Mycotoxin prevalence and heavy metal contamination of South African red meat

by

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“What’s done, is done.”

-Lady Macbeth

DECLARATION

Ethical clearance for the study was granted by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria. The certificate is attached as addendum D.

“I declare that the dissertation, which I hereby submit for the degree MSc Environmental Health at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another university”.

Signature ……

MM van Deventer

Date 29 October 2018

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ABSTRACT

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Introduction

Stunting is a national public health problem in South Africa affecting approximately 15.4% of children. Among the many possible causes of growth retardation is exposure to toxic substances by dietary means. Human exposure to mycotoxins and/or heavy metals has been linked to stunting. These two groups of food contaminants occur naturally in the environment, in the air and soil. Environmental factors such as climatic conditions, harvesting methods, storage- and transportation systems all provide multiple opportunities for mycotoxin and/or heavy metal contamination. Plants, as components of animal feed exposed to mycotoxins and heavy metals, become a pathway to contaminate meat and meat products.

Methods

The data used for this study is drawn from the monitoring and evaluation programme of the Department of Agriculture, Forestry and Fisheries – which screens heavy metal and mycotoxin contamination in meat – and from an original research study established at the University of Pretoria which studied mycotoxin contamination in red meat. The location and time of sampling from these two data sets were cross referenced with environmental conditions known to have an impact on contamination levels.

Results

The data in the two data sets were analysed. It was found that none of the beef and pork samples tested positive for mycotoxins. Two samples tested positive for heavy metals in 2012, namely lead (610 µg/kg) in Malmesbury and mercury (200 µg/kg) in Bela-Bela. No link could be made to any environmental factors as there were no positive mycotoxin results and no correlation could be found for the two positive incidences of heavy metal contamination.

Conclusion

As no sample tested positive for mycotoxins, no correlation between environmental factors and mycotoxin contamination could be established. Further to that, there was no correlation between heavy metal contamination levels and environmental factors.

Keywords: Environment, Heavy metals, Mycotoxins, Red meat.

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LIST OF ABBREVIATIONS

AF	Aflatoxin
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
AFM2	Aflatoxin M2
ARC	Agricultural Research Council
CODEX	Codex Alimentarius Commission
DAFF	Department of Agriculture, Forestry and Fisheries
DON	Deoxynivalenol
FAO	Food and Agricultural Organisation
FB	Fumonisin
HPLC	High performance liquid chromatography
HSRC	Human Science Research Council
ICP-MS	Inductively coupled plasma mass spectrometry
IUPAC	International Union for Pure and Applied Chemistry
LOQ	Limit of quantification
NDH	National Department of Health
NADPH	Nicotinamide adenine dinucleotide phosphate
OVI	Onderstepoort Veterinary Institute
OT	Ochratoxin
OTA	Ochratoxin A
PACA	Partnership for Aflatoxin Control in Africa
PPE	Porcine pulmonary oedema
ppm	parts per million (mg/ℓ or mg/kg)
SANHANES	South African National Health and Nutrition Examination Survey

SAVACG	South African Vitamin C Consultative Group
SAWS	South African Weather Service
SDG	Sustainable Development Goals
StatsSA	Statistics South Africa
UN	United Nations
UNICEF	United Nations International Children’s Emergency Fund
UP	University of Pretoria
WHO	World Health Organisation
ZEN	Zearalenone

CHAPTER ONE

The study in perspective

1.1 Background of the study

The triple burden of malnutrition – namely under-nutrition, overweight and obesity, and micronutrient deficiencies – is evident in South Africa. It is *inter alia* a result of the high level of food insecurity at household level among the population.[1] The most common form of under-nutrition found in children under five years of age in South Africa is stunting. Stunting is defined by the World Health Organization (WHO) as a height-to-age ratio value less than two standard deviations of the WHO Child Growth Standards median.[1]

Stunting affects more than 15% of children under five years of age in South Africa.[2] According to the South African National Health and Nutrition Examination Survey (SANHANES), the highest prevalence of stunting among South African children is in the age group null to three years.[1] Within this age group, 26.9% of boys and 25.9% of girls are stunted. Boys (age null to 14 years) living in rural informal areas are significantly more stunted than those living in urban formal areas (23.2 and 13.6%, respectively). For girls, the highest level of stunting is in urban informal areas (20.9%) and the lowest in urban formal areas (10.4%).[2-4] Stunting affects one in three boys and one in four girls in South Africa.

The Demographic and Health Survey of 2016 concluded that generally stunting decreases within higher wealth quintiles. In the highest wealth quintile, only 13% of children are stunted, increasing to 24% of children in the middle wealth quintile and reaching a high level of 36% of children in the lowest quintile. Furthermore, the data show that 34.6% of children living in urban areas and 40% of children living in non-urban areas are stunted.[5]

Globally, stunting rates are also alarmingly high, with 150.8 million (22.2%) of children under the age of five affected. The second Sustainable Development Goals (SDG) proposed by the United Nations calls for, among others goals, the end of stunting. The

goal is to eliminate stunting by the year 2025 and South Africa is a signatory to reaching this goal.[6]

Introducing meat as complementary feeding for children from six months of age is linked to reduced levels of stunting. Stunting levels are found to be lower in populations where meat is consumed between one and three times per week. This could be due to the beneficial nutritional value that meat offers. Iron, zinc and vitamin B_{12} are among the micronutrients that animal protein sources such as meat contribute to the diet,[7] however, consumption of contaminated meat could be a possible cause of stunting.

It is clear that stunting is caused by various factors, including diet and sanitary conditions interacting with one another, yet feeding practices and nutritional status of infants and children seem to be critical factors.[7] Toxic substances in the diet, such as mycotoxins and heavy metals, are believed to be one of the causes of stunting in young children.[8] The substances can enter the food chain through crops used for animal feed that have been exposed to environmental factors such as weather conditions that promote contamination.[9, 10]

1.1.1 Mycotoxins

Fungi producing mycotoxins are natural toxins that occur in the air and soil. Mycotoxins are classified as low molecular weight natural products produced as secondary metabolites by filamentous fungi.[11] Mycotoxin contamination is more likely to occur when plants are exposed to stress conditions such as insect damage, low soil fertility and extreme weather conditions.[12] This study focused on aflatoxin (AF), ochratoxin (OT), deoxynivalenol (DON) and zearalenone (ZEN). All four these mycotoxins can have serious health effects on humans.[13]

This study explored the effect of selected environmental factors on the occurrence of mycotoxins and its prevalence in red meat in South Africa.

1.1.2 Heavy metals

Heavy metal refers to a metallic element that has a relatively high density and high relative atomic weight, and is toxic or poisonous at low concentrations in the diet.[14] The four heavy metals including lead, arsenic, cadmium and mercury that pose a serious

threat to human health are studied. Cadmium and arsenic are both carcinogenic, whereas consumption of lead has been linked to stunting and mercury poisoning has been linked to cognitive and motor dysfunction.[14]

The effects of selected environmental factors on the occurrence of these heavy metals in red meat and how they can enter the food chain, are discussed.

1.2 Justification of the study

Meat is a nutrient-dense food and an important source of protein in the diet and as such can prevent stunting. Therefore, it is important to ensure that South African red meat is safe to eat and does not contribute to the levels of stunting in children currently experienced, as toxic substances possibly found in red meat have been linked to stunting. From Table 1-1, it can be seen that although the South African consumer does prefer chicken meat, there is also considerable consumption of beef and pork.

Table 1-1 The average consumption of meat by South African consumers[15]

Meat	Consumption (kg/capita/annum)
Chicken	42.0
Beef	13.5
Pork	5.2
Mutton/lamb	2.6
Total	63.3

For the University of Pretoria study, meat samples were collected from the Vhembe and Centane region, in the former Transkei. Home-grown maize samples from both these regions, have shown over many seasons to be contaminated with various mycotoxins.[16, 17] These mycotoxins include AF, OTA, DON and ZEN. Although this study did not test these regions' maize for mycotoxin contamination due to a lack of funding, it was reasonably assumed, due to past published findings, that home-grown maize will be contaminated. This contaminated maize formed part of the human and animal (ruminant and swine) daily diets. Figure 1-1 and 1-2 are photos of the home-grown maize taken in

the Vhembe district during the sampling of meat for this study. From these images it is clear that the maize in this district was contaminated with fungi.

Figure 1-1 Home grown maize in the Vhembe district photo 1[18]

Figure 1-2 Home grown maize in the Vhembe district photo 2[18]

1.3 Aim and objectives

1.3.1 Aims

The aims of the study were 1) to determine the prevalence of mycotoxins and heavy metal contamination in South African red meat, and 2) to establish the effect of selected environmental factors, such as weather conditions, on meat contamination by mycotoxins and heavy metals.

The project's specific objectives were to:

1. Determine the levels of mycotoxins in South African red meat obtained from rural areas.
2. Determine the current level of heavy metal contamination in South African red meat.
3. Establish a correlation between some selected environmental factors, including weather conditions, and the incidence of mycotoxins and heavy metals in red meat.

1.4 Presentation and structure of the dissertation

1.4.1 Chapter One: The study in perspective

This chapter provides a brief description of the study and the structure of the dissertation. The chapter also states the justification, aims and objectives of the study.

1.4.2 Chapter Two: Literature review

The literature review discuss both mycotoxins and heavy metals. The review focusses on AF, OT, DON, ZEN, lead, cadmium, mercury and arsenic. The literature covers the effects these substances can possibly have on human health; how they occasionally enter the food chain as influenced by some environmental factors; and what control strategies, regulations and monitoring systems regarding these substances are in place in South Africa.

1.4.3 Chapter Three: The occurrence of mycotoxins in red meat

Chapter three addresses the first and third objective of the study, in establishing any correlation between the prevalence of mycotoxins in meat and certain environmental factors. This aspect of the study was undertaken by analysing secondary data from two previous studies that focussed on rural South African red meat, bovine and porcine for AF, OTA, DON and ZEN (ZEN). The first study is from the UP where bovine and porcine samples were analysed for mycotoxins and the second from a monitoring study by the Department of Agriculture, Forestry and Fisheries (DAFF) which monitor South African meat for mycotoxin contamination. The results were then compared with environmental data (temperature and rainfall) received from the South African Weather Service, corresponding to the sampling period in order to establish if environmental factors could play a role in the risk of meat contamination by mycotoxins.

1.4.4 Chapter Four: The occurrence of heavy metals in red meat

Chapter Four addresses the second and third objectives of the study, namely to establish a correlation between the prevalence of heavy metals and seleced environmental factors. This was done by examining data from a monitoring study by the DAFF that analysed bovine and porcine meat for lead, arsenic, cadmium and mercury in relation to environmental data from the date of sampling to establish the role of environmental factors on the risk of heavy metal contamination of meat.

1.4.5 Chapter Five: Significance of the study, conclusion and recommendation

Chapter Five integrates the findings from the studies reported in Chapters Three and Four and ends with some concluding remarks based on these findings. This chapter summarises the findings and addresses the objectives of the study. Limitations and lessons learned are also discussed.

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CHAPTER TWO

Literature review

2.1 Introduction

South Africa is considered an example of a country experiencing nutrition transition, where under-nutrition and over-nutrition increasingly co-exist. At national level, stunting affects 15.4% of the children, with the highest prevalence in informal rural areas.[1-3] A child is regarded as being stunted when his/her height-to-age ratio is more than two standard deviations below the World Health Organization (WHO) Child Growth Standards median.[4] The highest prevalence of stunting among South African children is in the age group null to three years. In this age group, 26.9% of boys and 25.9% of girls are stunted.[5]

Various interrelated factors causing stunting in children are listed in Table 2-1. It is important to note that not all children are exposed to the same (or to all) factors, and at the same exposure level. The main causes of growth retardation are still disputed but they include, among others, exposure to toxic substances – some of which enter the human body through food. Prevalence of stunting can be reduced by an estimated 20% if better hygiene is practised and complementary feeding is improved, particularly if a multiple-micronutrient supplement is provided during the first 1000 days of life.[6]

Due to the current high prevalence of stunting affecting 26% of children under five years of age worldwide, and 36% of children under five years of age living in Africa in 2011, research on possible causes of stunting needs to be explored.[8] Since both mycotoxin and heavy-metal exposure were indicated as possible factors for stunting, it is important to study food as a possible cause.[9-11]

Many foods such as maize, peanuts and canned food products can be contaminated with mycotoxins and heavy metals. Possible exposure of both mycotoxins and heavy metals could be via consumption of red meat.[12, 13] Offal such as liver and kidney, in addition to animal products including milk and eggs, are the frequently consumed animal protein sources in the diets of rural communities.[1]

Table 2-1 Possible factors that can cause stunting in children[6]

Main factors	Sub-factors	Example
Household and family factors	Maternal factors	• Poor nutrition during pre-conception, pregnancy and lactation • Short maternal stature • Short birth spacing
	Home environment	• Inadequate child stimulation and activity • Food insecurity
Inadequate complementary feeding	Poor quality foods	• Poor micronutrient quality • Anti-nutrient content
	Inadequate practices	• Infrequent feeding • Inadequate feeding during and after illness • Thin food consistency
	Food and water safety	• Contaminated food and water (e.g. toxic substances such as mycotoxins and heavy metals) • Unsafe storage and preparation of foods
Breastfeeding	Inadequate practices	• Delayed initiation • Non-exclusive breastfeeding
Infection	Clinical and subclinical infection	• Malaria • Inflammation • Enteric infection

The National Food Consumption Survey of 1999 reported that meat and offal were consumed by 36 to 38% of one-to-five and six-to-nine year olds, respectively, and by 50 to 51% by those older than nine years of age.[2] From the Statistics South Africa (StatsSA) Income and Expenditure Survey 2010/11, it is clear that marginalised, middle-class and

more affluent consumers spent 22, 26 and 27% of their income, respectively, on meat products (including chicken, beef, pork and lamb).[14] Factors such as the current economic state of the country as well as rising unemployment levels will influence meat consumption in the coming decade. It is believed that meat consumption will continue to increase[15] – there was an estimated increase in beef consumption from 15% in the last decade to 24%. In the coming decade, pork consumption will rise by an estimated 23%, while consumption of lamb and mutton (being the most expensive red meat), is expected to increase by 11%.[15] The expected rise in meat consumption may likely be accompanied by an increased exposure to food contaminants including mycotoxins and heavy metals.

2.2 Mycotoxins

Mycotoxins are natural toxins that occur in the air and soil, and are of low molecular weight, produced as secondary metabolites by filamentous fungi.[16] Fungi that produce mycotoxins can be broadly classified into two categories: field fungi and storage fungi. Field fungi invade crops before harvest, and storage fungi only occur after harvesting.[17]

Factors that influence storage fungi, such as *Aspergillus candidus, Aspergillus flavus, Aspergillus niger* growth, include moisture content in the commodity and temperature of the environment. In the tropics, temperature and relative humidity conditions are ideal for storage fungi – and water activity then becomes the main determinant of fungal attack and proliferation,[17] producing attendant mycotoxins. The mycotoxins that will be discussed in this study are AF, OTA, DON and ZEN. Molecular representation of these four mycotoxins are illustrated in Figure 2-1.

