Cadmium and lead concentrations in livers and kidneys of cattle slaughtered at Grootfontein Abattoir in Namibia

by

Emmanuel Muchimbidziki Midzi

Submitted in partial fulfilment of the requirements for the degree Master of Veterinary Medicine (Hyg) in the Faculty of Veterinary Science, University of Pretoria

Year and date of submission: April 2012

ACKNOWLEDGEMENTS

Firstly, I would like to thank God for giving me the strength and resolve to see this work completed.

Secondly, I wish to acknowledge my promoter **Prof C.M.E. McCrindle** and co-promoter **Prof R.I. McCrindle**, for their committed and endless encouragement and guidance from start to end of this research work and writing. I also thank **Ms S. Lebogo** for enabling liaison with my promoters. Thank you very much.

Thirdly, I would like to thank my wife **Getrude** for her loving support and my children, **Tafadzwa, Tanatswa** and **Tadiwa** for bearing with me as I used family time to study and complete this work.

Fourthly, I acknowledge the following people whose support made this work a lot easier for me: **Dr A. Bishi** for epidemiological guidance; **Mr J. Ngenokesho** for assisting with specimens collection; **The Municipality of Grootfontein (Namibia)** and **Abattoir Personnel** for allowing me access to their facility and **the four butchers in Grootfontein** for allowing specimens collection.

Finally, I would like to acknowledge **Namibia Custom Smelters management** and **personnel** (Tsumeb – Namibia) for arranging a subsidised rate for analysis of the specimens for this study. I heartily thank you.

DEDICATION

I would like to dedicate this work to my wife **Getrude**, who inspired me in a very different way to undertake this project and supported me throughout.

DECLARATION

I, **Emmanuel Muchimbidziki Midzi**, declare that this dissertation, which I hereby submit for the degree M Med Vet (Hyg) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signed: _____

Date: _____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

CAC	:	Codex Alimentarius Commission of the FAO/WHO
[Cd]	:	Cadmium concentration
[Pb]	:	Lead concentration
FAO	:	Food and Agriculture Organisation of the United Nations
JECFA	:	Joint Expert Committee on Food Additives of FAO/WHO
mg kg^{-1}	:	milligrams per kilogram
ML	:	Maximum Limit
OIE	:	World Organization for Animal Health
PTWI	:	Provisional Tolerable Weekly Intake
PTMI	:	Provisional Tolerable Monthly Intake
WHO	:	World Health Organisation
WTO	:	World Trade Organisation

SUMMARY

Cadmium and lead concentrations in livers and kidneys of cattle slaughtered at Grootfontein Abattoir in Namibia

by

Emmanuel Muchimbidziki **Midzi**

Promoter: Professor C.M.E. McCrindle

Co-promoter: Professor R.I. McCrindlie

Department: Paraclinical Sciences

Degree: M Med Vet (Hyg)

The aim of this study was to determine the levels of cadmium (Cd) and lead (Pb) in livers and kidneys of cattle slaughtered at Grootfontein abattoir in Namibia. The study design was based on the epidemiological principles to detect a single animal whose kidneys or liver contained Cd or Pb residues.

The Grootfontein area of Namibia has extensive base-metal ore reserves, which were and are still extracted and processed in localities used as livestock pastures. Namibia is also an arid country which predominantly uses

borehole water for livestock and human consumption. These underground water bodies share the same space as base-metal ores.

The anthropogenic activities in this area under the existing geological and hydrogeological circumstances offer opportunities for Cd and Pb to enter the food chain. Entry of Cd and Pb in the food chain leads to bioaccumulation in cattle kidneys and livers to concentrations above Codex Alimentarius Commission (CAC) standards, creating a possible public health risk.

The CAC withdrew the maximum limit (ML) of 1mg kg^{-1} Cd in bovine kidneys and liver, but it has a provisional tolerable monthly intake (PTMI) of 0.025mg kg^{-1} human body weight. This CAC PTMI translates to a total exposure of 1.5mg Cd for a 60kg body weight person. The CAC ML for Pb in bovine offal is 0.5mg kg^{-1}, while its provisional tolerable weekly intake (PTWI) of 0.025mg kg^{-1} human body weight is under review.

This investigation intended to establish if Cd and Pb in the livers and kidneys of cattle slaughtered in the study area exceeded CAC human exposure limits.

Liver and kidney specimens were collected from 31 randomly sampled mature cattle (estimated over five years old based on incisor teeth examined post slaughter). The specimens were analysed at a local mine laboratory, which was the only facility available and capable of performing the tests. They were digested using wet-ashing (the oxidation procedure). All liver digestates were

analysed, while one kidney analyte was insufficient. Cd and Pb were measured using flame atomic absorption spectroscopy (FAAS). The detection limit (DL), which was the minimum metal concentration FAAS could measure was 0.2mg kg^{-1} for Cd and 1.1mg kg^{-1} for Pb. The laboratory could not refine the Pb DL which was more than twice the CAC ML.

All livers had Cd concentrations below 0.2mg kg^{-1}. One discarded kidney specimen was assigned a concentration below DL for analysis purposes. The Cd concentrations in 12 kidney specimens were below 0.2mg kg^{-1}, between 0.288 and 1.221mg kg^{-1} in 16 and above 1.5mg kg^{-1} (2.6 - 3.64mg kg^{-1}) in 3 specimens. The mean renal Cd concentration for the population (0.71±0.96mg kg^{-1}) was statistically lower than 1.5mg kg^{-1} ($p < 0.05$). Cd was therefore shown to be a chemical hazard for consumers of kidneys and a potential environmental hazard in the study area.

Pb was negative in all of the 31 liver specimens, while in all the 30 kidney specimen digestates it was detected at concentrations below 1.1mg kg^{-1}. This result confirmed the presence of Pb as a potential chemical hazard found in bovine kidneys. However, a more sensitive analytical method was required to assess Pb food chain and public health hazard parameters in the study area.

An epidemiological investigation of the study area using geographical information systems (GIS) to explore geographical factors that could have influenced exposure to Cd and Pb was done. While proximity to operational

and decommissioned mining ventures appeared to result in higher mean renal Cd concentrations, the influence was not statistically significant. Feedlot rearing also appeared to cause higher mean renal Cd concentrations but the impact was also not statistically significant.

It was concluded that Cd and Pb were chemical environmental contaminants which enter the animal and human food chain in the study area. A kilogram of bovine kidney-meat from approximately one in ten cattle (9.7%) carried more than 1.5mg Cd, exceeding the recommended CAC total dietary exposure for a 60-kg man. A mathematical model was used to estimate the risk of cattle with renal Cd concentration exceeding 1.5mg kg^{-1}, the expected number of cattle affected and the weight of meat entering the food chain. The estimated cattle population affected monthly was 5.95%, with a range of between 5 and 11 (mean = 8±4) cattle. These cattle were estimated to yield 8 to 18 (mean 13±6) kg bovine kidney-meat per month. A WHO standard-weight man who consumes a kilogram of kidney meat in this category in a month risks exposure to Cd doses beyond the CAC PTMI.

The epidemiological triad of interactions between the host (cattle), agent (Cd and Pb) and the environment (proximity to mines) was used in order to suggest risk mitigation options.

Recommendations from this study included advocacy on Cd and Pb in the food chain and developing partnerships with mining entities so that risk

mitigation and communication can be better coordinated. Consumers are advised to reduce their monthly intake of kidney meat as this organ tissue has higher levels that those in other bovine organs and tissues. The cattle in the study were mature breeding animals (>5 years) and it is likely that this factor increased the risk of higher levels in kidneys as temporal determinants for bioaccumulation of Cd are important. The kidneys of younger animals would thus be less likely to contain significant Cd levels. Lastly, use of mathematical modelling, to translate research findings into quantitative estimates useful for public health safety programs, is recommended.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background and research justification

Cadmium (Cd) and lead (Pb) are bioaccumulative heavy metals, which are toxic to livestock and humans. They naturally exist in the earth along with base-metal ores and become contaminants in the food chain as a result of natural and anthropogenic activities. Mining, ore smelting, weathering, erosion as well as household and industrial waste disposal are examples of natural and human activities resulting in the introduction of Pb and Cd into the biosphere. The chemical states of Cd and Pb may transform but they do not degrade, hence they persist in the environment.

The rate and extent of Cd and Pb entry into the food chain follows a clear dose-response, influenced by their levels at source, duration of exposure and chemical state at the sources (Kan & Meijer 2007). Once they enter the food chain they are poorly excreted, which allows them to accumulate, transfer and magnify (Kan & Meijer 2007). Animals and humans who occupy higher levels of the food chain, progressively bioaccumate Cd and Pb from plant and animal products they consume. Thus older exposed animals and humans suffer negative health effects which are overlapping, additive and escalating (Alonso, Benedito, Miranda, Castillo, Hernández & Shore 2000). To address these health concerns, the Food and Agriculture Organisation (FAO), together with the World Health Organisation (WHO), created the Joint Expert Committee on

Food Additives and Contaminants (JECFA) under CAC to deliberate standards and guidelines (WHO/FAO 2006b). The JECFA food standards are an outcome of risk analysis and they are reviewed through risk assessments.

Food chemical contaminant standards in the form of CAC MLs are used for harmonisation of the international food trade under the World Trade Organisation (WTO) to ensure consumers remain within provisional monthly and weekly tolerable intakes (WHO/FAO 2006b; WHO 2011). However, intra-territorial food trade is not governed by WTO food trade standards. Thus, consumption of Cd and Pb tainted foods is regarded as a growing public health concern, especially in developing countries, where testing to identify and exclude non-compliant food is seldom done for cost and food security reasons (Jarup 2003).

The Grootfontein area of Namibia has extensive base-metal ore reserves, which contain Cd and Pb (Plate 1.1). These ores were, and continue to be, mined and processed from both closed and active mines and processing plants (Plate 1.2). Base-metal ore mining and smelting / roasting, industrial activities and waste disposal are known causes of Cd and Pb release into the environment (air, soil and surface water), from where it enters the food chain (Berger & Cunha 1993; Tucker & ATSDR 2008).

Namibia is regarded the most arid country in Sub-Saharan Africa (Sweet & Burke 2006), but it has substantial underground water bodies (Plate 1.3). It is

known that interaction between ore-bearing rocks and percolating water can create and release soluble species of heavy metals, including Cd and Pb, into underground water bodies (Leung & Jiao 2006). The underground water bodies in Namibia are extracted using boreholes and the untreated water is used for livestock, crops and human consumption (Sweet & Burke 2006).

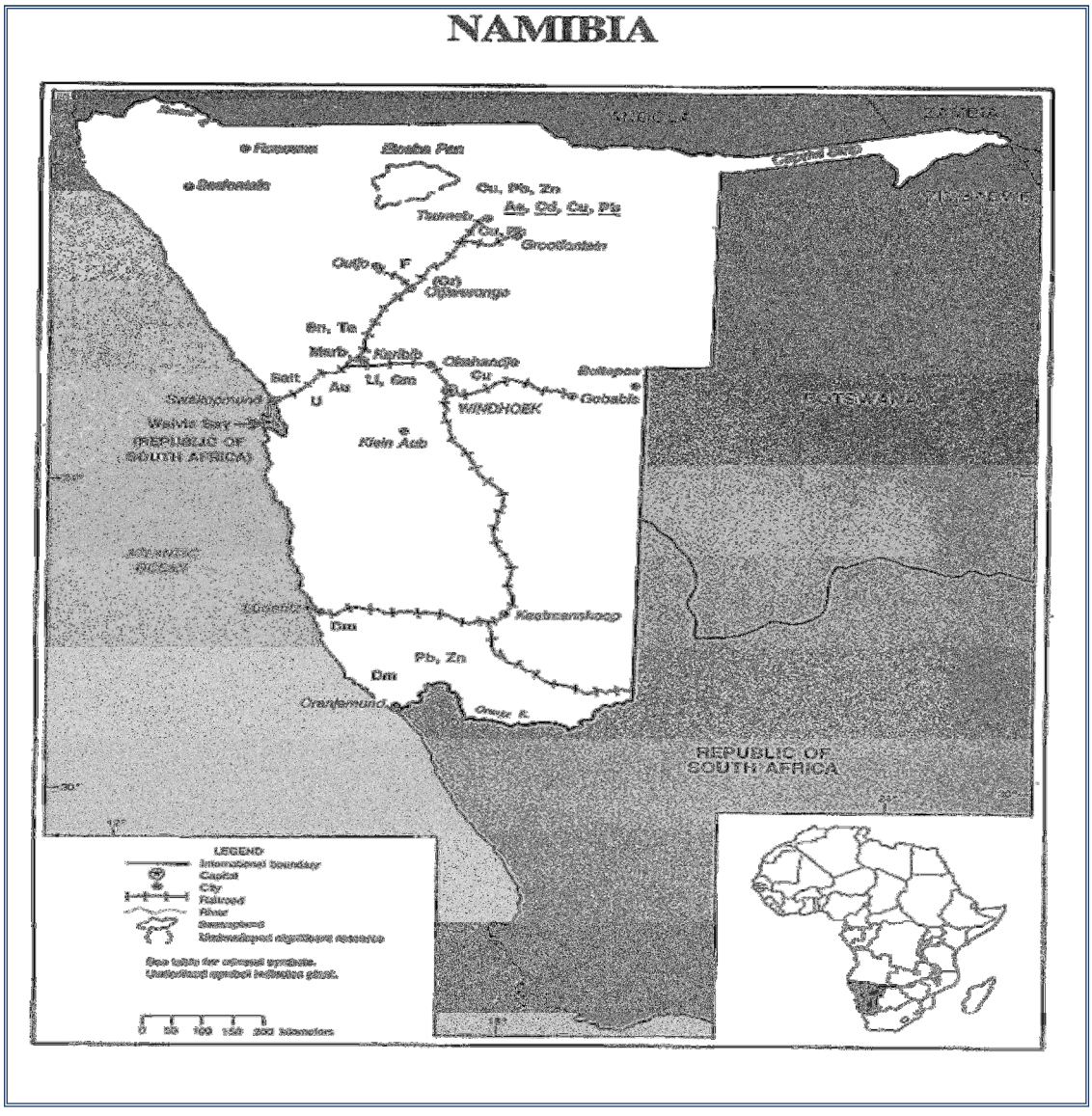

Plate 1.1 The geological map of Namibia: Base metal ore distribution[1]

[1] http://minerals.usgs.gov/minerals/pubs/country/maps/92289.gif

Plate 1.2 The mines and smelters maps: Grootfontein area of Namibia[2]

[2] Google Earth™ mapping service (2010) Google Earth 6.0.2 Out of Beta

The presence of suitable geological, hydro-geological and anthropogenic circumstances and activities in the Grootfontein area of Namibia can therefore give rise to single or a combination of sources and routes of Cd and Pb entry into the food chain.

It is known that kidneys and livers of livestock (including cattle) can accumulate Cd and Pb to concentrations to make consumption in sufficient quantities a public health concern (Forte & Bocca 2007). While no data is available regarding the Namibia annual per capita liver and kidney consumption, a number of low-income families are known to consume cattle kidneys and livers as a cheaper protein source (Norval - Personal communication 2006).

If these organs are contaminated with Cd and Pb, they can cause exposure to the metals at levels higher than the PTDI. Measuring the concentrations of Cd and Pb in kidneys and livers from cattle slaughtered at Grootfontein abattoir in Namibia enable comparison to CAC standards and guide exposure assessment.

No literature could be found which assessed the effects of existing geological, hydro-geological and anthropogenic circumstances and activities in the Grootfontein area of Namibia on Cd and / or Pb in the food chain. Livers and kidneys of cattle slaughtered at Grootfontein abattoir were analysed for Cd and Pb concentration using a locally available laboratory facility. This investigation

would enable formulation of risk mitigation and communication strategies for consumers of bovine offal in this part of Namibia.

