably because they are lactating, with Pb being released from bones along with calcium, as seen in free-ranging bears in this study and documented by Fuchs et al. (2021). Unfortunately, we do not have access to information on the lactation status of the adult female bears at the zoological institutions but some of them were living in the same enclosure as their cubs (zoological institutions, multiple personal communications). In contrast, the lower Pb concentration observed in adult males

could be due to them not lactating. If the exposure to Pb is the same in all adult bears, the whole-body burden of Pb might be higher in males as they cannot excrete Pb to the same extent as lactating females. Statistical testing on bigger group sample sizes is needed in order to explore blood Pb concentration differences between the different age and sex groups.

Cubs of the year are highly exposed to Pb through the milk they ingest from their mother (Fuchs et al., 2021). As they grow older and drink less milk, this source of exposure is reduced, as seen in the yearlings and the sub-adults.
Interestingly, the yearling and sub-adult free-ranging brown bears in this study did not show this pattern as the two groups had similar Pb concentrations. It would be interesting to analyse Pb concentration in milk from captive bears, along with mother and cub blood samples, to confirm whether it is, in fact, the mother's milk that is the source of the high Pb exposure in young captive bears. Additionally, more captive bears need to be tested to obtain an appropriate sample size that would allow for statistical testing of differences between the groups and between free-ranging and captive bears.

Blood Pb concentration was significantly higher in free-ranging than in captive bears. However, this study shows that Pb exposure is not exclusive to the free-ranging population of brown bears in Scandinavia. Captive bears should ideally have no access to ingestible Pb sources, but some zoological institutions occasionally feed their bears with donated meat from animals hunted with Pb-based ammunition (zoological institutions, multiple personal communications). Other institutions might have a "zero game meat feeding policy" to prevent Pb poisonings seen in other captive mammalian species fed game meat (Hivert et al., 2018). However, even though an institution can control what it feeds its animals now, bears move between institutions as part of international breeding programmes. Since Pb has a half-life in the body of up to several decades (Bergdahl & Skerfving, 2022), the exposure in the adults in this study could technically reflect ingestion of Pb sources that occurred several years ago at another institution. Another potential source of Pb exposure in captive bears is old cages painted with Pb-based paint, as documented in captive primates in the 1970s where ingestion of Pb paint chips led to poisonings and even mortality in some cases (Zook et al., 1973). Several of the zoological institutions in this study

mainly feed their bears commercial dog food (zoological institutions, multiple personal communications). Recent studies have demonstrated Pb concentrations above the European Union's maximum residue level in animal feed in multiple commercial dog food products (Pain et al., 2023; Zafalon et al., 2021), making this another possible source of ingestible Pb for captive bears.

Lastly, captive Scandinavian brown bears live closer to bigger cities and industrial areas than free-ranging bears. Therefore, there might be a higher risk of previous industrial activities contaminating their area (e.g., Pb from petrol contamination of the soil before Pb-based petrol was banned). Additionally, if the captive bears happen to live in an enclosure with high soil Pb contamination, they cannot escape this exposure, unlike a free-ranging bear.

Pb exposure continues to be a One Health issue (FAO et al., 2022; World Health Organization, 2022) and the sources of Pb exposure in free-ranging and captive Scandinavian brown bears are still undetermined.
As Pb bioaccumulates, monitoring the exposure and studying the source and health effects in top predatory and scavenging wildlife sentinel species like the brown bear will provide information on the environmental exposure and risk of negative health effects other animals face (Beeby, 2001).

References

Arnemo, J. M., & Evans, A. L. (2017). *Biomedical Protocols for Free-ranging Brown Bears, Wolves, Wolverines and Lynx*.

Beeby, A. (2001). What do sentinels stand for? *Environmental Pollution*, *112*(2), 285–298. https://doi.org/10.1016/S0269-7491(00)00038-5

Bergdahl, I. A., & Skerfving, S. (2022). Lead. In G. F. Nordberg & M. Costa (Eds.), *Handbook on the Toxicology of Metals: Fifth Edition: Vol. II: Specific Metals* (Fifth Edition, pp. 427–493). Academic Press. https://doi.org/10.1016/B978-0-12-822946-0.00036-2

Boesen, A. H., Thiel, A., Fuchs, B., Evans, A. L., Bertelsen, M. F., Rodushkin, I., & Arnemo, J. M. (2019). Assessment of the LeadCare® plus for use on Scandinavian brown bears (*Ursus arctos*). *Frontiers in Veterinary Science*, *6*, 285. https://doi.org/10.3389/fvets.2019.00285

Centers for Disease Control and Prevention. (2022). *Blood Lead Reference Value*. Childhood Lead Poisoning Prevention. https://www.cdc.gov/nceh/lead/data/blood-lead-reference-value.htm

Cornell University College of Veterinary Medicine. (2020). *eClinPath*. https://eclinpath.com/

EFSA Panel on Contaminants in the Food Chain (CONTAM). (2013). Scientific opinion on lead in food. *EFSA Journal*. https://doi.org/10.2903/j.efsa.2010.1570

Evans, G. O. (Ed.). (2009). *Animal Clinical Chemistry - A Practical Handbook for Toxicologists and Biomedical Researchers* (2nd ed.). CRC Press. https://doi.org/10.1201/9781420080124

FAO, UNEP, WHO, & WOAH. (2022). *One Health Joint Plan of Action (2022-2026). Working together for the health of humans, animals, plants and the environment*. https://doi.org/10.4060/cc2289en

Flora, S. J. S. (2014). Metals. In R. C. Gupta (Ed.), *Biomarkers in Toxicology* (pp. 485–519). Academic Press. https://doi.org/10.1016/B978-0-12-404630-6.00029-4

Fuchs, B., Thiel, A., Zedrosser, A., Brown, L., Hydeskov, H. B., Rodushkin, I., Evans, A. L., Boesen, A. H., Græsli, A. R., Kindberg, J., & Arnemo, J. M. (2021). High concentrations of lead (Pb) in blood and milk of free-ranging brown bears (*Ursus arctos*) in Scandinavia. *Environmental Pollution*, *287*, 117595. https://doi.org/10.1016/J.ENVPOL.2021.117595

Græsli, A. R., Fahlman, Å., Evans, A. L., Bertelsen, M. F., Arnemo, J. M., & Nielsen, S. S. (2014). Haematological and biochemical reference intervals for free-ranging brown bears (*Ursus arctos*) in Sweden. *BMC Veterinary Research*, *10*(1), 1–9. https://doi.org/10.1186/S12917-014-0183-X/TABLES/5

Hivert, L. G., Clarke, J. R., Peck, S. J., Lawrence, C., Brown, W. E., Huxtable, S. J., Schaap, D., Pemberton, D., & Grueber, C. E. (2018). High blood lead concentrations in captive Tasmanian devils (*Sarcophilus harrisii*): a threat to the conservation of the species? *Australian Veterinary Journal*, *96*(11), 442–449. https://doi.org/10.1111/avj.12753

IBM Corp. (2021). *IBM SPSS Statistics for Windows, Version 28*. IBM Corp.

Lentini, P., Zanoli, L., Granata, A., Signorelli, S. S., Castellino, P., & Dell'Aquila, R. (2017). Kidney and heavy metals - The role of environmental exposure (Review). *Molecular Medicine Reports*, *15*(5), 3413–3419. https://doi.org/10.3892/MMR.2017.6389/HTML

Ma, W.-C. (2011). Lead in mammals. In W. N. Beyer & J. P. Meador (Eds.), *Environmental Contaminants in Biota - Interpreting Tissue Concentrations* (Second Edition, pp. 595–608). CRC Press.

Martin, J., Basille, M., van Moorter, B., Kindberg, J., Allainé, D., & Swenson, J. E. (2010). Coping with human disturbance: spatial and temporal tactics of the brown bear (*Ursus arctos*). *Canadian Journal of Zoology*, *88*(9), 875–883. https://doi.org/10.1139/Z10-053

Pain, D. J., Green, R. E., Bates, N., Guiu, M., & Taggart, M. A. (2023). Lead concentrations in commercial dogfood containing pheasant in the UK. *Ambio*, *52*, 1339–1349. https://doi.org/10.1007/s13280-023-01856-x

Powolny, T., Scheifler, R., Raoul, F., Coeurdassier, M., & Fritsch, C. (2023). Effects of chronic exposure to toxic metals on haematological parameters in free-ranging small mammals. *Environmental Pollution*, *317*, 120675. https://doi.org/10.1016/J.ENVPOL.2022.120675

Ryser, A., Scholl, M., Zwahlen, M., Oetliker, M., Ryser-Degiorgis, M.-P., & Breitenmoser, U. (2005). A remote-controlled teleinjection system for the low-stress capture of large mammals. *Wildlife Society Bulletin*, *33*(2), 721–730. https://doi.org/10.2193/0091-7648(2005)33[721:ARTSFT]2.0.CO;2

Ryser-Degiorgis, M.-P. (2013). Wildlife health investigations: Needs, challenges and recommendations. *BMC Veterinary Research*, *9*, 223. https://doi.org/10.1186/1746-6148-9-223

Scandinavian Brown Bear Research Project (2023). *Skandinaviska Björnprojektet*. http://brownbearproject.com/

Söderberg, C., Rodushkin, Ilia, Johansson, A., & Kugelberg, F. C. (2023). Postmortem reference concentrations of 68 elements in blood and urine. *International Journal of Legal Medicine*, *137*, 655–669. https://doi.org/10.1007/S00414-023-02952-Z

Species360. (2021). *Zoological Information Management System*. https://zims.species360.org/

Stark, S., Winkelmann, B., Kluthe, C., Roigas, J., Querfeld, U., & Müller, D. (2007). Polycythemia and increased erythropoietin in a patient with chronic kidney disease. *Nature Clinical Practice Nephrology 2007 3:4*, *3*(4), 222–226. https://doi.org/10.1038/ncpneph0437

Stewart, I. B., & McKenzie, D. C. (2002). The human spleen during physiological stress. *Sports Medicine*, *32*(6), 361–369. https://doi.org/10.2165/00007256-200232060-00002/METRICS

Stockham, S. L., & Scott, M. A. (2008). *Fundamentals of Veterinary Clinical Pathology* (2nd ed.).

Van der Weele, T. J., & Mathur, M. B. (2019). Some desirable properties of the Bonferroni correction: is the Bonferroni correction really so bad? *American Journal of Epidemiology*, *188*(3), 617–618. https://doi.org/10.1093/AJE/KWY250

World Health Organization. (2020). Constitution of the World Health Organization. In *Basic documents* (Forty-ninth edition, pp. 1–19).

World Health Organization. (2022). *Lead poisoning*. https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health

Zafalon, R. V. A., Pedreira, R. S., Vendramini, T. H. A., Rentas, M. F., Pedrinelli, V., Rodrigues, R. B. A., Risolia, L. W., Perini, M. P., Amaral, A. R., de Carvalho Balieiro, J. C., Pontieri, C. F. F., & Brunetto, M. A. (2021). Toxic element levels in ingredients and commercial pet foods. *Scientific Reports*, *11*(1), 1–19. https://doi.org/10.1038/s41598-021-00467-4

Zook, B. C., Eisenberg, J. F., & McLanahan, E. (1973). Some factors affecting the occurrence of lead poisoning in captive primates. *Journal of Medical Primatology*, *2*(3–4), 206–217. https://doi.org/10.1159/000460323

Chapter 4: Lead (Pb) concentrations and associated pathology in tissues of Scandinavian brown bears (*Ursus arctos*)

Abstract

Lead (Pb) is toxic to wild mammals and other vertebrates. Previous studies have shown that the Scandinavian brown bear population is exposed to Pb. This study aimed to examine different tissue Pb concentrations and pathologies in Scandinavian free-ranging and captive brown bears (*Ursus arctos*). All 363 examined tissue samples from 82 free-ranging and captive Scandinavian brown bears contained Pb, confirming that both populations are exposed to Pb. The different tissue concentrations found in this study support that the Pb is likely distributed in the body in a three-compartment model, as seen in humans. However, no histopathological changes were identified on routine microscopic examination of liver, kidney and spinal cord tissues. Consequently, other ways to assess brown bear health effects from Pb exposure are needed. Tissues from hunted brown bears are an easy source to access for ongoing Pb monitoring of environmental health. However, tissues from hunted bears are likely to exceed the maximum allowed Pb concentrations in domestic mammalian products for human consumption, posing a risk to human health. Therefore, the author recommends against consuming or selling bear products obtained through the Swedish annual licensed hunt. Furthermore, this study supports the need for regulations on Pb in wildlife/game products.

Key words: histopathology, meat, offal, One Health, toxicology, wildlife population health

4.1 Introduction

Lead (Pb) is a non-essential, toxic element that acts as a surrogate for calcium in the body (Bergdahl & Skerfving, 2022). It is widely known for its toxic effects on human and animal health (Agency for Toxic Substances and Disease Registry (ATSDR), 2020; Ma, 2011, Chapter 2). In humans, toxicokinetic studies show that Pb in the body can be described with a three-compartment model of blood, soft tissues and bone (Bergdahl & Skerfving, 2022; Rabinowitz et al., 1973, 1976). Most of the Pb whole-body burden is in bone followed by soft tissues and blood, with biological half-lives varying from minutes in plasma to decades in cortical bone (Bergdahl & Skerfving, 2022).

The free-ranging Scandinavian brown bear (*Ursus arctos*) population is highly exposed to Pb as indicated by blood Pb concentration means between 78.9 and 96.6 µg/l (Chapter 3; Fuchs et al., 2021). Additionally, the captive population is also exposed to Pb as indicated by a mean blood Pb concentration of 60.2 µg/l (Chapter 3).
The presence of Pb in brown bear tissues has been demonstrated in several brown bear populations: Canadian (Pollock & St. Clair, 2020), Carpathian (Čelechovská et al., 2006; Žilinčár et al., 1992), Croatian (Bilandžić et al., 2012; Lazarus et al., 2014, 2017, 2020; Lazarus, Orct, et al., 2018; Lazarus, Sekovanić, et al., 2018; Skoko et al., 2023), Karelian (Medvedev, 1999), Polish (Skoko et al., 2023) and Russian (Medvedev, 1995). However, tissue Pb concentrations in the free-ranging Scandinavian brown bear population are unknown and no health investigations on tissues have been carried out in any brown bear population. Likewise, it is unknown whether Pb is present in tissues in the captive Scandinavian population and whether this is linked to any pathologies.

Since Pb is known to cause disease in the liver, kidney and neurological tissues, it is important to investigate potential Pb effects in these tissues (Chapter 2; Bergdahl & Skerfving, 2022; Ma, 2011). It has been shown that elevated alanine aminotransferase, total bilirubin and creatinine are correlated with increased blood Pb concentration, variables that can be indicative of liver and chronic kidney disease (Chapter 3). However, to further investigate the health effects of Pb in brown bears, tissues from both free-ranging and captive brown bears need to be examined for potential histopathological changes.

In addition to the importance of understanding the Pb distribution in the body for investigating potential health effects in the Scandinavian brown bear population, as seen in other wild mammals (Chapter 2), it is also vital information from a One Health perspective. With Pb bioaccumulating (Clemens, 2006; Radomyski et al., 2018), and the brown bear being a top predator, an omnivore and a scavenger (Wilson & Mittermeier, 2009), this species has great potential to act as a sentinel species for Pb exposure as a representative of ecosystem health. Moreover, as hundreds of Scandinavian brown bears are legally hunted in Sweden every year as a population control tool (Naturvårdsverket, 2023), it is crucial to know if edible parts of the animals pose a risk to human health. The hunters may choose to eat the bear parts themselves or sell them to a butcher, facilitating access to these products for the general public. In 2022, 624 brown bears were shot during the Swedish annual licensed hunt (Höök & Ågren, 2022).

This chapter aims to 1) determine the distribution of Pb in the body of free-ranging and captive brown bears; 2) investigate microscopic pathology changes in kidney, liver and spinal cord tissues related to Pb exposure; 3) assess if consumption of hunted brown bear products are likely to pose a risk to human health.

4.2 Material and methods

4.2.1 Free-ranging brown bears

The author sampled fifty-six brown bears shot during the Swedish annual licensed bear hunts in Gävleborg County in central Sweden (~61°N, 16°E) at the end of August in 2019-2021 (Table 4.1, Table 4.2). The county area is 80 % intensively managed coniferous forest with lakes and bogs being the other two main land cover types (Martin et al., 2010), and there are no industrial activities close to where the bears live. During the study period, the county had three ranger stations to which bears could be brought in for inspection after being shot (Länsstyrelsen Gävleborg, 2023; Regeringskansliet, 2022; Statens veterinärmedicinska anstalt, 2023). During the study period, only one ranger station per year was used to collect samples. However, the station received bears shot throughout the county. Hence, the location of the bear shooting did not predict which station the bear would be inspected at. Age was determined using premolar cementum annuli analysis (Matson et al., 1993) at a commercial laboratory (Matson’s Laboratory, Manhattan, MT, USA).

Table 4.1 Yearly distribution of sexes of 56 free-ranging Scandinavian brown bears (*Ursus arctos*) shot during the Swedish licensed annual hunts in 2019-2021 in Gävleborg County.

Sex	2019	2020	2021	Total
Males	11	8	9	28
Females	7	8	13	28
Total	18	16	22	56

Table 4.2 Distribution of age groups of 56 free-ranging Scandinavian brown bears (*Ursus arctos*) shot during the Swedish licensed annual hunts in 2019-2021 in Gävleborg County.

Age group[a]	Sex	No.	Total
Yearlings	Males	3	4
	Females	1	
Sub-adults	Males	12	24
	Females	12	
Adults[b]	Males	13	28
	Females	15	
Total		56	56

[a] Yearlings: 1 year old, sub-adults: 2-3 years old, adults: 4 or more years old. [b] Adult male age range: 4-7 years, adult female age range: 4-19 years.

Liver and kidney tissues were sampled from all 56 free-ranging brown bears with ceramic and plastic tools, whereas abdominal subcutaneous fat and abdominal skeletal muscle were sampled from 38 bears using metal tools (only sampled in 2020 and 2021 when additional funding for Pb analysis became available). All tools were cleaned and disinfected prior to use, and care was taken to avoid potential Pb nanoparticle contamination from the ammunition used (Kollander et al., 2017). For Pb analysis, liver parenchyma was sampled with a knife and forceps, ensuring no parts of the liver surface were included in the sample. For histopathological examination, a 0.5 cm thick piece of liver, including the liver surface, was collected.

The left or right kidney was sampled. For Pb analysis, the kidney cortex and medulla were carefully separated to ensure the other part did not contaminate the sample. For histopathological examination, a 0.5 cm thick kidney piece, including both cortex and medulla, was sampled. The kidney capsule was not included in samples for Pb analysis, but it was included in samples for histopathological examination.

Abdominal subcutaneous fat and abdominal skeletal muscle were sampled from a midline ventral abdominal incision using a scalpel blade and forceps for Pb analysis.

For Pb analysis, a minimum of 1 g of all soft tissue samples was placed in clear polypropylene containers with lids.

The hunters removed femoral (*n* = 24) and tibial (*n* = 4) bones up to 10 days after the bears were shot. The hunters removed the majority of the skeletal muscle and connective tissues around each bone and returned it to the Scandinavian Brown Bear Research Project's field station for storage at -20 °C. All bones were later thawed, and the remaining skeletal muscle and connective tissues were carefully removed using cleaned and disinfected ceramic knives and plastic forceps. Each bone was then cleaned with distilled water and wiped with laboratory paper towel. Mid-diaphyseal full-thickness samples of approximal 0.3 g were collected using a DREMEL® 3000 multitool and DREMEL® EZ SpeedClic Diamond Cutting Wheel (Dremel, Breda, the Netherlands). The cutting wheel was cleaned with distilled water and wiped with laboratory paper towel between each bone. The bone pieces were placed in clear polypropylene containers with lids.

4.2.2 Captive brown bears

Soft tissues and bone samples from 26 euthanised brown bears (14 males, 12 females) from six Swedish zoological institutions were collected (Appendix C). The samples were collected by either the attending zoo veterinarian (*n* = 22) between 2004 and 2021 or by the author (*n* = 4) in 2021 after the animal had been euthanised for another reason than this study (e.g., surplus animal euthanasia). Most of the samples were collected retrospectively. The organ sample collection during necropsies can therefore have differed from that done by the author. Also, the kidney cortex and medulla were not separated in the retrospective samples (*n* = 18). Liver, kidney cortex, kidney medulla, abdominal subcutaneous fat and abdominal skeletal muscle samples collected by the author were prepared as described above for the hunted bears. Additionally, the spinal cord was sampled with a clean and disinfected metal knife and forceps at the base of the cranium following the removal of the head. A 0.5 cm thick piece of the spinal cord was sampled and placed in a clear polypropylene container with a lid for

histopathological examination. For Pb analysis, a minimum of 1 g of spinal cord was placed in a clear polypropylene container with lid.

The attending veterinarian or the author removed the femoral and tibial bones during the necropsy. The majority of the skeletal muscle and connective tissues were removed prior to the bones being frozen at -20 °C on the same day as the necropsy. Thawing and preparation of the bone piece samples were undertaken as for the hunted free-ranging bears.

4.2.3 Lead (Pb) analysis

The 320 soft tissue samples from free-ranging and captive brown bears were stored at -20 °C and shipped frozen to an accredited laboratory (ALS Scandinavia AB, Luleå, Sweden) for Pb concentration analysis using sector field inductively coupled plasma mass spectrometry (ICP-SFMS, Söderberg et al., 2023). After the 0.3 g pieces were prepared, the 43 bone samples were stored at room temperature and shipped at room temperature to ALS Scandinavia. All samples were freeze-dried prior to the ICP-SFMS analysis and Pb concentrations were reported in µg/kg dry weight (d.w.).

For internal laboratory quality control, ALS Scandinavia analysed some samples twice. Using a conservative approach, the lowest Pb concentration for each sample was used for further analysis.

4.2.4 Histopathological screening

Histopathological samples of liver, kidney and spinal cord were placed in 1:10 tissue:formalin (4 % formaldehyde solution) in airtight containers sealed with Parafilm® M All-Purpose Laboratory Film (Amcor, Zürich, Switzerland) and stored at room temperature prior to processing.

Skeletal muscle, subcutaneous fat and bone tissues were not examined histologically due to limited funds available. Liver, kidney and central nervous tissues were prioritised as these were most likely to show pathologies (Chapter 2; Bergdahl & Skerfving, 2022; Ma, 2011).

Liver and kidney tissues from 38 free-ranging bears were analysed histologically: all bears hunted in 2019 (*n* = 18) as an initial screening, and later the 20 bears with the highest kidney cortex Pb concentration across 2020 and 2021 in an attempt to increase pathology detection success. Additionally, eight captive bears had their liver, kidney and spinal cord tissues analysed histologically.

As per routine process, the formalin-fixed tissues were embedded in paraffin by the Swedish National Veterinary Institute (SVA, Uppsala, Sweden). Sections of 3-4 µm were stained with Mayer's haematoxylin and eosin (Bancroft & Cook, 1984). Tissues from bears hunted in 2019 (*n* = 18) were screened by a veterinary pathologist at SVA. The author also screened these tissues, in addition to the remaining tissues (from 20 free-ranging and 8 captive bears), following instructions from the veterinary pathologist at SVA.

All samples were assessed for any microscopic lesions, including degenerative changes, vasculopathy and inclusion bodies.

All parts of the liver lobule, including the central vein, hepatic sinusoid, liver cell plates, and portal triad were examined, with special emphasis on detecting vascular damage, hepatocyte degeneration and fibrosis.

All parts of the kidney lobule, including renal cortex with renal corpuscles, proximal convoluted tubules and distal convoluted tubules, renal medulla with proximal straight tubules and distal straight tubules, and renal pelvis if present in the section were examined. Special attention was given to detecting pyknotic dark pink cells (as a sign of degeneration) and intranuclear inclusion bodies in proximal convoluted tubules, and interstitial fibrosis.

Both cross-sectional and sagittal sections of the spinal cord were processed for all eight captive bears. All parts, including the central canal, grey matter, white matter and meninges, were examined. Special attention was given to detecting oedema and degeneration.

4.2.5 Statistical analysis

To investigate if brown bears store Pb in the body in a similar matter as humans (three-compartment system, Bergdahl & Skerfving, 2022), paired-samples t-tests were used to determine whether there were significant mean Pb concentration differences between the different tissues in the free-ranging brown bears. Tibia samples were excluded from this analysis based on the low sample size (n = 4). The Bonferroni correction (p-value multiplied by the number of pairwise tests) was applied to avoid type I errors when running many comparisons (Van der Weele & Mathur, 2019).

Similarly, a paired-samples t-test was used to determine whether there was a significant mean Pb concentration difference between kidney mix (mix of kidney cortex and medulla) and liver in captive brown bears. The other captive bear tissues were excluded from this analysis based on the low sample sizes (n = 4 to 11).

Independent-samples t-tests were used to determine if there was a sex difference in liver Pb concentration in both free-ranging and captive bears. This tissue was chosen as it had the highest sample sizes for both sexes and both free-ranging and captive bears. There was homogeneity of variances for liver Pb concentrations for free-ranging (p = 0.183) and captive (p = 0.334) males and females, as assessed by Levene's test for equality of variances.