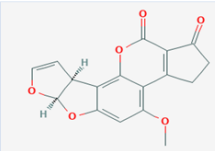
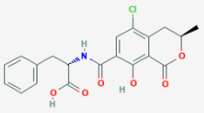
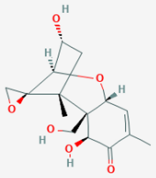
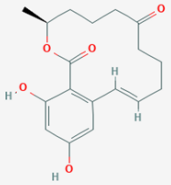
Mycotoxin	Molecular Formula	Molecular Weight	Structure
Aflatoxin B1	$C_{17}H_{12}O_6$	312.277 g/mol	
Ochratoxin A	$C_{20}H_{18}ClNO_6$	403.815 g/mol	
Deoxynivalenol	$C_{15}H_{20}O_6$	296.319 g/mol	
Zearalenone	$C_{18}H_{22}O_5$	318.369 g/mol	

Figure 2-1 Molecular formula, weight and structure of aflatoxin B1, ochratoxin A, deoxynivalenol and zearalenone [18-21]

AFs are secondary metabolites mainly by *Aspergillus flavus*, *Aspergillus parasiticus*, and occasionally other *Aspergillus* species.[22] AFs are currently the most important and well-known mycotoxins partially due to the fact it is very toxic and quantification methods are readily available. AF was the first mycotoxin to be discovered in 1961 in London when 100 000 turkeys died. The disease was named Turkey X. Subsequently it was discovered that the deaths were caused by feed that was contaminated with *Aspergillus flavus*, which was the fungus that produced the AF.[16] These fungal species are prevalent in food crops, particularly maize, giving rise to contaminated animal feed. These fungi can also produce AF during post-harvest conditions such as food storage, transportation, and processing.[16]

The best studied AF is AFB1. The reason being that it is produced in abundance by toxigenic strains and also regarded as the most potent natural carcinogen known. For people chronically infected with hepatitis B virus (common in Africa), AF consumption increases the risk of liver cancer.[22] The term aflatoxicosis is loosely used for diseases

caused after exposure to AF among humans and animals.[16] AFB1, AFB2, AFG1, and AFG2 are florescent under UV light (blue or green) and have relative chromatographic mobility during thin-layer chromatography. Another well know AF metabolite is AFM1 as it is found in milk. Cows that consume contaminated feed metabolically biotransform AFB1 to AFM1.[16]

DON is produced by the fusarium fungus, specifically *Fusarium graminearum* and *Fusarium culmorum*. DON is also known as vomotoxin due to its strong emetic effect after consumption.[23] It is a member of the trichothecenes family of mycotoxins and contains three free hydroxyl groups which are associated with its toxicity.[23] Due to its stability under high temperature, DON poses a food safety risk.[24] It was proven than DON is stable at 170 °C for 30 minutes, but degrades when cooking pasta due to leaching into the cooking water.[25] DON is water soluble and therefore there is no degradation when contaminated foodstuffs are fried in oil.[26]

OTA was discovered in a large screening project during 1965 on fungal metabolites with the aim to identify new mycotoxins. OTA is a metabolite from *Aspergillus ochraceus* and *Penicillium verrucosum*. Further research discovered that metabolites of other species *Aspergillus* are also part of the OTA family.[16] OTA has been linked to nephropathy in humans. Furthermore it has been identified as a human carcinogen. Studies have proven that OTA also causes kidney and liver cancer in laboratory animals.[27, 28]

ZEN is a secondary metabolite produced from *Fusarium graminearum*. It can also be classified as a nonsteroidal oestrogen or mycoestrogen. Molecularly this toxin significantly resembles the principal hormone produced by the human ovaries, 17 β-estradiol, and has been used to treat post-menopausal symptoms in women and in oral contraceptives.[16] ZEN does however have a poisoning effect on animals.[29] Between one and five ppm is sufficient to cause physiological responses in pigs.[30]

Classifications of mycotoxins differ according to the profession that categorizes the mycotoxins. This is due to their diverse chemical structure, their myriad biological effects, and their production by a wide number of fungal species.[16] For example, clinicians organize them according to the organ they affect, cell biologists arrange them into generic groups, organic chemists classify them according to their chemical structures, and mycologists classify them by the fungi that produce them.[16] Toxigenic and heterogeneous

mycotoxin metabolites are grouped together as they can lead to disease and death in human beings and in other vertebrates.

With the combination of climate, harvesting methods and storage and transporting systems in Africa, the continent offers a wide array of conditions that create an ideal environment promoting growth of mycotoxin-producing moulds.[31, 32] Increasingly poor harvesting methods and inadequate storage and transporting conditions are linked to the rapid growth in population and the subsequent demand for more food on the African continent.[32] Another factor is the early harvesting of crops, a common practice in Africa. This results in crops with a high moisture content which give fungi a longer window of opportunity to grow and produce mycotoxin during storage.[33]

It is also important to note that, on a continent such as Africa, food with visible mould is often consumed by both humans and animals. This is due to the fact that food is a scarce commodity and storage conditions are inadequate to protect crops and grains from fungal infection.[34] Some African governments have implemented regulations in an attempt to control mycotoxin contamination, especially AF contaminations. The Partnership for Aflatoxin Control in Africa (PACA) is working in 27 African countries. These countries are Benin, Botswana, Burkina Faso, Cameroon, Democratic Republic of the Congo, Egypt, Ethiopia, Gambia, Ghana, Kenya, Malawi, Mali, Mauritius, Mozambique, Nigeria, Rwanda, Senegal, Seychelles, South Africa, South Sudan, Sudan, Swaziland, Togo, Tunisia, Uganda, Zambia, Zimbabwe.[35] However, these regulations are mostly not implemented and controlled in rural areas where food is not produced for national supplies but mainly for subsistence farmers' own use.[34]

2.2.1 Agricultural practices and mycotoxin contamination

Occurrence of mycotoxins in plants is more likely when plants are exposed to stress conditions such as insect damage and pest infestation, low soil fertility and extreme weather conditions including drought.[35] Climatic aspects are the key agro-ecosystem forces that sustain fungal colonization and mycotoxin production.[36] Animal feed is often stored or transported under conditions that are favourable to mycotoxin development. Animals are exposed to mycotoxins mainly via consumption of contaminated feed. This is often only discovered when the animal is already showing signs of mycotoxicosis.[27]

Other possible routes of contamination include dermal, parental and aerosol exposure to mycotoxins.

It is known that most mycotoxins are metabolised in cattle, yet these metabolites are generally harmless to the animal.[37] Only one to six percent of consumed AFB1 by animals is transformed into AFM1, which is found in milk. For this reason AFM1 is currently regulated in milk.[38, 39] ZEN on the other hand is transformed into its hydroxyl-metabolite, α-ZEN, which is more toxic to cattle than ZEN.[37, 38]

Animal feeds become contaminated with mycotoxins by two pathways, namely the use of mycotoxin-contaminated crops during feed formulation, and storage under poor conditions. Destroying crops because they are contaminated with fungi can cause great economic loss and, for this reason, contaminated crops are often used as animal feed particularly among subsistence farmers. This creates problems for animals as they are exposed to a vast range of diseases caused by mycotoxin- contaminated feed.[16] Armbrecht *et al.* found reduced feed intake and slow weight gain in pigs exposed to AF through diet.[40] Negative growth effects were also observed in juvenile animals as a result of *in utero* exposure through maternal feed.[18] Table 2-2 summarises some of the toxic effects that different mycotoxins may have on swine and cattle.

Table 2-2 Possible health effects of mycotoxin on swine and cattle[22, 29, 41]

Mycotoxin	Possible effect on swine	Possible effect on cattle
Aflatoxin	• Intestinal haemorrhages • Damage of the kidneys • Pale and fatty liver • Porcine pulmonary oedema (PPE) • Increased water consumption • Fever • Diarrhoea • Blood in faeces and urine • Inflammation of the bladder and kidneys • Decreased performance • Immunosuppression • Pancreatic necrosis • Growth retardation	• Gastroenteritis • Intestinal haemorrhages • Impaired rumen function • Diarrhoea • Ketosis • Milk contamination • Decreased milk production • Mastitis

Mycotoxin	Possible effect on swine	Possible effect on cattle
Deoxynivalenol	• Reduced litter size • Feminisation • Stillbirths • Uterus cancer • Anorexia • Feed refusal	• Impaired thermoregulation • Mastitis and laminitis • Growth inhibition • Ketosis • Intestinal haemorrhages
Ochratoxin	• Intestinal haemorrhages • Damage of the kidneys • Pale and fatty liver • Porcine pulmonary oedema (PPE) • Increased water consumption • Fever • Diarrhoea • Blood in faeces and urine • Inflammation of the bladder / kidneys • Decreased performance • Immunosuppression • Pancreatic necrosis	• Increased water consumption • Increased urination • Permanent scarring of the kidneys
Zearalenone	• Irregular heats • Abortion • Pseudo pregnancy • Low conception rates • Ovarian cysts • Embryonic Loss • Tail necrosis • Nymphomania • Hypertrophy of the uterus • Shrunken udder • Stillbirths	• Irregular heats • Low conception rates • Ovarian cysts • Embryonic loss • Abortions • Low testicular development • Low sperm production

2.2.2 Mycotoxins and health

It is generally believed that mycotoxin exposure among humans and animals is more prevalent in Third World/developing countries, as it is more likely that the implementation of regulations regarding food handling and storage are not enforced.[16]

The first mycotoxin outbreak in Africa was in Ethiopia in 1978 due to *Claviceps purpurea* which is an ergot fungus. The second outbreak was acute aflatoxicosis in Kenya in 1981.[31] This was followed by another outbreak in Kenya in 2001 where 12 people died due to aflatoxicosis. The largest mycotoxin poisoning epidemic in the previous decade

was due to AF contaminated maize in 2004 in Kenya which resulted in 317 infected and 125 fatalities.[31]

Different mycotoxins have different effects on human health.[42] Mycotoxicosis is a result of poisoning by natural means. Symptoms of exposure vary due to the type of mycotoxin, level and duration of exposure, as well as the age, sex and health of the exposed individual. Furthermore, the severity of mycotoxicosis in humans can be enhanced by factors such as vitamin deficiency, energy deprivation, infectious-disease status and alcohol abuse.[16]

Mycotoxins have a range of detrimental effects on human health. It is speculated by some that the death of the first born sons in Egypt in Biblical times was due to mycotoxicoses.[41] Recent studies, and those carried out during the past decades, have demonstrated that fungi producing mycotoxin infestation of crops is a great concern across the African continent.[34]

Mycotoxicosis can be categorized as acute or chronic. The best-known outbreaks of mycotoxicosis were acute cases where there were rapid onsets and obvious toxic effects. Chronic mycotoxicosis, on the other hand, is caused by low-dose exposure over a prolonged period of time, leading to cancer and other irreversible health effects such as stunting.[16]

Clinical studies in Benin, Togo, Gambia, Ghana, Iran and Kenya reported an association between AF exposure and growth impairment (stunting) in children.[44-53] In Kenya, children are weaned with cereals. For this study 242 flour samples were taken together with the height and weight of children being weaned, 29% of the children were regarded as stunted. Of these samples 29% tested positive for aflatoxin contamination, some exceeding regulation limits. Exposure to AF begins early in the lives of many children worldwide. Children may be exposed to AF through maternal food intake *in utero*, breastfeeding, and weaning and post-weaning foods.[22] Detectable levels of serum or urinary AFs were noted in 85% to 100% of children in African countries, such as Gambia, Guinea, Kenya, Benin, Togo, and Senegal.[11, 44-46] Possible health effects of different mycotoxins on human health are presented in Table 2-3.

Table 2-3 Possible health effects of mycotoxins on humans[13, 46, 48]

Mycotoxin	Possible health effects
Aflatoxin	• Liver cancer • Stunting • Immune suppression • Enteropathy • Malabsorption of nutrients • Wasting (severe unintentional weight loss) • Stillbirths • Liver cirrhosis • Jaundice in new-borns • Kwashiorkor
Deoxynivalenol	• Nausea • Vomiting • Kidney damage • Lung cancer
Ochratoxin	• Balkan endemic nephropathy (fatal kidney disease)
Zearalenone	• Lipid peroxidation • Inhibit protein synthesis • Inhibit DNA synthesis • Exert genotoxic effects

2.2.3 Mycotoxins and red meat

Meat products can be contaminated with mycotoxins via two methods. Firstly, meat can become contaminated with mycotoxins if the animal is fed mycotoxin-contaminated feed. Secondly, meat can be contaminated during processing (such as air-dried meat products) and storage.[13]

In the early 1970's, it was documented that AF was found in organs and tissues swine (retention of 0.015 % AFB1) which have been fed AF contaminated feed at.[54] During the early 1980's, a method was developed to detect AF and was successfully applied to beef, pork, chicken meat and offal.[55] Determining mycotoxins in red meat products is different from determining mycotoxins in grain. There are a large number of interfering factors from the meat matrix such as the need to remove small proteins, peptides and phospholipids.[55] These processes differ in meat and grain.

OT can be found in meat products due to consumption of OTA contaminated feed by the animal. OT contamination occurs globally, but Africa and Europe are the regions where

this is a particular concern. Furthermore, it is challenging to analyse OT as it can be found in many food matrices.[56] ZEN was detected in 2001 in Austria in the muscle tissues of swine which have been fed mycotoxin contaminated oats. Liver samples were also taken from the same swine and ZEN was also found in these samples. The degree of glucoronidation for ZEN was 62% in liver and trace amounts were found in the muscle tissue.[57] Muscle tissue contamination of DON is less than that in crops but is a still a cause for concern.[58]

As it is evident from the literature, red meat can become contaminated with mycotoxins via consumption of contaminated diet by the animal the meat was obtained from.[59] Apart from the molecular structure of mycotoxins, various host factors influence the biotransformation and the extent of deposition of residues of mycotoxins in the muscle or organ tissue of the animal.[59]

AFB1 is considered the most hazardous mycotoxin and biotransformation finds place at a hepatic level.[59] It is metabolised by NADPH-dependent cytosolic enzymes resulting in aflatoxicol. Aflatoxicol is considered a non-detoxified and hazardous storage form with a toxicity equal to AFB1. Biotransformation in mammals can also result in AFB1-expoxide which is renowned for its cancerogenity.[59]

The AF metabolite can be inactivated by glutathione S-transferase. This detoxification pathway is important as several species might mediate resistance. Further detoxification might be mediated by the conjugation with sulphates and glucuronic acid.[60] However, studies have shown that the addition of aluminosilicate sorbents to swine feed results in a decrease of AFB1 in muscle tissue, but not in the liver or kidney.[61]

Second to AF, OTA is the most-studied mycotoxin with regard to foodstuff contamination. Ochratoxin can be detected in the blood plasma for a prolonged period of time due to its extended half-life period in several species.[62] OT has been detected in the kidneys and muscle tissue of swine, yet the concentration found in blood was much higher. This poses a threat to meat products such as sliced meat loaves and sausages, as some of these products include additives of pig blood or plasma. Ochratoxin A is also heat stable, thus raw and processed foodstuffs pose a threat of contamination. The extended retention period of ochratoxin is due to a strong serum albumin binding of ochratoxin A and the low proportion of free-floating toxins. [92] Bacterial metabolism in the gastrointestinal tract

of the rumen (of cattle and sheep) yields the less-toxic product ochratoxin α, a metabolite of ochratoxin A. No measurable transfer of ochratoxin A could be found in full-grown animals with healthy, well-developed digestive systems.[62]

In contrast with the extended retention of OT, ZEN undergoes fast biotransformation. This quick biotransformation in the animal, in combination with speedy excretion, via either bile or urine, significantly lowers the risk of contaminated meat or meat products. [59] The flexible molecular conformation of ZEN mimics natural 17β-oestradiol actions after binding to oestrogen receptors of target cells. The high oestrogen concentration in poultry blood has been one of the hypotheses why poultry is not very susceptible to ZEN.[63] ZEN is often combined with trichothecenes, due to its fungal origin, which brings to attention the consideration of underlying synergistic effects.[59]

DON is regarded the main fungal contaminant in wheat and maize in Canada, the United States of America, England and Southern Africa.[59] The epoxidal ring structure of DON is responsible for its toxicity and biological activity. The biotransformation occurs via hydrolysis, hydroxylation, glucuronidation and de-epoxidation. De-epoxidation is regarded as the best elimination pathway.[59] In rumen, DON losess toxicity due to de-epoxidation during the uptake stage. The metabolism of DON might be altered by the degenerative process in ruminal mucosa, seen in subacute ruminal acidosis.

2.2.4 Mycotoxins and the environment

Many environmental factors affect mycotoxin contamination as summarised in Table 2-4 Climatic conditions such as temperature and available moisture in the atmosphere are contributing factors both at pre- and post-harvest level.

Table 2-4 Factors affecting mycotoxin contamination in the food chain[64]

Factor	Example
Physical factors	• Moisture • Relative humidity • Temperature • Mechanical damage to crop post-harvest
Chemical factors	• Carbon dioxide • Oxygen • Composition of pesticides and fungicides
Biological factors	• Plant variety • Stress • Insects • Spore load

Mycotoxins produced by different fungal species require different optimal growth temperatures. AF producing fungi have the highest optimal growth temperature at 33 °C. DON and OTA can be produced by two fungal species with two different optimal growth temperatures.[65] It is clear that temperature is important when the growth of fungi producing mycotoxins is considered. This can be seen in Table 2-5.