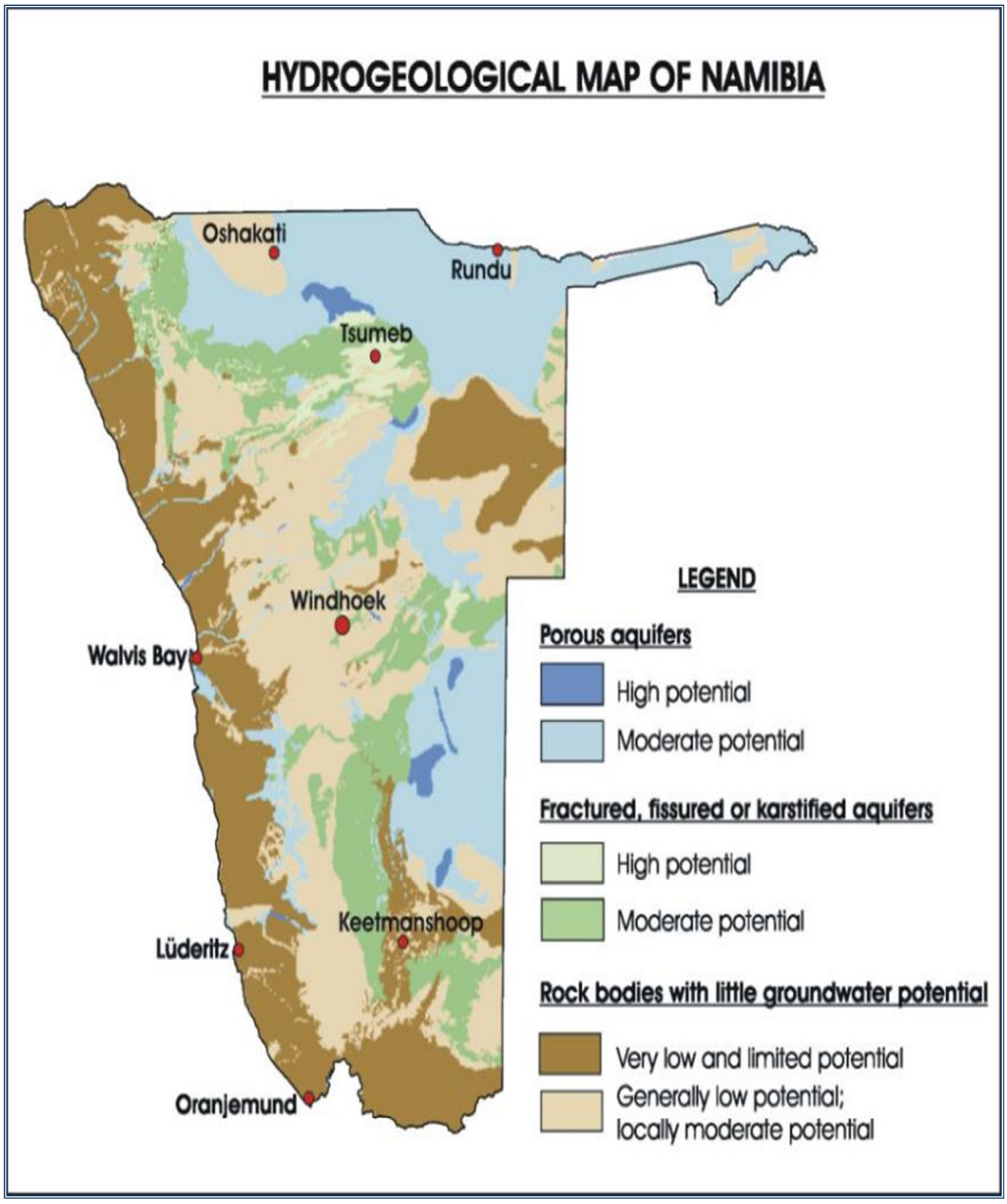

Plate 1.3 The hydrogeological map of Namibia[3]

[3] www.mme.gov.na/gsn/hydrogeologicalmapnammap.htm

1.2 Research questions

- Do kidneys and livers of cattle slaughtered at Grootfontein abattoir in Namibia contain the chemical hazards Cd and Pb?
- If present, what are the levels of Cd and Pb in livers and kidneys?
- How do the Cd and Pb levels compare to CAC standards?
- What is the likelihood of occurrence of cattle organs with organ Cd and Pb at concentrations that violate CAC standards or pose a recognised risk to consumers?

1.3 The research hypotheses

The null hypothesis was that existing geological, hydrogeological and athropogenic exposure factors in Grootfontein area of Namibia did not cause Cd and Pb to enter into kidneys and livers of cattle reared in the area to concentrations above CAC standards (H_o: μ_1 </= CAC threshold value). The alternative was that these exposure factors caused accummulation of Cd and Pb in livers and kidneys of the cattle reared in the area to concentration above their MLs in these organs (H_1: μ_1 > CAC threshold value).

1.4 Objectives

The following were the objectives of the study:

- To measure Cd and Pb concentrations in samples of kidneys and livers.
- To compare the measured organ Cd and Pb concentrations to their respective CAC standards.

- To use the MLs, PTMIs and PTMIs to estimate the prevalence of carcasses where levels of Cd and / or Pb were above published norms.
- To investigate environmental, agent and host determinants that influence uptake of Pb and Cd in the study area (exposure assessment).
- To suggest mitigation and communication strategies to reduce the risk to consumers.

1.5 Expected research benefits and future potential

It is expected that the outcomes of this research will:

- Indicate whether or not Cd and Pb are present in the organs of cattle slaughtered at Grootfontein abattoir in Namibia.
- Indicate the extent of Cd and Pb present in organs of cattle slaughtered at Grootfontein abattoir in comparison to CAC standards.
- Gauge whether local laboratory facilities used are suitable for future use to analyse these metals in meat products.
- Design communication strategies for risk mitigation by mines, cattle farmers and abattoirs in Grootfontein, to prevent or minimise entry of Pb and Cd into the cattle and/or human food chain.

1.6 Work plan

This research was conducted following a work plan with the following main activity areas and benchmarks. The time frames for achieving goals of each area of main activities were outcome based.

- Review the literature in regard to Pb and Cd in the environment and in meat and organs of cattle
- Obtain written consent from municipal authorities before samples are collected.
- Delimit the study area and define the study population.
- Collect, label, record, transport and store collected specimens.
- Submit specimens to the laboratory.
- Apply statistical analyses to laboratory results.
- Trace-back positive samples to the source herds or farms.
- Discuss results and conclude whether the null hypothesis was accepted or rejected.

CHAPTER 2

LITERATURE REVIEW

2.1 International regulatory framework for heavy metals in food

2.1.1 Regulatory bodies

CAC establishes food safety guidelines and standards through risk analyses or assessments (Joint FAO/WHO Food Standards Programme 2010). JECFA sets safety standards for food chemical hazards only, in the form of MLs, PTWIs or PTMIs (Codex Alimentarius Commission 2007, Joint FAO/WHO Food Standards Programme 2010).

An ML is an end-stage or safety quality control maximum concentration of a food-borne hazard legally allowed in foods for trade (WTO 2010). The PTWI and PTMI represent the maximum weight of a food-borne chemical hazard acquired per kg of human body weight while eating wholesome and nutritious food per week and month respectively (FAO/WHO 1997).

All food safety standards created by CAC are adopted by the WTO and applied under the Agreement on the Application of Sanitary and Phytosanitary Measures also known as the SPS Agreement to govern international trade in food commodities (WHO/FAO 2006b, WTO 2010). The SPS Agreement requires WTO member to accept for trade purposes, food items equalling CAC health or safety standards, yet avoiding unfair trade protectionism (Joint FAO/WHO Food Standards Programme 2010; WTO 2010).

2.1.2 CAC / WTO food safety standards for Cd and Pb

According to the Codex General Standard for Contaminants and Toxins in Food and Feed[4] definition, Cd and Pb are food chemical hazards. Thus, food safety standards are established and continue to be reviewed.

At the 36th Session of JECFA, work to create MLs for Cd in meat and offal products was halted because diet was deemed to contribute insignificantly to exposure (JECFA 2004). The Cd tolerable intake standard of 0.007mg kg^{-1} (PTWI) was revised and replaced with a PTMI of 0.025mg kg^{-1} (WHO 2011).

The CAC standard for Pb of 0.025mg kg^{-1} (PTWI) was withdrawn by the 73rd Session of JECFA as it was associated with decreased intelligence quotients in minors and high blood pressure in adults (WHO 2011). The current MLs for Pb in beef and bovine offal are 0.1mg kg^{-1} and 0.5mg kg^{-1} respectively.

The human body weight used for setting CAC safety standards for chemical hazards is 60kg (WHO 2011). Multiplying the CAC PTMI for Cd of 0.025mg kg-1 with the 60kg standard human body weight gives an exposure threshold of 1.5mg for the standard man. The exposure limits for Pb are under review.

Variability in food consumption patterns between and within countries justifies the need for each country to estimate its own Cd and Pb exposure risks and design dietary recommendations accordingly (GEMS 2003; WHO 2011).

[4] http://www.codexalimentarius.net/download/standards/17/CXS_193e.pdf

2.2 Cadmium

Cd is a toxic heavy metal found in Cd ore (Tucker & ATSDR 2008) or base metal ores as an impurity, from where it is also commercially extracted for industrial uses (Liu, Goyer & Waalkes 2008). It enters the soil, water and air through weathering of rocks, soil erosion and as well as dissolution or leaching of metal salts (Coynel, Schäfer, Dabrin, Girardot & Blanc 2007). The mining, roasting and smelting of base metal ores, industrial usage of Cd and disposal of industrial wastes also pollutes the environment (Jarup 2003; Liu *et al.* 2008; Tucker & ATSDR 2008).

Sewerage and household waste disposal as well as the use of metal contaminated phosphate fertilisers, are other anthropogenic sources of environmental contamination by Cd (Berger & Cunha 1993). Ground and surface water rarely carry high Cd levels unless contaminated by mining or industrial wastewater (Tucker & ATSDR 2008).

Most plants readily absorb and accumulate Cd from contaminated soil and water (Liu *et al*. 2008; Tucker & ATSDR 2008). The transfer rate from soil and water to the plants is dependent on the concentration of Cd and chemical or ionic status of the metal in the in soil and water (Sridhara Chary, Kamala & Samuel Suman Raj 2008). Low soil pH, high organic matter content and iron deficiency in sandy soil types, enhances the rate at which ionic Cd is absorbed by plants (Veltman, Huijbregts & Hendriks 2008; Peralta-Videa, Lopez, Narayan, Saupe & Gardea-Torresdey 2009). The potential of plants to absorb

Cd from the environment can be evaluated using a bioaccumulation factor, by dividing its concentration in plant materials by its concentration in the soil in which the plants grew (Peralta-Videa *et al.* 2009).

Cd that has entered plants is neither degraded nor excreted (Liu *et al*. 2008). It accumulates and becomes highly concentrated in leaves, fruits and seeds (Peralta-Videa *et al.* 2009). The primary accumulation of Cd in plant materials from soils starts the transfer process of the metal through all the trophic levels of the food chain (Tucker & ATSDR 2008).

Animal feed of plant origin is regarded as the highest animal exposure source for Cd, ahead of water and air (Figueroa 2008). Animals also obtain Cd as they eat contaminated soil and mineral supplements (Hooser 2007). Large dust particles tainted with Cd are trapped in the upper respiratory tract, from where they are coughed up and swallowed (Nordberg, Nogawa, Nordberg & Friberg 2007). Dust-borne Cd is absorbed directly from the lungs (Tucker & ATSDR 2008). From the gastro-intestinal tract, between 5 and 10% of the ingested Cd amount is absorbed (Liu *et al*. 2008). Cd uptake rates in the gastro-intestinal tract are enhanced by dietary deficiencies of iron, zinc, calcium, selenium, copper and proteins (Berger & Cunha 1993).

Females of reproductive age tend to have higher blood Cd concentrations compared to males, because females physiologically improve mineral absorption from the digestive system to meet the demand of pregnancy and

lactation (Liu *et al*. 2008). There is a body response to iron and calcium deficits associated with reproduction and lactation which inadvertently increase dietary Cd uptake (Nordberg *et al.* 2007; Liu *et al*. 2008).

Cd absorbed from the lungs and digestive system is transported through the body in blood bound to albumin and other large molecular weight proteins (Kan & Meijer 2007; Liu *et al*. 2008). Between 10 and 20% of the circulatory Cd is stored in the liver and in kidneys it is between 80 and 90%, while minor quantities are stored in bones (Hooser 2007; Kan & Meijer 2007; Liu *et al*. 2008). Hepatic Cd occurs as a Cd-metallothionein (a low-molecular weight protein) complex which freely circulates in the body (Liu *et al*. 2008). The Cd-metallothionein complexes enter a cycle of filtration, re-absorption, breakdown and reformation in the proximal renal tubular cell (Satarug & Moore 2004; Radostits, Gay, Hinchcliff & Constable 2007). This leads to retention of Cd in the kidneys and damage to kidney tissues (Liu *et al*. 2008).

Very small quantities of Cd are excreted from the body through urine and bile (as Cd-glutathione) in faeces (Liu *et al*. 2008). Renal excretion of Cd is very poor, due to recycling in proximal renal tubules (Satarug & Moore 2004). Increased dietary intake of selenium helps to improve the renal excretion of Cd. Selenium displaces Cd from metallothionein, forcing the Cd to bind to larger proteins, which are more readily lost from the kidneys (Berger & Cunha 1993).

The nephrotoxic effects of Cd derive from the recycling of Cd-metallothionein complexes in proximal renal tubules resulting in metallothionein and free Cd (Liu *et al*. 2008). The metallothionein is retained in the kidneys while free Cd stimulates further production of renal metallothionein. A vicious cycle is created, where by-products accumulate in and damage renal tubular cells, leading ultimately to renal failure (Liu *et al*. 2008). Cd also mimics, competes with, and displaces several essential trace elements during gastro-intestinal absorption (causing trace elements deficiency signs) (Berger & Cunha 1993; Liu *et al*. 2008). Its effects on proteins and enzymes explain Cd hepatotoxicity.

Livestock rarely show clinical signs of Cd poisoning, even when organ concentrations are above its CAC MLs for consumers (Hooser 2007). Evidence shows that adverse public health effects of Cd occur following exposure to doses previously considered safe (Satarug & Moore 2004; Liu *et al*. 2008). Chronic Cd poisoning resulting from the food chain is of public health concern (Hooser 2007).

The biological half-life of Cd is estimated between 20 and 30 years in humans (Liu *et al*. 2008). During this prolonged half-life, Cd causes continuously intoxication, as it is recycled between the bones, liver and kidneys (Satarug & Moore 2004). Chronic exposure to Cd escalates its toxic effects on kidneys and bone tissue with advancing age, increasing the chances of spontaneous fractures (Tucker & ATSDR 2008).

Cd induced renal failure in humans disturbs the homeostatic balance of calcium-phosphate and vitamin D resulting in hypercalciuria, osteomalacia, osteoporosis, renal stone formation, glycosuria, proteinuria, arthritis and teeth cavities (Jarup 2003; Liu *et al*. 2008). It is also reported to severely affect the reproduction endocrine system of women, by mimicking oestrogen probably as a result of protein and enzyme alteration (Peralta-Videa *et al.* 2009).

2.3 Lead

Pb is a highly toxic bluish-grey corrosion resistant metal which is ubiquitous in organic and inorganic divalent forms in the soil (Tarragó & ATSDR 2007; Liu *et al*. 2008). Contamination of the air, soil and water arises mainly from mining and disposal of industrial, military and household wastes (Tarragó & ATSDR 2007).

Mining releases inorganic Pb, which leaches into water under acidic conditions (Tarragó & ATSDR 2007) while burning fossil fuels predominantly releases organo-Pb compounds, which enter the food chain (Jarup 2003; Liu *et al*. 2008). Agricultural fungicides also introduce Pb into farm lands, where it becomes bio-available to plants and animals (Franco-Uría, López-Mateo, Roca & Fernández-Marcos 2009). Knowledge of the toxic nature of Pb has, however, led to a marked scaling down of its inclusion in various products (Jarup 2003; Liu *et al*. 2008).

Most Pb in the soil is immobilised and not very available for uptake by plants as it forms stable and insoluble complexes with organic matter (Peralta-Videa *et al.* 2009). The acidity of the environment determines the solubility and bioavailability of Pb salts, with the organic salts being more water-soluble and bio-available (Tarragó & ATSDR 2007).