A Welch t-test was run to determine if there was a difference in liver Pb concentration between free-ranging and captive brown bears due to the assumption of homogeneity of variances being violated, as assessed by Levene’s test for equality of variances (p = 0.028).

The analyses were carried out in IBM SPSS Statistics 28 (IBM Corp, 2021), with α set to 0.05. Box plots were created using R (R Core Team, 2023) and the package ggplot2 (Wickham, 2016).

4.2.6 Ethical approval

This study was approved by the School of Animal, Rural and Environmental Sciences Research Committee, Nottingham Trent University, Southwell, United Kingdom (ARE192009). This study only used samples from animals shot during the Swedish annual licensed hunts or euthanised in zoological institutions for reasons other than this study.

4.3 Results

4.3.1 Free-ranging brown bears

Pb was present in all 272 tissue samples analysed. The kidney cortex was the tissue with the highest Pb concentration, followed by tibia, femur and liver (Table 4.3; Figure 4.1).

Table 4.3 Tissue concentrations (µg/kg dry weight) of lead (Pb) from 56 free-ranging Scandinavian brown bears (*Ursus arctos*) shot during the Swedish annual licensed hunts in Gävleborg County in 2019, 2020 and 2021.

Tissue		*n*	Mean	SD	Median	Minimum	Maximum
Bone	femur	24	3,010.1	2,503.7	2,442.7	1,519.0	14,100.2
	tibia	4	3,802.3	1,371.3	3.790.1	2,159.3	5,469.6
Kidney	cortex	56	3,998.8	1,609.0	3.655.9	1.642.9	11,808.9
	medulla	56	373.8	158.5	353.9	130.9	944.4
Liver		56	1,748.0	866.6	1,547.6	455.2	5,519.0
Skeletal muscle		38	170.0	308.8	46.8	2.8	1,498.2
Subcutaneous fat		38	188.1	532.9	40.9	2.1	3,084.0

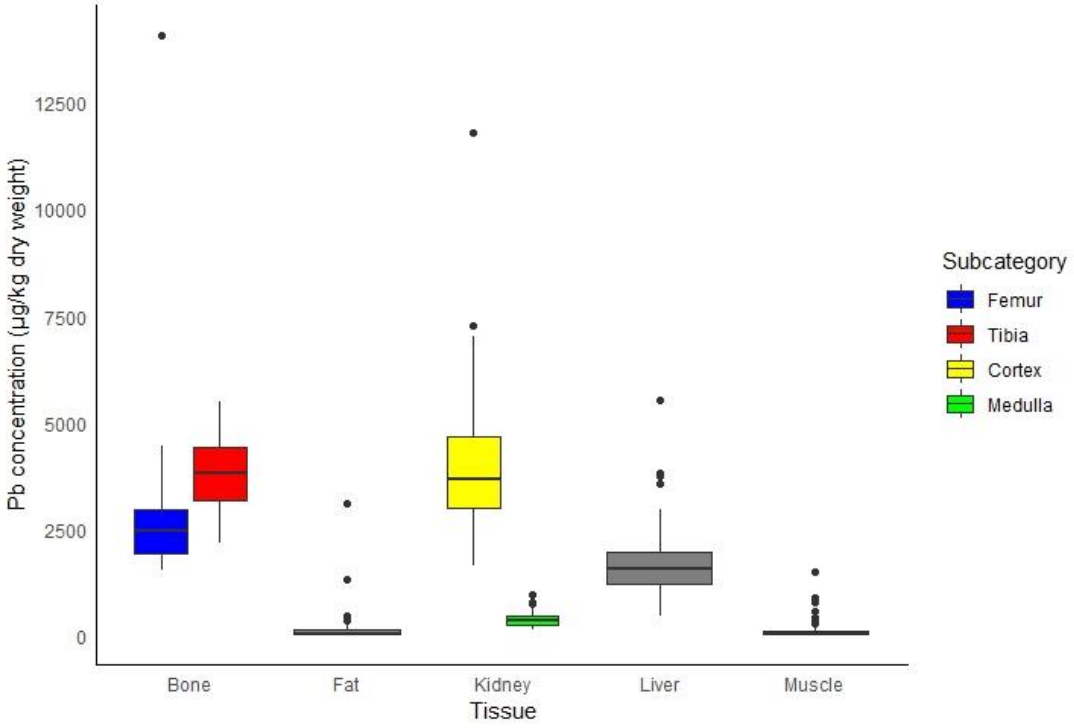

Figure 4.1 Box plot of tissue lead (Pb) concentrations (µg/kg dry weight) from 56 free-ranging Scandinavian brown bears (Ursus arctos) shot during the Swedish annual licensed hunts in Gävleborg County in 2019, 2020 and 2021.

Pb concentration differed significantly between all tissues, except between femur and kidney cortex, femur and liver, kidney medulla and subcutaneous fat, and skeletal muscle and subcutaneous fat (Table 4.4).

Table 4.4 Paired-samples t-tests between different tissue concentrations of lead (Pb) from 56 free-ranging Scandinavian brown bears (*Ursus arctos*) shot during the Swedish annual licensed hunts in Gävleborg County in 2019, 2020 and 2021. Bonferroni-adjusted *p*-value was applied. The *t*-value with the degrees of freedom in parentheses is followed by the Bonferroni-adjusted *p*-value. *t*-values were significant at the 0.05 level (two-tailed) and significant values are highlighted in bold. BL: adjusted p-value exceeded 1 (beyond limit).

Tissue/tissue	Femur	Kidney cortex	Kidney medulla	Liver	Skeletal muscle
Kidney cortex	*t*(23) = 1.673 *p* = BL				
Kidney medulla	***t*(23) = 5.380** ***p* = 0.015**	***t*(55) = -17.340** ***p* = 0.015**			
Liver	*t*(23) = -2.783 *p* = 0.165	***t*(55) = 11.287** ***p* = 0.015**	***t*(55) = -12.294** ***p* = 0.015**		
Skeletal muscle	***t*(17) = 4.469** ***p* = 0.015**	***t*(37) = -12.859** ***p* = 0.015**	***t*(37) = -3.124** ***p* = 0.045**	***t*(37) = 11.141** ***p* = 0.015**	
Subcutaneous fat	***t*(17) = 4.614** ***p* = 0.015**	***t*(37) = -12.363** ***p* = 0.015**	*t*(37) = 1.874 *p* = BL	***t*(37) = 10.791** ***p* = 0.015**	*t*(37) = 0.357 *p* = BL

There was no statistically significant difference in mean liver Pb concentration between males and females, $t(54) = 0.445$, $p = 0.658$.

No pathologies were identified through histological screening of the liver and kidney tissues.

4.3.2 Captive brown bears

Pb was present in all 91 tissues analysed. The kidney (mix of cortex and medulla) was the tissue with the highest Pb concentration, followed by kidney cortex, liver and femur (Table 4.5: Figure 4.2).

Table 4.5 Tissue concentrations (µg/kg dry weight) of lead (Pb) from 26 captive Scandinavian brown bears (*Ursus arctos*) euthanised between 2004 and 2021.

Tissue		n	Mean	SD	Median	Minimum	Maximum
Bone	femur	11	1,898.7	1,251.4	1.353.4	522.4	4,203.0
	tibia	4	1,074.0	311.4	1,012.4	784.5	1,486.7
Kidney	mix[a]	18	2,807.2	1,271.7	3,035.6	677.9	6,537.2
	cortex	8	2,578.9	1,646.9	1,848.6	1,062.8	5.136.4
	medulla	8	353.5	241.5	379.9	96.4	847.0
Liver		26	1,918.4	1809.7	1,562.0	331.2	9,411.7
Skeletal muscle		4	12.9	8.5	9.7	6.7	25.4
Spinal cord		8	27.5	23.7	15.1	10.5	76.8
Subcutaneous fat		4	8.1	5.7	7.7	2.6	14.5

[a] Mix of kidney cortex and kidney medulla in the sample.

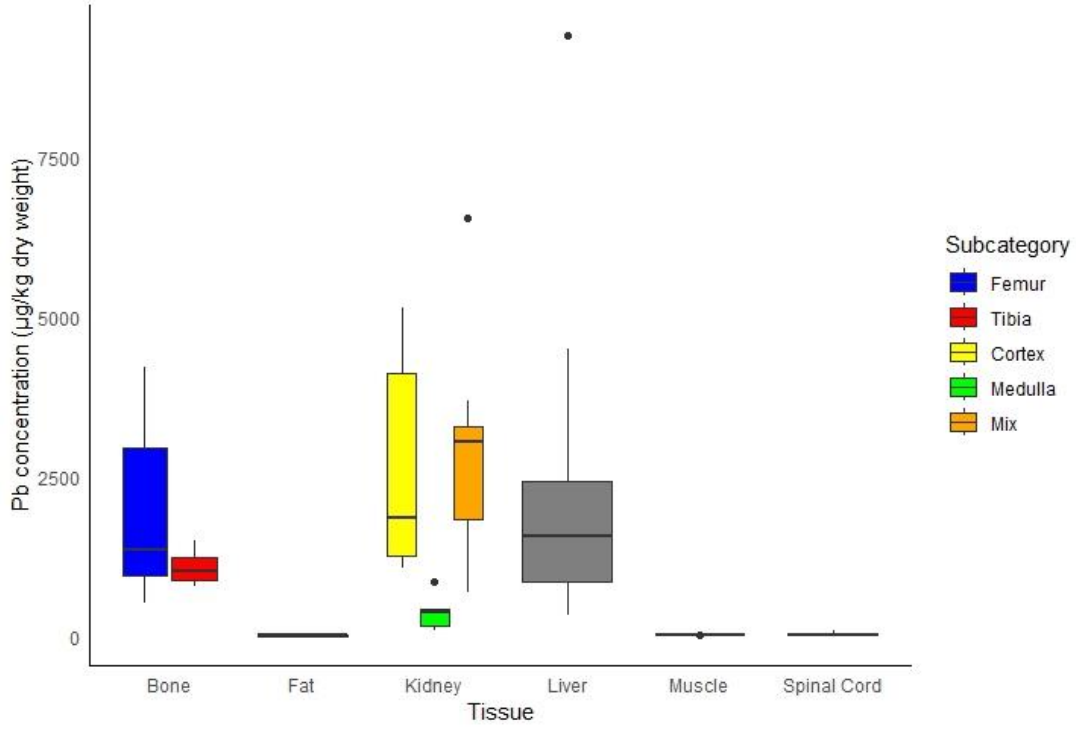

Figure 4.2 Box plot of tissue lead (Pb) concentrations (µg/kg dry weight) from 26 captive Scandinavian brown bears (*Ursus arctos*) euthanised between 2004 and 2021.

There was no significant mean Pb concentration difference between kidney mix and liver ($t(17) = -1.407$, $p = 0.177$) in the captive brown bears.

There was no statistically significant difference in mean liver Pb concentration between males and females, $t(24) = 0.219$, $p = 0.828$.

The Welch t-test showed that there was no significant difference in mean liver Pb concentration between free-ranging and captive brown bears, $t(30.449) = 0.456$, $p = 0.651$.

No pathologies were identified through histological screening of the liver, kidney and spinal cord tissues.

4.4 Discussion

4.4.1 Free-ranging brown bears

Consistent with the high blood Pb concentration already documented in the free-ranging brown bear population (Chapter 3; Fuchs et al., 2021), all tissues analysed also contained Pb. There was no statistically significant difference in liver Pb

concentration between males and females, indication that liver Pb storage is not dependent on sex. The findings, with the highest Pb concentrations detected in bone and kidney cortex, support the three-compartment model seen in humans (Bergdahl & Skerfving, 2022), but further details on toxicokinetics, including half-lives in brown bears, remain unknown.

In contrast to the blood variables study, which identified variables indicative of liver and chronic kidney disease (Chapter 3), no indication of disease was found when histologically screening the liver and kidney tissues for pathology.

It is possible that the Pb exposure is not high enough to cause microscopic changes to these organs. However, Pb has been shown to cause disease in these tissues at lower concentrations in wild *Rodentia* species (Ceruti et al., 2002; Tête et al., 2014). Therefore, it is possible that brown bears have a mechanism protecting them against negative health effects in these tissues. Further investigations into the negative effects of Pb in brown bears are needed. This could be histopathological examining of other tissues or correlating blood Pb concentration to other health variables. Recommendations for future studies are further discussed in Chapter 6.

Unfortunately, with the current setup of the Swedish annual licensed hunt, it takes several hours from when a bear is shot until the hunter presents it at a ranger station for inspection and sampling and by that time the blood will have started to coagulate and thereby no longer be suitable for accurate Pb analysis (Söderberg et al., 2023). Also, sampling of the central nervous system is not possible as most brown bears are hunted for trophies (hunters, multiple personal communications). Therefore, the hunters will only give permission for one midline ventral incision on the carcass, eliminating the possibility of sampling the central nervous system.

For future Pb exposure monitoring, training hunters to use filter paper for blood spot collection in the field right after the kill might be a way to obtain Pb concentrations from a large pool of animals each year (Rodríguez-Saldaña et al., 2021). However, such use of filter paper would require resources for training material to be created and shipped to a large group of hunters along with the filter paper equipment.

A proper toxicokinetic study in this species is unlikely to obtain ethical permission, as it would require multiple blood and tissue samples from the same individuals. Therefore, further insights into how Pb moves between different compartments in the body of brown bears are therefore difficult to obtain.

4.4.2 Captive brown bears

Pb was also detected in all 91 tissue samples from the captive brown bears. As seen in the free-ranging bears, there was no statistically significant difference in liver Pb concentration between males and females. There was no significant difference in liver concentration between free-ranging and captive bears, indicating similar Pb exposure in this tissue despite blood Pb concentrations being lower in captive than free-ranging bears.

Unfortunately, kidney tissues were sampled in various ways by the attending veterinarians (retrospective samples). This makes direct comparisons of different parts of the kidney between free-ranging (cortex and medulla sampled separately) and captive (often a mix of kidney parts sampled) brown bears impossible.

As discussed in Chapter 3, the captive brown bear population is likely exposed to Pb from some different sources (e.g., enclosures painted with Pb-based paints, Pb contaminated dog food) than the free-ranging bears (Pain et al., 2023; Zafalon et al., 2021; Zook et al., 1973).

No pathologies were detected in the liver, kidney and spinal cord tissues. Histopathological examination of brain tissues from captive bears might reveal pathologies in the central nervous system not present in the spinal cord tissue. However, as skulls are often saved intact it could be difficult to obtain brain tissues from captive bears.

4.4.3. Researching subclinical and chronic health effects of lead (Pb) exposure

Pb exposure has been shown to alter behaviours in some fish species (Alados & Weber, 1999; Li et al., 2019), and to decrease intelligence in humans (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2013). Captive bears could be the subject of a behavioural test indicative of cognitive ability level (e.g., similar to the test recently created for brown bears by Chambers & O'Hara (2023)). Such a test

could then be used to evaluate neurological health in relation to blood Pb concentration but comes with the need of knowing normal behaviour and/or cognitive ability level in bears not exposed to Pb. Additionally, resources to train captive bears for the behaviour test could be a cost-prohibiting factor. Evaluation of neurological health through behavioural testing in relation to Pb exposure is therefore likely far from being a possibility.

In eagles, Pb might have an indirect impact on survival through its effect on behaviour. Flight performance might be impaired in golden eagles (*Aquila chrysaetos*) exposure to Pb and thus increase the risk of mortality from other causes (Ecke et al., 2017). However, other studies on different eagle species could not find evidence that subclinical Pb concentrations are linked to “increased risk of trauma” (Isomursu et al., 2018) or “other causes of mortality” (Franson & Russell, 2014). When exposed to Pb, mallard (*Anas platyrhynchos*) ducklings experienced a reduction in body mass (Vallverdú-Coll et al., 2015), whereas a body mass reduction was not seen in western bluebird (*Sialia Mexicana*) and common quail (*Coturnix coturnix*) chicks (Fair & Myers, 2002; Fair & Ricklefs, 2002). Fritsch et al. (2019) showed that while lifetime breeding success of females decreases, the lifespan and survival probabilities increase with increasing Pb exposure in Eurasian blackbirds (*Turdus merula*).

These contrasting findings illustrate how difficult it is to investigate subclinical and chronic health effects in wildlife. It gets especially complicated when different studies classify health effects differently. In addition, ethical permits to conduct Pb toxicological effect studies in wildlife species are unlikely to be granted and subclinical effects therefore remain difficult to confirm.

4.4.4 One Health perspective

From a One Health perspective, it is concerning that Pb was detected in all the samples analysed. Tissues from shot Scandinavian brown bears are easily available every year in Sweden, thereby comprising a good source for Pb monitoring of environmental health (Clemens, 2006; Radomyski et al., 2018). However, at the same time, these tissues are likely to pose a threat to human health if consumed. While the European Union has set maximum Pb concentrations allowed in domestic animal meat and offal products, as well as

fishery products and bivalve molluscs, there are no Pb regulations on wildlife/game products (European Union, 2023; Thomas et al., 2020). Domestic mammalian meat (i.e., skeletal muscle from bovine animals, sheep and pigs) is allowed up to 100 µg Pb/kg (wet weight), and domestic mammalian offal up to 150-200 µg Pb/kg (wet weight, depending on species). Although it is not possible to directly compare dry weight and wet weight concentrations, the very high Pb concentrations detected in meat and especially offal from the hunted bears are likely to exceed the maximum allowed concentration set for domestic mammalian products (European Union, 2023). Furthermore, it is worth noting that meat and offal located in areas closer to the wound channel (i.e., ribs and heart) could potentially contain even higher concentrations of Pb (Kollander et al., 2017). Almost all (98 %; 55/56) hunted bears in this study were shot with Pb-based ammunition (unpublished data). The use of Pb-based ammunition to shoot the bear increases the risk to human health even further should any of these bear products be consumed. The hunted bears sampled in this study were shot with 2.7 bullets on average (unpublished data). A Pb-based rifle bullet leaves on average 2.8 g of metal in the body when fired (Stokke et al., 2017), and Pb fragments can be found up to 45 cm from the bullet entry site (Hunt et al., 2009). It is, therefore, advisable for hunters not to consume or sell any bear products. It is also desirable for the European Union to start regulating the allowed Pb concentrations in wildlife/game, as suggested by Thomas et al. (2020), to protect all consumers from this toxic element.

References

Agency for Toxic Substances and Disease Registry (ATSDR). (2020). *Toxicological profile for lead*. https://doi.org/http://dx.doi.org/10.15620/cdc:95222

Alados, C. L., & Weber, D. N. (1999). Lead effects on the predictability of reproductive behavior in fathead minnows (*Pimephales promelas*): A mathematical model. *Environmental Toxicology and Chemistry*, *18*(10), 2392–2399. https://doi.org/10.1002/ETC.5620181038

Bancroft, J. D., & Cook, H. C. (1984). *Manual of histological techniques*. Churchill Livingstone. https://doi.org/10.1002/PATH.1711450410

Bergdahl, I. A., & Skerfving, S. (2022). Lead. *Handbook on the Toxicology of Metals: Fifth Edition*, *II*, 427–493. https://doi.org/10.1016/B978-0-12-822946-0.00036-2

Bilandžić, N., Dežcrossed D Signek, D., Sedak, M., Crossed D Signokić, M., Šimić, B., Rudan, N., Brstilo, M., & Lisicin, T. (2012). Trace elements in tissues of wild carnivores and omnivores in Croatia. *Bulletin of Environmental Contamination and Toxicology*, *88*(1), 94–99. https://doi.org/10.1007/s00128-011-0449-y

Čelechovská, O., Literák, I., Ondruš, S., & Pospíšil, Z. (2006). Heavy metals in brown bears from the central European Carpathians. *Acta Veterinaria Brno*, *75*(4), 501–506. https://doi.org/10.2754/avb200675040501

Ceruti, R., Ghisleni, G., Ferretti, E., Cammarata, S., Sonzogni, O., & Scanziani, E. (2002). Wild rats as monitors of environmental lead contamination in the urban area of Milan, Italy. *Environmental Pollution*, *117*(2), 255–259. https://doi.org/10.1016/S0269-7491(01)00273-1

Chambers, H. R., & O'Hara, S. J. (2023). Problem-solving and spontaneous tool-using ability in European brown bears (*Ursus arctos arctos*). *Animal Behavior and Cognition*, *10*(1), 40–61. https://doi.org/10.26451/ABC.10.01.03.2023

Clemens, S. (2006). Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie*, *88*(11), 1707–1719. https://doi.org/10.1016/J.BIOCHI.2006.07.003

Ecke, F., Singh, N. J., Arnemo, J. M., Bignert, A., Helander, B., Berglund, Å. M. M., Borg, H., Bröjer, C., Holm, K., Lanzone, M., Miller, T., Nordström, Å., Räikkönen, J., Rodushkin, I., Ågren, E., & Hörnfeldt, B. (2017). Sublethal Lead Exposure Alters Movement Behavior in Free-Ranging Golden Eagles. *Environmental Science and Technology*, *51*(10), 5729–5736. https://doi.org/10.1021/acs.est.6b06024

EFSA Panel on Contaminants in the Food Chain (CONTAM). (2013). Scientific opinion on lead in food. *EFSA Journal*. https://doi.org/10.2903/j.efsa.2010.1570

European Union. (2023). *Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 (Text with EEA relevance)*.

Fair, J. M, & Myers, O. B. (2002). The ecological and physiological costs of lead shot and immunological challenge to developing western bluebirds. *Ecotoxicology, 11*, 199-20. https://doi.org/10.1023/A:1015474832239

Fair, J. M., & Ricklefs, R. E. (2002). Physiological, growth, and immune responses of Japanese quail chicks to the multiple stressors of immunological challenge and lead shot. *Archives of Environmental Contamination and Toxicology, 42*, 77-87. https://doi.org/10.1007/s002440010294

Franson, J. C., & Russell, R. E. (2014). Lead and eagles: demographic and pathological characteristics of poisoning, and exposure levels associated with other causes of mortality. *Ecotoxicology*, *23*(9), 1722–1731. https://doi.org/10.1007/S10646-014-1337-0/FIGURES/3

Fritsch, C., Jankowiak, Ł., & Wysocki, D. (2019). Exposure to Pb impairs breeding success and is associated with longer lifespan in urban European blackbirds. *Scientific Reports, 9*, 486. https://doi.org/10.1038/s41598-018-36463-4

Fuchs, B., Thiel, A., Zedrosser, A., Brown, L., Hydeskov, H. B., Rodushkin, I., Evans, A. L., Boesen, A. H., Græsli, A. R., Kindberg, J., & Arnemo, J. M. (2021). High concentrations of lead (Pb) in blood and milk of free-ranging brown bears (*Ursus arctos*) in Scandinavia. *Environmental Pollution*, *287*, 117595. https://doi.org/10.1016/J.ENVPOL.2021.117595

Höök, E., & Ågren, E. (2022). *Licensjakt björn 2022 - SVA Rapportserie 81/2022*.

Hunt, W. G., Watson, R. T., Oaks, J. L., Parish, C. N., Burnham, K. K., Tucker, R. L., Belthoff, J. R., & Hart, G. (2009). Lead bullet fragments in venison from rifle-killed deer: Potential for human dietary exposure. *PLoS ONE*, *4*(4). https://doi.org/10.1371/journal.pone.0005330

IBM Corp. (2021). *IBM SPSS Statistics for Windows, Version 28*. IBM Corp.

Isomursu, M., Koivusaari, J., Stjernberg, T., Hirvelä-Koski, V., & Venäläinen, E. R. (2018). Lead poisoning and other human-related factors cause significant mortality in white-tailed eagles. *Ambio*, *47*(8), 858–868. https://doi.org/10.1007/s13280-018-1052-9

Kollander, B., Widemo, F., Ågren, E., Larsen, E. H., & Loeschner, K. (2017). Detection of lead nanoparticles in game meat by single particle ICP-MS following use of lead-containing bullets. *Analytical and Bioanalytical Chemistry*, *409*(7), 1877–1885. https://doi.org/10.1007/S00216-016-0132-6/TABLES/3

Länsstyrelsen Gävleborg. (2023). *Licensjakt efter björn*. https://www.lansstyrelsen.se/gavleborg/djur/jakt-och-vilt/stora-rovdjur/licensjakt-efter-bjorn.html

Lazarus, M., Crnić, A. P., Bilandžić, N., Kusak, J., & Reljić, S. (2014). Cadmium, lead, and mercury exposure assessment among Croatian consumers of free-living game. *Arhiv Za Higijenu Rada i Toksikologiju*, *65*(3), 281–292. https://doi.org/10.2478/10004-1254-65-2014-2527

Lazarus, M., Orct, T., Reljić, S., Sedak, M., Bilandžić, N., Jurasović, J., & Huber, Đ. (2018). Trace and macro elements in the femoral bone as indicators of long-term environmental exposure to toxic metals in European brown bear (*Ursus arctos*) from Croatia. *Environmental Science and Pollution Research*, *25*(22), 21656–21670. https://doi.org/10.1007/s11356-018-2296-4

Lazarus, M., Orct, T., Sergiel, A., Vranković, L., Marijić, V. F., Rašić, D., Reljić, S., Aladrović, J., Zwijacz-Kozica, T., Zięba, F., Selva, N., & Huber. (2020). Metal(loid) exposure assessment and biomarker responses in captive and free-ranging European brown bear (*Ursus arctos*). *Environmental Research*, *183*, 109166. https://doi.org/10.1016/j.envres.2020.109166

Lazarus, M., Sekovanić, A., Orct, T., Reljić, S., Jurasović, J., & Huber, Đ. (2018). Sexual Maturity and Life Stage Influences Toxic Metal Accumulation in Croatian Brown Bears. *Archives of Environmental Contamination and Toxicology*, *74*(2), 339–348. https://doi.org/10.1007/s00244-017-0487-5

Lazarus, M., Sekovanić, A., Orct, T., Reljić, S., Kusak, J., Jurasović, J., & Huber, Đ. (2017). Apex predatory mammals as bioindicator species in environmental monitoring of elements in Dinaric Alps (Croatia). *Environmental Science and Pollution Research*, *24*(30), 23977–23991. https://doi.org/10.1007/s11356-017-0008-0

Li, X., Kong, H., Ji, X., Gao, Y., & Jin, M. (2019). Zebrafish behavioral phenomics applied for phenotyping aquatic neurotoxicity induced by lead contaminants of environmentally relevant level. *Chemosphere*, *224*, 445–454. https://doi.org/10.1016/J.CHEMOSPHERE.2019.02.174

Ma, W.-C. (2011). Lead in mammals. In W. N. Beyer & J. P. Meador (Eds.), *Environmental Contaminants in Biota - Interpreting Tissue Concentrations* (Second Edition, pp. 595–608). CRC Press.

