The effect of mycotoxin exposure on the growth of infants and young children in deep rural areas of the Eastern Cape Province, South Africa

RS Tshalibe

orcid.org/0000-0001-8979-7412

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Supervisor: Dr MJ Lombard
Co-supervisor: Dr H-M Burger
Co-supervisor: Dr C Taljaard

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PREFACE

I wish to express my profound gratitude to the following people who contributed greatly towards my work:

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To my brothers, sisters and family, thank you for your encouragement and being by my side throughout this PHD study.

My late remarkable mum and my dad who taught me to be hard working and be goal oriented, they moulded my character tremendously.

Finally, all the Glory goes to the Almighty God for his faithfulness, power, special grace and love throughout this endeavour.
ABSTRACT

Background

Malnutrition (especially undernutrition) is a global public health concern in developing countries such as South Africa (SA) and is predominately prevalent among infants and young children (IYC) less than 24 months of age. In rural, low-income communities of SA, the stunting prevalence is particularly high. These areas are usually subsistence farming communities that are mostly relying on maize as a staple food. Recent research indicated that environmental factors such as mycotoxin exposure are a possible contributing factor to impaired growth among children. Mycotoxins are toxic secondary metabolites produced by naturally occurring food-borne fungi. The mycotoxins present in the Eastern Cape (EC) are fumonisins (FB), deoxynivalenol (DON) and zearalenone (ZEA), aflatoxins (AF) are known to be absent. However, very little is known about the association between mycotoxin exposure and child growth and the complexity of confounding factors. The interaction between mycotoxin exposure and impaired growth could be of crucial importance in the reduction of morbidity and mortality amongst young children in rural areas of SA. Various factors influence growth causing undernutrition. These factors include amongst others, repeated infectious diseases, poor nutrient intake and poor sanitary infrastructure.

The overall aim of this thesis was to firstly determine multi-mycotoxin exposure levels of infants and young children and its effects on infant growth parameters in deep rural areas of the EC, and secondly to determine the effect (if any) that these mycotoxins have on the growth of these IYC. To achieve the aim of this study, the following specific objectives were identified: i) to describe the basic sociodemographic situation of households as well as general health and maize dietary intake of infants and young children; ii) to determine multi-mycotoxin exposure of children (0 - 24 months) in rural maize-subsistence farming areas of EC, South Africa; iii) to assess child growth indicators during the first 24 months of life and iv) to compare multi-mycotoxin exposure and infant and child growth at 0 - 12 months and 13 - 24 months.

Methods

The current study is a sub-study of the larger Philasana study. The primary aim of the PhilaSana study was to investigate the various factors influencing infant feeding and growth. This was in the form of a longitudinal, observational study and followed pregnant women and their infants up to the age of two years. The study was conducted in the Amatole District Municipality in the EC. A total of 234 infants and young children were included in the study, although not all of them were
followed up, due to availability on time of visit. Snowball sampling was used to identify possible participants.

The first article in this sub-study measures mycotoxin concentration levels and was thus a cross-sectional study design. The last two articles written in this sub-study used data collected at some of the PhilaSana time points and utilised as a longitudinal study design. Maize consumption of the IYC was determined with a quantitative food frequency questionnaire (the RAPP tool), which was designed and validated specifically for this Xhosa population. Once mean daily maize intake (cooked) was determined it was converted to raw maize intake based on recipes and ratios (raw: cooked) established during the development of the questionnaire. Once the raw maize intake of the IYC were obtained, the level of mycotoxin contamination in the raw maize was analysed. Thereafter, mycotoxin exposure was calculated and expressed as a probable daily intake (PDI, µgkg⁻¹ body weight day⁻¹). Growth of IYC was measured as weight and length / height. Current weight and length / height, head circumference (HC) and mid upper circumference (MUAC) z-scores were determined as well as birth anthropometric information as provided in the Road to Health Booklet (RTHb). Change in growth was determined by subtracting current z-score from the previous z-score or birth z-scores. This was conducted to determine the direction of growth (in other words is the infant growing at the required rate or not). WHO Anthro plus was used to determine z-scores. Furthermore, confounding factors such as health status (HIV and TB) of the children, food intake and socio-demographic factors were examined.

Results

The mean total FB, DON and ZEA levels for analysed home-grown maize samples were 1035, 24.5 and 31.0 µg kg⁻¹ respectively. Furthermore, mean daily maize intakes of children 0 - 24 months ranged from 1.6 g - 321 g day⁻¹. The mean probable daily intakes (PDI) of these children for total FB was above the PMDTI, while that of DON and ZEA were below the PMDTI.

Approximately 16% of the infants 0 – 12 months of age were stunted, however none of them were wasted or underweight. Furthermore, it was determined that infants were exposed to mean FB, DON and ZEA above the Provisional Maximum Tolerable Daily Intake (PMDTI). The mean length of infants exposed to high FB exposure levels was 4.4 cm shorter than the low exposed group, though they had a mean weight difference of only 0.3 g. ANCOVA results indicated that high FB exposure was significantly associated with LAZ, WAZ scores and reduction in length of infants 0 - 12 months.

Furthermore, 34% of the young children were stunted within the 13 - 24 age group, while none of the children were wasted and underweight. ANCOVA also showed a significant difference in WLZ
and LAZ changes with high FB exposure (p < 0.05). Linear regression further indicated that FB, DON, and ZEA exposure was associated with reduction in weight gain (g kg\(^{-1}\) day\(^{-1}\)).

**Conclusion**

The EC residents are predominately maize subsistent farmers. Maize samples analysed from the maize cobs collected in EC, had high levels of mycotoxins. Infants and young children from this area consume home-grown maize-based dishes such as soft porridge, *maheu* (fermented maize-meal) and maize meal. However, the home-grown maize in this area is contaminated with three mycotoxins, FB, DON and ZEA. The exposure levels of infants in this area was observed to be above the PMDTI, therefore posing serious health threats. Results concluded that FB exposure might be amongst the contributing factors of growth impairment in this area. The conclusion is in support of the notion that mycotoxin exposure results in impaired growth, due to poor appetite, reduced intestinal permeability and inflammatory reactions. The results are also in support of previous knowledge that z-scores and length of children are associated with high mycotoxin exposure. Furthermore, the results add on to explain further that growth changes are associated with mycotoxin exposure; therefore, growth rate is an issue not to be ignored regarding mycotoxin exposure. On the other hand, children were discovered to not be gaining sufficient weight in relation to their length, though they were gaining weight in relation to their current age. This alarming finding further explained growth impairment of the participants, the weight again was expected to be due to maize intake. Dietary diversification and safe complementary feeding are essential to curb growth impairment (especially in terms of length) and mycotoxin exposure amongst infants and children 0 - 24 months of age in EC.

**Key terms:** multi-mycotoxin exposure, children, risk assessment, maize-subsistence farming areas, growth impairment, malnutrition.
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<table>
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<tr>
<th>Term</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>Growth</td>
<td>A term used to refer to weight, length / height and where relevant mid upper arm circumference (MUAC) and head circumference (HC).</td>
</tr>
<tr>
<td>Change in growth</td>
<td>Determined by subtracting current z-score from the previous z-score (birth z-score or z-score at 0-12 months depending on the paper in concern). This was to determine a change in growth rate.</td>
</tr>
<tr>
<td>Growth rate</td>
<td>Determined by length change (growth per week, (cm week(^{-1})) and weight change (weight gain, (g kg(^{-1}) day(^{-1}))). This was calculated to determine if the exposed infants and young children grow at a slower rate than the unexposed.</td>
</tr>
<tr>
<td>Birth outcomes</td>
<td>The birth anthropometric information of the infants (weight, length / height and where relevant mid upper arm circumference (MUAC) and head circumference (HC).</td>
</tr>
<tr>
<td>Dietary intake</td>
<td>This was the habitual food intake of the young child; in this thesis it refers to the cooked maize intake which was utilised to calculate raw maize intake of the child per day.</td>
</tr>
<tr>
<td>General health</td>
<td>Only self-reported HIV and TB were included. Other health indicators were logistically difficult to measure.</td>
</tr>
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AF</td>
<td>Aflatoxin</td>
</tr>
<tr>
<td>CF</td>
<td>Complementary feeding</td>
</tr>
<tr>
<td>DON</td>
<td>Deoxynivalenol</td>
</tr>
<tr>
<td>EC</td>
<td>Eastern Cape</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agricultural Organisation</td>
</tr>
<tr>
<td>FB</td>
<td>Fumonisins</td>
</tr>
<tr>
<td>HAZ</td>
<td>Height-for-Age Z-scores</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>IUGRT</td>
<td>Intra-uterine growth retardation</td>
</tr>
<tr>
<td>IYCF</td>
<td>Infant and Young Child Feeding</td>
</tr>
<tr>
<td>JECFA</td>
<td>Joint FAO/WHO Expert Committee on Food Additives</td>
</tr>
<tr>
<td>NICHD</td>
<td>National Institute of Child Health and Human Development</td>
</tr>
<tr>
<td>OECD</td>
<td>Organisation for Economic Co-operation and Development</td>
</tr>
<tr>
<td>PEM</td>
<td>Protein energy malnutrition</td>
</tr>
<tr>
<td>PMCT</td>
<td>Prevention from mother to child transmission</td>
</tr>
<tr>
<td>PMTDI</td>
<td>Provisional Maximum Tolerable Daily Intake</td>
</tr>
<tr>
<td>SANHANES</td>
<td>South African National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>WAZ</td>
<td>Weight-for-Age Z-scores</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>WLZ</td>
<td>Weight-for-Length Z-scores</td>
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<tr>
<td>ZEA</td>
<td>Zearalenone</td>
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CHAPTER 1 INTRODUCTION

1.1 Rationale of the study

Malnutrition, especially undernutrition, remains a major health concern worldwide. It is the main underlying cause of death in children under 60 months of age, causing nearly half of all child deaths in the world (UNICEF, 2017). In Eastern and Southern Africa, 34.5% of children are undernourished (UNICEF, 2017). A large proportion of the undernutrition is related to stunting and wasting, resulting in, among others, delayed mental development, physiological effects, lower fat oxidation and hypertension in later life (UNICEF, 2017). Stunting is mostly associated with chronic undernutrition, while wasting is more often associated with acute undernutrition (Chen et al., 1980). Chronic undernutrition, as measured by stunting, has declined worldwide, however in 2016, 155 million children under the age of 60 months (UNICEF, 2017) were still affected. Acute undernutrition (wasting) affected 52 million children under 60 months (UNICEF, 2017).

Stunting and wasting are annually responsible for more than 2 million deaths and negatively affects the intellectual ability of children less than 60 months of age (Black et al., 2008). Children in sub-Saharan African countries such as South Africa are severely affected by chronic and acute undernutrition, especially in rural areas (Faber et al., 2005; Mamabolo et al., 2007). Many children (n = 1 188) in South Africa under five years died from acute malnutrition in 2016/17 (Massyn et al., 2017). Of the nine provinces, the Eastern Cape (EC) had the second highest percentage with 10.2% (n = 226) of children who passed away from severe and acute undernutrition (n = 1 188) (Massyn et al., 2017).

The first South African National Health and Nutrition Examination Survey (SANHANES-1) reported that approximately 26.9% of South African boys below the age of 36 months were stunted (< -2 SD (Height - for – Age (HAZ)) of which 9.9% were severely stunted (< -3 SD HAZ) (Shisana et al., 2013). In addition to this, 25.9% of girls under the age of 36 months were stunted (< -2 SD HAZ) of which 9.1% were severely stunted (< -3 HAZ) (Shisana et al., 2013). Of the boys in the Eastern Cape (EC), 21.6% were moderately stunted while 15.6% of the girls were moderately stunted (Shisana et al., 2013). Moderate stunting occurs when a child has a Z-score between -2 standard deviations (SD) and -3 SD (Opintan et al., 2010). It was reported that nationally 4.0% of the boys and 4.0% of the girls showed severe wasting. In the EC 1.6% of boys were wasted whilst 0.2% boys were severely wasted (Shisana et al., 2013). Amongst the girls, 3.2% were wasted whilst 1.1 % were severely wasted in EC (Shisana et al., 2013). However, the total sample size of this study is small and may not represent the severity of the undernutrition situation, especially in the rural areas.
Undernutrition is defined by growth standards. These are used to standardise a child’s growth parameters by comparing them with the median / average measure for children of the same age and gender (WHO, 1986). Taking age and gender into consideration, differences in growth parameters can be expressed in several ways such as SD units of either z-scores or a percentage of the median and percentiles (WHO, 1986; Onis, 2006). The z-score unit is defined as the difference between the value for an individual and median value of the reference population for the same age or height, divided by the SD (Wühl et al., 2002). The z-scores are currently used as the primary indicators for growth monitoring whereby delayed growth will result in faltering. Different cut-off values enable the different individual measurements to be converted into prevalence statistics. Cut-off values are used for identifying children suffering from undernutrition (Gibson, 2005).

Growth is measured by comparing length against the child’s age. Stunting is then defined as retarded skeletal growth due to long-term dietary inadequacies, repeated infections or both (Victora et al., 2008). In children less than 24 months of age stunting is measured by a length-for-age z-score (LAZ) less than -2 SD of the average (Lassi et al., 2013). Stunting dramatically increases during this period because of elevated requirements for a variety of nutrients required for rapid growth (Burgess, 2008). Weight-for-age z-scores (WAZ) denotes underweight in children less than 24 months of age, while weight-for-length (WLZ) z-score signifies wasting. Underweight is established by WAZ scores less than -2 SD and wasting as WLZ scores less than -2 SD.

The risk factors associated with undernutrition among rural South African children are diverse and complex. Socio-economic factors include food insecurity and poverty, infectious diseases (high HIV prevalence in South Africa), environmental (contaminated drinking water), poor sanitation infrastructure and psychosocial factors (Walker et al., 2007). South Africa is a developing country characterized by a rapid demographic and nutritional transition (Steyn et al., 2005). This transitional process includes the impact of urbanisation on cultural practices where more “westernized” practices either replace or coexist with the traditional. Exclusive breastfeeding for a period of six months has been recommended by the WHO (Fewtrell et al., 2007), although it is well known that infants in rural areas receive complimentary food from an early age (Mamabolo et al., 2004).

Parents should be advised to introduce complementary foods from six months and gradually increase frequency, consistency and variety of locally available foods (Ramirez-Avila et al., 2012). Nutrition education on preparation of these complementary foods is also essential (Ramirez-Avila et al., 2012). In most parts of South Africa, maize is the main cereal used as complementary food. In rural areas such as the EC, subsistence farming is a major source of
food security where the daily intake of maize is part of a culturally distinct dietary pattern and ethnic tradition (Lombard, 2014). In developing countries, such as those in sub-Saharan Africa, there are often limitations in the quality and quantity of available complementary foods (Shrimpton et al., 2001; Dewey & Adu-Afarwuah, 2008). Complementary feeding (CF) for infants refers to the timely introduction of safe and nutritious foods (at approximately six months of age) in addition to breastfeeding (Imdad et al., 2011). Complementary feeding should be appropriate and given in sufficient quantity (Kramer & Kakuma, 2007).

Undernutrition is more common in children residing in rural areas due to the poor socio-economic and environmental factors they are exposed to (Bain et al., 2014). Rural areas can be defined as pastoral landscapes, with unique demographic structures and settlement patterns, isolation, low population density, extractive economic activities, and distinct sociocultural milieus (Hart et al., 2005). These rural areas are usually based on subsistence farming with limited farming and storage methods.

Toxic secondary metabolites known as mycotoxins are produced by natural occurring food-borne fungi such as Fusarium spp (Pearson et al., 2002). The most important mycotoxins relevant to rural areas in the EC include FB, DON and ZEA (Shephard et al., 2013). Food contaminated by mycotoxins is considered a global public health priority and poses a threat to humans and animals as well as national economies in terms of industry and international maize exports (Bryden, 2007).

In recent years there has been a large interest in the role mycotoxins play in child growth faltering and thus stunting and wasting (Khlangwiset et al., 2011; Smith et al., 2012). Gut inflammation has been proposed as a possible mechanism linking mycotoxin exposure to poor child growth (Smith et al., 2012).

The underlying relationship between FB and stunting is important (Smith et al., 2012). In addition, the effect of DON exposure on growth in children has also not yet been studied. Exposure to DON in humans may cause gastroenteritis, growth faltering and immune toxicity (Turner et al., 2008). An ability to conduct accurate exposure assessment at the individual level is required to fully understand the potential health consequences for humans (Turner et al., 2008). Nevertheless, it is likely that DON has a negative effect on growth because of decreased food intake and reduced weight gain that has been observed in animal studies (Turner et al., 2008).

The 74th meeting of the Joint Food and Agricultural Organisation and the World Health Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA), provided a No
Observed Adverse Effect Level (NOAEL) of 0.2 mg kg⁻¹ body weight (bw) day⁻¹ and a safety factor of 100, as a group provisional maximum tolerable daily intake (PMTDI) for FB1, FB2 and FB3, alone or in combination, of 2 µg kg⁻¹ bw day⁻¹ (JECFA, 2012). The PMTDI for DON is 1 µg kg⁻¹ bw day⁻¹ and the PMTDI for ZEA is 0.5 µg kg⁻¹ bw day⁻¹ (JEFC, 2001; JEFCA, 2002).

The choice of method for conducting an exposure assessment is influenced by the purpose of the exposure assessment, the nature of the food chemical and the resources available for the study (Lambe, 2002). Deterministic methods estimate intakes of food chemicals in foods in a population, whereas probabilistic methods entail the advantage of estimating the probability with which different levels of intake will occur (Lambe, 2002). Probabilistic analysis permits the exposure assessor to model the variability (true heterogeneity) and uncertainty that may exist in the exposure variables, including food consumption data, thus examining the full distribution of possible resulting exposures (Lambe, 2002). However, the challenge of probabilistic modelling is the selection of appropriate modes of inputting food consumption data into the models (Lambe, 2002). Nonetheless, monitoring exposure to mycotoxins has become an integral part of ensuring the safety of the food supply (Lambe, 2002).

Using a deterministic approach (exposure was calculated based on known mycotoxin levels and raw maize intake), it was found that 12% of 215 infants who received a maize-based complementary food in Tanzania exceeded the PMTDI of 2 µg kg⁻¹ body weight of FB (Kimanya et al., 2010). Importantly, it was observed that at 12 months of age, the infants with FB exposures above the PMTDI were significantly shorter by 1.3 cm and lighter by 328 g than those with exposures below the limit. These findings suggest that FB intake is associated with growth impairment (Kimanya et al., 2012).

Currently very little is known about the role of mycotoxin exposure in the growth patterns among rural infants living in rural EC, South Africa (Lombard et al., 2014). The deterministic approach is relevant to this study due to the applicability to the study hypothesis, access and availability of analytical methods and resources. Evidence-informed data will therefore be valuable to address the overall high mycotoxin-exposure as well as its relation to growth impairment in vulnerable communities of South Africa.

1.2 The study setting and participants

The study was conducted in villages within the Amatole District Municipality, which is situated to the South of the EC of South Africa (Figure 1.1). These areas are deep rural areas, sparsely populated, and dominated by isiXhosa speaking individuals. The area covers a radius of 60 kilometres, with very little infrastructure. Accessibility to households is limited to a few
available gravel roads (Figure 1.2). Homes are scattered around the countryside and no formal address system exist or listings thereof. Because of these logistical challenges, participants are recruited within their villages via snowball sampling. The children in these areas rarely get access to medical attention from the distant clinics, as they must walk long distances to reach these. Reported child deaths in hospitals in this area recorded from during January – November 2009 were 347, making it the second highest in the Province (Province of the EC Health, 2010). However due to home deaths it can be assumed that this figure is higher, and some deaths were not reported.

Figure 1-1: District Municipalities in Eastern Cape Province (Source: Province of the Eastern Cape Health, 2010)

Figure 1-2: A typical subsistence farm in the rural Eastern Cape
1.3 Problem statement

The former Transkei region in the EC, is a deep rural area characterised by a high prevalence of poverty and poor infrastructure and therefore an increased risk of child wasting, and stunting exists (Shisana et al., 2013). Most of the inhabitants are dependent on government pensions and grants while migrant labourers provide an additional income (D’Haese & Van Huylenbroeck, 2005). Subsistence farming in South Africa is part of a culturally distinct dietary pattern and ethnic tradition in this area (Lombard et al., 2013; Lombard et al., 2014). A preliminary survey conducted in the study area amongst mothers and primary caregivers of infants indicated that they are depending on soft maize porridge as primary complementary food since home-grown maize is their subsistence crop (unpublished data). It has furthermore been well-documented that the home-grown maize in these rural areas contains extremely high levels of mycotoxins such as fumonisin B (FB), deoxynivalenol (DON) and zearalenone (ZEA) (Shephard et al., 2013, Burger et al., 2010).

The determination of mycotoxins exposure, which forms an integral part of the human risk assessment process, is of critical importance. The role of mycotoxins in child growth is grounded by epidemiological observations of the prevalence of stunting and the known high mycotoxin concentrations on the home-grown maize in this area. Currently very little is known about the mycotoxin exposure levels of infants and young children, nor about the role of mycotoxins exposure in the development of malnutrition. For instance, it is not known if mycotoxins have a direct or indirect (causing poor absorption, loss of appetite etc.) effect on undernutrition. Evidence-based information will therefore be valuable to address the overall high mycotoxin exposure as well as the high malnutrition rates in vulnerable communities in South Africa.

This study is a sub-study of a larger study, called PhilaSana, an isiXhosa name for “healthy infant”. The PhilaSana study is a longitudinal study following pregnant women and their infants until the age of 24 months. During this period, mothers and infants will be visited 4-6 times. The primary aim of PhilaSana is to investigate various factors associated with undernutrition in the Amatole District Municipality in the EC. Amongst these factors are mycotoxin exposure.

The aim and objectives of this sub-study include data collected under objectives 2, 3, 4 and 5 of the larger PhilaSana study. All methods used, and data collected for the sub-study have been described in detail in Chapter 3 of this thesis.
1.4  Aim

The aim of the study was to determine the exposure levels as well as the direct effect of multi-
mycotoxin exposure on infant and young child growth from birth to 24 months of age in deep
rural areas within the Amatole District Municipality of the EC.

1.5  Objectives

To achieve the aim of this study, the following specific objectives were identified:

- To describe the current sociodemographic situation of households as well as general
  health (HIV and TB) and maize intake of infants and young children;
- To determine multi-mycotoxin exposure of children (0 - 24 months) in rural maize-
  subsistence farming areas of EC, South Africa;
- To assess child growth indicators during the first 24 months of life;
- To compare multi-mycotoxin exposure and infant and child growth at 0 - 12 months
  and 13 - 24 months.

1.6  Ethical approval

The study was conducted according to the Helsinki declaration (World Medical Association,
2013) and the International Conference on Harmonisation guidelines (ICH steering committee,
1996). The study was approved by the Health Research Ethics Committee (HREC) of the
Faculty of Health Sciences, North-West University (Potchefstroom Campus) (NWU-00207-14-
S1) (Addendum 1). Before the onset of the study, goodwill permission was obtained from the
community leaders, including the local chief, headmen and traditional healers. Thereafter the
mothers signed informed consent before inclusion into the study.

1.7  Structure of the thesis

The thesis is organised in an article format according to the North–West University (NWU),
Potchefstroom campus guidelines. The thesis comprises of eight chapters. The NWU Harvard
referencing style was utilised for referencing in chapters one, two, three and eight of the
theses. Furthermore, references in chapters four, five and six were according to the relevant
journals. Reference lists were provided at the end of each chapter.

Chapter one provides a brief overview of the thesis, states the aim and objectives of the study
and elaborates on the research outputs emanating from the study. The chapter further outlines
the contributions of individual research team members.
Chapter two reviews literature on global guidelines of infant and young child feeding, prevalence of mycotoxins, biochemistry of mycotoxins, health effects of mycotoxins, measurement of mycotoxin exposure, determination of growth in children as well as the sociodemographic factors influencing development of infants and young children. It may seem as if some information is incomplete, however, literature regarding this topic is very limited.

Chapter three is an introductory chapter on the sociodemographic factors influencing growth of infants and young children.

Chapter four, five, six encompass the results output of this thesis in the format of research papers to be submitted to respective journals, these articles comply with the journal author guidelines. The articles will be submitted to World Mycotoxin Journal, Food and Chemical Toxicology Journal and Toxicology.

Chapter seven is a concluding chapter, which aims at explaining the mycotoxin and growth changes six monthly over a 24-month period. Due to large loss to follow-up, the available data is not sufficient to warrant a paper and thus it was added as a summary chapter.

Chapter eight summarises the study discussing the conclusions and recommendations for further study. The concluding chapter is structured according to the objectives of the study and will also identify the strengths and limitations of the study.

1.8 Research outputs of the study

Three papers will be submitted for approval to peer reviewed international journals. Paper 1 is currently under review at the World Mycotoxin Journal (see Addendum 2 for author information). This paper is the foundation of exposure data for paper 2 and 3 and thus these two will be submitted to review as soon as paper 1 is accepted. Paper 2 will be submitted to Food and Chemical Toxicology (Addendum 3) and paper 3 to Toxicology (Addendum 4). The papers were written according to the requirements of the individual journals.

Feedback of the study will be provided to the research participants. Results will be presented at National and International Congresses. To date, two of the papers have been presented at the 27th Congress of the Nutrition Society of South Africa and the 15th Congress of the Association for Dietetics in South Africa (Misty hills, Johannesburg), 5 – 7 September 2018 and the second edition of the World Public Health Nutrition Conference held in 2016 in Cape Town.

Information will also be disseminated to the communities via community meetings.
1.9 Contributions of members of the research team

Although the total research team is extensive and transdisciplinary, for this thesis only Miss Tshalibe, Dr Lombard, Dr Burger and Dr Taljaard participated in the data collection and writing of the thesis. Table 1.1 provides a summary of the role of everyone.

Table 1-1: Contributions of research team

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
<th>Role in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miss Ropafadzo S. Tshalibe</td>
<td>Centre of Excellence in Nutrition (CEN), North-West University, Potchefstroom campus</td>
<td>Involved in planning of the study, data collection, capturing and cleaning. Interpretation of results and statistical analysis. Primary author of the three papers. The individual also compiled the thesis.</td>
</tr>
<tr>
<td>Dr Martani J. Lombard</td>
<td>Centre of Excellence in Nutrition (CEN), North-West University, Potchefstroom campus</td>
<td>Supervisor of Miss Ropafadzo Tshalibe. Had a supervisory role in this study. Played a role in planning, execution, data interpretation and statistical analysis.</td>
</tr>
<tr>
<td>Dr Hester-Mari Burger</td>
<td>Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology</td>
<td>Co-Supervisor of Miss Ropafadzo Tshalibe. Had a supervisory role in this study. Played a role in planning, execution and data interpretation. She was also involved in the layout and writing of the papers.</td>
</tr>
<tr>
<td>Dr Christine Taljaard</td>
<td>Centre of Excellence in Nutrition (CEN), North-West University, Potchefstroom campus</td>
<td>Co-Supervisor of Miss Ropafadzo Tshalibe. Had a supervisory role in this study.</td>
</tr>
<tr>
<td>Prof Wentzel Gelderblom</td>
<td>Institute of Biomedical and Microbial Biotechnology, Cape</td>
<td>Technical expert</td>
</tr>
</tbody>
</table>
A special thanks goes to the fieldwork team (Figure 1.3).

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Gordon Shephard</td>
<td>Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology</td>
<td>Technical expert</td>
</tr>
<tr>
<td>Dr John R. Rheeder</td>
<td>Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology</td>
<td>Technical expert</td>
</tr>
</tbody>
</table>

**Figure 1-3:** The research team
1.10 References


World Medical Association declaration of Helsinki (1964), Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964. Amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975; 35th World Medical Assembly, Venice, Italy, October 1983; and the 41st, World Medical Assembly, Hong Kong, September 1989.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

Various factors influence the prevalence of undernutrition in Africa. Poor environmental conditions, overpopulation, poverty and food insecurity are amongst the major causes (Bain et al., 2014). Africa’s food insecurity and nutrition situation are worsening, mostly due to climate changes (Barrett & Maxwell, 2007). Several factors have contributed to this situation including exceptionally high population growth rates, political conflicts, climate changes and the endemic poverty in some regions (Ahmed & Cleeve, 2004). However, there is need to address the nutrition situation, especially related to the 2030 agenda for Sustainable Development Goal number 3, of attaining good health and well-being, especially child health across the globe (UN, 2017). The Universal Declaration of Human Rights (1948), Article 25, recognises the access to safe food as a basic human right and the United Nations Millennium Declaration (2000) also reaffirmed “the right of everyone to have access to safe and nutritious food” to prevent malnutrition (Wernaart et al., 2010).

Malnutrition, especially undernutrition, impacts negatively on human physical and cognitive development as well as the immune system (WHO, 2014). Undernutrition is often aggravated by poor infant and young child feeding and care practices, poor sanitation and hygiene, lack of access to education, quality health systems, safe drinking water, food borne infections, parasitic infestations and ingestion of harmful levels of contaminants due to unsafe food along the food chain (WHO, 2014). Undernutrition increases susceptibility to communicable and non-communicable diseases, thereby restricting the attainment of human potential and reducing productivity (WHO, 2014). Undernutrition also poses a high burden in the form of negative social and economic consequences to individuals, families, communities and states (WHO, 2014). In sub-Saharan Africa, the leading risk factors for death and disability adjusted life years are child and maternal undernutrition, unsafe sex, unsafe water sanitation, and poor personal hygiene (Murray, 2015). Below is the global undernutrition situation from 2000 – 2016 (Figure 2.1).
Globally undernutrition affects 22.9% of infants and children below 36 months of age (UNICEF, 2017). In Southern and Eastern Africa undernutrition affects 34.5% of infants and children below 36 months (UNICEF, 2017).

During this period chronic undernutrition (stunting) affected 155 million children under 60 months of age, whilst acute undernutrition (wasting) affected 52 million children under 60 months of age worldwide (UNICEF, 2017). Globally stunting and wasting accounted for 19 291 disability adjusted life years (DALYS) and 112 350 DALYS respectively among 188 countries during the period 1990 - 2013 (Murray, 2015). Undernutrition was the main underlying cause of death in children under 60 months of age, causing approximately 50% of all child deaths worldwide (UNICEF, 2017). Meanwhile, undernutrition dropped from being the top to the fourth leading cause of DALYS globally and accounted for 119 802 DALYS from 1990 - 2013 (Murray, 2015). Furthermore, the stunting rates declined by 10% from 32.7% to 22.9% from the year 2000 to 2016 (UNICEF, 2017). In Eastern and Southern Africa, the stunting rate declined from 45% to 34.5% (UNICEF, 2017).

Undernutrition accounts for 4.6% of under five deaths in South Africa (Massyn et al., 2017). Severe and acute undernutrition are also underlying factors in almost a third of childhood deaths in South Africa (Massyn et al., 2017). In South Africa, 1 188 children (< 5 years of age) died from acute undernutrition in 2016/17 (Massyn et al., 2017). Of these deaths, 10.2% were from the Eastern Cape (EC) Province (Massyn et al., 2017). South Africa is amongst the 34...
countries with the highest burden of stunting (Bhutta et al., 2013b). The South African National Examination Survey (SANHANES-1) reported that approximately 26.9% (n = 137) of boys below the age of 36 months were stunted of which 9.9% were severely stunted (Shisana et al., 2013). Of the girls, 25.9% (n = 143) girls were also stunted, of which 9.1% (n = 26) were severely stunted (Shisana et al., 2013).

Sub-optimal feeding practices during the complementary feeding period presents a threat to the survival and well-being of African children (Onofiok & Nnanyelugo, 1998). In sub-Saharan Africa, complementary foods are mainly watery cereal porridges of low energy and nutrient densities (Gibson et al., 1998). Complementary foods are often prepared, served and stored utilising conditions that expose the child to frequent infections (Kimmons, 1999). Older infants and young children are also often not given the care and attention needed for the selection of nutritious foods and the encouragement needed to eat foods in sufficient amounts to meet their energy and nutrient requirements (Nti & Lartey, 2007).

A large number of infants and children in sub-Saharan Africa are in subsistence farming areas, hence environmental toxins might influence their health. It is well-documented that complementary foods fed to infants are prone to mycotoxin exposure. Mycotoxins are toxic secondary metabolites produced by fungi on the food source (mostly maize and ground-nuts) that globally contaminate approximately 25% of cereal crops (Bryden, 2007). Cereal grains may be affected by fusarium mould strains due to inappropriate pre- and post-harvest agricultural practices along the food chain (Dowd, 1998). Fusarium mould is common in maize and groundnuts, which constitute a major portion of the diet in many developing countries (Wild & Gong, 2010). These moulds produce mycotoxins as secondary metabolites. Several of these toxins might be produced before harvest (aflatoxins (AF) and deoxynivalenol (DON), while others are produced mainly during postharvest stages (Fumonisins B (FB) and ochratoxin) (Bhat et al., 2010). As a result, various countries have put in place maximum tolerable limits concerning mycotoxins levels of agricultural produce (Wagacha & Muthomi, 2008).

Mycotoxins attract worldwide attention emanating from the significant economic losses associated with their impact on human health, animal productivity and trade (WHO, 2006; Wu, 2006). When mycotoxins are ingested, inhaled or absorbed through the skin, they eventually lead to lowered performance, immuno-suppression, impaired growth, various cancers or death in humans and animals (Wagacha & Muthomi, 2008). This however depends on the type, period and amount of exposure (Wagacha & Muthomi, 2008). Concurrently, an association has been epidemiologically suggested between consumption of FB contaminated maize and the high incidence of squamous cell oesophageal cancer in deep rural areas of the EC
(Shephard et al., 1992), North Eastern Italy (Franceschi et al., 1990), China (Li et al., 2001) and Iran (Kpodo & Bankole, 2008).

There is very little information available regarding the relationship between mycotoxin exposure (especially ZEA) and infant and young child growth. However, due to poor breastfeeding practices, infants and young children may be exposed to these mycotoxins from an early age. This literature review aims to explore the literature on mycotoxins and growth parameters, with emphasis on the mycotoxin concentration levels, exposure assessment, health effects due to exposure and factors affecting mycotoxin exposure.

### 2.2 Infant feeding

Globally, only 38% of infants zero to six months are exclusively breastfed (Chan, 2015). Concurrently sub-optimal breastfeeding increases the risk of mortality in the first 24 months of age (Black et al., 2013). Exclusive breastfeeding for the first six months of infants' lives saves can avert 13 - 15% of the 9 million deaths of children under 60 months old in the resource-poor settings of sub-Saharan Africa (Nkala & Msuya, 2011). Breastfeeding is further associated with improved cognitive function and influences educational attainment and income in adulthood (Victora et al., 2015).

Infant and young child feeding practices (IYCF) and the consequences thereof play a large role in obtaining sustainable socioeconomic development and poverty reduction (WHO, 2003). Appropriate IYCF feeding is crucial for child health, development and survival. The WHO (2009), developed guidelines for effective IYCF practices (WHO, 2003). These IYCF guidelines cover a range of practices and provide specific criteria for assessing progress of practices at population level. It further includes age-appropriate breastfeeding practices regarding timing, duration, and exclusivity, as well as information on timely and adequate introduction of optimal weaning and complementary foods (WHO, 2003). The indicators for assessing IYCF as outlined by WHO (2009), are amongst others the following: i) early initiation of breastfeeding, ii) exclusive breastfeeding up to 6 months of age, iii) continued breastfeeding until 12 months of age, iv) introduction of solid, semi-solid, or soft foods during complementary feeding and v) formula feeding (WHO, 2003).

### 2.3 Breastfeeding

Optimal infant feeding practices during the first six months of life are described as initiation of breastfeeding within the first hour after giving birth (Edmond et al., 2006) and exclusive breastfeeding for six months (WHO, 2003). Early initiation of breastfeeding is crucial as it ensures that the new-born receives colostrum which is rich in immune factors (Oddy, 2001).
The anti-microbial factors present in human milk encompass interferons, immunoglobulins, iron-binding proteins, polymorph nuclear leukocytes, macrophages and lymphocytes (Chandra, 1983). Colostrum is the most effective natural immune booster hence its importance (Uruakpa et al., 2002).