Table 2-5 Optimal growth temperatures of fungi producing mycotoxins[66-68]

Fungal Species	Mycotoxin	Optimal growth temperature (°C)
Aspergillus flavus	Aflatoxin	33
Fusarium verticillioides	Fumonisin	15 to 30
Fusarium graminearum	Deoxynivalenol	30
Fusarium graminearum	Zearalenone	12 to 18
Fusarium culmorum	Deoxynivalenol	26
Aspergillus ochraceus	Ochratoxin A	25 to 30
Penicillium verrucosum	Ochratoxin A	25

Furthermore, it is known that high levels of rainfall at the time of (or near) harvesting in warm regions leads to high concentrations of AF in many crops.[66] Lewis *et al.* reported that semi-arid to arid and drought conditions in tropical countries are also associated with higher levels of contamination.[67] High temperatures and high humidity are known to favour AF contamination post-harvest.[68]

Aspergillus ochraceus has a lower optimal growth moisture limit of between 15.5 and 16% in cereal grains, whereas *Aspergillus flavus* and *Penicillium verrucosum* have a higher optimal growth moisture limit of 17 to 18% and 16.5 to 20%, respectively.[68]

Soil moisture is often defined as the water content in the upper several meters of soil that is available for plant growth. The latest studies from Zurich have come to the conclusion that on average it rains most on days with high soil moisture. This is explained as moisture in the soil, the more water can evaporate, which increases the likelihood of precipitation.[69]

2.2.5 Mycotoxins: the South African picture

Mycotoxin contamination in cereals and peanuts has been studied and documented in South Africa. During 2001, peanut butter used in the school feeding scheme was found to be contaminated with levels of 271 µg/kg AF.[34] However, there is little data available in the public domain in South Africa with regard to mycotoxin levels in animal products, specifically with reference to meat. These foods are used to add variety to cereal-based staple diets. They represent a significant route of exposure for humans.[70]

In 2011, an outbreak of aflatoxicosis caused the deaths of over 220 dogs after the consume AF contaminated dog food. Concentrations ranged from below the limit of quantification (< 5 mg/kg) to 4946 mg/kg in 124 samples tested.[71] The South African government is currently regulating undesirable substances in animal feeds including the level of mycotoxins in some food products. Table 2-6 depicts the maximum content that is allowed in animal feed as regulated in Act no. 36 of 1947 on fertilizer, farm feeds, agricultural remedies and stock remedies.[72] The need for better regulation and control became evident after these reported incidents.

Table 2-6 Maximum allowable levels of mycotoxins in animal feed[72]

Substance	Farm feed	Maximum contents in mg/kg (ppm) in a farm feed with a moisture content of 120 g/kg
Aflatoxin B1	Feed ingredients	0.05
	Feed ingredients with the exception of groundnut, palm-kernel, cotton seed, maize and products derived from the processing thereof	0.02
	Complete farm feeds for cattle, sheep and goats	0.05
	Complete farm feeds for dairy cattle	0.005
	Complete farm feeds for calves and lambs	0.01
	Complete feeds for pigs and poultry (except young animals)	0.02
	Other complete farm feeds (including pets)	0.01
	Maize products intended for feedlot	0.3
	Supplement/concentrates for cattle, sheep and goats (except for dairy animals, calves and lambs)	0.05
Fumonisin B1	Horses and pets	5
	Pigs	10
	Beef and poultry	50
	Fish	10
Ochratoxin A	Feeding stuffs on full ration basis for pigs	0.05
	Feeding stuffs on full ration basis for poultry	0.2
Zearalenone	Feeding stuffs on full ration basis for sows and pigs	5
	Feeding stuffs on full ration basis for piglets	3
	Feeding stuffs on full ration basis for calves and dairy cattle	0.5

On the other hand, the restrictions on foodstuff suitable for human consumption are far less extensive. The Foodstuffs, Cosmetics and Disinfectants Act of 1972 only regulates AFB1, AFM1, ergot fungus and patulin.[39]

This Act states that foodstuffs are regarded contaminated, impure or decayed if:

- peanuts intended for further processing contain more than 15 µg/kg of total AF,
- all foodstuffs ready for human consumption contain more than 10 µg/kg of total AF, of which AFB1 is more than 5 µg/kg,
- milk that contains more than 0.05 µg/L of AFM1,
- wheat, rye, barley and oats which contain more than 0.05% (mass/mass) of *Ergot sclerotia,* and
- apple juice and apple juice ingredients in other beverages that contain more than 50 µg/L of patulin.[39]

It has been recommended that the South African government should equally regulate deoxynivalenol in maize and wheat flour, ochratoxin A in foodstuffs such as coffee and dried fruits, and fumonisins in maize-based products.[73] Reasons put forward for why mycotoxins legislation is excluded from foodstuffs include:

- the ignorance of farmers, the public, manufacturers and government about the existence of mycotoxins,
- the absence of regulations,
- 'dumping' of low-grade food on developing countries, and
- the consumption of contaminated products during food shortages.[73]

Obstacles faced in mycotoxin control are *inter alia* the cost associated with the monitoring programme, the level of training required for analysing the samples, as well as access to specialised equipment.

2.3 Heavy metals

Metals can be classified into four groups with regard to their health impact, namely micronutrients, essential, non-essential and toxic. Micronutrients such as copper, zinc, iron, calcium, magnesium and chromium are beneficial to human health.[75] It must be noted that these metals can become toxic when consumed in high amounts.[75, 76] Barium,

aluminium, lithium and zirconium are non-essential to human health, whereas tin and aluminium are regarded as less toxic.[77] The four heavy metals that are regarded as highly toxic are arsenic, cadmium, lead and mercury.[77]

Metals such as calcium, iron, copper, magnesium, zinc, cobalt and manganese are vital to life, although cells only need them in miniscule amounts. Table 2-7 depicts the beneficial metals, their benefits and dietary sources.

Table 2-7 Beneficial metals, their benefits and dietary sources[78]

Heavy metal	Metabolic need	Dietary source
Calcium	Critical for proper muscle and nerve function	• Dairy products • Broccoli • Figs • Sardines
Iron	Aids with the transportation of oxygen in the blood of the human body	• Meat • Beans • Spinach
Copper	Mops up dangerous highly reactive chemicals that have been linked to an increased risk of cancer and heart disease	• Lobster • Crabs • Beans • Nuts
Magnesium	Aids muscle contraction and relaxation	• Dark green leafy vegetables
Zinc	Plays a vital role in the tertiary structure of proteins. Important for controlling gene activity and regulating hormones	• Oysters • Chickpeas • Whole grains • Nuts
Cobalt	Forms the core of vitamin B12 and is important in the body for producing red blood cells	• Meat • Dairy products • Leafy green vegetables
Manganese	Breaks down fats, carbohydrates, and proteins in order to convert food into energy	• Whole grains • Cereal products

Heavy metal contamination of food, including animal source foods, is increasingly becoming an important aspect of food safety.[79] Heavy metals are defined as metals that have a specific weight of more than 5 g/cm^3.[80] These metals are regarded as

environmental pollutants due to their possible toxic effects on plants, animals and humans. Pollution may be the result of natural or anthropogenic sources.

2.3.1 Heavy metals and health

Heavy metals such as arsenic, cadmium, lead and mercury are not metabolised or broken down in the environment and thus accumulate in the food chain. They pose a number of threats to human health as some are potent carcinogens and or are mutagenic.[81] Table 2-8 shows some of the possible effects that lead, arsenic, cadmium and mercury can have on animal and human health when consumed.

Table 2-8 Health impacts of consumption of lead, arsenic, cadmium and mercury[82-86]

Heavy metal	Impact on animal health	Impact on human health
Lead	• Colic • Abortion • Opisthotonos • Salivation • Lacrimation • Paralysis	• Coma • Convulsions • Reduced IQ in children • Reduced attention span in children • Stunting
Arsenic	• Low body weight • Bloody diarrhoea • Hind limb paralysis • Blindness • Sloughing of skin	• Vomiting • Abdominal pain • Diarrhoea • Carcinogenic • Neurotoxicity • Diabetes • Pulmonary disease • Cardiovascular disease
Cadmium	• Anaemia • Retarded growth • Hyperphosphatemia • Osteoporosis • Loss of bone density • Kidney stones	• Bone disease • Lung impairment • Kidney dysfunction • Carcinogenic
Mercury	• Stomatitis • Pharyngitis • Diarrhoea • Blindness	• Insomnia • Memory loss • Cognitive and motor dysfunction • Kidney failure

2.3.1.1 Lead

Lead poisoning is of particular concern among young children, as it accumulates in the human body and affects multiple body systems. As with arsenic, lead is found in the Earth's crust and poses a significant public health problem around the world. Malnutrition is also of concern as the body absorbs more lead if other nutrients such as calcium are lacking in the diet. The WHO has identified lead as 1 of 10 chemicals of major public health concern as no amount of lead exposure is considered safe.[83]

A big health concern regarding lead exposure is the lead found in paint. Children that live in homes that were painted with lead containing paint showed blood lead concentrations of 20 µg/dℓ. The acceptable level of lead in blood is below 5 µg/dℓ. Exposure to lead can occur through inhalation of contaminated air or of ingestion of contaminated foods.[87, 88] The absorption of lead by the human body is however influenced by factors such as age and physiological status. It is estimated that absorption of lead through contaminated water can be as low as 35% for adults, yet 50% for children.[89] The human body absorbs lead firstly in the kidneys, then the liver and the rest is absorbed by other soft tissue such as the heart and brain.[89] Some of the early symptoms of lead poisoning of the central nervous system, which is the most vulnerable, include headaches, irritability and loss of memory.[88, 90] Prenatal exposure to lead has been found to be associated with reduced weight, preterm delivery and neuro-developmental abnormalities in the offspring.[24]

2.3.1.2 Mercury

Mercury is released from the Earth's crust into the environment from volcanic activity, weathering of rocks and human activities such as coal-fired power stations, industrial processes and waste incinerators. Toxicity of mercury varies in its various forms, organic and inorganic. Also varied are the effects on the human nervous system, digestive system, immune system, lungs, kidneys, skin and eyes. Exposure to mercury can adversely affect the development of the brains and nervous systems of babies.[74]

2.3.1.3 Arsenic

Inorganic arsenic is a component of the earth's crust and is naturally present at high levels in ground water across the globe. Chronic exposure to arsenic from drinking water, or food and/or crops that are irrigated with contaminated water, can lead to cancer and skin lesions. Well water in Bangladesh has widely been contaminated with arsenic, and

despite efforts to lower these levels, it is believed that 20 to 45 million people are still at risk of being exposed to arsenic contaminated water. Defining the disease caused by arsenic exposure proves to be difficult, as symptoms and signs vary among individuals, population groups and geographical areas.[82]

Numerous epidemiological studies have found relationships between arsenic exposure and cancer and other systemic health effects. All organs including cardiovascular, dermatological, nervous, hepatobiliary, renal, gastro-intestinal and respiratory systems are affected by arsenic exposure. Higher mortality rates due to cancer have been found in areas of high arsenic exposure.[91]

2.3.1.4 Cadmium

Cadmium enters the food chain through crops that are grown in contaminated soil. It is generally regarded that only between two to six percent of ingested cadmium is absorbed by the human body.[84] This absorption varies in accordance to the iron status of the individual and the form of cadmium in the consumed food. Cadmium can accumulate in the human kidney for 20 to 30 years and is known to produce respiratory tract problems and bone disease.[84] Vahter *et al* (1996) found that the nutritional status of individuals consuming cadmium rich foods, such as sunflower seeds, is a better determinant of cadmium exposure rather than the cadmium content in the foodstuff itself. The study found that women who are iron deficient show an increased uptake of cadmium from food.[92]

The route to human exposure includes cigarette smoke and ingestion of contaminated food. Absorbing cadmium through skin contact is rare.[93, 94] Cadmium is, however, present in trace amounts in foods such as leafy vegetables, potatoes, grains, liver, kidney and crustaceans.[95]

Various epidemiologic studies have shown an association between chronic exposure to low level cadmium and osteoporosis.[96-98] Cadmium levels in the human body can be tested in both urine and blood samples. However, blood tests for cadmium only show recent exposure, whereas urine samples will show the accumulation of cadmium in the human body.[99] Cadmium is regarded as a pulmonary and gastrointestinal irritant, which can be fatal. Acute exposure can include abdominal pain and vomiting.[100] Chronic

exposure to cadmium can lead to depressed levels of neurotransmitters such as norepinephrine, serotonin and acetylcholine. [101]

2.3.2 Heavy metals and red meat

Meat can become contaminated with heavy metals through direct exposure, drinking of polluted water or plants used as animal feed or feed component.[102, 103] Kan and Meijer reported that when an animal is exposed to heavy metals through diet, kidney rather than muscle tissue will show an increased level of heavy metals.[104] During 2003 in Spain, the kidney and liver tissues of animals were shown to contain high levels of toxic metals such as cadmium and lead. Levels of 59.6 µg/kg cadmium and 28.0 µg/kg lead were found in liver samples and 318 µg/kg cadmium and 20.2 µg/kg lead were found in kidney samples.[105]

It was found in Jamaica (in 2007) that the kidneys of cattle that were exposed to higher levels of cadmium and lead through diet, accumulated higher levels of these elements in the liver, and less in muscle tissue. Levels of 0.162 µg/g dry weight of lead and 10.1 µg/g dry weight cadmium was found in liver samples and 0.523 µg/g dry weight of lead and 6.71 µg/g dry weight cadmium was found in kidney samples.[106] Thus, consumption of the liver, kidney and bones of animals exposed to heavy metals through the diet poses a threat to human health and these parts should be discarded.[12]

MacLachlan, *et al* (2016)[107] analysed muscle, liver and kidney samples of 152 Australian sheep from different states for various trace elements including lead, mercury, arsenic and cadmium. The results are shown in Table 2-9.

From Table 2-9, it is clear that traces of lead were found in the kidney, liver and muscle tissues of Australian sheep. However, maximum acceptable levels of lead in sheep meat according to CODEX is 0.1 mg/kg, thus all samples were below the acceptable limit.

Mercury limits according to Codex is only regulated for water (0.001 mg/kg), salt (0.1 mg/kg) and fish (0.5 mg/kg).[108] The results reported in Table 2-9 indicate that only kidney samples had evident traces of mercury.

Table 2-9 Heavy metals (mg/kg) in Australian mutton and CODEX regulation[107, 108]

	Lead	Mercury	Arsenic	Cadmium
Kidney	0.057	0.01	0.01	0.85
Liver	0.04	0.00	0.01	0.28
Muscle tissue	0.01	0.00	0.01	0.00
CODEX acceptable levels	0.1	0.5 (for fish)	0.1 (for pork fat)	Not regulated for meat

Table 2-9 shows traces of arsenic in liver, kidney and muscle tissue (flank) of Australian mutton.[107] According to CODEX, the highest acceptable values for arsenic in pork fat is 0.1 mg/kg.[108]

Cadmium levels in red meat is not regulated by CODEX.[108] All levels of cadmium in sheep kidney and liver were below 1 mg/kg according to the results shown Table 2-9.[108]

2.3.3 Heavy metals: the South African picture

The South African Foodstuffs, Cosmetics and Disinfectants Act, 1972 regulates antimony, arsenic, cadmium copper, lead, mercury, tin and zinc in food products. Table 2-10 depicts the maximum levels for these heavy metals in red meat and processed meat.[109]

Table 2-10 Maximum allowable levels of arsenic, cadmium, lead and mercury in red meat and processed meat[72]

Heavy metal	Foodstuff	Maximum levels (mg/kg)
Arsenic	Meat and processed meat	1
Cadmium	Meat and processed meat	0.5 (level does not apply to liver and kidneys)
Lead	Meat and processed meat	0.1
	Meat and fat of poultry	0.1
	Cattle, edible offal	0.5
	Pig, edible offal	0.5
Mercury	N/A	Not regulated
Tin	Canned foods (including meat products)	250
	All uncanned meat and meat products	50

Heavy metals in animal source food, such as meat, is a possible concern in South Africa. In 2012, Ambushe *et al*[12] assessed levels of vanadium, chromium, manganese, strontium, cadmium, lead and uranium in bovine meat sourced from polluted areas in the North West province of South Africa. The study investigated levels of these metals in muscle, liver, kidney, fat and bone from bovine carcasses sampled at an abattoir in Tlokwe Local Municipality (now JB Marks). In that study, 0.51 and 0.16 mg/kg cadmium were recovered from kidney and liver samples respectively, as well as 0.23 and 0.11 mg/kg lead in kidney and liver samples respectively.[12] This is much higher than the maximum level for lead as per regulations. Currently, cadmium in meat is not regulated, but compared to the maximum level allowed for fish (2.0 mg/kg), it seems to be within acceptable levels.

2.3.4 Heavy metals and the environment

Heavy metals are released into the atmosphere via industrial emissions and the burning of fossil fuels. These emissions are captured in the atmosphere and brought down by rain to contaminate rivers, soil and ground water. This creates a pathway for plants to

become contaminated. Figure 2-2 illustrates a possible environmental pathway through which animals and crops can be exposed to heavy metals.