Martin, J., Basille, M., van Moorter, B., Kindberg, J., Allainé, D., & Swenson, J. E. (2010). Coping with human disturbance: spatial and temporal tactics of the brown bear (*Ursus arctos*). *Canadian Journal of Zoology*, *88*(9), 875–883. https://doi.org/10.1139/Z10-053

Matson, G., Van Daele, L., Goodwin, E., Aumiller, L., Reynolds, H., & Hristienko, H. (1993). *A laboratory manual for cementum age determination of Alaska brown bear first premolar teeth*. Alaska Department of Fish and Game Division of Wildlife Conservation.

Medvedev, N. (1995). Concentrations of cadmium, lead and sulphur in tissues of wild, forest reindeer from north-west Russia. *Environmental Pollution*, *90*(1), 1–5. https://doi.org/10.1016/0269-7491(94)00099-Y

Medvedev, N. (1999). Levels of heavy metals in Karelian wildlife, 1989-91. *Environmental Monitoring and Assessment*, *56*(2), 177–193. https://doi.org/10.1023/A:1005988511058

Naturvårdsverket. (2023). *Jakt på björn*. https://www.naturvardsverket.se/amnesomraden/jakt-och-vilt/jakt-pa-rovdjur/jakt-pa-bjorn/

Pain, D. J., Green, R. E., Bates, N., Guiu, M., & Taggart, M. A. (2023). Lead concentrations in commercial dogfood containing pheasant in the UK. *Ambio*, *52*, 1339–1349. https://doi.org/10.1007/s13280-023-01856-x

Pollock, S. Z., & St. Clair, C. C. (2020). Railway-associated attractants as potential contaminants for wildlife. *Environmental Management*, *66*(1), 16–29. https://doi.org/10.1007/s00267-020-01277-6

R Core Team. (2023). *R: A language and environment for statistical computing, Version 4.3.0* (4.3.0). R Foundation for Statistical Computing.

Rabinowitz, M. B., Wetherill, G. W., & Kopple, J. D. (1973). Lead Metabolism in the Normal Human: Stable Isotope Studies. *Science*, *182*(4113), 725–727. https://doi.org/10.1126/SCIENCE.182.4113.725

Rabinowitz, M. B., Wetherill, G. W., & Kopple, J. D. (1976). Kinetic analysis of lead metabolism in healthy humans. *The Journal of Clinical Investigation*, *58*(2), 260–270. https://doi.org/10.1172/JCI108467

Radomyski, A., Lei, K., Giubilato, E., Critto, A., Lin, C., & Marcomini, A. (2018). Bioaccumulation of trace metals in aquatic food web. A case study, Liaodong Bay, NE China. *Marine Pollution Bulletin*, *137*, 555–565. https://doi.org/10.1016/J.MARPOLBUL.2018.11.002

Regeringskansliet. (2022). *Förordning om ändring i jaktförordningen (1987:905)* (SFS 2022:1374).

Rodríguez-Saldaña, V., Fobil, J., & Basu, N. (2021). Lead (Pb) exposure assessment in dried blood spots using Total Reflection X-Ray Fluorescence (TXRF). *Environmental Research*, *198*, 110444. https://doi.org/10.1016/J.ENVRES.2020.110444

Skoko, B., Kovačić, M., Lazarus, M., Sekovanić, A., Kovačić, J., Sergiel, A., Zwijacz-Kozica, T., Reljić, S., Petrinec, B., Selva, N., Selva, N., & Huber, Đ. (2023). ^{90}Sr and stable element levels in bones of brown bears: long-term trends in bear populations from Croatia and Poland. *Environmental Science and Pollution Research*, *30*(12), 32507–32522. https://doi.org/10.1007/s11356-022-24397-4

Söderberg, C., Rodushkin, Ilia, Johansson, A., & Kugelberg, F. C. (2023). Postmortem reference concentrations of 68 elements in blood and urine. *International Journal of Legal Medicine*, *137*, 655–669. https://doi.org/10.1007/S00414-023-02952-Z

Statens veterinärmedicinska anstalt. (2023). *Björn*. https://www.sva.se/vilda-djur/rovdjur/bjorn/

Stokke, S., Brainerd, S., & Arnemo, J. M. (2017). Metal deposition of copper and lead bullets in moose harvested in Fennoscandia. *Wildlife Society Bulletin*, *41*(1), 98–106. https://doi.org/10.1002/wsb.731

Tête, N., Durfort, M., Rieffel, D., Scheifler, R., & Sánchez-Chardi, A. (2014). Histopathology related to cadmium and lead bioaccumulation in chronically exposed wood mice, *Apodemus sylvaticus*, around a former smelter. *Science of the Total Environment*, *481*(1), 167–177. https://doi.org/10.1016/j.scitotenv.2014.02.029

Thomas, V. G., Pain, D. J., Kanstrup, N., & Green, R. E. (2020). Setting maximum levels for lead in game meat in EC regulations: An adjunct to replacement of lead ammunition. *Ambio*, *49*(12), 2026–2037. https://doi.org/10.1007/S13280-020-01336-6/FIGURES/2

Vallverdú-Coll, N., López-Antia, A., Martinez-Haro, M., Ortiz-Santaliestra, M. E., Mateo, R. (2015). Altered immune response in mallard ducklings exposed to lead through maternal transfer in the wild. *Environmental Pollution, 205*, 350-356. https://doi.org/10.1016/j.envpol.2015.06.014

Van der Weele, T. J., & Mathur, M. B. (2019). Some desirable properties of the Bonferroni correction: is the Bonferroni correction really so bad? *American Journal of Epidemiology*, *188*(3), 617–618. https://doi.org/10.1093/AJE/KWY250

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag.

Wilson, D. E., & Mittermeier, R. A. (Eds.). (2009). *Handbook of the Mammals of the World: Carnivores* (Vol. 1). Lynx Edicions.

Zafalon, R. V. A., Pedreira, R. S., Vendramini, T. H. A., Rentas, M. F., Pedrinelli, V., Rodrigues, R. B. A., Risolia, L. W., Perini, M. P., Amaral, A. R., de Carvalho Balieiro, J. C., Pontieri, C. F. F., & Brunetto, M. A. (2021). Toxic element levels in ingredients and commercial pet foods. *Scientific Reports*, *11*(1), 1–19. https://doi.org/10.1038/s41598-021-00467-4

Žilinčár, J., Bystrica, B., Zvada, P., Kubín, D., & Hell, P. (1992). Die Schwermetallbelastung bei den Braunbären in den Westkarpaten. *Zeitschrift Für Jagdwissenschaft*, *38*(4), 235–243. https://doi.org/10.1007/BF02242838

Zook, B. C., Eisenberg, J. F., & McLanahan, E. (1973). Some factors affecting the occurrence of lead poisoning in captive primates. *Journal of Medical Primatology*, *2*(3–4), 206–217. https://doi.org/10.1159/000460323

Chapter 5: Hibernation increases blood lead (Pb) concentrations

Abstract

Currently, the effect of hibernation on blood lead (Pb) concentration is unknown. However, it is crucial to investigate this as different metabolic states might cause toxic element concentrations to either increase or decrease, potentially altering the risk of negative health effects. Whole blood Pb concentrations were measured in 15 sub-adult Scandinavian brown bears (*Ursus arctos*) during hibernation (February-March) and active state (June) of the same year. The concentration of Pb was significantly higher during hibernation compared to the active state ($Z = -3.294$, $p < 0.001$). The underpinning physiological process explaining why blood Pb concentration is higher during hibernation remains unclear. Additionally, it is still unknown whether higher blood Pb concentrations during hibernation pose an increased health risk to the individual brown bear, its foetuses, or cubs. Therefore, examination of health variables in relation to blood Pb concentration during different metabolic states is needed to investigate potential different health risks. This is the first evidence that a non-essential toxic element blood concentration differs during different metabolic states in any species.

Key words: Mammalia, torpor, toxicokinetics, toxicology, wildlife population health, winter sleep

5.1 Introduction

5.1.1 Hibernation

Several endothermic mammalian and avian species are classed as heterothermic species, meaning they can lower their body temperature in periods to conserve energy by expressing torpor (Geiser, 2011; Morales et al., 2021). This adaptation can increase their chance of survival during periods of food shortage or adverse environmental conditions. How much the body temperature is lowered, and for long the torpid state lasts, differ widely between species (Geiser, 2011).

The Scandinavian brown bear (*Ursus arctos*) is a top predator, omnivore and scavenger that hibernates (i.e., multiday torpor) for 6-7 months from October to May (Manchi & Swenson, 2005). In contrast to most mammalian species, the brown bear and other ursids use continuous torpor whereby there are no arousals during the hibernation phase, sometimes referred to as “winter sleep”. During this type of hibernation, the body temperature is only lowered by 3-5 °C (i.e., moderate hypothermia), and the animals survive this long inactive period using profound hypometabolism, where there is no intake of food or water and the animal does not defecate or urinate (Geiser, 2011; Hellgren, 1998; Manchi & Swenson, 2005). During the pre-hibernation phase (late summer to early autumn), individuals build up their fat reserves through a significant increase in feeding activity (hyperphagia), which can then be metabolised during the hibernation state (Manchi & Swenson, 2005). It has been shown that the Scandinavian brown bear uses environmental cues to initiate and physiological cues to terminates it hibernation phase (Evans et al., 2016a). The initiation of hibernation (i.e., entry into the den) is driven by environmental factors, particularly ambient temperature. A decreasing ambient temperature affects the bear’s activity level, heart rate and body temperature which all decrease prior to den entry. On the contrary, the end of the hibernation phase is driven by changes in physiological factors (e.g., increases in body temperature and metabolic rate).

It is unclear how brown bears cope with torpor for such an extended period without deteriorating. For example, humans in physically inactive states very quickly experience bone and muscle atrophy (Bloomfield, 1997). However, bears maintain their bone mass and density during hibernation and only have 10-20 % muscle fibre atrophy, attributed to intracellular water loss (Hellgren, 1998; McGee-

Lawrence et al., 2015). For American black bears (*Ursus americanus*) during hibernation, it has been suggested that water produced from fat metabolism replenishes water lost through respiration (Nelson, 1973). Nevertheless, increases in erythrocyte count, haemoglobin and haematocrit concentrations during hibernation (Græsli et al., 2015), suggest that dehydration is taking place during hibernation in Scandinavian brown bears.

5.1.2 The non-essential, toxic element lead (Pb)

Exposure to non-essential, toxic elements such as lead (Pb) is a major One Health issue with its large range of health implications on human, animal and environmental health (FAO et al., 2022). Pb is number two on The ATSDR 2022 Substance Priority List (Agency for Toxic Substances and Disease Registry, 2022) because Pb exposure has a negative effect on the nervous, renal, cardiovascular, haematological, immunological, reproductive and immune systems (Agency for Toxic Substances and Disease Registry, 2019). Additionally, according to the World Health Organization (WHO), there is no safe level of Pb exposure (World Health Organization, 2022).

The gradual reductions and later bans on Pb in petrol in developed countries since the 1970s have reduced the amount of Pb in the air (Bergdahl & Skerfving, 2022). Consequently, Pb exposure is now mainly via ingestion in humans (Bergdahl & Skerfving, 2022). In the case of wildlife, it is also expected that ingestion is the main exposure route for Pb in animals living far from industrial activities.

Despite a large amount of evidence that Pb and other non-essential elements negatively affect vertebrates (Pain et al., 2019), the toxicokinetics of these elements in wildlife remain unknown. Additionally, it is unknown whether their concentrations in blood differ in relation to metabolic state, which could potentially affect the associated health risk.

This study aims to determine whether whole blood Pb concentrations differed between hibernation and active state in Scandinavian brown bears. The findings from this study will be the first step in understanding how hibernation can affect non-essential, toxic element concentrations of any vertebrate species. This is

important as increases in blood Pb concentrations might relate to increased risks of negative health effects.

5.2 Materials and methods

5.2.1 Study area

The study area comprised of Dalarna and Gävleborg Counties in central Sweden (~61°N, 15°E), with the bears living far from industrial activities. The counties are mainly covered by intensively managed coniferous forest with bogs and lakes bogs being the other two main land cover types (Martin et al., 2010), There is usually snow cover from the end of October until late April, and mean daily temperatures range from −7°C in January to 15°C in July. The brown bear denning period in the study area is from October to May (Friebe et al., 2014).

5.2.2 Animals and captures

In the springs of 2017-2021, prior to this study, researchers from the Scandinavian Brown Bear Research Project (SBBRP) fitted 15 yearling brown bears (4 males, 11 females) with abdominal very high frequency (VHF) radio transmitter implants to allow their location to be monitored. For this study, these 15 individual bears were captured twice in the same year as sub-adults (2-3 years old). For statistical purposes, the two sexes were treated as one group, as all animals were sexually immature.

For the first capture, the bears were immobilised during their hibernation state (mid-February to early March) in 2019 (n = 7), 2020 (n = 3) and 2022 (n = 5), using a combination of ketamine, medetomidine and tiletamine-zolazepam for general anaesthesia (Arnemo & Evans, 2017; Evans et al., 2016b). Once recumbent, the bears were temporarily removed from their dens for blood sample collection. Whole blood samples were collected from the jugular vein using a vacutainer system while the bear was anaesthetised and in lateral or dorsal recumbency. Blood samples were collected in VACUETTE® 6 ml NH Trace Elements Sodium Heparin tubes (Greiner Bio-One International GmbH, Kremsmünster, Austria), frozen the same day, and stored at -20 °C until ready for analysis. Throughout the immobilisation period, intranasal oxygen supplementation was provided, and the animals' anaesthesia levels were closely monitored, including monitoring of rectal temperature, heart rate, breathing and reflexes. Additional ketamine was given

when warranted. After the sample collection, the bears were equipped with global positioning system (GPS) with VHF radio collars to locate them in the summer. Following blood sample collection and collar fitting, the bears were returned to their dens. Here, atipamezole was administered to antagonise medetomidine and thereby reduce the recovery time.

The same individual bears were re-captured 102-119 days later in early to mid-June, where they were immobilised by darting from a helicopter using a combination of medetomidine and tiletamine-zolazepam (Arnemo & Evans, 2017). Anaesthesia monitoring, blood sample collection and reversal procedures were the same as for the hibernation captures. To prevent haemolysis, the blood samples were stored with ice packs in the field, frozen the same day, and stored at -20 °C until ready for analysis.

5.2.3 Lead (Pb) analysis

Whole blood Pb concentrations were measured at an accredited laboratory (ALS Scandinavia AB, Luleå, Sweden) using sector field inductively coupled plasma mass spectrometry (ICP-SFMS, Söderberg et al. 2023). The concentrations were reported in µg/l wet weight. For the laboratory to evaluate its method's reproducibility, samples were analysed 1-4 times. Following a conservative approach, the lowest value measured across the repeated analyses was used for statistical analysis.

5.2.4 Statistical analysis

Wilcoxon signed-rank tests were used to determine if there was a significant difference in whole blood Pb concentrations between the hibernation and the active states. Statistical analyses were undertaken in IBM SPSS Statistics 28 (IBM Corp, 2021), with α set to 0.05. A paired observations box plot was created using R (R Core Team, 2023) and the package ggplot2 (Wickham, 2016).

5.2.5 Ethical approval

This study was approved by the Swedish Ethical Committee on Animal Research, Uppsala, Sweden (Dnr. 5.8.18-03376/2020), the Swedish Environmental Protection Agency, Stockholm, Sweden (NV-00741-18) and the School of Animal, Rural and Environmental Sciences Research Committee, Nottingham Trent

University, Southwell, United Kingdom (ARE192009). This study only used a portion of samples already collected by the SBBRP.

5.3 Results

The median whole blood Pb concentrations were significantly higher in brown bears during the hibernation state compared to the active state ($Z = -3.294$, $n = 15$, $p < 0.001$, Table 5.1, Figure 5.1). The median and mean concentration differences between the hibernation and active states were 30.105 and 42.031 µg/l, respectively. The relative median and mean differences were 42.0 % and 60.5 % higher, respectively, during hibernation compared to the active state.

Table 5.1 Whole blood concentrations (µg/l) of lead (Pb) in 15 sub-adult brown bears (*Ursus arctos*) captured twice in the same year (2019, 2020 or 2022) during the hibernation state and the following active state.

Hibernation state					Active state				
Median	**Mean**	**SD**	**Min.**	**Max.**	**Median**	**Mean**	**SD**	**Min.**	**Max.**
101.766	111.527	34.777	71.022	186.755	71.661	69.496	20.539	31.149	99.057

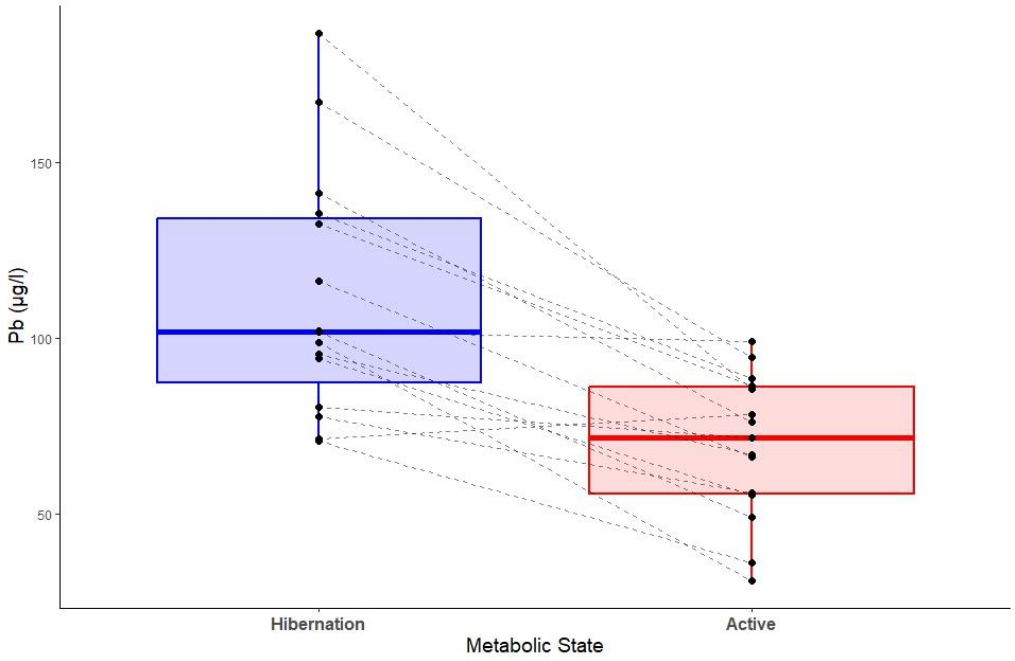

Figure 5.1 Paired observations box plot showing the temporal changes of whole blood lead (Pb) concentrations (µg/l) in 15 sub-adult brown bears (*Ursus arctos*) captured twice in the same year (2019, 2020 or 2022) during the hibernation state and the following active state.

5.4 Discussion

This study provides the first recorded evidence that hibernation affects toxic element concentrations in any animal species. The findings show that whole blood concentrations of Pb are significantly higher during the hibernation state than during the active state in brown bears.

5.4.1 Potential explanations of increased blood lead (Pb) concentrations during hibernation and health implications

Although non-essential element toxicokinetics in brown bears have not been studied, a potential factor explaining the significantly different blood Pb concentrations is dehydration, as dehydration during hibernation has been suggested to affect concentrations of some haematological variables in this species (Græsli et al., 2015). Unfortunately, indirect assessment of the dehydration status (e.g., using haematocrit concentration), was not conducted in this study. It is therefore unclear whether these 15 bears were significantly dehydrated during hibernation. However, analysis of other non-essential element concentrations on the same blood samples have shown that some of these elements do not vary in concentration between activity states (unpublished data). It is therefore unlikely, that dehydration would the only reason as to why the blood Pb concentration is significantly higher during hibernation compared to the active state.

In humans, Pb is mainly moving from the blood to the bones, and less so to the soft tissues, from which it has varying half-lives (i.e., months to decades, Bergdahl & Skerfving, 2022; Rabinowitz et al., 1976). It seems likely that during brown bears' active state, Pb will also move between the different compartments, have similar half-live and get excreted as seen in humans (i.e., a three-compartment model consisting of blood, soft tissues and bone): half-live of weeks to months in blood and soft tissues; years to decades in bone, excretion through urine and faeces (Chapter 4; Bergdahl & Skerfving, 2022; Rabinowitz et al., 1976). Additionally, Fuchs et al. (2021) documented that active state lactating brown bear females can excrete Pb through milk just as seen in humans (Bergdahl & Skerfving, 2022).

When no excretion is taking place (i.e., during the hibernation state), it is possible that whole-body non-essential element concentrations remain constant unless new sources of the elements get introduced into the body. Inhalation of Pb during hibernation in a den far from industrial activities must be considered unlikely. However, subcutaneous fat concentrations from hunted brown bears from this population shot in late-August show that Pb is present in this tissue, although at lower concentrations than in other tissues (*n* = 38, concentrations in µg/kg dry weight: mean = 188.076; SD = 532.904; median = 40.866; Chapter 4). With fat being the main energy source during hibernation (Geiser, 2011), it could also be that Pb whole blood concentrations increase during hibernation as Pb moves from fat to blood to a higher degree than during the active state. Therefore, the whole-body Pb concentration might stay the same throughout the hibernation period, but the blood Pb concentration increase during hibernation due to Pb moving from one body compartment to another (i.e., from soft tissue to blood).

Toxic elements should ideally not be present in a vertebrate's body as they can affect their health negatively (Agency for Toxic Substances and Disease Registry, 2022; World Health Organization, 2022). The median whole blood Pb concentrations found in this study were 8.5 and 6 times higher than the European Food Safety Authority's (EFSA) lower confidence limit benchmark doses of 12 µg/l for developmental neurotoxicity in children during hibernation and active states, respectively (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2013). This is in line with the high concentrations of Pb previously reported in this bear population from individuals captured in the spring (active state, Chapter 3; Fuchs et al., 2021).

However, how different metabolic states alter tissue and blood concentrations in different species, and how this again affects their health risk, remains unknown. Despite this, it is worth noting that brown bear foetuses are developing, and cubs are born and suckling during at least two hibernation periods (Friebe et al., 2014). Higher blood Pb concentrations in adult females during their hibernation state might therefore negatively affect foetus development and cub health as Pb passes over the placenta in humans and gets excreted in milk in both humans and bears (Bergdahl & Skerfving, 2022; Fuchs et al., 2021). Chapters 3 and 4 illustrate that the knowledge of Pb's health effects in brown bears is limited. As discussed in

Chapter 6, other variables need to be considered for evaluating the health status of individual bears in relation to Pb concentration during different metabolic states to determine if there is an increased health risk from Pb during hibernation.

5.4.2 Designing future studies to further understand the toxicokinetics of lead (Pb) during different metabolic states

To better understand the distribution of Pb in the body at different metabolic states, tissue biopsies of different organs would be needed in addition to blood samples. However, invasive sampling of anaesthetised wild animals comes with a series of ethical concerns. In addition, the nature of this study's experimental design, based on only two data points for each individual, precludes speculations of how the whole blood concentrations might change during the remaining part of the year. Unfortunately, logistics prohibit capturing live free-ranging bears in this population between June and February. Thus, utilising captive brown bears trained for voluntary blood sampling could be a way of studying whole blood concentration changes throughout the year. If it is not possible to get repeated samples from the same individuals, future studies could utilise both organ and blood samples taken opportunistically from free-ranging bears shot at different times of the year by hunters to get an idea of how Pb concentrations are changing throughout the year.