Vegetation growing next to mines and smelters accumulates high levels of Pb on the surfaces from dust and fumes, as well as within the parenchyma after absorption from soil (Radostits *et al.* 2007). Interstitial and surface Pb contamination can render vegetation or crops unsuitable for livestock feeding.

Feed or food, water, dust and fumes are the greatest sources for animal and human exposure (Liu *et al*. 2008). Larger particles of Pb are trapped by cilia in the respiratory tract, then coughed up and swallowed, to enter into the digestive system, where gastric acids solubilise and render them bioavailable (Thompson 2007). Organo-Pb compounds are much more bioavailable in the digestive system, especially if ingested on an empty stomach, due to lack of essential divalent ions that inhibit uptake (Tarragó & ATSDR 2007).

Pregnancy and lactation increases the efficiency of absorption of calcium, zinc and iron to meet physiological demand, which thus inadvertently increases dietary Pb uptake (Skerfving & Bergdahl 2007).

Some organo-Pb (tetraethyl and tetramethyl) compounds can be absorbed through the skin of animals, while fumes and fine particles are trapped in the alveoli and efficiently absorbed (Skerfving & Bergdahl 2007; Thompson 2007).

Absorbed Pb is predominantly reversibly attached to erythrocytes (Bradberry & Vale 2007; Liu *et al*. 2008). The erythrocyte-bound Pb is initially released and uniformly stored as active Pb in the liver and kidneys, regardless of animal gender (Jukna, Jukna & Siugzdaite 2006; Forte & Bocca 2007). It is later redistributed and stored in an inactive state, mainly in bones, with a minor portion in hair (Bradberry & Vale 2007; Radostits *et al.* 2007; Liu *et al*. 2008).

Active Pb binds to functional carboxyl, amino and sulphydyrl groups of proteins to disrupt the functionality of metallo-enzymes in biochemical and metabolic processes (Bradberry & Vale 2007; Liu *et al*. 2008). This occurs as Pb substitutes and mimics bivalent essential trace element ions, particularly multifunctional calcium (Bradberry & Vale 2007). The calcium substituting effects of Pb also affect the mineralisation of teeth (Skerfving & Bergdahl 2007; Liu *et al*. 2008).

Pb reduces the flexibility of erythrocytes, thus shortening their lifespan and disrupting the functions of haeme-synthetase, resulting in normocytic, hypochromic anaemia (Radostits *et al.* 2007). The neurological effects of Pb arise as it becomes a calcium surrogate which disrupts and alters the blood-brain barrier and affects synaptic transmissions (Liu *et al*. 2008).

Pb stored in bones is inert, although an on-going calcium interchange continuously transforms it to an active circulating form during pregnancy and lactation (Bradberry & Vale 2007; Skerfving & Bergdahl 2007; Tarragó & ATSDR 2007). Acidosis also promotes transformation of inert (osseous) Pb to active (soft-tissue-bound) Pb (Radostits *et al.* 2007). Active Pb can cross the placental barrier to accumulate in and affect the foetal brain (Thompson 2007; Liu *et al*. 2008).

In the kidneys, Pb initially causes reversible renal damage, which eventually becomes irreversible renal insufficiency due to interstitial fibrosis (Bradberry & Vale 2007). During pregnancy, Pb can cross the placental barrier resulting in reduced foetal viability, premature births, low birth weights and reduced intelligence quotient in children (Bradberry & Vale 2007; Skerfving & Bergdahl 2007). An acute high dose of Pb exposure is potentially fatal, while chronic low-level exposure results in accumulative subclinical poisoning. Chronic low-level Pb exposure causes dullness in animals, anorexia, blindness and hyperaesthesia (Radostits *et al.* 2007).

The majority of dietary Pb is lost in faecal matter, after forming insoluble complexes with organic matter (Radostits *et al.* 2007). Minute quantities of the absorbed Pb are excreted through the kidneys (75%) and the rest in bile, milk, sweat and hair (Bradberry & Vale 2007; Radostits *et al.* 2007; Liu *et al*. 2008).

Foods of animal origin seldom carry sufficient Pb concentrations to warrant public health concerns (Kan & Meijer 2007). The bioaccumulative nature of Pb and the possibility of multiple exposure sources however, justify the need for exposure minimisation strategies. This becomes even more pertinent because chelating agents used to manage acute Pb poisoning are not beneficial in managing chronic poisoning (Liu *et al*. 2008).

CHAPTER 3

MATERIALS AND METHODS

3.1 Sampling strategy

The sampling strategy was based on the study area, population at risk, sample size calculation and collection of specimens.

3.1.1 Authorisations

A written request (Annexure I) was made to the Grootfontein municipality to seek permission to collect specimens for this study. Written permission (Annexure II) was granted to collect specimens from cattle slaughtered for Country Wild and Vleiskor butcheries. Verbal consent was sort for specimens collection from cattle slaughtered for private individuals.

3.1.2 Study area

The study area included farms around the five mines and two smelters close to Grootfontein in Namibia, (See Plate 1.2 in Chapter 1). This represented the rough boundaries of the area from where cattle slaughtered at Grootfontein abattoir were expected to originate.

The abattoir was chosen for this research study as it slaughtered cattle from the surrounding farms and communal areas located near to closed and operating mines (See Plate 1.2 in Chapter 1). The abattoir is the only formal source of meat for over 25,000 people residing in the study area.

3.1.3 Study population

Three-year municipal records reviewed showed a monthly average cattle slaughter frequency that ranged between 88 and 184. All cattle slaughtered at the Grootfontein abattoir during the study period numbered 125 (14 male and 111 female), were included in the study.

The number of the ear tag, gender and age of each animal slaughtered were recorded on Daily Sampling Forms (Annexure III). Ages of all cattle slaughtered were estimated using dentition (Table 3.1), according to the Merck Veterinary Manual guidelines (Kahn, Line, Amstutz, Merck & Co. & Merial (Firm) 2006). It was impossible to establish the precise ages of cattle in the study population; however, age was estimated by examining the teeth.

Table 3.1 Aging cattle using dentition

Dental description	Age estimate
Fully erupted first permanent incisor (I1)	≤ 2 years old
Fully erupted second permanent incisor (I2)	≤ 2 $^1/_2$years old
Fully erupted third permanent incisor (I3)	≤ 3 $^1/_2$years old
Levelling I1 occlusal surface	≥ 5years old

This table was created using data from Merck Veterinary Manual (Kahn *et al.* 2006)

The age difference between younger and old breeding cattle was likely to influence results significantly; hence cattle were classified by age. All the cattle sampled were mature culled breeding stock whose ages were classified as at least 5 years old.

3.1.4 Specimens collection

Liver and kidney specimens were collected as described in detail below, from all slaughtered cattle, for measurement of Cd and Pb concentrations. These organs were targeted for analysis because they were food items (part of red offal) and have higher accumulation of the metals (Forte & Bocca 2007). About 4cm^3 of specimen tissue represented at least 100 grams of tissue, sufficient for the laboratory digestion and analysis.

Wedge-shaped specimens including only the cortex (more) and medulla (less) of the kidney were collected on the abattoir line. Higher metal concentrations were expected in the proximal renal tubular cells (cortex) compared to the medullary tissues. The tough connective tissue, from the pelvis of the kidneys, which is usually discarded either during cooking preparation or eating, was excluded during specimens' collection. V-shaped specimens were also excised from the right liver lobe starting from the edge inwards. The gall bladder and renal impressions were used to identify this lobe. Major veins or connective tissue were not expected towards the edge of the liver, hence no special efforts were made to avoid and exclude them.

3.1.5 Recording, labelling and storage

Each carcass was assigned a daily slaughter number (DSN), which was its position on the abattoir line. The DSN was inscribed on a pair of plastic zip-lock bags one with 'K', one with 'L' to represent kidney and liver respectively. Each

specimen was individually deposited in a specifically labelled brand new plastic zip-lock bag, which was closed for storage.

Specimens from male and female cattle were separated to create a male and female stratum in the sampling frame. Cattle in each stratum automatically acquired a cumulative slaughter number (CSN), which was the position in the pool of all cattle of the same gender in sampling order. Each CSN was used to replace its corresponding DSN on specimen containers / units within the male and female strata. The specimens were kept frozen at -19^{o}C to -23^{o}C before being sent for laboratory analysis.

3.1.6 Test sample size

For sample size calculations, the confidence level (β) used was 95% and it was estimated, in consultation with experts in the Epidemiology Section of the Ministry of Agriculture in Namibia, that the prevalence of positive cattle would be 8%. These figures were factored into the formula below by Thrusfield (2005) to compute the desired testing sample (n):

$$n = [1 - (1 - \beta\ (1/d)] * [(N - d/2)\ + 1/2]$$

In the formula, d is the expected number of affected cattle, found by multiplying the slaughtered cattle from which specimens were collected over 30 days ($N=125$) with the estimated prevalence (8%).

Proportional systematic random sampling from both the male and female strata was used to identify cattle from where paired kidney and liver specimens were sent for laboratory analysis. The sampling interval for both male and female strata was determined by dividing the total population slaughtered (*N=125*) by the desired sample size. The test sample size (*n*) calculated was 31 cattle.

The initial cow number (CSN) in the male and female sampling frames from which a specimen was collected, was randomised in Microsoft Office Excel®[5] using the formula below:

$$t = RAND\ () * (s - 1) + 1$$

In the formula, ***s*** was the stratum size and ***t*** was the CSN of the first unit selected in each stratum. A fourth sampling interval was used to select the rest of the units from each strata.

3.1.7 Trace-back of test sample cattle

Each animal carried an official identification ear tag, and these details were used to trace back cattle to the properties where rearing took place prior to slaughter, by querying a computerised livestock identification and traceability database. Trace-back enabled the investigation and analysis of environmental or spatial linkages between the properties where livestock were reared to old or new mine locations.

5 Microsoft Corporation (2008) Microsoft Excel [computer software] Microsoft, Redmond, Washington: USA.

3.2 Laboratory preparation of specimens

All laboratory analyses for Cd and Pb in kidney and liver specimens were done at Namibia Custom Smelters Laboratory at Tsumeb. The facility analyses copper ore for Weatherly International PLC mines in Namibia. All equipment utensils and chemicals used are listed in Table 3.2.

Specimens were digested using the oxidising technique described in Analytical Methods for Absorption Spectroscopy by PerkinElmer (1996), in preparation for Cd and Pb analysis. Five grams of specimen tissue were accurately weighed using a Mettler Toledo® AB204-S/FACT Analytical Balance. The weighed tissue materials were deposited in 125mL Erlenmeyer flasks together with glass beads. To the flask, 25mL of deionised water and 10mL of a 1 to 2 mixture of 65% nitric acid and 60% perchloric acid were also added. The deionised water was made using an Elgastat® B114 Deioniser.

The flask contents were then boiled until they became a clear solution called a analyte or analyte. Each analyte was then transferred into a 100mL volumetric flask and diluted to volume, using more deionised water and thoroughly mixed before undergoing analysis.

3.3 Analytical methods

Flame Atomic Absorption Spectrometry (FAAS) was used to quantify Cd and Pb in each analyte. An AAnalyst 200 Atomic Absorption Spectrometer made by PerkinElmer® was used. Deionised water (blank), Cd metal and Pb foil were

used to calibrate equipment using 'linear calibration through zero'. This is based on the fact that quantitative measurements in atomic absorption are based on Beer's Law, which states that concentration is proportional to absorbance (C = kA). Standard Cd and Pb solutions (1 gram per litre) were made by dissolving a gram of Cd metal and Pb foil in the least possible volume of nitric acid. This was further diluted to a litre with deionised water. The standard solutions were used as an analyte to generate the standard linear curve and an equation with a zero intercept (PerkinElmer 1996):

$$C = k0(-k1 * A)$$

In the formula, *C* was the metal concentration and *k0* was the reslope coefficient (concentration of the standard = 1.0) while k1 was a coefficient computed using the least squares method and *A* is the absorbance. Once calibrated, analytes were substituted for the standard solutions and the metal concentrations computed using the formula.

Quality control against matrix interferences was performed using the method of standard additions (PerkinElmer 1996). Matrix interferences occur when the viscosity, burning characteristics or surface tension of the sample analyte and standard differ resulting in either suppressed or enhanced the signal from the analyte. An aliquot made from homogenising all sample analytes was added to standard solutions used to calibrate equipment to introduce matrix interference during calibration (PerkinElmer 1996).

Table 3.2 Details of reagents and equipment used by the laboratory

Equipment / Reagent	Supplier	Address
60% perchloric acid	Merck KGaA	Germany
65% nitric acid	Merck KGaA	Germany
95% ethyl alcohol	SAARCHEM	South Africa
AAnalyst 200 Atomic Absorption System	PerkinElmer®	USA
AB204-S/FACT Analytical Balance	Mettler Toledo®	USA
Elgastat® USF B114 Deioniser	Veolia Water Solutions & Technologies	France
Cd metal	Merck KGaA	Germany
Electric hotplate	Defy®	South Africa
Glassware	Duran™ and Pyrex™	Germany
Pb foil	Merck KGaA	Germany

The detection limits (DL) or limits of detection (LOD) for Cd and Pb were 0.2mg kg^{-1} and 1.1mg kg^{-1} respectively. The DL was the smallest amount or quantity of Cd and Pb that could be detected and computed into a concentration using the FAAS calibration formula with reasonable certainty (Thomsen, Schatzlein & Mercuro 2003). FAAS is cheap, simple and reliable, hence it is commonly used to detect and measure metals in solid and aqueous samples (Matusiewicz & Krawczyk 2006). The working principle of FAAS is based on the fact that ground-state metals absorb light of specific wavelengths (PerkinElmer 1996).

During specimen analysis, metal ions in a solution were atomised using a flame before a known amount of light of specific wavelength for Cd and for Pb was supplied to the specimen chamber. If present, the atoms absorb amounts of wavelength specific light proportional to the number present. The difference in

intensity between the light at specimen chamber entry and exit is computed to give the concentration of the metals in each specimen (PerkinElmer 1996).

3.4 Results analyses

Kaplan-Meier[6] was applied using Microsoft Office Excel® to calculate the mean and standard deviation (SD) for the study population accounting for censored results (discarded and below DL). The population mean renal Cd concentration, SD and PTMI threshold was LOG transformed (=LOG ([Cd]) using Microsoft Office Excel® for hypotheses test. On-line one-sided Student ***t***-test[7] was used for hypothesis testing using 95% confidence (type 1 error probability of 0.05).

Renal Cd concentrations were sorted by gender of source animals to evaluate its effects. Each animal was also assigned a zone stating the approximate distance from the nearest mining activities to assess the effects of proximity. The results from 12 cattle from a feedlot were identified for comparison to the rest of the study population to evaluate the effects of husbandry practices.

The mean renal Cd concentration and SD for each subpopulation identified was calculated using the Kaplan-Meier method. Female cattle were expected to have renal Cd concentration higher than male cattle. Cattle pastured nearer mines were also expected to have higher exposure doses and consequently higher organ concentrations. Cattle reared in feedlots are generally fed fodder

6 http://www.practicalstats.com/nada/

7 http://in-silico.net/statistics/ttest/one-sample

from other areas compared to pastured cattle. The difference between the two husbandry practices was also evaluated.

LOG transformation (=LOG ([Cd]) using Microsoft Office Excel® was done to all mean renal Cd concentrations and SDs for each subpopulation. After LOG transformation, they were tested using on-line one-tailed ***t***-test for two unequal samples with unequal variance[8] at 95% confidence. The calculated ***t***-values were compared to the reference ***T***-table values. A calculated ***t***-value larger than the reference value signified statistical significance.

3.5 Risk estimation

The concentrations of Cd found in kidneys justified public exposure risk estimation. Kidneys are sold either homogenised in pies or without subdivision to individuals. Homogenisation of kidneys from many cattle dilutes the overall Cd exposure concentration. Individuals buying and consuming entire kidneys with high Cd concentrations are exposed to undiluted doses.