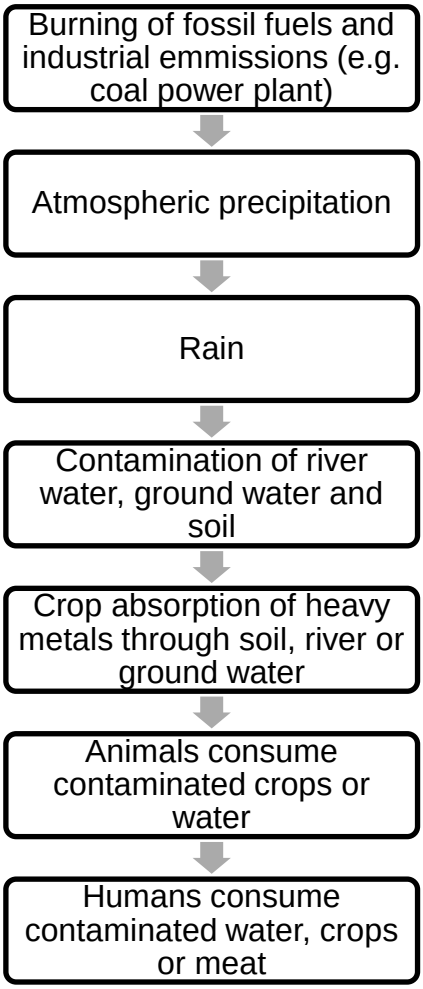


Figure 2-2 Simplified environmental pathway of heavy metal contamination in the food chain[110]

As is summarised in Figure 2-2, crops become contaminated with heavy metals due to various factors such as rainfall and the burning of fossil fuels. The heavy metals in the environment that end up in the feed of animals are of great concern since they can be compounded in meat. When high levels of heavy metals are found in the meat, organs and tissues of animals, there is a high risk that drinking water, as well as staple crops, will also be contaminated.[102,111]

2.4 Meat and the Sustainable Development Goals

The UN's 17 Sustainable Development Goals (SDGs), also known as the Global Goals, are a universal call by the United Nations with the aim to end poverty, protect the planet, and ensure that all people enjoy peace and prosperity. The 17 SDGs were put in place in January 2016 to replace the Millennium Development Goals.[110] The second goal entitled "End hunger, achieve food security and improved nutrition, and promote sustainable agriculture" aims, among other things, to end all forms of malnutrition by 2030. This target includes the internationally agreed upon target to end stunting in children under five years of age by 2025.[112]

Krebs, *et al* [113] (2011) found that the regular consumption of meat was associated with a significantly reduced risk for stunting. Furthermore, it was stated by this group that the irregular consumption of meat products increased the risk of all macronutrient deficiencies. Introducing red meat as complementary feeding can be nutritionally beneficial to older infants. Not only the micronutrients (such as zinc, iron and vitamin B^{12}) provided by meat are beneficial, but also the macronutrients such as protein.

Furthermore, the inclusion of meat in the diets of infants and toddlers has been recommended by the WHO.[114] Therefore, contamination levels of possible toxic substances indicated as possible factors that cause stunting, need to be monitored more extensively.

2.5 Conclusion

Mycotoxins and heavy metals both pose a health risk to humans when consumed through food in the diet. Not only are mycotoxins and heavy metals regarded as carcinogenic, but consumption of both have been linked to stunting which is a public health concern in South Africa. Furthermore, mycotoxins such as AF, OTA, DON and ZEN, and heavy metals such as cadmium, lead, arsenic and mercury, have been found in muscle and organ tissue of animals. Currently, the threat posed by these toxic contaminants in combination through the consumption of red meat is unknown, suggesting the need for this study to investigate the co-occurrence of these two groups of substances.

Both mycotoxins and heavy metals pose a health threat to animals and humans. One of these health threats is stunting. Only lead and aflatoxin have been positively linked to stunting in children. However, other heavy metals and mycotoxins have been linked to various growth retardations in animals. As stunting is not only a growth-related problem but also affects future mental development in children, possible pathways to toxic substances need to be examined. By identifying which foodstuffs pose a high or possible threat of contamination, governments can educate people in order to prevent possible exposure.

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CHAPTER THREE

OCCURRENCE OF MYCOTOXINS IN RED MEAT

3.1 Introduction

Mycotoxin-producing fungi are natural toxins that occur in the air and soil. They are classified as low molecular weight natural products produced as secondary metabolites by filamentous fungi.[1] Occurrence is more likely when plants are exposed to stress conditions such as insect damage, low soil fertility and extreme weather conditions.[2]

It is generally accepted that both humans and animals in Third World countries are more frequently exposed to mycotoxins, as it is more likely that the implementation of regulations regarding food handling and storage are not enforced.[1] The largest mycotoxin-poisoning epidemic in the previous decade (in 2004) was caused by AF- contaminated maize in Kenya.[3]

Given the temperate and humid conditions in large parts of Africa, coupled with inadequate harvesting methods and storage and transportation systems, the African continent offers a wide array of characteristics that create an enabling environment for mycotoxin-producing moulds to grow, as already discussed. [3]

Together with climatic conditions for moulds to grow and produce mycotoxins, it is also important to note that food with visible mould is often consumed in Africa. This is due to the fact that food is scarce and storage conditions are inadequate to protect crops and grains from fungal infection.[4] With this in mind, it can also be assumed that animals are also fed contaminated feeds. Some African governments have implemented regulations in an attempt to control mycotoxins, especially AF contamination.[4] These regulations, however, are mostly not implemented and actively controlled in rural areas where food is not produced for national food supplies but for own consumption.[4]

Different mycotoxins have different effects on human health. For example, AF damages the liver and represses the immune system.[5] Repressing the immune system gives way for other diseases and in practice only these get diagnosed and treated.[5] Furthermore, some sources suggest a connection between mycotoxin consumption and linear growth

retardation (stunting), as well as a connection between mycotoxin consumption and protein energy malabsorption.[6] Due to the high prevalence of stunting in developing countries such as South Africa, research needs to be conducted on the relationship, if any, between these conditions and the consumption of mycotoxins.

South Africa is considered an example of a country experiencing nutrition transition, where under-nutrition and over-nutrition increasingly co-exist. At national level, stunting is by far the most common nutritional disorder, affecting 15.4% of children, with the highest prevalence in informal rural areas.[7-9]

Studies have shown that the prevalence of stunting would be reduced by an estimated 20% by the following three measures: First, if better hygiene is practised, second if complementary feeding is improved, and third if a multiple micronutrient supplement is provided during pregnancy. The remaining causes of growth retardation remain unclear. These may include, among others, exposure to toxic substances, some of which are administered to the human body through food. Current research suggests that mycotoxin contamination is a likely cause of linear growth retardation in children.[10]

However, there is little data available in the public domain in South Africa with regard to mycotoxin levels in animal products, specifically with reference to meat. These foods represent a significant route of exposure for humans. On the other hand, the WHO has recommended that complementary feeding with red meat could possibly reduce stunting due to the nutritional benefits.[11]

Offal such as liver and kidney, as well as milk and eggs, are often the only protein sources in the diets of rural communities. The 1999 National Food Consumption Survey reported in their 24-hour recall and food frequency questionnaire, that milk and milk products were consumed by 45% of 1 to 5-year-olds and by 21% in the 10-year-old and above age group. [7] The meat and offal group of foods was consumed by 36 to 38% of 1 to 5 and 6 to 9-year-olds respectively, and by 50 to 51% in the older group. Eight to 14% of participants consumed an egg product. Between 40% and 60% of the children consumed vegetables and 12 to 18 % consumed fruit.[7]

Mycotoxins of particular concern, from the human health perspective, include the AF's from *Aspergillus*, OT from *Aspergillus* and *Penicillium*, DON and fumonisins (FB's), the

trichothecenes and ZEN from *Fusarium* species.[12] These fungal species are prevalent in food crops, particularly maize, giving rise to contaminated animal feed.[1] Meat from animals fed on contaminated feed and residues of mycotoxins are more likely to be contaminated. DON and OTA have been proven to remain stable during high temperatures (such as the cooking process), thus cooked meat can still contain a threat.

The objectives of this chapter are to determine the levels of mycotoxins in South African red meat obtained from rural areas and to establish a correlation between a few selected environmental factors, including weather conditions, and the incidence of mycotoxins in raw and cooked red meat.

3.2 Materials and methods

The prevalence and levels of mycotoxins in South African meat were determined based on findings from two separate studies.

3.2.1 Mycotoxin quantification – DAFF study

The first data set was from a monitoring study on bovine, ovine, poultry and porcine liver and kidney samples for mycotoxins (OT, AF and ZEN) analysed at the Veterinary Public Health, Department of Agriculture, Forestry and Fisheries (DAFF).[13] The data collected over a period of four years (from 2012 to 2016) in all nine provinces of South Africa was used. The meat samples were either pooled or single-sampled by a state veterinarian in the respective districts of sampling. The mycotoxin analyses were performed by the Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI) laboratory. ARC-OVI tested for AF, OT and ZEN in the samples with high performance liquid chromatography (HPLC). AF was tested according to method number RAFLA063 and OT and ZEN according to method numbers ROCHRA064 and RZEAR062, respectively.

3.2.2 Mycotoxin determination and quantification – University of Pretoria

The second data set was obtained from a current research project by the UP.[14] The project determined mycotoxin levels (AF, OT, ZEN and DON) in four cuts (two offal and two meat cuts) of two species (bovine, n = six and porcine, n = three).

3.2.2.1 Sampling

Four samples were taken from each carcass (n = four). Two organ samples (liver and kidney) were taken, one meat sample from the fore quarter (chuck) and one from the hind quarter (loin/thin flank). One kilogram of each was sampled. Porcine samples were collected from three animals from the Vhembe District in Limpopo (n = three). Bovine samples were collected from two regions, three animals in the Vhembe District, Limpopo and three animals from Transkei region, Eastern Cape (n = three/region). The samples were bought at registered abattoirs by a trained fieldworker. All the samples collected (n = 36) came from free-ranging animals of subsistence farmers which were not fed controlled feed. All animals were slaughtered during the summer of 2017. Once bought samples were frozen and kept at –4 °C to ensure the integrity of the samples. The frozen samples were transported overnight to the UP.

3.2.2.2 Sample preparation

1. The frozen samples were thawed overnight at room temperature. Chuck and loin/thin flank were deboned, cubed and minced. The liver and kidney samples were dissected to remove thick veins and tubes, then cubed and minced.
2. The bovine samples were halved in order to create 48 samples of 24 identical pairs.
3. One of each bovine sample pair (n = 24), as well as all porcine samples (n = 12) were packed in airtight freezer bags, labelled, and frozen for storage and transportation to the analytical laboratory.
4. The other bovine samples (n = 24) were cooked at 180 °C to an internal temperature of 70 °C. The cooked samples were weighed, packed in airtight freezer bags, labelled, and frozen for storage and transportation to the analytical laboratory.

3.2.2.3 Mycotoxin analyses

All samples were analysed by SGS Agri Food laboratory in Cape Town using their routine methods. Mycotoxins were tested with VICAM test kits for HPLC according to the following principles optimised for the specific laboratory conditions.

AF was tested using the AflaTest WB which is a quantitative method for AFB1, AFB2, AFG1, AFG2 and total AF. Firstly, samples were ground and weighed. Then samples

were mixed with an extraction solution (salt, methanol and water), blended and filtered. The extract was then applied to the AflaTest WB column bound with specific antibodies to which the AF binds. The column was washed to remove any impurities from the immunoaffinity column. Lastly, methanol was passed through the column to remove the AF from the antibodies and the methanol solution was injected into an HPLC system.[15] The Limit of Quantification (LOQ) was 1 µg/kg for AFB1, B2, G1, and G2 and 4 µg/kg total AF.

DON was tested with DONtest WB, a quantitative method to test samples for the presence of DON in parts per million. Samples were prepared by mixing with an extraction solution, blended and filtered. The extract was then applied to the DONtest WB column which contained DON antibodies to which the mycotoxin bound. The column was washed to get rid of any impurities from the immunoaffinity column. An eluting solution was passed through the column to remove DON from the antibodies and this eluting solution was then injected into and HPLC system.[16] The laboratory LOQ is 100 µg/kg for DON.

OTA was tested with OchraTest WB, a quantitative method for the detection of OTA in a variety of commodities. Samples were prepared by mixing with an extraction solution followed by blending and filtering. The extract was then added to the OchraTest WB column which contains OT antibodies. OT then bound to these antibodies. The column was then washed to rid the immunoaffinity column of impurities. Methanol was passed through the column to remove the OT from the antibodies and the methanol was injected to the HPLC system.[17] The laboratory LOQ is 1 ug/kg for OTA.

ZEN was tested with ZearalaTest WB, a quantification method for the detection of ZEN using an HPLC. The samples was prepared by mixing with an extraction solution that consisted of acetonitrile water (9:1, v/v), blending and filtering. The extract was applied to the ZearalaTest WB column which contained antibodies to which the ZEN bound. The column was washed to rid it of impurities. Methanol was passed through the column to release the zearalenone from the antibodies and the methanol was injected into the HPLC system.[18] The laboratory LOQ is 20 ug/kg for zearalenone.

This data set was analysed and discussed qualitatively.

3.2.3 Environmental factors – climatic conditions

Climatic conditions that are prone to influence mycotoxin contamination in plants are temperature and rainfall. Data for climatic conditions were investigated as follows: Historic data on temperature and rainfall was requested from the South African Weather Service (SAWS) for the areas where and dates within which the DAFF and the University of Pretoria performed sampling for mycotoxin analysis.

The data sets obtained from the DAFF and from the University of Pretoria, together with the weather data for the test periods, were discussed qualitatively. Furthermore, the weather data for the test periods for the University of Pretoria was analysed quantitatively to determine whether there was a statistical difference in the weather elements over a five-year period. The weather data was obtained for a five-year period (2013 to 2017) and a one-sample t-test ($\alpha = 0.05$) performed using Stata 14[19] to determine whether the average for the five-year period is significantly different to that of the average for the years 2005 to 2015. Table 3-1 depicts the null hypotheses.

3.3 Results and discussion

3.3.1 DAFF data set

The full data set is attached as Table 6-1 in annexure A.[13] From Table 3-1 and Table 6-1, it can be seen that all tests reported negative for all three mycotoxins across all species and samples. What is further apparent from Table 6-1 is that favourable conditions for growth of AF producing fungi including high temperatures and high rainfall[12] were observed in many cases. Temperatures close to or above the optimal growth temperature for AF of 33 °C[20], and an average rainfall of more than 1 mm per day were observed in five cases.

These incidences were 1) in November 2013, Lejweleputswa (Free State) had an average daily temperature of 31.24 °C and an average daily rainfall of 3.14 mm, 2) in December 2013 at Siyanda (Northern Cape), an average daily temperature of 35.03 °C and an average daily rainfall of 1.06 mm were noted, 3) in Bloemfontein (Free State) in January 2014 with an average daily temperature of 32.07 °C and an average daily rainfall of 1.8 mm, 4) in February 2014, Camperdown (KwaZulu Natal) and Modimole (Limpopo)

both showed favourable temperatures of 31.17 °C and 33.57 °C, respectively, and average daily rainfall of 4.11 mm and 3.61 mm respectively, 5) in December 2014, Waterberg (Limpopo) had an average daily temperature of 30.36 °C and an average daily rainfall of 5.55 mm. Despite the fact that ideal climatic conditions were observed none of the meat samples tested positive for AF contamination during the monitoring period (2012 – 2015).

Table 3-1 Summary of mycotoxin, rainfall and temperature data from the monitoring and evaluating programme by DAFF (2012-2015)

Species	Province	Rainfall range (mm)	Temp range (°C)	Ochratoxin	Aflatoxin	Zearalenone
Poultry Bovine Ovine	Northern Cape	0-1.67	1.81-35.03	Negative	Negative	Negative
Poultry Bovine Ovine	Western Cape	0-6.05	1.82-32.19	Negative	Negative	Negative
Poultry Bovine Ovine	Eastern Cape	0.07-4.88	0.55-32.07	Negative	Negative	Negative
Poultry Bovine Ovine	Gauteng	0-2.45	3.59-31.19	Negative	Negative	Negative
Poultry Bovine Ovine	Limpopo	0-9.91	9.49-33.57	Negative	Negative	Negative
Poultry Bovine Ovine	Mpumalanga	0.04-2.4	8.58-29.32	Negative	Negative	Negative
Poultry Bovine Ovine	North West	0-17.47	1.81-32.26	Negative	Negative	Negative
Poultry Bovine Ovine	Free State	0-5.24	0.55-32.07	Negative	Negative	Negative
Poultry Bovine Ovine	KwaZulu Natal	0.11-5.16	2.54-32.13	Negative	Negative	Negative

Aspergillus ochraceus and *Penicillium verrucosum,* which are the fungal species responsible for OT contamination, both have a lower optimal growth temperature of 25 to 30 °C and 25 °C, respectively.[20] These temperatures, paired with an optimal growth

moisture limit of between 15.5 to 16% and 16.5 to 20%, create an optimal for OT producing fungi to grow.[12]

Average maximum daily temperatures between 25 °C and 30 °C with and average daily rainfall of more than 1 mm were observed a number of times during the sampling periods as reported in Table 6-1.