References

Agency for Toxic Substances and Disease Registry. (2019). *Lead Toxicity*. Case Studies in Environmental Medicine. https://www.atsdr.cdc.gov/csem/leadtoxicity/cover-page.html

Agency for Toxic Substances and Disease Registry. (2022). *The ATSDR 2022 Substance Priority List*. https://www.atsdr.cdc.gov/spl/index.html#2022spl

Arnemo, J. M., & Evans, A. L. (2017). *Biomedical Protocols for Free-ranging Brown Bears, Wolves, Wolverines and Lynx*.

Bergdahl, I. A., & Skerfving, S. (2022). Lead. In G. F. Nordberg & M. Costa (Eds.), *Handbook on the Toxicology of Metals: Fifth Edition: Vol. II: Specific Metals* (Fifth Edition, pp. 427–493). Academic Press. https://doi.org/10.1016/B978-0-12-822946-0.00036-2

Bloomfield, S. A. (1997). Changes in musculoskeletal structure and function with prolonged bed rest. *Medicine and Science in Sports and Exercise*, *29*(2), 197–206. https://doi.org/10.1097/00005768-199702000-00006

EFSA Panel on Contaminants in the Food Chain (CONTAM). (2013). Scientific opinion on lead in food. *EFSA Journal*. https://doi.org/10.2903/j.efsa.2010.1570

Evans, A. L., Singh, N. J., Friebe, A., Arnemo, J. M., Laske, T. G., Fröbert, O., Swenson, J. E., & Blanc, S. (2016a). Drivers of hibernation in the brown bear. *Frontiers in Zoology, 13*, 7. https://doi.org/10.1186/s12983-016-0140-6

Evans, A. L., Singh, N. J., Fuchs, B., Blanc, S., Friebe, A., Laske, T. G., Frobert, O., Swenson, J. E., & Arnemo, J. M. (2016b). Physiological reactions to capture in hibernating brown bears. *Conservation Physiology*, *4*(1), 61. https://doi.org/10.1093/CONPHYS/COW061

FAO, UNEP, WHO, & WOAH. (2022). *One Health Joint Plan of Action (2022-2026). Working together for the health of humans, animals, plants and the environment*. https://doi.org/10.4060/cc2289en

Friebe, A., Evans, A. L., Arnemo, J. M., Blanc, S., Brunberg, S., Fleissner, G., Swenson, J. E., & Zedrosser, A. (2014). Factors Affecting Date of Implantation, Parturition, and Den Entry Estimated from Activity and Body Temperature in Free-Ranging Brown Bears. *PLOS ONE*, *9*(7), e101410. https://doi.org/10.1371/JOURNAL.PONE.0101410

Fuchs, B., Thiel, A., Zedrosser, A., Brown, L., Hydeskov, H. B., Rodushkin, I., Evans, A. L., Boesen, A. H., Græsli, A. R., Kindberg, J., Kindberg, J., & Arnemo, J. M. (2021). High concentrations of lead (Pb) in blood and milk of free-ranging brown bears (*Ursus arctos*) in Scandinavia. *Environmental Pollution*, *287*, 117595. https://doi.org/10.1016/j.envpol.2021.117595

Geiser, F. (2011). Hibernation: Endotherms. *ELS*. https://doi.org/10.1002/9780470015902.A0003215.PUB2

Græsli, A. R., Evans, A. L., Fahlman, Å., Bertelsen, M. F., Blanc, S., & Arnemo, J. M. (2015). Seasonal variation in haematological and biochemical variables in free-ranging subadult brown bears (*Ursus arctos*) in Sweden. *BMC Veterinary Research*, *11*(1), 1–9. https://doi.org/10.1186/S12917-015-0615-2/TABLES/5

Hellgren, E. C. (1998). Physiology of Hibernation in Bears. *Ursus*, *10*, 467–477.

IBM Corp. (2021). *IBM SPSS Statistics for Windows, Version 28*. IBM Corp.

Manchi, S., & Swenson, J. E. (2005). Denning behaviour of Scandinavian brown bears *Ursus arctos*. *Wildlife Biology*, *11*(2), 123–132. https://doi.org/10.2981/0909-6396(2005)11[123:DBOSBB]2.0.CO;2

Martin, J., Basille, M., van Moorter, B., Kindberg, J., Allainé, D., & Swenson, J. E. (2010). Coping with human disturbance: spatial and temporal tactics of the brown bear (*Ursus arctos*). *Canadian Journal of Zoology*, *88*(9), 875–883. https://doi.org/10.1139/Z10-053

McGee-Lawrence, M., Buckendahl, P., Carpenter, C., Henriksen, K., Vaughan, M., & Donahue, S. (2015). Suppressed bone remodeling in black bears conserves energy and bone mass during hibernation. *Journal of Experimental Biology*, *218*(13), 2067–2074. https://doi.org/10.1242/JEB.120725

Morales, J. O., Walker, N., Warne, R. W., & Boyles, J. G. (2021). Heterothermy as a mechanism to offset energetic costs of environmental and homeostatic perturbations. *Scientific Reports*, *11*, 19038. https://doi.org/10.1038/s41598-021-96828-0

Nelson, R. A. (1973). Winter sleep in the black bear. A physiologic and metabolic marvel. *Mayo Clinic Proceedings*, *48*(10), 733–737.

Pain, D. J., Mateo, R., & Green, R. E. (2019). Effects of lead from ammunition on birds and other wildlife: A review and update. *Ambio*, *48*(9), 935–953. https://doi.org/10.1007/s13280-019-01159-0

R Core Team. (2023). *R: A language and environment for statistical computing, Version 4.3.0* (4.3.0). R Foundation for Statistical Computing.

Rabinowitz, M. B., Wetherill, G. W., & Kopple, J. D. (1976). Kinetic analysis of lead metabolism in healthy humans. *The Journal of Clinical Investigation*, *58*(2), 260–270. https://doi.org/10.1172/JCI108467

Söderberg, C., Rodushkin, Ilia, Johansson, A., & Kugelberg, F. C. (2023). Postmortem reference concentrations of 68 elements in blood and urine. *International Journal of Legal Medicine*, *137*, 655–669. https://doi.org/10.1007/S00414-023-02952-Z

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag.

World Health Organization. (2022). *Lead poisoning*. https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health

Chapter 6: Discussion

This chapter synthesises the findings of the thesis and addresses the objectives described in Chapter 1. Ideas for future research are provided and discussed.

6.1 Synopsis

The global systematic literature review (Chapter 2) documented that while Pb exposure was reported in 153 wild mammalian species, a lot remains unknown about Pb exposure in this animal class. Only 6 % of the published studies were conducted in developing countries, where the Pb environmental contamination might be highest. Additionally, there was a tendency to study herbivorous and omnivorous species that were easy to access, leaving the predatory and scavenging species less studied, despite they might be the most interesting from a One Health perspective. Finally, very little is known about the health effects of Pb exposure in wild mammals illustrated by only 25 % of the studies investigating Pb health effects and, of these, only 62 % finding any health effects. As expected, all health effects identified in the studies were negative.

Chapters 3 and 4 showed that the Pb exposure in free-ranging Scandinavian brown bears might be linked to blood variables indicative of liver and chronic kidney disease (i.e., haemoglobin, alanine aminotransferase, total bilirubin and creatinine), yet histopathological changes in tissues were not detected. The captive brown bear population in Scandinavia is also exposed to Pb and shows similar blood concentration patterns as the free-ranging bears. The high blood Pb concentrations in cubs and lactating females is likely from Pb being excreted in milk, meaning the cubs are exposed to Pb from an early age when suckling, and in the lactating females Pb is moving with calcium from bone tissue into the blood. Just as for the free-ranging bears, the examination of captive bear tissues also failed to detect any histopathological changes. From a One Health perspective however, most important were the findings of high Pb concentrations in bear products consumed by humans. This highlights that Pb continues to be of great One Health concern and that, currently, it is not advisable to eat any bear products from Swedish brown bears.

The results from Chapter 5 showed that Scandinavian brown bears have significantly higher blood Pb concentrations during hibernation than when they are active. Whether the bears are more at risk of the negative health effects of Pb during hibernation or if they possess mechanisms to protect them, remain unknown.

6.2 The Scandinavian brown bear (*Ursus arctos*) as a sentinel for lead (Pb) exposure monitoring

Sentinel animals are being used as biological monitors in many ways (Beeby, 2001). For a species to be a good sentinel species for Pb exposure monitoring, it would need to fulfil two requirements: 1) the species should be a good representation of the environment it lives in, from a Pb context, and 2) samples from the species should be easy to access. The Scandinavian brown bear fulfils both of these requirements.

As Pb bioaccumulates in both plants and animals (Clemens, 2006; Radomyski et al., 2018), studying animals at high trophic levels provides information about Pb exposure from the environment and lower trophic levels. The Scandinavian brown bear has a varied diet from being a top predator, a scavenger and an omnivore (Wilson & Mittermeier, 2009) and it can thereby act as an indicator species for many different parts of the food web (Beeby, 2001). Additionally, the species has a long lifespan of approximately 30 years (Chapron et al., 2009; Schwartz et al., 2003), meaning that the same individuals can be followed over long periods of time for continuing sampling.

In Sweden, the brown bear belongs to the state (“statens vilt”) meaning that the government is responsible for monitoring the health of the free-ranging population and examining all carcasses (Regeringskansliet, 2022). This work is coordinated by the Swedish National Veterinary Institute (SVA) which delegates part of this work to the county boards during the annual licensed bear hunt (Höök & Ågren, 2022; Naturvårdsverket, 2023). This results in hundreds of brown bears from across the species’ geographical range in Sweden being sampled already for different purposes, including ongoing biobanking of samples. Additionally, the brown bear is a commonly kept species in Nordic zoological institutions with 56 animals across 13 institutions as of 09 June 2023 (Species360, 2023). The

mission of the European Association of Zoos and Aquaria (EAZA) includes species conservation through scientific research and EAZA has started to establish biobanks of samples to assist with this part of the mission (European Association of Zoos and Aquaria, 2023b, 2023a).

Consequently, samples from both free-ranging and captive Scandinavian brown bears are easily accessible for research. Utilising samples from animals shot or anaesthetised for other reasons reduces the overall number of research animals needed, supportive of the important 3R principles for more ethical wildlife research (NC3Rs, 2023), as already applied in this thesis.

The brown bear can also be a good species to study if interested in retrospective Pb exposure monitoring. Hair samples from free-ranging brown bears could easily be collected using hair traps (Woods et al., 1999), but it would be difficult to standardise where on the body the hair is sampled from. However, as a traditional game and trophy species, pelts have been saved in many museums across the globe and are potentially available for environmental contaminant monitoring (Naturhistoriska riksmuseet, 2023). If a reliable and repeatable method to test brown bear hair for Pb can be developed (Sela et al., 2007), this has great potential for studying historical Pb exposure from across the species' large geographical range currently covering three continents (McLellan et al., 2017). Overall, the Scandinavian brown bear is an excellent sentinel species for monitoring Pb exposure.

6.3 Health effects of lead (Pb) in Scandinavian brown bears (*Ursus arctos*)

Chapters 3 and 4 showed that despite four blood variables indicative of liver and chronic kidney disease were correlated with blood Pb concentration, no histopathological changes were detected. Without knowing the normal blood variable reference ranges for free-ranging Scandinavian brown bears that have not been exposed to Pb, it is not possible to determine if the increased concentrations of haemoglobin, alanine aminotransferase, total bilirubin and creatinine variables are a direct result of the Pb exposure (i.e., causation has not been established).

There are three possible explanations as to why no definite negative health effects of Pb exposure were detected in the Scandinavian brown bears: 1) the Pb exposure is not high enough to cause disease, 2) the correct negative health effect has not been studied during the thesis, and 3) the bears have a way of protecting themselves from the harmful effects of Pb.

Histopathological changes in the kidney have been detected in free-ranging brown rats (*Rattus norvegicus*) with a kidney Pb concentration of 42 µg/kg wet weight (Ceruti et al., 2002). Likewise, histopathological changes in the liver have been detected in free-ranging long-tailed field mice (*Apodemus sylvaticus*) with a liver Pb concentration of 90 µg/kg dry weight (Tête et al., 2014). As the kidney and liver tissue concentrations found in Chapter 4 are much higher than these (means ranging from 353.5 to 3,998.8 µg/kg dry weigh) it is likely that the Pb exposure in Scandinavian brown bears is indeed high enough to cause negative health effects if the species does not have protective mechanisms to prevent such health effects.

Although the Scandinavian brown bear population is considered “healthy” by the governmental agency in charge of wildlife disease surveillance, SVA (National Veterinary Institute (SVA), 2022), it is possible that the health effects in this species caused by Pb exposure has just not been investigated yet. Ideas for future studies are discussed in section 6.6.

The brown bear might have one or more mechanisms to protect themselves from Pb’s negative health effects. Scandinavian brown bears have significantly higher blood taurine concentrations than humans (Stenvinkel et al., 2013), and this amino acid has been demonstrated to act as an antioxidant in rats intentionally poisoned with Pb (Gürer et al., 2001). However, serum taurine and whole blood Pb concentrations are not correlated in free-ranging Scandinavian brown bears (unpublished data). If taurine is in fact protecting brown bears against Pb’s negative health effects, it might just need to be present at a certain concentration to protect the bears, and not necessarily be correlated with blood Pb concentration. Another potential protective mechanism of interest is superoxide dismutase as the activity of this antioxidative enzyme has a positive correlation with blood Pb concentration in brown bears in Croatia and Poland (Lazarus et al., 2020). In another taxonomic kingdom, it has been shown that some terrestrial

plants (e.g., bryophytes) possess mechanisms to reduce the impact the harmful effects of Pb and other toxic, non-essential elements (Stanković & Sabovljević, 2018). This is done by activating different exclusion mechanisms that prevents the toxic element from entering the plant tissue. Whether such exclusion mechanisms exist in the animal kingdom is unknown.

6.4 One Health perspective

It is not new that Pb exposure is a major One Health issue (FAO et al., 2022; World Health Organization, 2019). Despite the banning of Pb in some products such as petrol, there is still a long way to go before Pb is banned in all anthropogenic activities and thereby reducing the environmental exposure as much as possible. Studies of mosses in Sweden over 40 years (1975-2015) have shown a decrease of 96 % in Pb concentration from 1975 to 2015 as a direct result of the ban on Pb in petrol in the 1990s (Danielsson & Karlsson, 2016). Yet, Pb-based paints are still present in the majority of countries worldwide (World Health Organization, 2022) and very few places have made legally-binding controls on Pb-based ammunition. California was the first federal state in 2019, and Denmark will be the first country in 2024 to ban all Pb-based hunting ammunition (Miljøministeriet, 2022; State of California, 2013). More bans like this are hopefully on their way in other places to prevent Pb from being scattered around in the environment by hunters (European Chemicals Agency, 2023).

The results from Chapter 4 showed that Pb exposure through consumption of free-ranging Scandinavian brown bear products pose a risk to human health. The study showed that the bear meat and offal already contain Pb (means ranging from 170.0 to 3,998,8 µg/kg dry weight) before the animal is shot by a hunter. These concentrations are likely to be higher than the concentrations of Pb allowed in domestic mammalian products for human consumption (European Union, 2023). That 98 % of the hunters then add even more Pb to these edible products by shooting the bears with Pb-based ammunition is indeed worrisome (unpublished data). Once again, the author would like to stress that eating bear products originating from the Swedish free-ranging brown bears cannot be recommended, and the author supports the need for regulation on toxic element concentrations allowed in wildlife/game products as already suggested by other researchers (Thomas et al., 2020). The results from Chapters 3 and 4 will add evidence to the

current body of literature on Pb exposure and are expected to influence game managers and policymakers worldwide regarding the regulation of Pb-based ammunition to reduce the Pb environmental contamination and to improve human health.

6.5 Study limitations

Although a large number of bears were included in this thesis, Pb analysis of blood and tissues from additional bears from different age and sex groups would get the group sample sizes big enough to perform statistical testing.

Pb concentrations in different tissues could be analysed from the hunted bears to further investigate the distribution in the body, although accessing central nervous tissue is not possible at the moment as only a ventral midline incision can be made at the ranger inspection station. Slightly more decomposed tissues might be available for sampling when the butchering is taking place but the logistics for such sampling is currently not in place.

In terms of accuracy of the Pb concentrations measured, one concern could be that some of the captive brown bear tissue samples were stored at -20 °C for over 15 years prior to being analysed for Pb (Chapter 4). Long term studies investigating stability of elements are rare, but it has been shown that Pb in blood is stable at -20 °C for at least 36 months (Tevis et al., 2018). The tissue samples in Chapter 4 were all stored in closed plastic containers or bags, and we can therefore assume that the Pb concentration at the time of measurement is close to identical as the concentration at time of sampling. Additionally, as it was shown that 4 °C and -20° C perform equally well as -70 °C in terms of Pb stability in blood (Tevis et al., 2018), future Pb studies can reduce their energy usage by raising the sample storage temperature.

6.6 Future research recommendations

Some key questions have arisen from this thesis: 1) is Pb affecting the health of Scandinavian brown bears? 2) do Scandinavian brown bears have a mechanism to protect themselves against Pb? 3) does Pb whole-body concentration fluctuate over the year? 4) does a “normal” Scandinavian brown bear in terms of Pb

exposure exist? and 4) what Pb concentrations in Scandinavian brown bears would we find in areas with no hunting?

It is likely that we still have not investigated the right variable to detect a negative health effect from Pb, and it remains unknown if brown bears possess a protective mechanism against Pb's health effects. For example, we do not know if the bears' neurological development or lifetime reproductive success are affected, or whether their immune systems are compromised, thereby making them more prone to infectious diseases. Antioxidant, neurological health and long-term reproductive success studies are ideas for future research.

One enzyme of interest would be δ-aminolevulinic acid dehydratase (ALAD), as blood and tissue concentrations of this antioxidant have been demonstrated to decrease in several avian species (Descalzo et al., 2021; Martinez-Haro et al., 2011) and in some *Rodentia* and *Eulipotyphla* species (Mouw et al., 1975; Stansley & Roscoe, 1996) when they were exposed to Pb.

Cognitive ability testing (Chambers & O'Hara, 2023) and continuing blood Pb studies over long periods of time in captive bears would be also valuable and help us answers questions about Pb fluctuations and long-term neurological health effects.

Studying Pb concentrations in relation to health variables during different metabolic states (i.e., hibernation versus active state) is crucial to determine if hibernation increases the risk of health effects from Pb in cubs of the year, yearlings, sub-adults and adults. Unfortunately, for human safety, it is currently only possible to capture sub-adults during hibernation (i.e., no adult bear present). However, as the first study showing that hibernation increases the concentration of a toxic element, there might be other hibernating species easier to capture, that studies like this can be carried out in. In this geographical region, the western European hedgehog (*Erinaceus europaeus*) and the raccoon dog (*Nyctereutes procyonoides*) could be considered (IUCN, 2023).

If we can find a way to get blood and tissue samples from the same animals at different times a year, this could help to establish Pb's toxicokinetics in the

species. However, this very invasive type of study is currently unlikely in both free-ranging and captive individuals from an ethical perspective. The use of filter paper to collect blood samples from free-ranging bears could provide us with information of Pb concentrations in the few bears shot outside of the Swedish annual licensed hunt season, and thereby help to investigate potential further fluctuations of Pb throughout the year (Rodríguez-Saldaña et al., 2021).

For all further research, it is important to remember that until we have populations, both free-ranging and captive, that are much less exposed to Pb, we will not be able to confirm the Pb concentrations of 'normal' Scandinavian brown bears.

We will hopefully see a reduction in Pb-based ammunition and other products in the near future. The European Chemicals Agency has already proposed a restriction on the use of Pb in hunting, sports shooting and fishing to reduce the Pb environmental emissions with 72 % over 20 years (European Chemicals Agency, 2023). With ongoing studies on the same brown bear population, we would be able to detect if this affects the level of Pb exposure and potentially, over time, get to a stage where we can get an idea of what "normal"/non-Pb-exposed bears are like.

Finally, it is crucial to note that different brown bear populations have been shown to be exposed to additional toxic elements (i.e., arsenic, cadmium and mercury) (Fuchs et al., 2023), so future studies in Scandinavian brown bears could benefit from investigating health effects with attention to the potential cocktail effect several toxic elements can have when combined (Bopp et al., 2018).

Taking a One Health approach to all future research on toxic elements is recommended, as Pb is just one of many aspects of environmental pollution affecting human, animal and environmental health (Sleeman et al., 2019).
It might be that in order to understand the health effects in Scandinavian brown bears, we need to look more closely at how Pb can impact behaviour and body mass and thereby indirectly affect survival as seen in some bird species (Ecke et al., 2017; Vallverdú-Coll et al., 2015). And perhaps we even need to look outside the animal kingdom for inspiration, such as studying different Pb exclusion mechanisms found in plants (Stanković & Sabovljević, 2018).

6.7 Conclusions

On a global scale, wild mammals across many taxa are exposed to and affected by Pb. Studying mammals at high trophic levels, like the Scandinavian brown bear, is recommended to get a good representation of the environment. The Scandinavian brown bears, both free-ranging and captive, are exposed to Pb, yet how Pb exactly affects their health requires further investigations. Blood Pb concentration varies with metabolic states which might alter the risk of health effects throughout the year.

As Pb continues to be a One Health issue, monitoring Pb exposure in the Scandinavian brown bear is recommended as samples are easy to access and products from the hunted individuals are used for human consumption. Currently, these products cannot be recommended for human consumption. The findings in this thesis support the need for more controls on Pb in anthropogenic products, including ammunition, and continuing Pb exposure monitoring in a One Health context.

References

Beeby, A. (2001). What do sentinels stand for? *Environmental Pollution*, *112*(2), 285–298. https://doi.org/10.1016/S0269-7491(00)00038-5

Bopp, S. K., Barouki, R., Brack, W., Dalla Costa, S., Dorne, J. L. C. M., Drakvik, P. E., Faust, M., Karjalainen, T. K., Kephalopoulos, S., van Klaveren, J., Kolossa-Gehring, M., Kortenkamp, A., Lebret, E., Lettieri, T., Nørager, S., Rüegg, J., Tarazona, J. V., Trier, X., van de Water, B., … Bergman, Å. (2018). Current EU research activities on combined exposure to multiple chemicals. *Environment International*, *120*, 544–562. https://doi.org/10.1016/J.ENVINT.2018.07.037

Ceruti, R., Ghisleni, G., Ferretti, E., Cammarata, S., Sonzogni, O., & Scanziani, E. (2002). Wild rats as monitors of environmental lead contamination in the urban area of Milan, Italy. *Environmental Pollution*, *117*(2), 255–259. https://doi.org/10.1016/S0269-7491(01)00273-1

Chambers, H. R., & O'Hara, S. J. (2023). Problem-solving and spontaneous tool-using ability in European brown bears (*Ursus arctos arctos*). *Animal Behavior and Cognition*, *10*(1), 40–61. https://doi.org/10.26451/ABC.10.01.03.2023

Chapron, G., Wielgus, R., Quenette, P. Y., & Camarra, J. J. (2009). Diagnosing mechanisms of decline and planning for recovery of an endangered brown bear (*Ursus arctos*) population. *PLOS ONE*, *4*(10), e7568. https://doi.org/10.1371/JOURNAL.PONE.0007568

Clemens, S. (2006). Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie*, *88*(11), 1707–1719. https://doi.org/10.1016/J.BIOCHI.2006.07.003

Danielsson, H., & Karlsson, G. P. (2016). *Metaller i mossa 2015 - Rapport C 204*.

Descalzo, E., Camarero, P. R., Sánchez-Barbudo, I. S., Martinez-Haro, M., Ortiz-Santaliestra, M. E., Moreno-Opo, R., & Mateo, R. (2021). Integrating active and passive monitoring to assess sublethal effects and mortality from lead poisoning in birds of prey. *Science of The Total Environment*, *750*, 142260. https://doi.org/10.1016/J.SCITOTENV.2020.142260

Ecke, F., Singh, N. J., Arnemo, J. M., Bignert, A., Helander, B., Berglund, Å. M. M., Borg, H., Bröjer, C., Holm, K., Lanzone, M., Miller, T., Nordström, Å., Räikkönen, J., Rodushkin, I., Ågren, E., & Hörnfeldt, B. (2017). Sublethal Lead Exposure Alters Movement Behavior in Free-Ranging Golden Eagles. *Environmental Science and Technology*, *51*(10), 5729–5736. https://doi.org/10.1021/acs.est.6b06024

European Association of Zoos and Aquaria. (2023a). *About us*. https://www.eaza.net/about-us/eaza/

European Association of Zoos and Aquaria. (2023b). *Research*. https://www.eaza.net/conservation/research/

European Chemicals Agency. (2023). *Lead in shot, bullets and fishing weights*. https://echa.europa.eu/hot-topics/lead-in-shot-bullets-and-fishing-weights

European Union. (2023). *Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 (Text with EEA relevance)*.