The risk of bovine kidneys with more than 1.5mg Cd per kilogram of meat being released into the food chain was estimated using a mathematical model. An Event Pathway (Figure 3.1) leading to release of kidney meat with over 1.5mg kg^{-1} Cd was developed. The initiating event was the slaughter of cattle at the abattoir (L_1). The probability that FAAS would detect Cd in the organs was designated P_1. The probability that FAAS detects Cd above DL was P_2, while

8 http://in-silico.net/statistics/ttest/two-sample

P_3 was the probability that FAAS detects Cd at organ levels exceeding 1.5mg kg^{-1}. All the probabilities came from the results. ***P_1, P_2***, and ***P_3*** must all occur one after another for it to be known that kidney meat carrying 1.5mg kg^{-1} or more Cd enters the food chain. The probabilities were therefore multiplied to establish risk (***R***).

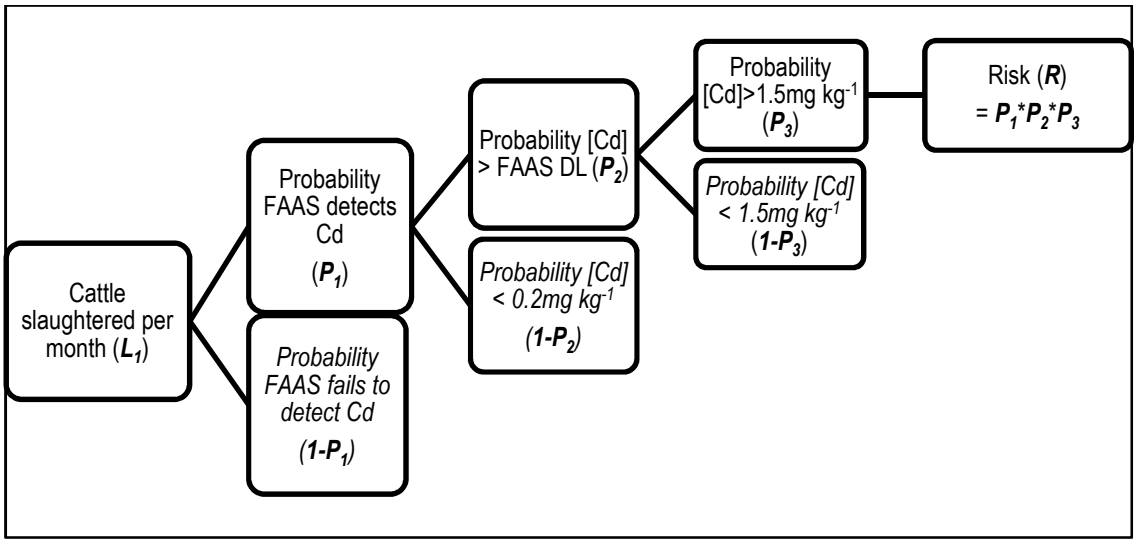

Figure 3.1 Event Pathway for renal Cd risk calculation: This pathway followed the events culminating in the detection of Cd exceeding 1.5mg kg^{-1} using FAAS. The risk (***R***) was an intersection of ***P_1, P_2***, and ***P_3***, hence they were multiplied.

The calculated risk was converted to expected number of cattle affected (***ENCA***) by multiplying it with the expected slaughter population (***ESP***) of cattle monthly. The ***ESP*** was established from municipal records over a three year period. The records showed that the number of cattle slaughtered monthly ranged from 88 to 184 (mean = 136±68) cattle. The ***ENCA*** was multiplied with the expected weight of two bovine kidneys to convert it to the weight of kidney

meat with more than 15mg kg^{-1} Cd entering the food chain monthly. The average weight of two mature cattle kidneys is about 1.6kg (Jones 1981).

3.6 Methodology for risk mitigation

The epidemiological triad is a well-known way to examine causality through interaction between determinants linked to host, agent and environment (Center for Disease Control (US) 1992, Thrusfield 2005). A similar approach, whose application is depicted in Figure 3.2, can be used to manage risks at each level of these interactions for chemical environmental food contaminants.

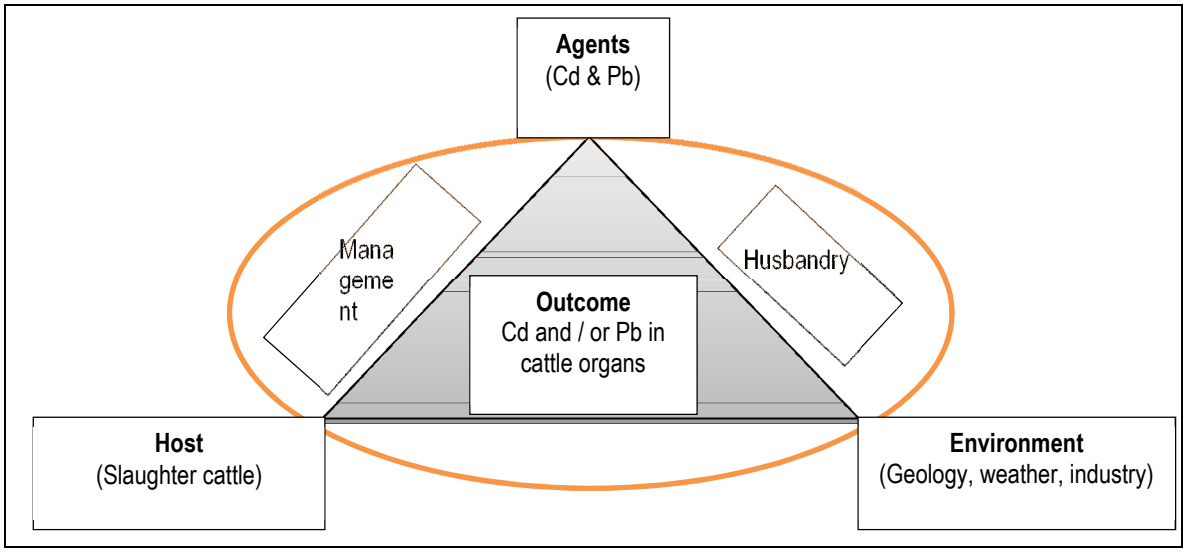

Figure 3.2 Epidemiological triad: Depiction of the relationship between the agents, host and environment used to understand and apply interventions that could reduce heavy metal concentrations in cattle organs in the study area.

The host, agent and environmental variables affecting entry and accumulation of Cd and Pb in cattle livers and kidneys were qualitatively analysed to develop mitigation options available at farm level in the study area to minimise Cd and Pb food chain entry. Cd and Pb bioavailability and biochemical determinants in

relation to the cattle age, gender and physiology were discussed in Chapter 2. The environmental determinants (land use, rain / water, streams, feeding, air / wind, mining and production systems) were investigated to assess mitigation options available at farm level.

CHAPTER 4

RESULTS

4.1 Introduction

The first section of this chapter is a tabulated list showing gender, age and trace back (farm of origin GPS co-ordinates and designated identification) of cattle in the study population. The GPS locations of all farms were plotted.

Thereafter the results of analysis for Cd and then Pb in kidneys and livers are presented graphically and tabulated. The population and subpopulation means and SDs with hypothesis testing results are then presented.

Lastly, this chapter presents the results of the estimation of risk and weight of kidney meat carrying Cd at concentrations exceeding 1.5mg kg^{-1} entering the food chain. The mathematical model used to estimate risk and expected weight of kidney meat is also presented in this section.

4.2 Population structure

The study population of thirty-one cattle consisted of four (12.9%) male and twenty-seven (87.1%) female cattle (Table 4.1). All the male cattle were entire (not castrated). They had a full complement of permanent incisors in varying states of wearing and neck exposure and this was used to estimate age. It was determined that the minimum age of cattle slaughtered was five years.

Table 4.1 Study population gender, age and trace back (GPS co-ordinates)

Case	Gender	Origin*	GPS Co-ordinates (*) South	East	Age
1	F	A	-19.11	17.48	>5yrs
2	F	A	-19.11	17.48	>5yrs
3	F	B	-19.18	16.48	>5yrs
4	F	C	-19.33	17.40	>5yrs
5	M	D	-19.18	18.11	>5yrs
6	F	E	-19.42	17.33	>5yrs
7	F	E	-19.42	17.33	>5yrs
8	F	F	-19.48	17.11	>5yrs
9	F	G	-19.28	18.11	>5yrs
10	F	G	-19.28	18.11	>5yrs
11	M	H	-19.32	18.07	>5yrs
12	F	H	-19.32	18.07	>5yrs
13	F	H	-19.32	18.07	>5yrs
14	F	H	-19.32	18.07	>5yrs
15	F	H	-19.32	18.07	>5yrs
16	F	H	-19.32	18.07	>5yrs
17	M	H	-19.32	18.07	>5yrs
18	F	H	-19.32	18.07	>5yrs
19	F	H	-19.32	18.07	>5yrs
20	F	H	-19.32	18.07	>5yrs
21	F	H	-19.32	18.07	>5yrs
22	F	H	-19.32	18.07	>5yrs
23	F	I	-19.48	18.18	>5yrs
24	F	I	-19.48	18.18	>5yrs
25	F	J	-20.15	17.11	>5yrs
26	F	K	-20.22	16.48	>5yrs
27	M	L	-19.03	19.26	>5yrs
28	F	M	-18.56	17.41	>5yrs
29	F	N	-19.44	18.19	>5yrs
30	F	N	-19.44	18.19	>5yrs
31	F	N	-19.44	18.19	>5yrs

Key: Origin*: The origins of each animal was designated an alphabetical letter and GPS co-ordinates (*) without minutes were recorded to protect their true identities.

This population structure was consistent with the practice in the study area where culled mature breeding cattle are slaughtered for local use, while weanlings are exported live to South Africa or slaughtered for export to Europe.

4.3 Trace back to farms of origin

The cattle were traced back to fourteen farms identified 'A' to 'N' and the number from each farm recorded (Table 4.1). Each farm contributed one to twelve cattle (mean=2 ±3; mode=1). The ovoid study area of commercial and communal areas was roughly 120km long by 80km wide (Plate 4.1).

4.4 Cadmium results

In all liver specimens, Cd was below DL (0.2mg kg^{-1}) while in kidney specimens it was measurable (Table 4.2). Twelve cattle (38.7%), comprising two males (16.7%) and ten females (83.3%), had Cd levels below the DL. Another twelve cattle (38.7%) of which one (8.3%) was male and eleven female (91.7%) had levels between 0.29 and 0.94mg kg^{-1}. Four cattle (12.9%) had renal Cd between 1.10 and 1.22mg kg^{-1}, while the other three cattle (9.7%), having renal Cd concentrations between 2.6 and 3.64mg kg^{-1}.

The distribution of renal Cd concentrations observed (Figure 4.1) was skewed to the left. More cattle kidneys had lower renal Cd concentrations than those with higher. The linear regression coefficient (r^2 = 0.87) for the log transformed renal Cd concentrations was very strong (Figure 4.2).

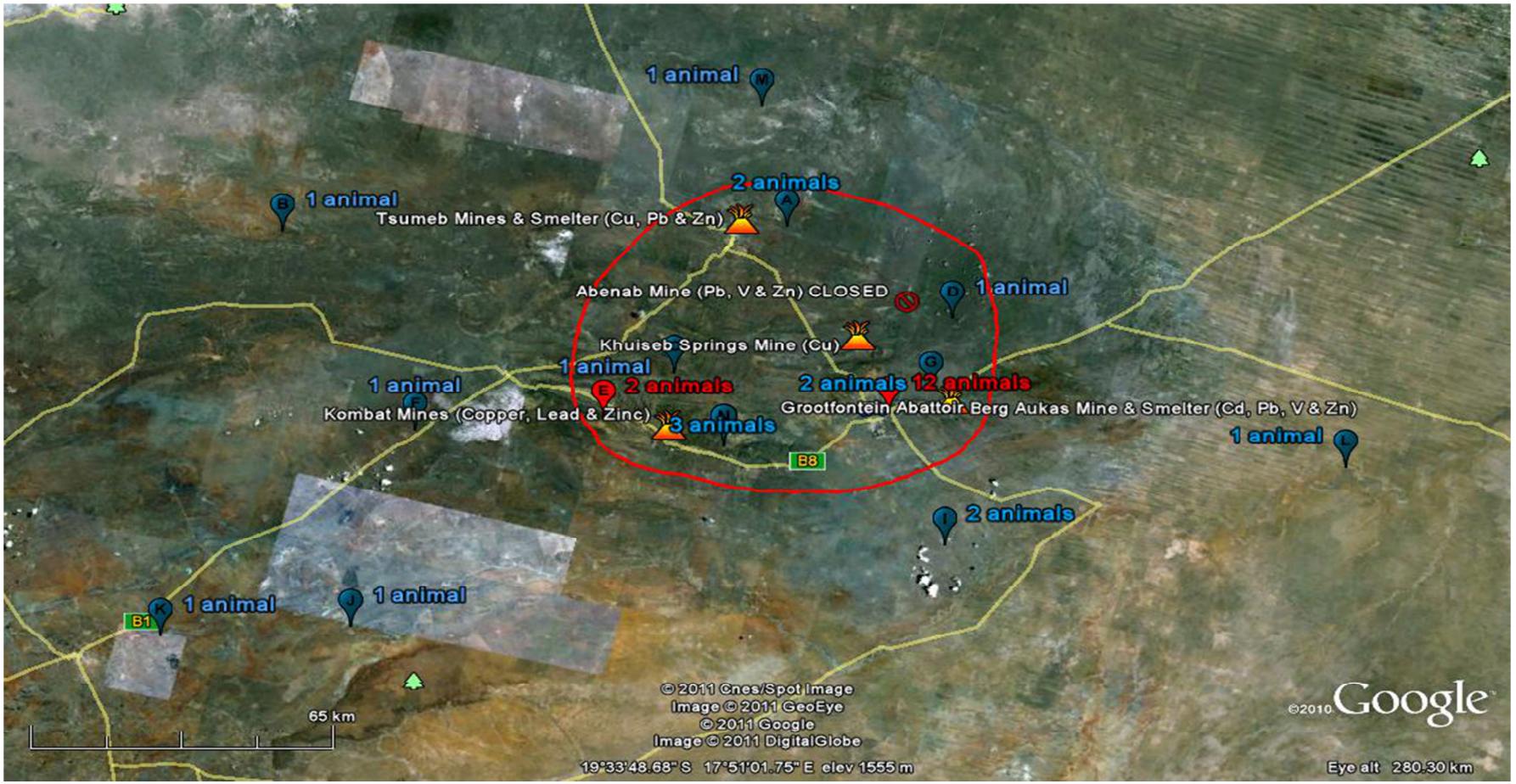

Plate 4.1 Study population spatial distribution map: Farms of origin (red and blue bubbles) were lettered A to N, while mines were pink triangles. The red circle roughly demarcates the farming area within a 20km radius of mining facilities[9].

[9] Google (Firm) (2005). *Google Earth*™ [Mountain View, CA], Google. http://earth.google.com/.