These incidences were 1) in December 2012 at Heidelberg (Gauteng), an average maximum daily temperature of 26.93 °C and an average daily rainfall of 3.95 mm were recorded 2) Middelburg (Mpumalanga) received 1.05 mm average daily rainfall and recorded an average daily maximum temperature of 29.32 °C in February 2013 3) in March 2013 Amathole (Eastern Cape) reported an average daily rainfall of 3.14 mm and recorded an average daily maximum temperature of 28.36 °C 4) during November 2013 Ehlanzeni (Mpumalanga) received 2.4 mm average daily rainfall with a recorded average daily maximum temperature of 26.28 °C 5) in January 2014 these conditions were observed in Ditsobotla and Ngaka Modiri Molema, both in the North West, as well as in Mopani (Limpopo) and Vrede (KwaZulu Natal) 6) Joe Gqabi (Eastern Cape) reported average daily rainfall of 4.72 mm and average daily maximum temperature of 25.61 °C in February 2014, 7) Amatole (Eastern Cape) and Mooi River (KwaZulu Natal) both reported favourable conditions with average daily rainfall of 3.88 mm and 1.78 mm respectively, and an average daily maximum temperature of 27.26 °C and 26.75 °C respectively in February 2014, 8) Swartruggens (North West) received an average daily rainfall of 2.58 mm and recorded daily average maximum temperature of 27.84 °C in November 2014, 9) December 2014 showed favourable conditions in both Lesedi (Gauteng) and Mopani (Limpopo), 10) during 2015, favourable conditions occurred twice in February, in Harry Gwala (Eastern Cape) and Camperdown (KwaZulu Natal), with an average daily maximum temperature of 26.06 °C and 27.92 °C respectively, and an average daily rainfall of 4.88 mm and 5.16 mm respectively, 11) In 2015 Mangaung (Free State) reported an average daily maximum temperature of 28.39 °C and an average daily rainfall of 2.54 mm. Despite the fact that ideal climatic conditions were observed none of the meat samples tested positive for OTA contamination during the monitoring period (2012 – 2015).

Fusarium graminearum produces both ZEA and DON. However, the optimal growth temperature for the production of ZEA is 25 °C, which is in contrast with the optimal growth temperature of 29 to 30 °C for DON production by fungi.[15]

This optimal temperature for ZEN was observed seven times as reported in Table 6-1 namely 1) in May 2012 Delmas (Gauteng) recorded an average daily maximum temperature of 24.71 °C, 2) Cullinan (Gauteng) recorded an average daily maximum temperature of 24.64 °C in August 2012, 3) Wolwehoek (Free State) reported an average daily maximum temperature of 24.83 °C in December 2012, 4) Joe Gqabi (Eastern Cape) reported an average daily maximum temperature of 25.61 °C in February 2014, 5) in December 2014 both Cramond (KwaZulu Natal) and Lesedi (Gauteng) reported favourable temperatures of an average daily maximum temperature of 24.71 and 25.46 °C respectively, 6) Mangaung (Free State) reported an average daily maximum temperature in May 2015 of 24.95 °C. Despite the fact that ideal climatic conditions were observed none of the meat samples tested positive for ZEN contamination during the monitoring period (2012 – 2015).

What is interesting to note is that during June 2015, an extreme cold front was recorded at Thabo Mofutsanyana (Free State), Boshof (Northern Cape), Harrismith (Free State), Bonnievale (Western Cape) and Wesselsbron (Free State), with temperatures ranging 2.33 to 3.13 °C. The temperature reported in June 2015 was lower by as much as 18.39 °C compared to the 2014 daily maximum temperatures ranging from 17.94 to 21.34 °C.

One of the possible reasons why none of the samples tested positive for mycotoxin contamination could include the low accuracy and outdated method of detection used by the routine analytical laboratory commissioned by DAFF.

3.3.2 University of Pretoria data set

The full data set is attached as Table 7-1 in Annexure B.[14] Table 3-3 is a summary of this data set. From Table 3-2 and Table 7-1, it can be seen that optimal growth temperature (33 °C) for AF were not met in January, March or April 2017 in the Transkei or Vhembe district. Daily average rainfall of 6.60 and 1.44 mm were reported in January and March, respectively. This should be noted as high rainfall near or during harvest time is normally

associated with high concentrations of AF contamination in crops.[16] Despite the fact that ideal climatic conditions were observed none of the meat samples tested positive for AF contamination during the study period (2017).

Table 3-2 Summary of the University of Pretoria data set of mycotoxins, rainfall and temperature (2017)

Species	Province	Rainfall (mm) (min-max)	Temp (°C) (min-max)	OTA (µg/kg)	AF (µg/kg)	DON (µg/kg)	ZEN (µg/kg)
Bovine Porcine	Limpopo	1.44 - 6.60	7.71-28.18	<1	<4	<100	<20
Bovine Porcine	Eastern Cape	0.48 - 0.48	12.47-25.6	<1	<4	<100	<20

LOD and LOQ: OTA <1, AF <4, DON <100, ZEN <20

As already discussed, good conditions for the fungal species responsible for OT were reported in the Vhembe district in Table 7-1. In January 2017, the same district reported an average daily maximum temperature of 27.45 °C and an average daily rainfall of 6.60 mm, and in April 2017, an average daily maximum temperature of 28.18 °C with an average daily rainfall of 1.44 mm. Despite the fact that ideal climatic conditions were observed none of the meat samples tested positive for OTA contamination during the study period (2017).

Optimal temperature of 30 °C for *Fusarium graminearum* to produce DON was not reported in 2017. However, in January 2016, the Vhembe district reported an average maximum daily temperature of 31.09 °C.[20] Optimal temperature of 25 °C for ZEN production by *Fusarium graminearum* was reported in the Transkei in April 2017 with an average daily temperature of 25.6 °C. Despite the fact that ideal climatic conditions were observed none of the meat samples tested positive for ZEN and DON contamination during the study period (2017).

From Table 7-1, it can also be seen that there are no differences reported in results for the raw and cooked samples from the UP study.

Table 3-3 Average daily rainfall, maximum and minimum temperature for the location and five year period of sampling by the University of Pretoria data set and the previous five year period

Location	Province	Month	2008-2012 Average	2013-2017 Average	p –value
Average daily rainfall (mm)					
Vhembe	Limpopo	January	3.01	3.48	0.70
Vhembe	Limpopo	March	2.59	1.45	0.18
Transkei	Eastern Cape	April	1.55	0.97	0.17
Average daily maximum temperature (°C)					
Vhembe	Limpopo	January	26.40	28.11	0.09
Vhembe	Limpopo	March	27.20	27.42	0.84
Transkei	Eastern Cape	April	23.40	24.94	0.13
Average daily minimum temperature (°C)					
Vhembe	Limpopo	January	20.60	17.58	0.19
Vhembe	Limpopo	March	18.80	15.82	0.11
Transkei	Eastern Cape	April	13.80	11.95	0.06

Table 3-3 shows the average daily rainfall, maximum and minimum temperature for the location and five year period of sampling by the UP data set and the previous five year period. There were no significant differences between the averages of the two periods. The literature has proven that climatic conditions such as temperature and rainfall can have an effect on the occurrence of mycotoxin contamination. If a significant difference in these climatic conditions arises, meat samples should be analysed for mycotoxin contamination.

From the literature discussed in chapter two, it is also evident that the bio-transformation of each mycotoxin is different for each animal species, as well as in blood, muscle and

organ tissues. Although no positive results were found for mycotoxin contaminated meat it does not, exclude the fact that the growth of the animal could have been influenced (stunted growth). However, this was not investigated in the study.

3.4 Conclusions and recommendations

In a country such as South Africa, where stunting and various other malnutrition conditions are prevalent, it is important to continue to assess the mycotoxin intake among humans and animals to determine high risk areas. Favourable environmental conditions for mycotoxin producing fungi to thrive, such as optimal growth temperatures and rainfall have been met in the areas where red meat was sampled. The two data sets under discussion in this chapter concluded that none of the meat samples tested were contaminated with AF, OTA, DON and/or ZEN.

Although the results from these studies suggests that the DAFF monitoring and evaluating programme is adequate the availability of new detection methods with sophisticated equipment may in fact deliver different results. It is therefore recommended that a further study be conducted where different analytical methods are compared in order to ascertain if it will yield the same results, keeping in mind the different food matrixes.

It is important to monitor the prevalence of mycotoxins in meat on a continuous basis. Mycotoxins pose a serious threat to human health, and from the literature it is clear that meat can become contaminated at various points in the food processing chain. More research is needed on the mycotoxin metabolite including masked metabolites in animals and other mycotoxins that could not be analysed, due to the absence of analytical standards in order to expand and increase the effectiveness of monitoring programmes.

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CHAPTER FOUR

OCCURRENCE OF HEAVY METALS IN RED MEAT

4.1 Introduction

The global public health concern regarding heavy metals has increased in recent years. The reason for this is that human exposure has increased drastically as a result of increased widespread use of heavy metals for agricultural, industrial, domestic and technological application.[1] Metallic elements that have a relatively high density compared to water are regarded as heavy metals.[2] Well-known examples are lead, mercury and cadmium.[9] Metalloids such as arsenic are also regarded as heavy metals because of the assumption that heaviness and toxicity are interlinked.[3]

High consumption of specific trace metals can be toxic.[4] Human exposure to heavy metals is most likely industrial or domestic effluents, combustion, bushfires, decomposition of chemical fertilizers and pesticides. Dietary exposure is also possible.[5] At low levels of exposure, arsenic, cadmium, lead and mercury can induce multiple-organ damage as they are systemic poisons.[6] For the purpose of this study, these four heavy metals and their occurrence in red meat were studied.

Although heavy metals occur naturally in the earth's crust, increased human exposure is a result of anthropogenic activities such as mining and smelting, as well as the domestic and agricultural use of metal and metal compounds.[7-10] Environmental contamination also includes atmospheric deposition, including rain, snow and fog and industrial sources such as coal power plants.[11-12] According to Sloss and Smith (2000), heavy metals that are released into the environment by coal power stations include mercury, arsenic and cadmium.[13]

As with other heavy metals, lead appears naturally in the earth's crust, yet anthropogenic activities such as burning of fossil fuels and mining release high concentrations into the atmosphere. In 2004 it was estimated that 1.52 million metric tons of lead were used in industrial applications in the United States and that 83% of that was for the production of lead-acid batteries.[14, 15]

Mercury is found in nature in three forms, namely elemental, inorganic and organic. Each form has its own toxicity profile.[16] Elemental mercury can be found in liquid form at room temperature. Methylmercury is its organic state and is most frequently encountered in the environment. Methylmercury is formed by the methylation of inorganic mercury found in soil and water.[17]

Arsenic is detected in low concentrations in all environmental matrices. It is estimated that millions of people are chronically exposed to arsenic. Exposure can be through ingesting contaminated water or food sources, inhalation and dermal contact.[18]

Cadmium is considered a heavy metal that is both an environmental and an occupational concern. It is widely distributed in the earth's crust.[19] The commercial use of cadmium was reduced in developing countries due to the environmental risk.[20]

The objectives of the chapter are to determine the current level of heavy metal contamination (lead, mercury, arsenic and cadmium) in South African red meat and establish a correlation between some selected environmental factors, including weather conditions, and the incidence of heavy metals in red meat.

4.2 Materials and methods

The prevalence of heavy metal levels in South African meat was determined by analysing the analytical data from a monitoring study of bovine, ovine, poultry and porcine liver and kidney samples for lead, arsenic, cadmium and mercury. This analytical data was provided by Veterinary Public Health, Department of Agriculture Forestry and Fisheries (DAFF). [21] The data was collected over a period of five years (from 2012 to 2016) in all nine provinces of South Africa. The meat samples were either pooled samples or single samples obtained by the state veterinarian in the district of sampling. Heavy metal analyses were performed by the Agricultural Research Council - Onderstepoort Veterinary Institute (ARC-OVI) laboratory. ARC-OVI tested for lead, arsenic, cadmium and mercury using inductively coupled plasma mass spectrometry (ICP-MS).[21]

One of the climatic conditions that influences heavy metal contamination in plants is rainfall. Historic data on rainfall was requested from the South African Weather Service

(SAWS) for the areas and periods when DAFF collected samples for monitoring. Other environmental factors that were investigated are the areas in which DAFF collected samples to determine if a coal power plant is in a 50 km radius.

The weather data for the two scenario's where meat tested positive for heavy metal contamination, Malmesbury (610 µg/kg lead in bovine liver) and Bela-Bela (200 µg/kg mercury in porcine liver), was analysed and discussed quantitatively. The weather data was obtained for a five year period (2013 to 2017) and a one sample t-test ($\alpha = 0.05$) performed, with Stata 14[48], to determine whether the average for the five year period was significantly different than that of the average for the years 2008 to 2012.

4.3 Results and discussion

The data received from DAFF together with the environmental data is presented in Table 8-1 of Addendum C. [21] Table 4-1 provides a summary of the national chemical residue programme and environmental data (Table 8-1). From Table 8-1, it can be seen that only two samples tested positive for heavy metal contamination. The first incidence was reported in bovine liver (610 µg/kg lead) from Malmesbury, Western Cape sampled in May 2012. The area where the sample was taken from is not close to a coal power station, nor was there increased rainfall from 2011. In fact the average daily rainfall reduced from 1.89 to 0.06 mm. Located slightly over 50 km south west of Malmesbury is Koeberg Nuclear Power Station, yet unlike fossil fuel-fired power stations (coal), nuclear power stations do not produce air pollution while operating.[22]

Table 4-1 Summary of the national chemical residue programme by DAFF as well as rainfall and coal power station proximity

Species	Province	Rainfall (mm) (min-max)	Coal power station (<50 km)	Results
Poultry Bovine Ovine Porcine	Northern Cape	0-3.36	No	Negative
Poultry Bovine Ovine Porcine	Western Cape	0.70-5.47	No	610 µg/kg lead
Poultry Bovine Ovine Porcine	Eastern Cape	0.07-2.01	No	Negative
Poultry Bovine Ovine Porcine	Gauteng	0.01-4.07	Yes	Negative
Poultry Bovine Ovine Porcine	Limpopo	0.28-9.91	No	200 µg/kg mercury
Poultry Bovine Ovine Porcine	Mpumalanga	0-5.39	Yes	Negative
Poultry Bovine Ovine Porcine	North West	0-3.36	No	Negative
Poultry Bovine Ovine Porcine	Free State	0-8.5	Yes	Negative
Poultry Bovine Ovine Porcine	KwaZulu Natal	0.07-5	No	Negative

The second incidence reported was found in September 2012 in Bela-Bela in Limpopo province. Here porcine liver tested positive for 200 µg/kg mercury. Bela-Bela is not located near a coal power plant, yet the average daily rainfall increased from 2011 to 2012 from 0 to 1.66 mm.

Table 4-2 depicts significant difference of the average daily rainfall for May 2013 to 2017 and May 2008 to 2012 in Malmesbury (Western Cape) and September 2013 to 2017 and September 2008 to 2012 in Bela-Bela (Limpopo). The statistics was done on Stata 14.[24] These where the two locations where meat tested positive for heavy metals. From Table 4-2 it is clear that there was no significant difference observed between the mean daily rainfall for the period between 2013 and 2017. As no significant difference was found for these areas, no correlation can be made.

Table 4-2 Average daily rainfall over a five year period and significant difference in areas were heavy metal contamination was found

Location	Provence	Month	2008 – 2012 Average	2013 – 2017 Average	p - value
Malmesbury	Western Cape	May	1.16	0.97	0.62
Bela-Bela	Limpopo	September	0.39	0.57	0.67

Neither of the positive incidences occurred near coal power stations. However, the burning of fossil fuels, responsible for the emission of heavy metals, are not limited to coal power stations only, but can also occur as a result of other industrial processes such as smelting and refining. The proximity of these industrial plants were not investigated in this study.