Diarrhoea and related infectious diseases accounted for 69.3% DALYS in 2013 (Murray, 2015). Infants aged zero to five months who are not breastfed have seven-fold and five-fold increased risks of death from diarrhoea and pneumonia respectively, compared with infants who are exclusively breastfed (Doherty et al., 2011). During the first six months of age, non-exclusive breastfeeding may result in a more than two-fold increased risk of dying from diarrhoea and pneumonia (Doherty et al., 2011).

Mullany et al., (2008) indicated in a study in Southern Nepal, a rural area in South Asia, that 3.4% (n = 771) were breastfed within the first hour after birth. Furthermore, Mullany et al., (2008), indicated that a total of 99.7% (n = 23 164) of infants in a rural area in Southern Nepal, were confirmed to have ever been breastfed, the exact time at which they initiated breastfeeding was estimated for 98.6% (n = 22 838) infants. Partial breastfeeding (i.e. combined breastfeeding with other milk-based fluids and / or solids) was the most common established breastfeeding pattern in this setting 72.6% of infants). In a study by Rohner et al., (2013), amongst 1 784 children in the Philippines in five urban areas, early initiation of breastfeeding and current breastfeeding was reported for approximately half of children 6 to 23 months of age (Rohner et al., 2013).

In South Africa, fluids and food such as cereals are introduced to infants as early as three to four weeks after birth as reported in a study by Sibeko et al., (2004), conducted in a peri-urban area amongst 115 mothers (Sibeko et al., 2005). Goosen et al., (2014), reported in their study that 77% (n = 108) of their mothers-initiated breastfeeding during the first six months with only 5% (n = 5) of doing so within the first hour after giving birth (Goosen et al., 2014). Fourteen per cent of the mothers, who were HIV positive were reported not to be breastfeeding (Goosen et al., 2014). Goosen et al., (2014), established that EBF during the first six months of life was a rare practice in these low-income communities (Goosen et al., 2014). It was concluded that water, on-prescription medicines and formula milk and / or food were introduced at an early age (Goosen et al., 2014). At the time of the study, 10% (n = 11) of mothers who initiated breastfeeding discontinued breastfeeding (Goosen et al., 2014), mostly before their infants were three months of age (Goosen et al., 2014). Six per cent (n = 8) of the mothers breastfed exclusively at the time the study was conducted Goosen et al., (2014). Ninety-four per cent (n = 132) applied suboptimal breastfeeding practices: breastfed partially, 31% (n = 43), did not breastfeed, 36% (n = 51), breastfed predominantly, 27% (n = 38) and the rest of the mothers
EBF (Goosen et al., 2014). Concurrently all mothers who never initiated breastfeeding or who discontinued breastfeeding (31%, n = 43) gave formula milk as replacement feed (Goosen et al., 2014).

Cumulative evidence regarding benefits from breastfeeding to the growth, development and health of a child exists, hence mothers are recommended to breastfeed (Polit & Beck, 2008). Breast milk contains immunoglobulin A (IgA) antibodies which help directly or indirectly with anti-inflammatory response in a child who is breastfed (Oddy, 2002). Breast milk contains lactoferrin and oligosaccharides which are essential as protective factors against microbial infection (Newburg, 2000). Furthermore, cytokines and growth factors are present in breast milk thereby contributing to the active stimulation of the child’s immune system. Additionally, breastfeeding is associated with reduced risk of infectious diseases (Duijts et al., 2010). Quality of breast milk of mothers is affected by their nutrient intake which contains iodine, vitamin A and all the other micronutrients that are essential for infant development (Black et al., 2008).

In a study by Kramer et al., (2001), breastfeeding was found to have a protective effect against gastrointestinal tract (GIT) and respiratory infection in developing countries as well as low income countries (Kramer et al., 2001a). Despite the advantageous effect of EBF, a study done in Malawi showed no association between exclusive breastfeeding and height-for age z-scores (HAZ) (Espo et al., 2002). Onyango et al., (1998) however concluded that though breastfeeding is regarded as the universal practice during the first twelve months, inadequate dietary practices thereafter affects the child’s growth negatively (Onyango et al., 1998). It can be concluded that breastfeeding coupled with proper complementary feeding is important for child growth and disease prevention during early life.

2.4 Complementary feeding

Complementary feeding is defined as the process starting when breast milk alone is no longer enough to meet nutritional requirements of infants, and therefore other foods and liquids are essential along with breast milk (Dewey, 2001). The complementary feeding period regarded as the period from 6 - 24 months is a crucial period marked with sensitivity to stunting and irreversible consequences such as death. The child is also vulnerable to diarrhoea and their physical and mental strength are at risk during this time (UNICEF, 2009).

Complementary foods are often of lesser nutritional quality and safety than breast milk. In addition, it is often given in insufficient amounts and can displace breast milk (WHO, 2002). Gastric capacity limits the amount of food that an infant or young child can consume during
each meal. Repeated infections reduce appetite and increase the risk of inadequate intakes. Infants are particularly vulnerable during the transition period when complementary feeding begins. Ensuring the nutritional and safety needs of infants are met requires that complementary foods be timely, adequate and safe (WHO & UNICEF., 2003):

- timely – meaning that complementary foods are introduced when the need for energy and nutrients exceeds what can be provided through exclusive and frequent breastfeeding;

- adequate – complementary foods should provide sufficient energy, protein and micronutrients to meet a growing child’s nutritional needs;

- safe – complementary foods are hygienically stored and prepared, and fed with clean hands using clean utensils and not bottles and teat

Complementary feeding should be introduced at the age of six months (Daelmans et al., 2009). There is no doubt that complementary feeding practices affect the nutritional status, health and growth of children (Steyn et al., 1993). Inadequate complementary feeding as recognised by the Innocenti Declaration on Infant and Young Child Feeding (2005) pose significant threats to the child’s health (UNICEF, 2005). Poor weaning practices can lead to stunted growth, results in delayed motor and mental development, immune incompetence, frequent attacks of diarrhoeal disease, protein energy malnutrition, micronutrient deficiencies, and interferes with physical and intellectual status in adulthood (Hendricks & Badruddin, 1992; Martorell, 1993).

Lower risk of malnutrition is anticipated, if infants and young children are fed according to the stipulated feeding frequency. Timely solid food introduction is important for dietary diversity and is associated with reduced probability of underweight and stunting (Marriott et al., 2012). In a study conducted in Zambia and Ethiopia, timely introduction of solid, semi-solid or soft food was significantly associated with higher HAZs in Zambia and marginally associated with HAZs in Ethiopia (Disha et al., 2012). Poor food quality in exception of quantity negatively impacts infant and young child growth and development (Allen et al., 1992).

Poor nutrition triggers vulnerability to infection for it weakens the immune system and infection aggravates poor nutrition (Stillwaggon, 2002). In general, infections influence body size and growth through their effects on metabolism and nutrition (WHO, 1986). On the other hand, it cannot be ruled out that genetic factors play a role in stunting in different population groups (WHO, 1986).
Poor nutrition accelerates incidence, severity and length of infection as it reduces the body’s ability to fight infection (Dewey & Begum, 2011). Symptoms that accompany infections such as loss of appetite, diarrhoea, fever, and reduced food intake, may cause poor nutrient absorption, resulting in nutrient losses and altered metabolism characterised by high levels of catabolism (Dobbinson, 2002). These then contribute to additional weight loss and eventually growth faltering (Dobbinson, 2002).

Poor nutrition because of inadequate complementary feeding puts the infant or child at risk of other infections. These infections such as malaria, pneumonia and tuberculosis (TB) have contributed to growth faltering in children; however, diarrhoea plays a major role as it is associated with nutrient loss, malabsorption of nutrients, and anorexia with catabolism (Caulfield et al., 2004). Various studies indicated that the prevalence of diarrhoea, fever and/or malaria was associated with increased growth faltering (Bonhoeffer et al., 2000, Rowland et al., 1988, Wamani et al., 2006). From a review of maternal and child nutrition done by Black et al., (2008), stunting increased with each episode of diarrhoea and at 24 months increased by a factor of 1.05 with each episode of the diarrhoeal infection. However, growth faltering did not worsen if the child was already stunted in the first six months of age. Similarly, the duration of fever and diarrhoea was found to be associated with incidences of growth faltering amongst children 6-18 months in Malawi (Chikhungu, 2013). Inappropriate complementary feeding, such as a large consumption of maize based foods may result in inflammation and enteropathy. In a study amongst children in rural areas in Zimbabwe it was suggested that extensive enteropathy occurs during infancy and that low-grade inflammation may result in growth faltering during infancy (Prendergast et al., 2014).

Adequate nutrition during infancy and early childhood is fundamental to the development of each child’s human potential (Dewey, 2001). Inadequate dietary intake leads to undernutrition which includes deficiencies of macronutrients and micronutrients (Latham, 1997). On the other hand, it can also be due to hunger and poverty which leads to food insecurity in households (Tanumihardjo et al., 2007). During infancy and childhood, a rapid growth spurt is expected to occur to the extent that inadequate intake of food would result in malnutrition which encompasses stunting, wasting and underweight. However, the most common dietary deficiencies in developing countries are diets low in protein (especially animal) and micronutrients, most commonly vitamin A, zinc, iron and iodine (Ramakrishnan et al., 2009).

Ensuring adequate nutrition during complementary feeding is a global health priority, though meeting nutritional needs of 6-24-month-old children is challenging (Dewey, 2013). Sub-optimal complementary feeding in children can be due to inadequate practices and food and water safety (Kimani-Murage et al., 2011). Inadequacy of practices includes infrequent
feeding, excessively diluted feeds with low energy density, inadequate feeding during illness, providing insufficient quantities and practising non-responsive feeding (Umeta et al., 2003).

A cross-sectional study was conducted by Faber et al., (2014), to investigate the nutrient density of complementary food consumed by children aged 6 – 24 months, in three age categories (6 – 11 months, 12 – 17 months and 18 – 24 months) from an urban (n = 158) and a rural (n = 158) area, in the KwaZulu-Natal Province of South Africa (Faber et al., 2014). Amongst breastfed children, nutrient density of the complementary diet was adequate for protein, vitamin A and vitamin C. Additionally the diet was inadequate in zinc for 100% of the children. Greater than 80% of children consumed complementary food which was inadequate in calcium, iron and niacin. Between 60% - 80% of the breastfed children consumed a complementary diet inadequate in vitamin B6 and riboflavin. Urban / rural differences in nutrient density for animal and plant protein, cholesterol and fibre occurred in 18 – 24-month-old children.

2.5 Subsistence farming and staple foods in Africa

Subsistent farming is usually practised in resource poor areas, were the inhabitants mainly consume maize as the staple food. Maize and groundnuts were for instance recognised as the major dietary staples in Africa (Gong et al., 2003). The maize produced by these farmers is considered as a cereal crop. Cereal grains and associated by-products constitute important sources of energy and protein for all classes of farm livestock and human beings (Placinta et al., 1999). However, improper storage of the maize in open granaries exposes them to moulds which then produce toxins named mycotoxins. When cereal grains and animal feeds are colonised by moulds there is a significant risk of contamination with the secondary metabolites of these fungi ultimately in human beings due to consumption of the cooked cereal grains and by-products of the farm livestock.

In most parts of sub-Saharan Africa, maize is the main cereal used in complementary food (Faber, 2005). Unfortunately, maize is very vulnerable to contamination by fumonisins (FB), deoxinevelenol (DON) and zearalenone (ZEA) mycotoxins (Miller, 2002; Van Der Westhuizen et al., 2003, Kimanya et al., 2009). Weaning results in a marked increase in exposure, as mycotoxins-contaminated household foods begin to be consumed, and this change in diet may be associated with growth faltering, particularly stunting (Gong, 2003).

Cereal grains may be contaminated by mycotoxins in two ways; fungi growing as pathogens on plants pre-harvest or growing saprophytically on stored grains post-harvest (Glenn, 2007). Sorting of the maize to remove the moulds has been advocated in order to reduce mycotoxin
exposure in these areas (van der Westhuizen et al., 2011). Exposure risk to humans is either directly through cereal grains or indirectly through farm livestock (kidney, liver, milk and eggs) (Fokunang et al., 2006). Cereal grains are consumed in the complementary foods by children in maize subsistence areas in sub-Saharan Africa. Therefore, many individuals are not only malnourished but are also chronically exposed to high levels of mycotoxins in their diet as postulated by Gong et al., (2003).

Factors that contribute to mycotoxin contamination of food and feed in Africa are environmental, socio-economic and related to food production (Wagacha & Muthomi, 2008). Environmental conditions, especially high humidity and temperatures, favour fungal proliferation resulting in contamination of food (Wagacha & Muthomi, 2008). Additionally, tropical conditions such as high temperatures and moisture, monsoons, unseasonal rains during harvest and flash floods lead to fungal proliferation and production of mycotoxins (Bhat & Vasanthi, 2003). Hepworth et al., 2012, also postulated that DON is a ubiquitous contaminant of cereal crops in temperate regions of the world (Hepworth et al., 2012).

Concurrently, poor aeration in the houses and dirty floors may promote fungal growth on wet maize kernels (Wagacha & Muthomi, 2008). Poor aeration and dirty floors are common in the huts present in rural areas. Pre-harvest practices; time of harvesting; handling of produce during harvesting; moisture levels at harvesting, transportation, marketing and processing as well as insect damage contribute to mycotoxin contamination (Wagacha & Muthomi, 2008). The moisture levels at harvesting of grain in rural areas is not scientifically controlled, therefore fungal proliferation is expected under humid conditions.

Maize meal porridge is the most common type of food introduced to infants and children in rural areas, whilst commercial infant cereal is commonly introduced in urban areas of South Africa (Steyn et al., 1993; Sibeko et al., 2005). Unfortunately, mycotoxins are relatively resistant to cooking and processing hence, basic food preparation procedures do not remove mycotoxins safely from the complementary foods (Rao et al., 1982). Additionally, several Fusarium mycotoxins are found in combination in infested cereal grains (Tajima et al., 2002). Children are more vulnerable to mycotoxin exposure than adults as they tend to live longer and hence the chronic diseases develop over a longer period. As chronic diseases develop over a longer period, the infants and children will suffer from the effects of mycotoxin exposure for longer than adults. Their organs are also too immature to detoxify the poisons from fungi.
2.6 Mycotoxins

It is well known that various biological factors may impair growth (Black et al., 2008). In terms of health, the most important mycotoxins are the trichothecenes (including DON (vomitoxin), ZEA and FB (Luongo et al., 2008, Turner & Pasturel, 2013). The term mycotoxin emerged in 1962 in the aftermath of an unusual veterinary crisis near London, England during which approximately 100,000 turkeys died. The turkey X disease was associated with groundnut meal, contaminated with secondary metabolites from Aspergillus flavus (AF) (Zain, 2011). This raised awareness to scientists that other mould metabolites might also be deadly (Bennett & Klich, 2003). The term mycotoxin literally means poison produced by fungi (Wagacha & Muthomi, 2008). The importance of a mycotoxin lies in the target and the concentration of the metabolite. Mycotoxins are toxic to vertebrates in minute concentrations (Bennett, 1987). Mycotoxins are diverse in nature, from simple C4 compounds such as moniliformin, to complex substances such as the phomopsins (Dinis et al., 2007).

More than 300 mycotoxins have been identified this far, though scientific attention is focused on those that have been proven to be carcinogenic (cancer causing) and/or toxic (Zain, 2011). The fusarium mould strains are the mycotoxins of essence to this study. Fusarium moulds are frequently encountered in more temperate regions of the world (Jackson & Bullerman, 1999). Additionally, fusarium mould strains produce FB, DON, and ZEA (Smith et al., 2012).

2.6.1 Fumonisins

Fumonisins include three similar groups (FB1, FB2 and FB3) (Miller, 2002; Fandohan et al., 2005). Fumonisins 1 and 2 are cancer-promoting metabolites of Fusarium proliferatum and Fusarium verticillioides that have a long-chain hydrocarbon unit which plays a role in toxicity (Marasas et al., 2004). Kumar et al., (2008), highlighted that the food-borne mycotoxins are likely to be of greatest significance in Africa and other tropical, developing countries (Kumar et al., 2008). Fumonisins are the major mycotoxin contaminants found in maize from Tanzania (Kimanya et al., 2008, Shephard et al., 2013a) for instance.

The 56th meeting of the Joint Food and Agricultural Organisation and the World Health Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA), provided a No Observed Adverse Effect Level (NOAEL) of 0.2 mg kg\(^{-1}\) body weight day\(^{-1}\) and a safety factor of 100. As a group, provisional maximum tolerable daily intake (PMTDI) for FB1, FB2 and FB3, alone or in combination is thus set at 2 µg kg\(^{-1}\) body weight day\(^{-1}\) (FAO/WHO, 2001).

Ecological studies in the deep rural areas of the EC in South Africa showed that FB contamination was more prevalent in maize consumed by people in the southern part
compared with the northern part of the region. This correlates with oesophageal cancer (OC) rates which are amongst the highest in the world (Marasas, 2001; Wild & Gong, 2010).

Fumonisins further inhibits the uptake of folic acid via the folate receptor and has been implicated in the high incidence of neural tube defects in rural populations in these deep rural areas (EC, South Africa) and Northern China (Marasas et al., 2004). A study amongst infants below six months by Magoha et al., in Tanzania indicated that exposure to FB analysed using a deterministic approach (exposure calculations based on maize intake and mycotoxin exposure concentrations of maize), ranged from 0.005 - 0.88 μg/kg of bw. These levels were below the PMDTI of 2 μg/kg bw per day (Magoha et al., 2014).

2.6.2 Zearalenone

Zearalenone is a mycotoxin produced by F. graminea-rum and other Fusarium moulds by means of maize, wheat, barley, oats and sorghum as substrates (Zain, 2011). It is a non-steroidal compound that exhibits oestrogen-like activities in certain farm animals (cattle, pigs and sheep), (Zain, 2011). The mycotoxin ZEA has been linked to scabby grain toxicosis in the USA, China, Japan and Australia (Zain, 2011). In children, ZEA bears on the reproductive system affecting hyperoestrogenism (Kuiper-Goodman, 1991). Zearalenone may affect steady child growth and development (Kuiper-Goodman, 1991). However, very little information is available regarding ZEA risk assessment and its effect on infant and young child growth (Lombard et al., 2013).

2.7 Measurement of mycotoxins

2.7.1 Fumonisins

Enzyme-linked immunosorbent assays (ELISA) were developed for analysis of Fusarium mycotoxins (Thongrussamee et al., 2008). However, in global terms, high-pressure liquid chromatography and gas chromatography with mass spectrometry have now emerged as the methods of choice, largely replacing earlier techniques based on thin-layer chromatography (Wang et al., 1995; Yamashita et al., 1995). However, as stated earlier, this is a very expensive method and thus not always viable. In a study by Kimanya et al., 2010, FB1 was determined following the procedure by Sydenham et al., (1990), FB2 and FB3 in the maize flour were determined by using a liquid chromatography (LC) method based on Samapundo et al., (2006).

In a study by Hepworth et al., (2012), an LC/MS was utilised for exposure assessment of DON in pregnant women (Hepworth et al., 2012). A urinary biomarker for total DON was determined
as previously described by Turner et al., (2008). Results were based on standard calibration with added internal standard. Urinary creatinine, used to normalise urinary biomarker determinations, was determined according to the standard alkaline-picrate method and modified for a 96-well plate format (Shephard et al., 2013a).

Recently Shephard et al., (2013a) validated a method where the urinary multi-mycotoxin levels can currently be assessed by liquid chromatography mass spectroscopy (LC/MS) utilising the dilute shoot method. This is done following the mycotoxin extraction process.

Recently in a study by Shephard et al., (2013a), in the EC Province, South Africa, (mostly a maize subsistence area), female participants donated their first morning urine. This was analysed both with a sample clean up (single and multiple biomarker) and by a dilute and shoot multi biomarker method (Shephard et al., 2013a).

Fumonisin B1 was determined as samples were rerun (analysed more than once), with a dilution ratio of 1:1 to enhance detection limits (Shephard et al., 2013b). Chromatographic separation was achieved on a Waters Atlantis T3 column (150 -3.0 mm, 3 lm) eluted with a gradient of water / acetonitrile acidified with 0.1% acetic acid at a flow of 600 IL/min (Shephard et al., 2013b). Results were corrected for apparent recovery based on relative responses of liquid standards and spiked samples (Shephard et al., 2013b).

As pointed out by Shephard et al., (2013b), urinary FB1 was separately determined using a tailor-made single target method as previously described by Turner et al., (2008). Briefly, a stable-isotope internal standard (d6-FB1) was added to diluted urine, which was cleaned-up on a Waters Oasis MAX- SPE cartridge (Shephard et al., 2013b). After washing with ammonium hydroxide (5%, 2 mL) and methanol (2 mL), the mycotoxin and internal standard were eluted with 2% formic acid in methanol (2 mL). Thereafter the eluate was dried down and reconstituted in methanol / water using a ratio (1:1, 200 IL). Analysis was performed on a Waters LC-Quattro Micro MS system using a Phenomenex (Torrance, CA, USA) C18 (150 -4.6 mm, 5 lm) reversed phase column and an acetonitrile/water/formic acid gradient as mobile phase pumped at a flow rate of 1 mL/min. Mass spectrometry (MS) / Mass spectrometry (MS) conditions have been described previously by Turner et al., 2008. Results were based on standard calibration with added internal standard, which compensated for both recovery losses and matrix effects (Shephard et al., 2013b).
2.7.2 Deoxinevalenol

Deoxynivalenol is a partial water-soluble toxin and exposure can be assessed by the urinary biomarker DON glucuronide during the chromatographic analysis (Meky et al., 2003).

In a study by Shephard et al., (2013a) samples were briefly spiked with a stable-isotope internal standard (13C-DON) and treated with b-glucuronidase at 37 °C for 18 hours, after which they were diluted and cleaned-up on a Vicam DON test IAC. After elution with methanol (4 mL), the eluate was evaporated to dryness and reconstituted in water / ethanol with a ratio of 90:10, 250 IL.

Urine samples collected in the rural areas of the EC Province, South Africa indicated the presence of DON (100%; mean 20.4 ± 49.4 ng/mg creatinine) after hydrolysis with b-glucuronidase (Shephard et al., 2013b). Shepherd et al., (2013a), utilised the newly developed multi-biomarker method with b-glucuronidase and immunoaffinity clean-up method and determined the presence of ZEA (100%; 0.529 ± 1.60 ng / mg creatinine), FB1 (96%; 1.52 ± 2.17 ng / mg creatinine), a-ZEA (92%; 0.614 ± 1.91 ng / mg creatinine), DON (87%; 11.3 ± 27.1 ng / mg creatinine), b-ZEA (75%; 0.702 ± 2.95 ng / mg creatinine) and ochratoxin A (98%; 0.041 ± 0.086 ng / mg creatinine) (Shephard et al., 2013b). These findings demonstrate the value of multi-biomarker methods in measuring exposures in populations exposed to multiple mycotoxins although it is still being validated and very expensive. This is the first finding of the presence of DON and ZEA in this rural area (Shephard et al., 2013b).

2.8 Mycotoxin exposure levels in home-grown maize

Fumonisin concentrations were determined in ready-to-cook maize flour in a study by Kimanya et al., 2010 in Tanzania (Kimanya et al., 2010). Two packages of maize-based flour collected during the two 24-hour dietary recalls, were opened and the contents were thoroughly mixed by using a laboratory mixer to constitute a composite sample (Kimanya et al., 2010). From the thoroughly mixed maize flour, 15 g of flour was taken and analysed for FB1, FB2 and FB3, (Kimanya et al., 2010).

To evaluate suitability of the method, blank samples of maize flour were spiked with FB1 at concentrations ranging from 50 - 150 μg/kg (Kimanya et al., 2010). The average recovery value for FB1 was 84% (four samples, Recovery of sample determination (RSD) of 15.45%) (Kimanya et al., 2010). The flour samples were also spiked with FB2 and FB3 each at levels from 100 - 300 μg / kg. The average recovery was 86% (four samples, RSD of 9.16%) and 85% (four samples, RSD of 7.91 FB2 and FB3) respectively (Kimanya et al., 2010).
The limit of detection (LOD) for FB1 was 20 μg / kg and for FB2 or FB3 was 18 μg / kg. The LOD for the method was based on the mean value of the blank readings plus three standard deviations. The findings were corrected for recovery (Kimanya et al., 2010).

Fumonisin exposure assessment, for each of the children who consumed maize, was performed using the total FB (B1 + B2 + B3) contamination data determined in the study and maize consumption data reported in Kimanya et al., (2009). In the study by Kimanya et al., (2010) the child's average maize consumption reported by Kimanya et al., (2009) was adjusted by multiplying it with his / her weekly frequency (number of days in a week) of maize consumption, divided by seven. This adjustment was done to obtain a better estimate of the habitual maize intake of the infants than what would be obtained from the repeat 24-hour dietary recall alone.

At the end of the study by Kimanya et al., (2010), infants who consumed maize were categorized into two groups; the low and high exposure groups. The low exposure group comprised infants who consumed maize that contained undetectable FB and those exposed to FB levels below the PMTDI of 2 μg/kg bw. The high exposure group comprised infants exposed to FB levels above 2 μg/kg bw. The PMTDI limit was derived by Joint FAO / WHO Expert Committee on Food Additives based on nephrotoxicity of these toxins in rodents and recommended for FB1, FB2 and FB3 alone or in combination (Soriano & Dragacci, 2004; FAO-WHO, 2012). The Joint FAO / WHO Expert Committee on Food Additives recommends a PMDTI limit of 2 μg/kg bw μg/kg bw on the basis of NOAEL for renal lesions in male rats, which is approximately one-third of the lowest observable adverse effect level (FAO/WHO, 2001). In the evaluation the NOAEL was 200 μg/kg bw/day but a safety factor of 100 was used to account for species and interspecies sensitivities thus deriving the PMTDI limit of 2 μg/kg bw (FAO-WHO, 2012). Kimanya et al., (2010) chose to use this cut-off value to group the infants because international and national agencies responsible for formulation of food safety standards use this limit to determine the PMTDI for FB in maize; suggesting that being under PMTDI is safer than being above the limit.

2.9 Risk assessment and exposure levels

Fumonisin exposure risk assessments performed for communities relying on maize in South Africa and Tanzania showed that consumption of maize containing FB concentrations above 155 μg/kg can result in exposure above the provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg body weight (bw) (Kimanya et al., 2008). In West Africa, high FB levels of 640 μg/kg in maize from Benin were detected (Doko et al., 1995). Concurrently, Bankole & Adebambo (2004), detected 65 – 1,830 μg/kg (mean 390 μg/kg) FB from Nigeria (Bankole &
Adebanjo, 2004). These levels of mycotoxin presence will result in exposure well above the PMTDI of 2 μg/kg-1 bw/day-1 as set by the joint FAO/WHO Expert Committee on Food Additives (JECFA, 2012).

In a study by Wagacha & Muthomi, 2008, all samples of home-brewed Xhosa maize beer in South Africa were positive for FB1, (ranging from 38 – 1 066 ng/ml with a mean of 281 ng/ml). Total FB (1, 2 and 3) ranged from 43 – 1 329 ng/ml, with a mean of 369 ng/ml (Wagacha & Muthomi, 2008).

In terms of FB, a study conducted in Tanzania, 12% (n = 26) of the 215 infants who received a maize-based complementary food in Tanzania exceeded the PMTDI of 2 µkg⁻¹ body weight (Kimanya et al., 2010). This was conducted with a deterministic approach which is less accurate than the newly discovered urinary biomarker. Although the biomarker is more ideal to use, it is still being validated and is much more expensive than the deterministic approach (Kimanya et al., 2010). Deterministic methods estimate intakes of food chemicals that may occur in a population (Lambe, 2002).

In a study by Hepworth et al., (2012) in the United Kingdom, women aged 16 – 44 years (n = 85) provided a urine sample for DON analysis during their last trimester of pregnancy and completed a food-frequency questionnaire (FFQ) (Hepworth et al., 2012). The urinary DON biomarker was detected in all measured samples (geometric mean (GM) = 10.3 ng DON mg⁻¹ creatinine, range = 0.5 - 116.7 ng mg⁻¹). Levels of DON were higher in women of South Asian origin (GM: 15.2 ngmg⁻¹; 95% CI = 10.7 - 21.5 ng mg⁻¹) compared with non-South Asians (GM = 8.6 ng mg⁻¹; 95% CI = 6.6 - 11.8 ngmg⁻¹), p = 0.02.

Estimated DON intake from FFQ data and typical levels of DON contamination of food suggested that the results were due to higher levels of exposure from bread consumption, particularly daily intake of DON from chapattis in South Asians (estimated mean = 2.4 mg day⁻¹; 95% CI = 1.2 - 3.7 mg day⁻¹) compared with non-South Asians (estimated mean = 0.2 mg day⁻¹; 95% CI = 0 - 0.4 mg day⁻¹). This finding was the first biomarker demonstration of DON in pregnant women, and urinary DON levels were the highest ever recorded.

2.10 Socioeconomic status as a determinant of mycotoxin exposure

Socioeconomic status (SES) can be defined as a multidimensional construct that includes measures of economic resources in addition to social factors such as power, prestige and hierarchical social status (Hackman & Farah, 2009). Measurement of SES is complicated and controversial, though, the most common indicators are income, education and occupation, or some combination thereof (Hackman & Farah, 2009). The SES of children individually has not
yet been established, and thus their status is best measured by the SES of their parents or caregivers, which can affect developmental outcomes. This is ultimately independent of their achieved SES later in life (Hackman & Farah, 2009).

The SES of most inhabitants of sub-Sahara Africa predominantly exposes them to consumption of mycotoxin contaminated products, either directly or indirectly (at various points along the food chain) (Wagacha & Muthomi, 2008). Socioeconomic status can affect mycotoxin exposure when maize is traditionally stored in granaries, though storage of improperly dried maize inside homes. The storage of maize mainly occurs during periods of food shortage, which may facilitate contamination of maize with mycotoxins (Wagacha & Muthomi, 2008). Furthermore, individuals revert from proper sorting of the maize kernels with the intention of serving the available maize. In addition, there seems to be a correlation between SES of most inhabitants in sub-Sahara countries and exposure to mycotoxins.

In a study by Adejumo et al., (2013), the SES of mothers significantly influenced AF exposure levels from staple cereals (Adejumo et al., 2013). Aflatoxin is a naturally occurring mycotoxin produced by two types of mould: Aspergillus flavus and Aspergillus parasiticus (Yin et al., 2008). The SES of lactating mothers also significantly influenced their dietary exposure and the exposure risk of the infants to AF (Adejumo et al., 2013). The SES of mothers determines what they consume, as the SES affects the dietary diversity of these individuals. Therefore, mothers consume a diet that is cereal based. Thereafter the infants will be exposed to mycotoxins in utero.

Similarly, Gong et al., 2003, conducted a study amongst children aged 9 - 60 months in Benin and Togo. This indicated that SES might also be expected to correlate with poor food quality and higher AF-alb (albumin) levels, though no significant effect was observed.

2.11 Socioeconomic factors as determinants of stunting

Three key socioeconomic variables defined at both community and family levels are of special interest; maternal education, household wealth, and community socioeconomic status (SES). Following recent works of Filmer & Pritchett, (2001), Fotso & Kuate-Defo, (2005), constructed two complementary socioeconomic indexes using principal components analysis: (i) Household wealth index that captures a household's possessions, type of drinking water source, toilet facilities and flooring material; and (ii) Community socioeconomic index, defined from the proportion of households having access to clean water and to electricity, as well as the proportion of wage earners and of educated adults (level of education primary or higher) (Fotso & Kuate-Defo, 2005).
Understandably, the community socioeconomic index is designed to represent the broad socioeconomic ecology of the surrounding area in which families live (Diez Roux, 2001). Maxwell et al., (1996) & Duncan et al., (1998) pointed out that it is indeed necessary to consider the characteristics of the immediate environment or community where people live, besides the usual rural–urban location of residence and household-level socioeconomic factors. The proportion of wage earners, on the other hand, is designed to capture the job opportunities in the community. Overall, these three socioeconomic variables capture both household and community attributes, and, at the household level, distinguish between the purely economic and the social dimensions of SES (Fotso & Kuate-Defo, 2006). The sociodemographic factors included by Mamabolo et al., (2005) were the mother’s educational status, occupation, marital status, age and parity, and infant’s age of weaning. The father’s education and mother's occupation were also included in Fotso’s study in 2006. These socioeconomic factors have not always been incorporated in other studies, on the assumption that they usually correlate strongly with household income, (Mosley & Chen, 1984). However, in contrast, a study by Magadi et al., (2003), showed that both variables had independent effects on child nutritional status. In some societies of the developing world, the husband generally makes decisions regarding fertility, contraception and use of health care services, so that certain behaviours and practices which may affect child health and nutrition depend on the father, and specifically, on his level of education (Fotso & Kuate-Defo, 2005). However, in another study, it was pointed out that, there is an independent relationship between SES and child malnutrition and hence largely accounts for the differences in child malnutrition by location of residence (Kanjilal et al., 2010). On the other hand, there is evidence that SES affects child malnutrition as propounded by Fotso & Kuate-Defo, (2006). However, malnutrition is caused by many factors, including inter-generational factors. Therefore, it must be tackled in a multidisciplinary manner. Hence it can be concluded that child malnutrition may either differ or not differ in rural and urban areas after controlling for SES. This is mainly because some intervention strategies may be viable theoretically, but implementation may be difficult due to practicability and underlying causes of child malnutrition (such as intergenerational causes).

Childhood socioeconomic status (SES) is associated with cognitive achievement throughout life (Hackman & Farah, 2009). The relevance of SES to cognitive neuroscience lies in its surprisingly strong relationship to cognitive ability as measured by IQ and school achievement beginning in early childhood (Hackman & Farah, 2009).

Hertzman, 1999, offers the hypothesis that systemic differences in the quality of early environments, in terms of stimulation and emotional and physical support, will affect the
sculpting and neurochemistry of the central nervous system in ways that will adversely affect cognitive, social, and behavioural development (Hertzman, 1999). Little research has been completed on this hypothesis, though research shows that anthropometric indicators of undernutrition during infancy predict cognitive performance in middle childhood and adolescence (Bradley & Corwyn, 2002).

Food variety and dietary diversity, which are associated with nutritional status of South African children, are limited in poor communities in South Africa (Steyn et al., 2006). Additionally, it was pointed out that evidence amongst younger children indicates that disproportionate male undernutrition occurs in households with low socioeconomic status; in better off households, the gender difference then disappears (Monyeki et al., 2008). In a study by Mamabolo et al., 2005, in the Central Region of Limpopo, SA 85% (n=161) of the children from families with more than five people per household, 21% already had younger siblings, 19% were from families with ≥ 3 people, and a large proportion (71%) were breastfed for 12 months (Mamabolo et al., 2005). Most of the mothers were single (61%) whilst 88% of them had secondary education (Mamabolo et al., 2005). Ninety per cent of the children saw their mothers daily, but 67% did not live in the same household as their fathers (Mamabolo et al., 2005). The level of unemployment of the mothers was 67% (Mamabolo et al., 2005). Mothers were the primary caregivers (74%). Twenty-six per cent of the children lived in either traditional houses or shacks (Mamabolo et al., 2005). The above findings were supported in a study that pointed out that poverty and associated health, nutrition, and social factors prevent at least 200 million children in developing countries from attaining their developmental potential (Walker et al., 2007).