FAO, UNEP, WHO, & WOAH. (2022). *One Health Joint Plan of Action (2022-2026). Working together for the health of humans, animals, plants and the environment*. https://doi.org/10.4060/cc2289en

Fuchs, B., Joly, K., Hilderbrand, G. V., Evans, A. L., Rodushkin, I., Mangipane, L. S., Mangipane, B. A., Gustine, D. D., Zedrosser, A., Brown, L., & Arnemo, J. M. (2023). Toxic elements in arctic and sub-arctic brown bears: Blood concentrations of As, Cd, Hg and Pb in relation to diet, age, and human footprint. *Environmental Research*, *229*, 115952. https://doi.org/10.1016/J.ENVRES.2023.115952

Gürer, H., Özgünes, H., Saygin, E., & Ercal, N. (2001). Antioxidant effect of taurine against lead-induced oxidative stress. *Archives of Environmental Contamination and Toxicology*, *41*(4), 397–402. https://doi.org/10.1007/S002440010265/METRICS

Höök, E., & Ågren, E. (2022). *Licensjakt björn 2022 - SVA Rapportserie 81/2022*.

IUCN. (2023). *The IUCN Red List of Threatened Species. Version 2022-2.* https://www.iucnredlist.org

Lazarus, M., Orct, T., Sergiel, A., Vranković, L., Marijić, V. F., Rašić, D., Reljić, S., Aladrović, J., Zwijacz-Kozica, T., Zięba, F., Selva, N., & Huber. (2020). Metal(loid) exposure assessment and biomarker responses in captive and free-ranging European brown bear (<i>Ursus arctos>/i>). *Environmental Research*, *183*, 109166. https://doi.org/10.1016/j.envres.2020.109166

Martinez-Haro, M., Green, A. J., & Mateo, R. (2011). Effects of lead exposure on oxidative stress biomarkers and plasma biochemistry in waterbirds in the field. *Environmental Research*, *111*(4), 530–538. https://doi.org/10.1016/J.ENVRES.2011.02.012

McLellan, B. N., Proctor, M. F., Huber, D., & Michel, S. (2017). *Ursus arctos* (amended version of 2017 assessment). The IUCN Red List of Threatened Species 2017: e.T41688A121229971.

Miljøministeriet. (2022). *Bekendtgørelse om våben og ammunition der må anvendes til jagt m.v. - BEK nr 971 af 21/06/2022*.

Mouw, D., Kalitis, K., Anver, M., Schwartz, J., Constan, A., Hartung, R., Cohen, B., & Ringler, D. (1975). Lead. Possible toxicity in urban vs rural rats. *Archives of Environmental Health*, *30*(6), 276–280. https://doi.org/10.1080/00039896.1975.10666699

National Veterinary Institute (SVA). (2022). *Wildlife disease surveillance in Sweden 2021 - SVA report series 76:2022*.

Naturhistoriska riksmuseet. (2023). *Environmental Contaminant Monitoring*. https://www.nrm.se/en/forskningochsamlingar/miljoforskningochovervakning/miljogiftsovervakning.932.html

Naturvårdsverket. (2023). *Jakt på björn*. https://www.naturvardsverket.se/amnesomraden/jakt-och-vilt/jakt-pa-rovdjur/jakt-pa-bjorn/

NC3Rs. (2023). *The 3Rs*. https://nc3rs.org.uk/who-we-are/3rs

Radomyski, A., Lei, K., Giubilato, E., Critto, A., Lin, C., & Marcomini, A. (2018). Bioaccumulation of trace metals in aquatic food web. A case study, Liaodong Bay, NE China. *Marine Pollution Bulletin*, *137*, 555–565. https://doi.org/10.1016/J.MARPOLBUL.2018.11.002

Regeringskansliet. (2022). *Förordning om ändring i jaktförordningen (1987:905)* (SFS 2022:1374).

Rodríguez-Saldaña, V., Fobil, J., & Basu, N. (2021). Lead (Pb) exposure assessment in dried blood spots using Total Reflection X-Ray Fluorescence (TXRF). *Environmental Research*, *198*, 110444. https://doi.org/10.1016/J.ENVRES.2020.110444

Schwartz, C. C., Keating, K. A., Reynolds III, H. V., Barnes Jr., V. G., Sellers, R. A., Swenson, J. E., Miller, S. D., McLellan, B. N., Keay, J., McCann, R., Smith, R., & Herrero, S. (2003). Reproductive maturation and senescence in the female brown bear. *Ursus*, *14*(2), 109–119.

Sela, H., Karpas, Z., Zoriy, M., Pickhardt, C., & Becker, J. S. (2007). Biomonitoring of hair samples by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). *International Journal of Mass Spectrometry*, *261*(2–3), 199–207. https://doi.org/10.1016/J.IJMS.2006.09.018

Sleeman, J. M., Richgels, K. L. D., White, C. L., & Stephen, C. (2019). Integration of wildlife and environmental health into a One Health approach. *Revue Scientifique et Technique de l'OIE, 38*(1), 91-102. https://doi.org/10.20506/rst.38.1.2944

Species360. (2023). *Zoological Information Management System*. Species Holding. https://zims.species360.org/Main.aspx

Stanković, J. D., & Sabovljević, M. S. (2018). Bryophytes and heavy metals: a review. *Acta Botanica Croatia, 77*(2), 109-118. https://doi.org/10.2478/botcro-2018-0014

Stansley, W., & Roscoe, D. E. (1996). The uptake and effects of lead in small mammals and frogs at a trap and skeet range. *Archives of Environmental Contamination and Toxicology*, *30*(2), 220–226. https://doi.org/10.1007/BF00215801

State of California. (2013). *An act to amend Section 3004.5 of the Fish and Game Code, relating to hunting - Assembly Bill No. 711*.

Stenvinkel, P., Fröbert, O., Anderstam, B., Palm, F., Eriksson, M., Bragfors-Helin, A.-C., Qureshi, A. R., Larsson, T., Friebe, A., Zedrosser, A., Josefsson, J., Svensson, M., Sahdo, B., Bankir, L., & Johnson, R. J. (2013). Metabolic changes in summer active and anuric hibernating free-ranging brown bears (*Ursus arctos*). *PLOS ONE*, *8*(9), e72934. https://doi.org/10.1371/JOURNAL.PONE.0072934

Tête, N., Durfort, M., Rieffel, D., Scheifler, R., & Sánchez-Chardi, A. (2014). Histopathology related to cadmium and lead bioaccumulation in chronically exposed wood mice, *Apodemus sylvaticus*, around a former smelter. *Science of the Total Environment*, *481*(1), 167–177. https://doi.org/10.1016/j.scitotenv.2014.02.029

Tevis, D. S., Jarrett, J. M., Jones, D. R., Cheng, P. Y., Franklin, M., Mullinex, N., Caldwell, K. L., & Jones, R. L. (2018). Assessing the stability of Cd, Mn, Pb, Se, and total Hg in whole human blood by ICP-DRC-MS as a function of temperature and time. *Clinica Chimica Acta*, *485*, 1–6. https://doi.org/10.1016/J.CCA.2018.05.043

Thomas, V. G., Pain, D. J., Kanstrup, N., & Green, R. E. (2020). Setting maximum levels for lead in game meat in EC regulations: An adjunct to replacement of lead ammunition. *Ambio*, *49*(12), 2026–2037. https://doi.org/10.1007/S13280-020-01336-6/FIGURES/2

Vallverdú-Coll, N., López-Antia, A., Martinez-Haro, M., Ortiz-Santaliestra, M. E., Mateo, R. (2015). Altered immune response in mallard ducklings exposed to lead through maternal transfer in the wild. *Environmental Pollution, 205*, 350-356. https://doi.org/10.1016/j.envpol.2015.06.014

Wilson, D. E., & Mittermeier, R. A. (Eds.). (2009). *Handbook of the Mammals of the World: Carnivores* (Vol. 1). Lynx Edicions.

Woods, J. G., Paetkau, D., Lewis, D., Mclellan, B., Proctor, M., & Strobeck, C. (1999). Genetic tagging of free-ranging black and brown bears. *Wildlife Society Bulletin*, *27*, 616–627.

World Health Organization. (2019). *Lead poisoning and health*. https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health

World Health Organization. (2022). *Legally-binding controls on lead paint*. The Global Health Observatory. https://www.who.int/data/gho/data/themes/topics/indicator-groups/legally-binding-controls-on-lead-paint

Appendix A

Taxonomic search terms (27 orders, 162 families, 1296 genera) used in the global systematic literature review (Chapter 2).

mammal OR mammals OR mammalian OR Mammalia OR Afrosoricida OR Carnivora OR Cetartiodactyla OR Chiroptera OR Cingulata OR Dasyuromorphia OR Dermoptera OR Didelphimorphia OR Diprotodontia OR Eulipotyphla OR Hyracoidea OR Lagomorpha OR Macroscelidea OR Microbiotheria OR Monotremata OR Notoryctemorphia OR Paucituberculata OR Peramelemorphia OR Perissodactyla OR Pholidota OR Pilosa OR Primates OR Proboscidea OR Rodentia OR Scandentia OR Sirenia OR Tubulidentata OR Abrocomidae OR Acrobatidae OR Ailuridae OR Anomaluridae OR Antilocapridae OR Aotidae OR Aplodontiidae OR Atelidae OR Balaenidae OR Balaenopteridae OR Bathyergidae OR Bovidae OR Bradypodidae OR Burramyidae OR Caenolestidae OR Callitrichidae OR Calomyscidae OR Camelidae OR Canidae OR Capromyidae OR Castoridae OR Caviidae OR Cebidae OR Cercopithecidae OR Cervidae OR Chaeropodidae OR Cheirogaleidae OR Chinchillidae OR Chlamyphoridae OR Chrysochloridae OR Cistugidae OR Craseonycteridae OR Cricetidae OR Ctenodactylidae OR Ctenomyidae OR Cuniculidae OR Cyclopedidae OR Cynocephalidae OR Dasypodidae OR Dasyproctidae OR Dasyuridae OR Daubentoniidae OR Delphinidae OR Diatomyidae OR Didelphidae OR Dinomyidae OR Dipodidae OR Dugongidae OR Echimyidae OR Elephantidae OR Emballonuridae OR Equidae OR Erethizontidae OR Erinaceidae OR Eschrichtiidae OR Eupleridae OR Felidae OR Furipteridae OR Galagidae OR Geomyidae OR Giraffidae OR Gliridae OR Herpestidae OR Heteromyidae OR Hippopotamidae OR Hipposideridae OR Hominidae OR Hyaenidae OR Hylobatidae OR Hypsiprymnodontidae OR Hystricidae OR Indriidae OR Iniidae OR Kogiidae OR Lemuridae OR Lepilemuridae OR Leporidae OR Lipotidae OR Lorisidae OR Macropodidae OR Macroscelididae OR Manidae OR Megadermatidae OR Megalonychidae OR Mephitidae OR Microbiotheriidae OR Miniopteridae OR Molossidae OR Monodontidae OR Mormoopidae OR Moschidae OR Muridae OR Mustelidae OR Myocastoridae OR Myrmecobiidae OR Myrmecophagidae OR Mystacinidae OR Myzopodidae OR Nandiniidae OR Natalidae OR Neobalaenidae OR Nesomyidae OR Nesophontidae OR

Noctilionidae OR Notoryctidae OR Nycteridae OR Ochotonidae OR Octodontidae OR Odobenidae OR Ornithorhynchidae OR Orycteropodidae OR Otariidae OR Palaeopropithecidae OR Pedetidae OR Peramelidae OR Petauridae OR Petromuridae OR Phalangeridae OR Phascolarctidae OR Phocidae OR Phocoenidae OR Phyllostomidae OR Physeteridae OR Pitheciidae OR Platacanthomyidae OR Platanistidae OR Pontoporiidae OR Potoroidae OR Prionodontidae OR Procaviidae OR Procyonidae OR Prolagidae OR Pseudocheiridae OR Pteropodidae OR Ptilocercidae OR Rhinocerotidae OR Rhinolophidae OR Rhinopomatidae OR Sciuridae OR Solenodontidae OR Soricidae OR Spalacidae OR Suidae OR Tachyglossidae OR Talpidae OR Tapiridae OR Tarsiidae OR Tarsipedidae OR Tayassuidae OR Tenrecidae OR Thryonomyidae OR Thylacinidae OR Thylacomyidae OR Thyropteridae OR Tragulidae OR Trichechidae OR Tupaiidae OR Ursidae OR Vespertilionidae OR Viverridae OR Vombatidae OR Ziphiidae OR Abditomys OR Abeomelomys OR Abrawayaomys OR Abrocoma OR Abrothrix OR Acerodon OR Acinonyx OR Acomys OR Aconaemys OR Acrobates OR Addax OR Aegialomys OR Aepeomys OR Aepyceros OR Aepyprymnus OR Aeretes OR Aeromys OR Aethalops OR Aethomys OR Ailuropoda OR Ailurops OR Ailurus OR Akodon OR Alcelaphus OR Alces OR Alionycteris OR Allactaga OR Allactodipus OR Allenopithecus OR Allocebus OR Allochrocebus OR Allocricetulus OR Alouatta OR Alticola OR Amblysomus OR Ametrida OR Ammodillus OR Ammodorcas OR Ammospermophilus OR Ammotragus OR Amorphochilus OR Amphinectomys OR Anathana OR Andalgalomys OR Andinomys OR Anisomys OR Anomalurus OR Anonymomys OR Anotomys OR Anoura OR Anourosorex OR Antechinomys OR Antechinus OR Anthops OR Antidorcas OR Antilocapra OR Antilope OR Antrozous OR Aonyx OR Aotus OR Aplodontia OR Apodemus OR Apomys OR Aproteles OR Arabitragus OR Arborimus OR Archboldomys OR Arctictis OR Arctocebus OR Arctocephalus OR Arctogalidia OR Arctonyx OR Ardops OR Arielulus OR Ariteus OR Artibeus OR Arvicanthis OR Arvicola OR Asellia OR Aselliscus OR Atelerix OR Ateles OR Atelocynus OR Atherurus OR Atilax OR Atlantoxerus OR Atopogale OR Auliscomys OR Austronomus OR Avahi OR Axis OR Babyrousa OR Baeodon OR Baiomys OR Baiyankamys OR Balaena OR Balaenoptera OR Balantiopteryx OR Balionycteris OR Bandicota OR Barbastella OR Bassaricyon OR Bassariscus OR Bathyergus OR Batomys OR Bauerus OR Bdeogale OR Beamys OR Beatragus OR Belomys OR Berardius OR Berylmys

OR Bettongia OR Bibimys OR Bison OR Biswamoyopterus OR Blanfordimys OR Blarina OR Blarinella OR Blarinomys OR Blastocerus OR Boneia OR Boromys OR Bos OR Boselaphus OR Brachiones OR Brachylagus OR Brachyphylla OR Brachytarsomys OR Brachyteles OR Brachyuromys OR Bradypus OR Brassomys OR Brotomys OR Brucepattersonius OR Bubalus OR Budorcas OR Bullimus OR Bunolagus OR Bunomys OR Burramys OR Cabassous OR Cacajao OR Caenolestes OR Calcochloris OR Callibella OR Callicebus OR Callimico OR Callistomys OR Callithrix OR Callorhinus OR Callosciurus OR Callospermophilus OR Calomys OR Calomyscus OR Caloprymnus OR Caluromys OR Caluromysiops OR Calyptophractus OR Camelus OR Canis OR Cannomys OR Cansumys OR Caperea OR Capra OR Capreolus OR Capricornis OR Caprolagus OR Capromys OR Caracal OR Cardiocranius OR Cardioderma OR Carlito OR Carollia OR Carpitalpa OR Carpomys OR Carterodon OR Caryomys OR Casinycteris OR Castor OR Catagonus OR Catopuma OR Cavia OR Cebuella OR Cebus OR Centronycteris OR Centurio OR Cephalopachus OR Cephalophus OR Cephalorhynchus OR Ceratotherium OR Cercartetus OR Cercocebus OR Cercopithecus OR Cerdocyon OR Cerradomys OR Cervus OR Chacodelphys OR Chaerephon OR Chaeropus OR Chaetocauda OR Chaetodipus OR Chaetomys OR Chaetophractus OR Chalinolobus OR Cheirogaleus OR Cheiromeles OR Chelemys OR Cheracebus OR Chibchanomys OR Chilomys OR Chilonatalus OR Chimarrogale OR Chinchilla OR Chinchillula OR Chionomys OR Chiroderma OR Chiromyscus OR Chironax OR Chironectes OR Chiropodomys OR Chiropotes OR Chiruromys OR Chlamyphorus OR Chlorocebus OR Chlorotalpa OR Chodsigoa OR Choeroniscus OR Choeronycteris OR Choeropsis OR Choloepus OR Chrotogale OR Chrotomys OR Chrotopterus OR Chrysochloris OR Chrysocyon OR Chrysospalax OR Cistugo OR Civettictis OR Cloeotis OR Clyomys OR Coccymys OR Coelops OR Coendou OR Coleura OR Colobus OR Colomys OR Condylura OR Conepatus OR Congosorex OR Conilurus OR Connochaetes OR Cormura OR Corynorhinus OR Coryphomys OR Craseonycteris OR Crateromys OR Cratogeomys OR Cremnomys OR Cricetomys OR Cricetulus OR Cricetus OR Crocidura OR Crocuta OR Crossarchus OR Crossomys OR Crunomys OR Cryptochloris OR Cryptomys OR Cryptonanus OR Cryptoprocta OR Cryptotis OR Ctenodactylus OR Ctenomys OR Cuniculus OR Cuon OR Cuscomys OR Cyclopes OR Cynictis OR Cynocephalus OR Cynogale OR Cynomops OR Cynomys OR Cynopterus OR Cystophora OR Cyttarops OR Dacnomys OR

Dactylomys OR Dactylonax OR Dactylopsila OR Dama OR Damaliscus OR Dasycercus OR Dasykaluta OR Dasymys OR Dasyprocta OR Dasypus OR Dasyuroides OR Dasyurus OR Daubentonia OR Delanymys OR Delomys OR Delphinapterus OR Delphinus OR Deltamys OR Dendrogale OR Dendrohyrax OR Dendrolagus OR Dendromus OR Dendroprionomys OR Deomys OR Dephomys OR Dermanura OR Desmalopex OR Desmana OR Desmodilliscus OR Desmodillus OR Desmodus OR Desmomys OR Diaemus OR Dicerorhinus OR Diceros OR Diclidurus OR Dicrostonyx OR Didelphis OR Dinaromys OR Dinomys OR Diomys OR Diphylla OR Diplogale OR Diplomesodon OR Diplomys OR Diplothrix OR Dipodomys OR Dipus OR Distoechurus OR Dobsonia OR Dolichotis OR Dologale OR Dorcatragus OR Dorcopsis OR Dorcopsulus OR Dremomys OR Dromiciops OR Dryadonycteris OR Drymoreomys OR Dryomys OR Dugong OR Dusicyon OR Dyacopterus OR Dymecodon OR Echimys OR Echinops OR Echinosorex OR Echiothrix OR Echymipera OR Ectophylla OR Eidolon OR Eira OR Elaphodus OR Elaphurus OR Elephantulus OR Elephas OR Eligmodontia OR Eliomys OR Eliurus OR Ellobius OR Emballonura OR Enchisthenes OR Enhydra OR Eoglaucomys OR Eolagurus OR Eonycteris OR Eospalax OR Eothenomys OR Eozapus OR Episoriculus OR Epixerus OR Epomophorus OR Epomops OR Eptesicus OR Equus OR Eremitalpa OR Eremodipus OR Eremoryzomys OR Erethizon OR Erignathus OR Erinaceus OR Eropeplus OR Erophylla OR Erythrocebus OR Eschrichtius OR Eubalaena OR Euchoreutes OR Euderma OR Eudiscoderma OR Eudiscopus OR Eudorcas OR Eulemur OR Eumetopias OR Eumops OR Euneomys OR Euoticus OR Eupetaurus OR Euphractus OR Eupleres OR Euroscaptor OR Euryoryzomys OR Euryzygomatomys OR Eutamias OR Exilisciurus OR Falsistrellus OR Felis OR Felovia OR Feresa OR Feroculus OR Fossa OR Fukomys OR Funambulus OR Funisciurus OR Furipterus OR Galago OR Galagoides OR Galea OR Galemys OR Galenomys OR Galeopterus OR Galictis OR Galidia OR Galidictis OR Gardnerycteris OR Gazella OR Genetta OR Geocapromys OR Geogale OR Geomys OR Georychus OR Geoxus OR Gerbilliscus OR Gerbillurus OR Gerbillus OR Giraffa OR Glaucomys OR Glauconycteris OR Glironia OR Glirulus OR Glis OR Glischropus OR Globicephala OR Glossophaga OR Glyphonycteris OR Glyphotes OR Golunda OR Gorilla OR Gracilimus OR Gracilinanus OR Grammomys OR Grampus OR Graomys OR Graphiurus OR Gulo OR Gyldenstolpia OR Gymnobelideus OR Gymnuromys OR Habromys OR Hadromys OR Haeromys OR Halichoerus OR Halmaheramys OR

Handleyomys OR Hapalemur OR Hapalomys OR Haplonycteris OR Harpiocephalus OR Harpiola OR Harpyionycteris OR Heimyscus OR Helarctos OR Heliophobius OR Heliosciurus OR Helogale OR Hemibelideus OR Hemicentetes OR Hemiechinus OR Hemigalus OR Hemitragus OR Herpailurus OR Herpestes OR Hesperoptenus OR Heterocephalus OR Heterogeomys OR Heterohyrax OR Heteromys OR Heteropsomys OR Hexolobodon OR Hippocamelus OR Hippopotamus OR Hipposideros OR Hippotragus OR Histiotus OR Histriophoca OR Hodomys OR Holochilus OR Hoolock OR Hoplomys OR Huetia OR Hyaena OR Hybomys OR Hydrictis OR Hydrochoerus OR Hydrodamalis OR Hydromys OR Hydropotes OR Hydrurga OR Hyemoschus OR Hyladelphys OR Hylaeamys OR Hylobates OR Hylochoerus OR Hylomys OR Hylomyscus OR Hylonycteris OR Hylopetes OR Hyomys OR Hyorhinomys OR Hyosciurus OR Hyperacrius OR Hyperoodon OR Hypogeomys OR Hypsignathus OR Hypsiprymnodon OR Hypsugo OR Hystrix OR Ia OR Ichneumia OR Ichthyomys OR Ictidomys OR Ictonyx OR Idionycteris OR Idiurus OR Indopacetus OR Indri OR Inia OR Iomys OR Irenomys OR Isolobodon OR Isoodon OR Isothrix OR Isthmomys OR Jaculus OR Juliomys OR Juscelinomys OR Kadarsanomys OR Kannabateomys OR Kerivoula OR Kerodon OR Kobus OR Kogia OR Komodomys OR Kunsia OR Laephotis OR Lagenodelphis OR Lagenorhynchus OR Lagidium OR Lagorchestes OR Lagostomus OR Lagostrophus OR Lagothrix OR Lagurus OR Lama OR Lamottemys OR Lampronycteris OR Laonastes OR Lariscus OR Lasionycteris OR Lasiopodomys OR Lasiorhinus OR Lasiurus OR Latidens OR Lavia OR Leggadina OR Leimacomys OR Lemmiscus OR Lemmus OR Lemniscomys OR Lemur OR Lenomys OR Lenothrix OR Lenoxus OR Leontocebus OR Leontopithecus OR Leopardus OR Leopoldamys OR Lepilemur OR Leporillus OR Leptailurus OR Leptomys OR Leptonychotes OR Leptonycteris OR Lepus OR Lestodelphys OR Lestoros OR Liberiictis OR Lichonycteris OR Limnogale OR Limnomys OR Lionycteris OR Lipotes OR Lissodelphis OR Lissonycteris OR Litocranius OR Lobodon OR Lonchophylla OR Lonchorhina OR Lonchothrix OR Lontra OR Lophiomys OR Lophocebus OR Lophostoma OR Lophuromys OR Lorentzimys OR Loris OR Loxodonta OR Loxodontomys OR Lundomys OR Lutra OR Lutreolina OR Lutrogale OR Lycalopex OR Lycaon OR Lyncodon OR Lynx OR Lyroderma OR Macaca OR Macroderma OR Macrogalidia OR Macroglossus OR Macronycteris OR Macrophyllum OR Macropus OR Macroscelides OR Macrotarsomys OR Macrotis OR Macrotus OR Macruromys