From Table 8-1, it can be seen that seven areas sampled are close to coal power stations. All of the samples however, tested negative for detectable levels of heavy

metals. As the samples tested negative, no correlation could be made between environmental factors and prevalence of heavy metals in red meat.

Five of these sampled areas are within a 50 km radius of Camden power station in Ermelo (Mpumalanga), all in the Gert Sibane district. Camden power station was commissioned in April 1967 and mothballed (decommissioned but kept in good condition) in 1990. In 2003 it was decided by Eskom's board to bring the power station back into full service.[23] Delmas (Gauteng) is located 46 km from Kendal power station, which is the largest dry-cooled power station in the world, using significantly less water than conventional coal power stations. Negative results were found for heavy metals in samples from Delmas (Gauteng). Sampling in Metsimaholo (Free State), which is 20 km from Lethabo power station, also showed negative results. [23] Once again, as these samples tested negative no correlation could be made between environmental factors and prevalence of heavy metals in red meat.

The dispersion of heavy metals due to the burning of fossil fuel is influenced by wind speed and direction. The area which is polluted will then be father away. The quality of the coal could also influence the amount of contaminants released as well as the complete combustion of the coal.

4.4 Conclusions and recommendations

Heavy metals such as arsenic, cadmium, lead and mercury are generally present in the environment. From the literature it is evident that meat products are prone to become contaminated with these heavy metals. All of these four heavy metals mentioned pose a threat to human health including stunting in children.

From the data in Table 8-1 it is evident that since 2012 South African red meat has not tested above accepted levels for any heavy metal contaminant as was reported by the DAFF monitoring and evaluating programme. This is valuable information in a developing country such as South Africa where great emphasis is placed on the contribution of red meat to alleviate iron and zinc deficiency. Red meat is recommended by the WHO as complementary feeding to children due to its unique nutritional benefit.[45] A study by

Ambushe *et al* (2012) found accumulated traces of lead in kidney samples of cattle sourced from the North West Province. Further screening of heavy metals in South African meat is recommended especially in areas were water quality is questionable.[47] As wind plays an important role in air pollution, dispersion models of industrial plants and coal power stations need to be investigated in future studies in order to determine areas possibly affected by pollution.

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CHAPTER FIVE

SIGNIFICANCE OF THE STUDY, CONCLUSION AND RECOMMENDATION

5.1 Introduction

Mycotoxins and heavy metals occur naturally in the air and soil. Dietary exposure to both these two groups of substances have been linked to childhood stunting.[1-3] Although the prevalence of stunting has decreased globally, it still affects more than 20% of children. The highest proportion of stunted children is in Africa, where 35.6% of children under five years of age are stunted.[4] The sole cause of stunting remains under speculation but it is believed that exposure to toxic substances, such as heavy metals and mycotoxins in the diet, are contributing factors.[5]

Red meat can become contaminated (with both mycotoxins and heavy metals) during processing and packing. The main pathway of contamination is, however, through animal feeds. Animals consume contaminated feed and residues of these contaminants end up in the animal tissues.[6, 7] Crops become contaminated by the transfer of mycotoxins and heavy metals through soil, ground water and river water.[8] Mycotoxins can also contaminate crops during transportation and storage. Climatic aspects form a key agro-ecosystem force that promotes fungal colonisation and mycotoxin production.[9, 10]

The South African National Food Consumption Survey (by means of a 24-hour food recall and food- frequency questionnaire) found that 36% of one to five year olds consume meat and meat products.[11] The Income and Expenditure Survey from Statistics South Africa showed that all consumer groups spend on average more than 20% of their income on meat and meat products.[12] Both these are evidence that South Africans consume red meat on a regular basis and therefore red meat could be a source of exposure to mycotoxins and heavy metals.

5.2 Significance of the study

Stunting affects more than 15 % of all children under the age of 5 in South Africa.[13.] Possible causes need to be investigated.[13] Complementary feeding of meat to children from age 6 months is recommended by the WHO.[14] As meat could be a possible pathway for toxic substances to enter the diet, investigation into its safety is of great importance. Furthermore, South Africa has recently undersigned to participate in the second SDG as set out by the UN with a main indicator of eliminating the prevalence of stunting by 2025. It is important to determine whether South African red meat is contaminated with toxins, and is therefore a possible cause of stunting.

This study (Chapter Three) concluded that no meat samples tested positive for mycotoxin contamination. No other studies could be found on mycotoxin contaminated meat in South Africa. Heavy metal contaminated meat was found in in two samples in this study (Chapter Four). In Malmesbury 610 µg/kg lead was detected in bovine liver and in Bela-Bela 200 µg/kg mercury was detected in porcine liver. However, the contamination levels were still within acceptable limits as set out by the regulations. Furthermore, environmental conditions, such as climate (rainfall and temperature) and/or proximity of coal power stations, can have an effect on mycotoxin or heavy metal contamination of food sources, although no correlation was found in this study.

5.3 Conclusion

Red meat and red meat products are consumed by all socio-economic groups in South Africa. It is therefore important to know whether the health risk it poses is of great concern, since South Africa is facing the triple burden of malnutrition. From literature it has been reported that red meat and red meat products can become contaminated with mycotoxins (AF, OT, DON and ZEN) and heavy metals (cadmium, lead, arsenic and mercury). It was also noted that these two groups of toxic substances pose a real threat to human health development.

The fact that consumption of contaminate feed can be the reason animal source foodstuff is contaminated by mycotoxins is underlined by a previous study detecting AFM1 in South

African retail milk. It was concluded that the milk became contaminated due to the ingestion of contaminated feed as high levels of AFB1 was detected in the samples.

Chapter Three focused on the occurrence of mycotoxins in red meat. The chapter investigated the contamination of meat by four mycotoxins that were considered carcinogenic. These mycotoxins can have a serious health impact on both humans and animals. Stunting in children has been linked to aflatoxin exposure through the diet and other mycotoxins have caused growth retardation in animals.

Environmental factors could possibly have a consequence on the contamination, as this can have an effect on crops which are consumed by animals. As mentioned in Chapter Three, recent studies have found mycotoxins in home grown maize from both regions where samples for the UP study were collected. However, both studies found no traceable amounts of mycotoxins in muscle tissue or organs of ruminants and swine. Possible explanations could be that contaminated feed was not ingested and/or the bio-transformation of mycotoxins during the metabolic process of the animal, resulted in the findings of the study.

Chapter Four investigated the occurrence of heavy metals in South African meat. Although only two incidences in the DAFF monitoring and evaluation programme reported positive for heavy metal contamination (610 µg/kg lead in bovine liver and 200 µg/kg mercury in porcine liver), it does not rule out the fact that meat can be a contributor to heavy metal exposure in humans. Another study found accumulated traces of lead in kidney samples of cattle sourced from the North West province.

As mentioned, stunting is a national public health problem in South Africa. Harmful exposure to toxic substances and inadequate nutrition during pregnancy can result in preterm delivery which is a contributing factor to stunting in early childhood. During early childhood growth faltering may begin at three to five months and become more dominant from six to 18 months. Determining if food sources are contributing to stunting due to contamination with mycotoxins and heavy metals can contribute to improved understanding of the occurrence of stunting in both humans and animals.

5.4 Limitations of the study

Determining the occurrence of mycotoxins and heavy metals in South African red meat proved to be difficult. The monitoring and evaluation programme by DAFF is not consistent and thus no pattern is evident alongside historical weather data. As no specific sampling plan is followed by DAFF, instances where meat can be contaminated might have been over looked. The DAFF monitoring programme monitor only four mycotoxins currently.

The data set from the UP that tested four mycotoxins in tissue samples of pork and cattle reported no trace amounts of mycotoxins. The data could have been more meaningful if tests on local crops were also performed in the areas of sampling. If this was done then some conclusion could have been made regarding the carry-over of mycotoxins into red meat. Due to a lack of funding, the sample size of the study was small and the samples where not tested for heavy metals.

5.5 Recommendations

Further research regarding the impact that environmental factors, such as rainfall and temperature, may have on the possible mycotoxin and heavy metal contamination of red meat and red meat products is needed. This is proven by the fact that in Chapter Three, it was seen that there was a significant difference in maximum daily temperatures in a region where peanut butter was contaminated with AF in 2001. Chapter Four also showed a significant difference in rainfall in an area where meat tested positive for mercury contamination.

Further studies are recommended to determine whether environmental factors, including climate change, can have an effect on contamination of food used as feed or a feed component. An early warning system to test commodities prone to contamination should be implemented in areas where significant differences in maximum daily temperatures are observed. As fungi growth and mycotoxin contamination are dependent on ideal environmental conditions, these should be monitored and analysis should be done accordingly.

To enhance the monitoring and evaluation programme, it is suggested that more mycotoxins, including masked metabolites, need to be analysed. As technology develops and methods improve such as the liquid chromatography-tandem mass spectrometry LC-MS/MS results should be compared to ensure that current methods used routinely by DAFF are still valid for the food matrix. Also, sample areas for detection of heavy metals in animal tissues should be more carefully selected to include areas where it is known that water is contaminated with heavy metals. In order to determine whether red meat can be assumed as a low risk commodity further studies are recommended.

5.6 References

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ADDENDUM A: TABLE 6-1

Table 6-1 DAFF mycotoxins monitoring and evaluation programme data with rainfall and temperature data for time of sampling and year before sampling

Species	Sample Type	District	Province	Month	Year	Weather Station	Mycotoxin			Year = 0			Year = –1		
							Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (mg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (°C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Poultry	Liver	Delmas	G	5	2012	Spring	Neg	Neg	Neg	0.01	6.36	24.71	0.17	5.35	19.37
Bovine	Liver	Mafikeng	NC	6	2012	Mafikeng Wo	Neg	Neg	Neg	0.40	4.64	20.07	0.85	3.63	20.36
Poultry	Liver	Worcester	WC	6	2012	Worcester-Aws	Neg	Neg	Neg	2.53	6.57	17.51	3.05	7.27	16.60
Ovine	Liver	Motheo	FS	7	2012	Bloemfontein Wo	Neg	Neg	Neg	0.36	2.55	17.59	0.29	3.49	16.59
Poultry	Liver	Mopani	L	7	2012	Thohoyandou Wo	Neg	Neg	Neg	0.00	9.49	24.15	0.58	8.01	21.81
Poultry	Liver	Mopani	L	7	2012	Thohoyandou Wo	Neg	Neg	Neg	0.00	9.49	24.15	0.58	8.01	21.81
Poultry	Liver	Bapsfontein	G	8	2012	Irene Wo	Neg	Neg	Neg	0.14	5.93	21.39	0.14	5.93	21.39
Poultry	Liver	Atlantis	WC	8	2012	Malmesbury	Neg	Neg	Neg	2.30	5.08	17.13	1.39	5.76	19.69
Poultry	Liver	Camperdown	KZN	8	2012	Oribi Airport	Neg	Neg	Neg	3.08	10.09	23.23	0.51	8.57	22.23
Poultry	Liver	Paarl	WC	8	2012	Paarl	Neg	Neg	Neg	4.68	5.93	16.39	1.92	7.17	19.70

Species	Sample Type	District	Province	Month	Year	Weather Station	Mycotoxin			Year = 0			Year = –1		
							Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (mg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (°C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Porcine	Liver	Bela Bela	M	8	2012	Warmbad Towoomba	Neg	Neg	Neg	0.01	6.72	27.78	0	5.46	25.66
Bovine	Liver	Cullinan	G	8	2012	Wonderboom Airport	Neg	Neg	Neg	0	6.08	24.63	0.28	4.82	22.89
Bovine	Liver	Jankempdorp	NC	9	2012	Taung	Neg	Neg	Neg	0.26	7.85	26.32	0.01	8.23	29.34
Bovine	Liver	Kenneth Kaunda	NW	10	2012	Klerksdorp	Neg	Neg	Neg	0.14	13.68	29.90	0.66	11.43	28.67
Bovine	Liver	Fezile Dabi	G	10	2012	Kroonstad	Neg	Neg	Neg	0.94	12.32	28.27	0.48	10.81	27.91
Ovine	Liver	Dr Ruth Segomotsi Mompati	NW	10	2012	Vryburg	Neg	Neg	Neg	0.50	11.75	32.26	0	8.16	29.80
Poultry	Liver	Malmesbury	WC	12	2012	Malmesbury	Neg	Neg	Neg	0	16.26	31.88	0.26	13.81	28.39
Poultry	Liver	Klipheuwel	WC	12	2012	Paarl	Neg	Neg	Neg	0.03	19.14	32.19	0.22	15.35	28.28
Bovine	Liver	Heidelberg	G	12	2012	Springs	Neg	Neg	Neg	3.95	16.46	26.93	6.27	16.93	27.71
Ovine	Liver	Wolwehoek	FS	12	2012	Vereeniging	Neg	Neg	Neg	5.24	14.00	24.83	4.75	15.15	27.36
Ovine	Liver	Velddrif	WC	2	2013	Langebaanweg Aws	Neg	Neg	Neg	0.35	15.11	27.86	0.06	14.95	27.60

Species	Sample Type	District	Province	Month	Year	Weather Station	Mycotoxin			Year = 0			Year = –1		
							Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (mg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (°C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Bovine	Liver	Malmesbury	WC	2	2013	Malmesbury	Neg	Neg	Neg	0.86	15.93	31.38	0.06	15.10	30.89
Bovine	Liver	Malmesbury	WC	2	2013	Malmesbury	Neg	Neg	Neg	0.86	15.93	31.38	0.06	15.10	30.89
Porcine	Liver	Malmesbury	WC	2	2013	Malmesbury	Neg	Neg	Neg	0.86	15.93	31.38	0.06	15.10	30.89
Ovine	Liver	Bojanola	NW	2	2013	Pilanesberg	Neg	Neg	Neg	17.47	23.22	23.90	1.52	19.10	33.00
Porcine	Liver	Middleburg	M	2	2013	Witbank	Neg	Neg	Neg	1.05	13.72	29.32	1.37	15.50	29.82
Ovine	Liver	Amathole	EC	3	2013	Dohne - Agr	Neg	Neg	Neg	3.14	14.30	28.36	3.41	13.90	26.11
Ovine	Liver	Darling	WC	5	2013	Geelbek	Neg	Neg	Neg	0.17	11.55	26.45	0.86	5.96	20.11
Ovine	Liver	Siyanda	NC	5	2013	Upington Wo	Neg	Neg	Neg	0	8.20	26.17	0.01	6.87	26.46
Ovine	Liver	Ceres	WC	5	2013	Worcester-Aws	Neg	Neg	Neg	0.46	7.82	22.36	0.17	6.87	21.17
Porcine	Liver	Riebeeck west	WC	6	2013	Malmesbury	Neg	Neg	Neg	4.86	6.09	18.86	2.48	6.83	18.59
Poultry	Liver	Atlantis	WC	6	2013	Malmesbury	Neg	Neg	Neg	4.86	6.09	18.86	2.48	6.83	18.59
Poultry	Liver	Malmesbury	WC	6	2013	Malmesbury	Neg	Neg	Neg	4.86	6.09	18.86	2.48	6.83	18.59
Porcine	Kidney	Bronkhorst-spruit	G	6	2013	Witbank	Neg	Neg	Neg	0	3.59	21.30	0.03	3.46	19.89

Species	Sample Type	District	Province	Month	Year	Weather Station	Mycotoxin			Year = 0			Year = –1		
							Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (mg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (°C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Bovine	Liver	Lejweleputswa	FS	7	2013	Glen College Aws	Neg	Neg	Neg	0.21	0.55	21.20	0.25	1.21	19.56
Bovine	Liver	Vryburg	NW	7	2013	Vryburg	Neg	Neg	Neg	0	2.15	22.78	0	0.11	21.07
Bovine	Liver	Bronkhorst-spruit	G	7	2013	Witbank	Neg	Neg	Neg	0	3.68	20.28	0	4.14	20.95
Porcine	Liver	Amajumba	KZN	8	2013	Newcastle	Neg	Neg	Neg	0.26	8.72	24.11	0.13	9.34	26.10
Poultry	Liver	Camperdown	KZN	8	2013	Oribi Airport	Neg	Neg	Neg	0.34	9.28	24.41	3.08	10.09	23.23
Poultry	Liver	Delmas	G	8	2013	Springs	Neg	Neg	Neg	0.07	4.89	22.33	0.06	4.29	22.91
Bovine	Liver	Dr. Ruth Sepati Mogomotsi	NW	8	2013	Vryburg	Neg	Neg	Neg	0	1.81	23.23	0	4.20	24.94
Bovine	Kidney	Dr. Ruth Sepati Mogomotsi	NW	8	2013	Vryburg	Neg	Neg	Neg	0	1.81	23.23	0	4.20	24.94
Bovine	Liver	Cullinan	G	8	2013	Wonderboom Airport	Neg	Neg	Neg	0.04	4.71	22.95	0	6.08	24.63
Bovine	Liver	Kimberly	NW	10	2013	Kimberley Wo	Neg	Neg	Neg	0.51	8.96	28.91	0.88	9.39	29.32
Poultry	Liver	Malmesbury	WC	10	2013	Malmesbury	Neg	Neg	Neg	0.94	9.01	25.08	0.29	9.71	24.13