2.12 Health effects of mycotoxins

Chronic high exposure levels of mycotoxins may be a larger public health problem than realised (Hesseltine, 1986). Children have more future years of life than adults, hence have more time to develop chronic diseases that may be triggered by early environmental exposures (Sherif et al., 2009). Mycotoxins are important in the context of child health in sub-Saharan Africa, because they are prevalent along the food chain and may have substantial negative health consequences. Concurrently mycotoxins have been associated with several chronic and acute human diseases (Bryden, 2007). Though mycotoxins have been linked to human illnesses, direct connection and correlations have rarely been made (Bryden, 2007). General symptoms of mycotoxicosis in humans include vomiting, diarrhoea and other associated gastro-intestinal problems often attributed to various other health issues such as bacterial infection (Bhat et al., 2010, Smith et al., 2012).
Bhat et al., (2010), indicated that apart from being highly toxic, some mycotoxins are also linked to the incidence of certain types of cancers and neurological disorders (Bhat et al., 2010). Fumonisin reduces uptake of folate in different cell lines, and thus consumption of FB has been implicated in neural tube defects (NTD) (Zain, 2011). Concurrently FB exposure has been associated with increased risk of NTD (Missmer et al., 2006) and retarded growth in animals (Dilkin et al., 2003; Oswald et al., 2003). Fumonisin has been associated with self-reported abdominal pain and diarrhoea in an outbreak of foodborne disease associated with high FB intake in India (Bhat et al., 1997). The research on the relationship between FB exposure and growth of children is unclear and lagging presently.

Ueno et al., (1997), reported FB contamination in maize samples from Haimen, a high hepatocellular carcinoma (HCC) incidence area, with levels 10 - 50-fold higher than in Penlai, a low HCC risk area (Ueno et al., 1997). The report clearly indicated a possible association between FB exposure and HCC incidence. Subsequently, other studies have reported high FB levels in maize from high oesophageal cancer (OC) incidence areas compared with low (Li et al., 2001; Sun et al., 2007, Burger et al., 2010, Shephard et al., 2013). Other reports have associated maize consumption with high OC incidence, but did not consider FB exposure specifically, e.g. in Italy (Franceschi et al., 1990). Sydenham et al., (1990) and Chu & Li (1994), reported an association of FB exposure and OC incidence. These studies overall are consistent with the hypothesis that FB exposure may be associated with OC risk.

It has been shown that FB inhibits ceramide synthase, which ultimately inhibits sphingolipid metabolism (Bouhet & Oswald, 2007). Complex sphingolipids are vital to the cell membrane integrity. Disruption in this biosynthetic pathway could affect intestinal epithelial cell viability and proliferation, modification of cytokine production, and modulation of intestinal barrier function (Bouhet & Oswald, 2007). When intestinal absorption barriers develop, nutrition absorption is quickly affected and can with chronic exposure result in growth impairment (Smith et al., 2012). Fumonisin B is believed to be the most dominant of the FB toxins and may cause decreased expression of local pro-inflammatory cytokines and disruption of sphingolipid metabolism (Smith et al., 2012).

Bouhet et al., (2006) reported that FB exposure led to a decrease in the trans-epithelial electric resistance (a marker of increased epithelial permeability). This was conducted on intestinal porcine epithelial cells. Furthermore, Bouhet et al., (2006), reported that FB exposed pigs may have a down regulation of the local IL-8 measured in the intestine. This suggest that FB affects mucosal immunity, which may increase enteric infection risk (Bouhet et al., 2006, Smith et al.,
It is thus expected that FB may cause elevated intestinal permeability because of the disruptions in the sphingolipid metabolism. This then inevitably increases the risk of infection because of the altered mucosal immunity (Bouhet et al., 2006).

Infants and young children are also at risk of exposure to DON, through the maize based complementary foods consumed (Turner, 2010). However, the experimental analysis of DON in these foods has not occurred due to the recent development of the DON biomarker (Smith et al., 2012). Nonetheless, it is likely that DON has a negative effect on growth in infants and young children based on what was observed in animal studies (Rotter et al., 1995; Rotter, 1996; Pestka, 2010). High DON exposure levels induced in mice and rats resulted in impaired gastric emptying and gut mobility and thus poor weight gain (Fioramonti et al., 1993, Amuzie & Pestka, 2009). Additionally, DON has been associated with diarrhoea and vomiting in humans after consumption of mould damaged wheat in India (Bhat et al., 1989).

2.13 Growth faltering

Growth faltering begins in-utero or soon after birth, is pronounced in the first 12 - 18 months, and could continue to approximately 40 months of age, after which it levels off (Grantham-McGregor et al., 2007). Some catch-up growth might take place, but most stunted children remain stunted (Grantham-McGregor et al., 2007).

The supply of nutrients to the foetus influences foetal growth tremendously (Barker, 2001). The mother’s body composition and size, her nutrient stores, dietary patterns, the transport of nutrients to the placenta and transfer across the placenta influence foetal growth (Barker, 2001). When demand for nutrients exceeds its supply, undernutrition is triggered (Barker, 2001). Concurrently the supply may either be low or high when the mother is thin or starving or when the placenta fails it ultimately becomes low, or demand may be high due to rapid growth of the foetus (Barker, 2001).

Occurrence of undernutrition in utero causes some foetuses to fail to develop appropriate homeostatic responses and die; some will develop responses that will allow growth to continue at the same normal growth rate; while others will develop homeostatic responses that will ensure survival but the growth rates of some tissues and systems will be retarded (Hales & Barker, 2001). This last group of foetuses with retarded systems will be at risk of coronary heart disease and other disorders in adult life (Hales & Barker, 2001).

The foetus eventually adapts to undernutrition by changing its metabolism characterized by catabolism which would cause the foetus to consume its own substrate for energy and also at the same time the foetus would lower its metabolism, thereby retarding the rate of growth
resulting in disproportionate organ sizes and tissues (Hales & Barker, 2001). These adaptations to undernutrition permanently alter the structure and function of the body (Hales & Barker, 2001). Foetuses with systems and organs which would have undergone retarded growth may fail to detoxify mycotoxins during infancy. Hormone production and tissue sensitivity to these hormones are also affected (Hales & Barker, 2001). Undernutrition in late gestation may lead to reduced growth of the kidney which is developing rapidly at that time (Hales & Barker, 2001). Reduced replication of kidney cells may permanently reduce cell numbers, because after birth there seems to be no capacity for renal cell division to ‘catch-up’ (Hales & Barker, 2001). This ultimately predisposes the child to chronic diseases such as diabetes and kidney failure (Hales & Barker, 2001).

2.13.1 Determination of growth in children

There are various ways of assessing the nutritional status of infants and young children up to 24 months of age. Children’s growth patterns for instance are amongst the tools used to assess a population’s nutritional status (De Onis et al., 2006). Concurrently nutritional status is assessed using clinical signs, biochemical indicators or anthropometry (Gibson, 2005). The anthropometric approach is the most commonly used and is advantageous compared to the other methods of assessing nutritional status in children less than 24 months of age (Zere & McIntyre, 2003). Anthropometric measures are non-invasive, less costly and easy to obtain compared to clinical signs and biochemical indicators (Gibson, 2005). The anthropometric indicators are constructed using data on the children's age, height / length and weight (De Onis et al., 2006). Three key anthropometric measures calculated from the age, height and weight data are weight-for-height, height-for-age and weight-for-age (Gibson, 2005). These measures are expressed in the form of z-scores, which compare a child's weight and height with those of similar children from a reference healthy population (Gibson, 2005).

The child’s anthropometric measurements include weight, length, head circumference (HC) and mid-upper arm circumference (MUAC) (Mamabolo et al., 2005). Of importance in anthropometric measurements is the length-for-age (LAZ) z-score. Length-for-age is utilised as a measure of failure to thrive and can thus be indicative of stunting or chronic undernutrition (Gibson, 2005). For children up to 24 months of age recumbent length is measured instead of height (Gibson, 2005). However, weight can be measured with a baby scale (Mamabolo et al., 2005).

The conventional cut-off points of z-scores, undernutrition in its various forms are defined in Table 2.1.
Weight-for-height, which indicates a combination of WAZ and HAZ, has often been used instead of HAZ to measure nutrition adequacy in infants and young children (WHO, 1986; Grantham-McGregor et al., 2007). It was reported that 4.0% of the boys and 4.0% of the girls were severely stunted in EC. In the same Province, 1.6% of the boys were wasted whilst 0.2% (n=1) of the boys were severely wasted (Shisana et al., 2013). Amongst the girls, 3.2% (n=9) were wasted whilst 1.1% were severely wasted in EC (Shisana et al., 2013).

It is widely accepted that anthropometry is the appropriate tool for determining the nutritional status of children (WHO, 1986). It is essential for statistical purposes that measurements of a study population be related to the reference population by standard deviation scores (Z scores) (WHO, 1986). The basic measurements to be considered in anthropometry are age, weight and length whilst indices are combinations of measurements for example weight and age (WHO, 1986). The term indicator relates to the use or application of indices and the indicator is often constructed from the indices for example WAZ or HAZ are indicators of wasting and stunting respectively (WHO, 1986; Onis, 2006).

Wasting indicates a deficit in tissue and fat mass compared with the amount expected in a relative healthy child of the same height or length and may result either from failure to gain weight or from actual weight loss whilst stunting signifies a reduction in skeletal muscle growth (WHO, 1986; Onis, 2006). The growth rate of infants may be reduced from birth, but a significant degree of stunting, representing the cumulative retarded growth, may not be evident for a couple of years (WHO, 1986). Stunting is frequently associated with poor overall economic conditions, especially mild to moderate, chronic or repeated infections, as well as inadequate nutrient intake (WHO, 1986). The prevalence of wasting is greatest between 12 - 24 months of age, when dietary deficiencies are common and diarrhoeal diseases more frequent and tends to decrease later with age (WHO, 1986). By contrast, the prevalence of stunting increases over time up to the age of 24 or 36 months and then tends to level off thereafter (WHO, 1986).

The use of the standard deviation (SD) with the height-for-age as a predictor of stunting in children has been recommended (De Onis & Blössner, 2003). Furthermore, it is of paramount
importance to focus on the window of opportunity during the first 1 000 days of a child’s life when stunting could still be reversible. This is also the period when rapid pathological changes marked by linear growth occurs (Prendergast et al., 2014). This method of assessing malnutrition through environmental pathogenesis on growth retardation is promising in that it predicts the child at risk of malnutrition unlike the LAZs which screens children who are already stunted (Peterson et al., 2013). More research is required in monitoring child growth patterns from conception aiming to put into action any interventions that prevent stunting (De Onis & Blössner, 2003).

The use of birth weight as a predictor of infant growth has been suggested in some studies. Animal studies showed that different patterns of foetal growth had different effects on the relative size of organs at birth, even though overall body size may be the same (Barker, 2001). This emphasizes the severe limitation of birth weight as a measure of foetal growth (Hales & Barker, 2001). Low birth weight (LBW) has been defined by the World Health Organization (WHO) as weight at birth less than 2 500 g (WHO, 2011). The global prevalence of LBW is 15.5%, indicating that approximately 20.6 million such infants are born each year, 96.5% of them in developing countries. There is significant variation in LBW rates across the United Nations regions, with the highest incidence in South-Central Asia (WHO, 2011). Low birth weight can be a consequence of preterm birth (defined as birth before 37 completed weeks of gestation), or due to small size for gestational age (SGA, defined as weight for gestation < 10th percentile), or both (Kramer et al., 2001b). In addition, depending on the birth weight reference used, a variable but small proportion of LBW infants born at term and are not SGA (Karlberg & Albertsson-Wikland, 1995). Intrauterine growth restriction, defined as a slower than normal rate of foetal growth, is usually responsible for SGA (WHO, 2011). Low birth weight thus defines a heterogeneous group of infants: some are born at term but are SGA, and some are both born early and SGA (WHO, 2011). Being born with LBW is generally recognized as a disadvantage for the infant (Bahl, 2006). These infants are at higher risk of early growth retardation, infectious disease, developmental delay and death during infancy and childhood (WHO, 2011).

Child stunting, wasting and IUGR are responsible for more than 2 million deaths and 20% of DALYS among children less than 60 months of age (Black et al., 2008). Chronic malnutrition in the form of stunting has decreased from 40% to 33% in developing countries between 1980 and 2000 (Black et al., 2008). However, it is still a major public health concern, with a third of all children below 60 months of age being stunted worldwide (Black et al., 2008). Childhood malnutrition was the leading risk of DALYS in 2013 in sub-Saharan Africa (Murray, 2015). In South Africa, a greater proportion of younger children are stunted (21% - 48%) rather than
underweight (8% -15%) (Faber, 2001; Mamabolo et al., 2005; Steyn et al., 2005; Monyeki et al., 2008). In a study conducted by Mamabolo et al., (2005) in the rural villages of Limpopo, South Africa, 48% of the children were stunted, 9% underweight, 1% wasted, 18% overweight and 24% obese (Mamabolo et al., 2005). Stunting is thus an important public health problem for children living in environments with poverty, poor nutrition and high prevalence of infectious diseases such as those in developing countries (Vella et al., 1994; Adair & Guilkey, 1997).

2.13.2 Factors affecting growth and development of children

Maternal short stature and low body mass index (BMI) in mothers during pregnancy and lactation of less than 18.5 kg/m² was associated with stunting in 43.8% of the children, underweight (42.2%) and resulted in 22% wasted children (Wollo, 2005). Maternal short stature is due to chronic deficiencies in energy and macronutrients has an indication on the birth outcome of a child as well as the delivery methods (Møller & Lindmark, 1997). Mothers with a short stature have a risk factor of caesarean birth and low BMI affects IUGR which is a risk factor during the neonatal period and has later effects in infants (Black et al., 2008). Infection also plays a major role on the nutrient quality of breast milk (Black et al., 2008).

A study conducted in Zimbabwe concluded that stunting begins in utero and is associated with low maternal immune system function and lowered immunoglobulin factor 1 (IGF-1) levels at birth (Prendergast et al., 2014). If extensive enteropathy or recurrent enteric illness occurs during infancy these antibodies will be released and are classified as low-grade antibodies and low-grade chronic inflammation response may result in infant growth faltering (Prendergast et al., 2014). It was discovered that inflammatory markers were higher in cases in comparison to the control group from 6 weeks of age and were associated with lower levels of IGF-1 throughout infancy (Prendergast et al., 2014). The study conclusively suggested that stunting is influenced by both maternal and infant factors. Therefore, during pregnancy, maternal nutritional and inflammatory status may impact the child’s growth, resulting in IUGR and low birth weight. After birth, low-grade inflammation early in life is associated with stunting (Prendergast et al., 2014).

- **Health care system**

The health care system is responsible for the identification and screening of children exposed to inadequate growth and development (Valadez et al., 1996). Health facilities therefore play an important role in the community as they provide services that deal with child care and feeding, preventive and treatment opportunities. Concurrently overburdened health systems with few trained professionals have too little time for individual counselling of caregivers on
appropriate feeding practices (WHO & UNICEF, 2003). Assessment of stunting is ultimately a problem in a setting where there is a lack of skills and time to do the assessments and absence of counselling for a specific child may result, therefore defeating the purpose of assessment, as they won’t be problem rectification (Onis et al., 2012).

- **Water and Sanitation and Environment**

Environmental contamination has a direct effect on the growth of exploring infants and toddlers who crawl, are learning to walk and putting anything in their mouths (Zeitlin et al., 1995). Floors and the surrounding contaminated environment affect the children’s health and consequently their growth (Tamburlini et al., 2002). Proper disposal of faeces, removal of animal waste and hand hygiene are sensitive at this age. Insufficient access to clean, safe water may therefore serve as a barrier to hygiene practices and safe preparation of complementary foods (Stewart et al., 2013), encouraging microbial proliferation and occurrence of diarrhoeal diseases. The environment also includes population density, degree of urbanisation and climate change as these factors may contribute to worsening rates of malnutrition (Stewart et al., 2013). More population-dense areas and places with a lower degree of urbanisation tend to worsen malnutrition rates. The rise of temperature by 2-2.5 °C substantially increases stunting due to malnutrition that is projected to occur especially in sub-Saharan Africa and South Asia and with a rise to 4°C it will become worse (World Bank, 2014). As the temperatures increase there is a tendency of increased loss of nutrients from the body through dehydration, therefore increasing stunting rates.

The safety of food and water relates to the infection that results in stunted growth (Dewey & Meyers, 2011). Safe water sources, storage of water and hand washing practices contribute to the reduced child’s risk of diarrhoea and other factors that contribute to stunted growth (Checkley et al., 2004). The peak incidence of diarrhoeal disease is during the second six months of infancy, as the intake of complementary foods increases, and feeding bottles are a particularly important route of transmission of pathogens (Dewey, 2001). Food preparation techniques and storage also have a greater influence on child growth as it causes diarrhoea if microbial contamination and growth is favoured (Kimmons, 1999). Recently there has been renewed interest in the effects of mycotoxins in child growth (Wild & Gong, 2010). Toxins may be produced from moulds found on grains such as maize which is consumed by infants as complementary feeding which are affected by fungi (Smith et al., 2012). Concurrently gut inflammation may be a mechanism that links mycotoxins to poor child growth (Smith et al., 2012).
• **Maternal education**

Several studies have supported the evidence that the mother's schooling is a stronger determinant of child welfare (Bicego & Boerma, 1993; Defo, 1996). Moreover, when education is widespread, it can influence not only the behaviour of the women themselves, but can additionally permeate the whole community, transforming the group norms and opening the door to modern health practices (Lalou & LeGrand, 1997). This justifies the inclusion of education in the community socioeconomic factors (Fotso & Kuate-Defo, 2006). Maternal education affects the health of the children as well as nutritional practices inside households (Akhter, 2012). Formal education of the mother and father results in improved protective caregiving behaviours, sanitation, vitamin A intake, completing childhood immunisation and the use of iodised salt (Semba *et al.*, 2008). This observation was consistent in both the urban and rural areas and there was no difference in practices because of informal education which took at least three years. Meanwhile a high level of education cannot eliminate the stunting of the whole generation since stunting is an outcome of complex causal factors and is intergenerational (Semba *et al.*, 2008).

Education of caregiving practices instead of formal education will be more helpful in reducing stunting over a long-time and reduce mortality rates (Semba *et al.*, 2008). Improving knowledge and skill building is necessary to improve complementary feeding practices (Piwoz *et al.*, 2003).

• **Agriculture**

Farming trends that involve mono-cropping and heavy dependence on grains contribute to reduced dietary diversity and result in possible micronutrient deficiencies (Jorgensen & Gault, 2012). Additionally, mono-cropping and heavy dependence on grains increases exposure to mycotoxins. Efforts should be directed at the agriculture sector to produce high quality foods such as for example the orange flesh sweet potato that is being introduced in Mozambique and Uganda to improve vitamin A status of the population (Low *et al.*, 2007; Hotz *et al.*, 2012). Small livestock rearing also improves diet and may provide critical nutrients such as protein in a child’s diet (Tontisirin *et al.*, 2002). However, increased access to food may be insufficient if not paired with a behaviour change component that puts knowledge into practice by ensuring that high quality foods are fed to young children.

• **Cultural beliefs**

How food is fed and who feeds the child is as important as the quantity and quality of foods (Birch & Fisher, 1998). Cultural beliefs, knowledge and perceptions influence food behaviours.
Food taboos and methods of food preparation that may affect the child’s health also play a role in following the recommended feeding practices. These beliefs are influenced by the society that surrounds the primary caregiver (Stewart et al., 2013). The society includes husbands, brothers, sisters, older siblings, mothers-in-law, grandmothers, neighbours including extended family within the household and within the community, as well as the health care professionals who provide child feeding and care practices to the caregiver (Mugivhi, 2010). Men in the household are the ones responsible for the resources available in the household for they are in control of household finances and resources and hence influence choices over the types of food to be purchased in a household (Coutsoudis et al., 2000). Hence, female-headed families lacking a male figure tend to suffer tremendously. Since women are the primary caregivers, female empowerment is an important factor that underlies child growth and development as well as involvement of the mother’s social support network in complementary feeding programmes is particularly important to be considered (Stewart et al., 2013).

- Economic stability

Economic instability due to government and other power structures, markets and services result in increases in food prices and it negatively influences household nutrition (Lovendal & Knowles, 2006). High food prices reduce the probability of micronutrient intake, particularly inadequacies in food intake and high food prices reduce the probability of micronutrient intake in poor households (Maxwell & Smith, 1992).

The consumption of locally available foods as complementary feeding which can be affordable to everyone should therefore be encouraged. Community infrastructure constraints may also contribute negatively to market, product prices and accessibility, so it is necessary to reduce such barriers for they also affect complementary feeding practices recommendations (Stewart et al., 2013).

2.14 Relationship between mycotoxin exposure and physical growth

There has been renewed interest in the role of mycotoxins, in child growth faltering (Khlangwiset et al., 2011; Smith et al., 2012). Child growth faltering is an important public health issue as it has effects leading to the death of children (Shrimpton et al., 2001). Exposure to these toxins occurs via maize and groundnuts contaminated with fungi during production, storage or food processing (Wagacha & Muthomi, 2008). Concurrently, Smith et al., 2012, proposed that gut inflammation may be one of the mechanisms linking mycotoxin exposure to poor child growth (Smith et al., 2012). In another study by Gong et al., (2002), AF exposure
was shown to correlate with impaired growth in children, (Gong et al., 2002). Additionally, Okoth & Ohingo, (2005), elaborated that the number of children who were wasted and were being fed on flour contaminated with mycotoxins was highly significant (p = 0.002).

The environmental toxins produced by moulds are deadly because they result in inflammatory reactions and the occurrence of precancerous cells. Toxins in the body lead an inflammatory response, which is a fundamental type of response by the body to disease and injury and results in swelling or tumour (Hotamisligil, 2006) (Figure 2.2). Neoplastic reactions may result, whereby abnormal cells which are difficult for the immune system to destroy occur, these may clone and later become precancerous (Teng et al., 2008). An autoimmune response whereby the body fights against its own cells might also occur (Teng et al., 2008). Below is an illustration of how toxins may lead to inflammatory response, autoimmune response and neoplastic reactions.

![Illustration of how toxins may lead to inflammatory response, autoimmune response and neoplastic reactions](image)

Inflammatory reactions are linked to growth faltering, as observed in earlier animal studies (Smith et al., 2012a) However, growth faltering is a major public health problem affecting infants and young children in Tanzania (Kimanya et al., 2010). Among children of less than 60 months of age, growth failure, as measured by rates of stunting, underweight and wasting, stands at 38, 22 and 3%, respectively in 2004 - 2006 in Tanzania (Kimanya et al., 2010). In comparison to other African countries, infants in Tanzania experience growth retardation during the period of introduction of complementary foods (Kimanya et al., 2010). However,
studies linking mycotoxin exposure to infant and young child growth in Sub-Saharan Africa are limited (Lombard, 2014).

Maize from Africa contains fumonisins at concentrations which can be as high as 10 000 µg/kg (Shephard et al., 1995; Doko et al., 1996; Kedera et al., 1999; Nikiema et al., 2004; Fandohan et al., 2005; Kimanya et al., 2008; Kimanya et al., 2009). Fumonisin exposure assessments performed for communities relying on maize in South Africa and Tanzania showed that consumption of maize containing fumonisins concentrations above 155 µg/kg can result in exposure above the provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg body weight (bw) (Kimanya et al., 2008). A fumonisin exposure assessment for infants consuming maize-based foods in Tanzania, (Kimanya et al., 2009) showed previously that the infants are at a very high risk (up to 24%) of exceeding the PMTDI. Using fumonisin exposure as a continuous variable, it was demonstrated that there is a dose-effect relationship between exposure and growth in length, although not significant (Kimanya et al., 2010). Plotting the predicted values (using the mixed analysis) showed a definite decrease in length with increase in fumonisin exposure (Kimanya et al., 2010). The predicted values are influenced by many zero values and the skewed distribution of the exposure values (Kimanya et al., 2010). Entering exposure as a dichotomous variable using 2 µg/kg bw cut-off level, exposure was associated with a well-pronounced and significant difference in growth between the high and the low exposure groups (Kimanya et al., 2010). One hundred and sixty-six children recruited between the ages of 6 - 14 months were observed to have a negative association between fumonisin exposure and LAZ scores (Shirima et al., 2015).

In Benin and Togo, researchers reported a striking association between mycotoxin exposure and growth faltering, whereby over a period of eight months, children exposed to the highest level of AF had a 2 cm lower height gain than those exposed to the lowest levels (Gong et al., 2004). There was a significant correlation between HAZ scores and both measures of AF-alb (p = 0.009) for AF-alb, p = 0.001 for mean AF-alb over three survey points), but there was no significant correlation between WHZ scores and AF-alb (Gong et al., 2004). Below is a conceptual framework explaining the mechanism behind mycotoxin exposure and growth (Figure 2.3) (Smith et al., 2013).
Mycotoxin exposure results in the inhibition of protein synthesis and increased intestinal permeability. The increased intestinal permeability results in loss of nutrients and therefore affecting growth in animals. The failure to uptake nutrients when the intestine is altered also leads to growth impairment. Inhibition of protein synthesis as applied to AF and DON results in growth impairment. Inhibition of protein synthesis can also result in physical alterations to the intestine, leading to malabsorption of nutrients and impaired intestinal barrier function, like the pathology in EE (Smith et al., 2012). Increase in systemic cytokines (DON); and inhibition of ceramide synthase (FB) was observed to also occur in animals, leading to impaired growth (Smith et al., 2012). Increases in systemic cytokines can lead to impaired hepatic protein synthesis and reduced production of IGF-ALS and IGF-1. Deoxynivalenol, inhibits protein synthesis, affecting several important proteins, such as claudin-4, SGLT1, GLUT5, L-serine transporters, IGF-1, and IGF-ALS. Claudin-4 is essential in the proper functioning of tight junctions, and reduced expression of claudin-4 elevates intestinal permeability (Smith et al.,
Reduced expression of SGLT1, GLUT5, and L-serine transporters lead to glucose-galactose malabsorption and impaired reabsorption of water in the colon may cause diarrhoea, which could affect intestinal permeability as well as the uptake of key nutrients such as copper and zinc. Insulin growth factor-1 mediates the effects of growth hormone, which is required for linear growth; the ALS forms a ternary complex with IGF-1 and its major binding protein (IGFBP3) (Smith et al., 2012). Fumonisin-induced inhibition of ceramide synthase affects sphingolipid metabolism, which negatively affects the cellular wall and may result in increased intestinal permeability directly or by inhibiting regeneration of the epithelial barrier. (Smith et al., 2012).

2.14.1 Fumonisin and stunting

Dilkin et al., (2003), found that pigs fed FB alone or combined with FB had decreased food consumption and body weight. There was an interactive effect of the two toxins, which indicates that the combination of FB and AF may have a greater impact on growth than either component alone (Dilkin et al., 2003). This finding was particularly interesting, as grain is often contaminated with multiple mycotoxins and it is common for AF and FB to coexist in the same food source (Kimanya et al., 2008). Kimanya et al., (2010) observed that children with FB intakes greater than the PMTDI were significantly shorter and lighter than children with FB intakes less than the PMTDI. The major causal pathways suggested are decreased food intake and the inhibition of sphingolipid metabolism, which could lead to the degradation of the epithelial barrier and stimulation of an inflammatory immune response (Kimanya et al., 2010). Importantly in Kimanya’s study, it was observed that at 12 months of age, the infants with FB exposures above the PMTDI were significantly shorter by 1.3 cm and lighter by 328 g than those with exposures below the limit, indicating possible future stunting (Kimanya et al., 2010). These findings suggest that chronic FB exposure might be associated with growth retardation Shirima et al., (2013) and that further research is required.

2.14.2 Deoxynivalenol and stunting

It is likely that DON has a negative effect on growth of children, because of decreased food intake and reduced weight gain that has been observed in animal studies (Turner et al., 2012). The PMTDI for DON was established based on an observed reduction in weight gain in rodents, (Smith et al., 2012). Rotter et al., (1995), found that pigs fed grain contaminated with DON had an estimated 20% lower feed intake and a 13% lower weight gain than the control group and suggested that DON induces feed refusal in pigs. In a study by Amuzie & Pestka, (2009), it was found that DON intake in mice induced a decrease in circulating levels of IGF-1, an important mediator of the growth hormone axis, and hepatic IGF acid-labile subunit
(IGFALS), which forms a complex with circulating IGF-1. Such data therefore suggest a direct mechanism by which mycotoxin ingestion may reduce linear growth. No study to date has assessed the combined effects of multiple mycotoxins on stunting (Smith et al., 2015).

The results of studies conducted with animals on DON exposure reported reduced nutrient absorption (Dewey & Adu-Afarwuah, 2008, Smith et al., 2012), inhibition of protein synthesis (McKay et al., 2010, Smith et al., 2012), inhibition of sphingolipid synthesis (Smith et al., 2012, Roumen & Goris, 1993) and food refusal (Smith et al., 2012, Roumen & Goris, 1993). However, studies in humans to date have not come up with such results which indicate the likelihood of growth impairment. Therefore, is it an area of concern in research.

2.15 Approaches in reducing undernutrition

The current evidence bases for interventions to improve child undernutrition have been comprehensively reviewed as part of the Lancet Nutrition Series (Bhutta et al., 2013a). Some interventions are designed way after recent advances in research and technology have emerged, therefore becoming irrelevant in the current research atmosphere. Recent advances to deal with mycotoxin exposure resulting in undernutrition include, detoxifying mycotoxins using degradation enzymes and utilisation of non-toxigenic fungal strains (Alberts et al., 2017). Evidence-based research on ways of making interventions relevant to Africa, through identifying priorities in countries is essential. Other interventions, however, have been promoted for years but are still being implemented in only a few areas or not at all, and may not even be implemented in countries where the interventions are included in national policies and plans (Victora et al., 2005). Broad food system policies that can contribute to longer term eradication of the undernutrition burden are scarce in these countries. Focusing on agricultural and food system policies on human health and nutrition goals is an under-exploited opportunity with great potential (Bryce et al., 2008).

2.16 Interventions for prevention of mycotoxins

It is unfortunate that developing countries tend to have climatic conditions (humid conditions) that encourage mould growth and mycotoxin formation and hence are faced with much greater problems while at the same time having fewer resources to detect, control and reduce the extent of contaminated food (Sherif et al., 2009). Reductions in exposure will surely serve to protect vulnerable populations while the full extent of the health burden is clarified. Notwithstanding the need for a better evidence-base on mycotoxins and health, given the existing experimental, human epidemiology and mechanistic data, a reduction in human exposure to these toxins is considered a priority (Wild & Gong, 2010). When the economic
benefit of less contaminated crops is coupled with improved health the reasons to act are compelling (Wild & Gong, 2010). The response, however, needs to be a concerted one, including international agencies, governments and non-governmental organizations; to address what remains a largely and rather shamefully ignored global health issue (Wild & Gong, 2010). It is imperative for African countries to strengthen nationwide surveillance, increase food and feed inspections to ensure food safety, and local education and assistance to ensure that food grains and animal feeds are harvested correctly, dried completely, and stored properly (Wagacha & Muthomi, 2008). Other possible interventions include biological control, chemical control, breeding for resistance as well as surveillance and awareness creation and legislation, (Wagacha & Muthomi, 2008). WHO (2006), has put plans in place to focus on field projects, strengthening surveillance and raising awareness and educating consumers on matters related to mycotoxins in Africa among others (Wagacha & Muthomi, 2008).

Intervention strategies to reduce occurrence and exposure to mycotoxins can be done at the individual or community level (Bhat & Vasanthi, 2003). At Individual level attempting to change diets to avoid risky foods such as maize is essential (Bhat & Vasanthi, 2003). In EC, this may not be viable due to the economic status of the individuals, that limits affordability of diversified foods, therefore simple sorting (to remove mouldy kernels) and washing of maize may be advocated (van der Westhuizen et al., 2011). Physical sorting of contaminated cereal grains could also be useful. In communities, mycotoxin formation in crops can be limited to pre-harvest measures: through good agricultural practices such as rotating crops, irrigating to eliminate drought stress, controlling weeds, cultivating mould-resistant stocks, and introducing biocontrols such as non-mycotoxigenic fungal strains (Bhat & Vasanthi, 2003). Post-harvest measures constitute rapid drying by mechanical means and keeping crops dry. Sorting out contaminated grains by physical means, by colour, and washing with water will also reduce mycotoxins (Bhat & Vasanthi, 2003). Chemical methods of detoxification include ammonisation processes (Bhat & Vasanthi, 2003). When appropriate agricultural practices are not implemented, these fungal compounds are endowed with toxic effects to human beings and have detrimental health effects on infants and children (Placinta et al., 1999).

It is of importance to focus on high-risk agricultural commodities such as maize pre- and post-harvest in maize subsistence areas among high-risk population groups for selected mycotoxins hence yielding the greatest public health benefit (Bhat & Vasanthi, 2003). Monitoring human population groups for diseases attributable to mycotoxins, coupled with implementing appropriate prevention and control measures, encompassing decontamination and detoxification, would ensure a food chain free from mycotoxins (Bhat & Vasanthi, 2003).
2.17 Conclusion

The wide range of the causal factors of stunting and their impact on the mother during pregnancy as well as directly on the child makes it very challenging to model expected returns from individual interventions. Evidence from large-scale interventions, working towards stunting reduction programs is inadequate and decisions must therefore depend on what is biologically credible including evidence from research-based trials (Black et al., 2008).

Messages in line with positive complementary feeding practices integrate hygiene and sanitation. Targeting health behaviour practices that are more closely related to nutrition, such as hand-washing before food preparation and before feeding young children and use of clean water sources, will better guarantee impact on the interventions implemented (Checkley et al., 2004). The contextual conditions driving causal factors of child growth may need more creative programming approaches in alternative sectors (Lutter et al., 2013).

An enabling environment needs to be built to address more distal factors causing stunting (Black et al., 2013). Conclusively it is imperative that critical evaluation of the intervention strategies is done to streamline the sustainability, cultural acceptability, economic feasibility, ethical implication, and overall effectiveness of potential interventions (Wagacha & Muthomi, 2008). The other challenge is the fact that they are also several factors involved in malnutrition and it may be difficult to target them all at one goal.
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CHAPTER 3 OVERALL METHODS AND CHARACTERISTICS OF THE STUDY POPULATION

3.1 Introduction

The aim of this chapter is to describe the overall methods used and to provide basic descriptive information of the study area and the participants. In the chapters that follow, some of the participants will be excluded in the analysis based on the inclusion and exclusion criteria of that specific research paper.

3.2 PhilaSana study design, sample size and methods

This sub-study (PhD thesis) was part of a larger study (the PhilaSana study). Since the research has never been conducted in South Africa, the larger study was considered as a pilot study to determined future research. The larger study was in the form of a longitudinal, cohort study design using systematic and snowball sampling to recruit pregnant women and mother / caregiver and infant pairs at various villages within the pre-selected area. The large PhilaSana study followed infants during their first 1 000 days of life (conception to 24 months) as well as their mothers. The study therefore included pregnant isiXhosa-speaking mothers, aged 16 to 45 years of age.

The primary aim of the large study is to determine factors associated with infant and young child malnutrition in deep rural areas such as the Eastern Cape.

With the support of a biostatistician, it was decided to have a sample size of 120 mother and infant pairs. This was based on logistics and compliance rates of previous studies conducted in these rural areas that have shown a participant compliance of 80% with a sample size of between 100 - 120 participants. Due to the large number of logistical problems, determining a specific sample size proved difficult.

Mother/ caregiver infant pairs were followed for the first 1 000 days of the infant’s life. During these 1 000 days data was collected in six consecutive visits. The first visit was conducted during pregnancy (ideally the third trimester) to determine the mother’s current health status as well as her dietary patterns during pregnancy. After birth, the mother-infant pair were visited another five times to determine the mother and infant’s health status as well as their dietary patterns and feeding practices. In situations where the mother left for an urban area and left the infant in the rural area the caregiver will be considered the “mother.” Data from the infant continued to be collected.
During the six visits, data was collected in the form of questionnaires, anthropometric measurements, urine samples, blood samples, breast milk samples and home-grown maize samples. Samples were collected at mother, infant and household level.

For Paper 1, the sub-study cross-sectional data will be used to provide information on the mycotoxin levels and maize exposure. For Paper 2 and 3 longitudinal data will be used to determine the association of exposure and growth.

3.2.1 Study area

The study was conducted in villages within two deep rural sections of the Amatole District Municipality in the EC (Figure 3.1). The municipality consist of 26% of the population of the EC of which 39% are urban and 61% rural (Province of the Eastern Cape Health, 2010). There is a population estimate of 1 856 381 people in 23 593 km2 (Province of the Eastern Cape Health, 2010). According to the Provincial Department of Health the estimated unemployment rate is 53.3% and the illiteracy rate 29.1%.