Table 4.2 Cd results (mg kg^{-1} wet weight) in kidneys and livers

Case	Gender	Kidney [Cd]	Liver [Cd]	Distance to nearest mine
1	F	<DL	<DL	0 – 10 km
2	F	0.493	<DL	0 – 10 km
3	F	<DL	<DL	>71 km
4	F	0.718	<DL	0 – 10 km
5	M	<DL	<DL	0 – 10 km
6	F	0.936	<DL	11 – 20 km
7	F	1.221	<DL	11 – 20 km
8	F	<DL	<DL	41 – 50 km
9	F	0.568	<DL	0 – 10 km
10	F	0.824	<DL	0 – 10 km
11	M	Discarded	<DL	11 – 20 km
12	F	<DL	<DL	11 – 20 km
13	F	0.288	<DL	11 – 20 km
14	F	0.569	<DL	11 – 20 km
15	F	0.610	<DL	11 – 20 km
16	F	0.760	<DL	11 – 20 km
17	M	1.096	<DL	11 – 20 km
18	F	1.119	<DL	11 – 20 km
19	F	1.133	<DL	11 – 20 km
20	F	2.600	<DL	11 – 20 km
21	F	3.444	<DL	11 – 20 km
22	F	3.641	<DL	11 – 20 km
23	F	<DL	<DL	21 – 30 km
24	F	0.549	<DL	21 – 30 km
25	F	<DL	<DL	>71 km
26	F	0.719	<DL	>71 km
27	M	0.753	<DL	61 – 70 km
28	F	<DL	<DL	31 – 40 km
29	F	<DL	<DL	0 – 10 km
30	F	<DL	<DL	0 – 10 km
31	F	<DL	<DL	0 – 10 km

Key: <DL: Below the FAAS detection limit of 0.2mg kg^{-1}
Discarded: Not analysed because sample was spilt and remainder was not enough to analyse

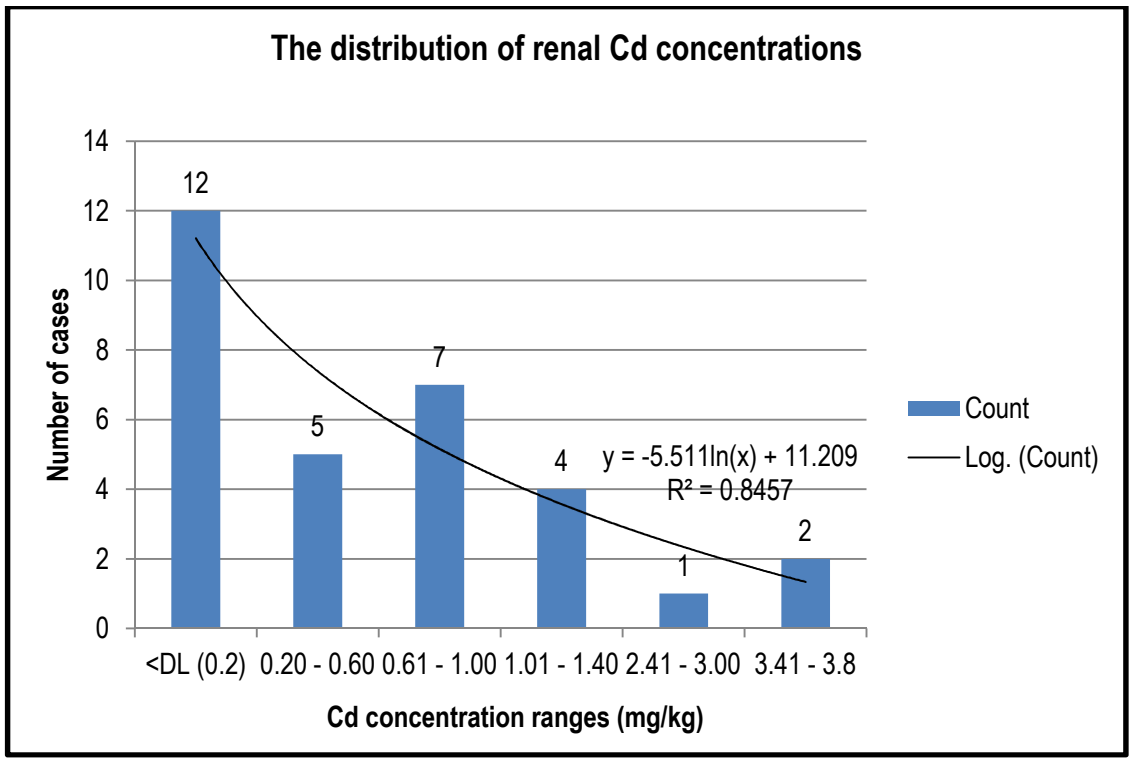

Figure 4.1 Histogram of grouped renal [Cd] against number of cases. The distribution is skewed to the left with a left tail. Most cattle had low renal Cd concentrations with very few cases on the extreme right.

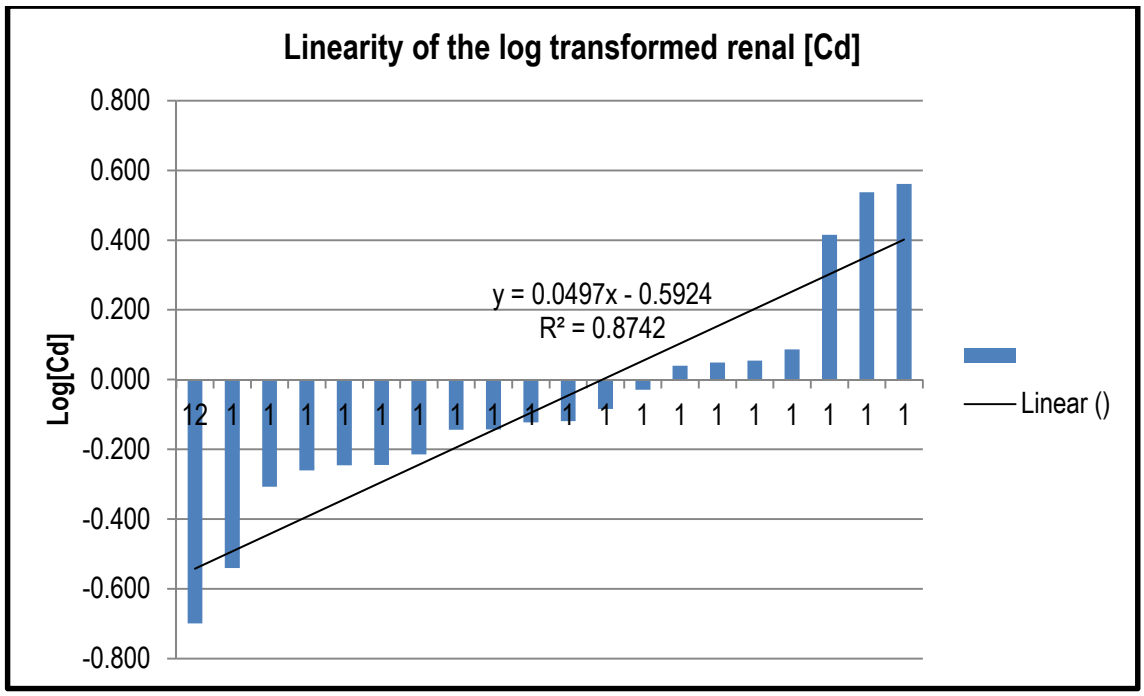

Figure 4.2 Linear regression graph of log transformed renal [Cd] showing positive correlation.

4.5 Lead results

Table 4.3 Pb results (mg kg^{-1} wet weight) in kidneys and livers

Case	Gender	Kidney [Pb]	Liver [Pb]	Distance to nearest mine
1	F	<DL	ND	0 – 10 km
2	F	<DL	ND	0 – 10 km
3	F	<DL	ND	>71 km
4	F	<DL	ND	0 – 10 km
5	M	<DL	ND	0 – 10 km
6	F	<DL	ND	11 – 20 km
7	F	<DL	ND	11 – 20 km
8	F	<DL	ND	41 – 50 km
9	F	<DL	ND	0 – 10 km
10	F	<DL	ND	0 – 10 km
11	M	Discarded	ND	11 – 20 km
12	F	<DL	ND	11 – 20 km
13	F	<DL	ND	11 – 20 km
14	F	<DL	ND	11 – 20 km
15	F	<DL	ND	11 – 20 km
16	F	<DL	ND	11 – 20 km
17	M	<DL	ND	11 – 20 km
18	F	<DL	ND	11 – 20 km
19	F	<DL	ND	11 – 20 km
20	F	<DL	ND	11 – 20 km
21	F	<DL	ND	11 – 20 km
22	F	<DL	ND	11 – 20 km
23	F	<DL	ND	21 – 30 km
24	F	<DL	ND	21 – 30 km
25	F	<DL	ND	>71 km
26	F	<DL	ND	>71 km
27	M	<DL	ND	61 – 70 km
28	F	<DL	ND	31 – 40 km
29	F	<DL	ND	0 – 10 km
30	F	<DL	ND	0 – 10 km
31	F	<DL	ND	0 – 10 km

Key: <DL: Below the FAAS detection limit of 1.1mg kg^{-1}; ND: Not detected;
Discarded: Not analysed because sample was spilt and remainder was not enough to analyse.

The results presented in Table 4.3 show that Pb was detected at levels below the DL (1.1mg kg^{-1}) in all kidneys. It was not detected in all liver specimens. No statistical analyses were done as there were quantitative outcomes for Pb in neither livers nor kidneys.

4.6 Renal Cd results statistics

4.6.1 Renal Cd averages

The mean renal Cd concentrations for the study population, as well as female and male subpopulations are summarised in Table 4.4. One-sided ***t***-test ($t_{0.05\ (1),\ 30\ =\ 1.697}$) on the log transformed population mean of 0.71±0.96mg kg^{-1} against the hypothetical mean of 1.5 yielded a ***t***-value of 3.757. The population mean renal Cd concentration was therefore statistically lower than the CAC threshold PTMI threshold.

Table 4.4 Population and gender-based renal Cd results summary (mg kg^{-1} wet weight)

Gender	*n*	Range	Mean [Cd] (Log [Cd])	SD (Log SD)
Female	27	[<DL; 3.641]	0.75 (0.375)	±1.00 (0.5)
Male	4	[<DL; 1.096]	0.46 (0.163)	±0.68 (0.333)
Overall	**31**	**[<DL; 3.641]**	**0.71 (0.352)**	**±0.96 (0.481)**

Key: ***n*** = group size; Log[Cd] = 1.5+log[Cd] to ensure log transformation was a positive value

Since female cattle were expected to have higher renal Cd concentrations than males, the log transformed gender-based means were subjected to a one-sided ***t***-test for two samples with unequal variance ($t_{0.05\ (1),\ 29\ =\ 1.699}$). The calculated ***t***-

value was 0.816. This meant that although the female cattle mean renal Cd concentration seemed higher than males', the two were statistically equal.

4.6.2 Renal Cd averages at farms of origin

From GPS mapping, ten-kilometre (imaginary) concentric zones were created around mining centres, with the furthest zone more than 71km away. Proximity to mines was expected to be directly organ Cd concentrations. Through trace-back all cattle were allocated a matching approximate distance from mines (Table 4.2, Table 4.3 and Figure 4.3).

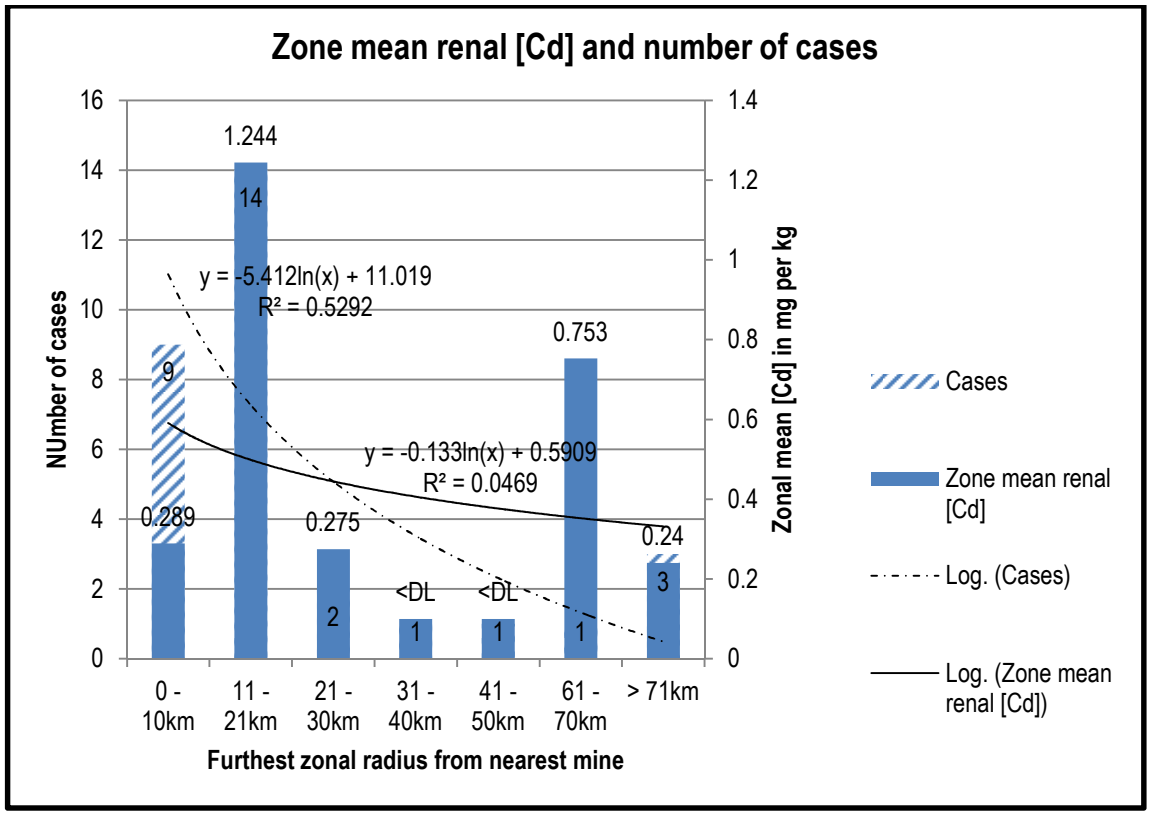

Figure 4.3 Renal [Cd] by zoned distance from nearest mines: The majority of the cattle came from within 30km of mines. The apparent highest mean renal Cd concentration was from cattle reared in the zone 11 to 20km from the nearest mine. A feedlot which fattens old cattle was the main source of cattle in the 11 to 20km zone.

The numbers of cattle originating from each zone are shown in Figure 4.3. The majority of the cattle were from farms located within 30km of mining activities. Within zones, the mean renal Cd concentrations were: 0.289mg kg^{-1} (0-10km); 1.244mg kg^{-1} (11-20km); 0.275mg kg^{-1} (21-30km); <DL (31-40km); <DL (41-50km); 0.753mg kg^{-1} (61-70km) and 0.24mg kg^{-1} (>71km). A feedlot was located in the 11 – 20km zone, with the apparent highest mean renal Cd concentration. The feedlot, which fattens old cattle, was the main source of cattle (85.7%) in this zone. Exposure durations for feedlot cattle were however expected to be relatively short after arrival from farms further away from mines.

The feedlot was located in a valley downstream from a mine while all other source farms were normal extensive cattle ranches. The log transformed mean renal Cd concentration for cattle originating from the feedlot (1.27±1.28mg kg^{-1}) was computed and compared to that of the rest of the cattle (0.36±0.43mg kg^{-1}) using a one-sided ***t***-test for two samples with unequal variance ($t_{0.05\ (1),\ 29} = 1.699$). The calculated ***t***-value of 1.038 was smaller than the reference ***t***-value. Though the mean for the feedlot appeared higher than the rest of the cattle sources, the difference was statistically insignificant.

4.7 Estimation of renal Cd risk

The monthly Cd exposure threshold for a 60kg-man was established as 1.5mg in Section 2.1.2. Considering that some cattle kidneys in this research were found with Cd concentrations exceeding 1.5mg kg^{-1}, it was necessary to perform public health exposure risk estimate (***RE***). An assumption that a 60kg-

man may eat a kilogram or more kidney meat from a bovine with renal Cd concentration above 1.5mg kg^{-1} formed the basis of the risk estimation. The renal Cd findings of this research provided affirmative responses to questions in the Decision Tree (Figure 4.4).