OR Madoqua OR Madromys OR Makalata OR Malacomys OR Malacothrix OR Mallomys OR Mammelomys OR Mandrillus OR Manis OR Margaretamys OR Marmosa OR Marmosops OR Marmota OR Martes OR Massoutiera OR Mastacomys OR Mastomys OR Maxomys OR Mazama OR Megadendromus OR Megaderma OR Megadontomys OR Megaerops OR Megaloglossus OR Megalomys OR Megaoryzomys OR Megaptera OR Megasorex OR Melanomys OR Melasmothrix OR Meles OR Mellivora OR Melogale OR Melomys OR Melonycteris OR Melursus OR Menetes OR Mephitis OR Meriones OR Mesechinus OR Mesembriomys OR Mesocapromys OR Mesocricetus OR Mesomys OR Mesophylla OR Mesoplodon OR Metachirus OR Micaelamys OR Mico OR Microakodontomys OR Microcavia OR Microcebus OR Microdillus OR Microdipodops OR Microgale OR Microhydromys OR Micromys OR Micronomus OR Micronycteris OR Microperoryctes OR Micropotamogale OR Micropteropus OR Microryzomys OR Microsciurus OR Microtus OR Millardia OR Mimetillus OR Mimon OR Mindomys OR Miniopterus OR Miopithecus OR Mirimiri OR Mirounga OR Mirza OR Mirzamys OR Mogera OR Molossops OR Molossus OR Monachus OR Monodelphis OR Monodon OR Monophyllus OR Monticolomys OR Mops OR Mormoops OR Mormopterus OR Moschiola OR Moschus OR Mosia OR Mungos OR Mungotictis OR Muntiacus OR Murexia OR Muriculus OR Murina OR Mus OR Muscardinus OR Musonycteris OR Musseromys OR Mustela OR Mydaus OR Mylomys OR Myocastor OR Myodes OR Myoictis OR Myomimus OR Myomyscus OR Myonycteris OR Myoprocta OR Myopterus OR Myopus OR Myosciurus OR Myosorex OR Myospalax OR Myotis OR Myrmecobius OR Myrmecophaga OR Mysateles OR Mystacina OR Mystromys OR Myzopoda OR Naemorhedus OR Nandinia OR Nanger OR Nannosciurus OR Nannospalax OR Nanonycteris OR Napaeozapus OR Nasalis OR Nasua OR Nasuella OR Natalus OR Neacomys OR Neamblysomus OR Necromys OR Nectogale OR Nectomys OR Nelsonia OR Neodon OR Neofelis OR Neofiber OR Neohylomys OR Neomicroxus OR Neomonachus OR Neomys OR Neonycteris OR Neophascogale OR Neophoca OR Neophocaena OR Neopteryx OR Neoromicia OR Neotamias OR Neotetracus OR Neotoma OR Neotomodon OR Neotomys OR Neotragus OR Neovison OR Nephelomys OR Nesokia OR Nesolagus OR Nesomys OR Nesophontes OR Nesoromys OR Nesoryzomys OR Nesotragus OR Neurotrichus OR Neusticomys OR Nilgiritragus OR Nilopegamys OR Ningaui OR Niviventer OR Noctilio OR Nomascus OR Noronhomys OR Notiomys OR Notiosorex OR Notocitellus OR

Notomys OR Notopteris OR Notoryctes OR Nyctalus OR Nyctereutes OR Nycteris OR Nycticebus OR Nycticeinops OR Nycticeius OR Nyctiellus OR Nyctimene OR Nyctinomops OR Nyctomys OR Nyctophilus OR Ochotona OR Ochrotomys OR Octodon OR Octodontomys OR Octomys OR Odobenus OR Odocoileus OR Oecomys OR Oenomys OR Okapia OR Olallamys OR Oligoryzomys OR Ommatophoca OR Ondatra OR Onychogalea OR Onychomys OR Orcaella OR Orcinus OR Oreamnos OR Oreoryzomys OR Oreotragus OR Ornithorhynchus OR Orthogeomys OR Orycteropus OR Oryctolagus OR Oryx OR Oryzomys OR Oryzorictes OR Osgoodomys OR Otaria OR Otocolobus OR Otocyon OR Otolemur OR Otomops OR Otomys OR Otonycteris OR Otonyctomys OR Otopteropus OR Otospermophilus OR Ototylomys OR Ourebia OR Ovibos OR Ovis OR Oxymycterus OR Ozimops OR Ozotoceros OR Pachyuromys OR Pagophilus OR Paguma OR Palaeopropithecus OR Palawanomys OR Pan OR Panthera OR Pantholops OR Papagomys OR Papio OR Pappogeomys OR Paracrocidura OR Paracynictis OR Paradipus OR Paradoxurus OR Paraechinus OR Paragalago OR Parahyaena OR Parahydromys OR Paraleptomys OR Paramelomys OR Parantechinus OR Paranyctimene OR Parascalops OR Parascaptor OR Parastrellus OR Paratriaenops OR Paraxerus OR Pardofelis OR Paremballonura OR Parotomys OR Paruromys OR Pattonomys OR Paucidentomys OR Paulamys OR Pecari OR Pectinator OR Pedetes OR Pelea OR Pelomys OR Pennatomys OR Pentalagus OR Penthetor OR Peponocephala OR Perameles OR Perimyotis OR Perodicticus OR Perognathus OR Peromyscus OR Peropteryx OR Peroryctes OR Petaurillus OR Petaurista OR Petauroides OR Petaurus OR Petinomys OR Petrodromus OR Petrogale OR Petromus OR Petromyscus OR Petropseudes OR Phacochoerus OR Phaenomys OR Phaiomys OR Phalanger OR Phaner OR Pharotis OR Phascogale OR Phascolarctos OR Phascolosorex OR Phataginus OR Phenacomys OR Philander OR Philantomba OR Philetor OR Phloeomys OR Phoca OR Phocarctos OR Phocoena OR Phocoenoides OR Phodopus OR Phoniscus OR Phylloderma OR Phyllomys OR Phyllonycteris OR Phyllops OR Phyllostomus OR Phyllotis OR Physeter OR Piliocolobus OR Pipistrellus OR Pithecheir OR Pithecheirops OR Pithecia OR Plagiodontia OR Planigale OR Platacanthomys OR Platalina OR Platanista OR Platymops OR Platyrrhinus OR Plecotus OR Plecturocebus OR Plerotes OR Podogymnura OR Podomys OR Podoxymys OR Poecilogale OR Poelagus OR Pogonomelomys OR Pogonomys OR Poiana OR Poliocitellus OR Pongo OR

Pontoporia OR Porcula OR Potamochoerus OR Potamogale OR Potorous OR Potos OR Praomys OR Presbytis OR Priodontes OR Prionailurus OR Prionodon OR Prionomys OR Procapra OR Procavia OR Procolobus OR Procyon OR Proechimys OR Proedromys OR Prolagus OR Prolemur OR Prometheomys OR Promops OR Pronolagus OR Propithecus OR Prosciurillus OR Proteles OR Protochromys OR Protoxerus OR Psammomys OR Pseudantechinus OR Pseudoberylmys OR Pseudocheirus OR Pseudochirops OR Pseudochirulus OR Pseudohydromys OR Pseudois OR Pseudomys OR Pseudorca OR Pseudoryx OR Pseudoryzomys OR Ptenochirus OR Pteralopex OR Pteromys OR Pteromyscus OR Pteronotus OR Pteronura OR Pteropus OR Ptilocercus OR Pudu OR Puma OR Punomys OR Pusa OR Pygathrix OR Pygeretmus OR Pygoderma OR Rangifer OR Raphicerus OR Rattus OR Ratufa OR Redunca OR Reithrodon OR Reithrodontomys OR Rhabdomys OR Rhagomys OR Rheithrosciurus OR Rheomys OR Rhinoceros OR Rhinolophus OR Rhinonicteris OR Rhinophylla OR Rhinopithecus OR Rhinopoma OR Rhinosciurus OR Rhipidomys OR Rhizomys OR Rhogeessa OR Rhombomys OR Rhynchocyon OR Rhynchogale OR Rhyncholestes OR Rhynchomeles OR Rhynchomys OR Rhynchonycteris OR Rhyneptesicus OR Romerolagus OR Rousettus OR Rubrisciurus OR Rucervus OR Rungwecebus OR Rupicapra OR Rusa OR Ruwenzorisorex OR Saccolaimus OR Saccopteryx OR Saccostomus OR Saguinus OR Saiga OR Saimiri OR Salanoia OR Salinomys OR Salpingotulus OR Salpingotus OR Santamartamys OR Sapajus OR Sarcophilus OR Sauromys OR Saxatilomys OR Scalopus OR Scapanulus OR Scapanus OR Scapteromys OR Scaptochirus OR Scaptonyx OR Sciurillus OR Sciurocheirus OR Sciurotamias OR Sciurus OR Scleronycteris OR Scolomys OR Scoteanax OR Scotinomys OR Scotoecus OR Scotomanes OR Scotonycteris OR Scotophilus OR Scotorepens OR Scotozous OR Scutisorex OR Sekeetamys OR Selevinia OR Semnopithecus OR Setifer OR Setirostris OR Setonix OR Sicista OR Sigmodon OR Sigmodontomys OR Simias OR Sminthopsis OR Smutsia OR Solenodon OR Solisorex OR Solomys OR Sommeromys OR Sooretamys OR Sorex OR Soricomys OR Soriculus OR Sotalia OR Sousa OR Spalacopus OR Spalax OR Speothos OR Spermophilopsis OR Spermophilus OR Sphaerias OR Sphaeronycteris OR Spilocuscus OR Spilogale OR Srilankamys OR Steatomys OR Stenella OR Steno OR Stenocephalemys OR Stenoderma OR Stochomys OR Strigocuscus OR Sturnira OR Styloctenium OR Stylodipus OR Submyotodon OR Suncus OR Sundamys OR Sundasciurus OR

Surdisorex OR Suricata OR Sus OR Syconycteris OR Sylvicapra OR Sylvilagus OR Sylvisorex OR Symphalangus OR Synaptomys OR Syncerus OR Syntheosciurus OR Tachyglossus OR Tachyoryctes OR Tadarida OR Taeromys OR Talpa OR Tamandua OR Tamias OR Tamiasciurus OR Tamiops OR Tanyuromys OR Tapecomys OR Taphozous OR Tapirus OR Tarsipes OR Tarsius OR Tarsomys OR Tasmacetus OR Tateomys OR Tatera OR Taterillus OR Taxidea OR Tayassu OR Tenrec OR Tetracerus OR Thainycteris OR Thallomys OR Thalpomys OR Thamnomys OR Thaptomys OR Theropithecus OR Thomasomys OR Thomomys OR Thoopterus OR Thrichomys OR Thryonomys OR Thylacinus OR Thylamys OR Thylogale OR Thyroptera OR Tlacuatzin OR Tokudaia OR Tolypeutes OR Tomopeas OR Tonatia OR Tonkinomys OR Toromys OR Trachops OR Trachypithecus OR Tragelaphus OR Tragulus OR Transandinomys OR Tremarctos OR Triaenops OR Trichechus OR Trichosurus OR Trichys OR Trinomys OR Trinycteris OR Trogopterus OR Tryphomys OR Tscherskia OR Tupaia OR Tursiops OR Tylomys OR Tylonycteris OR Tympanoctomys OR Typhlomys OR Uranomys OR Urocitellus OR Urocyon OR Uroderma OR Uromys OR Uropsilus OR Urotrichus OR Ursus OR Vampyressa OR Vampyriscus OR Vampyrodes OR Vampyrum OR Vandeleuria OR Varecia OR Vernaya OR Vespadelus OR Vespertilio OR Vicugna OR Viverra OR Viverricula OR Voalavo OR Volemys OR Vombatus OR Vormela OR Vulpes OR Waiomys OR Wallabia OR Wiedomys OR Wilfredomys OR Wyulda OR Xenomys OR Xenothrix OR Xenuromys OR Xeromys OR Xeronycteris OR Xerospermophilus OR Xerus OR Zaedyus OR Zaglossus OR Zalophus OR Zapus OR Zelotomys OR Zenkerella OR Ziphius OR Zygodontomys OR Zygogeomys OR Zyzomys

Appendix B

The 183 studies included in the global systematic literature review (Chapter 2).

al Sayegh Petkovšek, S., Kopušar, N., & Kryštufek, B. (2014). Small mammals as biomonitors of metal pollution: A case study in Slovenia. *Environmental Monitoring and Assessment*, *186*(7), 4261–4274. https://doi.org/10.1007/s10661-014-3696-7

Alleva, E., Francia, N., Pandolfi, M., de Marinis, A. M., Chiarotti, F., & Santucci, D. (2006). Organochlorine and heavy-metal contaminants in wild mammals and birds of Urbino-Pesaro Province, Italy: An analytic overview for potential bioindicators. *Archives of Environmental Contamination and Toxicology*, *51*(1), 123–134. https://doi.org/10.1007/s00244-005-0218-1

Amici, A., Danieli, P. P., Russo, C., Primi, R., & Ronchi, B. (2012). Concentrations of some toxic and trace elements in wild boar (*Sus scrofa*) organs and tissues in different areas of the Province of Viterbo, Central Italy. *Italian Journal of Animal Science*, *11*(4), e65. https://doi.org/10.4081/ijas.2011.e65

Amuno, S., Niyogi, S., Amuno, M., & Attitaq, J. (2016). Heavy metal bioaccumulation and histopathological alterations in wild Arctic hares (Lepus arcticus) inhabiting a former lead-zinc mine in the Canadian high Arctic: A preliminary study. *Science of the Total Environment*, *556*, 252–263. https://doi.org/10.1016/j.scitotenv.2016.03.007

Andreani, G., Cannavacciuolo, A., Menotta, S., Spallucci, V., Fedrizzi, G., Carpenè, E., & Isani, G. (2019). Environmental exposure to non-essential trace elements in two bat species from urbanised (Tadarida teniotis) and open land (Miniopterus schreibersii) areas in Italy. *Environmental Pollution*, *254*, 113034. https://doi.org/10.1016/J.ENVPOL.2019.113034

Anzolin, D. G., Sarkis, J. E. S., Diaz, E., Soares, D. G., Serrano, I. L., Borges, J. C. G., Souto, A. S., Taniguchi, S., Montone, R. C., Bainy, A. C. D., Bainy, A. C. D., & Carvalho, P. S. M. (2012). Contaminant concentrations, biochemical and hematological biomarkers in blood of West Indian manatees Trichechus manatus from Brazil. *Marine Pollution Bulletin*, *64*(7), 1402–1408. https://doi.org/10.1016/j.marpolbul.2012.04.018

Beernaert, J., Scheirs, J., Leirs, H., Blust, R., & Verhagen, R. (2007). Non-destructive pollution exposure assessment by means of wood mice hair. *Environmental Pollution*, *145*(2), 443–451. https://doi.org/10.1016/j.envpol.2006.04.025

Bennett, P. M., Jepson, P. D., Law, R. J., Jones, B. R., Kuiken, T., Baker, J. R., Rogan, E., & Kirkwood, J. K. (2001). Exposure to heavy metals and infectious disease mortality in harbour porpoises from England and Wales. *Environmental Pollution*, *112*(1), 33–40. https://doi.org/10.1016/S0269-7491(00)00105-6

Bilandžić, N., Sedak, M., Crossed D Signokić, M., & Šimić, B. (2010). Wild boar tissue levels of cadmium, lead and mercury in seven regions of continental Croatia. *Bulletin of Environmental Contamination and Toxicology*, *84*(6), 738–743. https://doi.org/10.1007/s00128-010-9999-7

Bilandžić, N., Sedak, M., Vratarić, D., Perić, T., & Šimić, B. (2009). Lead and cadmium in red deer and wild boar from different hunting grounds in Croatia. *Science of the Total Environment*, *407*(14), 4243–4247. https://doi.org/10.1016/j.scitotenv.2009.04.009

Binkowski, Ł. J., Merta, D., Przystupińska, A., Sołtysiak, Z., Pacoń, J., & Stawarz, R. (2016). Levels of metals in kidney, liver and muscle tissue and their relation to the occurrence of parasites in the red fox in the Lower Silesian Forest in Europe. *Chemosphere*, *149*, 161–167. https://doi.org/10.1016/j.chemosphere.2016.01.099

Biser, J. A., Vogel, L. A., Berger, J., Hjelle, B., & Loew, S. S. (2004). Effects of heavy metals on immunocompetence of white-footed mice (Peromyscus leucopus). *Journal of Wildlife Diseases*, *40*(2), 173–184. https://doi.org/10.7589/0090-3558-40.2.173

Boesen, A. H., Thiel, A., Fuchs, B., Evans, A. L., Bertelsen, M. F., Rodushkin, I., & Arnemo, J. M. (2019). Assessment of the LeadCare® plus for use on Scandinavian brown bears (*Ursus arctos*). *Frontiers in Veterinary Science*, *6*, 285. https://doi.org/10.3389/fvets.2019.00285

Borchert, E. J., Leaphart, J. C., Bryan, A. L., & Beasley, J. C. (2019). Ecotoxicoparasitology of mercury and trace elements in semi-aquatic mammals and their endoparasite communities. *Science of the Total Environment*, *679*, 307–316. https://doi.org/10.1016/j.scitotenv.2019.04.326

Bordeleau, S., Asselin, H., Mazerolle, M. J., & Imbeau, L. (2016). ‘Is it still safe to eat traditional food?’ Addressing traditional food safety concerns in aboriginal communities. *Science of the Total Environment*, *565*, 529–538. https://doi.org/10.1016/j.scitotenv.2016.04.189

Brumbaugh, W. G., Mora, M. A., May, T. W., & Phalen, D. N. (2010). Metal exposure and effects in voles and small birds near a mining haul road in Cape Krusenstern National Monument, Alaska. *Environmental Monitoring and Assessment*, *170*(1–4), 73–86. https://doi.org/10.1007/s10661-009-1216-y

Bryan, C. E., Christopher, S. J., Balmer, B. C., & Wells, R. S. (2007). Establishing baseline levels of trace elements in blood and skin of bottlenose dolphins in Sarasota Bay, Florida: Implications for non-invasive monitoring. *Science of the Total Environment*, *388*(1–3), 325–342. https://doi.org/10.1016/j.scitotenv.2007.07.046

Burco, J., Myers, A. M., Schuler, K., & Gillin, C. (2012). Acute lead toxicosis via ingestion of spent ammunition in a free-ranging cougar (*Puma concolor*). *Journal of Wildlife Diseases*, *48*(1), 216–219. https://doi.org/10.7589/0090-3558-48.1.216

Cappelli, J., Frasca, I., García, A., Landete-Castillejos, T., Luccarini, S., Gallego, L., Morimando, F., Varuzza, P., & Zaccaroni, M. (2020). Roe deer as a bioindicator: preliminary data on the impact of the geothermal power plants on the mineral profile in internal and bone tissues in Tuscany (Italy). *Environmental Science and Pollution Research*, *27*(29), 36121–36131. https://doi.org/10.1007/S11356-020-09708-X/TABLES/6

Čelechovská, O., Literák, I., Ondruš, S., & Pospíšil, Z. (2006). Heavy metals in brown bears from the central European Carpathians. *Acta Veterinaria Brno*, *75*(4), 501–506. https://doi.org/10.2754/avb200675040501

Ceruti, R., Ghisleni, G., Ferretti, E., Cammarata, S., Sonzogni, O., & Scanziani, E. (2002). Wild rats as monitors of environmental lead contamination in the urban area of Milan, Italy. *Environmental Pollution*, *117*(2), 255–259. https://doi.org/10.1016/S0269-7491(01)00273-1

Chan, H. M., Kim, C., Khoday, K., Receveur, O., & Kuhnlein, H. V. (1995). Assessment of dietary exposure to trace metals in Baffin Inuit food. *Environmental Health Perspectives*, *103*(7–8), 740–746. https://doi.org/10.1289/ehp.95103740

Chen, Y.-P., Liu, Q., Ma, Q.-Y., Maltby, L., Ellison, A. M., & Zhao, Y. (2018). Environmental toxicants impair liver and kidney function and sperm quality of captive pandas. *Ecotoxicology and Environmental Safety*, *162*, 218–224. https://doi.org/10.1016/j.ecoenv.2018.07.008

Chiari, M., Cortinovis, C., Bertoletti, M., Alborali, L., Zanoni, M., Ferretti, E., & Caloni, F. (2015). Lead, cadmium and organochlorine pesticide residues in hunted red deer and wild boar from northern Italy. *Food Additives & Contaminants: Part A*, *32*(11), 1867–1874. https://doi.org/10.1080/19440049.2015.1087058

Chmiel, K. M., & Harrison, R. M. (1981). Lead content of small mammals at a roadside site in relation to the pathways of exposure. *Science of the Total Environment, The*, *17*(2), 145–154. https://doi.org/10.1016/0048-9697(81)90180-7

Cooke, J. A., Andrews, S. M., & Johnson, M. S. (1990). Lead, zinc, cadmium and fluoride in small mammals from contaminated grassland established on fluorspar tailings. *Water, Air, and Soil Pollution*, *51*(1–2), 43–54. https://doi.org/10.1007/BF00211502

Corsolini, S., Focardi, S., Leonzio, C., Lovari, S., Monaci, F., & Romeo, G. (1999). Heavy metals and chlorinated hydrocarbon concentrations in the red fox in relation to some biological parameters. *Environmental Monitoring and Assessment*, *54*(1), 87–100. https://doi.org/10.1023/A:1005974014029

Curi, N. H. D. A., Brait, C. H. H., Filho, N. R. A., & Talamoni, S. A. (2012). Heavy metals in hair of wild canids from the Brazilian Cerrado. *Biological Trace Element Research*, *147*(1–3), 97–102. https://doi.org/10.1007/s12011-011-9303-7

da Silva, J., de Freitas, T. R. O., Heuser, V., Marinho, J. R., Bittencourt, F., Cerski, C. T. S., Kliemann, L. M., & Erdtmann, B. (2000). Effects of chronic exposure to coal in wild rodents (Ctenomys torquatus) evaluated by multiple methods and tissues. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *470*(1), 39–51. https://doi.org/10.1016/S1383-5718(00)00094-2

Damek-Poprawa, M., & Sawicka-Kapusta, K. (2003). Damage to the liver, kidney, and testis with reference to burden of heavy metals in yellow-necked mice from areas around steelworks and zinc smelters in Poland. *Toxicology*, *186*(1–2), 1–10. https://doi.org/10.1016/S0300-483X(02)00595-4

Danieli, P. P., Serrani, F., Primi, R., Ponzetta, M. P., Ronchi, B., & Amici, A. (2012). Cadmium, lead, and chromium in large game: A local-scale exposure assessment for hunters consuming meat and liver of wild boar. *Archives of Environmental Contamination and Toxicology*, *63*(4), 612–627. https://doi.org/10.1007/s00244-012-9791-2

Demesko, J., Markowski, J., Demesko, E., Słaba, M., Hejduk, J., & Minias, P. (2019). Ecotype Variation in Trace Element Content of Hard Tissues in the European Roe Deer (Capreolus capreolus). *Archives of Environmental Contamination and Toxicology*, *76*(1), 76–86. https://doi.org/10.1007/s00244-018-0580-4

D'Havé, H., Scheirs, J., Mubiana, V. K., Verhagen, R., Blust, R., & de Coen, W. (2005). Nondestructive pollution exposure assessment in the European hedgehog (Erinaceus europaeus): I. Relationships between concentrations of metals and arsenic in hair, spines, and soil. *Environmental Toxicology and Chemistry*, *24*(9), 2356–2364. https://doi.org/10.1897/04-597R.1

Dip, R., Stieger, C., Deplazes, P., Hegglin, D., Müller, U., Dafflon, O., Koch, H., & Naegeli, H. (2001). Comparison of heavy metal concentrations in tissues of red foxes from adjacent urban, suburban, and rural areas. *Archives of Environmental Contamination and Toxicology*, *40*(4), 551–556. https://doi.org/10.1007/s002440010209

Diters, R. W., & Nielsen, S. W. (1978). Lead poisoning of raccoons in Connecticut. *Journal of Wildlife Diseases*, *14*(2), 187–192. https://doi.org/10.7589/0090-3558-14.2.187

Dudarev, A. A., Chupakhin, V. S., Vlasov, S. V., & Yamin-Pasternak, S. (2019). Traditional diet and environmental contaminants in coastal chukotka III: Metals. *International Journal of Environmental Research and Public Health*, *16*(5). https://doi.org/10.3390/ijerph16050699

Dupont, A., Siebert, U., Covaci, A., Weijs, L., Eppe, G., Debier, C., de Pauw-Gillet, M.-C., & Das, K. (2013). Relationships between in vitro lymphoproliferative responses and levels of contaminants in blood of free-ranging adult harbour seals (Phoca vitulina) from the North Sea. *Aquatic Toxicology*, *142–143*, 210–220. https://doi.org/10.1016/j.aquatox.2013.08.014

Durkalec, M., Nawrocka, A., Krzysiak, M., Larska, M., Kmiecik, M., & Posyniak, A. (2018). Trace elements in the liver of captive and free-ranging European bison (Bison bonasus L.). *Chemosphere*, *193*, 454–463. https://doi.org/10.1016/j.chemosphere.2017.11.050

Durkalec, M., Szkoda, J., Kolacz, R., Opalinski, S., Nawrocka, A., & Zmudzki, J. (2015). Bioaccumulation of lead, cadmium and mercury in roe deer and wild boars from areas with different levels of toxic metal pollution. *International Journal of Environmental Research*, *9*(1), 205–2012. https://doi.org/10.22059/ijer.2015.890

Eira, C., Torres, J., Vingada, J., & Miquel, J. (2005). Concentration of some toxic elements in Oryctolagus cuniculus and in its intestinal cestode Mosgovoyia ctenoides, in Dunas de Mira (Portugal). *Science of the Total Environment*, *346*(1–3), 81–86. https://doi.org/10.1016/j.scitotenv.2004.11.014

Engel, G., O'Hara, T. M., Cardona-Marek, T., Heidrich, J., Chalise, M. K., Kyes, R., & Jones-Engel, L. (2010). Synanthropic primates in Asia: Potential sentinels for environmental toxins. *American Journal of Physical Anthropology*, *142*(3), 453–460. https://doi.org/10.1002/ajpa.21247

Ertl, K., Kitzer, R., & Goessler, W. (2016). Elemental composition of game meat from Austria. *Food Additives and Contaminants: Part B Surveillance*, *9*(2), 120–126. https://doi.org/10.1080/19393210.2016.1151464