The area is a deep rural, characterised by limited infrastructure. Rural areas are pastoral landscapes, with unique demographic structures and settlement patterns, isolation, low population density, extractive economic activities, and distinct sociocultural milieus (Hart et al., 2005). Accessibility to households is limited to a few available gravel roads. Homes are scattered along the countryside and no formal addresses exist or listings thereof.

![A landscape photo of rural Eastern Cape](image)

Figure 3-1 A landscape photo of rural Eastern Cape
3.3 Methods

3.3.1 Participants

This sub-study used information collected during the large study and focused on data collected after birth (0 – 23 months). Information provided in the rest of this methods section only focused on data collected that was relevant to this sub-study.

Inclusion criteria (for sub-study)

- Infants born to mothers previously included in the study;
- Infants within the relevant age group of the study.

Exclusion criteria (for sub-study)

- Infants that were seriously ill for any clinical reasons.
- Mothers / caregiver who (for whatever reason) was not able to provide information regarding the infant’s care.

3.3.2 Recruitment

Before the onset of the research project the relevant traditional leaders and key stake holders were contacted telephonically, and the necessary arrangements were made. On the first day of the research project various traditional leaders such as the local chief and headmen as well as the traditional healers were approached, and the study was explained to them. Upon their permission, messengers (mostly young boys) were used to send messages to all households in the relevant area about a community meeting. The following day (at the community meeting) the project was explained to the residents (male and female) in easy to understand isiXhosa. This task was given to a specially trained field worker who has conducted similar research projects in the area (with the former PROMEC Unit of the MRC as well as with University Stellenbosch) is known to the residents and the local traditional leaders. Once everybody was clear (especially the senior males in the villages) about the aims and objectives of the study, as well as the inclusion and exclusion criteria, the study procedure was explained, at a local area (usually the school, shop or outside the clinic). A suitable area was identified where willing volunteers fitting the inclusion criteria convened the following day. Due to the lack of public transport and infrastructure (in terms of the existence of roads) participants walked to the venue (as this is their only means of transport). However, opportunity was provided for volunteers to be collected at home (or the nearest road) by the research team. A field worker was appointed for this specific task. This was then considered the first data collection day.
(thus participants had a day to consider participation). Volunteers (pregnant mothers) were then given individual written informed consent at the assembly point at a separate, independent data collection point from an independent field worker. Fieldworkers informed mothers to tell other mothers in their area about the study and that they could come the following day.

3.3.1 Questionnaires

Questionnaires have been developed or adapted from various sources to measure specific information. All questionnaires were completed by standardised research assistants and fieldworkers.

3.3.1.1 Demographic questionnaire

A standard demographic questionnaire (Addendum 2), developed and field tested by the PROMEC Unit of the MRC was used to describe the demographics of the participants. This questionnaire elicits information including age, gender, language, location, medical history, cultural practices, smoking habits, alcohol consumption, ownership of household assets, productive assets, animals, sources of domestic water, type of sanitation, crowding (number of household members per sleeping room), occupation and education.

3.3.1.2 Socio demographic questionnaire

A socio demographic questionnaire (Addendum 3), drawing on field tested socio demographic questionnaires was used to collect socio demographic data.

3.3.1.3 Semi-quantitative food frequency questionnaire

The necessary dietary information was obtained from the mother/caregiver of each infant or child. To assess food intake, a previously validated and culturally specific dietary assessment tool (Addendum 7) was used (Lombard et al., 2013, Lombard et al., 2014). The RAPP (Ratio and Portion size Photo) tool was developed and validated among rural people living in this area. This dietary assessment tool included a semi-quantitative food frequency questionnaire with life-size pictures of food items and dishes that are most likely consumed by this rural population. Data regarding infant food consumption was collected during each visit.

The already validated questionnaire was based on adult habitual diet and portion sizes. Previously collected data from infants in this area (from 120 24 hour recalls) was used to determine infant and young child weaning and complementary dishes. Infant and child portion
sizes photographs were taken by a professional photographer and the current adult questionnaire was adapted to only include the infant dishes.

The tool was used to assess usual intake over a month of children living in the two areas. The questionnaire was administered for children 6 - 24 months as well as children younger than 6 months who were not exclusively breastfed.

3.3.2 Anthropometry

Trained research assistants/ fieldworkers conducted anthropometric assessments of the infants and young children. The measurements were conducted according to the WHO and ISAK standards. An average of two measurements were taken, as well as a third measurement if two measurements deviated by > 0.5 cm, to ensure accuracy and a third if two previous measurements differ by too great extent.

The following anthropometric measures were taken for each infant and young child (0 – 24 months):

- Length (cm), measured to the nearest 0.1 cm. Infants w;
- Weight (kg), measured to the nearest 0.1 kg;
- Head circumference (cm), measured to the nearest 0.1 cm;
- Mid-upper arm circumference (cm), measured to the nearest 0.1 cm.

The following anthropometric measures was taken for mothers:

- Weight (kg), measured to the nearest 0.1 kg;

Weight was measured to the nearest 0.1 kg with a portable scientific scale (Seca 876, Seca 334 WLRK). Length of infants and young children (< 24 months) was measured with a baby length-measuring mat. Mid-upper arm and head circumferences measured to the nearest 0.1 cm was measured with a MUAC band and head circumference tape (Seca 212). Weight-for-age z score (WAZ); length-for-age z score (LAZ); and weight-for-length z score (WLZ) were calculated with Anthro Plus.

3.3.3 Maize samples

Multi-mycotoxin (total FB (FB1 + FB2 + FB3), DON and ZEA) contamination levels in maize and cooked maize-based complementary foods was analysed. A collection of raw maize and cooked maize-based complementary foods was collected. During recruitment, mothers was asked to donate a sample of their home-grown maize. Those donating maize were
compensated with a commensurate amount of commercial maize meal or rice. Maize samples collected were frozen at -20°C for later dispatch to the laboratory.

3.3.4 Quality control

Questionnaires were checked for completeness by a primary investigator within 24 hours after collection. Any incomplete data were identified and completed. Every evening after completion of the day’s fieldwork, a short fieldwork meeting was conducted where general problems that occurred during the day were discussed.

3.3.5 Ethics Review Committee

The study was conducted according to the Helsinki declaration and the ICH & MRC guidelines, and the protocol was submitted for approval by the Health Research Ethics Committee of the Faculty of Health Sciences, North-West University.

The scientific validity of the study was approved by all the collaborators involved as well as three independent researchers within the School of Physiology, Nutrition and Consumer Sciences. Thereafter the study was submitted for ethical approval to the Health Research Ethics Committee of North-West University, Potchefstroom Campus and the Faculty of Health and Wellness Ethics Committee, CPUT.

3.3.6 Goodwill permission

Before the onset of the study permission was obtained from the relevant stakeholders and community leaders, including the local chief, headmen and traditional healers.

Participant: Information and voluntary participation

On the first day of the research projects various traditional leaders such as the local chief and headmen as well as the traditional healers were approached, and the study was explained to them. Upon their permission, messengers (mostly young boys) were used to send messages to all households in the relevant area about a community meeting. The following day (at the community meeting) the project was explained to the residents (male and female) in easy to understand isiXhosa. This task was allocated to a specially trained field worker who has conducted similar research projects in the area (with the PROMEC Unit of the MRC as well as with University Stellenbosch) and who is known to the residents and the local traditional leaders. Once everybody is clear (especially the senior males in the villages) about the aims and objectives of the study, as well as the inclusion and exclusion criteria and study procedure, a local area (usually the school, shop or outside the clinic) was identified where willing
volunteers fitting the inclusion criteria convened the following day. This was then the first data collection day. Volunteers were given individual informed consent and data collection commenced.

The study was conducted at central locations in the villages or at participants’ homes (depending on logistics). All informed consent was taken by an independent field worker and data collection was conducted without the presence of household members, community members or unnecessary fieldworkers.

Mothers younger than 16 - 18 years gave assent while their mothers (grandmothers of the infants) provided consent. However, if the mother (younger than 18 years) refused assent then refusal will be accepted regardless if the grandparents gave written consent.

Participants were fully informed in their home language of isiXhosa about the (i) objectives of the study, (ii) the use of the results and (iii) the benefits of the study in a simplistic manner / language level grade 8. This was explained verbally as well as with a written informed consent form. Willing participants signed an informed consent form, also in their own language. Participants gave separate written consent for participating in the overall data collection and for any photographic material that might be taken during the study. Two copies of the consent forms were signed by the participant as well as an independent witness – one for the possession of the participant and one for the researcher. Completed consent forms were kept separate from the completed questionnaires – during the fieldwork as well as in storage after exiting from the study.

Participants were informed verbally as well as in the information form of their right to withdraw at any stage with no consequence to themselves. Participants further received a participation number and remained anonymous during data processing and interpretation of results.

All participants were informed that they may refuse to participate in the project, and that refusal to participate would not in any way compromise them. Participants were further informed that all information obtained during the study were handled in a confidential manner, to ensure they always remain anonymous, including when results of the study are published in scientific journals or presented at scientific congresses. Due to the total lack of infrastructure, privacy in the area (deep rural) cannot fully be assured. However, all efforts were made by the research team to ensure privacy. Results from the study will be published without individual identification.
3.3.7 Expertise, skills and legal competencies

The fieldworkers working on the project had been involved in various projects in the area and other areas. They all have extensive training in written informed consent (local leaders as well as participants) and assent, data collection, questionnaire completion, anthropometry and maize collection as well as infant and young child feeding. Additional training was given before the onset of the study to further their experience and understanding of the procedures of this specific project.

3.3.8 Incentive and Reimbursement for participants

Participants each received a small food parcel to the value of R50 after completion of each visit as a token of appreciation. No participant received any financial incentive, due to the high degrees of poverty in this area.

3.3.9 Storage and archiving of data and samples

After completion of data collection (each separate visit), information from each participant was captured on Excel. All hard copies of questionnaires are kept in a locked office (that of the PI) at NWU for 7 years with a “do not destroy until 2023” label. Only relevant research personnel will have access to these questionnaires. Information captured on Excel was password protected and only relevant research personnel would once again have access to the data. Results from the study will be published without individual identification.

3.3.10 Legal authorisation

Since this project commenced outside local assembly points no authorisation was applied for to the Department of Health.

3.4 Demographic information of participants

In total 234 mothers / caregivers of infant and young children (0 - 24 months old) were interviewed regarding their household situation in the larger PhilaSana study. At least 64% (n = 151) of the included mothers / caregivers had no, or very limited education (< Grade 6) and none of the included mothers / caregivers were employed. Sixty-seven percent (n = 158) received a child grant or old age grant. In terms of water and sanitation 44% (n = 104) make use of the river / dam while rest have water tanks. Lastly, 66% (n = 156) used pit toilets while the rest used the bush.
A sample of 138 infants, (50% male) were age 0 - 12 months. Seventy-one (51%) participants were between the ages of 0 - 5 months. The sociodemographic information of the infants’ aged 0 - 12 months is presented in Table 1. Most primary caregivers acquired only primary education (58.1%) while 28% had secondary education. All the mothers were unemployed. Only 23% (n = 32) of the caregivers reported to be receiving child support and 67% (n = 93), did not receive any child support. A larger number of the households 30.9% (n = 41) obtained water from the river and only 0.7% (n = 1), had a borehole. Most of the households 70.5% (n = 101) did not own flush toilets. Lastly, most of the participants spend approximately 26 US dollars on food weekly.

3.4.2 Demographic information of children 12 - 24 months

The sociodemographic information of the children aged 13 - 24 months is presented in table 2. Many caregivers 25.9% (29) had no education at all, while 30.4% (34) had secondary education. About half of the households 46.2% (52) received child support, while only 17 (19%) did not receive child support. Only 5.4% (6) of the caregivers were employed, while the majority 80.4% (90) were unemployed. A larger number of the households 13.4% (n = 15) obtained water from the river and only 9.8% (n = 11), obtained water from a tank. The majority of the households 60.8% (n = 68) did not have flush toilets while only, 2.7% (3) had flush toilets. Most of the participants spend approximately 26 US dollars on food weekly.
Table 3-1: The basic socio demographic information of households with infants 0 – 12 months old

<table>
<thead>
<tr>
<th>Sociodemographic</th>
<th>n</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Education level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;Std 6/&lt;Grd 8-10</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td>Std 6-8/Grd 8-10</td>
<td>47</td>
<td>33.8</td>
</tr>
<tr>
<td>Std 9-10/Grd 11-12</td>
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</tr>
<tr>
<td>None</td>
<td>4</td>
<td>2.9</td>
</tr>
<tr>
<td><strong>Employment status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unemployed</td>
<td>111</td>
<td>80</td>
</tr>
<tr>
<td><strong>Child support</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td>No</td>
<td>93</td>
<td>67</td>
</tr>
<tr>
<td><strong>Water source</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borehole / well</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Communal tap</td>
<td>40</td>
<td>29</td>
</tr>
<tr>
<td>Jojo tank</td>
<td>16</td>
<td>11.5</td>
</tr>
<tr>
<td>River/dam</td>
<td>41</td>
<td>30.9</td>
</tr>
<tr>
<td><strong>Sanitation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bucket/Pot</td>
<td>4</td>
<td>2.9</td>
</tr>
<tr>
<td>Bush</td>
<td>2</td>
<td>1.4</td>
</tr>
<tr>
<td>Pit/VP</td>
<td>92</td>
<td>66.2</td>
</tr>
<tr>
<td>Flush toilet</td>
<td>3</td>
<td>2.1</td>
</tr>
</tbody>
</table>

n=sample size
Std=Standard
Grd=Grade
VP = Pit toilet
Table 3-2: The basic socio demographic information of households with young children 13 - 24 months old

<table>
<thead>
<tr>
<th>Sociodemographic</th>
<th>n</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Education level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;Std 6/&lt;Grd 8-10</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Std 6-8/Grd 8-10</td>
<td>14</td>
<td>12.5</td>
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<tr>
<td>Std 9-10/Grd 11-12</td>
<td>34</td>
<td>30.4</td>
</tr>
<tr>
<td>None</td>
<td>29</td>
<td>25.9</td>
</tr>
<tr>
<td><strong>Employment status</strong></td>
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<td></td>
</tr>
<tr>
<td>Employed</td>
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<td>5.4</td>
</tr>
<tr>
<td>Unemployed</td>
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<td>80.4</td>
</tr>
<tr>
<td><strong>Child support</strong></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>52</td>
<td>46.4</td>
</tr>
<tr>
<td>No</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td><strong>Water source</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borehole / well</td>
<td>-</td>
<td>--</td>
</tr>
<tr>
<td>Communal tap</td>
<td>11</td>
<td>9.8</td>
</tr>
<tr>
<td>Jojo tank</td>
<td>16</td>
<td>14.3</td>
</tr>
<tr>
<td>River/dam</td>
<td>15</td>
<td>13.4</td>
</tr>
<tr>
<td><strong>Sanitation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bucket/Pot</td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td>Bush</td>
<td>26</td>
<td>23.3</td>
</tr>
<tr>
<td>Pit/VP</td>
<td>37</td>
<td>33</td>
</tr>
<tr>
<td>Flush toilet</td>
<td>3</td>
<td>2.7</td>
</tr>
</tbody>
</table>

n=sample size  
Std=Standard  
Grd=Grade  
VP = Pit toilet

**3.5 Discussion**

The current study revealed that the community was socio-economically disadvantaged and therefore children were at high risk of poor nutritional status. Taking into consideration, the high unemployment rate, low education levels and high dependency on social grants, these are vibrant indicators of poor socio-economic status. The poor sanitation, especially the lack of flush toilets is a health hazard to the community. Although most of the households had access to electricity, wood was also largely used as a fuel source for cooking. Additionally, the community had an inaccessible road infrastructure, with household sparsely located along the
countryside. This was also an indication of poor infrastructure of the community. The findings of this study are consistent with description of rural areas in South Africa.

With the low socio-economic status of this community, it was not surprising that stunting was highly prevalent amongst the children, regardless of gender and age. However, there was a higher prevalence of stunting in older children, aged 12 - 24 months of age. Stunting in populations is often associated with, low education levels, poor sanitation and high unemployment rates (Akhter, 2012; Dewey & Begum, 2011; Stewart et al., 2013). The low socioeconomic status also played a pivotal role in the high mycotoxin exposures above the PMDTI of the infants, FB (53.1%) and children (53.6%). There was a higher prevalence of mycotoxin exposure in the older children, aged, 12 - 24 months of age, in comparison to what Kimanya et al., 2010 observed in Tanzania. Socio-economic status of populations often affects mycotoxin exposure as postulated (Wagacha & Muthomi, 2008).
3.6 Reference


CHAPTER 4 PAPER 1: MULTIMYCOTOXIN EXPOSURE OF CHILDREN (0 – 24 MONTHS) IN RURAL MAIZE SUBSISTANCE FARMING AREAS OF EASTERN CAPE, SOUTH AFRICA

Paper 1 has already been submitted to the World Mycotoxin Journal for review. The paper is in the format of the author guidelines (Addendum 2). The paper focuses on objective 2 of the sub-study, including maize consumption and mycotoxin exposure of infants and young children 0 – 24 months of age.
Multi-mycotoxin exposure of children (0 - 24 months) in rural maize-subsistence farming areas of Eastern Cape, South Africa

R.S. Tshalibe¹, J.P. Rheeder², J.F. Alberts², C. Taljaard-Krugell¹, W.C.A. Gelderblom², G.S. Shephard², M.J. Lombard¹ and H-M. Burger²

¹ Centre of Excellence for Nutrition (CEN), Faculty of Health Sciences, North-West University, Potchefstroom Campus, Private Bag X6001, 2520, South Africa

²Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology, PO Box 1906, Bellville, 7535, South Africa

Corresponding author: H-M Burger Tel: +27 (21) 959 4393; burgerh@cput.ac.za

Running header: Mycotoxin exposure of young children in rural Eastern Cape, South Africa
Abstract

In South Africa, child malnutrition is highly prevalent, among children below 24 months from rural areas in the Eastern Cape (EC) Province, where maize is mostly used as complementary food. Previous studies conducted in parts of EC have indicated high levels of fumonisin B (FB) mycotoxins in home-grown maize and co-occurrence with other Fusarium mycotoxins such as deoxynivalenol (DON) and zearalenone (ZEA). A cross-sectional study of children below 24 months was conducted in rural maize-subsistence farming areas in Centane, EC to determine mycotoxin exposures. Home-grown maize samples (n=171) were collected from households in the study area and analysed by LC-MS/MS for FB, DON and ZEA. Food intakes of 129 children were quantified using a validated quantified food frequency questionnaire (QFFQ). Individual raw maize consumption was calculated using recipes from the QFFQ. Probable daily intakes (PDIs) for each mycotoxin were determined using a deterministic approach and were compared to the respective mycotoxins’ provisional maximum tolerable daily intake (PMTDI). The mean total FB (sum of fumonisins B₁, B₂ and B₃), DON and ZEA levels in home-grown maize were 1035, 24.5 and 31.0 µg/kg. Mean daily cooked maize intakes of children ranged from 1.60 - 321 g/day and increased with age. The mean PDIs for total FB, DON and ZEA were 8.4, 0.2 and 0.3 µg/kg body weight (bw)/day, respectively. Exposures stratified by age indicated persistent high mean PDIs for total FB, above the PMTDI of 2 µg/kg bw/day, ranging between 5.0 - 11.6 µg/kg bw/day. Mean exposure to DON and ZEA were below their relevant PMTDIs (1 and 0.5 µg/kg bw/day, respectively). Individually, 81% and 13% of children had exposures above the PMTDI for total FB and for ZEA, respectively. Results confirm the magnitude of FB exposure among vulnerable groups from rural maize subsistence farming areas in EC. Dietary diversification is recommended.

KEYWORDS: mycotoxin exposure, children, risk assessment, fumonisin, deoxynivalenol, zearalenone, maize
4.1 Introduction

Globally, chronic (stunting) and acute (wasting) child malnutrition affects 155 million and 52 million children under 60 months of age, respectively (UNICEF, 2017). South Africa has one of the high burdens of stunting and wasting (Massyn et al., 2017; UNICEF, 2017). The national level for boys and girls below the age of three years are 26.9% and 25.9% for stunting, respectively, while 3.8% of the boys and 1.5% of the girls experienced wasting (Shisana et al., 2013). The various factors that influence development of malnutrition include economic, demographic, and cultural changes, poverty, poor nutrition, infectious diseases, early introduction of complementary foods and environmental factors (Mamabolo et al., 2004; Mamabolo et al., 2005; UNICEF Conceptual Framework, WHO, 2011). However, very little is known about the role of certain naturally occurring toxins such as mycotoxins and the association with child malnutrition and underdevelopment.

Mycotoxins are toxic metabolites produced by fungi and are known to contaminate a large variety of crops including cereals, groundnuts and maize (Wild and Gong, 2010). They are associated with several adverse health effects in humans (Zain et al., 2011). There is a need for human mycotoxin risk assessment, due to the occurrence of these food contaminants in crops from especially rural subsistence farming areas in low- and middle-income countries (Sherif et al., 2009). In this regard, home-grown maize from Eastern Cape (EC) Province, South Africa is known to be contaminated by mycotoxins, particularly fumonisins (FB), and to a lesser extent deoxynivalenol (DON) and zearalenone (ZEA) (Shephard et al., 2013a). Of these, FB has consistently remained the major mycotoxin relevant to rural subsistence maize farming communities in the EC (Burger et al., 2010; Shephard et al., 2007, 2013a). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has determined provisional maximum tolerable daily intakes (PMDTIs) for total FB (sum of fumonisins B₁, B₂ and B₃) of 2 µg/kg body weight (bw)/day and for DON and ZEA of 1 µg/kg bw/day and 0.5 µg/kg bw/day, respectively (JECFA, 2001, 2002, 2012). Exposures below the PMDTI levels are considered safe, although the combined adverse biological effects of individual mycotoxins have not been established (Alassane-Kpembi and Oswald, 2016).

Chronic consumption of mycotoxins such as DON or DON and FB in combination, has been suggested to modulate child growth by inducing a poor appetite, gut impairment, inflammatory diarrhoea, decreased nutrient absorption and systematic immune activation (Lombard, 2014; Smith et al., 2012). The exposure to FB has also been associated with a decrease in complex
sphingolipids, glycerolphospholipids and cholesterol, which are essential for cell membrane integrity, resulting in possible intestinal epithelial cell proliferation, disruption of cytokine production and modulation of the intestinal barrier function (Riedel et al., 2016; Smith et al., 2012).

Complementary foods consumed by children living in rural areas of South Africa are mostly maize-based, since most households are involved in subsistence farming (Lombard et al., 2013; Mamabolo et al., 2004). In rural EC, the primary complementary or weaning food is a thin maize porridge (Lombard et al., 2013). Mycotoxin exposure assessment is therefore of importance, especially among the vulnerable populations such as children living in these areas. In resource-poor countries, which lack up-to-date national dietary survey data and continued mycotoxin surveillance, the deterministic approach to risk assessment remains the most feasible alternative (Burger et al., 2014). In Africa, several mycotoxin exposure assessments among infants and young children, consuming complementary foods made from maize, sorghum and groundnuts have been published. Studies conducted in countries such as Tanzania, Benin, Nigeria, Togo, Kenya, Egypt and Guinea using various methodological approaches, mostly focused either on one mycotoxin such as aflatoxin whereas others included additional mycotoxins such as FB, DON and ZEA (Adetunji et al., 2017; Gong et al., 2003; Kiarie et al., 2016; Kimanya et al., 2010, 2012; Magoha et al., 2014; Shrima et al., 2015; Okoth et al., 2005; Polychronaki et al., 2008).

In South Africa, most studies have focused on FB exposure in adults, indicating the presence of urinary FB, DON and ZEA in EC using multi-mycotoxin urinary biomarkers (Shephard et al., 2013b). Overall, all the studies conducted among adults using a deterministic method, consistently showed mean exposure levels for FB, four times higher than its PMTDI of 2 µg/kg bw/day (Bolger et al., 2001; Bulder et al., 2012; Burger et al., 2014; Shephard et al., 2007). In a study conducted by Shephard et al. (2007), exposure to FB was assessed for children aged 1 - 9 years and 10 - 17 years and showed probable daily intakes of 2.7 - 35.9 and 1.96 - 17.1 µg/kg bw/day, respectively.

Studies on multiple mycotoxin exposure of children in South Africa are therefore of importance to understand the magnitude of the problem and the possible link with adverse health outcomes. The current study is aimed at determining maize intakes among rural children aged 0 - 24 months and to conduct an exposure assessment for FB, DON and ZEA using detailed measured
maize consumption patterns and relevant toxin levels in home-grown maize and to characterize the risk by comparison with their respective PMTDIs.

4.2 Methods

4.2.1 Study design and population

A cross-sectional study design, using systematic and snowball sampling, was used to identify households with home-grown maize and children aged 0 - 24 years from rural maize-subsistence farming households in Centane, Amatole District Municipality, EC. Any children not permanently residing in the area were excluded. Snowball sampling was utilised due to the scattered nature of the sparsely populated villages within the District. Systematic sampling was of importance as the villages are not easily accessible, therefore there was need of a random starting point, although the time of sampling was predetermined and fixed. Household consuming home-grown maize were thus identified at the onset of the study. One-hundred and twenty-nine children consuming home-grown maize were included in the study, while exclusively breast-fed infants were excluded.

Household socio-economic status was obtained from a socio-economic questionnaire including ethnicity, household income, expenditure and maternal/caregiver education levels. Feeding practices of children and health status information were obtained from mothers or caregivers. All data were collected from trained voluntary caregivers after informed and signed consent.

4.2.2 Maize collection

Home-grown maize (traditional landrace variety and commercial hybrids) was collected simultaneously to the survey, from households from which the study sample was drawn, based on availability of the maize. Whole maize kernel samples (± 2 kg per sample) were randomly collected from visibly healthy lots, which the householders themselves had sorted and were destined for human consumption. Samples were kept in clearly marked linen bags and were stored in the laboratory at 4°C prior to analysis.

4.2.3 Maize consumption by children

Trained interviewers obtained child food consumption information from the mothers or caregivers. The Ratio and Portion Size Photo (RAPP) tool (a validated culturally specific dietary assessment method determining habitual dietary intake of adults residing in this area)
was utilised (Lombard et al., 2014). The ratios (of maize and other ingredients) and portion sizes in the tool were adapted for child intake based on 150, 24-hour recalls (infants and young children (0 - 24 months) obtained before the start of this study (Lombard et al., 2014).

The tool consists of three portion size photographs per traditional dish and a semi-quantitative food frequency questionnaire (QFFQ), focusing on intake during the past month. Monthly intake of cooked maize was converted to a daily intake followed by calculating raw maize intakes, using recipes obtained during the development of the RAPP tool (Lombard et al., 2014).

4.2.4 Multi-mycotoxin analysis

Methanol, acetonitrile, formic acid (HPLC grade) and Whatman filter paper were obtained from Merck (NJ, USA). Water for all experiments was successively purified by reverse osmosis and a Milli-Q water purification system (Millipore, MA, USA).

Pure analytical standards of FB$_1$, FB$_2$, FB$_3$ (purity >95%) were prepared at the Institute of Biomedical and Microbial Biotechnology of the Cape Peninsula University of Technology, South Africa, according to the methods of Cawood et al. (1991) and Gelderblom et al. (1993). ZEA and DON analytical standards were obtained from Sigma-Aldrich (Merck, Darmstadt, Germany). Individual stock solutions of the fumonisin standards (0.1 mg/ml) were prepared in acetonitrile-H$_2$O (1:1) and of zearalenone and deoxynivalenol (0.1 mg/ml) in acetonitrile. Aliquots of the stock solutions were used to prepare a working solution containing (i) fumonisins and deoxynivalenol at individual concentrations of 5 µg/ml and (ii) zearalenone (250 ng/ml) in acetonitrile-H$_2$O (1:1). For compiling matrix-matched calibration curves, at least five working standard dilutions were prepared with blank maize matrix extract, prepared as described below using blank control maize.

Fumonisin B, DON and ZEA were extracted from maize according to the method of Sewram et al. (2003) with minor modifications. Briefly, 100 ml of extraction solvent [methanol: acetonitrile: water (25:25:50; v/v/v)] was added to ground maize kernels (10 g) and placed on a shaker (80 rpm) for 20 min. The extracts were subsequently centrifuged at 500 g for 10 min at 4°C. The supernatant (20 ml) was diluted (1:1) with methanol: water (25:75), filtered (Whatman No 4-filter paper) and analysed by LC-MS/MS. FAPAS (London, England) quality control reference maize samples (Cat no T22110QC and T22133QC), containing the mycotoxins in expected concentration ranges, were included.
Quantification of FB$_1$, FB$_2$, FB$_3$, DON and ZEA in maize extracts was performed at the Mass Spectrometry Unit of the Central Analytical Facility of Stellenbosch University, South Africa. The mycotoxins were separated on a reversed-phase BEH C$_{18}$ column (2.1x100 mm; particle size 1.7 µm; Waters, Milford, MA, USA) and analysed with positive electrospray ionisation (ESI) (Capillary voltage 3.5 kV; Cone voltages: FB, 50 V; DON, 35 V; ZEA, 20 V) in the multiple reaction monitoring (MRM) mode in a Waters Acquity Ultra Performance Liquid Chromatograph (UPLC) coupled to a Waters Xevo TQ tandem quadrupole mass spectrometer. Eluent A was water and eluent B was acetonitrile, both containing 0.1% formic acid. The elution gradient consisted of an initial mobile phase composition (2% B) held constant for 0.5 min, followed by a linear gradient to 40% B within 7 min and to 70% B over 3 min, followed by a 1-minute wash step at 100% B and finally a 3-minute column re-equilibration back to 2% B for a total run time of 15 minutes. The flow rate of the mobile phase was 0.35 ml/min. For each compound, one precursor and two product ions were monitored, one product ion for quantification and one for confirmation (Table 1). A calibration curve consisting of five matrix-matched standards for each mycotoxin was used to compensate for matrix effects in the analysis. The analytical method was validated for limit of detection (LOD), recovery and repeatability (Table 2).

**Table 1 Flow rate of the mobile phase and precursors for each mycotoxin**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Cone Voltage (V)</th>
<th>Precursor Ion (Collision Energy)</th>
<th>Quantifier Ion (Collision Energy)</th>
<th>Qualifier Ion (Collision Energy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fumonisin B$_1$</td>
<td>50</td>
<td>722.3 (40)</td>
<td>334.3 (40)</td>
<td>352.3 (38)</td>
</tr>
<tr>
<td>Fumonisin B$_2$ and B$_3$</td>
<td>50</td>
<td>706.3 (40)</td>
<td>318.3 (40)</td>
<td>336.3 (40)</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>35</td>
<td>397.1 (15)</td>
<td>203.2 (15)</td>
<td>231.2 (12)</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>20</td>
<td>319.1 (23)</td>
<td>185.0 (23)</td>
<td>187.0 (19)</td>
</tr>
</tbody>
</table>
Table 2 Validation of analytical method with limit of detection, recovery and repeatability

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LOD(^1) (µg/kg)</th>
<th>Spike level (µg/kg)</th>
<th>Recovery (%)</th>
<th>RSDr(^2) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fumonisin B(_1)</td>
<td>0.6</td>
<td>1060</td>
<td>84</td>
<td>2</td>
</tr>
<tr>
<td>Fumonisin B(_2)</td>
<td>0.6</td>
<td>925</td>
<td>66</td>
<td>4</td>
</tr>
<tr>
<td>Fumonisin B(_3)</td>
<td>0.6</td>
<td>520</td>
<td>79</td>
<td>1</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>3.7</td>
<td>684</td>
<td>78</td>
<td>4</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>1.0</td>
<td>232</td>
<td>95</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^1\) LOD: Limit of detection; \(^2\) RSDr: Relative standard deviation for repeatability.

4.2.5 Mycotoxin exposure

Mycotoxin exposures for total fumonisins (FB\(_1\) + FB\(_2\) + FB\(_3\)), DON and ZEA were determined using mycotoxin concentrations obtained from the analysis. In instances where the mycotoxin levels observed were below the Lower limit of quantification (LOD), the actual LOD value of the method was used as an upper bound scenario. Probable daily intake (PDI) (µg/kg bw/day) was calculated by multiplying individual total daily raw maize intake (g/day) with mean mycotoxin concentration of total FB, DON and ZEA (µg/kg) divided by bw (kg). The PMTDIs for each mycotoxin were used to indicate low (< PMTDI) or high (≥ PMTDI) exposure.

4.2.6 Anthropometry

Trained research assistants conducted weight assessments of the children. Measurements were conducted according to WHO and UNICEF Standards (available at http://www.who.int/childgrowth/training/en). Averages of two measurements were taken, as well as a third measurement if the two measurements deviated by greater than 0.5 kg. Weight was measured to the nearest 0.1 kg with a calibrated portable baby scientific scale (Seca 334, United Kingdom).

4.2.7 Statistical Analysis

Statistical analyses were conducted with SPSS version 25. Data were tested for normality using the Shapiro-Wilk Test. Quantitative data included means, median, ranges, minimum, maximum, confidence intervals and standard deviation (SD). Two categories were utilised to categorise mycotoxin exposure, “low exposure” (<PMTD) and “high exposure” (≥PMTDI).
4.2.8 Ethical approval

Ethical approval was obtained from the Health Research Ethics Committee of North-West University (Potchefstroom Campus), South Africa (NWU-00207-14-S1). The study was conducted according to the Helsinki Declaration (World Medical Association, 2013) and the International Conference on Harmonisation and Meta Population Research Centre guidelines (ICH steering committee, 1996). Written informed consent was obtained from each mother in their home language and goodwill permission was obtained from relevant community leaders.

4.3 Results

4.3.1 Mycotoxin levels of maize samples

The highest upper bound mean contamination level was for total FB (1035 µg/kg), whereas mean contamination levels for DON and ZEA were much lower at 24.5 µg/kg and 31.0 µg/kg, respectively (Table 3).

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Positive (% &gt; LOD)</th>
<th>Mean± SD¹ (µg/kg)</th>
<th>Median (µg/kg)</th>
<th>Range (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>100%</td>
<td>1035 ± 2633</td>
<td>83.2</td>
<td>0.6 – 15590</td>
</tr>
<tr>
<td>Fumonisin B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>18%</td>
<td>24.5 ± 41.8</td>
<td>20.0</td>
<td>3.7 - 403.4</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>14%</td>
<td>31.0 ± 152.2</td>
<td>1.0</td>
<td>1.0 – 1523</td>
</tr>
</tbody>
</table>

¹Upper bound mean with non-detect samples allocated the respective LOD; ²SD: Standard deviation

4.3.2 Participants

A total of 129 children were included, with 58% (n = 75) male, and 4% (n = 5) ≤ 6 months old. In terms of maternal/caregiver education, 15% (n = 19) of the mothers/caregivers had no formal education, 16% (n = 21) attained grade 1 - 5, while 68% (n = 87) finished grade 8 - 12 and only 2% (n = 2) attained a tertiary education. Seventy-three percent (n = 94) of the mothers/caregivers received child support grants. Household water was mostly obtained from the river (47%; n = 60), 32% (n = 42) from communal taps and 21% (n = 27) used personal
water tanks. The majority used pit latrines (65%; n = 84), whereas 32% (n = 41) used the bush and 3% (n = 4) had flush toilets. Unemployment of mothers/caregivers was 94% (n = 121).

### 4.3.3 Feeding practices

Most children (88%; n = 113) received home-grown maize soft porridge and the remainder received other home-grown maize-based dishes such as samp, stiff porridge and crumbly porridge. Total raw maize daily intake mostly originated from soft porridge (1.1 - 252 g/day), crumbly porridge (2.3 - 266 g/day), stiff porridge (2.2 - 288 g/day) and samp (2.3 - 60 g/day). Maize-based complementary food consumption among children as young as one month was observed. Table 4 provides a summary of raw maize consumption according to age groups and indicates increased maize consumption with age.