Species	Sample Type	District	Province	Month	Year	Weather Station	Mycotoxin			Year = 0			Year = –1		
							Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (mg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (°C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Poultry	Liver	Wellington	WC	10	2013	Paarl	Neg	Neg	Neg	1.71	11.90	24.27	0.48	12.10	22.96
Bovine	Liver	Lejweleputswa	FS	11	2013	Glen College Aws	Neg	Neg	Neg	3.14	11.68	31.24	0.59	13.00	28.81
Ovine	Liver	Fezile Dabi	FS	11	2013	Kroonstad	Neg	Neg	Neg	0	13.75	29.43	0.75	13.76	30.14
Ovine	Liver	Ehlanzeni	M	11	2013	Kruger M Int. Air.	Neg	Neg	Neg	2.40	16.49	26.28	3.63	15.73	24.86
Bovine	Liver	Cramond	KZN	12	2013	Cedara	Neg	Neg	Neg	4.07	13.26	22.94	2.81	13.02	23.08
Bovine	Liver	Gert Sibande	M	12	2013	Ermelo Wo	Neg	Neg	Neg	5.63	12.88	22.23	2.88	12.88	23.89
Bovine	Liver	Heidelberg	G	12	2013	Springs	Neg	Neg	Neg	5.14	13.58	23.25	3.95	16.46	26.93
Ovine	Liver	Siyanda	NC	12	2013	Upington Wo	Neg	Neg	Neg	1.06	18.47	35.03	1.35	16.94	33.57
Poultry	Liver	Wolseley	WC	12	2013	Worcester-Aws	Neg	Neg	Neg	0.01	16.16	31.11	0	16.97	30.61
Ovine	Liver	Bloemfontein	FS	1	2014	Bloemfontein - Stad	Neg	Neg	Neg	1.80	16.35	32.07	1.85	16.83	31.69
Bovine	Liver	Ditsobotla	NW	1	2014	Lichtenburg	Neg	Neg	Neg	3.54	15.82	29.37	0.62	16.66	28.95
Poultry	Kidney	Ngaka Modiri Molema	NW	1	2014	Lichtenburg	Neg	Neg	Neg	3.54	15.82	29.37	0.62	16.66	28.95

Species	Sample Type	District	Province	Month	Year	Weather Station	Mycotoxin			Year = 0			Year = –1		
							Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (mg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (°C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Poultry	Liver	Mopani	L	1	2014	Thohoyandou Wo	Neg	Neg	Neg	9.91	20.10	28.43	19.51	19.84	28.53
Ovine	Liver	Vrede	KZN	1	2014	Vrede	Neg	Neg	Neg	1.44	14.75	27.94	3.06	14.54	26.74
Ovine	Liver	Joe Gqabi	EC	2	2014	Barkly East	Neg	Neg	Neg	4.72	11.05	25.61	2.51	9.24	26.12
Porcine	Liver	Boland	WC	2	2014	Cape Town Wo	Neg	Neg	Neg	0.08	18.06	28.20	1.34	17.23	26.36
Bovine	Liver	Amatole	EC	2	2014	Dohne - Agr	Neg	Neg	Neg	3.88	14.35	27.26	2.44	14.99	29.53
Bovine	Liver	Fezile Dabi	G	2	2014	Kroonstad	Neg	Neg	Neg	0	16.67	27.80	0.35	15.42	31.39
Porcine	Liver	Mooi River	KZN	2	2014	Mooi River	Neg	Neg	Neg	1.78	13.69	26.75	4.04	12.49	27.04
Poultry	Liver	Camperdown	KZN	2	2014	Oribi Airport	Neg	Neg	Neg	4.11	19.05	31.17	5.39	16.34	27.88
Porcine	Liver	Modimolle	L	2	2014	Warmbad Towoomba	Neg	Neg	Neg	3.61	18.62	33.57	3.09	17.24	34.78
Poultry	Liver	Lejweleputswa	FS	5	2014	Glen College Aws	Neg	Neg	Neg	0.37	3.14	23.84	0	3.88	24.75
Poultry	Liver	Germiston	G	5	2014	Johannesburg Int Wo	Neg	Neg	Neg	0.14	9.34	20.92	0.50	8.53	19.88
Bovine	Liver	Joe Gqabi	EC	6	2014	Barkly East	Neg	Neg	Neg	0.01	2.17	13.41	0.07	1.15	14.81

Species	Sample Type	District	Province	Month	Year	Weather Station	Mycotoxin			Year = 0			Year = –1		
							Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (mg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (°C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Poultry	Liver	Naledi	FS	6	2014	Bloemfontein Wo	Neg	Neg	Neg	0	2.93	18.48	0	2.22	19.18
Ovine	Liver	De Aar	NC	6	2014	De Aar Wo	Neg	Neg	Neg	0.07	1.81	15.94	0.03	2.43	16.84
Poultry	Liver	Pretoria	G	6	2014	Pretoria Eendracht	Neg	Neg	Neg	0.08	5.23	20.69	0	4.31	22.14
Poultry	Liver	Pretoria	G	6	2014	Pretoria Eendracht	Neg	Neg	Neg	0.08	5.23	20.69	0	4.31	22.14
Bovine	Liver	Thabo Mofutsanyana	FS	7	2014	Bethlehem Wo	Neg	Neg	Neg	0	3.37	16.72	0	0.35	17.61
Bovine	Liver	Amathole	EC	7	2014	Dohne - Agr	Neg	Neg	Neg	0.07	3.32	18.19	0.47	6.38	19.45
Bovine	Liver	Gert Sibande	M	7	2014	Ermelo Wo	Neg	Neg	Neg	0	3.94	17.44	0	3.94	17.44
Ovine	Kidney	Ehlanzeni	M	7	2014	Kruger M Int. Air.	Neg	Neg	Neg	0.04	8.58	21.83	0.56	11.12	21.98
Bovine	Liver	Bronkhort-spruit	G	7	2014	Witbank	Neg	Neg	Neg	0	5.08	22.32	0	3.68	20.28
Porcine	Liver	Dargle	KZN	8	2014	Cedara	Neg	Neg	Neg	0.11	6.51	22.75	0.46	4.96	21.45
Porcine	Liver	Viljoenskroon	FS	8	2014	Klerksdorp	Neg	Neg	Neg	0.01	5.94	23.07	0.03	3.97	22.51
Poultry	Liver	Camperdown	KZN	8	2014	Oribi Airport	Neg	Neg	Neg	0.18	10.05	24.18	0.34	9.28	24.41

Species	Sample Type	District	Province	Month	Year	Weather Station	Mycotoxin			Year = 0			Year = –1		
							Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (mg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (°C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Poultry	Liver	Mopani	L	8	2014	Thohoyandou Wo	Neg	Neg	Neg	0	11.12	26.30	0.28	11.12	25.59
Bovine	Liver	Bronkhort-spruit	G	8	2014	Witbank	Neg	Neg	Neg	0.01	8.58	24.37	0.11	4.60	20.57
Bovine	Liver	Cullinan	G	8	2014	Wonderboom Airport	Neg	Neg	Neg	0	4.77	23.66	0.04	4.71	22.95
Bovine	Liver	Amathole	EC	10	2014	Dohne - Agr	Neg	Neg	Neg	2.01	7.81	21.29	3.45	8.13	23.24
Poultry	Liver	Francis Baard	NC	10	2014	Kimberley Wo	Neg	Neg	Neg	0.18	10.67	29.72	0.51	8.96	28.91
Poultry	Liver	Malmesbury	WC	10	2014	Malmesbury	Neg	Neg	Neg	0.08	9.69	28.67	0.94	9.01	25.08
Bovine	Liver	Groblershoop	NC	10	2014	Upington Wo	Neg	Neg	Neg	0.43	13.30	32.41	0.26	11.65	30.81
Ovine	Liver	Gert Sibande	L	11	2014	Ermelo Wo	Neg	Neg	Neg	3.58	11.92	4.37	3.58	11.92	24.37
Ovine	Liver	Swartruggens	NW	11	2014	Rustenburg	Neg	Neg	Neg	2.58	16.18	27.84	1.51	17.10	32.04
Bovine	Liver	Cramond	KZN	12	2014	Cedara	Neg	Neg	Neg	3.73	13.86	24.71	4.07	13.26	22.94
Bovine	Liver	Lesedi	G	12	2014	Grand Central	Neg	Neg	Neg	2.45	15.39	25.46	3.18	15.10	24.45
Ovine	Liver	Fezile Dabi	G	12	2014	Kroonstad	Neg	Neg	Neg	0.92	15.98	30.18	4.26	15.19	27.13

Species	Sample Type	District	Province	Month	Year	Weather Station	Mycotoxin			Year = 0			Year = –1		
							Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (mg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (°C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Bovine	Liver	Waterberg	L	12	2014	Marken	Neg	Neg	Neg	5.55	18.37	30.36	2.97	19.00	29.03
Porcine	Liver	Waterberg	L	12	2014	Marken	Neg	Neg	Neg	5.55	18.37	30.36	2.97	19.00	29.03
Poultry	Liver	Mopani	L	12	2014	Thohoyandou Wo	Neg	Neg	Neg	7.09	19.92	29.38	7.30	18.60	26.87
Poultry	Liver	Worcester	WC	12	2014	Worcester-Aws	Neg	Neg	Neg	0	15.45	30.06	0.01	16.16	31.11
Poultry	Liver	Frances Baard	NC	1	2015	Kimberley Wo	Neg	Neg	Neg	0.99	17.55	34.39	1.65	17.71	34.02
Bovine	Liver	Durbanville	WC	2	2015	Cape Town Wo	Neg	Neg	Neg	0.11	15.23	26.28	0.08	18.06	28.20
Ovine	Liver	De Aar	NC	2	2015	De Aar Wo	Neg	Neg	Neg	0.63	14.22	31.41	2.55	16.91	31.90
Porcine	Liver	Harry Gwala	EC	2	2015	Ixopo	Neg	Neg	Neg	4.88	15.20	26.06	1.54	16.76	27.88
Porcine	Liver	Kamberg	KZN	2	2015	Klerksdorp	Neg	Neg	Neg	1.56	15.28	32.13	0.71	17.60	29.38
Bovine	Liver	Fezile Dabi	G	2	2015	Kroonstad	Neg	Neg	Neg	0.24	14.62	31.19	0	16.67	27.80
Ovine	Liver	Velddrif	WC	2	2015	Langebaanweg Aws	Neg	Neg	Neg	0.02	13.98	26.66	0.04	16.29	29.58
Porcine	Liver	Swartland	WC	2	2015	Malmesbury	Neg	Neg	Neg	0	12.63	30.75	0.05	16.22	33.35
Poultry	Liver	Camperdown	KZN	2	2015	Oribi Airport	Neg	Neg	Neg	5.16	17.65	27.92	4.11	19.05	31.17

Species	Sample Type	District	Province	Month	Year	Weather Station	Mycotoxin			Year = 0			Year = –1		
							Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (mg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (°C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Bovine	Liver	Wellington	WC	2	2015	Paarl	Neg	Neg	Neg	0.06	16.34	30.28	0.14	18.73	32.65
Poultry	Liver	Mangaung	FS	3	2015	Bloemfontein Wo	Neg	Neg	Neg	2.54	12.95	28.39	2.08	13.05	26.82
Poultry	Liver	Mangaung	FS	5	2015	Bloemfontein Wo	Neg	Neg	Neg	0.21	2.48	24.95	0.26	2.66	22.81
Bovine	Liver	Prieska	WC	5	2015	Prieska	Neg	Neg	Neg	0	8.08	27.23	1.43	8.22	23.78
Bovine	Liver	Kgetleng	NW	5b	2015	Rustenburg	Neg	Neg	Neg	0	8.78	27.96	0.06	8.25	24.66
Ovine	Liver	Van Rhynsdorp	WC	5	2015	Vredendal	Neg	Neg	Neg	0.20	9.12	26.01	0.63	9.92	25.69
Bovine	Liver	Thabo Mofutsan-yana	FS	6	2015	Bethlehem Wo	Neg	Neg	Neg	1.05	2.21	2.33	0	1.82	17.94
Bovine	Liver	Boshof	NC	6	2015	Kimberley Wo	Neg	Neg	Neg	1.67	2.84	3.13	0.03	0.75	18.92
Bovine	Liver	Harrismith	FS	6	2015	Klerksdorp	Neg	Neg	Neg	0.33	2.54	2.95	0.20	1.59	21.34
Porcine	Liver	Bonnievale	WC	6	2015	Tygerhoek	Neg	Neg	Neg	6.05	1.82	2.57	2.99	4.14	18.86
Poultry	Liver	Wesselsbron	FS	6	2015	Welkom	Neg	Neg	Neg	1.17	2.06	2.62	0	0.75	19.88
Bovine	Liver	Francis Baard	NC	7	2015	Kimberley Wo	Neg	Neg	Neg	0.17	3.69	19.95	0	0.10	18.55

Species	Sample Type	District	Province	Month	Year	Weather Station	Mycotoxin			Year = 0			Year = –1		
							Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (mg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (°C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Poultry	Liver	Stellenbosch	WC	7	2015	Strand	Neg	Neg	Neg	5.39	8.76	16.55	3.86	8.35	17.51
Bovine	Liver	Bronkhorst-spruit	G	7	2015	Witbank	Neg	Neg	Neg	0.03	3.84	19.55	0	5.08	22.32
Porcine	Liver	Middelburg	NW	7	2015	Witbank	Neg	Neg	Neg	0.03	3.84	19.55	0	5.08	22.32

G - Gauteng, WC – Western Cape, NC – Northern Cape, EC – Eastern Cape, L – Limpopo, M – Mpumalanga, NW – North West, FS – Free State, KZN – KwaZulu Natal, Neg – negative.

ADDENDUM B: TABLE 7-1

Table 7-1 University of Pretoria mycotoxin analyses with rainfall and temperature data for time of sampling and year before sampling.