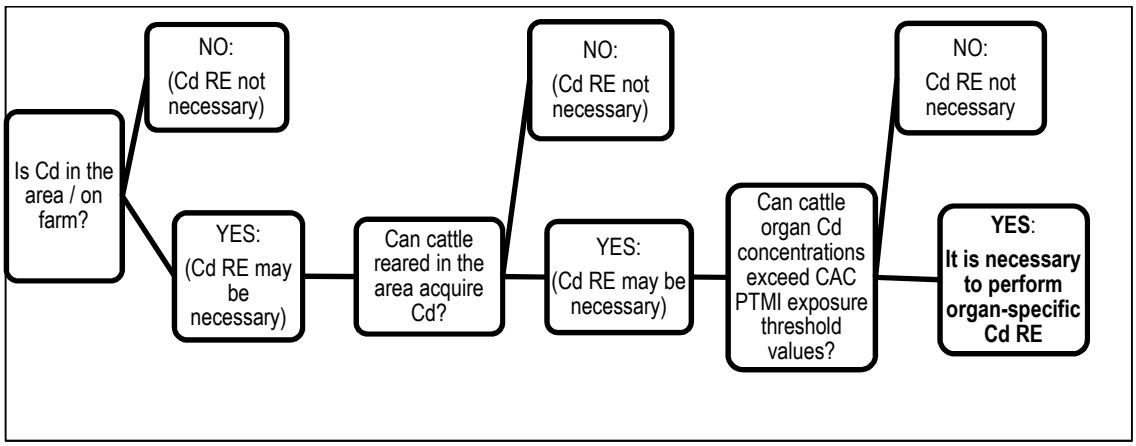

Figure 4.4 Cd food chain entry decision tree: Used to justify Cd risk estimation

The renal Cd findings from Section 4.4 and Table 4.2 were used to establish numerical values of P_1, P_2 and P_3 for the Event Pathway in Section 3.5 and populate Figure 4.5. The probability that FAAS detects Cd in a kidney analyte (P_1) was considered 100%. Cd was detected in all specimens except the one discarded. The probability that the detected renal Cd concentration was above the DL (P_2) was 61.3%. This was established by dividing the number specimens with quantified renal Cd concentrations (19) by the total specimens analysed (31). Lastly the probability that a specimen would have renal Cd concentration above 1.5mg kg^{-1} (P_3) was 9.7% (divide number of specimens with Cd concentration above 1.5mg kg^{-1} (3) by the total number of specimens tested (31). The resultant risk (100%*61.3%*9.7%) was 5.95% per month.

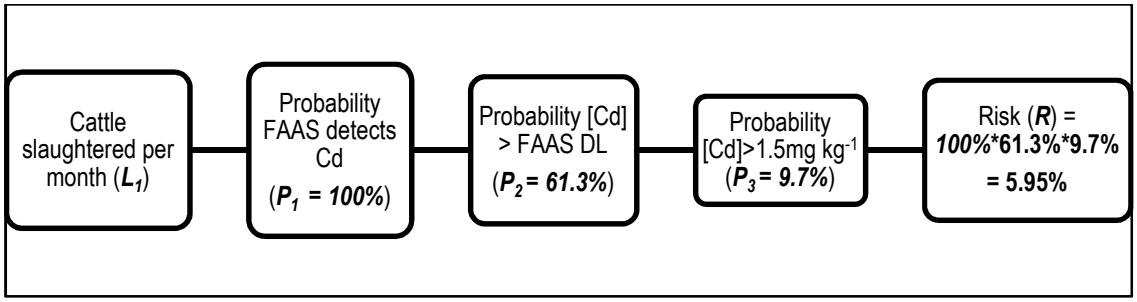

Figure 4.5 The Cd risk Event Pathway with probabilities of interest: Calculated risk (***R***) was 5.95%

A mathematical model (Table 4.5) was used to convert risk (***R***) to weight of kidney meat yielded per month. The estimated risk (***R*** = 5.95%) was convert as explained in Section 3.5 to the expected number of cattle affected (***ENCA***).

Table 4.5 Renal Cd risk estimation mathematical model

Unit label	Label	Value	Source
Risk	(R)	5.95%	Event Pathway
Expected slaughter population	(ESP)	88 – 184 (mean: 136±68)	Municipal records
Expected number of cattle affected	(ENCA)	5 – 11 (mean: 8±4)	R*ESP
Expected kidney weights	(EKW)	1.6kg	(Jones 1981)
Estimated kidney meat yield per month		**8 – 18 (mean: 13±6)**	**ENCA*EKW**

The ***ENCA*** were between 5 and 11 (mean = 8±4) monthly. The weight of meat with Cd concentration exceeding 1.5mg kg^{-1} was established by multiplying the monthly ***ENCA*** with the expected weight of two kidneys from one animal (1.6kg). Thus, 8 to 18 (mean = 13±6) kg of bovine kidney meat with Cd concentration over 1.5mg kg^{-1} were estimated to enter the food chain from Grootfontein abattoir monthly.

CHAPTER 5

DISCUSSION

5.1 Introduction

This study confirmed the presence of Cd and Pb in livers and kidneys of cattle slaughtered at Grootfontein abattoir in Namibia. As was seen in Chapter 4, only the levels of Cd in kidneys gave rise to concern. Renal Cd risk estimation was therefore performed using an organ concentration of 1.5mg kg^{-1} as a critical value for a 60kg-man on the assumption that such a man consumes a kilogram of kidney meat monthly.

5.2 Study population structure

The local municipal abattoir was the only source of cattle liver and kidney specimens used in this research. The population size and gender composition of the study population matched historical slaughter data trends for the abattoir.

The presence of exclusively mature cattle (more than five years old) in the study population suggests they were culled reproductive cattle. The production systems in the study area primarily aim to produce weaners between 8 and 15 months of age, which are exported live, for feedlot purposes, to South Africa, or oxen (±30 months old) which are slaughtered for European Union (EU) beef exports (Mendelsohn 2006). Old (culled) breeding cows and bulls are sold to local butchers and slaughtered at Grootfontein abattoir for local consumption.

Between 10 and 15% of the less productive breeding cattle population is culled annually in Namibia (Mendelsohn 2006).

Increasing age is a positive predictor for renal Cd and Pb concentrations in cattle (Nriagu, Boughanen, Linder, Howe, Grant, Rattray, Vutchkov & Lalor 2009; Prankel, Nixon & Phillips 2004). Also previous work on renal concentrations in cattle concluded that there is positive correlation between age and the concentrations of Cd and Pb in organs (Alonso *et al.* 2000; Canty, Lane & More 2009).

The study population could be regarded as being biased towards older cattle (OIE 2010), but it correctly represents the population slaughtered for beef consumption in the study area. It could not however, be directly assessed whether cattle age had an influence on the results of this study as no young cattle were sampled. However positive correlation between the age and renal Cd levels could be indirectly inferred as routine monitoring on young cattle (±30 months) slaughtered for export in Namibia has never shown the metal at concentrations that violated EU standards.

5.3 Laboratory resources and competencies

The locally available laboratory facility capable of analysing and measuring heavy metals in biological products used FAAS as an analytical method. Although the method confirmed the presence of Cd and Pb in the bovine organs sampled, it was not sensitive enough to accurately measure Pb

because the DL was too high. The analytical method did however, measured Cd, which allowed comparison of the residue levels found to CAC standards. Given the challenges regarding availability of laboratory and technical capacity to test food chemicals in the study area, FAAS can be a useful screening test (Denny 1987). Specimens where heavy metals are detected can undergo secondary testing using more costly and sensitive equipment and techniques.

5.4 Cadmium in kidneys and livers

The presence of Cd at concentrations below the DL in all cattle livers and with over 60% of the kidneys above the DL was not unexpected. Bioaccumulation is higher in the kidneys because of recycling of the Cd metallothionein in kidneys unlike in the liver (Liu *et al*. 2008). While there was found to be transfer of Cd from the environment and accumulation in the kidneys sampled, the mean organ concentration was found to be lower than the CAC PTMI threshold value of 1.5mg. People who eat homogenised kidney meat were unlikely to get Cd dietary exposure doses that violated CAC PTMI. The average renal Cd concentrations in this study (0.71±0.96mg kg^{-1}) were higher than those found by Jukna *et al.* (2006) in Lithuania (0.17±0.01mg kg^{-1}) but lower than those by Nriagu *et al.* (2009) in Jamaica (7.92µg g^{-1}).

The higher renal Cd concentrations in female compared to male cattle found in this study were also observed by previous authors (Jukna *et. al.* 2006). This could be physiologically explained. Higher physiological demand for minerals for reproduction in females is met through up-regulated assimilation, especially

during pregnancy and lactation (Skerfving & Bergdahl 2007). The end result is more efficient mineral absorption, including Cd, leading to a net result of higher Cd bioaccumulation (Nordberg *et al.* 2007; Liu *et al*. 2008).

From eating a kilogram of kidney tissue sourced from this cattle population per month, a person who weighs 60kg has a 9.7% chance of dietary Cd above the CAC PTMI 0.025mg kg^{-1}. On the other hand, eating a kilogram of homogenised kidney tissue in a month contributes below 50% of the 60kg man PTMI.

The multiple sources of exposure (soil, water, dust, meat, vegetable etc.) and exposure routes (oral, dermal and respiratory) imply that the prevalence of cattle with Cd in kidneys at concentrations potentially harmful to public health in the area may actually be higher than 9.7%.

The presence of high levels of Cd in soil, vegetation and underground water in the study area has been confirmed (Mapani, Ellmies, Kamona, Kříbek, Majer, Knésl, Pašava, Mufenda & Mbingeneeko 2010). However, human exposure assessment in the study area is a prerequisite for deciding the appropriate level of protection (maximum level) for Cd in bovine kidneys for Namibia.

5.5 Lead in kidneys and livers

The detection of Pb in the kidneys of all cattle in this study implies its presence in the food chain. The magnitude of the human exposure risk relative to CAC recommended limits, from eating kidneys could not be assessed as the FAAS

technique DL were insufficient to measure concentrations of Pb in tissue samples.

Although Pb was not detected in the livers of cattle sampled in this study, the high FAAS DL hinders making inferences on the human exposure risk from eating the livers given the PTWI of 0.025mg kg^{-1} (WHO 2011) is low. Results from a more sensitive analytical method are required to decide whether offal concentrations of Pb in the study area pose a public health concern or not, and guide the decision whether or not risk mitigation is needed.

5.6 Estimated renal Cd risk

Using the sensitivity of the available analytical laboratory and Cd population parameters established through this research, the risk of having bovine kidneys with concentrations exceeding 1.5mg kg^{-1} was 5.95%. This risk was equivalent to 13 ±6kg of kidney meat with Cd levels above CAC standards entering the food chain from Grootfontein abattoir.

Some families and individuals in the study area routinely eat bovine kidneys for breakfast and enjoy kidney pies (Norval - Personal communication 2006). As long as homogenised kidney meat from several different animals is consumed, it is unlikely for consumers to violate the CAC PTMI. However, a single individual who buys and consumes a full cattle kidney alone has a risk of Cd exposure at doses that violate the CAC PTMI.

When looking at the overall Cd exposure, other additional Cd exposure risk, sources in the study area, like water, dust and vegetables (Mapani *et. al.* 2010), should be considered. Arid conditions and long dry seasons experienced in Namibia (Mendelsohn 2006) could promote Cd exposure from dust entering the lungs. These possibilities for extra and probably concurrent Cd exposures may significantly escalate Cd dietary exposure rates for consumers through an additive effect from several concomitant sources. .

5.7 Namibian renal Cd appropriate level of protection

There are no Cd standards for beef and bovine offal in Namibia. However, the appropriate Cd renal maximum limit in bovine kidneys can be established after exposure assessment that evaluates all possible human exposure sources and routes (WHO/FAO 2006a). Consumption data (WHO/FAO 2006b) on national, regional and even ethnic exposure sources could be used to determine the likely quantities of Cd consumed by individuals.

Multiplying the Cd concentration in each exposure source by the quantity of each source consumed monthly, the monthly source-specific Cd weight is determined. The source-specific Cd weights are added to obtain the expected total Cd weight an individual acquires from all exposure sources per month.

5.8 Renal Cd risk mitigation options

Cd in cattle kidneys at concentrations of public health concern justifies exploring risk management options and communication strategies to assist

bringing the metal levels in the food chain in the area to an acceptable level. The risk of Cd in kidneys of cattle in the study area originates from interactions of determinants in the epidemiological triad linked to the agent (Cd), host (cattle) and the environment (farm location, husbandry, water and feeding). Intervention aimed at attributes which interact with the cattle, Cd and environmental to minimise Cd in the food chain from livestock grazing systems offer risk mitigation options (Merry 1988; OIE 2006).

While this study confirmed the presence of public health hazards Cd and Pb in the food chain, clarification of exposure circumstances in this study area still requires further research (Bilandžić, Sedak, Vratarić, Perić & Šimić 2009). However, even without exact knowledge about the exposure circumstances, the OIE (2006) gives several points in livestock production systems at which control can be instituted to minimise the entry of chemical hazards that compromise food safety. The generic environmental contaminants minimisation strategies recommended by the OIE can be adapted, modified accordingly and applied to minimise entry and bioaccumulation of Cd in kidneys and livers of cattle reared in the study area.

5.8.1 Appropriate land use

It is critical to know the heavy metal contamination levels of soil, plants and water within the vicinity of mines, dumps and smelters before using the land for agriculture (John & Leventhal 1995). The presence and bioavailability of Cd in

the cattle rearing habitat of the Grootfontein area of Namibia was confirmed through detection and measurement in organs of cattle in this study.

Trace-back and mapping showed that cattle reared closer to current and historical mining sites (within 20km) accumulated higher Cd concentrations in kidneys compared to those further (more than 20km) away. Vegetation, soil, dust, and underground water of the study area are all possible Cd exposure sources for cattle via the oral or respiratory routes (Canty *et. al.* 2009).

The decision to venture into cattle ranching at farms close to mining ventures should be cognisant of the potential of heavy metals entering the food chain. Cattle ranching at farms in close proximity to mining activities should thus entail profiling soil, water, air and vegetation for heavy metals and chemicals that influence Cd bioavailability (Merry 1988).

5.8.2 Pasture and grazing

The arid climatic conditions of Namibia favour extensive beef cattle ranching under bush encroached and overgrazed conditions (Mendelsohn 2006). Geophagia associated with overgrazing can expose cattle to Cd (Merry 1988). Livestock can consume up to 44% of their seasonal dry matter intake in soil (Smith, Abrahams, Dagleish & Steigmajer 2009). Pasture management at cattle ranches where the soil Cd concentration is high must minimise overgrazing and thus geophagia. Overgrazing can be avoided by bush clearing to improve grass cover combined with correct stocking densities and rotation intervals.

The natural forage and fodder which nourishes cattle can be a Cd exposure source (Jukna *et. al.* 2006; Mendelsohn 2006). It is thus vital to know the Cd content of various plants eaten by cattle so that mitigation to minimise exposure or assimilation can be implemented.

5.8.3 Supplementary feeding

Salt blocks and rock phosphate-based supplementary licks are extensively used in Namibia because it is phosphate deficient (Sweet & Burke 2006). Salt blocks put on the ground leach salt and eventually encourage cattle to ingest Cd contaminated soil (Abrahams 2011). Placing rock salt blocks on non-absorbent pedestals protected from rain, would reduce geophagia on areas the blocks are placed.

Rock phosphate which is used in the manufacture of supplementary phosphate lick for livestock may also be Cd-contaminated (Merry 1988) thus exposure risk can be reduced by exclusively using registered products which are routinely analysed for metal content. Farmers can also provide cattle with zinc, copper, selenium, calcium and iron as strategic dietary supplements. These bivalent mineral supplements, in addition to their physiological value, also have capacity to potentially lower the dietary Cd uptake through competitive absorption (Berger & Cunha 1993).

Cattle that scavenge supplementary grain or feed spilling from feeding troughs may inadvertently ingest Cd contaminated soil around feeding areas (Merry 1988). Supplementary stock feeds and licks should therefore be fed from under-filled troughs with sufficient feeding space to minimise feed spillage. Additionally, feeding troughs may also be set on concrete platforms so that if spillage occurs, soil is not ingested. This is intervention is important when providing feed supplements for both feedlot and pasture reared livestock.

5.8.4 Livestock watering

Namibia is an arid country that relies mainly on underground water for livestock during most of the year (Mendelsohn 2006). The underground water has subsequent to our study, been found to have high levels of dissolved zinc, lead and cadmium, which is reflective of the chemistry of ore rocks in the area (Mapani *et. al.* 2010). It is therefore ideal to seasonally monitor the concentrations of Cd in the water used for livestock. High copper and sulphur concentrations in Cd contaminated borehole water offers protection to livestock (Merry 1988). Similarly, copper sulphate granules may be added to Cd contaminated water to provide similar protection.

5.9 Sustainability strategy for risk mitigation

5.9.1 Advocacy

Advocacy groups could be formed (Malayang III 2002) in the study area to engage farmers, mines and all other parties interested in the risk of Cd in the

food chain. Advocacy should establish government support so that policy can be created to guide a comprehensive Cd risk analysis project in Namibia.