Falandysz, J., Szymczyk-Kobrzyńska, K., Brzostowski, A., Zalewski, K., & Zasadowski, A. (2005). Concentrations of heavy metals in the tissues of red deer (Cervus elaphus) from the region of Warmia and Mazury, Poland. *Food Additives and Contaminants*, *22*(2), 141–149. https://doi.org/10.1080/02652030500047273

Ferreira, A. F., Garcia, P. V., Camarinho, R., & Rodrigues, A. S. (2015). Volcanogenic pollution and testicular damage in wild mice. *Chemosphere*, *132*, 135–141. https://doi.org/10.1016/j.chemosphere.2015.03.017

Flache, L., Czarnecki, S., Düring, R.-A., Kierdorf, U., & Encarnação, J. A. (2015). Trace metal concentrations in hairs of three bat species from an urbanized area in Germany. *Journal of Environmental Sciences*, *31*, 184–193. https://doi.org/10.1016/j.jes.2014.12.010

Fritsch, C., Cosson, R. P., Cœurdassier, M., Raoul, F., Giraudoux, P., Crini, N., de Vaufleury, A., & Scheifler, R. (2010). Responses of wild small mammals to a pollution gradient: Host factors influence metal and metallothionein levels. *Environmental Pollution*, *158*(3), 827–840. https://doi.org/10.1016/j.envpol.2009.09.027

Fuchs, B., Thiel, A., Zedrosser, A., Brown, L., Hydeskov, H. B., Rodushkin, I., Evans, A. L., Boesen, A. H., Græsli, A. R., Kindberg, J., Kindberg, J., & Arnemo, J. M. (2021). High concentrations of lead (Pb) in blood and milk of free-ranging brown bears (*Ursus arctos*) in Scandinavia. *Environmental Pollution*, *287*, 117595. https://doi.org/10.1016/j.envpol.2021.117595

Gamberg, M., & Braune, B. M. (1999). Contaminant residue levels in arctic wolves (Canis lupus) from the Yukon Territory, Canada. *Science of the Total Environment*, *243–244*, 329–338. https://doi.org/10.1016/S0048-9697(99)00408-8

Gašparík, J., Binkowski, Ł. J., Jahnátek, A., Šmehýl, P., Dobiaš, M., Lukáč, N., Błaszczyk, M., Semla, M., & Massanyi, P. (2017). Levels of Metals in Kidney, Liver, and Muscle Tissue and their Influence on the Fitness for the Consumption of Wild Boar from Western Slovakia. *Biological Trace Element Research*, *177*(2), 258–266. https://doi.org/10.1007/S12011-016-0884-Z/TABLES/5

Gasparik, J., Massányi, P., Slamecka, J., Fabis, M., & Jurcik, R. (2004). Concentration of Selected Metals in Liver, Kidney, and Muscle of the Red Deer (*Cervus elaphus*). *Journal of Environmental Science and Health, Part A*, *39*(8), 2105–2111. https://doi.org/10.1081/ESE-120039378

Gbogbo, F., Rainhill, J. E., Koranteng, S. S., Owusu, E. H., & Dorleku, W.-P. (2020). Health Risk Assessment for Human Exposure to Trace Metals Via Bushmeat in Ghana. *Biological Trace Element Research*, *196*(2), 419–429. https://doi.org/10.1007/s12011-019-01953-7

Gerofke, A., Ulbig, E., Martin, A., Müller-Graf, C., Selhorst, T., Gremse, C., Spolders, M., Schafft, H., Heinemeyer, G., Greiner, M., Lahrssen-Wiederholt, M., & Hensel, A. (2018). Lead content in wild game shot with lead or non-lead ammunition – does “state of the art consumer health protection” require non-lead ammunition? *PLoS ONE*, *13*(7). https://doi.org/10.1371/journal.pone.0200792

Getz, L. L., Verner, L., & Prather, M. (1977). Lead concentrations in small mammals living near highways. *Environmental Pollution (1970)*, *13*(2), 151–157. https://doi.org/10.1016/0013-9327(77)90099-4

Gil-Jiménez, E., Mateo, R., de Lucas, M., & Ferrer, M. (2020). Feathers and hair as tools for non-destructive pollution exposure assessment in a mining site of the Iberian Pyrite Belt. *Environmental Pollution*, *263*. https://doi.org/10.1016/j.envpol.2020.114523

Griesel, S., Kakuschke, A., Siebert, U., & Prange, A. (2008). Trace element concentrations in blood of harbor seals (Phoca vitulina) from the Wadden Sea. *Science of the Total Environment*, *392*(2–3), 313–323. https://doi.org/10.1016/j.scitotenv.2007.10.062

Guerrero-Castilla, A., Olivero-Verbel, J., & Marrugo-Negrete, J. (2014). Heavy metals in wild house mice from coal-mining areas of Colombia and expression of genes related to oxidative stress, DNA damage and exposure to metals. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *762*, 24–29. https://doi.org/10.1016/j.mrgentox.2013.12.005

Habran, S., Pomeroy, P. P., Debier, C., & Das, K. (2013). Changes in trace elements during lactation in a marine top predator, the grey seal. *Aquatic Toxicology*, *126*, 455–466. https://doi.org/10.1016/j.aquatox.2012.08.011

Harding, L. E., Harris, M. L., & Elliott, J. E. (1998). Heavy and trace metals in wild mink (Mustela vison) and river otter (Lontra canadensis) captured on rivers receiving metals discharges. *Bulletin of Environmental Contamination and Toxicology*, *61*(5), 600–607. https://doi.org/10.1007/s001289900803

Hermoso de Mendoza García, M., Hernández Moreno, D., Soler Rodríguez, F., López Beceiro, A., Fidalgo Álvarez, L. E., & Pérez López, M. (2011). Sex- and age-dependent accumulation of heavy metals (Cd, Pb and Zn) in liver, kidney and muscle of roe deer (*Capreolus capreolus*) from NW Spain. *Journal of Environmental Science and Health, Part A*, *46*(2), 109–116. https://doi.org/10.1080/10934529.2011.532422

Hernández-Moreno, D., de La Casa Resino, I., Fidalgo, L. E., Llaneza, L., Soler Rodríguez, F., Pérez-López, M., & López-Beceiro, A. (2013). Noninvasive heavy metal pollution assessment by means of Iberian wolf (Canis lupus signatus) hair from Galicia (NW Spain): A comparison with invasive samples. *Environmental Monitoring and Assessment*, *185*(12), 10421–10430. https://doi.org/10.1007/s10661-013-3341-x

Hinck, J. E., Cleveland, D., Brumbaugh, W. G., Linder, G., & Lankton, J. (2017). Pre-mining trace element and radiation exposure to biota from a breccia pipe uranium mine in the Grand Canyon (Arizona, USA) watershed. *Environmental Monitoring and Assessment*, *189*(2). https://doi.org/10.1007/s10661-017-5765-1

Hivert, L. G., Clarke, J. R., Peck, S. J., Lawrence, C., Brown, W. E., Huxtable, S. J., Schaap, D., Pemberton, D., & Grueber, C. E. (2018). High blood lead concentrations in captive Tasmanian devils (*Sarcophilus harrisii*): a threat to the conservation of the species? *Australian Veterinary Journal*, *96*(11), 442–449. https://doi.org/10.1111/avj.12753

Hoekstra, P. F., Braune, B. M., Elkin, B., Armstrong, F. A. J., & Muir, D. C. G. (2003). Concentrations of selected essential and non-essential elements in arctic fox (*Alopex lagopus*) and wolverines (*Gulo gulo*) from the Canadian Arctic. *Science of the Total Environment*, *309*(1–3), 81–92. https://doi.org/10.1016/S0048-9697(02)00684-8

Hoff, G. L., Bigler, W. J., & McKinnon, J. G. (1977). Heavy metal concentrations in kidneys of estuarine raccoons from Florida. *Journal of Wildlife Diseases*, *13*(1), 101–102. https://doi.org/10.7589/0090-3558-13.1.101

Honda, K., Tatsukawa, R., Itano, K., Miyazaki, N., & Fujiyama, T. (1983). Heavy metal concentrations in muscle, liver and kidney tissue of striped dolphin, stenella coeruleoalba, and their variations with body length, weight, age and sex. *Agricultural and Biological Chemistry*, *47*(6), 1219–1228. https://doi.org/10.1080/00021369.1983.10863383

Horai, S., Minagawa, M., Ozaki, H., Watanabe, I., Takeda, Y., Yamada, K., Ando, T., Akiba, S., Abe, S., & Kuno, K. (2006). Accumulation of Hg and other heavy metals in the Javan mongoose (*Herpestes javanicus*) captured on Amamioshima Island, Japan. *Chemosphere*, *65*(4), 657–665. https://doi.org/10.1016/j.chemosphere.2006.01.078

Ieradi, L. A., Cristaldi, M., Mascanzoni, D., Cardarelli, E., Grossi, R., & Campanella, L. (1996). Genetic damage in urban mice exposed to traffic pollution. *Environmental Pollution*, *92*(3), 323–328. https://doi.org/10.1016/0269-7491(95)00109-3

Jarzyńska, G., & Falandysz, J. (2011). Selenium and 17 other largely essential and toxic metals in muscle and organ meats of Red Deer (*Cervus elaphus*) -

Consequences to human health. *Environment International*, *37*(5), 882–888. https://doi.org/10.1016/j.envint.2011.02.017

Johnson, M. S., Roberts, R. D., Hutton, M., & Inskip, M. J. (1978). Distribution of Lead, Zinc and Cadmium in Small Mammals from Polluted Environments. *Oikos*, *30*(1), 153. https://doi.org/10.2307/3543536

Kakuschke, A., Valentine-Thon, E., Griesel, S., Fonfara, S., Siebert, U., & Prange, A. (2005). Immunological impact of metals in harbor seals (Phoca vitulina) of the North Sea. *Environmental Science and Technology*, *39*(19), 7568–7575. https://doi.org/10.1021/es0505200

Kakuschke, A., Valentine-Thon, E., Griesel, S., Gandrass, J., Luzardo, O. P., Boada, L. D., Peña, M. Z., González, M. A., Grebe, M., Pröfrock, D., Erbsloeh, H. B., Kramer, K., Fonfara, S., & Prange, A. (2010). First health and pollution study on harbor seals (Phoca vitulina) living in the German Elbe estuary. *Marine Pollution Bulletin*, *60*(11), 2079–2086. https://doi.org/10.1016/j.marpolbul.2010.07.011

Kålås, J. A., Ringsby, T. H., & Lierhagen, S. (1995). Metals and selenium in wild animals from Norwegian areas close to Russian nickel smelters. *Environmental Monitoring and Assessment*, *36*(3), 251–270. https://doi.org/10.1007/BF00547905

Kalisinska, E., Lanocha-Arendarczyk, N., Kosik-Bogacka, D., Budis, H., Podlasinska, J., Popiolek, M., Pirog, A., & Jedrzejewska, E. (2016). Brains of native and alien mesocarnivores in biomonitoring of toxic metals in Europe. *PLoS ONE*, *11*(8). https://doi.org/10.1371/journal.pone.0159935

Kannan, K., Agusa, T., Evans, T. J., & Tanabe, S. (2007). Trace element concentrations in livers of polar bears from two populations in Northern and Western Alaska. *Archives of Environmental Contamination and Toxicology*, *53*(3), 473–482. https://doi.org/10.1007/s00244-007-0018-x

Kierdorf, H., Åberg, G., & Kierdorf, U. (2008). Lead concentrations and lead and strontium stable-isotope ratios in teeth of European roe deer (*Capreolus capreolus*). *European Journal of Wildlife Research*, *54*(2), 313–319. https://doi.org/10.1007/S10344-007-0152-9/TABLES/1

Kierdorf, H., & Kierdorf, U. (2000). Roe deer antlers as monitoring units for assessing temporal changes in environmental pollution by fluoride and lead in a German forest area over a 67-year period. *Archives of Environmental Contamination and Toxicology*, *39*(1), 1–6. https://doi.org/10.1007/S002440010072

Kierdorf, H., & Kierdorf, U. (2002). Reconstruction of a decline of ambient lead levels in the Ruhr area (Germany) by studying lead concentrations in antlers of roe deer (*Capreolus capreolus*). *Science of The Total Environment*, *296*(1–3), 153–158. https://doi.org/10.1016/S0048-9697(02)00073-6

Kocan, A. A., Shaw, M. G., Edwards, W. C., & Eve, J. H. (1980). Heavy metal concentrations in the kidneys of white-tailed deer in Oklahoma. *Journal of Wildlife Diseases*, *16*(4), 593–596. https://doi.org/10.7589/0090-3558-16.4.593

Kolesarova, A., Slamecka, J., Jurcik, R., Tataruch, F., Lukac, N., Kovacik, J., Capcarova, M., Valent, M., & Massanyi, P. (2008). Environmental levels of cadmium, lead and mercury in brown hares and their relation to blood metabolic parameters. *Journal of Environmental Science and Health, Part A*, *43*(6), 646–650. https://doi.org/10.1080/10934520801893741

Komarnicki, G. J. K. (2000). Tissue, sex and age specific accumulation of heavy metals (Zn, Cu, Pb, Cd) by populations of the mole (*Talpa europaea* L.) in a central urban area. *Chemosphere*, *41*(10), 1593–1602. https://doi.org/10.1016/S0045-6535(00)00018-7

Kottferová, J., & Koréneková, B. (1998). Distribution of Cd and Pb in the tissues and organs of free-living animals in the Territory of Slovakia. *Bulletin of Environmental Contamination and Toxicology*, *60*(1), 171–176. https://doi.org/10.1007/s001289900607

Kuiters, A. T. (1996). Accumulation of cadmium and lead in red deer and wild boar at the Veluwe, the Netherlands. In *Veterinary Quarterly* (Vol. 18, pp. 134–135). Taylor & Francis Group . https://doi.org/10.1080/01652176.1996.9694715

Ladouceur, E. E. B., Kagan, R., Scanlan, M., & Viner, T. (2015). Chronically embedded lead projectiles in wildlife: A case series investigating the potential for lead toxicosis. *Journal of Zoo and Wildlife Medicine*, *46*(2), 438–442. https://doi.org/10.1638/2015-0026R.1

Langlois, C., & Langis, R. (1995). Presence of airborne contaminants in the wildife of northern Québec. *Science of the Total Environment*, *160–161*(C), 391–402. https://doi.org/10.1016/0048-9697(95)04372-8

Lanocha, N., Kalisinska, E., Kosik-Bogacka, D. I., Budis, H., & Noga-Deren, K. (2012). Trace metals and micronutrients in bone tissues of the red fox *Vulpes vulpes* (L., 1758). *Acta Theriologica*, *57*(3), 233–244. https://doi.org/10.1007/s13364-012-0073-1

Lanocha, N., Kalisinska, E., Kosik-Bogacka, D. I., Budis, H., Sokolowski, S., & Bohatyrewicz, A. (2013). Comparison of metal concentrations in bones of long-living mammals. *Biological Trace Element Research*, *152*(2), 195–203. https://doi.org/10.1007/s12011-013-9615-x

Lanszki, J., Orosz, E., & Sugár, L. (2009). Metal levels in tissues of Eurasian otters (*Lutra lutra*) from Hungary: Variation with sex, age, condition and location. *Chemosphere*, *74*(5), 741–743. https://doi.org/10.1016/j.chemosphere.2008.10.022

Larter, N. C., & Nagy, J. A. (2000). A comparison of heavy metal levels in the kidneys of High Arctic and mainland caribou populations in the Northwest Territories of Canada. *Science of the Total Environment*, *246*(2–3), 109–119. https://doi.org/10.1016/S0048-9697(99)00418-0

Law, R. J., Jones, B. R., Baker, J. R., Kennedy, S., Milne, R., & Morris, R. J. (1992). Trace metals in the livers of marine mammals from the Welsh coast and the Irish Sea. *Marine Pollution Bulletin*, *24*(6), 296–304. https://doi.org/10.1016/0025-326X(92)90590-3

Lazarus, M., Crnić, A. P., Bilandžić, N., Kusak, J., & Reljić, S. (2014). Cadmium, lead, and mercury exposure assessment among Croatian consumers of free-living game. *Arhiv Za Higijenu Rada i Toksikologiju*, *65*(3), 281–292. https://doi.org/10.2478/10004-1254-65-2014-2527

Lazarus, M., Orct, T., Blanuša, M., Vicković, I., & šoštarić, B. (2008). Toxic and essential metal concentrations in four tissues of red deer (***Cervus elaphus***) from Baranja, Croatia. *Food Additives & Contaminants: Part A*, *25*(3), 270–283. https://doi.org/10.1080/02652030701364923

Lazarus, M., Orct, T., Reljić, S., Sedak, M., Bilandžić, N., Jurasović, J., & Huber, Đ. (2018). Trace and macro elements in the femoral bone as indicators of long-term environmental exposure to toxic metals in European brown bear (*Ursus arctos*) from Croatia. *Environmental Science and Pollution Research*, *25*(22), 21656–21670. https://doi.org/10.1007/s11356-018-2296-4

Lazarus, M., Orct, T., Sergiel, A., Vranković, L., Marijić, V. F., Rašić, D., Reljić, S., Aladrović, J., Zwijacz-Kozica, T., Zięba, F., Selva, N., & Huber. (2020). Metal(loid) exposure assessment and biomarker responses in captive and free-ranging European brown bear (*Ursus arctos*). *Environmental Research*, *183*, 109166. https://doi.org/10.1016/j.envres.2020.109166

Lazarus, M., Sekovanić, A., Orct, T., Reljić, S., Jurasović, J., & Huber, Đ. (2018). Sexual Maturity and Life Stage Influences Toxic Metal Accumulation in Croatian Brown Bears. *Archives of Environmental Contamination and Toxicology*, *74*(2), 339–348. https://doi.org/10.1007/s00244-017-0487-5

Lazarus, M., Sekovanić, A., Orct, T., Reljić, S., Kusak, J., Jurasović, J., & Huber, Đ. (2017). Apex predatory mammals as bioindicator species in environmental monitoring of elements in Dinaric Alps (Croatia). *Environmental Science and Pollution Research*, *24*(30), 23977–23991. https://doi.org/10.1007/s11356-017-0008-0

Lazarus, M., Vicković, I., Šoštarić, B., & Blanuša, M. (2005). Heavy metal levels in tissues of red deer (*Cervus elaphus*) from eastern Croatia. *Archives of Industrial Hygiene and Toxicology*, *56*(3), 233–240.

Levengood, J. M., & Heske, E. J. (2008). Heavy metal exposure, reproductive activity, and demographic patterns in white-footed mice (*Peromyscus leucopus*) inhabiting a contaminated floodplain wetland. *Science of the Total Environment*, *389*(2–3), 320–328. https://doi.org/10.1016/j.scitotenv.2007.08.050

Lewis, L. A., Poppenga, R. J., Davidson, W. R., Fischer, J. R., & Morgan, K. A. (2001). Lead toxicosis and trace element levels in wild birds and mammals at a firearms training facility. *Archives of Environmental Contamination and Toxicology*, *41*(2), 208–214. https://doi.org/10.1007/s002440010239

Lind, Y., Bignert, A., & Odsjö, T. (2006). Decreasing lead levels in Swedish biota revealed by 36 years (1969–2004) of environmental monitoring. *Journal of Environmental Monitoring*, *8*(8), 824–834. https://doi.org/10.1039/B517867C

Lourenço, J., Pereira, R., Gonçalves, F., & Mendo, S. (2013). Metal bioaccumulation, genotoxicity and gene expression in the European wood mouse (*Apodemus sylvaticus*) inhabiting an abandoned uranium mining area. *Science of the Total Environment*, *443*, 673–680. https://doi.org/10.1016/j.scitotenv.2012.10.105

Ludolphy, C., Kierdorf, U., & Kierdorf, H. (2021). Lead concentrations in antlers of European roe deer (*Capreolus capreolus*) from an agricultural area in Northern Germany over a 119-year period - a historical biomonitoring study. *Environmental Science and Pollution Research*, *28*(40), 56069–56078. https://doi.org/10.1007/S11356-021-14538-6/TABLES/2

Ludolphy, C., Kierdorf, U., & Kierdorf, H. (2022). Antlers of European roe deer (*Capreolus capreolus*) as monitoring units to assess lead pollution in a floodplain contaminated by historical metal ore mining, processing, and smelting in the Harz Mountains, Germany. *Environmental Pollution*, *302*. https://doi.org/10.1016/j.envpol.2022.119080

Marques, C. C., Sánchez-Chardi, A., Gabriel, S. I., Nadal, J., Viegas-Crespo, A. M., & da Luz Mathias, M. (2007). How does the greater white-toothed shrew, *Crocidura russula*, responds to long-term heavy metal contamination? - A case study. *Science of the Total Environment*, *376*(1–3), 128–133. https://doi.org/10.1016/j.scitotenv.2007.01.061

Martin, A., Müller-Graf, C., Selhorst, T., Gerofke, A., Ulbig, E., Gremse, C., Greiner, M., Lahrssen-Wiederholt, M., & Hensel, A. (2019). Comparison of lead levels in edible parts of red deer hunted with lead or non-lead ammunition. *Science of The Total Environment*, *653*, 315–326. https://doi.org/10.1016/J.SCITOTENV.2018.10.393

Martin, P. A., McDaniel, T. V., Hughes, K. D., & Hunter, B. (2011). Mercury and other heavy metals in free-ranging mink of the lower Great Lakes basin, Canada, 1998-2006. *Ecotoxicology*, *20*(7), 1701–1712. https://doi.org/10.1007/s10646-011-0763-5

Martiniaková, M., Omelka, R., Jančová, A., Stawarz, R., & Formicki, G. (2011). Concentrations of selected heavy metals in bones and femoral bone structure of bank (*Myodes glareolus*) and common (*Microtus arvalis*) voles from different polluted biotopes in Slovakia. *Archives of Environmental Contamination and Toxicology*, *60*(3), 524–532. https://doi.org/10.1007/s00244-010-9545-y

Massányi, P., Tataruch, F., Slameka, J., Toman, R., & Jurík, R. (2003). Accumulation of lead, cadmium, and mercury in liver and kidney of the brown hare (*Lepus europaeus*) in relation to the season, age, and sex in the West Slovakian Lowland. *Journal of Environmental Science and Health, Part A*, *38*(7), 1299–1309. https://doi.org/10.1081/ESE-120021127

McKinnon, J. G., Hoff, G. L., Bigler, W. J., & Prather, E. C. (1976). Heavy metal concentrations in kidneys of urban gray squirrels. *Journal of Wildlife Diseases*, *12*(3), 367–371. https://doi.org/10.7589/0090-3558-12.3.367

Medvedev, N. (1995). Concentrations of cadmium, lead and sulphur in tissues of wild, forest reindeer from north-west Russia. *Environmental Pollution*, *90*(1), 1–5. https://doi.org/10.1016/0269-7491(94)00099-Y

Medvedev, N. (1999). Levels of heavy metals in Karelian wildlife, 1989-91. *Environmental Monitoring and Assessment*, *56*(2), 177–193. https://doi.org/10.1023/A:1005988511058

Milton, A., Cooke, J. A., & Johnson, M. S. (2003). Accumulation of lead, zinc, and cadmium in a wild population of Clethrionomys glareolus from an abandoned lead mine. *Archives of Environmental Contamination and Toxicology*, *44*(3), 405–411. https://doi.org/10.1007/s00244-002-2014-5

Milton, A., Johnson, M. S., & Cooke, J. A. (2002). Lead within ecosystems on metalliferous mine tailings in Wales and Ireland. *Science of the Total Environment*, *299*(1–3), 177–190. https://doi.org/10.1016/S0048-9697(02)00253-X

Morales, J. S. S., Rojas, R. M., Pérez-Rodríguez, F., Casas, A. A., & López, M. A. A. (2011). Risk assessment of the lead intake by consumption of red deer and wild boar meat in southern Spain. *Food Additives and Contaminants - Part A*, *28*(8), 1021–1033. https://doi.org/10.1080/19440049.2011.583282

Morris, R. J., Law, R. J., Allchin, C. R., Kelly, C. A., & Fileman, C. F. (1989). Metals and organochlorines in dolphins and porpoises of Cardigan Bay, West Wales. *Marine Pollution Bulletin*, *20*(10), 512–523. https://doi.org/10.1016/0025-326X(89)90140-9

Moses, S. K., Whiting, A. v., Bratton, G. R., Taylor, R. J., & O'Hara, T. M. (2009). Inorganic nutrients and contaminants in subsistence species of Alaska: linking wildlife and human health. *International Journal of Circumpolar Health*, *68*(1), 53–74. https://doi.org/10.3402/ijch.v68i1.18294

Mouw, D., Kalitis, K., Anver, M., Schwartz, J., Constan, A., Hartung, R., Cohen, B., & Ringler, D. (1975). Lead. Possible toxicity in urban vs rural rats. *Archives of Environmental Health*, *30*(6), 276–280. https://doi.org/10.1080/00039896.1975.10666699

Mulero, R., Cano-Manuel, J., Ráez-Bravo, A., Pérez, J. M., Espinosa, J., Soriguer, R., Fandos, P., Granados, J. E., & Romero, D. (2016). Lead and cadmium in wild boar (*Sus scrofa*) in the Sierra Nevada Natural Space (southern Spain). *Environmental Science and Pollution Research*, *23*(16), 16598–16608. https://doi.org/10.1007/s11356-016-6845-4