Species	Sample Type	Raw/Cooked	District	Province	Month	Year	Weather Station	Mycotoxin				Year = 0			Year = –1		
								Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (µg/kg)	Deoxynivalenol (µg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Bovine	Liver	R	Vhembe	L	1	2017	Thohoyandou Wo	<1	<4	<20	<100	6.60	19.58	27.45	2.11	20.30	31.09
Bovine	Kidney	R	Vhembe	L	1	2017	Thohoyandou Wo	<1	<4	<20	<100	6.60	19.58	27.45	2.11	20.30	31.09
Bovine	Chuck	R	Vhembe	L	1	2017	Thohoyandou Wo	<1	<4	<20	<100	6.60	19.58	27.45	2.11	20.30	31.09
Bovine	Thin flank	R	Vhembe	L	1	2017	Thohoyandou Wo	<1	<4	<20	<100	6.60	19.58	27.45	2.11	20.30	31.09
Bovine	Liver	C	Vhembe	L	1	2017	Thohoyandou Wo	<1	<4	<20	<100	6.60	19.58	27.45	2.11	20.30	31.09
Bovine	Kidney	C	Vhembe	L	1	2017	Thohoyandou Wo	<1	<4	<20	<100	6.60	19.58	27.45	2.11	20.30	31.09
Bovine	Chuck	C	Vhembe	L	1	2017	Thohoyandou Wo	<1	<4	<20	<100	6.60	19.58	27.45	2.11	20.30	31.09

Species	Sample Type	Raw/Cooked	District	Province	Month	Year	Weather Station	Mycotoxin				Year = 0			Year = –1		
								Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (µg/kg)	Deoxynivalenol (µg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Bovine	Thin flank	C	Vhembe	L	1	2017	Thohoyandou Wo	<1	<4	<20	<100	6.60	19.58	27.45	2.11	20.30	31.09
Porcine	Liver	R	Vhembe	L	3	2017	Thohoyandou Wo	<1	<4	<20	<100	1.44	7.71	28.18	1.92	19.40	29.92
Porcine	Kidney	R	Vhembe	L	3	2017	Thohoyandou Wo	<1	<4	<20	<100	1.44	7.71	28.18	1.92	19.40	29.92
Porcine	Chuck	R	Vhembe	L	3	2017	Thohoyandou Wo	<1	<4	<20	<100	1.44	7.71	28.18	1.92	19.40	29.92
Porcine	Thin flank	R	Vhembe	L	3	2017	Thohoyandou Wo	<1	<4	<20	<100	1.44	7.71	28.18	1.92	19.40	29.92
Bovine	Liver	R	Transkei	EC	4	2017	Umthatha Wo	<1	<4	<20	<100	0.48	12.47	25.6	1.65	12.29	27.01
Bovine	Kidney	R	Transkei	EC	4	2017	Umthatha Wo	<1	<4	<20	<100	0.48	12.47	25.6	1.65	12.29	27.01
Bovine	Chuck	R	Transkei	EC	4	2017	Umthatha Wo	<1	<4	<20	<100	0.48	12.47	25.6	1.65	12.29	27.01
Bovine	Thin flank	R	Transkei	EC	4	2017	Umthatha Wo	<1	<4	<20	<100	0.48	12.47	25.6	1.65	12.29	27.01
Bovine	Liver	C	Transkei	EC	4	2017	Umthatha Wo	<1	<4	<20	<100	0.48	12.47	25.6	1.65	12.29	27.01

Species	Sample Type	Raw/Cooked	District	Province	Month	Year	Weather Station	Mycotoxin				Year = 0			Year = –1		
								Ochratoxin (µg/kg)	Aflatoxin (µg/kg)	Zearalenone (µg/kg)	Deoxynivalenol (µg/kg)	Rainfall (mm)	Min temp (°C)	Max temp (C)	Rainfall (mm)	Min temp (°C)	Max temp (°C)
Bovine	Kidney	C	Transkei	EC	4	2017	Umthatha Wo	<1	<4	<20	<100	0.48	12.47	25.6	1.65	12.29	27.01
Bovine	Chuck	C	Transkei	EC	4	2017	Umthatha Wo	<1	<4	<20	<100	0.48	12.47	25.6	1.65	12.29	27.01
Bovine	Thin flank	C	Transkei	EC	4	2017	Umthatha Wo	<1	<4	<20	<100	0.48	12.47	25.6	1.65	12.29	27.01

EC – Eastern Cape, L – Limpopo; R – Raw, C – Cooked, LOD and LOQ: OTA: <1, AF<4, DON<100, ZEN<20

ADDENDUM C: TABLE 8-1

Table 8-1 DAFF heavy metal monitoring and evaluation programme data with rainfall and temperature data for time of sampling and year before sampling

Specie	Sample Type	District	Province	Month	Year	Weather Station	Results	Year = 0	Year = –1	Coal power station
								Rainfall (mm)	Rainfall (mm)	<50 km
Ovine	Liver	Dr Kenneth Kaunda	NC	5	2012	Klerksdorp	Neg	0	1.47	No
Bovine	Liver	Malmesbury	WC	5	2012	Malmesbury	Positive for Lead = 610 µg/kg	0.06	1.89	No
Poultry	Liver	Delmas	G	5	2012	Springs	Neg	0.01	0.17	Yes
Ovine	Liver	Ehlanzeni	M	6	2012	Kruger Mpumalanga Int. Air	Neg	0	0.11	No
Ovine	Kidney	Motheo	FS	7	2012	Bloemfontein Wo	Neg	0.36	0.29	No
Poultry	Liver	Atlantis	WC	7	2012	Malmesbury	Neg	1.50	0.53	No
Ovine	Liver	Paarl	WC	7	2012	Paarl	Neg	4.46	1.26	No
Poultry	Liver	Camperdown	KZN	9	2012	Oribi Airport	Neg	5.00	1.34	No
Poultry	Liver	Mopani	L	9	2012	Thohoyandou Wo	Neg	2.30	0.02	No

Specie	Sample Type	District	Province	Month	Year	Weather Station	Results	Year = 0	Year = –1	Coal power station
								Rainfall (mm)	Rainfall (mm)	<50 km
Porcine	Liver	Bela-Bela	L	9	2012	Warmbad Towoomba	Positive for Mercury (Hg) = 200 µg/kg	1.66	0	No
Poultry	Liver	Worcester	WC	9	2012	WorcesterAws	Neg	0.37	0.23	No
Poultry	Liver	Keneth Kaunda	NW	10	2012	Klerksdorp	Neg	0.14	0.66	No
Bovine	Liver	Swartruggens	NW	10	2012	Rustenburg	Neg	2.94	1.93	No
Ovine	Liver	DR Ruth Segomotsi Mompati	NW	10	2012	Vryburg	Neg	0.50	0	No
Bovine	Liver	Caledon	WC	11	2012	Hermanus	Neg	0.23	0.65	No
Ovine	Liver	Willowdene	FS	11	2012	Jhb Bot Tuine	Neg	1.04	0.01	No
Bovine	Liver	Douglas	NC	11	2012	Kimberley Wo	Neg	0.65	1.37	No
Bovine	Liver	Ladysmith	KZN	11	2012	Ladysmith	Neg	1.33	0.81	No
Porcine	Liver	Mooi Rivier	KZN	11	2012	Mooi River	Neg	2.75	3.13	No

Specie	Sample Type	District	Province	Month	Year	Weather Station	Results	Year = 0	Year = –1	Coal power station
								Rainfall (mm)	Rainfall (mm)	<50 km
Porcine	Liver	Pretoria	G	11	2012	Pretoria Eendracht	Neg	4.07	1.84	No
Ovine	Liver	Vrede	KZN	11	2012	Vrede	Neg	0.99	0.51	No
Ovine	Liver	Kimberly	NC	1	2013	Kimberley Wo	Neg	1.59	1.35	No
Bovine	Liver	Gert Sibande	M	4	2013	Ermelo Wo	Neg	3.92	0.73	Yes
Poultry	Liver	Darling	WC	5	2013	Geelbek	Neg	1.08	0.86	No
Poultry	Liver	Atlantis	WC	5	2013	Malmesbury	Neg	1.23	0.75	No
Porcine	Liver	Mooi Rivier	KZN	5	2013	Mooi River	Neg	1.57	0.01	No
Poultry	Liver	Camperdown	KZN	5	2013	Oribi Airport	Neg	0.86	0.41	No
Porcine	Liver	Paarl	WC	5	2013	Paarl	Neg	2.46	1.57	No
Ovine	Liver	Velddrif	WC	6	2013	Langebaanweg	Neg	1.59	1.76	No
Poultry	Liver	Paarl	WC	6	2013	Paarl	Neg	5.47	3.76	No
Poultry	Liver	Darling	WC	7	2013	Geelbek	Neg	0.75	1.87	No
Bovine	Liver	Lejweleputswa	FS	7	2013	Glen College Aws	Neg	0.21	0.25	No

Specie	Sample Type	District	Province	Month	Year	Weather Station	Results	Year = 0	Year = –1	Coal power station
								Rainfall (mm)	Rainfall (mm)	<50 km
Ovine	Liver	Paarl	WC	7	2013	Paarl	Neg	3.61	4.46	No
Ovine	Liver	Mopani	L	7	2013	Thohoyandou Wo	Neg	0.28	0.00	No
Bovine	Liver	Kgetleng River	NW	8	2013	Rustenburg	Neg	0	0	No
Ovine	Liver	Metsimaholo	FS	8	2013	Vereeniging	Neg	0.41	0.05	Yes
Ovine	Liver	Dr Ruth Segomotsi Mompati	NW	8	2013	Vryburg	Neg	0	0	No
Bovine	Liver	Lejweleputswa	FS	11	2013	Glen College Aws	Neg	3.14	0.59	No
Bovine	Liver	Olifanthoek	NC	11	2013	Kathu	Neg	0.23	0.21	No
Bovine	Liver	Paarl	WC	11	2013	Paarl	Neg	3.50	0.30	No
Bovine	Liver	Heidelberg	G	11	2013	Springs	Neg	3.60	2.85	No
Bovine	Liver	Overberg	WC	11	2013	Struisbaai	Neg	3.09	0.23	No
Poultry	Liver	Botshabelo	FS	1	2014	Bloemfontein Wo	Neg	0.48	1.41	No

Specie	Sample Type	District	Province	Month	Year	Weather Station	Results	Year = 0	Year = –1	Coal power station
								Rainfall (mm)	Rainfall (mm)	<50 km
Bovine	Liver	Darling	WC	1	2014	Geelbek	Neg	0.17	0.17	No
Ovine	Liver	Darling	WC	1	2014	Geelbek	Neg	0.17	0.17	No
Porcine	Kidney	Kroonstad	FS	1	2014	Kroonstad	Neg	1.11	1.41	No
Ovine	Liver	Madibeng	NW	1	2014	Mafikeng Wo	Neg	3.36	0.79	No
Poultry	Liver	Atlantis	WC	1	2014	Malmesbury	Neg	1.51	0.14	No
Poultry	Liver	Camperdown	KZN	1	2014	Oribi Airport	Neg	2.01	3.08	No
Poultry	Liver	Stellenbosch	WC	1	2014	Strand	Neg	1.63	0.57	No
Poultry	Liver	Weltevreden	WC	1	2014	Strand	Neg	1.63	0.57	No
Bovine	Liver	Hartswater	NC	1	2014	Taung	Neg	0.05	3.05	No
Poultry	Liver	Mopani	L	1	2014	Thohoyandou Wo	Neg	9.91	19.51	No
Bovine	Kidney	Gert Sibande	M	2	2014	Ermelo Wo	Neg	2.68	3.14	Yes
Bovine	Liver	Senekal	FS	2	2014	Ficksburg	Neg	1.08	1.27	No
Porcine	Liver	Modimolle	L	2	2014	Warmbad Towoomba	Neg	3.61	3.09	No

Specie	Sample Type	District	Province	Month	Year	Weather Station	Results	Year = 0	Year = –1	Coal power station
								Rainfall (mm)	Rainfall (mm)	<50 km
Porcine	Liver	Baynesfield	KZN	5	2014	Oribi Airport	Neg	0.07	0.86	No
Poultry	Liver	Camperdown	KZN	5	2014	Oribi Airport	Neg	0.07	0.86	No
Bovine	Liver	Thabo Mofutsanyana	FS	7	2014	Bethlehem Wo	Neg	0	0	No
Poultry	Liver	Mangaung	FS	7	2014	Bloemfontein Wo	Neg	0	0.08	No
Bovine	Liver	Amathole	EC	7	2014	Dohne Agr	Neg	0.07	0.47	No
Ovine	Liver	Gert Sibande	M	7	2014	Ermelo Wo	Neg	0	0	Yes
Ovine	Liver	Fezile Dabi	FS	7	2014	Kroonstad	Neg	0	0	No
Ovine	Liver	Aliwal North	EC	8	2014	AliwalNorth Plaatkop	Neg	0.36	0.03	No
Bovine	Liver	Amathole	EC	10	2014	Dohne Agr	Neg	2.01	3.45	No
Bovine	Liver	Amathole	EC	10	2014	Dohne Agr	Neg	2.01	3.45	No
Poultry	Liver	Francis Baard	NC	10	2014	Kimberley Wo	Neg	0.18	0.51	No
Ovine	Liver	Kgetleng	NW	10	2014	Rustenburg	Neg	1.03	1.93	No

Specie	Sample Type	District	Province	Month	Year	Weather Station	Results	Year = 0	Year = –1	Coal power station
								Rainfall (mm)	Rainfall (mm)	<50 km
Bovine	Kidney	Gordonia	NC	10	2014	Upington Wo	Neg	0.43	0.26	No
Bovine	Liver	ZF Mcgawu	NC	10	2014	Upington Wo	Neg	0.43	0.26	No
Ovine	Liver	DRSM	NW	10	2014	Vryburg	Neg	0.29	0.32	No
Ovine	Liver	Namakwa	NC	11	2014	Brandvlei	Neg	1.10	0	No
Ovine	Liver	Namakwa	NC	11	2014	Brandvlei	Neg	1.10	0	No
Bovine	Liver	Lejweleputswa	FS	11	2014	Glen College Aws	Neg	8.50	3.14	No
Bovine	Liver	Lesedi	G	11	2014	Grand Central	Neg	1.84	1.34	No
Ovine	Liver	Paarl	WC	11	2014	Paarl	Neg	1.46	3.50	No
Bovine	Liver	Overberg	WC	11	2014	Struisbaai	Neg	1.13	3.09	No
Bovine	Liver	Christiana	NC	11	2014	Taung	Neg	3.36	1.07	No
Bovine	Liver	ZF Mcgawu	NC	11	2014	Upington Wo	Neg	1.14	0.05	No
Bovine	Liver	Gert Sibande	M	12	2014	Ermelo Wo	Neg	3.59	5.63	Yes
Bovine	Liver	Gert Sibande	M	12	2014	Ermelo Wo	Neg	3.59	5.63	Yes

Specie	Sample Type	District	Province	Month	Year	Weather Station	Results	Year = 0	Year = –1	Coal power station
								Rainfall (mm)	Rainfall (mm)	<50 km
Bovine	Liver	Klipheuwel	WC	12	2014	Paarl	Neg	0.15	0.06	No
Ovine	Liver	Mangaung	FS	1	2015	Bloemfontein Wo	Neg	0.81	0.48	No
Poultry	Liver	Mangaung	FS	1	2015	Bloemfontein Wo	Neg	0.81	0.48	No
Porcine	Liver	Winelands	WC	1	2015	Cape Town Wo	Neg	0.44	0.75	No
Porcine	Liver	Viljoenskroon	FS	1	2015	Klerksdorp	Neg	2.72	0.55	No
Bovine	Liver	Velddrif	WC	1	2015	Langebaanweg Aws	Neg	0.36	0.92	No
Poultry	Liver	Malmesbury	WC	1	2015	Malmesbury	Neg	0.42	1.51	No
Bovine	Liver	Waterberg	L	1	2015	Marken	Neg	0.71	1.53	No
Poultry	Liver	Camperdown	KZN	1	2015	Oribi Airport	Neg	2.94	2.01	No
Ovine	Liver	De Aar	NC	2	2015	De Aar Wo	Neg	0.63	2.55	No
Bovine	Liver	Nkonyale	KZN	2	2015	Greytown	Neg	5.39	2.31	No
Poultry	Liver	Mopani	L	2	2015	Thohoyandou Wo	Neg	4.30	2.99	No
Poultry	Liver	Malmesburg	WC	5	2015	Malmesbury	Neg	0.70	1.77	No

Specie	Sample Type	District	Province	Month	Year	Weather Station	Results	Year = 0	Year = –1	Coal power station
								Rainfall (mm)	Rainfall (mm)	<50 km
Poultry	Liver	Camperdown	KZN	5	2015	Oribi Airport	Neg	0.09	0.07	No
Bovine	Liver	Thabo Mofutsanyana	FS	6	2015	Bethlehem Wo	Neg	1.05	0	No
Bovine	Liver	Bronkhorstspruit	G	6	2015	Witbank	Neg	0.05	0.31	No
Poultry	Liver	Mangaung	FS	7	2015	Bloemfontein Wo	Neg	0.50	0	No
Ovine	Liver	Fezile Dabi	FS	7	2015	Kroonstad	Neg	0.21	0	No
Poultry	Liver	Paarl	WC	7	2015	Paarl	Neg	4.58	5.22	No
Ovine	Liver	Moorreesburg	WC	7	2015	Porterville	Neg	2.17	2.63	No
Ovine	Liver	Namakwa	NC	9	2015	Brandvlei	Neg	0.12	0.02	No
Poultry	Liver	Fezile Dabi	FS	9	2015	Kroonstad	Neg	0.70	0.01	No
Porcine	Liver	Nottingham	KZN	9	2015	Mooi River	Neg	0.12	0.02	No

KZN – KwaZulu Natal, L – Limpopo, M Mpumalanga, FS – Free State, NC – Northern Cape, WC – Western Cape, G – Gauteng, EC – Eastern Cape, NW – North West, Neg – negative.

ADDENDUM D: ETHICAL CLEARANCE CERTIFICATE

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 03/14/2020.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

28/09/2017

Approval Certificate
New Application

Ethics Reference No: 435/2017

Title: Mycotoxin prevalence and heavy metal contamination of South African red meat

Dear Ms Maricia van Deventer-Schoeman

The **New Application** as supported by documents specified in your cover letter dated 22/09/2017 for your research received on the 22/09/2017, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 27/09/2017.

Please note the following about your ethics approval:
- Ethics Approval is valid for 1 year
- Please remember to use your protocol number (**435/2017**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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