5.9.2 Research and monitoring

There is virtually no data and research on Cd in the food chain in Namibia which makes it problematic to give science-based opinions on the risk level for Cd and appropriate levels of protection. Research can be used to guide decision making on food safety with regards to Cd. Seasonal measurement of Cd levels in vegetation and underground water would also inform when and how to intervene to minimise the entry of Cd in the food chain (John & Leventhal 1995).

Mines active in the area may also be encouraged to conduct regular quality testing of water and air to monitor Cd impact and trends in the environment (Codex Alimentarius Commission 2007). Laboratory screening of meat for heavy metals is ideal to identify and remove contaminated meat from the food chain (Figueroa 2008). Testing and excluding meat from the food chain is never or seldom done, due to practical and cost reasons (Kan & Meijer 2007). However, mines in the study area have personnel and laboratory equipment capable of measuring metals in organic and inorganic materials. Partnerships could be established between public health authorities, mining companies and butchers to facilitate cheaper and easier monitoring of Cd concentrations in meat products produced in the area.

5.9.3 Risk communication

The Cd mitigation strategy in the study area can only be successful if awareness is created amongst all stakeholders (Codex Alimentarius Commission 2007). Existing and prospective cattle farmers in the area should be sensitised to the risk of Cd presence of in the environment, the risk of food chain entry and consequences of presence in food.

A Cd message pamphlet with basic and simple to understand information can be created (McCrindle 2004). The pamphlet would carry basic information defining Cd and Pb, and possible sources of exposure. Consequences of exposure must also be given and explained in simple terms. The pamphlets should also provide the target audience with sources where further information regarding Cd may be obtained. The main message would be to consume kidney meat from cattle reared in the study area in moderation (less than a kilogram per month). Butchers could also be encouraged to mix kidney meat from multiple animals in order to achieve Cd dilution.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Heavy metals Cd and Pb were considered to be present and bioavailable in the environment where cattle in this study were reared. It was concluded that Cd in kidneys of some mature cattle in this study accumulated to concentrations that violated public health safety standards, although the mean level of all samples studied was below CAC ML. Consumption of kidney meat from mature culled breeding cattle reared in this area of Namibia is a cause of public health concern regarding Cd, The risk is probably significantly higher in kidney meat from the older than those of younger cattle as a result of bioaccumulation linked to age. The consumption of liver from these cattle was concluded to be of minimal public health concern as the concentrations were below the CAC ML. Cattle reared closer to mines and cows had apparently higher renal Cd concentrations than those reared further away from mines and male cattle respectively, but the difference from the mean was not found to be significant.

Although the presence of Pb in the food chain from cattle kidneys was confirmed, no conclusions could be made about its public health implications due to the poor sensitivity of the FAAS technique employed in determinations.

6.2 Recommendations

A wide and comprehensive Cd risk analysis in the study area is recommended since a proportion of cattle kidneys analysed showed levels of Cd exceeding the international human dietary exposure limits. The Cd risk analysis would be expected to establish the metal concentrations in current human exposure sources and source specific exposure contributions in order to create a country specific appropriate level of protection.

It is recommended to use more sensitive analytical techniques to measure Pb in the cattle kidneys and liver as FAAS was not sufficiently sensitive, although adequate as a screening method.

Advocacy on Cd in the food chain to create awareness and generate interest in all stakeholders including farmers, consumers, the state and the mining industry is recommended. Health and environmental authorities could also be sensitised to the risk of cumulative Cd toxicity and more in depth exposure assessment of environmental sources and people at risk be initiated.

It is also recommended that risk communication be initiated through pamphlets with information about Cd dietary exposure risk and dietary recommendations for consumers who eat large amounts of kidney meat.

Use of mathematical modelling to translate research findings into quantitative reports for public health heavy metal risk mitigation is recommended.

CHAPTER 7

REFERENCES

1. Abrahams P.W. 2011. Involuntary soil ingestion and geophagia: A source and sink of mineral nutrients and potentially harmful elements to consumers of earth materials. *Applied Geochemistry* doi:10.1016/j.apgeochem.2011.05. 003.
2. Alonso M.L., Benedito J.L., Miranda, M., Castillo C., Hernández J. & Shore, R.F. 2000. Arsenic, cadmium, lead, copper and zinc in cattle from Galicia, NW Spain. *The Science of the Total Environment,* 246: 237 - 248.
3. Berger L.L. & Cunha T.J. 1993. *Salt and trace minerals for livestock, poultry and other animals*. Alexandria, Virginia: Salt Institute.
4. Bilandžic N., Sedak M., Vrataric D., Peric T. & Šimic B. 2009. Lead and cadmium in red deer and wild boar from different hunting grounds in Croatia. *Science of the Total Environment,* 407: 4243 - 4247.
5. Bradberry S. & Vale A. 2007. Lead. *Medicine,* 35: 627 - 628.
6. Canty M.J., Lane E.A. & More S.J. 2009. Study 5: Kidney cadmium concentrations in cattle from the index farm, 2003-2005 and 2009. *Epidemiological studies of poor animal performance on a farm in Castlecomer, Co. Kilkenny. A contribution to the work of a national inter-agency group by the Centre for Veterinary Epidemiology and Risk Analysis, UCD Veterinary Sciences Centre, University College Dublin. A report prepared for the Minister of Agriculture, Fisheries and Food,*

December 2009. Dublin, Ireland: University College Dublin and the Department of Agriculture, Fisheries and Food.

7. Center for Disease Control (U.S.). 1992. *Principles of epidemiology: An introduction to applied epidemiology and biostatistics: Self-study course 3030-G*. Atlanta, Georgia: Dept. of Health, Education, and Welfare, Public Health Service, Center for Disease Control.
8. Codex Alimentarius Commission. 2007. *Working principles for risk analysis for food safety for application by governments*. Rome: World Health Organization & Food and Agriculture Organization of the United Nations.
9. Coynel A., Schäfer J., Dabrin A., Girardot N. & Blanc G. 2007. Groundwater contributions to metal transport in a small river affected by mining and smelting waste. *Water Research,* 41: 3420 - 3428.
10. Denny P. 1987. Monitoring of Heavy Metals - A Proposed Strategy for Developing Countries, in *Lead, mercury, cadmium and arsenic in the environment*, edited by Hutchinson T. C., & Meema, K. M. Chichester: J. Wiley and Sons, 343 - 347.
11. FAO/WHO. 1997. Joint FAO/WHO Expert Consultation on the Application of Risk Management to Food Safety Matters. *Risk management and food safety: Report of a Joint FAO/WHO Consultation, Rome, Italy, 27 - 31 January 1997.* Rome: Food and Agriculture Organization of the United Nations.
12. Figueroa B.E. 2008. Are more restrictive food cadmium standards justifiable health safety measures or opportunistic barriers to trade? An

answer from economics and public health. *Science of the Total Environment,* 389: 1- 9.

13. Forte G. & Bocca B. 2007. Quantification of cadmium and lead in offal by SF-ICP-MS: Method development and uncertainty estimate. *Food Chemistry,* 105: 1591 - 1598.
14. Franco-Uría A., López-Mateo C., Roca E. & Fernández-Marcos M.L. 2009. Source identification of heavy metals in pastureland by multivariate analysis in NW Spain. *Journal of hazardous materials,* 165: 1008 - 1015.
15. GEMS. 2003. *GEMS/Food regional diets: Regional per capita consumption of raw and semi-processed agricultural commodities*. Geneva: Food Safety Unit, Programme of Food Safety and Food Aid, World Health Organization.
16. Hooser S.B. 2007. Cadmium, in *Veterinary toxicology basic and clinical principles,* 1st edition, edited by R.C. Gupta. New York: Academic Press, 422 - 426.
17. Jarup L. 2003. Hazards of heavy metal contamination. *British Medical Bulletin,* 68: 167 - 182.
18. JECFA. 2004. *Report of the 36th Session of the Codex Committee on Food Additives and Contaminants, Rotterdam, the Netherlands, 22 - 26 March 2004.* Geneva: Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Accessed 28 January 2010 from: www.codexalimentarius.net/download/report/614/al04_12e.pdf.

19. John D.A. & Leventhal J.S. 1995. Bioavailability of metals, in: *Preliminary compilation of descriptive geoenvironmental mineral deposit models,* edited by E.A. Du Bray & Geological Survey (U.S.). Denver, Colorado: U.S. Dept. of the Interior, U.S. Geological Survey: [Open-File Reports Section, distributor], 10 - 18.

20. Joint FAO/WHO Food Standards Programme. 2010. *Codex Alimentarius Commission Procedural manual*, 19th edition. Rome: World Health Organisation & Food and Agriculture Organisation.

21. Jones S.D.M. 1981. Offal growth in young and mature dairy cows. *Canadian Journal of Animal Science,* 61: 607 - 611.

22. Jukna C., Jukna V., & Siugzdaite J. 2006. Determination of heavy metals in viscera and muscles of cattle. *Bulgarian Journal of Veterinary Medicine*, 9(1): 35 - 41.

23. Kahn, C.M., Line, S., Amstutz, H.E., Merck & Co., & Merial (Firm). 2006. *The Merck veterinary manual*. New Jersey: Whitehouse Station, Merck.

24. Kan C.A. & Meijer, G.A.L. 2007. The risk of contamination of food with toxic substances present in animal feed. *Animal Feed Science and Technology,* 133: 84 - 108.

25. Leung C. & Jiao J.J. 2006. Heavy metal and trace element distributions in groundwater in natural slopes and highly urbanized spaces in Mid-Levels area, Hong Kong. *Water Research,* 40: 753 - 767.

26. Liu J., Goyer R.A. & Waalkes M.P. 2008. Toxic Effects of Metals, in *Casarett and Doull's Toxicology: The Basic Science of Poisons,* 7th edition, edited by C.D. Klassen. New York: McGraw-Hill, 932 - 947.

27. Malayang III B.S. 2002. Consumer Advocacy, Food Safety and the Environment in a Developing Tropical Economy: The Case of the Philippines. *Proceedings of the International Workshop: Food Safety Management in Developing Countries, CIRAD-FAO 11 - 13 December 2000, Montpellier, France*, edited by E. Hanak, E. Boutrif, P. Fabre & M. Pineiro. Montpellier, France: CIRAD-FAO, CIRAD CD-ROM.

28. Mapani B., Ellmies R., Kamona F., Kříbek B., Majer V., Knésl I., Pašava J., Mufenda M. & Mbingeneeko F. 2010. Potential human health risks associated with historic ore processing at Berg Aukas, Grootfontein area, Namibia. *Journal of African Earth Sciences*, 58(4): 634 - 647.

29. Matusiewicz H. & Krawczyk M. 2006. Determination of cadmium and lead in reference materials by volatile species generation with in-situ trapping flame atomic absorption spectrometry. *Microchemical Journal,* 83: 17.

30. McCrindle C.M.E. 2004. Veterinary Extension and Communication. *Lecture notes for post graduate veterinary extension and communication module*. Published by The University of Pretoria.

31. Mendelsohn J.M. 2006. *Farming systems in Namibia*. Windhoek: RAISON (Research & Information Services of Namibia).

32. Merry R.H. 1988. Investigations on cadmium in South Australia: rainfall, soils, cereals, pastures and soil-plant relations. *Proceedings No.2. Cadmium Accumulations in Australian Agriculture: National Symposium, Canberra, 1 - 2 March 1988*, edited by J. Simpson & W. Curnow. Canberra: Australian Government Publishing Service, 62 - 79.

33. Nordberg G. F., Nogawa K, Nordberg M. & Friberg L. T. 2007. Cadmium, in *Handbook on the toxicology of metals*, 3rd edition, edited by G.F, Nordberg, B.A. Fowler, M. Nordberg & L. Friberg. Amsterdam, Boston: Academic Press, 445 - 486.

34. Norval A. 2006. *Personal communication*. Former state Veterinarian for Grootfontein area and Director of Veterinary Services (Namibia).

35. Nriagu J., Boughanen M., Linder A., Howe A., Grant C., Rattray R., Vutchkov M. & Lalor G. 2009. Levels of As, Cd, Pb, Cu, Se and Zn in bovine kidneys and livers in Jamaica. *Ecotoxicology and Environmental Safety,* 72: 564 - 571.

36. OIE. 2006. Guide to good farming practices for animal production food safety. (Animal Production Food Safety Working Group). *Scientific and Technical Review,* 25, 823 - 36.

37. OIE. 2010. Animal health surveillance (Chapter 1.4), in: *Terrestrial animal health code*. Paris: World Organization for Animal Health.

38. Peralta-Videa J.R., Lopez M.L., Narayan M., Saupe G. & Gardea-Torresdey J. 2009. The biochemistry of environmental heavy metal uptake by plants: Implications for the food chain. *The International Journal of Biochemistry & Cell Biology,* 41: 1665 - 1677.

39. PerkinElmer. 1996. Analytical methods for atomic absorption spectroscopy. Norwalk: Perkin Elmer.

40. Prankel S.H., Nixon R.M. & Phillips C.J.C. 2004. Meta-analysis of feeding trials investigating cadmium accumulation in the livers and kidneys of sheep. *Environmental Research,* 94: 171 - 183.

41. Radostits O.M., Gay, C.C., Hinchcliff K.W., Constable P.D., (Eds). 2007. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*, 10th edition. Edinburgh: Elsevier Saunders.

42. Satarug S. & Moore M.R. 2004. Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. *Environmental Health Perspectives,* 112: 1099 - 1103.

43. Skerfving S. & Bergdahl I.A. 2007. Lead, in: *Handbook on the toxicology of metals*, 3rd edition, edited by G.F, Nordberg, B.A. Fowler, M. Nordberg & L. Friberg. Amsterdam, Boston: Academic Press, 599 - 643.

44. Smith K.M., Abrahams P.W., Dagleish M.P. & Steigmajer J. 2009. The intake of lead and associated metals by sheep grazing mining-contaminated floodplain pastures in mid-Wales, UK: I. Soil ingestion, soil-metal partitioning and potential availability to pasture herbage and livestock. *Science of the Total Environment*, 407: 3731 - 3739.

45. Sridhara Chary N., Kamala C.T. & Samuel Suman Raj D. 2008. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotoxicology and Environmental Safety,* 69: 513 - 524.

46. Sweet J. & Burke A. 2006. Namibia, In: *Country Pasture / Forage Resources Profiles.* Rome: Food and Agriculture Organization of the United Nations.

47. Tarragó O. & ATSDR. 2007. *Lead Toxicity*: *Case Studies in Environmental Medicine (CSEM).* Atlanta: Agency for Toxic Substances and Disease Registry, (U.S.).

48. Thompson L. 2007. Lead, in: *Veterinary toxicology basic and clinical principles,* 1st edition, edited by R.C. Gupta. New York: Academic Press, 438 - 441.

49. Thomsen V., Schatzlein D. & Mercuro D. 2003. Limits of Detection in Spectroscopy. *Spectroscopy December 2003,* 18 (12): 112 – 114, Accessed 6 February 2012 from: www.bioforensics.com/conference07/LOD/LODs.pdf.

50. Thrusfield M.V. 2005. *Veterinary Epidemiology*. Ames, Iowa: Blackwell Science.

51. Tucker P.G. & ATSDR. 2008. *Cadmium Toxicity: Case Studies in Environmental Medicine (CSEM).* Atlanta: Agency for Toxic Substances and Disease Registry, (U.S.).

52. Veltman K., Huijbregts M.A.J. & Hendriks A.J. 2008. Cadmium bioaccumulation factors for terrestrial species: Application of the mechanistic bioaccumulation model OMEGA to explain field data. *Science of the Total Environment,* 406: 413 - 418.

53. WHO. 2011. Evaluation of Certain Food Additives and Contaminants: Seventy-third Report of the Joint FAO/WHO Expert Committee on Food Additives, In: *World Technical Report Series 960*. Geneva: Food and Agriculture Organisation & World Health Organisation.

54. WHO/FAO. 2006a. *Food safety risk analysis: a guide for national food safety authorities*. Rome: World Health Organization & Food and Agriculture Organization of the United Nations.