#### Table 4 Raw maize consumption according to age groups

<table>
<thead>
<tr>
<th>Age group (months)</th>
<th>n</th>
<th>Mean ± SD(^{1}) (g/day)</th>
<th>Median (g/day)</th>
<th>Range (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 6</td>
<td>5</td>
<td>37.8 ± 37.6</td>
<td>21.9</td>
<td>8.8 - 97.5</td>
</tr>
<tr>
<td>7 – 12</td>
<td>48</td>
<td>69.9 ± 70.4</td>
<td>36.0</td>
<td>3.4 – 266</td>
</tr>
<tr>
<td>13 – 18</td>
<td>41</td>
<td>69.6 ± 64.6</td>
<td>15.5</td>
<td>1.60 – 309</td>
</tr>
<tr>
<td>19 - 24</td>
<td>35</td>
<td>128 ± 74</td>
<td>115</td>
<td>2.5 – 321</td>
</tr>
</tbody>
</table>

\(^{1}\) SD: Standard deviation

### 4.3.4 Mycotoxin exposure of children aged 0 - 24 months

The upper-bound mean probable daily intakes (PDIs) for total FB among children stratified by age were above the PMTDI, whereas DON and ZEA exposure where below their respective PMTDIs across all ages (Table 5). Further stratification of children according to high exposure (PDI ≥ PMTDI) and low exposure (PDI < PMTDI) indicated that for total FB, 81% (n = 105), were in the high exposure (≥ 2 µg/kg bw/day) group and 13% (n = 17) were in the high ZEA exposure (≥ 0.5 µg/kg bw/day) group (Table 6). Exposure to DON was below the PMTDI for all children.
Table 5 Mycotoxin exposure of infants according to age groups

<table>
<thead>
<tr>
<th>Age group (months)</th>
<th>N</th>
<th>Mean (± SD) Probable Daily Intake (Range) (µg/kg bw/day)</th>
<th>Total FB</th>
<th>DON</th>
<th>ZEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 6</td>
<td>5</td>
<td>5.0 ± 4.5 (1.2 - 11.1)</td>
<td>0.1 ± 0.1 (0.0 - 0.3)</td>
<td>0.2 ± 0.1 (0.0 - 0.3)</td>
<td></td>
</tr>
<tr>
<td>7-12</td>
<td>48</td>
<td>7.7 ± 7.5 (0.4 - 30.6)</td>
<td>0.2 ± 0.2 (0.0 - 0.7)</td>
<td>0.2 ± 0.2 (0.0 - 0.9)</td>
<td></td>
</tr>
<tr>
<td>13 - 18</td>
<td>41</td>
<td>7.0 ± 6.1 (0.2 - 26.0)</td>
<td>0.2 ± 0.2 (0.0 - 0.6)</td>
<td>0.2 ± 0.2 (0.0 - 1.0)</td>
<td></td>
</tr>
<tr>
<td>19 - 24</td>
<td>35</td>
<td>11.6 ± 7.0 (0.3 - 27.6)</td>
<td>0.3 ± 0.2 (0.0 - 0.7)</td>
<td>0.4 ± 0.2 (0.0 - 0.8)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>129</td>
<td>8.4 ± 7.1 0.2 - 30.6</td>
<td>0.2 ± 0.2 0.0 - 0.7</td>
<td>0.3 ± 0.2 0.0 - 0.9</td>
<td></td>
</tr>
</tbody>
</table>

PMDTIs: FB = 2 µg/kg bw/day, DON = 1 µg/kg bw/day, ZEA = 0.5 µg/kg bw/day

Table 6 Mycotoxin exposure according to provisional maximum tolerable daily intakes

<table>
<thead>
<tr>
<th>Total FB</th>
<th>DON</th>
<th>ZEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>24 (19)</td>
<td>105 (81)</td>
<td>129 (100)</td>
</tr>
</tbody>
</table>

4.4. Discussion

The present study examined multi-mycotoxin exposure (or PDI) among children 0 - 24 months using the relevant home-grown maize-based complementary food consumption patterns including contamination levels in home-grown maize.

4.4.1 Feeding practices of children

This study indicated that children younger than 6 months (n = 5) are already receiving maize-based soft porridge as complementary food. This is in contrast with the WHO recommendations of exclusive breast feeding for infants younger than 6 months (WHO, 2007).
Similarly, studies conducted in specific areas of South Africa have shown that substantial number of infants are indeed consuming a complementary dish within their first month (Mamabolo et al., 2004). Studies conducted in Vhembe district and in central districts of Limpopo Province (LP), South Africa indicated that high percentages of infants aged 3 months already consumed maize-based foods (43.2% and 36.9%, respectively) (Mushaphi and Mbenyane, 2008; Mamabolo et al., 2004). In KwaZulu-Natal Province, Faber et al., (2005) showed that 82% of 391 infants aged 2 - 6 months consumed maize-based soft porridge twice a day. Similar results were obtained amongst infants aged 6 - 11, 12 - 17 and 18 - 24 months from a more recent study conducted in KwaZulu-Natal (Faber et al., 2014). A study conducted in the north of Tanzania also reported early complementary feeding (before 6 months) and showed that 80% of the infants aged 3 months had already been exposed to foods other than breastmilk (plain maize, mixed cereal, food from millet, wheat and cow’s milk) (Magoha et al., 2014). The maize consumption of 8.8 - 97.5 g/day of infants 0 - 6 months in the current study exceeded the intakes in a study conducted among Tanzanian infants, of the same age group consumed (0.57 - 37.50 g/day) also at risk of exposure to mycotoxins.

The dietary assessment was performed using a validated RAPP tool (Lombard et al., 2014). The shortcomings of the QFFQ of this particular tool is the fact that self reported individual consumptions may be overestimated by the participants, leading to a slight overestimation of the mycotoxin exposure levels. However the deterministic approach which utilises the dietary assessment is still the standard practice, in reference to previous studies (Bulder et al., 2012, Burger et al., 2010 and Burger et al., 2014).

4.4.2  Mycotoxin exposure

Contamination levels observed in home-grown maize collected from rural households showed high levels of FB, a result consistent with previous studies in this area (Burger et al., 2010; Shephard et al., 2007, 2013a). The lower levels of DON and ZEA are similar to those observed in South African commercial ‘Special’ maize meal (Burger et al., 2013) and consistent with a previous multi-mycotoxin study on maize collected in this area (Shephard et al., 2013a).

The mycotoxin exposure among children in the current study highlights and confirms the magnitude and predominance of FB as an important public health concern in this vulnerable group. Apart from the mean PDI for FB that is up to four times higher than its PMTDI, the study also showed an additional risk of exposure to ZEA, although to a much lesser extent. The
low levels of contamination of the local maize with DON is of a lesser concern, although occasional exposures above the PMTDI may occur for the high maize consumers with a maize intake at the upper end of the contamination range found in this study. Therefore, a deterministic mean PDI may mask high exposure subgroups within a study sample. A previous study conducted among children (1 - 9 years) from a similar area in South Africa showed that those from Centane (southern EC) had a higher PDI for FB of 14.4 µg/kg bw/day (Shephard et al., 2007). In contrast, children 1 - 9 years from Bizana in the northern EC, had a much lower PDI (6.6 µg/kg bw/day) than the current study population, whereas adolescents (10-17 years) in Centane had comparable exposures of 8.2 µg/kg bw/day.

The FB exposure levels found in this study are within the range observed from previous deterministic studies conducted among adults from the same area in which PDIs ranging between 8.2 - 8.5 µg/kg bw/day were also observed (Burger et al., 2010; Shephard et al., 2007).

Only a limited number of other studies in infants and young children have been reported across Africa. In Tanzania, exposure to FB among infants 0 - 6 months old was much lower than the current study, ranging from 0.005 - 0.88 µg/kg bw/day (Magoha et al., 2014). In another Tanzanian study among 18 - 24 month old infants the corresponding PDIs ranged between 0.2 - 26.4 µg/kg bw/day (Kimanya et al., 2014). In contrast, a study conducted in Nigeria observed much higher FB-related PDI’s amongst infants 0 - 48 months (12.4 µg/kg bw/day) and children 5 - 12 years, (8.3 µg/kg bw/day ) (Adetunji et al., 2017). The PDI’s for DON and ZEA among the Nigerian infants and children were higher compared to the current study, ranging between 0.3 to 0.6 µg/kg bw/day (DON) and 0.1 and 0.3 µg/kg bw/day (ZEA), respectively (Adetunji et al., 2017).

Overall in the current study, 88% of children had FB exposure above the PMTDI, whereas the studies in Tanzania showed much lower proportions ranging from 0 - 56% above the PMTDI (Kimanya et al., 2010, Kimanya et al., 2012, Kimanya et al., 2014; Magoha et al., 2014). The current study indicated no children were at risk for exposure to DON, whereas a Tanzanian study conducted by Kimanya et al., (2014) amongst children aged 18 - 24 months showed a high percentage (66%) were at risk of high DON exposure. The current study utilised a validated method for determination of FB, DON and ZEA.

Despite the lack of clear causative mechanisms between exposure to mycotoxins such as FB, DON and ZEA, and health effects in infants and young children, the potential health risks posed
by exposure to these mycotoxins has received international attention from global health authorities such as the WHO (WHO, 2014). The contribution of FB exposure and to a lesser extent ZEA as seen in the current study, is expected to impact negatively on children’s health or exacerbate existing health conditions (Smith et al., 2012). To address this public health concern, exclusive breastfeeding for infants younger than 6 months and breastfeeding for up to 2 years of age should remain the healthiest and most affordable and reliable approach across South Africa. This, together with cultural-specific intervention strategies such as simple sorting and washing of maize prior to consumption, which are known to reduce FB levels, are therefore essential to ensure healthier and safer food for infants and young children during complementary and weaning periods (Van der Westhuizen et al., 2011).

4.5 Conclusions

Although this study included only a small sample size, it provides evidence for high fumonisins exposure among vulnerable population groups such as rural infants and young children exposed to multiple-mycotoxins in South Africa. A small percentage of infants were exposed at an early stage (before six months) and exposure increases with age. Further studies should investigate the effect of these exposures on the growth of infants and young children. A follow-up paper will be published that focus on the relationship between mycotoxin exposure and growth (especially stunting) of infants 0 - 12 of age.

4.6 Conflict of interest

The authors declare conflict of interest.

4.7 Acknowledgements

The authors would like to acknowledge the cooperation of the participants who made this study possible and the field workers who played a pivotal role in data collection. Midlands State University, Zimbabwe is acknowledged for giving Ropafadzo Tshalibe the opportunity to be involved in this study.

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CHAPTER 5 PAPER 2: MULTI-MYCOTOXIN EXPOSURE AND INFANT (0-12 MONTHS) GROWTH IN DEEP RURAL AREAS OF THE EASTERN CAPE, SOUTH AFRICA

Paper 2 will be submitted to Food and Chemical Toxicology for review. The paper is in the format of the author guidelines (Addendum 2). The paper focuses on objective 3 of the sub-study, including the association between maize consumption, mycotoxin exposure and growth of infants 0 – 12 months of age. This age group was selected due to their changing eating habits (from breastfeeding to complementary food). Older children (12 – 24 months) were excluded from this paper since they consume larger food portions and may have different growth patterns than infants. These age groups also make it more comparable to other similar studies.
MULTI-MYCOTOXIN EXPOSURE AND INFANT (0 - 12 MONTHS) GROWTH IN DEEP RURAL AREAS OF THE EASTERN CAPE, SOUTH AFRICA

Ropafadzo S. Tshalibe¹, Hester-Mari Burger², Christine Taljaard-Krugell¹, Johanna F. Alberts², Gordon S. Shephard², Martani J. Lombard¹

¹ Centre of Excellence for Nutrition (CEN), North-West University, Potchefstroom Campus, Faculty of Health Sciences, Private Bag X6001, 2520, South Africa

² Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology, PO Box 1906 Bellville, 7535, South Africa

Ropafadzo S. Tshalibe¹ (tshaliberopafadzo1984@gmail.com), Hester-Mari Burger² (BurgerH@cput.ac.za), ChristineTaljaard¹ (nienatal@gmail.com), Martani J Lombard¹ (Tani.Lombard@nwu.ac.za), Johanna F. Alberts² (AlbertsH@cput.ac.za) and Gordon S. Shephard² (gshephard@mweb.co.za).

Corresponding author. Martani Lombard: Tel:+27 (18) 299 2085

E-mail address: Tani.Lombard@nwu.ac.za
ABSTRACT

Background: Undernutrition is high amongst infants 0 - 12 months in South Africa (SA) and especially rural Eastern Cape (EC). In most EC subsistence farming households, the primary complementary foods are maize-based. However, home-grown maize in rural EC has high levels of mycotoxins [fumonisins (FB), deoxynivalenol (DON) and zearalenone (ZEA)]. These high levels of mycotoxins may influence growth. This study aims to explore the association between mycotoxin exposure and growth impairment.

Method: Fifty-one infants consuming only home-grown maize were included in the longitudinal study. World Health Organisation (WHO) z-scores for weight-for-age (WAZ), length-for-age (LAZ) and weight-for-length (WLZ) were used to determine growth velocity from birth to current weight. Average daily raw maize intake was determined with a validated culturally specific food frequency questionnaire. Exposures were estimated as probable daily intakes (PDI) and assessed with respect to provisional maximum tolerable daily intake (PMTDI).

Results: Geometric mean FB PDI was 6.3 µg kg⁻¹ bw day⁻¹ (> PMTDI) while DON and ZEA were 1.2 and 1.2 µg kg⁻¹ bw day⁻¹ (> PMTDI), respectively. The prevalence of stunting of the infants in this cohort was observed to be 16%. ANCOVA showed that infants exposed to FB levels above the provisional maximum intake levels had a mean length 4.4 cm shorter than their counterparts. Furthermore, ANCOVA showed significant differences in LAZ (p value < 0.01), WAZ (p value < 0.05) z-scores and length (p value < 0.001) of infants exposed to low FB exposure as compared to high FB exposure. Change in LAZ also indicated a negative growth velocity of infants within the high FB exposure group.

Conclusion: The length change of infants is a gradual process. The drop in LAZ from birth to current age were observed in the high FB exposed group and calls for a follow-up study of these infants beyond 12 months to determine long-term exposure and the effect on growth.

KEYWORDS: mycotoxin exposure, undernutrition, maize and growth
5.1 Background

Child stunting and wasting are globally a public health problem in low- and middle-income countries (Black et al., 2008). Growth faltering such as mentioned above has a severe impact on long-term physical development (WHO, 2014). Growth faltering is further linked to a variety of factors such as poor nutrition, poor hygiene, low socioeconomic status, political instability, repeated infectious diseases, and environmental toxins (Black et al., 2008; UNICEF Conceptual Framework, WHO, 2011).

Mycotoxins are secondary metabolites produced by fungi found on maize (FAO, 1991). Important mycotoxins known to affect human health include aflatoxins (AF), fumonisins (FB), deoxynivalenol (DON) and zearalenone (ZEA). In terms of infant growth, some evidence exists that links higher AF exposure to higher stunting prevalence, however very little information is available on the role of FB, DON and ZEA on infant growth (Lombard, 2014).

In rural areas, such as the Eastern Cape (EC), infants often consume maize-based complementary foods early (submitted article, Tshalibe et al., 2019a, Lombard, 2014). The population mainly comprises subsistence farmers and it is well-documented that home-grown maize contains high levels of FB and lower levels of DON and ZEA (Shephard et al., 2013, submitted article, Tshalibe et al., 2019a). These mycotoxins may play a role in the prevalence of poor growth among children from rural maize-subsistence areas in EC (Lombard, 2014). Infants are more susceptible to toxins than adults, because of their lower body weight, higher metabolic rate, lower ability to detoxify and incomplete development of organs and tissues (Cano-Sancho et al., 2009).

Previous studies on infant mycotoxin exposures (AF and FB) and growth impairment risk have been conducted in Africa. In Kisumu District in Kenya, it was found that children 3 - 36 months (n = 242) were wasted, wasting of these children was also significantly associated with AF contaminated gruel (mixture of grains) and / or flour consumption (p = 0.002) (Okoth and Ohingo, 2004). Furthermore, in Tanzania, the LAZ and WAZ scores of infants (6 - 12 months) consuming high amounts of FB (> PMDTI) (n = 215), were significantly lower than the low exposure group (Kimanya et al., 2010). Shrima et al., (2015) studied 166 infants aged 6 - 14 months in Tanzania. Those exposed to high levels of FB exposure were predominately stunted (LAZ below -2 score). The proportion of infants with LAZ scores below -2 z-score was 44% at recruitment, 55% at 6 months and 56% at 12 months after recruitment (Shrima et al., 2015).

Raw maize consumption in terms of frequency and portion size is high amongst infants 0-24 months in EC, SA (submitted article, Tshalibe et al., 2019a). Intakes above 100 grams per day
were reported in a recent study (submitted article, Tshalibe et al., 2019). This study also indicated high FB probable daily intakes (PDIs) above the provisional maximum tolerable daily intake (PMTDI) of 2 µg kg⁻¹ body weight (bw) day⁻¹, with DON and ZEA below their PMTDIs of 1 and 0.5 µg kg⁻¹ bw day⁻¹, respectively (JECFA, 2012, 2001, 2002). The current study will therefore, focus on determining a possible association between three mycotoxins (FB, DON and ZEA) and infant growth parameters.

5.2 Methods

5.2.1 Study design and Population

The study utilised a longitudinal study design and it is part of a larger infant and young child feeding project among children aged 0 – 24 months, the PhilaSana project. For the current study only, infants aged 0 – 12 were included and those (n = 51) consuming specifically home-grown maize exclusively or in combination with breastmilk, formula or other foods. Infants exclusively breastfed were excluded from this study. Infants were recruited from various villages in the Centane, Amatole District Municipality in EC using systematic and snowball sampling due to low population density and poor infrastructure. Household socio-economic status and ethnicity information was obtained, as well as, employment status and education level of mothers/caregivers.

Ethical approval for the study was obtained from the Human Research Ethics Committee of North-West University (Potchefstroom Campus), South Africa (NWU-00207-14-S1). The study was conducted according to the Helsinki declaration (World Medical Association, 2013) and the International Conference on Harmonisation and Metapopulation Research Centre guidelines (ICH steering committee, 1996).

5.2.2 Maize intake

Trained interviewers obtained infant food consumption information from the mothers/caregivers. The RAPP (Ratio and Portion Size Photo) tool (validated culturally specific dietary assessment method that determines habitual dietary intake of people living in this area) was used (Lombard et al., 2014). The tool consists of a semi-quantitative food frequency questionnaire (FFQ) and portion sized photographs. It was adapted for infant and young children’s intakes based on 150 24-hour recalls (infants and young children 0 - 24 months). This was obtained before the onset of the study. Three food portion size photographs per dish were also developed and validated before the onset of the study. The tool is designed to determine intake for the past month and converted to a daily average consumption of each food item or dish consumed (Lombard et al., 2014). Amount of cooked maize consumed in a
day was determined by the portion size (in grams) multiplied by the number of portions consumed in a day. Monthly intake was then determined by consumption frequency per week or per month multiplied by intake at a time. Monthly intake was divided by 28 days to give a mean daily intake of cooked maize meal.

The following formula was used:

\[
\text{Daily mean intake} = \frac{(\text{Portion of cooked maize} \times \text{number of portions} \times \text{frequency per week} \times 4)}{28 \text{ days}}
\]

Mean daily intake of cooked maize was then converted to raw maize according to recipes obtained during the development of the tool (Lombard et al., 2014).

5.2.3 Mycotoxin exposure assessment

Infant mycotoxin exposure was assessed using a deterministic approach and expressed as probable daily intake (Lombard, 2014) using mycotoxin levels in home-grown maize. The mean total FB (FB1 + FB2 + FB3) DON and ZEA levels were obtained from the analysis of maize collected from maize-subsistence farming households in Centane (Tshalibe et al., In Press a). Mean contamination levels of maize for FB were 1035.0 µg kg\(^{-1}\), DON 24.5 µg kg\(^{-1}\) and ZEA 31.0 µg kg\(^{-1}\) respectively.

5.2.4 Growth measures of infants

Date of birth, sex and birth anthropometric (length, weight, head circumference (HC) and mid-upper arm circumference (MUAC), as well as gestational age information, were retrieved from clinical cards (infant clinic cards with birth anthropometric information, recorded at birth at the clinics). Thereafter, trained research assistants conducted anthropometric assessments of the infants. Measurements were taken according to WHO and UNICEF Standards (available at http://www.who.int/child_growth/training/en). Averages of two measurements (weight and length) were taken, as well as a third measurement if there was a deviation of more than 0.5 cm, for length and 0.5 kg for weight. Weight was measured to the nearest 0.1 kg with a calibrated portable scientific scale (Seca, 334), manufactured by Seca, United Kingdom. Length was measured with a baby length-measuring mat.

Anthropometric data was captured in the WHO Anthroplus version 1.0.4 (2007), (www.who.int/childgrowth/software/en, 2011). Undernutrition was determined using SD from the WHO Z-scores (Onis, 2006). Chronic undernutrition is measured as length-for-age (LAZ) below -2 standard deviation (SD) of the WHO growth standards (z-scores), while wasting is weight-for-length (WLZ) below -2 SD and underweight, weight-for-age (WAZ) below -2 SD.
Infants with a z-score between -2 and -3 SD LAZ were classified as moderately stunted and those ≤ -3 SD were severely stunted. Infants with WLZ or WAZ -2 SD < -3 SD were considered moderately wasted and underweight respectively. Infants with an SD ≤ -3 SD WAZ and WLZ were classified as severely underweight and wasted. Infants with a z-score above 4 or below -4 SD were excluded as possible measurement errors.

5.2.5 Statistical analysis

Data were captured and cleaned in Excel. Statistical analyses were conducted with SPSS version 25. Data were tested for normality using the Shapiro-Wilk Test. The mycotoxin data were not normally distributed, and thus log-transformed for statistical analyses. Geometric means were reported for FB, DON and ZEA in all text and tables.

Two categories were utilised to categorise mycotoxin exposure, “low exposure” (PDI < PMTDI,) and “high exposure” (PDI ≥ PMTDI,).

Individual infant multi-mycotoxin exposure was compared with the infant’s growth parameters (current length, current weight, current WAZ (SD), WLZ (SD), and LAZ (SD) z-scores as well WAZ, LAZ and WLZ changes. Change in Z-score (from birth to 12 months) was calculated to determine if one group had a slower growth rate (indicating a trend towards stunting or wasting) than another. Changes in Z-scores were calculated as current z-score (12 months) – birth z-score (0 months).

Quantitative data included means / geometric mean, SD, medians; minimum and maximum ranges were used as descriptive measures. Infants were subdivided according to exposure level. Independent t - test was used to detect any statistically significant differences between the different groups. ANCOVA, with adjustment of covariates (age, gender, education of mother, HIV and TB status of infants) was also utilised to detect the statistical differences between the different groups. Linear regression was conducted to determine the association between mycotoxin exposure and growth of infants. Lastly, the infant's exposure were categorised according to the percentage exposed above the relevant PMTDI for total FB, DON and ZEA.

5.3 RESULTS

5.3.1 Participants

A sample of 51 infants, (55%, n = 28 males) participated in the study. Most infants were 7 - 12 months old (92%, n = 47). In terms of maternal / caregiver education, 20% (n = 10) had no formal education, 20% (n = 10) attained grade 1 - 5, while 60% finished grade 8 - 12 (n = 31).
Most of the mothers / caregivers, 73% (n = 37) received child grants. Approximately half of the households 43% (n = 23), obtained water from the river, while 28% (n = 14) used communal tap water and 29% (n = 15), had their own tanks. Sixty-seven percent (n = 34) used pit toilets, while 33% (n = 17) used the bushes. A high percentage of the mothers / caregivers were unemployed, 92% (n = 47) with the local hotel being the only source of work in the area.

5.3.2 Maize intake

The maize-based foods consumed by infants in the current study were mostly soft porridge, *maheu* (fermented maize-meal drink) and crumbly maize meal. The raw maize consumption ranged from 3.4 to 338.8 g day$^{-1}$, with a mean raw intake of 74.8 g day$^{-1}$.

5.3.3 Mycotoxin exposure

The geometric mean PDIs for total FB, DON and ZEA were 6.3, 1.2 and 1.2 µgkg$^{-1}$ bw day$^{-1}$ respectively (Table 1). Most of the infants, 80% (n = 41), were in the high FB exposure group (≥ PMTDI of 2 µgkg$^{-1}$ bw day$^{-1}$).

**Table 1 Geometric mean probable daily intakes for total FB, DON and ZEA**

<table>
<thead>
<tr>
<th>PDI (n = 51)</th>
<th>Median IQR (25th - 75th)</th>
<th>Geometric Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total FB</td>
<td>4.4 (2.5 - 11.1)</td>
<td>6.3 (2.5)</td>
</tr>
<tr>
<td>DON</td>
<td>0.1 (0.1 - 0.3)</td>
<td>1.2 (1.2)</td>
</tr>
<tr>
<td>ZEA</td>
<td>0.1 (0.1 - 0.3)</td>
<td>1.2 (1.2)</td>
</tr>
</tbody>
</table>

FB = fumonisins, DON = deoxynivalenol, ZEA = zearalenone

PDI = Probable daily intake

PMDTI: FB = 2 µgkg$^{-1}$ bw day$^{-1}$, DON = 1 µgkg$^{-1}$ bw day$^{-1}$, ZEA = 0.5 µgkg$^{-1}$ bw day$^{-1}$

IQR - Interquartile range

PDI = µgkg$^{-1}$ bw day$^{-1}$

5.3.4 Birth information and anthropometric measurements of the infants

Out of a total sample of 51 infants, birth weight information was available for only 41 infants (80%). Missing values were due to the high number of home births. None of the infants had a birth weight < 2 500 g. Birth length information was available for 46 infants, of these 46 infants, 14% (n = 6) had a birth length (BL) below the normal average WHO world standards of healthy
infants. The gestational age of 11% (n = 6) of the infants was below 38 weeks, regarded as being premature.

None of the 51 infants were wasted or underweight (Z-score < -2 SD), whereas 16%, (n = 8) of the infants were stunted (Z-score < -2 SD).

5.3.5 Mycotoxin exposure in relation to growth of infants

Independent t-tests showed no significant differences in birth outcomes of infants exposed to low mycotoxins and those exposed to high FB and DON exposure (Table 2). Therefore, if there was exposure during pregnancy, it did not by any means affect the growth of infants at birth.

Table 2 Differences in growth parameters at birth according to PMTDI

<table>
<thead>
<tr>
<th>Birth Parameters</th>
<th>FB &lt; PMDTI</th>
<th>FB &gt; PMDTI</th>
<th>ZEA &lt; PMDTI</th>
<th>ZEA &gt; PMDTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>3.4 (0.6)</td>
<td>3.3 (0.4)</td>
<td>3.3 (0.5)</td>
<td>3.4 (0.4)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>48.8 (4.1)</td>
<td>48.7 (5.4)</td>
<td>49.1 (5.2)</td>
<td>47.6 (4.9)</td>
</tr>
<tr>
<td>WLZ</td>
<td>0.7 (2.0)</td>
<td>-1.0 (2.6)</td>
<td>-1.1 (2.6)</td>
<td>0.2 (2.3)</td>
</tr>
<tr>
<td>LAZ</td>
<td>-0.5 (2.4)</td>
<td>-0.3 (2.5)</td>
<td>0.2 (2.6)</td>
<td>-0.8 (2.1)</td>
</tr>
<tr>
<td>WAZ</td>
<td>0.2 (1.4)</td>
<td>-0.1 (0.9)</td>
<td>-0.4 (1.0)</td>
<td>0.2 (0.8)</td>
</tr>
</tbody>
</table>

FB = fumonisins, ZEA = zearalenone
WLZ = Weight-for-Length Z-score, LAZ = Length-for-Age Z-score,
WAZ = Weight-for-Age Z-score, PMTDI = Provisional maximum tolerable daily intake.

Independent t-test was performed at 5% significance levels. However, no significant differences between the weight of infants in the high FB and high ZEA groups and low exposure groups (Table 3) were observed. DON results were not included as there were none in the high exposure group. There were also no significant differences in length, WLZ, LAZ and WAZ of infants within the low and high exposure groups for both FB and ZEA.
Table 3 Means, standard deviation and independent t-tests results of mycotoxin exposure in relation to growth indicators of infants 0-12 months of age.

<table>
<thead>
<tr>
<th>Growth</th>
<th>Low FB</th>
<th>High FB</th>
<th>Low ZEA</th>
<th>High ZEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
<td>n</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>10</td>
<td>9.1 (1.0)</td>
<td>41</td>
<td>9.1 (1.3)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>10</td>
<td>72.8 (4.4)</td>
<td>41</td>
<td>70.0 (4.4)</td>
</tr>
<tr>
<td>WAZ</td>
<td>10</td>
<td>0.7 (0.9)</td>
<td>41</td>
<td>0.5 (1.0)</td>
</tr>
<tr>
<td>WLZ</td>
<td>10</td>
<td>0.8 (1.13)</td>
<td>41</td>
<td>1.0 (0.9)</td>
</tr>
<tr>
<td>LAZ</td>
<td>10</td>
<td>0.5 (1.5)</td>
<td>41</td>
<td>-0.3 (1.6)</td>
</tr>
</tbody>
</table>

WAZ = Weight-for-age Z-score, WLZ = Weight-for-length Z-score, LAZ = Length-for-age Z-score

Data was adjusted for various confounding factors, including age, gender, HIV, TB, maternal / caregiver education and household water source relative to growth and exposure are reported in Table 4. After adjusting for confounding factors, the total sample size was reduced because of the adjustment. The infants in the high FB exposure group were shorter than those within the low FB exposure group. There was a statistically significant association between the lower length of infants and high FB exposure. WAZ scores of infants within the high FB exposure group were significantly different from the low FB exposed group; while there was only a slight difference in WAZ scores for the high ZEA exposed infants and the low ZEA exposed groups. A marked difference in infant WLZ scores was observed between the low and high exposed FB and ZEA exposure groups, though there was no statistical association. A significant difference in LAZ score was also noticed between infants with low FB exposure compared with the high FB exposure group.
Table 4 Linear regression results for adjusted data for the association between growth and mycotoxin exposures

<table>
<thead>
<tr>
<th>Growth</th>
<th>Low FB</th>
<th>High FB</th>
<th>Low ZEA</th>
<th>High ZEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
<td>n</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>9</td>
<td>9.2 (1.1)</td>
<td>34</td>
<td>8.9 (1.2)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>9</td>
<td>73.8 (3.4)</td>
<td>34</td>
<td>69.4 (4.4) ***</td>
</tr>
<tr>
<td>WAZ</td>
<td>9</td>
<td>0.8 (0.9)</td>
<td>34</td>
<td>0.4 (1.0) *</td>
</tr>
<tr>
<td>WLZ</td>
<td>9</td>
<td>0.7 (1.1)</td>
<td>34</td>
<td>0.9 (0.8)</td>
</tr>
<tr>
<td>LAZ</td>
<td>9</td>
<td>0.8 (1.3)</td>
<td>34</td>
<td>-0.4 (1.5) **</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, HIV, TB, education and water source of mycotoxin exposure in relation to growth

WAZ=Weight-for-age Z-score, WLZ=Weight-for-length Z-score, LAZ=Length-for-age Z-score

* P value < 0.05, ** P value < 0.01, *** P value < 0.001

Infants within the high FB and ZEA exposure group had a higher change in WLZ as compared to the low exposed group, as shown in Table 5. High fumonisin exposure had a negative LAZ score change as opposed to a positive LAZ change for high ZEA exposure. The results also indicate a lower positive WAZ change with high FB as opposed to no difference in WAZ change for high and low ZEA exposure groups. However, none of these were statistically significant.

Table 5 T-test results of Z scores of infant’s growth compared to mycotoxin exposures

<table>
<thead>
<tr>
<th>Growth</th>
<th>Low FB</th>
<th>High FB</th>
<th>Low ZEA</th>
<th>High ZEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
<td>n</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>WLZ</td>
<td>5</td>
<td>0.1 (1.4)</td>
<td>31</td>
<td>1.9 (2.3)</td>
</tr>
<tr>
<td>LAZ</td>
<td>8</td>
<td>1.2 (1.7)</td>
<td>34</td>
<td>-0.1 (2.8)</td>
</tr>
<tr>
<td>WAZ</td>
<td>8</td>
<td>0.6 (1.18)</td>
<td>32</td>
<td>0.5 (1.2)</td>
</tr>
</tbody>
</table>

WLZ = Weight-for-length Z-score, LAZ = Length-for-age Z-score, WAZ = Weight-for-age Z-scores

Data was adjusted for age, gender, HIV, TB, maternal / caregiver education and water source of mycotoxin exposure and compared to change in growth as shown in Table 6. Changes in z-scores were calculated as current z-score compared with birth z-score. None of the growth
changes were statistically significant when comparing infants stratified by high or low mycotoxin exposure. There was a higher positive WLZ change with high FB exposure, and lower in WLZ change with high ZEA exposure. High fumonisin exposure had a negative LAZ score change and a positive change in LAZ change for ZEA exposure. Lastly, there was a lower WAZ change with high FB and ZEA exposure, however, none of these were significant differences.

Table 6 Linear regression results for the change in Z-scores and mycotoxin exposures

<table>
<thead>
<tr>
<th>Change in growth</th>
<th>Low FB</th>
<th>High FB</th>
<th>Low ZEA</th>
<th>High ZEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Mean (SD)</td>
<td>n</td>
<td>Mean (SD)</td>
<td>n</td>
</tr>
<tr>
<td>WLZ</td>
<td>5</td>
<td>0.1 (1.4)</td>
<td>25</td>
<td>1.9 (2.5)</td>
</tr>
<tr>
<td>LAZ</td>
<td>7</td>
<td>1.0 (1.66)</td>
<td>28</td>
<td>-0.1 (2.95)</td>
</tr>
<tr>
<td>WAZ</td>
<td>7</td>
<td>0.4 (1.2)</td>
<td>27</td>
<td>0.3 (1.1)</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, HIV, TB, education and water source

WLZ = Weight-for-length Z-score, LAZ = Length-for-age Z-score, WAZ = Weight-for-age

5.4. Discussion

Exclusive breastfeeding practices up to six months of age are very limited in the EC (Tshalibe et al., 2019 unpublished). Infants are also often given complementary and weaning foods at an early age (less than six months) in South Africa (SA) (Sibeko et al., 2005). The mycotoxins associated with maize grown in subsistence farming areas in the EC include FB, DON and ZEA (Shephard et al., 2013, Burger et al., 2010) and have been implicated in the development of various health problems such as increase in systemic cytokines and food refusal and impaired nutrient uptake (DON), inhibition ceramide synthase (FB), leading to impaired growth (Smith et al., 2012). Unfortunately, very little is known about chronic exposure of FB, DON and ZEA among infants and particularly its association with stunting (Lombard et al., 2014).

This study, therefore, explored mycotoxin exposure of infants 0 - 12 months in relation to their growth. The need to investigate the effect of exposure on infant growth surfaces from the high stunting prevalence and high mycotoxin concentrations of the home-grown maize in EC.

In Tanzania, average maize intake of 8 g day⁻¹, (n = 143), among infants less than six months were observed (Magoha et al., 2014). Furthermore, in Tanzania, mean intakes of 43 g day⁻¹, were obtained (n = 254) amongst infants aged 6 - 8 months of age (Kimanya et al., 2012). Similarly, Kimanya et al., (2010), also observed lower maize intakes of 32 g day⁻¹, amongst
infants aged 6 - 12 months of age, \( n = 215 \), in Tanzania. Although it can be argued that the infants in this study were older, therefore the justification of consuming more cooked raw maize with 92% being 7-12 months old.

Infants have more future years of life than adults, hence have more time to develop chronic diseases that may be triggered by early environmental and dietary exposures (Sherif et al., 2009). Chronic undernutrition also develops over time, which makes exposure to mycotoxins at an early age, an important public health challenge.