Niemi, A., Venäläinen, E. R., Hirvi, T., & Valtonen, M. (1993). Heavy metals in muscle, liver, and kidney from Finnish elk in 1980–81 and 1990. *Bulletin of Environmental Contamination and Toxicology*, *50*(6), 834–841. https://doi.org/10.1007/BF00209947

Norheim, G., Skaare, J. U., & Wiig, Ø. (1992). Some heavy metals, essential elements, and chlorinated hydrocarbons in polar bear (*Ursus maritimus*) at Svalbard. *Environmental Pollution*, *77*(1), 51–57. https://doi.org/10.1016/0269-7491(92)90157-6

North, M. A., Lane, E. P., Marnewick, K., Caldwell, P., Carlisle, G., & Hoffman, L. C. (2015). Suspected lead poisoning in two captive cheetahs (*Acinonyx jubatus jubatus*) in South Africa, in 2008 and 2013. *Journal of the South African Veterinary Association*, *86*(1). https://doi.org/10.4102/jsava.v86i1.1286

Ogle, M. C., Scanlon, P. F., Kirkpatrick, R. L., & Gwynn, J. v. (1985). Heavy metal concentrations in tissues of mink in Virginia. *Bulletin of Environmental Contamination and Toxicology*, *35*(1), 29–37. https://doi.org/10.1007/BF01636476

O'Hara, T. M., George, J. C., Blake, J., Burek, K., Carroll, G., Dau, J., Bennett, L., McCoy, C. P., Gerard, P., & Woshner, V. (2003). Investigation of heavy metals in a large mortality event in caribou of northern Alaska. *Arctic*, *56*(2), 125–135. https://doi.org/10.14430/arctic608

O'Hara, T. M., Hanns, C., Bratton, G., Taylor, R., & Woshner, V. M. (2006). Essential and non-essential elements in eight tissue types from subsistence-hunted bowhead whale: nutritional and toxicological assessment. *International Journal of Circumpolar Health*, *65*(3), 228–242. https://doi.org/10.3402/ijch.v65i3.18108

Pankakoski, E., Hyvärinen, H., Jalkanen, M., & Koivisto, I. (1993). Accumulation of heavy metals in the mole in Finland. *Environmental Pollution*, *80*(1), 9–16. https://doi.org/10.1016/0269-7491(93)90003-7

Parelho, C., Bernardo, F., Camarinho, R., Rodrigues, A. S., & Garcia, P. (2016). Testicular damage and farming environments - An integrative ecotoxicological link. *Chemosphere*, *155*, 135–141. https://doi.org/10.1016/j.chemosphere.2016.04.043

Parker, G. H., & Hamr, J. (2001). Metal levels in body tissues, forage and fecal pellets of elk (*Cervus elaphus*) living near the ore smelters at Sudbury, Ontario. *Environmental Pollution*, *113*(3), 347–355. https://doi.org/10.1016/S0269-7491(00)00183-4

Pedersen, S., & Lierhagen, S. (2006). Heavy metal accumulation in arctic hares (*Lepus arcticus*) in Nunavut, Canada. *Science of the Total Environment*, *368*(2–3), 951–955. https://doi.org/10.1016/j.scitotenv.2006.05.014

Penrose, B., MacIntosh, A. E., Parbhakar-Fox, A., Smith, L. B. E., Sawyer, T., D'Agnese, E., & Carver, S. (2022). Heavy metal wombats? Metal exposure pathways to bare-nosed wombats (*Vombatus ursinus*) living on remediated tin mine tailings. *Science of the Total Environment*, *835*. https://doi.org/10.1016/j.scitotenv.2022.155526

Pereira, R., Pereira, M. L., Ribeiro, R., & Gonçalves, F. (2006). Tissues and hair residues and histopathology in wild rats (*Rattus rattus* L.) and Algerian mice (*Mus spretus* Lataste) from an abandoned mine area (Southeast Portugal). *Environmental Pollution*, *139*(3), 561–575. https://doi.org/10.1016/j.envpol.2005.04.038

Pokorny, B., Glinšek, A., & Ribarič-Lasnik, C. (2004). Roe deer antlers as a historical bioindicator of lead pollution in the Šalek Valley, Slovenia. *Journal of Atmospheric Chemistry*, *49*(1), 175–189. https://doi.org/10.1007/S10874-004-1223-X

Pokorny, B., Jelenko, I., Kierdorf, U., & Kierdorf, H. (2009). Roe deer antlers as historical bioindicators of lead pollution in the vicinity of a lead smelter, Slovenia. *Water, Air, and Soil Pollution*, *203*(1–4), 317–324. https://doi.org/10.1007/S11270-009-0014-Z/TABLES/3

Pokorny, B., & Ribarič-Lasnik, C. (2000). Lead, cadmium, and zinc in tissues of roe deer (*Capreolus capreolus*) near the lead smelter in the Koroška region (northern Slovenia). *Bulletin of Environmental Contamination and Toxicology*, *64*(1), 20–26. https://doi.org/10.1007/s001289910004

Pollock, S. Z., & St. Clair, C. C. (2020). Railway-associated attractants as potential contaminants for wildlife. *Environmental Management*, *66*(1), 16–29. https://doi.org/10.1007/s00267-020-01277-6

Poole, K. G., Elkin, B. T., & Bethke, R. W. (1998). Organochlorine and heavy metal contaminants in wild mink in Western Northwest Territories, Canada. *Archives of Environmental Contamination and Toxicology*, *34*(4), 406–413. https://doi.org/10.1007/s002449900337

Pulscher, L. A., Gray, R., McQuilty, R., Rose, K., Welbergen, J. A., & Phalen, D. N. (2021). Evidence of chronic cadmium exposure identified in the critically endangered Christmas Island flying-fox (*Pteropus natalis*). *Science of the Total Environment*, *766*, 144374. https://doi.org/10.1016/j.scitotenv.2020.144374

Pulscher, L. A., Gray, R., McQuilty, R., Rose, K., Welbergen, J., & Phalen, D. N. (2020). Investigation into the utility of flying foxes as bioindicators for environmental metal pollution reveals evidence of diminished lead but significant cadmium exposure. *Chemosphere*, *254*, 126839. https://doi.org/10.1016/J.CHEMOSPHERE.2020.126839

Quakenbush, L., & Citta, J. J. (2009). Trace element concentrations in bearded seals (*Erignathus barbatus*) near Red Dog Mine compared to other locations in Alaska. *Journal of Marine Biology*, *2009*, 275040. https://doi.org/10.1155/2009/275040

Ráez-Bravo, A., Granados, J. E., Cano-Manuel, F. J., Soriguer, R. C., Fandos, P., Pérez, J. M., Pavlov, I. Y., & Romero, D. (2016). Toxic and essential element concentrations in Iberian ibex (*Capra pyrenaica*) from the Sierra Nevada Natural Park (Spain): Reference intervals in whole blood. *Bulletin of Environmental Contamination and Toxicology*, *96*(3), 273–280. https://doi.org/10.1007/s00128-015-1711-5

Rappaport, S. C., Strebel, R. F., Garner, E., & Craston, D. (1975). Lead insult to the dentition in the leopard. *Pharmacology and Therapeutics in Dentistry*, *2*(3–4), 201–207.

Read, H. J., & Martin, M. H. (1993). The effect of heavy metals on populations of small mammals from woodlands in Avon (England); with particular emphasis on metal concentrations in *Sorex araneus* L. and *Sorex minutus* L. *Chemosphere*, *27*(11), 2197–2211. https://doi.org/10.1016/0045-6535(93)90132-O

Reglero, M. M., Monsalve-González, L., Taggart, M. A., & Mateo, R. (2008). Transfer of metals to plants and red deer in an old lead mining area in Spain. *Science of the Total Environment*, *406*(1–2), 287–297. https://doi.org/10.1016/j.scitotenv.2008.06.001

Reynolds, K. D., Schwarz, M. S., McFarland, C. A., McBride, T., Adair, B., Strauss, R. E., Cobb, G. P., Hooper, M. J., & McMurry, S. T. (2006). Northern pocket gophers (*Thomomys talpoides*) as biomonitors of environmental metal contamination. *Environmental Toxicology and Chemistry*, *25*(2), 458–469. https://doi.org/10.1897/05-130R1.1

Rintala, R., Venäläinen, E. R., & Hirvi, T. (1995). Heavy metals in muscle, liver, and kidney from finnish reindeer in 1990-91 and 1991-92. *Bulletin of Environmental Contamination and Toxicology*, *54*(1), 158–165. https://doi.org/10.1007/BF00196283

Roberts, R. D., Johnson, M. S., & Hutton, M. (1978). Lead contamination of small mammals from abandoned metalliferous mines. *Environmental Pollution (1970)*, *15*(1), 61–69. https://doi.org/10.1016/0013-9327(78)90061-7

Rogers, T. A., Bedrosian, B., Graham, J., & Foresman, K. R. (2012). Lead exposure in large carnivores in the greater Yellowstone ecosystem. *The Journal of Wildlife Management*, *76*(3), 575–582. https://doi.org/10.1002/jwmg.277

Rogival, D., Scheirs, J., De Coen, W., Verhagen, R., & Blust, R. (2006). Metal blood levels and hematological characteristics in wood mice (*Apodemus sylvaticus* L.) along a metal pollution gradient. *Environmental Toxicology and Chemistry; Oxford*, *25*(1), 149–157. https://doi.org/10.1897/04-659R1.1

Rush, S. A., Borgå, K., Dietz, R., Born, E. W., Sonne, C., Evans, T., Muir, D. C. G., Letcher, R. J., Norstrom, R. J., & Fisk, A. T. (2008). Geographic distribution of selected elements in the livers of polar bears from Greenland, Canada and the United States. *Environmental Pollution*, *153*(3), 618–626. https://doi.org/10.1016/j.envpol.2007.09.006

Sánchez-Chardi, A., Marques, C. C., Nadal, J., & da Luz Mathias, M. (2007). Metal bioaccumulation in the greater white-toothed shrew, *Crocidura russula*, inhabiting an abandoned pyrite mine site. *Chemosphere*, *67*(1), 121–130. https://doi.org/10.1016/j.chemosphere.2006.09.009

Sánchez-Chardi, A., Ribeiro, C. A. O., & Nadal, J. (2009). Metals in liver and kidneys and the effects of chronic exposure to pyrite mine pollution in the shrew Crocidura russula inhabiting the protected wetland of Doñana. *Chemosphere*, *76*(3), 387–394. https://doi.org/10.1016/j.chemosphere.2009.03.036

Sanderson, G. C., & Thomas, R. M. (1961). Incidence of Lead in Livers of Illinois Raccoons. *The Journal of Wildlife Management*, *25*(2), 160. https://doi.org/10.2307/3798674

Santiago, D., Motas-Guzmán, M., Reja, A., María-Mojica, P., Rodero, B., & García-Fernández, A. J. (1998). Lead and cadmium in red deer and wild boar from Sierra Morena Mountains (Andalusia, Spain). *Bulletin of Environmental Contamination and Toxicology*, *61*(6), 730–737. https://doi.org/10.1007/s001289900822

Sato, I., Yamauchi, K., & Tsuda, S. (2016). Long-Term Survey of Cadmium and Lead Contamination in Japanese Black Bears Captured in Iwate Prefecture, Japan. *Bulletin of Environmental Contamination and Toxicology*, *97*(6), 806–812. https://doi.org/10.1007/s00128-016-1942-0

Sauer, R. M., Zook, B. C., & Garner, F. M. (1970). Demyelinating encephalomyelopathy associated with lead poisoning in nonhuman primates. *Science*, *169*(3950), 1091–1093. https://doi.org/10.1126/science.169.3950.1091

Schroder, J. L., Basta, N. T., Payton, M., Wilson, J. A., Carlson, R. I., Janz, D. M., & Lochmiller, R. L. (2003). Ecotoxicological risks associated with land treatment of petrochemical wastes. I. Residual soil contamination and bioaccumulation by cotton rats (*Sigmodon hispidus*). *Journal of Toxicology and Environmental Health - Part A*, *66*(4), 305–325. https://doi.org/10.1080/15287390306365

Shlosberg, A., Bellaiche, M., Regev, S., Gal, R., Brizzi, M., Hanji, V., Zaidel, L., & Nyska, A. (1997). Lead toxicosis in a captive bottlenose dolphin (*Tursiops truncatus*) consequent to ingestion of air gun pellets. *Journal of Wildlife Diseases*, *33*(1), 135–139. https://doi.org/10.7589/0090-3558-33.1.135

Skerratt, L. F., Speare, R., Berger, L., & Winsor, H. (1998). Lyssaviral infection and lead poisoning in black flying foxes from Queensland. *Journal of Wildlife Diseases*, *34*(2), 355–361. https://doi.org/10.7589/0090-3558-34.2.355

Skibniewski, M., Skibniewska, E. M., & Kośla, T. (2015). The content of selected metals in muscles of the red deer (*Cervus elaphus*) from Poland. *Environmental Science and Pollution Research*, *22*(11), 8425–8431. https://doi.org/10.1007/s11356-014-4007-0

Sobota, S., Baranowska-Bosiacka, I., Gutowska, I., Kupiec, M., Dusza, E., Machoy, Z., & Chlubek, D. (2011). Biomonitoring of lead and fluoride contamination in forests using chemical analysis of hard tissues of roe deer (*Capreolus capreolus* L.). *Polish Journal of Environmental Studies*, *20*(2), 435–443.

Srebočan, E., Janicki, Z., Crnić, A. P., Tomljanović, K., Šebečić, M., & Konjević, D. (2012). Cadmium, lead and mercury concentrations in selected red deer (*Cervus elaphus* L.) tissues from north-eastern Croatia. *Journal of Environmental Science and Health, Part A*, *47*(13), 2101–2108. https://doi.org/10.1080/10934529.2012.695994

Stansley, W., & Roscoe, D. E. (1996). The uptake and effects of lead in small mammals and frogs at a trap and skeet range. *Archives of Environmental Contamination and Toxicology*, *30*(2), 220–226. https://doi.org/10.1007/BF00215801

Stavros, H.-C. W., Bonde, R. K., & Fair, P. A. (2008). Concentrations of trace elements in blood and skin of Florida manatees (*Trichechus manatus latirostris*). *Marine Pollution Bulletin*, *56*(6), 1221–1225. https://doi.org/10.1016/j.marpolbul.2008.03.035

Stavros, H.-C. W., Bossart, G. D., Hulsey, T. C., & Fair, P. A. (2007). Trace element concentrations in skin of free-ranging bottlenose dolphins (*Tursiops truncatus*) from the southeast Atlantic coast. *Science of the Total Environment*, *388*(1–3), 300–315. https://doi.org/10.1016/j.scitotenv.2007.07.030

Syakalima, M. S., Choongo, K. C., Chilonda, P., Ahmadu, B., Mwase, M., Onuma, M., Sugimoto, C., Tsubota, T., Fukushi, H., Yoshida, M., Yasuda, J., & Nakazato, Y. (2001). Bioaccumulation of lead in wildlife dependent on the contaminated environment of the Kafue flats. *Bulletin of Environmental Contamination and Toxicology*, *67*(3), 438–445. https://doi.org/10.1007/s001280143

Tête, N., Afonso, E., Bouguerra, G., & Scheifler, R. (2015). Blood parameters as biomarkers of cadmium and lead exposure and effects in wild wood mice (*Apodemus sylvaticus*) living along a pollution gradient. *Chemosphere*, *138*, 940–946. https://doi.org/10.1016/j.chemosphere.2014.12.031

Tête, N., Durfort, M., Rieffel, D., Scheifler, R., & Sánchez-Chardi, A. (2014). Histopathology related to cadmium and lead bioaccumulation in chronically exposed wood mice, *Apodemus sylvaticus*, around a former smelter. *Science of the Total Environment*, *481*(1), 167–177. https://doi.org/10.1016/j.scitotenv.2014.02.029

Tête, N., Fritsch, C., Afonso, E., Coeurdassier, M., Lambert, J.-C., Giraudoux, P., & Scheifler, R. (2013). Can Body Condition and Somatic Indices be Used to Evaluate Metal-Induced Stress in Wild Small Mammals? *PLoS ONE*, *8*(6). https://doi.org/10.1371/journal.pone.0066399

Travis, E. K., Watson, P., & Dabek, L. (2012). Health assessment of free-ranging and captive Matschie's tree kangaroos (*Dendrolagus matschiei*) in Papua New Guinea. *Journal of Zoo and Wildlife Medicine*, *43*(1), 1–9. https://doi.org/10.1638/2010-0082.1

Venäläinen, E. R., Niemi, A., & Hirvi, T. (1996). Heavy metals in tissues of Hares in Finland, 1980-82 and 1992-93. *Bulletin of Environmental Contamination and Toxicology*, *56*(2), 251–258. https://doi.org/10.1007/s001289900038

Vermeulen, F., D'Havé, H., Mubiana, V. K., Van den Brink, N. W., Blust, R., Bervoets, L., & De Coen, W. (2009). Relevance of hair and spines of the European hedgehog (*Erinaceus europaeus*) as biomonitoring tissues for arsenic and metals in relation to blood. *Science of the Total Environment*, *407*(5), 1775–1783. https://doi.org/10.1016/j.scitotenv.2008.10.039

Wagemann, R. (1989). Comparison of heavy metals in two groups of ringed seals (*Phoca hispida*) from the Canadian Arctic. *Canadian Journal of Fisheries and Aquatic Sciences*, *46*(9), 1558–1563. https://doi.org/10.1139/f89-198

Wagemann, R., Snow, N. B., Lutz, A., & Scott, D. P. (1983). Heavy metals in tissues and organs of the narwhal (*Monodon monoceros*). *Canadian Journal of Fisheries and Aquatic Sciences*, *40*(S2), s206–s214. https://doi.org/10.1139/f83-326

Way, C. A., & Schroder, G. D. (1982). Accumulation of lead and cadmium in wild populations of the commensal rat, Rattus norvegicus. *Archives of Environmental Contamination and Toxicology*, *11*(4), 407–417. https://doi.org/10.1007/BF01056066

Welch, W. R., & Dick, D. L. (1975). Lead concentrations in tissues of roadside mice. *Environmental Pollution (1970)*, *8*(1), 15–21. https://doi.org/10.1016/0013-9327(75)90068-3

Wieczorek-Dąbrowska, M., Tomza-Marciniak, A., Pilarczyk, B., & Balicka-Ramisz, A. (2013). Roe and red deer as bioindicators of heavy metals contamination in north-western Poland. *Chemistry and Ecology*, *29*(2), 100–110. https://doi.org/10.1080/02757540.2012.711322

Wintle, N. J. P., Martin-Wintle, M. S., Zhou, X., & Zhang, H. (2018). Blood Lead Levels in Captive Giant Pandas. *Bulletin of Environmental Contamination and Toxicology*, *100*(1), 59–63. https://doi.org/10.1007/s00128-017-2221-4

Wolkers, H., Wensing, T., & Groot Bruinderink, G. W. T. A. (1994). Heavy metal contamination in organs of red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*) and the effect on some trace elements. *Science of the Total Environment, The*, *144*(1–3), 191–199. https://doi.org/10.1016/0048-9697(94)90438-3

Woolf, A., Smith, J. R., & Small, L. (1982). Metals in livers of white-tailed deer in Illinois. *Bulletin of Environmental Contamination and Toxicology*, *28*(2), 189–194. https://doi.org/10.1007/BF01608574

Woshner, V. M., O'Hara, T. M., Bratton, G. R., & Beasley, V. R. (2001). Concentrations and interactions of selected essential and non-essential elements in ringed seals and polar bears of arctic Alaska. *Journal of Wildlife Diseases*, *37*(4), 711–721. https://doi.org/10.7589/0090-3558-37.4.711

Wren, C. D., Fischer, K. L., & Stokes, P. M. (1988). Levels of lead, cadmium and other elements in mink and otter from Ontario, Canada. *Environmental Pollution*, *52*(3), 193–202. https://doi.org/10.1016/0269-7491(88)90003-6

Zalewski, K., Falandysz, J., Jadacka, M., Martysiak-Żurowska, D., Nitkiewicz, B., & Giżejewski, Z. (2012). Concentrations of heavy metals and PCBs in the tissues of European beavers (*Castor fiber*) captured in northeastern Poland. *European Journal of Wildlife Research*, *58*(4), 655–660. https://doi.org/10.1007/s10344-012-0613-7

Zook, B. C., Eisenberg, J. F., & McLanahan, E. (1973). Some factors affecting the occurrence of lead poisoning in captive primates. *Journal of Medical Primatology*, *2*(3–4), 206–217. https://doi.org/10.1159/000460323

Zook, B. C., Sauer, R. M., & Garner, F. M. (1970). Lead poisoning in Australian fruit bats (*Pteropus poliocephalus*). *Journal of the American Veterinary Medical Association*, *157*(5), 691–694.

Zook, B. C., Sauer, R. M., & Garner, F. M. (1972). Lead poisoning in captive wild animals. *Journal of Wildlife Diseases*, *8*(3), 264–272. https://doi.org/10.7589/0090-3558-8.3.264

Appendix C

Table showing the samples taken from 40 captive brown bears (*Ursus arctos*) from 8 zoological institutions in Sweden and Denmark. Blood samples from 25 bears are included in Chapter 3 and tissue samples from 26 bears are included in Chapter 4.

Zoological institution	ID	Blood[a]	Liver	Kidney mix	Kidney cortex	Kidney medulla	Spinal cord	Skeletal muscle	Subcutaneous fat
Borås Djurpark (Sweden)	Lupin		X		X	X	X		
	Viol		X		X	X	X		
Järvzoo (Sweden)	Lisa	TE							
	JZM20001	TE	X		X	X	X	X	X
	JZM20002	TE	X		X	X	X	X	X
	JZM20003	TE	X		X	X	X	X	X
Kolmårdens Djurpark (Sweden)	KM01	LH	X	X					
	KM02	LH	X	X					
	KM03		X	X					
	KM04		X	X					
	KM05		X	X					
	KM06		X	X					
	KM07		X	X					
	KM08		X	X					
	KM09		X	X					
	KM10		X	X					
	KM11		X	X					
	KM12		X	X					

	Nanna	LH								
	Astrid	LH								
Kolmårdens Djurpark (Sweden)	Andrea	LH								
	Idun	LH								
	Thor	LH								
	Freja1	LH								
Lycksele Djurpark (Sweden)	Iris	TE	X			X	X	X	X	X
Orsa Rovdjurspark (Sweden)	Kaboom	TE	X	X						
	Knall	LH	X	X						
	Krut	TE	X	X						
	Helge		X	X						
	Hedvig		X	X						
	Inez		X	X						
	Freja2	TE								
	Bambam	TE								
Skansen (Sweden)	Miska	EDTA	X			X	X	X		
	LB	EDTA	X			X	X	X		
Skånes Djurpark (Sweden)	Ester	EDTA								
	Glok	EDTA								
	Koda	EDTA								
	Kiwi	EDTA								
Zoologisk Have København (Denmark)	Rode	EDTA								
Total	**40**	**25**	**26**	**18**	**8**	**8**	**8**	**4**	**4**

[a] TE: trace element whole blood test tube; LH: lithium heparin whole blood test tube; EDTA: ethylenediaminetetraacetic acid whole blood test tube.

Appendix D

Data used to create an equation to convert whole blood lithium heparin lead (Pb) concentrations (LH-Pb, µg/l) to whole blood trace element Pb concentrations (TE-Pb, µg/l). Both blood test tubes were taken from 21 free-ranging and 7 captive brown bears (*Ursus arctos*) in 2021.

ID	Date	LH-Pb	TE-Pb
Jaervzoo_JZM20001	08/09/2021	34.351	34.860
Jaervzoo_JZM20002	08/09/2021	66.425	63.593
Jaervzoo_JZM20003	08/09/2021	69.766	71.802
Jaervzoo_Lisa	19/05/2021	145.958	142.333
Lycksele_Iris	27/08/2021	15.727	15.564
Orsa_Bambam	15/06/2021	36.675	45.146
Orsa_Freja	15/06/2021	38.745	40.628
W1304	10/06/2021	89.311	73.817
W1418	11/06/2021	144.850	132.409
W1505	11/06/2021	99.264	94.398
W1509	14/05/2021	110.454	100.896
W1707	11/05/2021	96.075	101.269
W1819	16/05/2021	39.292	35.232
W1908	12/05/2021	96.717	85.956
W1913	27/05/2021	100.391	92.840
W1914	12/05/2021	109.334	99.272
W2007	14/05/2021	70.335	66.811
W2012	13/05/2021	151.449	145.649
W2013	13/05/2021	97.252	91.680
W2015	13/05/2021	75.329	79.090
W2025	12/05/2021	132.836	131.692
W2026	12/05/2021	123.445	118.742
W2030	27/05/2021	138.575	135.071
W2107	28/05/2021	114.504	112.723
W2115	13/05/2021	208.639	215.991
W2116	14/05/2021	99.873	86.329
W2118	16/05/2021	110.179	113.865
W2120	10/06/2021	84.760	90.388

Trace element concentration = (0.9743 x lithium heparin concentration) - 0.4658