55. WHO/FAO. 2006b. *Understanding the Codex Alimentarius*. Rome: World Health Organization & Food and Agriculture Organization of the United Nations.

56. WTO. 2010. Sanitary and Phytosanitary Measures, in: *The WTO Agreements Series.* Geneva: World Trade Organisation.

CHAPTER 7

REFERENCES

1. Abrahams P.W. 2011. Involuntary soil ingestion and geophagia: A source and sink of mineral nutrients and potentially harmful elements to consumers of earth materials. *Applied Geochemistry* doi:10.1016/j.apgeochem.2011.05. 003.
2. Alonso M.L., Benedito J.L., Miranda, M., Castillo C., Hernández J. & Shore, R.F. 2000. Arsenic, cadmium, lead, copper and zinc in cattle from Galicia, NW Spain. *The Science of the Total Environment,* 246: 237 - 248.
3. Berger L.L. & Cunha T.J. 1993. *Salt and trace minerals for livestock, poultry and other animals*. Alexandria, Virginia: Salt Institute.
4. Bilandžic N., Sedak M., Vrataric D., Peric T. & Šimic B. 2009. Lead and cadmium in red deer and wild boar from different hunting grounds in Croatia. *Science of the Total Environment,* 407: 4243 - 4247.
5. Bradberry S. & Vale A. 2007. Lead. *Medicine,* 35: 627 - 628.
6. Canty M.J., Lane E.A. & More S.J. 2009. Study 5: Kidney cadmium concentrations in cattle from the index farm, 2003-2005 and 2009. *Epidemiological studies of poor animal performance on a farm in Castlecomer, Co. Kilkenny. A contribution to the work of a national inter-agency group by the Centre for Veterinary Epidemiology and Risk Analysis, UCD Veterinary Sciences Centre, University College Dublin. A report prepared for the Minister of Agriculture, Fisheries and Food,*

December 2009. Dublin, Ireland: University College Dublin and the Department of Agriculture, Fisheries and Food.

7. Center for Disease Control (U.S.). 1992. *Principles of epidemiology: An introduction to applied epidemiology and biostatistics: Self-study course 3030-G*. Atlanta, Georgia: Dept. of Health, Education, and Welfare, Public Health Service, Center for Disease Control.
8. Codex Alimentarius Commission. 2007. *Working principles for risk analysis for food safety for application by governments*. Rome: World Health Organization & Food and Agriculture Organization of the United Nations.
9. Coynel A., Schäfer J., Dabrin A., Girardot N. & Blanc G. 2007. Groundwater contributions to metal transport in a small river affected by mining and smelting waste. *Water Research,* 41: 3420 - 3428.
10. Denny P. 1987. Monitoring of Heavy Metals - A Proposed Strategy for Developing Countries, in *Lead, mercury, cadmium and arsenic in the environment*, edited by Hutchinson T. C., & Meema, K. M. Chichester: J. Wiley and Sons, 343 - 347.
11. FAO/WHO. 1997. Joint FAO/WHO Expert Consultation on the Application of Risk Management to Food Safety Matters. *Risk management and food safety: Report of a Joint FAO/WHO Consultation, Rome, Italy, 27 - 31 January 1997.* Rome: Food and Agriculture Organization of the United Nations.
12. Figueroa B.E. 2008. Are more restrictive food cadmium standards justifiable health safety measures or opportunistic barriers to trade? An

answer from economics and public health. *Science of the Total Environment,* 389: 1- 9.

13. Forte G. & Bocca B. 2007. Quantification of cadmium and lead in offal by SF-ICP-MS: Method development and uncertainty estimate. *Food Chemistry,* 105: 1591 - 1598.
14. Franco-Uría A., López-Mateo C., Roca E. & Fernández-Marcos M.L. 2009. Source identification of heavy metals in pastureland by multivariate analysis in NW Spain. *Journal of hazardous materials,* 165: 1008 - 1015.
15. GEMS. 2003. *GEMS/Food regional diets: Regional per capita consumption of raw and semi-processed agricultural commodities*. Geneva: Food Safety Unit, Programme of Food Safety and Food Aid, World Health Organization.
16. Hooser S.B. 2007. Cadmium, in *Veterinary toxicology basic and clinical principles,* 1st edition, edited by R.C. Gupta. New York: Academic Press, 422 - 426.
17. Jarup L. 2003. Hazards of heavy metal contamination. *British Medical Bulletin,* 68: 167 - 182.
18. JECFA. 2004. *Report of the 36th Session of the Codex Committee on Food Additives and Contaminants, Rotterdam, the Netherlands, 22 - 26 March 2004.* Geneva: Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Accessed 28 January 2010 from: www.codexalimentarius.net/download/report/614/al04_12e.pdf.

19. John D.A. & Leventhal J.S. 1995. Bioavailability of metals, in: *Preliminary compilation of descriptive geoenvironmental mineral deposit models,* edited by E.A. Du Bray & Geological Survey (U.S.). Denver, Colorado: U.S. Dept. of the Interior, U.S. Geological Survey: [Open-File Reports Section, distributor], 10 - 18.

20. Joint FAO/WHO Food Standards Programme. 2010. *Codex Alimentarius Commission Procedural manual*, 19th edition. Rome: World Health Organisation & Food and Agriculture Organisation.

21. Jones S.D.M. 1981. Offal growth in young and mature dairy cows. *Canadian Journal of Animal Science,* 61: 607 - 611.

22. Jukna C., Jukna V., & Siugzdaite J. 2006. Determination of heavy metals in viscera and muscles of cattle. *Bulgarian Journal of Veterinary Medicine*, 9(1): 35 - 41.

23. Kahn, C.M., Line, S., Amstutz, H.E., Merck & Co., & Merial (Firm). 2006. *The Merck veterinary manual*. New Jersey: Whitehouse Station, Merck.

24. Kan C.A. & Meijer, G.A.L. 2007. The risk of contamination of food with toxic substances present in animal feed. *Animal Feed Science and Technology,* 133: 84 - 108.

25. Leung C. & Jiao J.J. 2006. Heavy metal and trace element distributions in groundwater in natural slopes and highly urbanized spaces in Mid-Levels area, Hong Kong. *Water Research,* 40: 753 - 767.

26. Liu J., Goyer R.A. & Waalkes M.P. 2008. Toxic Effects of Metals, in *Casarett and Doull's Toxicology: The Basic Science of Poisons,* 7th edition, edited by C.D. Klassen. New York: McGraw-Hill, 932 - 947.

27. Malayang III B.S. 2002. Consumer Advocacy, Food Safety and the Environment in a Developing Tropical Economy: The Case of the Philippines. *Proceedings of the International Workshop: Food Safety Management in Developing Countries, CIRAD-FAO 11 - 13 December 2000, Montpellier, France*, edited by E. Hanak, E. Boutrif, P. Fabre & M. Pineiro. Montpellier, France: CIRAD-FAO, CIRAD CD-ROM.

28. Mapani B., Ellmies R., Kamona F., Kříbek B., Majer V., Knésl I., Pašava J., Mufenda M. & Mbingeneeko F. 2010. Potential human health risks associated with historic ore processing at Berg Aukas, Grootfontein area, Namibia. *Journal of African Earth Sciences*, 58(4): 634 - 647.

29. Matusiewicz H. & Krawczyk M. 2006. Determination of cadmium and lead in reference materials by volatile species generation with in-situ trapping flame atomic absorption spectrometry. *Microchemical Journal,* 83: 17.

30. McCrindle C.M.E. 2004. Veterinary Extension and Communication. *Lecture notes for post graduate veterinary extension and communication module*. Published by The University of Pretoria.

31. Mendelsohn J.M. 2006. *Farming systems in Namibia*. Windhoek: RAISON (Research & Information Services of Namibia).

32. Merry R.H. 1988. Investigations on cadmium in South Australia: rainfall, soils, cereals, pastures and soil-plant relations. *Proceedings No.2. Cadmium Accumulations in Australian Agriculture: National Symposium, Canberra, 1 - 2 March 1988*, edited by J. Simpson & W. Curnow. Canberra: Australian Government Publishing Service, 62 - 79.

33. Nordberg G. F., Nogawa K, Nordberg M. & Friberg L. T. 2007. Cadmium, in *Handbook on the toxicology of metals*, 3rd edition, edited by G.F, Nordberg, B.A. Fowler, M. Nordberg & L. Friberg. Amsterdam, Boston: Academic Press, 445 - 486.

34. Norval A. 2006. *Personal communication*. Former state Veterinarian for Grootfontein area and Director of Veterinary Services (Namibia).

35. Nriagu J., Boughanen M., Linder A., Howe A., Grant C., Rattray R., Vutchkov M. & Lalor G. 2009. Levels of As, Cd, Pb, Cu, Se and Zn in bovine kidneys and livers in Jamaica. *Ecotoxicology and Environmental Safety,* 72: 564 - 571.

36. OIE. 2006. Guide to good farming practices for animal production food safety. (Animal Production Food Safety Working Group). *Scientific and Technical Review,* 25, 823 - 36.

37. OIE. 2010. Animal health surveillance (Chapter 1.4), in: *Terrestrial animal health code*. Paris: World Organization for Animal Health.

38. Peralta-Videa J.R., Lopez M.L., Narayan M., Saupe G. & Gardea-Torresdey J. 2009. The biochemistry of environmental heavy metal uptake by plants: Implications for the food chain. *The International Journal of Biochemistry & Cell Biology,* 41: 1665 - 1677.

39. PerkinElmer. 1996. Analytical methods for atomic absorption spectroscopy. Norwalk: Perkin Elmer.

40. Prankel S.H., Nixon R.M. & Phillips C.J.C. 2004. Meta-analysis of feeding trials investigating cadmium accumulation in the livers and kidneys of sheep. *Environmental Research,* 94: 171 - 183.

41. Radostits O.M., Gay, C.C., Hinchcliff K.W., Constable P.D., (Eds). 2007. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*, 10th edition. Edinburgh: Elsevier Saunders.

42. Satarug S. & Moore M.R. 2004. Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. *Environmental Health Perspectives,* 112: 1099 - 1103.

43. Skerfving S. & Bergdahl I.A. 2007. Lead, in: *Handbook on the toxicology of metals*, 3rd edition, edited by G.F, Nordberg, B.A. Fowler, M. Nordberg & L. Friberg. Amsterdam, Boston: Academic Press, 599 - 643.

44. Smith K.M., Abrahams P.W., Dagleish M.P. & Steigmajer J. 2009. The intake of lead and associated metals by sheep grazing mining-contaminated floodplain pastures in mid-Wales, UK: I. Soil ingestion, soil-metal partitioning and potential availability to pasture herbage and livestock. *Science of the Total Environment*, 407: 3731 - 3739.

45. Sridhara Chary N., Kamala C.T. & Samuel Suman Raj D. 2008. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotoxicology and Environmental Safety,* 69: 513 - 524.

46. Sweet J. & Burke A. 2006. Namibia, In: *Country Pasture / Forage Resources Profiles.* Rome: Food and Agriculture Organization of the United Nations.

47. Tarragó O. & ATSDR. 2007. *Lead Toxicity*: *Case Studies in Environmental Medicine (CSEM).* Atlanta: Agency for Toxic Substances and Disease Registry, (U.S.).

48. Thompson L. 2007. Lead, in: *Veterinary toxicology basic and clinical principles,* 1st edition, edited by R.C. Gupta. New York: Academic Press, 438 - 441.

49. Thomsen V., Schatzlein D. & Mercuro D. 2003. Limits of Detection in Spectroscopy. *Spectroscopy December 2003,* 18 (12): 112 – 114, Accessed 6 February 2012 from: www.bioforensics.com/conference07/LOD/LODs.pdf.

50. Thrusfield M.V. 2005. *Veterinary Epidemiology*. Ames, Iowa: Blackwell Science.

51. Tucker P.G. & ATSDR. 2008. *Cadmium Toxicity: Case Studies in Environmental Medicine (CSEM).* Atlanta: Agency for Toxic Substances and Disease Registry, (U.S.).

52. Veltman K., Huijbregts M.A.J. & Hendriks A.J. 2008. Cadmium bioaccumulation factors for terrestrial species: Application of the mechanistic bioaccumulation model OMEGA to explain field data. *Science of the Total Environment,* 406: 413 - 418.

53. WHO. 2011. Evaluation of Certain Food Additives and Contaminants: Seventy-third Report of the Joint FAO/WHO Expert Committee on Food Additives, In: *World Technical Report Series 960*. Geneva: Food and Agriculture Organisation & World Health Organisation.

54. WHO/FAO. 2006a. *Food safety risk analysis: a guide for national food safety authorities*. Rome: World Health Organization & Food and Agriculture Organization of the United Nations.

55. WHO/FAO. 2006b. *Understanding the Codex Alimentarius*. Rome: World Health Organization & Food and Agriculture Organization of the United Nations.

56. WTO. 2010. Sanitary and Phytosanitary Measures, in: *The WTO Agreements Series.* Geneva: World Trade Organisation.

CHAPTER 8

ANNEXURES

Annexure I: Sampling request letter

Telfax: +264 67 242140
Cell: +264 81 2026144
E-mail: mkdzi@mweb.com.na

P. O. Box 81
Grootfontein
Namibia
9000

05 November 2007

To: The CEO
Grootfontein Municipality
P. O. Box 23
Grootfontein

RE: SAMPLING CATTLE CARCASSES FOR HEAVY METAL TESTING PROJECT

Dear Sir

I, Dr Midzi, the State Veterinarian intend to conduct the above referred Research Project towards my Master Degree in Veterinary Public Health with the University of Pretoria.

This project intends to gauge the level of presence or absence of heavy metals (lead, chromium etc) in beef processed at Grootfontein Municipal Abattoir. These items the project intends to look for are considered a major public concern internationally, yet we have not done anything to assess if the local population of this town is at risk.

As you will appreciate, Namibia has extensive mineral ores, and uses a lot of underground water for human and livestock consumption. These are potential sources of exposure of animals and man to the toxic heavy metals.

This research will attempt to establish if animals and are exposed to heavy metals, which eventually can become transferred to man eating their meat. The project's finding will justify or allay fears regarding heavy metals in beef supplied to local consumers through four butcheries slaughtering cattle at Grootfontein Municipal abattoir. Final this research will try to influence national policy change regarding meat safety and processing.

To be able to conduct the research, I write requesting for the Municipality's permission to collect, approximately, eight cattle carcasses (kidney and liver samples) from all cattle slaughtered at Grootfontein Municipal Abattoir over a one month period.

Thanking you in advance for your anticipated help,

Midzi, Emmanuel M
State Veterinarian, Grootfontein

Annexure II: Sampling consent letter

Municipality of Grootfontein

Town Clerk's Office

Ref. No. 17/9/3

✉ 23
☎ (067) 243101
Fax (067) 242930
Grootfontein
Namibia

16 November 2007

Dr. E. M. Midzi
P.O. Box 81
GROOTFONTEIN
Republic of Namibia

Dear Dr. Midzi

ABATTOIR MAINTENANCE:

Your letter with regard to drug and heavy metal sampling from cattle carcasses received on 6 November 2007 has reference.

Your request was submitted to Council and it was resolved as follows on Management Committee Meeting of 13 November 2007, Item 189: -

1. *that Council approve the presence of Dr. Midzi, the local State Veterinarian at the Grootfontein Municipal Abattoir for one month to sample kidneys and livers for antibiotics and heavy metal for his research project.*

2. *that Council is indemnified in case of any accident or injury to Dr. Midzi the local State Veterinarian, while sampling kidneys and livers at the Grootfontein Municipal Abattoir.*

3. *that only kidneys and livers of Country Wild and Vleiskor be sampled (free of charge) in the presence of either the Environmental Health Practitioner, Mr. M. Aribeb or the SE: Environmental Health, Mr. J. Colesky.*

Sampling from other carcasses could also take place, provided written authorization has been obtained from the owner of the animals/carcasses and submitted to the Grootfontein Municipality.

Yours faithfully

CHIEF EXECUTIVE OFFICER

JC/jc

Annexure III: Daily Sampling Form used

Place: Grootfontein Municipal Abattoir

Daily sample #	Brand	Ear Tag #	Sex		Incisors			Cumulative sample #
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	
			F	M	2	4	6	