The geometric mean exposure (based on the PDI) to FB obtained in this study was three times above the PMDTI of 2 \( \mu \text{gkg}^{-1} \text{bw day}^{-1} \) (JEFCA, 2012). In contrast, in Tanzania, lower FB PDIs were obtained, ranging from 0.005 to 0.88 \( \mu \text{gkg}^{-1} \text{bw day}^{-1} \) amongst younger infants less than six months of age (Magoha et al., 2014). However, higher PDI's were obtained in Tanzania among infants aged 6 - 12 months of age (Kimanya et al., 2010). The predominately high FB exposures obtained in the current study can be masked, by the small sample size. A smaller sample size limits the validity of the results obtained, as one might have sampled mainly those consuming high amounts of daily cooked maize. These levels of exposure risk found in this study are similar to those obtained in adult studies from the same area (Burger et al., 2010, Shephard et al., 2007).

Results indicated that the geometric means for DON and ZEA were above their PMDTI's of 1 \( \mu \text{gkg}^{-1} \text{bw day}^{-1} \) and 0.5 \( \mu \text{gkg}^{-1} \text{bw day}^{-1} \) (JEFCA, 2001, JEFCA, 2002).

A lower percentage (16%) of stunted infants was obtained in this study as compared to Shisana et al., 2013, results from EC. In contrast Shisana et al., 2013 also obtained slightly higher wasting rates, above one percent in comparison 0 % in this study (Shisana et al., 2013).

There were no significant differences in birth outcomes between the low and high FB and ZEA exposure groups and therefore birth outcomes were excluded as possible confounding factors in this study. Deoxynivalenol and ZEA, were found not to be associated with any growth parameters in this study, mostly related to the low contamination level in home-grown maize. As the children grow older, and consume more maize, it is therefore possible that the exposures can increase.

Infants exposed to FB exposure above the PMDTI were significantly shorter by 4 cm, as compared to those exposed below the PMDTI, after adjusting for confounding factors. This length difference is larger compared to what was observed in Tanzania. This study showed a significant decrease in length four times that reported by Kimanya et al., in 2010. However, of
significance, is the fact that weight-for-age z-scores were statistically associated with high FB exposure.

Data was adjusted for age, gender, HIV, TB, maternal / caregiver education and water source. Adjusted data indicated a decrease in LAZ score of approximately one Z-score, which is clinically significant since it indicates a negative direction and a possible trend towards stunting in the future. This was also statistically significant, confirming the trend towards future stunting. The adjusted data showed a statistical significance between the low and high exposed groups, in comparison to the lack of statistical significance observed in the unadjusted data. Primarily the change in z-score indicates that the infant LAZ scores shifted from the positive towards the negative z-scores. In contrast to this study, Shrima et al., 2015, observed that FB exposure was only negatively associated with LAZ but no other z-scores at 6 months (p = 0.016) and at 12 months from recruitment (p = 0.014). However, in this study, FB concentrations were negatively associated with both LAZ and WAZ z-scores after adjusting for confounders. This study’s results indicated a statistically significant difference between the high and low exposed groups after confounder adjustment.

Although the infants were picking up weight in terms of length, as demonstrated by higher WLZ and WLZ change (birth to current) of infants within the high FB exposure group (before and after adjusting for confounders), there is a significant drop in LAZ and LAZ change (before and after adjusting for confounders), explaining the decrease in growth rate. The decrease in LAZ score was of statistical significance, although the increase in WLZ and WLZ change, as well as LAZ change, did not reach a statistical significance.

Findings from this study align with evidence regarding the potential effect of FB on growth impairment (Smith et al., 2012). These findings have raised a concern that FB may induce intestinal enteropathy, a subclinical condition of the small intestine, characterised by reduced absorptive capacity, poor appetite, inflammatory reactions and increased intestinal permeability, therefore mediating stunting, as previously observed in animals. (Smith et al., 2012).

This study accounted for some possible confounding factors such as age, gender, HIV, TB, maternal / caregiver education and water source and mother’s education. Our study predicted that FB might have a direct effect on chronic health or stunting and underweight, but not wasting after 12 months of age. This study is similar to that of Chen et al., 2018, where it was shown that FB is a significant risk factor in length, and weight after adjusting for covariates. These findings suggest that FB exposure plays a pivotal role in growth impairment. A follow
up study including older infants is therefore essential, as stunting occurs over a longer period, hence these children may be affected to a large extent.

The small sample size of infants included in this study was due to the sparsely populated rural area which was not easily accessible, the poor infrastructure and logistical problems of not being able to stay in the area for a long period of time as it has financial constraints. The ongoing nutrition transition and changes in agricultural practices and access to home-grown maize might have impacted negatively on home-grown maize consumption patterns of infants. The deterministic approach of FB, DON and ZEA exposure at six monthly time points within the twelve months is an estimation of acute exposure and not chronic, however since contaminated home-grown maize is eaten daily these results provide valuable information to evaluate risk. Furthermore, determining exposure at specific time points, while following up children also affected the exposure results obtained by Chen et al., 2018 in Tanzania negatively. Following up infants at specific time points does not account for the exact amount of maize consumed daily, as the individual may misreport what was consumed in your absence, due to the time lapse.

This study is in line with responsible consumption and production, as postulated by the sustainable development goal number 12 (UN, 2017). Since complementary foods consumed by infants are prone to high mycotoxin levels it is important to recommend culture specific nutrition education on exclusive breastfeeding, safe and nutritious weaning and complementary food, including mycotoxin lowering strategies to reduce exposure.

Lastly, although the results for this study were adjusted for various factors, including child health (HIV and TB exposure), it is advised that the secondary effects mycotoxins have on infants, such as inflammatory response, infectious diarrhoea and reduced appetite be investigated in more depth. In addition to this it is important to mention that although the data was adjusted for some confounders, not all could have been taken into account. Thus, it is important to realise that malnutrition is a complicated condition with many variables (such as low energy and/or protein intake and weaning practices). Follow-up research should also identify additional factors that contribute to the malnutrition in this area and to determine the strength of the associations.

5.5 Conclusion

Mycotoxin exposure, especially FB, is a contributing factor of impaired growth amongst infants 0 - 12 months of age in EC. Negative associations were found between mycotoxin exposure and current growth. The drop in LAZ amongst children exposed to high FB exposure was
statistically significant. Although the change in z-score was not low enough to clinically diagnose stunting (≤-2) z-score; it is an indication of the possible risk of stunting in the future. A further drop in LAZ change may indicate a significant difference when children in the later age groups are exposed for longer periods. A follow up study with older children, (who consume larger amounts of maize), should investigate this in more depth.

5.6 Conflict of interest

The authors declare no conflicts of interest.

5.7 Author contribution

Miss Tshalibe contributed towards study design and data collection, statistical analysis and quality control of questionnaires. She was also primarily responsible for article writing. Dr Burger was involved in study design and data collection. She was also involved in sourcing of funding and article compilation. Dr Taljaard-Krugell was involved in article compilation. Prof Gelderblom and Dr Shephard contributed to data interpretation and writing of the paper. Dr Lombard contributed to study design, funding and data management. She also assisted in article compilation and statistical analysis.

5.8 Acknowledgements

The authors would like to acknowledge the cooperation of the participants who made this study successful. The field workers played a pivotal role in data collection.

5.9 Funding

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Role of funding sources The funding sources were not involved at all in the project design, data collection, result analysis, interpretation or conclusions.
5.10 References


CHAPTER 6 PAPER 3: MULTI-MYCOTOXIN EXPOSURE AND CHILD (13 – 24 MONTHS) GROWTH IN DEEP RURAL AREAS OF EASTERN CAPE PROVINCE, SOUTH AFRICA

Paper 3 will be submitted to the Toxicology for review. The paper is in the format of the author guidelines (Addendum 3). The paper focuses on objective 3 of the Philasana sub-study, it explores the association between maize consumption, mycotoxin exposure and growth of children 13 – 24 months of age. Children older than 24 months and younger than 12 months were excluded from this paper since they consume different food portions and may have different growth patterns with the children in question.
Multi-mycotoxin exposure and child (13 - 24 months) growth in deep rural areas of Eastern Cape Province, South Africa

Ropafadzo S. Tshalibe¹, Hester-Mari Burger², Christine Taljaard-Krugell¹, Johanna F. Alberts², Gordon S. Shephard², Martani J. Lombard¹

¹ Centre of Excellence for Nutrition (CEN), North-West University, Potchefstroom Campus, Faculty of Health Sciences, Private Bag X6001, 2520, South Africa

² Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology, PO Box 1906 Bellville, 7535, South Africa

Ropafadzo S. Tshalibe¹ (tshaliberopafadzo1984@gmail.com), Hester-Mari Burger² (BurgerH@cput.ac.za), ChristineTaljaard-Krugell¹ (nienatal@gmail.com), Martani J Lombard¹ (Tani.Lombard@nwu.ac.za) and Johanna F. Alberts² (AlbertsH@cput.ac.za) and Gordon S. Shephard² (gshephard@mweb.co.za).

Corresponding author. Martani Lombard: Tel:+27 (18) 299 2085

E-mail address: Tani.Lombard@nwu.ac.za
ABSTRACT

Background: In the Eastern Cape (EC) Province 21.6% of boys and 15.6% of the girls below 36 months of age were stunted (Shisana et al., 2013). In deep rural areas of Eastern Cape (EC), South Africa (SA), maize is generally used as weaning food. It is further well known that the maize produced by these subsistence farmers has high levels of mycotoxins. These mycotoxins are well known to be associated with health problems. It has also been speculated that mycotoxins may result in growth impairment.

Method: For in-depth understanding of the mycotoxin exposure (including fumonisins (FB), deoxynivanol (DON) and zearalenone (ZEA) in relation to growth, a total of 70 children were recruited. World Health Organisation (WHO) z-scores for weight-for-age (WAZ), length-for-age (LAZ) and weight-for-length (WLZ) were utilised in determining physical growth. Average daily maize intake was determined with a culturally specific food frequency questionnaire and converted to raw maize intake. Mycotoxin exposure was then calculated based on the average daily raw maize intake and the known mycotoxin level per kilogram raw maize.

Results: Results showed high mean mycotoxin exposures of 7.8, 1.2 and 1.3 µgkg\(^{-1}\) bw day\(^{-1}\) for FB, DON and ZEA, respectively. Thirty-four percent of the children in this cohort were stunted. ANCOVA showed a significant difference in WLZ and LAZ changes with high FB exposure (p < 0.05). Linear regression also indicated that high FB exposure (p = 0.04), DON (p = 0.04) and ZEA (p = 0.04) was associated with reduced weight gain gkg\(^{-1}\) day\(^{-1}\).

In conclusion, FB exposure was associated with growth impairment. Further research is needed to examine whether a threshold dose of multi-mycotoxin exposure exists that could result in child growth impairment.

KEYWORDS: mycotoxin exposure, undernutrition, rural, infant growth and Eastern Cape
6.1 Introduction

Children who are poorly nourished between 13-24 months of age are at increased risk of impaired development, poor growth, and could later in life experience diminished work capacity and chronic diseases (Dewey & Begum, 2011). Moreover, many children in subsistence farming areas in sub-Saharan Africa are exposed to mycotoxin-contaminated complementary foods and these mycotoxins have been associated with poor nutrition intake (Smith et al., 2012). In South Africa (SA), the Eastern Cape (EC) is characterised by the production of home-grown maize known to be highly contaminated by mycotoxins such as fumonisins (FB) in addition to lower levels of deoxynivalenol (DON) and zearalenone (ZEA) (Shephard et al., 2013). Mycotoxins are toxic metabolites produced by fungi and may result in adverse health effects in humans (Zain et al., 2011). The 74th meeting of the Joint Food and Agricultural Organisation and the World Health Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA) determined a provisional maximum tolerable daily intake (PMTDI) for FB of 2 µg kg\(^{-1}\) bw day\(^{-1}\) (JEFCA, 2012). The PMTDIs for DON and ZEA are 1 µg kg\(^{-1}\) bw day\(^{-1}\) and 0.5 µg kg\(^{-1}\) bw day\(^{-1}\) respectively (JECFA, 2001 and 2002).

In rural EC, children mainly consume thin maize porridge contaminated by multiple mycotoxins (submitted article, Tshalibe et al., 2019\(^a\)). These children also have short breastfeeding periods of not more than 12 months (unpublished data, Tshalibe et al., 2019\(^b\)). With the early introduction of complementary foods prepared from home-grown maize, exposure to mycotoxins are increased and may pose a risk of growth impairment. Exposure to mycotoxins such as FB above the PMDTI, have been associated with impaired child growth (Smith et al., 2012; unpublished data, Tshalibe et al., 2019\(^b\)).

Previous studies on child mycotoxin exposures (Aflatoxin (AF) and FB) and growth impairment risk have been conducted worldwide. In Bhaktapur, Nepal, a study among children aged 15, 24 and 36 months, (n = 85) showed that chronic exposure to AF was not significantly associated with weight – for - age (WAZ), length – for - age (LAZ) and weight – for - length (WLZ) scores (Mitchell et al., 2017). However, in Benin, West Africa, amongst children aged 16-37 months, (n = 200), a strong negative correlation (p = 0.0001) between length and AF-alb was observed (Gong et al., 2003). Furthermore, in Tanzania, children at 12 months of age, (n = 215) consuming FB above the PMDTI were reported to be shorter by 1.3 cm and 328 g lighter, these results reported that high FB exposure was significantly associated with growth impairment (Kimanya et al., 2010). In EC infants younger than 12 months were significantly shorter by approximately 4 cm and LAZ was observed to be significantly associated with high FB exposure, (n = 51) (unpublished data, Tshalibe et al., 2019\(^b\)).
This study explores the relationship between multi-mycotoxin exposure (FB, DON and ZEA) and child growth (13 - 24 months of age) within the EC.

6.2 Methods

6.2.1 Study design

This study was conducted in the form of a longitudinal study on young child feeding as part of the PhilaSana project. Children aged 13 - 24 months, were recruited from various villages in Centane, Amatole District Municipality in the EC. Any children who do not reside permanently in the area were excluded. Snowball sampling was utilised to sample mothers with infants and young children below the age of 24 months due to low population density and inaccessible road infrastructure. Systematic sampling was also used as the mothers were supposed to be visited while their children were 13 – 24 months of age. In addition to child feeding practices, health status and anthropometry, household socio-economics, as well as the employment status and education level of mothers/caregivers were obtained.

Ethical approval for the study was obtained from the Human Research Ethics Committee of North-West University (Potchefstroom Campus), South Africa (NWU-00207-14-S1). The study was conducted according to the Helsinki declaration (World Medical Association, 2013) and the International Conference on Harmonisation and Metapopulation Research Centre guidelines (ICH steering committee, 1996). Signed informed consent was obtained after goodwill permission and getting authorisation from the community leaders to carry out the study.

6.2.2 Anthropometric measures

Date of birth, gender and birth anthropometric (length, weight, head circumference (HC), mid-upper arm circumference (MUAC) and gestational age information was obtained from the road to health cards. Trained research assistants conducted anthropometric assessments of the children. Measurements were taken according to WHO and UNICEF Standards (available at http: //www. who.int/child growth/training/en). Means of two measurements (weight and length) were taken, as well as a third measurement if there was a deviation of more than 0.5 cm, for length and 0.5 kg for weight. Weight was measured to the nearest 0.1 kg with a calibrated portable scientific scale (Seca, 334). Length was measured with a baby length - measuring mat.

Anthropometric data was captured into the WHO Anthroplus version 1.0.4 (2007), (www.who.int/childgrowth/software/en, 2011). Growth was determined using SD from the
WHO Z-scores (Onis, 2006). Children with a Z-score between -2 and -3 SD LAZ were classified as moderately stunted and those ≤ -3 SD as severely stunted. Children with WLZ or WAZ -2 SD < -3 SD were considered moderately wasted and underweight respectively. Children with an SD ≤ -3 SD for WAZ and WLZ were classified as severely underweight and wasted. However, children with a z-score above 4 or below -4 SD were excluded due to possible measurement errors.

6.2.3 Maize intake

Trained interviewers collected child food consumption information from the mothers / caregivers. The RAPP (Ratio and Portion Size Photo) tool, a validated culturally specific dietary assessment method determining habitual maize intake of people living in rural areas in the EC was utilised (Lombard et al., 2014). This tool consists of a semi-quantitative food frequency questionnaire (FFQ) and portion size photographs. It was adapted for young children’s intakes based on 150 24-hour recalls (young children 0 - 24 months). Three food portion size photographs per dish were developed and validated (Lombard et al., 2013). The RAPP tool is designed to determine intake for the past month and converted to a daily average consumption of each food item or dish consumed (Lombard et al., 2014). Amount of maize consumed in a day was determined by the portion size (in grams) multiplied by the number of portions consumed in a day. Monthly intake was then determined by consumption frequency per week or per month multiplied by intake at a time. Thereafter, monthly intake was divided by 28 days to give a mean daily intake of cooked maize meal (unpublished data, Tshalibe et al., 2019b).

Mean daily intake of consumed maize was then converted to raw maize according to recipes obtained during the development of the tool (Lombard et al., 2014).

6.2.4 Mycotoxin exposure

Risk of mycotoxin exposure of children was determined according to maize contamination levels. Total FB (FB1+ FB2 + FB3) DON and ZEA levels were obtained from the analysis of home-grown maize collected from Centane with mean contamination levels of 1035.0 µgkg⁻¹ for total FB, for DON: 24.5 µgkg⁻¹ and 31.0 µgkg⁻¹ for ZEA (submitted article, Tshalibe et al., 2019a). Lastly, the deterministic method was utilised to determine mycotoxin exposure and expressed as a probable daily intake (PDI). Thereafter, the children were grouped according to exposure categories, “low exposure” (PDI < PMTDI), and “high exposure” (PDI ≥ PMTDI) for each mycotoxin.
6.2.5 Statistical analysis

Data were captured and cleaned in Excel. Statistical analysis was conducted with Statistical Package for Social Sciences Software (SPSS) version 25. Data were tested for normality using Shapiro-Wilk Test. The mycotoxin data were not normally distributed, and thus log-transformed for statistical analysis.

Individual child multi-mycotoxin exposure was compared with the child’s growth parameters, using linear regression, (current length, current weight, and current WAZ (SD), WLZ (SD), and LAZ (SD) z-scores as well change in WAZ, LAZ and WLZ. Change in Z-score was calculated to determine if one group had a slower growth rate (indicating a trend towards stunting or wasting) than another. Changes in z-scores were calculated as current z-score (24 months) – previous z-score (13 months).

Quantitative data included means / geometric mean, SD, medians; minimum and maximum ranges were used as descriptive measures. Children were subdivided according to their exposure levels. Independent t-tests were used to detect statistically significant differences between the different groups. ANCOVA, with adjustment of covariates (age, gender and HIV status of child) were also utilised to detect statistical differences between the different groups. Linear regression was performed to determine whether there was an association between mycotoxin exposure and change in z-scores of the children. Lastly, children were categorised according to mycotoxin exposure above its relevant PMTDI.

6.3 Results

6.3.1 Participants

A sample of 70 children, (34%, n = 24 females) were included in the study. Most children were 13 - 17 months old (59%, n = 41). Maternal/ caregiver education indicated that, 17% (n = 12) had no formal education, 16% (n = 11) attained grade 1 - 5, while the majority, 66% finished grade 8 - 12 (n = 46) and only 1% (n = 1) had tertiary education. A large percentage of the mothers/ caregivers, 69% (n = 48) received child support grants. Furthermore, approximately half of the households 54% (n = 37), obtained water from the river, while 29% (n = 20) used communal tap water and 17% (n = 13), had their own tank. Fifty-eight percent (n = 40) used pit toilets, while 38% (n = 26) used the bush and only 4% (n = 3). A high percentage of the mothers / caregivers were unemployed, 96% (n = 67) with the local hotel being the only source of work in the study area.
6.3.2 Birth information of the children

Birth weight information was available for 57 children (81%). Two of the children had a birth weight < 2500 g. Birth length information was available for 57 children, 13% (n = 7) of these children had their birth length (BL) below the normal average, WHO world standards of healthy children. Missing values of birth information were due to the large number of home births. The gestational age of 9% (n = 5) was below 38 weeks and thus regarded as premature.

6.3.3 Anthropometric information of the children

A high percentage of the children were stunted 34% (n = 24). None of the children in this age group were wasted or underweight.

6.3.4 Maize intake

Maize based foods consumed by children included soft porridge, *maheu* (fermented maize-meal drink) and crumbly maize meal. The daily cooked maize consumption ranged from 1.6 to 321.0 g per child day$^{-1}$, with a mean intake of 97 g day$^{-1}$, some of these young children were still receiving breast milk in addition to complementary food.

6.3.5 Mycotoxin exposure

The geometric mean exposure levels (PDIs) for FB, DON and ZEA all higher than their PMDTIs. Of the 70 included children, only 14% (n = 10) were in the low exposure (0 – 2 µgkg$^{-1}$ bw day$^{-1}$), and 86 % (n = 60) in the high (≥ 2 µgkg$^{-1}$ bw day$^{-1}$) FB group. Table 1 presents the medians and the geometric mean exposures to FB, DON and ZEA.

Table 1 Medians and geometric mean of mycotoxin exposure

<table>
<thead>
<tr>
<th>Mycotoxin (PDI) (n = 70)</th>
<th>Median IQR (25th - 75th)</th>
<th>Geometric Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total FB</td>
<td>7.5 (4.3 – 12.6)</td>
<td>7.8 (2.1)</td>
</tr>
<tr>
<td>DON</td>
<td>0.2 (0.1 - 0.3)</td>
<td>1.2 (1.1)</td>
</tr>
<tr>
<td>ZEA</td>
<td>0.2 (0.1 – 0.4)</td>
<td>1.3 (1.2)</td>
</tr>
</tbody>
</table>

FB = fumonisins, DON = deoxynivanenol, ZEA = zearalenone

PDI = Probable daily intake

PMDTI = Provisional maximum tolerable daily intake, FB = 2 µgkg$^{-1}$ bw day$^{-1}$, DON = 1 µgkg$^{-1}$ bw day$^{-1}$, ZEA = 0.5 µgkg$^{-1}$ bw day$^{-1}$

Probable daily intake, in µgkg$^{-1}$ bw day$^{-1}$
6.3.6 Mycotoxin exposure and growth of children

No significant differences were observed between the birth outcomes of children in the high FB and ZEA group, in comparison to those in the low FB and ZEA groups, therefore there was no growth impairment due to mycotoxin exposure in utero (Table 2).

No significant differences in growth parameters were observed between children with the high FB and ZEA exposure group as compared to those within the low FB and ZEA exposed groups. Based on the results and analysis performed, DON was excluded since there were no children within the high exposure group. Slight differences in parameters such as length and LAZ were observed among children in the high FB exposure group as compared to those in the low exposure groups. Whereas children in the high ZEA exposure group, showed a slight difference in weight, length, and LAZ as compared to the low exposure group, however, this was not significant (Table 3).

Table 2 Growth parameters of infants exposed to low and high mycotoxin levels at birth

<table>
<thead>
<tr>
<th>Birth outcome</th>
<th>Total FB</th>
<th>ZEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; PMDTI</td>
<td>&gt;PMDTI</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>3.0 (0.3)</td>
<td>3.2 (0.6)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>48.7 (2.9)</td>
<td>48.5 (4.3)</td>
</tr>
<tr>
<td>WLZ</td>
<td>-1.6 (0.3)</td>
<td>-1.0 (2.5)</td>
</tr>
<tr>
<td>LAZ</td>
<td>0.2 (0.4)</td>
<td>-0.2 (1.7)</td>
</tr>
<tr>
<td>WAZ</td>
<td>-0.7 (0.6)</td>
<td>-0.3 (1.21)</td>
</tr>
</tbody>
</table>

FB = fumonisins, ZEA = zearalenone
WLZ = Weight-for-Length Z-score, LAZ = Length-for-Age Z-score, WAZ = Weight-for-Age Z-score
P value =0.05

Figure 1 shows that the second largest group of the children are stunted and exposed to FB above the PMDTI. Furthermore, the pie chart shows that the least number of children are not stunted and not exposed to FB above the PMTDI.
Table 3 Growth indicators of infants with different exposure levels

<table>
<thead>
<tr>
<th>Growth Indicators</th>
<th>Low FB</th>
<th>High FB</th>
<th>Low ZEA</th>
<th>High ZEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
<td>n</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>10</td>
<td>10.2 (1.2)</td>
<td>60</td>
<td>10.9 (1.4)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>10</td>
<td>76.0 (4.5)</td>
<td>60</td>
<td>77.4 (4.5)</td>
</tr>
<tr>
<td>WAZ</td>
<td>10</td>
<td>0.1 (1.2)</td>
<td>60</td>
<td>0.2 (0.8)</td>
</tr>
<tr>
<td>WLZ</td>
<td>10</td>
<td>0.6 (1.1)</td>
<td>60</td>
<td>1.1 (1.1)</td>
</tr>
<tr>
<td>LAZ</td>
<td>10</td>
<td>-1.0 (1.3)</td>
<td>60</td>
<td>-1.3 (1.4)</td>
</tr>
</tbody>
</table>

**WAZ** = Weight-for-age Z-score, **WLZ** = Weight-for-length Z-score, **LAZ** = Length-for-age Z-score

P value=0.05

Data has been adjusted for confounding factors, including age, gender and HIV and is reported in Table 4. No significant statistical differences in growth parameters of children within the high FB and ZEA exposure as compared to the children not exposed.
Table 4 Statistical differences in growth parameters of children with different exposure levels

<table>
<thead>
<tr>
<th>Growth Indicators</th>
<th>Low FB</th>
<th>High FB</th>
<th>Low ZEA</th>
<th>High ZEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
<td>n</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>6</td>
<td>10.8 (1.4)</td>
<td>32</td>
<td>10.5 (1.2)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>6</td>
<td>77.2 (4.9)</td>
<td>32</td>
<td>77.3 (4.6)</td>
</tr>
<tr>
<td>WAZ</td>
<td>6</td>
<td>0.3 (1.2)</td>
<td>32</td>
<td>0.1 (0.75)</td>
</tr>
<tr>
<td>WLZ</td>
<td>6</td>
<td>1.0 (1.1)</td>
<td>32</td>
<td>0.8 (0.8)</td>
</tr>
<tr>
<td>LAZ</td>
<td>6</td>
<td>1.0 (1.2)</td>
<td>32</td>
<td>-1.0 (1.2)</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, HIV, of mycotoxin exposure in relation to growth

WAZ = Weight – for - age Z-score, WLZ = Weight - for-length Z-score, LAZ = Length – for - age Z-score

Table 5 shows independent t-test results for Z-score changes from 13 – 24 month Z-scores of the low and high exposed groups. No significant changes in Z-scores were observed between the high and low FB and ZEA exposure groups.

Table 5 Independent t-test results for Z-score changes from 13 – 24 month Z-scores of the low and high exposed groups

<table>
<thead>
<tr>
<th>Growth</th>
<th>Low FB</th>
<th>High FB</th>
<th>Low ZEA</th>
<th>High ZEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
<td>n</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>WLZ change</td>
<td>5</td>
<td>2.8 (3.0)</td>
<td>49</td>
<td>1.9 (2.4)</td>
</tr>
<tr>
<td>LAZ change</td>
<td>5</td>
<td>-1.2 (4.1)</td>
<td>49</td>
<td>-1.0 (2.3)</td>
</tr>
<tr>
<td>WAZ change</td>
<td>8</td>
<td>-0.1 (1.0)</td>
<td>54</td>
<td>0.5 (1.4)</td>
</tr>
</tbody>
</table>

WLZ = Weight-for-length Z-score, LAZ = Length-for-age Z-score, WAZ = Weight-for-age Z-scores
Change in Z-score data has been adjusted for confounding factors, including age, gender and HIV and is reported in Table 6 below. Means, SD and ANCOVA results were adjusted in relation to mycotoxin exposure and change in growth. Changes in Z-scores were calculated as current Z-score the Z-score at 13 - 24 months. A statistically significant difference in both WLZ change and LAZ changes with high FB exposure was observed. Notably, FB and ZEA high exposure resulted in a negative LAZ change whereas there was a slight difference in WAZ change because of high FB exposure, although these changes were not statistically significant.

Table 6 Adjusted data of Z-score changes from birth to 12 months for those exposed and not exposed

<table>
<thead>
<tr>
<th>Growth</th>
<th>Low FB</th>
<th>High FB</th>
<th>Low ZEA</th>
<th>High ZEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
<td>N</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>WLZ change</td>
<td>2</td>
<td>4.2 (5.0)</td>
<td>27</td>
<td><strong>1.4 (2.4)</strong> *</td>
</tr>
<tr>
<td>LAZ change</td>
<td>2</td>
<td>1.9 (5.7)</td>
<td>27</td>
<td><strong>0.7 (1.4)</strong> *</td>
</tr>
<tr>
<td>WAZ change</td>
<td>4</td>
<td>-0.2 (1.1)</td>
<td>34</td>
<td>0.4 (1.4)</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, HIV, of mycotoxin exposure in relation to change in growth

WLZ = Weight – for - length Z-score, LAZ = Length – for - age Z-score, WAZ = Weight – for - age Z-scores

* P value < 0.05, ** P value < 0.01, *** P value < 0.001

6.4 Discussion

This study explored the risk of mycotoxin exposure of children 13 - 24 months in relation to their growth status. High mycotoxin exposure is thought to to reduce child growth due to the co-occurrence of stunting and high mycotoxin concentrations in home-grown maize from rural areas in the EC. Currently very little is documented about the role of mycotoxin exposure in the development of undernutrition among rural children. Unpublished data indicated that amongst infants 0 - 12 months in EC, length and LAZ, as well as WAZ, were significantly associated with high FB exposure (unpublished data, Tshalibe et al., 2019 b).

This study observed high stunting rates of approximately 34%, although none of the children were wasted or underweight. In contrast Shisana et al., 2013, South African national report obtained lower percentages of stunted children in EC. However, Shisana et al., 2013 observed wasting and underweight rates slightly above one percent (Shisana et al., 2013). Lower
stunting rates of 16% were obtained amongst infants 0 - 12 months of age (unpublished data, Tshalibe et al., 2019 b). These lower stunting rates in the 0 - 12 age group are expected, as stunting occurs over time.

High raw maize intake levels of approximately 100 g day\(^{-1}\) higher were observed in the current study in comparison with other studies such as those from Tanzania. The maize intake consumed per day in this study was 1.6 – 321 g day\(^{-1}\). Lower maize flour consumption of 16 - 254 g day\(^{-1}\) was observed amongst children 18 - 24 months of age in Kikelewa village, Tanzania (n = 41) (Kimanya et al., 2014). In contrast to this study, lower mean maize intake of 12.1, 8.9 and 7.9 g/kg b.w amongst children 12-22 months from Nyabula, Kigwa and Kikelewa districts in Tanzania were observed (Shrima et al., 2013). The dietary assessment method utilised was the 24-hour recall, in contrast to the QFFQ utilised in the current study (Shrima et al., 2013). However, this study utilised a validated QFFQ, specifically for people residing in EC (Lombard et al., 2013; Lombard et al, 2014).

The geometric mean exposure to FB obtained in this study was three and a half times above the PMDTI of 2 µgkg\(^{-1}\) bw day\(^{-1}\) (JEFCA, 2012). Lower PDI's were obtained in Tanzania among children aged 18 - 24 months of age ranging 0.19 - 26.37 µgkg\(^{-1}\) bw day\(^{-1}\) (Kimanya et al., 2014). The mean and median for FB obtained in this study were higher than the geometric mean of 6.3 µgkg\(^{-1}\) bw day\(^{-1}\) and median of 4.4 µgkg\(^{-1}\) bw day\(^{-1}\) amongst infants 0 - 12 months of age from a previous study in this area (unpublished data, Tshalibe et al., 2019 b). As children get older, their portion sizes increase, and thus chronic exposure increases, especially if they have a limited dietary diversity.

Results from this study indicated that the geometric mean for DON and ZEA were also above their PMDTI’s of 1 µgkg\(^{-1}\) bw day\(^{-1}\) and 0.5 µgkg\(^{-1}\) bw day\(^{-1}\) (JEFCA, 2001, JEFCA, 2002). Similar results were obtained in the earlier study of infants 0 - 12 study, the DON and ZEA PMDTIs were also above their PMDTs (unpublished data, Tshalibe et al., 2019 b). While 70% of the children were exposed to FB above the PMDTI in this study, the FB percentage exposure is bound to increase beyond 24 months when the children consumed larger amounts of complementary food.

Twenty six percent of the children exposed to FB above the PMDTI were stunted, confirming that exposure above the PMDTI contributes to stunting. In contrast to the 0 - 12-month group, children exposed to high FB did not reflect a shorter length in comparison to the low FB exposure group, instead, the length of the high exposed children was slightly higher after adjusting for confounding factors. The results of this study are in contrast with what was obtained in the previous study amongst infants 0 - 12 months of age, where infants exposed
to high FB were shorter by approximately 4 cm (unpublished data, Tshalibe et al., 2019b). A study conducted in Tanzania showed that high FB exposed infants were shorter by 1.3 cm. (Kimanya et al., 2010). No association between length, current Z-scores and high mycotoxin exposure were shown, as opposed to the previous 0 - 12-month study (unpublished data, Tshalibe et al., 2019b). Although a smaller percentage of children were exposed above the PMTDI for ZEA (high exposure group) and no children were in the high DON exposure group, a chronic low level of exposure is still anticipated due to their maize-based staple diet. The exposure to high ZEA was not associated with current growth indicators and these findings are similar to what Mitchell et al., 2017 obtained for DON, after adjusting for covariates (education, age, energy adjusted zinc, iron, vitamin A). This study was however not able to include energy intake, zinc, iron and vitamin A as confounding factors. Therefore, FB remains the critical mycotoxin of concern in contributing to growth impairment, to date.

Lower weight difference amongst high FB exposed children was obtained in this study as compared to a study by Kimanya et al., (2010). Kimanya et al., 2010 reported that 26 infants above 12 months consuming FB above the PMDTI were lighter by 328 g (Kimanya et al., 2010). Children in the current study were lighter by about 0.3 g, with high FB exposure. The slight difference in the weight of children within the low and high exposed groups in the current study is expected. The differences in the weight of the children within the high and low exposure groups might have been attributed by differences in children (in this sample) as well as diet differences. This weight finding is also in support of what was obtained previously in the 0 - 12 months age group (unpublished data, Tshalibe et al., 2019b). In contrast Chen et al., 2018 in Haydom, Tanzania, found that FB exposure was associated with underweight (p < 0.0053), amongst children less than 36 months. Similarly, the 0 - 12 months study also concluded that the FB exposure was associated with underweight (unpublished data).

However, results from this study indicated a linear association of FB, DON and ZEA exposure and weight gain g kg⁻¹day⁻¹, an indication that the children were picking up weight with higher maize consumption (p < 0.05). However, of significance, is the fact that high exposure to FB was found to be statistically associated with growth velocity (WLZ and LAZ) changes. These results indicate that the children’s growth impairment due to high FB exposure was evident at (p-value < 0.05). However, these results must be interpreted with caution, considering the small sample size. Current LAZ scores also shifted more towards the negative z-scores with high mycotoxin exposure. Furthermore, the children in this age group had a negative mean LAZ, as compared to the 0 - 12-month group (unpublished data, Tshalibe et al., 2019b), indicating the development of stunting. More alarming is that, the children were not growing well as denoted by negative LAZ change, in addition to not picking up weight in terms of length.
as demonstrated by the decrease in WLZ change of children within the high FB exposure group. Values of LAZ changes and WLZ changes of children within the high exposed group were statistically different from those within the low FB exposure group, p-values less than 0.05. Furthermore, a substantial number of children in the current study exposed to FB above the PMDTI were also stunted. These results are in support of what Chen et al., 2018 observed in Tanzania, where it was concluded that there was a dose-effect response, greater FB exposure results in increased stunting risk. Findings from this study, support evidence regarding the potential mechanistic effect of FB based on experimental studies, suggesting that FB could affect growth impairment (Smith et al., 2012).

Fumonisin B may induce intestinal enteropathy, a subclinical condition of the small intestine, characterised by reduced absorptive capacity and increased intestinal permeability, therefore mediating stunting (Smith et al., 2012). This study accounted for some possible confounding factors such as HIV of children, gender, birth information and age of children and showed that FB has a direct effect on chronic health or stunting and wasting, but not underweight. These findings suggest that FB exposure plays a pivotal role in growth impairment in the long term.

It is therefore critical that further in-depth research is done to investigate the effect of multiple mycotoxins on child growth and development and the associated mechanisms. Furthermore, since data was only adjusted for a small number of confounding factors, it is important that further research is conducted to determine the association between other factors such as energy and protein intake, and malnutrition.

6.5 Conclusions

Mycotoxin exposure (FB) may be a contributing factor of growth impairment amongst children 13 - 24 months of age in EC. There was an association between high FB exposure and growth changes of the children. Reduced weight gain was also associated with high FB, DON and ZEA exposure. A follow up study tracking the growth of children within the different age groups in relation to the mycotoxin exposure is of paramount importance. Growth impairment also occurs over a longer period; hence these children may be affected to a greater extent as they grow.

6.6 Conflict of interest

The authors declare no conflict of interest.
6.7  Author contribution

Miss Tshalibe contributed towards study design and data collection, statistical analysis and quality control of questionnaires. She was also primarily responsible for article writing. Dr Burger was involved in study design and data collection. She was also involved in sourcing of funding and article compilation. Dr Taljaard-Krugell was involved in article compilation. Prof Gelderblom and Dr Shephard contributed to data interpretation and writing of the paper. Dr Lombard contributed to study design, funding and data management. She also assisted in article compilation and statistical analysis.

6.8  Acknowledgements

The authors would like to acknowledge the cooperation of the participants who made this study successful. The field workers played a pivotal role in data collection.

6.9  Funding

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Role of funding sources  The funding sources were not involved at all in the project design, data collection, result analysis, interpretation or conclusions.
6.10 References


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CHAPTER 7: OVERALL DISCUSSION AND CONCLUSIONS:
MYCOTOXIN EXPOSURE AND CHANGE IN GROWTH OVER 24 MONTHS

The following chapter is a concluding chapter and therefore is not in an article format like the previous chapters. The chapter explains how growth and mycotoxin exposure changed within the four six monthly time points. It focuses on objective 4 of the sub-study.
7.1 Introduction

A major component of child care is the set of practices caregivers employ to provide complementary foods to children in their first twenty-four months of life (PAHO/WHO, 2003). The mothers of the children in Eastern Cape (EC) must ensure that they are administering complementary food which is safe to the children to curb mycotoxin exposure. If the complementary food is prepared from mycotoxin contaminated grains or cereals, children will be more prone to growth impairment. Households in Centene, EC are maize subsistent farmers and the complementary food prepared for the young children is in the form of soft porridge and crumbly maize-meal (submitted article, Tshalibe et al., 2019a). However, the maize produced by these subsistence farmers is often stored in open granaries encouraging mould growth and thereafter toxins termed mycotoxins are produced. There has been a notion that these mycotoxins may result in growth impairment due to increased intestinal permeability and reduced uptake of nutrients as observed in animal studies (Smith et al., 2012a). The incidence of growth impairment increases within the second year and may increase/continue? further thereafter (unpublished data, Tshalibe et al., 2019c). However exclusive breastfeeding remains the safest and nutritious approach way beyond twenty-four months of age (Dewey, 2001). The aim of this chapter was to determine whether the growth rate of infants and young children changes within six monthly intervals over a period of twelve months. The chapter will give a clear picture of how low and high mycotoxin exposure changes within six monthly time intervals.

7.2 Methods

7.2.1 Study population

This study was a longitudinal study design, using systematic and snowball sampling as recruitment. One hundred and twenty children (0 - 24 months of age), consuming home-grown maize and breast milk or formula milk at various villages in the Amatole District in the Eastern Cape (EC) were recruited between January 2015 and December 2016. Inclusion criteria for participation included mothers planning to remain in the study area for at least six months after enrolment. Information on the date of birth of the infants was obtained from their road to health booklet and recorded. Trained interviewers obtained information about the child namely food consumption, infant feeding practices and general health status, from their mothers or caregivers.
7.2.2 Anthropometry

Trained research assistants conducted anthropometric assessments of the children. The anthropometric measurements were obtained six monthly in a follow-up study. The follow up visits were done while the infants and young children were in (0 - 6), (7 - 12), (13 - 17) and (18 – 24) months age ranges. Measurements were conducted according to WHO and United Nations International Children’s Emergency Fund Standards (available at http://www.who.int/child growth/training/en). An average of two measurements were taken, as well as a third measurement if the two measurements deviated by >0.5cm. Anthropometric data was captured into the WHO Anthroplus version 1.0.4 (2007), for analysis (www.who.int/childgrowth/software/en, 2011). Under-nutrition was determined using SD from the Z scores (Onis, 2006). Thus, children with a Z score below -2SD or underweight, stunting/wasting below -2SD were classified as moderately or severely malnourished. Length - for - age Z scores (stunting), weight - for - length (wasting) and weight - for – age (underweight) were determined from the anthropometric data obtained.

Trained research assistants conducted anthropometric assessments of the young children. Measurements were conducted according to WHO and United Nations International Children’s Emergency Fund Standards (available at http://www.who.int/child growth/training/en). An average of two measurements (weight, length, head circumference (HC) and mid-upper arm circumference (MUAC) were taken, as well as a third measurement if the two measurements deviated by more than 0.5cm. Weight was measured to the nearest 0.5 kg with a portable scientific scale (Seca, 334). Length was measured with a baby length-measuring mat. Mid-upper arm circumference and head circumference measured to the nearest 0.1 cm was measured with a MUAC band and HC tape (Seca, 212). Anthropometric data was captured into the WHO Anthroplus version 1.0.4 (2007), for analysis (www.who.int/childgrowth/software/en, 2011). Under-nutrition was determined using SD from the WHO Z- scores (Onis, 2006). Young children with a Z- score between -2 and -3 SD LAZ were classified as moderately stunted and those below -3 SD were severely stunted. Young children with WLZ <-2 SD and > -3 SD were considered moderately underweight and below -3 SD severely wasted.

7.2.3 Dietary assessments

Dietary maize consumption information was collected using a validated dietary assessment method. The Ratio and Portion Size Photo (RAPP) tool which is a culturally specific dietary assessment method that determines the habitual dietary intake of Xhosa individuals living in this area was utilised (Lombard et al., 2013). It consists of life size photographic pictures of
the types of foods mostly consumed and a semi-quantitative food frequency questionnaire, on
the dietary intake of the children for the past month (Lombard et al., 2014). The RAPP tool
focused on cooked maize dishes. Amount of cooked maize consumed at a time was
determined by the portion size (in grams) multiplied by the number of portions consumed a
day. Monthly intake was determined by consumption frequency per week or per month
multiplied by intake at a time. Monthly intake was divided by 28 days to give a mean daily
intake of cooked maize meal (Lombard et al., 2014). Mean daily intake of cooked maize was
converted to raw maize according to recipes obtained during the development of the RAPP
tool (Lombard et al., 2014).

7.2.4 Mycotoxin exposure

The mean total DON, FB and ZEA were obtained from the analysis of ± 2 kg homegrown (HG)
maize collected from each household with available home-grown maize from an earlier study
in the same area (Submitted article, Tshalibe et al/2019 a). The mycotoxin concentration is the
amount of mycotoxin on one kg of raw maize. The following concentrations were shown:
1035.0 µg kg⁻¹, DON 24.5 µg kg⁻¹ and ZEA 31.0 µg kg⁻¹ respectively. Lastly, the deterministic
method was utilised to determine mycotoxin exposure in an earlier study (unpublished data,
Tshalibe et al., 2019a). Mycotoxin exposure is measured as individual probable daily intakes
(PDIs) are calculated by multiplying total daily raw maize intake (g/day) with mycotoxin
concentration (µg/kg) divided by body weight (kg). The provisional maximum tolerable daily
intakes (PMTDIs) for each mycotoxin was utilised to indicate high exposure (PD1 > PMTDI).
The 56th meeting of the Joint Food and Agricultural Organisation and the World Health
Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA), provided a No
Observed Adverse Effect Level (NOAEL) of 0.2 mg kg⁻¹ body weight day⁻¹ and a safety factor
of 100, as a group PMTDI for FB, alone or in combination, of 2 µg kg⁻¹ body weight day⁻¹
(JECFA, 2012). The PMTDI for DON is 1 µg kg⁻¹ body weight day⁻¹ and ZEA is 0.5 µg kg⁻¹ body
weight day⁻¹ (JECFA, 2001, JEFCA, 2002).

7.2.5 Statistical analysis

Shapiro-Wilks’ test indicated that the mycotoxin data was not normally distributed. Descriptive
data included means, standard deviation, and 95% Confidence Intervals were presented in
tables and graphs.

Two categories were utilised to analyse the data, “low exposure” (< PMTDI) and “high
exposure” (> PMTDI).
Ethical approval for the study was obtained from the Human Research Ethics Committee of North-West University (Potchefstroom Campus), South Africa (NWU-00207-14-S1). The study was carried out according to the Helsinki declaration (World Medical Association declaration of Helsinki, 1964), and the International Conference on Harmonisation and Metapopulation Research Centre guidelines. Thereafter the mothers signed written informed consent.

7.3 Results

One-hundred and twenty young children consuming home-grown maize and breast milk, or formula were included in this study. Of the included children n = 4 were within the 0 - 6 age group, n = 46 were aged 7 - 12 months, n = 40 were aged 13 to 18 months and n = 30 were in the 19 - 24-month age group. The infant and young children were then split into different Z-score categories. Only data for the undernourished children was reported. The stunting, wasting and underweight of the young children in the different age groups are shown in Table 1. Table 1 shows data of only young children below -2 Z-scores (stunted, wasted and underweight). Table 1 below shows data of only young children below -2 Z-scores with the 0 - 24 age group. The number of infants and young children with the 0-24-month age group below -2 Z-scores were stunted (n = 31, 24%), wasted (n = 0, 0%) and underweight (n = 0, 0%).

Table 1 Growth parameters of the children in the different age groups, only young children below -2 z-scores

<table>
<thead>
<tr>
<th>Age category (months)</th>
<th>Stunting mean (SD)</th>
<th>Stunted n (%)</th>
<th>Wasting mean (SD)</th>
<th>Wasted n (%)</th>
<th>Underweight mean (SD)</th>
<th>Underweight n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6</td>
<td>-0.5 (2.8)</td>
<td>2 (50)</td>
<td>1.3 (1.03)</td>
<td>0 (0)</td>
<td>0.8 (1.40)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>7-12</td>
<td>-1.5 (1.5)</td>
<td>6 (13)</td>
<td>0.9 (0.95)</td>
<td>0 (0)</td>
<td>0.5 (0.98)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>13-18</td>
<td>-1.1 (1.4)</td>
<td>11 (28)</td>
<td>1.0 (1.01)</td>
<td>0 (0)</td>
<td>0.3 (0.93)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>19-24</td>
<td>-1.4 (1.3)</td>
<td>12 (41)</td>
<td>1.02 (1.2)</td>
<td>0 (0)</td>
<td>0.2 (0.90)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Stunted= LAZ < -2 (SD), Wasted= WLZ < -2 (SD), Underweight= WAZ < -2 (SD)
Figure 1, 2 and 3 show the number of young children exposed to FB, DON and ZEA below and above the PMDTI in the different age groups. The 7 - 12-month age group had the highest number of young children above the PMDTI for FB exposure. While the 0 - 6-month age group had the least number of young children above the PMDTI for FB exposure. None of the children were exposed to DON above the PMDTI as shown in figure 2.

Figure 1 Number of children exposed to FB

PMDTI = Provisional Maximum Tolerable Daily intake

Figure 2 Number of children exposed to different levels of DON
Figure 3 Number of children exposed to different levels of ZEA

PMDTI = Provisional Maximum Tolerable Daily Intake

7.4 Discussion

In Nepal 85 children less than 36 months of age were followed. The prevalence of stunting in the 9-11 month age group was 14%, which is similar to the 7 - 12-month age group (13%) from this study (Mitchell et al., 2017). The prevalence of stunting within the 0 - 6, 13 - 18 and 19 - 24-month group were higher compared to the previous results in Eastern Cape (EC) (Shisana et al., 2013). The South African National Examination Survey (SANHANES-1) reported that 26.9% (n = 137) of boys below the age of 36 months were stunted (< -2 SD LAZ) of which 9.9% were severely stunted (< -3 SD LAZ) (Shisana et al., 2013). Of the girls 25.9% (n = 143) were stunted (-2 SD LAZ), of which 9.1% (n = 26) were severely stunted (Shisana et al., 2013). Although the sample size in this study was slightly smaller than that included by Shisana et al., 2013.

Data obtained in our study suggests that stunting increases with age; stunting rate among children 13 - 18 months was reported to be 28 % and 19 - 24 months age group had a rate of 41 %. The mean LAZ score of -1.4 obtained in this study amongst young children 19-24 months is slightly higher to what was obtained from young children at 24 months of age (Mitchell et al., 2017). However, the mean LAZ from the current study was lower than the mean of -2.5 obtained at 24 months of age in Tanzania (Chen et al., 2018).
Childhood stunting is associated with an elevated risk of child morbidity and mortality (Black et al., 2004) with approximately, 8 - 10 million children deaths occurring globally in children under five years annually, with 90 % occurring in low- and middle-income countries (LMIC) (Jones et al., 2003). One thousand one hundred and eighty eight South African under-fives died from acute malnutrition in 2016/17 (Massyn et al., 2017). Furthermore, a large percentage, 226 (10.2%), of the children who died from severe acute malnutrition were from the EC (Massyn et al., 2017). The results obtained in this study are similar to what was previously stated that between the ages of 6 - 24 months of age stunting is at its peak in LMIC due to the quality of the complementary foods (Dewey & Adu-Afarwuah, 2008).

The stunting rates in our study might have been lower than the actual stunting rates as the area is sparsely populated and some places were inaccessible due to the road infrastructure. The number of loss to follow-up children impacted on the sample size as well as the statistical power of the results obtained.

Typical weaning foods in rural EC consist of soft porridge, crumbly maize-meal, steamed bread and maheu. The early introduction of other cereals or grains as complementary foods could have contributed to mycotoxin exposure, though our study streamlined to maize-based mycotoxin exposure. Mycotoxin exposure above and below the PMDTI was lowest amongst young children 0-6 months, because this age group consumes lower amounts of complementary feeding. The exposure to FB, DON and ZEA is lower within the 0-6 age group as compared to the 7 – 12-month group, the exposure is likely to increase after 24 months of age. Therefore, any amount of mycotoxin exposure is of health concern.

7.5 CONCLUSION

Growth impairment of young children was observed to increase with the increase in age. However, mycotoxin exposure above and below the PMDTI was lowest within the 0 - 6 age group and highest within the 7 - 12-month age group. The exposure dropped in the 19 - 24 age group extraordinarily. The mycotoxin exposure is likely to increase after twenty-four months of age, therefore a follow up study beyond this age group is vital. Furthermore, as these children grow, their portion sizes will increase and thus the ratio of mycotoxin to body weight will increase.
7.6 References


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CHAPTER 8: OVERARCHING DISCUSSION

8.1 Introduction

The overall aim of this thesis was to determine the association between multi-mycotoxin exposure and infant and young child growth from birth to 24 months using the deterministic approach. The study was limited to analysis of only home-grown maize, therefore commercial based foods were excluded. The study aimed at describing the sociodemographic information of the households, to determine multi-mycotoxin exposure levels (Fumonisins (FB), Deoxynivalenol (DON) and Zearalenone (ZEA) of children (0 - 24 months), to compare mycotoxin exposure and infant and child growth (0 - 12 months) and (13 – 24) months and compare the long-term anthropometric growth parameters and mycotoxin exposure patterns during the first 24 months of life.

The determination of mycotoxin exposure forms an integral part of the human risk assessment process and is of critical importance. The role of mycotoxins in child growth stems from epidemiological observations of the high prevalence of stunting (Shisana et al., 2013) and the known high mycotoxin concentrations on the home-grown maize in EC, SA (Shepherd et al., 2013; Burger et al., 2010). Currently very little is known about the role of mycotoxin exposure in the development of stunting among rural infants and young children living in the EC, SA. Evidence-based information will, therefore, be valuable to address the overall high mycotoxin exposure as well as the high stunting rates in vulnerable communities in EC.

Aflatoxin influences various phases of infant and young child growth, but it is not clear what the influence of, FB, DON and ZEA mycotoxins are on growth in EC, SA (Lombard, 2014). While FB, DON and ZEA mycotoxins contaminate numerous grains, both in the field and during storage (Goertz et al., 2010). Smith et al., (2012), speculated that mycotoxin exposure affects the gut health and results in growth impairment, as indicated in animal studies. Deoxynivalenol, has several effects such as decreased nutrient absorption (Yunus et al., 2012), impairment of protein synthesis and reduced expression of claudin-4, a protein critical to the proper functioning of the tight junctions that regulate intestinal permeability (Pinton et al., 2010; Van De Walle et al., 2010). High levels of DON result in severe and persistent diarrhoea and can therefore lead to dehydration and loss of appetite (Dewey & Mayers, 2011). Children may therefore be exposed to multi-mycotoxin exposure emanating from mycotoxin contaminated maize dishes. This study aimed to elaborate on the magnitude and direction of mycotoxin exposure and effect on young child growth (0 - 24 months) in EC. To the best of the authors’ knowledge, no such studies have been carried out in this regard in the area.
Despite frequent high-profile incidents such as acute poisoning outbreaks, mycotoxins have not been widely prioritized from a public health perspective in low-income countries (Wild & Gong, 2010). Mycotoxins furthermore have not been investigated broadly in relation to infant and young child growth. In cases where nontoxic levels have been met, it has been largely driven by the need to meet stringent import regulations on mycotoxin contamination in the high-income countries of the world rather than to protect the population consuming the contaminated crops locally (Wild & Gong, 2010). It also has to be taken into consideration that regulatory mycotoxin national limits are not applicable to the EC community (Shephard et al., 2019). A recent study conducted by Shephard et al., 2019, outlined that the new South African Maximum levels (ML) for raw maize (4000 μg/kg) affect approximately 13% of the crop. Furthermore, children residing in the subsistent areas of EC consume maize as a staple complementary food hence import regulations of mycotoxins will not safeguard them against exposure. In addition, the consumption by young children is higher relative to their body weight, hence they may require an ML of 100 μg/kg at 95% consumption rate (Shephard et al., 2019). Moreover, subsistent farmers in EC lack access to modern grinding mills, henceforth their maize is basically hammer milled (Shephard et al., 2019). The effect of this hammer milling, virtually becomes the transfer of all the fumonisin from the raw maize to the consumed maize (Shephard et al., 2019). Regardless, of the fact that approximately 25% of the Centane home-grown maize crop is above the respective (4000 μg/kg), contamination level (Shephard et al., 2019). The reasons for the inadequate action to tackle the problem of mycotoxins in low-income countries are undoubtedly complex and incompletely researched (Wild, 2007). Furthermore, maize consumption is predominant in many African countries (Shephard et al., 2019). Intervention strategies such as advocacion for dietary diversity may assist in doing away with the health risk problem of mycotoxins at hand (Shephard et al., 2019).

The 56th meeting of the Joint Food and Agricultural Organisation and the World Health Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA), provided a No Observed Adverse Effect Level (NOAEL) of 0.2 mg/kg⁻¹ body weight day⁻¹ and a safety factor of 100, as a group provisional maximum tolerable daily intake (PMTDI) for FB, alone or in combination, of 2 μg/kg⁻¹ body weight day⁻¹ (JECFA, 2012). The hazard characterisation of 800 , using a safety factor of 1000, suggests a maximum tolerable daily intake for FB1 of 0.8μg/kg body weight per day, which is lower than 2μg/kg body weight per day established by JECFA derived from rodent nephrotoxicity (Shephard et al., 2019). The PMTDI for DON is 1 μgkg⁻¹ body weight day⁻¹while that of ZEA is 0.5 μgkg⁻¹ body weight day (JEFCA, 2001; JEFCA, 2002).
8.2 Sociodemographic situation, general health status and dietary intake of young children

The risk factors associated with undernutrition among infants and young children in SA are diverse and complex. Findings of this study concluded that most of the inhabitants of the EC, use the bushes as toilets, the majority are unemployed and very few attained tertiary education. Amongst, young children less than 24 months of age, we also investigated whether the socioeconomic status, health status and feeding practices affected the growth of the children as well as mycotoxin using linear regression analysis model. The finding of this study was that the health status of the children was associated with the growth status and mycotoxin exposure to a less extent. Therefore, the health status of the young children was considered as a confounding factor for growth retardation in analysis. The higher maize consumption of the young children contributed to elevated mycotoxin exposure. Our study also supported the fact from previous work that the education level of the mothers is a confounding factor in the growth of young children. Our study also indicated failure to comply with WHO recommendations of exclusive breastfeeding at six months of age. Despite the fact that, exclusive breastfeeding (EBF) for a period of six months has been recommended by the WHO (Fewtrell et al., 2007). Suboptimal breastfeeding accounted for 44 203 DALYS from 1990-2013 globally in 188 countries studied (Murray, 2015). The finding of the current study, of consumption of complementary maize as early as three months was also in line with previous findings, as infants consumed maize and other foods within the first and third month of age (Magoha et al., 2014; Mamabolo et al., 2004). At three months of age, 80% of the infants in Tanzania had already been exposed to foods other than breastmilk (Magoha et al., 2014). This is an interesting finding which calls for further investigation of the feeding practices of infants less than six months of age in EC, SA. In light of this, exclusive breastfeeding during the first six months of life protects the infants against morbidity and mortality (Victora et al., 2016; WHO, 2001).

8.3 Mycotoxin levels of home-grown maize kernels from EC and exposure levels of young children less than 24 months of age

This study determined the mycotoxin levels of maize kernels in EC. These mycotoxin levels were utilised in the deterministic approach, to ascertain whether the young children were above their Provisional Maximum tolerable daily intakes (PMDTIs) for the respective mycotoxins. We determined the mycotoxin levels of FB, DON and ZEA in home-grown maize from rural EC using LC-MS/MS, secondly, consumption amounts of children aged 0 - 24 months was assessed with food frequency questionnaires (FFQs) and lastly multi-mycotoxin risk assessment among children (comparison of PDIs with PMDTIs). Multi-mycotoxin
exposure assessments among infants and young children in high-risk areas of Africa are limited. Therefore, this study was of importance, as early multi-mycotoxin exposure due to the consumption of maize-based complementary foods and the possible link between infant adverse health outcomes has not been investigated in SA. The mycotoxin levels of home-grown maize from EC were predominantly high. The maize consumption of the young children, as well the mycotoxin exposure levels increased with age, six monthly. The FB exposure levels of the young children in this study were also high, above the PMDTIs, while DON and ZEA were below their PMDTs. In addition, a large percentage of the young children were exposed to FB above the PMDTI. The results of FB exposure obtained in this study were higher than the results from Tanzania (Kimanya et al., 2014; Magoha et al., 2014). Findings of this study support the notion that children in maize subsistence areas are prone to mycotoxin exposure, also the fact that maize in EC, SA has high mycotoxin levels (Burger et al., 2010).

8.4 Growth and mycotoxin exposure of the young children 0 - 12 months of age

In Eastern and Southern Africa 34.5% of children are still undernourished (UNICEF, 2017). Furthermore, a large part of the undernutrition is related to stunting and wasting (WHO, 2014). However, in 2016 stunting affected 155 million children below the age of 36 months, while wasting affected 52 million children below the age of 60 months, globally (UNICEF, 2017). Globally 22.9 % of the children under 60 months are stunted (UNICEF, 2017). Therefore, the growth of children and mycotoxin exposure were determined in the current study. These measurements helped to explain the relationship between growth and mycotoxin exposure of the young children. A prevalence of 16% stunting amongst the young children 0 - 12 months in EC, SA was observed. None of the infants were wasted or underweight.

The South African National Examination Survey (SANHANES-1) reported that approximately 26.9% (n = 137) of boys below the age of 36 months were stunted ( < -2SD LAZ) of which 9.9% were severely stunted ( < -3SD LAZ) (Shisana et al., 2013). Of the girls 25.9% (n = 143) girls were also stunted ( -2SD LAZ), of which 9.1 % (n = 26) were severely stunted ( < -3LAZ) (Shisana et al., 2013). In the EC 21.6% of the boys and 15.6 % of the girls were stunted (Shisana et al., 2013). Stunting occurs when a child has a Z-score < -2 SD. In the EC 1.6% of the boys were wasted whilst 0.2% (n = 1) were severely wasted (Shisana et al., 2013). Amongst the girls 3.2 %( n = 9) were wasted whilst 1.1% were severely wasted in the EC (Shisana et al., 2013). The geometric mean exposure (based on the PDI) to FB obtained was far above the PMDTI of 2 µgkg⁻¹ bw day⁻¹(JECFA, 2012). In addition, findings indicated that the geometric mean for DON and ZEA were also above their PMDTI’s of 1 µgkg⁻¹ bw day⁻¹ and 0.5 µgkg⁻¹ bw day⁻¹ (JEFCA, 2001, JEFCA, 2002).
In this study, infants 0 - 12 months of age were shorter by 4.4 cm and lighter by only 0.3 g with high FB exposure. Furthermore, length of the infants (0 - 12 months) was also associated with FB exposure, on adjustment of confounding factors (age, gender, HIV, TB, education and water source), after adjusting for confounding factors. These results are in line with Kimanya et al., 2010, importantly in that study, it was observed that at 12 months of age, the infants with FB exposure above the PMDTI were significantly shorter by 1.3 cm. In contrast to Kimanya’s study infants exposed to FB above the PMDTI were far lighter by 328 g, compared to those with exposures below the PMDTI (Kimanya et al., 2010). Results on the relationship between growth parameters (scores) and mycotoxin exposure, indicated that LAZ and WAZ, were associated with dietary FB exposure among infants 0 - 12 months, after adjusting for confounding factors (age, gender, HIV, TB, education and water source). These findings suggest that FB intake is associated with growth retardation (Kimanya et al., 2012). The conclusion that WAZ was associated with FB exposure, is in line with the results obtained in Tanzania by Chen et al., 2018, in Haydon, Tanzania amongst infants 0 - 12 months of age. It was observed that FB exposure was negatively associated with WAZ scores (non detectable samples excluded, p = 0.005), (Chen et al., 2018).

8.5 Relationship between anthropometric growth parameters and dietary mycotoxin of children 13-24 months of age

The stunting rates observed in this study amongst children 13 - 24 months were high (34%) compared to the results obtained previously in EC, SA (Shisana et al., 2013). None of the young children were either wasted or underweight. The geometric mean exposure to FB obtained in this study was three and a half times above the PMDTI 2 µgkg\(^{-1}\) bw day\(^{-1}\). Results from this study indicated that the geometric mean for DON and ZEA were also above their PMDTI’s of 1 µgkg\(^{-1}\) bw day\(^{-1}\) and 0.5 µgkg\(^{-1}\) bw day\(^{-1}\) (JEFC, 2001, JEFCA, 2002). Of scientific significance is the fact that growth velocity (WLZ and LAZ changes), were associated with high FB exposure amongst older children 13 - 24 months of age. Our results indicated a great impact on growth impairment with high FB exposure, as measured by growth velocity (Z-score changes over time). This is a novel finding and it clearly supports the notion that growth impairment is associated with mycotoxin exposure (Smith et al., 2012). Fumonisin B may induce intestinal enteropathy, a subclinical condition of the small intestine, characterised by reduced absorptive capacity, poor appetite and increased intestinal permeability, therefore mediating stunting (Smith et al., 2012b). However, young children in this study slightly lost weight (0.3 g), with high FB exposure. Young children exposed to FB, DON and ZEA above the PMDTI dropped in LAZ, WAZ and WLZ scores in the current study. The findings of our study are in contrary with what Magoha et al., 2014 observed, rate of stunting and underweight
was lower in infants who were exposed to fumonisins (Magoha et al., 2014). There was an association between weight change (g kg\(^{-1}\) day\(^{-1}\)) and mycotoxin exposure amongst young children 13 - 24 months of age, in the current study. This finding indicated high maize consumption rates.

8.6 Growth and mycotoxin changes over 24 months

Our results indicated that the percentage of stunted young children increased with age over the 24-month period, with the 19 - 24-month age group having the highest stunting prevalence. This finding was quite normal as stunting develops and increases over time. It also brought to the authors attention that the stunting rates in EC are predominately high, which is a public health concern. None of the children were either wasted or underweight. In terms of mycotoxin exposure, the highest number of young children exposed above the PMDTI (FB and ZEA) were within the 7 – 12 age group. While the least number were within the 0 – 6-month age group. These findings meant that, although infants consume maize below six months of age, the number is quite low. However, it is an alarming finding as these infants are supposed to be exclusively breast fed within that age group, according to WHO recommendations. None of the children in all the age groups were exposed to DON above the PMDTI.

8.7 Implications and perspectives

The results of this PhD study contributed to the body of knowledge by answering some questions regarding the relationship between mycotoxin exposure and infant and young child growth. However, stunting rates are alarming, to the extent that it is vital to further investigate the causes thereof. The percentage of children exposed to FB was high in this study, as well as the exposure above the PMDTI. However, it must be taken into consideration that the community understudy is not well developed to detoxify these toxins, therefore it is vital to promote dietary diversity, to lower mycotoxin exposure. Mycotoxin exposure also has an economical effect, due to the lower agricultural output.

The findings of this longitudinal study agree to some extent with the hypothesis that mycotoxin exposure is related to growth impairment, as the LAZ scores of the young children were observed to deteriorate towards the negative side (below zero), with increase in mycotoxin exposure. There was also an association between length, LAZ and WAZ scores, z-score changes (LAZ and WLZ) and mycotoxin exposure. Our results are also in support of the fact that maize fed young children tend to have a higher weight again. There was an association between weight change (g kg\(^{-1}\) day\(^{-1}\)) and mycotoxin exposure amongst young children in this study.
8.8 Conclusion

A small sample size of young children was observed to be consuming home-grown maize with breast-milk, formula or other foods. These were thus included in the study. The lower number of children consuming home-grown maize was linked to transition to the western diet, which promotes higher intake of commercial maize. Transition to western diet also results in more commercial maize, therefore this notion may support the fact that children 13 - 24 months consumed less of the home-grown maize than expected. However, the maize samples from this area were observed to have high FB, DON and ZEA levels on analysis. Results of this study also concluded that LAZ and WAZ scores were associated with high FB exposures, amongst infants 0 - 12 months of age. Furthermore, shorter length was also associated with high FB exposure. It was also observed that high FB exposure resulted in shorter length of 4.4 cm amongst infants 0 - 12 months of age. Another interesting finding was that growth velocity (WLZ changes and LAZ changes) were associated with high FB exposure, in the 13 - 24 age group. Weight gain (g kg⁻¹day⁻¹) was observed to be associated with FB, DON and ZEA, indicating weight gain due to high consumption of maize complementary foods.

8.9 Recommendations

The researchers plan to explore intervention strategies of reducing mycotoxin exposure in the rural EC, SA.

Furthermore, the researchers would like to investigate the underlying causes of stunting in EC, SA. This arises from the fact that undernutrition is a global issue presently.

Researchers also intend to conduct experimental studies, to gain insight into the threshold of mycotoxin exposure that causes growth impairment, as well as the rate at which the growth impairment occurs. This of significance as some of the growth impairment may occur in utero, therefore, an experimental study following up animals from pregnancy to birth until 24 months of age, would reflect the rate at which the growth impairment occurs.

Further research can be done to fully understand how mycotoxins influence indirect factors, such as appetite, infectious diarrhoea and inflammation and henceforth resulting in growth impairment.

The researchers also plan on analysing mycotoxins in breast milk samples.

A follow up study tracking the growth of children within the different age groups in relation to mycotoxin exposure is essential.
It is important to recommend culture specific nutrition education on exclusive breastfeeding, weaning and complementary food, but also on possible mycotoxin lowering strategies to reduce exposure, to the community.

8.10 Limitations

The study included some limitations and various factors such as the small sample size should be taken into account when interpreting the data.

Use of the deterministic approach (Food Frequency Questionnaires (FFQs) tend to over report intake), (mother / caregiver might not have been there during every meal), although this was the ideal method as they are no biomarkers for the mycotoxins understudy expect for Aflatoxin.

The research did not manage to include energy intake and micronutrient intake as possible confounding factors of the study.

Measurement of mean mycotoxin levels of maize (this can be different within samples and between samples),

The large numbers of home births and the lack of medical facilities made it difficult to obtain infant birth anthropometry;

The birth anthropometric information was obtained from the road to health cards, therefore it is unclear what procedures were used and how it influenced results,

It was assumed that the mycotoxins were not present in the breastmilk,

Lastly, the deterministic approach as used in this study is less accurate than using biomarkers to study the health implications of mycotoxin exposure. Due to logistical reasons, urinary analyses were not possible for this study. However, a study is currently underway to compare the urinary exposure with the deterministic approach, to better interpret results.

For a better understanding of the growth impairment effects of DON, FB and ZEA a larger study would be vital, as all confounding factors will be controlled for.
8.11 References


ANNEXURES

Addendum 1: Ethics approval letters from NWU HREC

Private Bag X6001, Potchefstroom South Africa 2520
Tel: 018 299-1111/2222
Web: http://www.nwu.ac.za

Faculty of Health Sciences

Health Sciences Ethics Office for Research, Training and Support

Health Research Ethics Committee (HREC)

Tel: 018-285 2291

Email: Wayne.Towers@nwu.ac.za

14 November 2016

Dr MJ Lombard Nutrition

Dear Dr Lombard

APPROVAL OF YOUR APPLICATION TO CONVERT TO A LARGER STUDY BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00207-14-A1

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

Study title: PhilSana Project
Study leader/supervisor: Dr MJ Lombard

The Health Research Ethics Committee (HREC) has reviewed your request to convert the umbrella study entitled "PhiliSana" to a larger study. The applicant has made the requested changes to the satisfaction of the HREC, however, it is requested that in future, the applicants must please indicate any changes made with a yellow highlight, to make review of the changes easier. The HREC approves the conversion request, however, we do request that more information be given on page 37 of the proposal about what is meant by the term "relevant research personnel". Please indicate exactly which research members have access to what aspect of the data. We request that once you have made this correction, that you please send all the updated final application documents (all the documents e.g. cover letter, executive summary, proposal, application form, informed consent form (if applicable) etc. without any track changes/highlights) with the required signatures, electronically to Ethics-HRECAppl@nwu.ac.za and bring the hard copies of these documents to the office of Ms Carolien van Zyl (Building G16, Room 138A). We also request that (if applicable) the applicants please bring the informed consent documentation to the office of Ms Carolien van Zyl (Building G16, Room 138A) to receive the official stamp.

Please inform us immediately if there are any amendments required to your study. If there are any queries, please let us know at your earliest convenience.

Yours sincerely

Dr Wayne Towers  Prof Minrie Greeff
HREC Chairperson  Ethics Office Head
Addendum 2: Guidelines for authors of World Mycotoxin Journal

Guidelines for authors of *World Mycotoxin Journal*

Scope

*World Mycotoxin Journal* is a peer-reviewed scientific journal with only one specific focus of the science of mycotoxins. The journal contains original research papers and critical reviews on mycotoxins, together with opinions, a calendar of forthcoming mycotoxin-related events, and book reviews.

*World Mycotoxin Journal* takes a multidisciplinary approach, and it focuses on a broad spectrum of issues:

- International developments and regulatory issues
- The economic impact of mycotoxins
- The latest information on major mycotoxins and emerging problems in the food and feed chain
- Human and animal nutrition and health effects
- Latest discoveries in mycotoxin toxicology and toxicokinetics
- Trends in modelling and prediction of mycotoxin formation
- Strategies for pre- and postharvest prevention and control
- Application of genomics in mycotoxin research
- Molecular biology for control of mycotoxigenic fungi
- Decontamination and detoxification solutions
- New developments in mycotoxin sampling analysis and analytical quality assurance, including reference materials
- Worldwide cases of occurrence and exposure to mycotoxins

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Indicate the type of manuscript. Your manuscript must contain title, author(s) and affiliation(s) and the requirements for the manuscript type (see the Table below).

Your paper should be within the range of pages given for the type of manuscript (see the Table below). Contact the editorial office if your manuscript exceeds the page range.
The maximum size in Manuscript Central for your manuscript, including figures and additional files is 50 Mb. If you need more space please contact the editorial office.

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² Should be submitted to the editor-in-chief.

³ Please contact the editor-in-chief before submission.
General

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Title: bold, sentence case, and no longer than 20 words.

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Affiliations: all sentence case; affiliations include the full address of all authors and including the e-mail address of the corresponding author.

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The abstract should be clear on itself and not containing more than 300 words.

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Do not mention references in the abstract.

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Use a maximum of two heading levels:

Level 1: Boldface, sentence case.

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Commas are used for numbers greater than 1000. Ordinal numbers less than 10 are preferably spelled out. Periods are used for decimals. Use a 0 before the decimal point for numbers below 1 (e.g. 0.005).

Authors should use SI units. Abbreviations should be used for all units; units should be given as kg/ha rather than kg ha$^{-1}$.

Other abbreviations should be given in full on first use and are followed by the abbreviation in parentheses.

Numerical values should be given in figures.

Manufacturer or supplier names and location (city and country) are given for special chemicals, software, equipment and other products.

Use single quotation marks in the text.

Numbered lists should be provided with Arabic numbers or lower case alphabet. Use a period after the number or letter (e.g. 1. or a.). Unnumbered lists should be provided with bullets.

Do not indent paragraphs. Use the tab function to place words at a certain position in the text, not spaces.

**Footnotes**

Footnotes should be avoided. If absolutely necessary, they should be numbered in the text, indicated by superscript numbers.

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Appendices appear after the references; must contain a title and should be numbered when more than one.

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Avoid large tables. Tables should fit within the journal size (maximum size per page 20×27 cm). Landscape format is acceptable only by exception; this will be decided by the publisher.

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Tables should be included in the text at the right place.

Tables should be clear without reading the text. Column headings should be brief and clear.

Any necessary explanations essential for understanding the table should be given as a footnote at the bottom of the table. Use either numbers or letters for footnotes.

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We do not recommend using the Word 2007, 2008, 2010 or 2011 equation editor. This can in some cases result in display errors. Instead, use the legacy equation editor in word (Insert menu; select insert object; select Microsoft equation) or use MathType (recommended).

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References concerning submitted, but not yet accepted manuscripts, unpublished data or ‘personal communications’ should not be cited in the reference list, but may be mentioned in the text as (unpublished data) or (Initials + Family name, personal communications).

Work accepted for publication, but not yet published or first published online should be referred to as ‘in press’. If possible provide a DOI for these manuscripts.

In the text, refer to the author’s name (without initials) and year of publication. Publications from the same authors in a single year should use a, b, etc.

If reference is made to a publication written by more than two authors, the name of the first author should be followed by ‘et al.’. Use ‘and’ and not ‘&’ for two authors.

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References cited together in the text should be arranged alphabetically.

All publications cited in the text should be presented in an alphabetical list of references at the end of the manuscript (no numbering).

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**Ethics**

Authors should ensure that the work described is entirely original. If authors use the work and/or words of others this should be cited or quoted appropriately. Plagiarism in all its forms is unacceptable.

It is accepted that authors sometimes need to manipulate images for clarity, however, manipulation for purposes of deception or fraud will be seen as scientific ethical abuse.

If plagiarism or manipulation is identified, manuscripts will either not be considered for publication or if already published the paper will be withdrawn from the electronic version of the journal.

*World Mycotoxin journal* requires that all authors disclose any potential sources of conflict of interest.

Authors need to confirm that all the research meets the ethical guidelines, including adherence to the legal requirements (for human and animal studies) of the study country.
Mycotoxin names and abbreviations in World Mycotoxin Journal

For **masked mycotoxin terminology** the proposal of Rychlic *et al.* (2014) in Mycotoxin Research 30: 197-205, must be used.

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<td>Cyclopiazonic acid</td>
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<td>Fumonisin B₃</td>
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<td>Moniliformin</td>
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<td>Zearalenone</td>
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<td>Mycophenolic acid</td>
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Size and arrangement of figures

Provide figures approximately at the size at which they will be printed.

Try to keep figures compact and clear.

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Arial is the recommended font for all texts in artwork.
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Do not use 3-D graphs when there is no scientific reason to do so.

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DESCRIPTION
Food and Chemical Toxicology (FCT), an internationally renowned journal, that publishes original research articles and reviews on toxic effects, in animals and humans, of natural or synthetic chemicals occurring in the human environment with particular emphasis on food, drugs, and chemicals, including agricultural and industrial safety, and consumer product safety. Areas such as safety evaluation of novel foods and ingredients, biotechnologically-derived products, and nanomaterials are included in the scope of the journal. FCT also encourages submission of papers on inter-relationships between nutrition and toxicology and on in vitro techniques, particularly those fostering the 3 Rs.

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Authors must clearly and briefly identify what novel toxic effect (s) or toxic mechanism (s) of the chemical are being reported and what their significance is in the abstract. Furthermore, sufficient doses should be included in order to provide information on NOAEL/LOAEL values.

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Food and Chemical Toxicology (FCT), an internationally renowned journal, aspires to publish original research articles and reviews on toxic effects, in animals or humans, of natural or synthetic chemicals occurring in the human environment with particular emphasis on food, drugs, and chemicals, including agricultural and industrial safety, and consumer product safety. Areas such as safety evaluation of novel foods and ingredients, biotechnologically-derived products, and nanomaterials are included in the scope of the journal. FCT also encourages submission of papers on inter-relationships between nutrition and toxicology and on in vitro techniques, particularly those fostering the 3 Rs.

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toxicity, including molecular biology. Mechanisms underlying toxic phenomena.

Toxicological examinations of specific chemicals or consumer products, both those showing adverse effects and those demonstrating safety, that meet current standards of scientific acceptability.

Manuscripts concerning materials/substances of only local interest for which the chemical composition of the material/substance is not clearly defined will not be considered. Manuscripts addressing only pharmacological properties, or only potentially beneficial effects using in vitro or in vivo systems, are not within the scope of the journal.

FCT is committed to the highest standards. Only papers that have not been previously published, that fit in the above mentioned scope, and that have been reviewed by experts in the field prior to publication will be accepted. Cover letters must state that the paper is new and original and not under consideration for publication elsewhere. Papers pending in other journals will not be considered. Co-authors should be individuals who have contributed substantially to the content of the papers.

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The Journal’s main purpose is the publication of papers reporting and interpreting original unpublished toxicological research, particularly studies promoting an understanding of the mechanisms underlying toxic effects or improvements in methods for predicting adverse effects. Papers reporting the toxicological examination of specific foods, chemicals or consumer products will be published, irrespective of the positive or negative nature of the results, provided the tests and reporting meet current standards of acceptability. In addition, Short Communications will also be considered, as will concise interpretative Reviews of toxicological topics of contemporary significance. Letters to the Editor will be limited to comments on contributions already published in the journal; if a letter is accepted, a response (for simultaneous publication) will be invited from the authors of the original contribution. All Letters to the Editor should be submitted to the Editor in Chief, Jose L. Domingo through the online submission system of the Journal.

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Addendum 4: Guidelines to Authors for Toxicology

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GUIDE FOR AUTHORS

INTRODUCTION

Toxicology, an international journal, publishes only the highest quality original research and critical reviews dealing with the adverse effects of xenobiotics on the health of humans and animals. The goal of the journal is to advance the scientific understanding of mechanisms of toxicity. Emphasis will be placed on toxic effects observed at relevant exposures, which have direct impact on safety evaluation and risk assessment. All manuscripts published in Toxicology are subject to rigorous peer review.
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1.1 (then 1.1.1, 1.1.2, …), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

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Results should be clear and concise.

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The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

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**Addendum 5: Questionnaires**

**EPIDEMIOLOGICAL QUESTIONNAIRE**

<table>
<thead>
<tr>
<th>Study No.</th>
<th>Date of interview</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interviewer</td>
<td>Date of birth</td>
</tr>
</tbody>
</table>

Age ___________________________  Gender: Male □1  Female □2

---

(6) Where were you born?

<table>
<thead>
<tr>
<th>GP</th>
<th>N Prov</th>
<th>Mpum</th>
<th>NW</th>
<th>FS</th>
<th>E Cape</th>
<th>W Cape</th>
<th>N Cape</th>
<th>KZN</th>
<th>Other:</th>
</tr>
</thead>
</table>

In the area you were born was it Urban, Rural, or on a Farm, or in a Squatter camp or in a Hostel?

| | | | | | | | | | |

(8) Have you lived in any of these areas and for how long did you stay in this area?

<table>
<thead>
<tr>
<th>GP</th>
<th>N Prov</th>
<th>Mpum</th>
<th>NW</th>
<th>FS</th>
<th>E Cape</th>
<th>W Cape</th>
<th>N Cape</th>
<th>KZN</th>
<th>Other:</th>
</tr>
</thead>
</table>

| yrs | Yrs | yrs | yrs | Yrs | yrs | yrs | yrs | yrs | yrs |

In the area you have lived was it Urban, Rural, a Farm, a Squatter camp or in a Hostel?

| | | | | | | | | | |

(9) Have you worked in any of these provinces?

<table>
<thead>
<tr>
<th>GP</th>
<th>N Prov</th>
<th>Mpum</th>
<th>NW</th>
<th>FS</th>
<th>E Cape</th>
<th>W Cape</th>
<th>N Cape</th>
<th>KZN</th>
<th>Other:</th>
</tr>
</thead>
</table>

What kind of work did you do and for how long have you worked there? (Use table below)
Choose the right option and fill in space above regarding question 9 (What kind of work)

1. Gold mine
2. Asbestos mine
3. Other mine
4. Glass / brick / Tile factory
5. Asbestos factory
6. Car repair / making shop
7. Foundry
8. Chemical factory
9. Tyre / Rubber factory
10. Building site
11. A farm
12. Transport industry
13. A dusty factory
14. Other:

(10) What is the highest standard you passed at school?

<table>
<thead>
<tr>
<th></th>
<th>SubA Grade 1</th>
<th>SubB Grade 2</th>
<th>Std 1 Grade 3</th>
<th>Std 2 Grade 4</th>
<th>Std 3 Grade 5</th>
<th>Std 4 Grade 6</th>
<th>Std 5 Grade 7</th>
<th>Std 6 Grade 8</th>
<th>Std 7 Grade 9</th>
<th>Std 8 NTC 1 Grade 10</th>
<th>Std 9 NTC 2 Grade 11</th>
<th>Std 10 NTC 3 Grade 12</th>
<th>Univ / Tech</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
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<td></td>
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</tbody>
</table>

(11) What is the highest standard your wife / husband passed at school?

(12) Mark this box if not married ☐
(13) Describe the usual or past occupation of your wife / husband ………………………………

(14) What language do you speak most at home? 1st…………………………2nd………………

(15) What language does/did your father speak? 1st…………………………2nd………………

(16) What language does/did your mother speak? 1st…………………………2nd………………

<table>
<thead>
<tr>
<th>Zulu</th>
<th>Xhosa</th>
<th>Sotho</th>
<th>Pedi</th>
<th>Tswana</th>
<th>Venda</th>
<th>Swazi</th>
<th>Shangaan</th>
<th>Ndebele</th>
<th>English</th>
<th>Afrikaans</th>
</tr>
</thead>
</table>

(If other, please specify)

(17) Where do you normally cook food? Inside□ Outside□ Inside and Outside□

(18) What type of fuel do you mostly use in your house for cooking?

<table>
<thead>
<tr>
<th>Wood</th>
<th>Mealie</th>
<th>Cob</th>
<th>Gas</th>
<th>Paraffin</th>
<th>Electricity</th>
<th>Other (specify):</th>
<th>None</th>
</tr>
</thead>
</table>

(19) What type of fuel do you mostly use in your house for keeping warm?

<table>
<thead>
<tr>
<th>Wood</th>
<th>Mealie</th>
<th>Cob</th>
<th>Gas</th>
<th>Paraffin</th>
<th>Electricity</th>
<th>Other (specify):</th>
<th>None</th>
</tr>
</thead>
</table>

(20) Where did you (or your family) normally cook food 20 years ago?

<table>
<thead>
<tr>
<th>Inside □</th>
<th>Outside□</th>
<th>Inside and Outside□</th>
</tr>
</thead>
</table>

(21) What type of fuel did you mostly use in your house for cooking 20 years ago?

<table>
<thead>
<tr>
<th>Wood</th>
<th>Mealie</th>
<th>Cob</th>
<th>Gas</th>
<th>Paraffin</th>
<th>Electricity</th>
<th>Other (specify):</th>
<th>None</th>
</tr>
</thead>
</table>

(22) What type of fuel did you mostly use in your house for keeping warm 20 years ago?

<table>
<thead>
<tr>
<th>Wood</th>
<th>Mealie</th>
<th>Cob</th>
<th>Gas</th>
<th>Paraffin</th>
<th>Electricity</th>
<th>Other (specify):</th>
<th>None</th>
</tr>
</thead>
</table>
(23) In any of the houses you lived in, did the smoke ever make your eyes water?

Yes, more than 5 years □ No □

(24) Have you ever smoked cigarettes or a pipe regularly?

Yes (now) □ In the past □ Never □

If ‘Yes’ (or ‘Yes, in the past’), in the past 5 to 10 years, how many would you usually smoke in a day?

Number of per day: Cigarettes □ Hand rolled cigarettes □ Pipes □

If ‘Yes’ (or ‘Yes, in the past’), how old were you when you first started smoking regularly?

□ years old

(25) Are you still smoking

Yes □ No □

(26) If you smoked in the past when did you stop?

□ years old Duration □

(27) Have you ever used snuff regularly?

Yes (now) □ In the past □ Never □

If yes, in the past 5 to 10 years, how often would you use snuff each day? □ times per day

(28) Have you ever chewed tobacco, regularly?

Yes (now) □ In the past □ Never □

If yes, in the past 5 to 10 years, how often would you chew tobacco each day? □ times per day

(29) If you are a current smoker (or smoked in the past) does (or did) the smoking from the cigarettes/pipe make (made) you cough?

Yes (now) □ In the past □ Never □

If yes, how often? □ (Daily 1; Weekly 2; Monthly 3; Seldom 4)

(31) How often do you have a drink containing alcohol?

Never □ Monthly □ Once a week □ 2-3 Times a week □ 4 - 6 times a week □ Everyday □

(32) How many drinks containing alcohol do you have on a particular day when you are drinking?

1 or 2 □ 3 or 4 □ 5 or 6 □ 7 to 9 □ 10 or more □
(33) Mark the appropriate option

<table>
<thead>
<tr>
<th>Beer</th>
<th>Spirits</th>
<th>Wine/sherry</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home-made</td>
<td>Commercial beer</td>
<td>Homemade</td>
<td>Commercial</td>
</tr>
<tr>
<td>Umqoboti □</td>
<td>Juba □</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nininanini □</td>
<td>Nininanini □</td>
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<tr>
<td>Indlovu □</td>
<td>Indlovu □</td>
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<tr>
<td>Ingqotovu □</td>
<td>Ingqotovu □</td>
<td></td>
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</tr>
<tr>
<td>If other, specify:</td>
<td>If other, specify:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| How many times per week?     |         |             |       |
| What container do you use?   |         |             |       |

- Mug1, Can2, Beer dumpy3, 1litre Beer bottle4, Wine carton/box5, Communal container6, Other7, Don't know8

<table>
<thead>
<tr>
<th>Age start:</th>
<th>yrs</th>
<th>yrs</th>
<th>yrs</th>
<th>yrs</th>
<th>yrs</th>
<th>yrs</th>
<th>yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>How long have you been drinking?</td>
<td>yrs</td>
<td>yrs</td>
<td>yrs</td>
<td>yrs</td>
<td>yrs</td>
<td>yrs</td>
<td>yrs</td>
</tr>
</tbody>
</table>

(34) Have you ever felt you should cut down on your drinking? Yes □ No □
(35) Have people ever annoyed you by criticizing your drinking?  
Yes ☐  No ☐

(36) Have you ever felt bad or guilty about your drinking?  
Yes ☐  No ☐

(37) Have you ever had a drink first thing in the morning to steady your nerves or get rid of a hangover?  
Yes ☐  No ☐

(38) Have you ever been pregnant?  
Yes ☐  No ☐

(39) If yes, how many times have you been pregnant?  
☐

(40) If yes, how many times have you had a miscarriage/still births/abortion?  
☐

(41) If yes, how many of your children were born alive?  
☐

(42) Have you ever had a child, still borne or alive with a birth defect?  Yes ☐  No ☐  Don’t know ☐

(43) Do you know of any blood female relatives (mother, sister etc.) who had a child born with a birth defect?  
Yes ☐  No ☐  Don’t know ☐

(45) Have you ever had your blood pressure measured before today?  Yes ☐  No ☐

If yes, were you told that it was high?  
Yes ☐  No ☐  WOMEN ONLY: Yes, only during pregnancy ☐

(46) Have you ever had high blood sugar (diabetes)?  Yes ☐  No ☐  Don’t know ☐

(47) Has any blood relative had cancer?  Yes ☐  No ☐  Don’t know ☐

* (Remember family tree)
If yes, then Who ........................................... Type of cancer .............................................

Who .......................................................... Type of cancer .............................................

Who .......................................................... Type of cancer .............................................

(48) Do you own?

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Number of sheep:</td>
<td>Number of cattle:</td>
<td>Number of chickens:</td>
<td>Number of goats:</td>
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<tr>
<td></td>
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</tbody>
</table>

Livestock – pigs?  
Number of pigs: 

Livestock – any other?  
Number of: 

(49) Do you suffer from heartburn?  
Yes □ No □  How often? □ (Daily 1; Weekly 2; Monthly 3; Seldom 4)

How long have you been suffering from heartburn?  
□ □ months or □ □ years

(50) Do you have any problem with swallowing?  
Yes □ No □

How long have you been having swallowing complaints? □ □ months or □ □ years

(51) Have you ever used alternative (herbal or traditional) medicine on a regular basis (more than once a week)? Yes □ No □

For what reason do/ did you use this medicine? .................................................................

From whom did you get this alternative medicine?  
□

(Pharmacist 1; Medical Doctor 2; Traditional Healer 3; Homeopath 4; Home-made 5; Other 6)

(52) Do you practice self-induced vomiting? Yes □ No □

How often? □ (Daily 1; Weekly 2; Monthly 3; Seldom 4)

For how long have you been practising self induced vomiting? □ □ months or □ □ years
What method do you use? □ [Emetic; Instrument (e.g. finger; feather); Both]

(Emetic is a special mixture you swallow to make you ill or nauseas)

If Yes, describe the emetic? .................................................................

(53) Do you suffer from any disease at present (chronic or acute)? Yes □ No □

If, yes what? ................................................

(54) Is anybody in your family affected by or died from any disease other than cancer e.g.: heart condition?

Yes □ No □ (Remember family tree)

If yes, then who ........................................ Type of disease.................................

Who ...................................................... Type of disease.................................

Who ...................................................... Type of disease.................................
Food frequency questionnaire - Infant

Study No. __________________

Date of interview __________________

Interviewer _________________

Date of birth ______________________

<table>
<thead>
<tr>
<th>Type of food</th>
<th>Yes / No</th>
<th>Home (H) / bought (B)</th>
<th>Portion size</th>
<th>Portions at a time</th>
<th>Less than a month</th>
<th>Amount per month</th>
<th>Amount per week</th>
<th>Amount per day</th>
<th>Total per month</th>
<th>Total per day</th>
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</thead>
<tbody>
<tr>
<td>White bread</td>
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<tr>
<td>Brown bread</td>
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<tr>
<td>Baked bread</td>
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<tr>
<td>Steamed bread</td>
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<tr>
<td>Dumplings</td>
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<td>Other</td>
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<td>Type of food</td>
<td>Yes/No</td>
<td>Home (H) / bought (B)</td>
<td>Portion size</td>
<td>Portions at a time</td>
<td>Less than a month</td>
<td>Amount per month</td>
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<tr>
<td>CEREALS</td>
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<td>Soft porridge</td>
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<tr>
<td>Stiff pap</td>
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<tr>
<td>Crumbly pap</td>
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<td>Samp</td>
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</tr>
<tr>
<td>Type of food:</td>
<td>Yes / No</td>
<td>Home (H) / bought (B)</td>
<td>Ratio</td>
<td>Portion size</td>
<td>Portions at a time</td>
<td>Less than a month</td>
<td>Amount per month</td>
<td>Amount per week</td>
<td>Amount per day</td>
<td>Total per month</td>
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<td>MIXED DISHES</td>
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<tr>
<td>Maize meal + Imifino</td>
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<tr>
<td>Maize meal + Spinach</td>
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<tr>
<td>Maize meal + Pumpkin</td>
<td>1:2</td>
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<td>1:3</td>
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<td>3:1</td>
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* Standing height is about 0.7 cm less than recumbent length (don’t subtract this amount, just indicate measured height / length)
ISIVUMELWANO SOKUZIBANDAKANYA KUPHANDO: Komama abubudala obungaphezu kweminyaka eyi 18.

ISIHLOKO SOVAVANYO

Uvavanyo ulibizwa nogukaba NguPhilasana: Kufunwa ukwaziwa ngobudlelwane obukhoyo noluphando phakathi kwe mytoxin nokuchaphazeleka kwamasana, kwabatwana abakhulayo basezilalini zase Empumakoloni, eMzantsi Afrika

REFERENCE NUMBERS: NWU-00207-14-S1

UMPHANDI OMKHULU

Dr Martani Lombard

IDILESI

Room 149, G16
Igumbi 149, G16
North-West University
Kwi Dyunivesithi yoMntla -Ntshona
Faculty of Health Sciences
Kwicandelo lwempilo nobunzululwazi
Private Bag X6001
Potchefstroom
2522

INOMBOMBOLO MFONOMFONO:

018 299-2085
**Molweni**

Singabaphandi abasuka kwi Dyunivesithi yaseMtlal-Ntshona siphanda ngobume bemphilo nesondlo somama kunye netsana.

Uyamenywa wena nosana lwakoho ukuba nithabathe inxaxheba kwivavanyo lophando i Philasana.

Uyacelwa ukuba uthatbathe ixesha lakho ukuba ufunde inkukachaezikule fomu malunga nophando.


**Lungantoni oluphando?**

Oluphando luzakuqhutywa kwilali ezikufutshane Mazeppa Bay nase Qolora kufuphi nolwandle. Sifuna ukubona ukuba umbona ukhunta kangakananina, kwaye usana lithi liwutye kuze chaphazele njani ukukhula kosana lwakho. Siyakuthi sibandwendwele omama netsana abathe bathabatha inxaxheba kasithandathu eminyakeni emibini. (Owokuqalq iyakuba phambi kukoza isokhosana)

Isizathu sokuba senze oluphando:

Kufuna ulwazi ukuba usana lwakho litye ukutya okunjani nokungakani kwinyangang eQgithileyo sakuthi sikubonise ifanekiso ukwenza oko

Siqonde nemeko yekhaya nendlela utyiwa ngayo ukuthi ichapehezela kanjani usana.

Sakuthi silandele indlela usana olukhula ngayo ndlela yokutya kwiminyaka emibni yokuqala kubom besana.

Sakukhangela umbona lowo niwutyawo ukuba unokukhunta na, ungangakani umbona enithe nawutya eminyakeni emibini. Ukuze sibone oku siyakunyanzeleka ukuba sithabathe isampile zomchamo, ubusi Iwebele kunye nokutyawo.

Sakukhangela izizathu ezenza ukuba isana lingakhuli ngendlela etyiwa ngayo ukuthi ichapahezela kanjani usana.

Kungani wena nosana nimenywe ukuba nithabathe uinxaxheba?

Uyamenywa wena nosana ukuba nithabathe inxaxheba, kuba ukhulelwe uzakufuna usana kungekudala. Kwaye kuba ungumhlali wase Mazeppa Bay okanye eQolora

Abantu abenelungelo lokuthabatha inxaxheba:

Abantu alungele uphando nabgantu abaneminyaka eyi 18-45

Akusayi kuthabatha inxaxheba xa:

Xa uneminyaka engaphansi kwe 16, xa undwendwele indawo, okanye umtwana wakho ezelwe kudala.

Iyintoni inxaxheba yakho?

Ulindelwe ukuba ukwazi ukuba ukuthetha nabaphandini kathathu nognyaka (kwiminyaka emibini) malunga nalembandela elandelayo:

ngobume bakho bempilo kunye nobasa lwakho (izinto ezinjenenge HIV, isifo sephephakunye notywala)

ngendlela enitya ngayo wena nosana lwakho

nentlobo yombona enuwutyayo wena nosana,
Ulindeleke usinike imvume yokuba sithabathe ubungakanani bakho bomzimba wena nosana wakho maxawonke sikundwendwele

ulindeleke usinike usine umchamo owakho kunye nosana lakho (kathathu ngonyaka)

ukuba uyancancisa uyacelwa ukuba isiphe igcuntswana lobisi lebele (kuknanye)

ulindeleke ukuba isiphe amatisipuni ambini engazi osana lwakho kabini uphando lusaqhubeka, ukuze sikwazi ukukhangelu intshologwane kagawulayo.

Uyukusixelela xa ufunza ukuba usana lakho liwavanyelwe inthsologgwane kagawulayo okanye lumgenziwa uvavanyo kwaye nokuba uyazifuna iziphumo okanye awuzifuni.

Ulindeleke ukuba isiphe ikomityi/imagi ezibini zombona eleke sindwendwel iikhaya lakho. Siyawubuya sikunike imbona wakho ukuze ungashotelwa ngumbona wokutyala.

Yintoni enizoyifumana wena nosana lwakho ngoluphando?


Oluphando lubalulekile kakhulu ngoba liyakuthi lusinike lwazu ulunzulu malungu namosana angakhuli ngedlela ebefanele akhula ngayo. Olulwazi ke lakthi lusincede ukufundisa abongikazi nomama bengingqi. Xa amasana ekhula kakhle akasayikugula kaninzi kodwa bakufunda kakauhle esikolweni.

Bukhona na ubungozi xa uthabatha inxaxheba koluphando?

**Kuyakwenzeko ntoni xa ufumanisa ukungaphatheki kakuhle kuba kungenxa yoluphando?**

Ukungaphatheki kakuhle kungenzeka qha mhlawumbe xakuthatzyatha igazi esaneni. Kodwa ke ligcutswana nje lelegi elifana nje nokuholathywa yinaliti. Okunye ingakukunxinyiswa kosana inabukeni elithile elikhongozela uchamo esaneni mhlambe lingamenza akhaphatheki kakuhle kodwa alisayi kulilimaza usana.

Mhlambe kungakho ukungaphatheki kakuhle xa ufanele isiphe umchamo wakho kunye nebisi lebele ungaphathwa zintlone. Asisayi kuvumela nabanina ukuba abesegumbini elinye na xawuzasinika isample zomchamo nebisi lebele. Eminye yemibuzo nayo ingenza into yokuba ungaphathethi kakuhle kodwa ke xa ungathandi ukuyiphendulu wamkelelikile ukuxelala umntu ozakube ekubuza imibuzo ukuba uwuzuwuphendulu lowombozo ongakuphathi kakuhle, kwaye kwamkelelele.

*Xa ufuna ukubanengxoxo nabaphandi emva kokubonana nabo malungano kucacisela kwezinto ongaziqondiyo wamkelelele ukidibana nomongikazi okanye umphandi.*

**Ngubani ozakubona olwazi othe wasipha lona?**

Akekho ozakuba olwazi lokuba wena ungubani ngoba wonke umntu othabatha inxaxheba uzakunikwa inombolo azakubizwa ngayo ngumhlandi kubonana ozakube olwazi. Maxesha onke kuzakusetwyniswa inombolo leyo ozakube uiyphiwe hayi igama laXa. Nesample zakho zego, zomchamo nobisi izi zivakubona nenombolo kubonana.

*Xasichaza imiphumela yophando sakuthetha siquke bonke abantu asisayikuchaza ubume bakho wedwa. Ngabaphandi kubonana abenelungelo lokubona ulwazi lophando kwaye namaphepha ayakuxixelwa endaweni ekhulselelele yelo kei ofisi ka Dr Lombard. Naxa Sifaka o-ulwazi ekhompetheni kuyakubakho inombolo esetyenziswayo ukuvula inkcuchacha zophando, ngabaphandi kubonana abenelungelo lokuvula ifi yiile ezo.*

**Ngamanye amaxesha iziponsas zophando (uRhulumente womZantsi Afrika) kunye namalungu**

eqoqo lwemithetho lophando ayakwazi ukuwafuna amaphepha kuba befunqa ukuqinisekisa ukuba abekwe eligcinweni olulungilele. Amaphepha ophando akugcinwa iminyaka eyi15.

**Uwusayi kubhatalwa ngokuthabatha inxaxheba kuphando?**

Akusayikubhatalwa ngokuthabatha inxaxheba kodwa akhona amqithiqithana oyakuthi uwafuna eleke sikundwemwele. Kwakuthi ke xasesifikilelele ukupheleni kophando siyakuthi siniphe isipho esincinci sengubo njengombulele kodwa kuyakwenzeka oko emva kweminyaka emibini.
Akusayikubhataliswa mali ngoku thatha inxaxheba.

**Kukhona ofuna ukukwazi okungokunye?**

Xa kukho into ongayiqondiyo nazi inombolo zomxeba zika Dr Lombard.

Ungaphinde unxibelelwano neliqoqo lwemithetho IweZobinzulu-Iwazi nonkosikazi Carolien van Zyl at 018 299 2094; carolien.vanzyl@nwu.ac.za ingakumbi xa unsesikhalazo okanye okungakwenelisiyo malungananabaphandi.

Uyakuthi ufumane zonke inkcukhacha zakho zophando.

**Uzakwazi njani ngenkcukhacha zophando**

Inkcukhacha uyakuthi uzifumane roqo emva kokubabesikkhe sakundwendwela nasekupheleni kophando uyuzifuna Ku Dr Lombard.

**Isiqinisekiso salowo uthabatha inxaheba**

Uyakuthi usayine ngezantsi Mna ___________________________ ndiyavuma ukuzibandakanya mna nosana lwam koluphando lePhilaSana. lobudlelwane phakathi kwe myxin nokuchaphazeleka kwamasana, kwabatwana abakhulayo basezilalini zase Empumakoloni, eMzantsi Afrika

**Ndiyaqisekisa ukuba:**

Ndilufundile olulwazi ngophando nefomu ebhalwe ngolwimi lwam endilivayo kwaye ndacacelwa konke

Ithuba lokubuza imibubozo kubaphandi ngabendingakuqondi ndilinikiwe, nomphandi uuyiphendule yonke imibuzo yam.

Ndikuqonda ukuthabatha inxaxheba kwam koluphando kukuthanda kwam ngaphandle kokunyanzelwa

Ndiyaqonda ukuthabatha inxaxheba kwam koluphando kungokuthanda kwam akusosinyanzelo

Ndingayeka nanini ndithanda akuzuba nasiphene kum.

Ndingacelwa ukuba ndiyeye phambi kwexasha ukuba umphandi ubona kundilungele oko, okanye andilandeli imigaqo yophando njengoko besivumelene.

Ndiyavuma/andivumi ukuba umphandi angavavaya usan lwam ukuba linayo intshologwane ka gawulayo (Sayina)
Ndiyavuma/andivumi ukwazi ngeziphumo zikagawulayo malunga nosana lwam: ____________________
(sayina)

Ndiyavuma/andivumi ukuba umphandi athabathe ifoto azi sebenzisekuphando ezinosana lwam okanye igama lakhe azibonise abanye abaphandi abakwenzileyo kuphando ____________________
(sayina)

________________________

Isayinwe e                   ngosukulwesi                  20

________________________

Isityikityo somthathinxaxheba     Isityikityo sengqina

Isiqinisekiso salowo ofumene inkcukhacha zophando

________________________

Mna(Igama)                  Ndiyaqinisekisa ukaba

Ndimchazele lonke ulwazi olusemqulwini malunga nophando u .......................................................... 

Ndimxelele ukuba abuze imibuzo, uthabathe ixesha lakhe phambi kokuphendula 

Ndiqinisekile ukuba uyakuqonda konke okumalunga nophando njengoko be ndichazele 

Ndiyisebenzisile/kangendisebenzise itoliki

________________________

Isayinwe e                   ngosukulwesi                  20...

70
Ndimchazele lonke ulwazi olusemqulwini

I explained the information in this document to ............................................

I encouraged him/her to ask questions and took adequate time to answer them.

Ndimxelele ukuba abuze imibuzo, uthabathe ixesha lakhe phambi kokuphendula

Ndiqinisekile ukuba uyakuqonda konke okumalunga nophando njengoko be ndichazele

Ndiyisebenzisile/kangendisebenzise itoliki


Isayinwe e ngosukulwesi 20


Isityikityo somthathinxaxheba Isityikityo sengqina
PARTICIPANT INFORMATION LEAFLET AND WRITTEN ASSENT FORM:

Mothers younger than 18 years

TITLE OF THE RESEARCH PROJECT:

The PhilaSana Project: The relationship between mycotoxin exposure and infant and young child growth amongst infants from deep rural areas of the Eastern Cape Province, South Africa – a Pilot Study

REFERENCE NUMBERS: NWU-00207-14-S1

PRINCIPAL INVESTIGATOR:

Dr Martani Lombard

ADDRESS:

Room 149, G16
North-West University
Faculty of Health Sciences
Private Bag X6001
Potchefstroom
2522

CONTACT NUMBER:

018 299-2085
Good day

We are researchers from the North-West University and we look at the health and nutrition of mothers and their babies.

You are being invited to take part in a research study that forms part of the PhilaSana project. Please take some time to read the information in this form, which will explain the details of this project. Please ask the researcher any questions about any part of this project that you do not fully understand. It is very important that you are happy and that you clearly understand what this research is about and how you can be part of it. Also, taking part in the study is completely your choice and you are free to say no. If you say no, this will not affect you negatively in any way. You are also free to stop being part of the study at any point, even if you do agree to take part.

This study has been approved by the Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (NWU-00207-14-S1) (Potchefstroom Campus) and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki and the ethical guidelines of the National Health Research Ethics Council. It might be necessary for the research ethics committee members or relevant authorities to inspect the research records.

What is this research study all about?

This study will be done in villages around Mazeppa Bay and Qolora by Sea. We want to see if there is any mold on the maize you and your baby eat and if this mold cause your baby to grow less than he/she should. We will visit the mothers and their babies that take part in the study six times in two years (of which the first one will be before your baby is born).

If you agree to take part in the study, we will ask you questions about you and your baby’s health and the food you eat. We will also look at you and your baby’s body measurements (what you and the baby weigh, how tall you and the baby are as well the size of your waist and hips and the size of the baby’s head and his/her upper arm). We will also get some urine from you and the baby and do some small blood tests for the baby. The blood tests will include different things to see if your baby is healthy and growing as he/ she should and will include a test for HIV test. If you decide to breast feed, we will ask you to express some of your breast milk (just once). The people who will do the study will be experienced health researchers trained in nutrition and health. 120 mothers and their babies will be part of the study.
The purpose of this study is to:

look at ways to know what and how much you and the baby have eaten during the last month with food photographs;

understand the different reasons (such as your household circumstances, health and diet) that influence the growth of the baby during the first two years;

follow the baby’s growth related health reasons and food patterns during the first two years of the baby’s life;

see if the maize you and the baby eat has any mold on, and how much of the maize you and the baby take in during the two years. To see this we will take some urine, breast milk and food samples.

see if there are any health reasons that cause the baby to grow less than other babies in the first two years.

Why have you been invited to participate?

You have been invited to participate because you are now pregnant and will have a baby soon. Also because you live in either Mazeppa Bay or Qolora by Sea.

You have also complied with the following inclusion criteria:

You are in the right age group for the study (16 – 45 years).

You will not be part of the study if:

You are younger than 16 years

Older than 45 years;

Only visiting the area;

The baby is born too early.

What will your responsibilities be?

You will be expected to talk to us three times a year (for two years) about:

your health (such as HIV, TB and alcohol),

your eating habits,
the maize you eat,

You will be expected to allow us to measure your body every time we visit (your length, height and the area around your waist);

You will be expected to give us some of your urine every time we visit (three times a year);

If you decide to breastfeed you will be expected to give us a little bit of your breast milk (once);

*You will be expected to give us 2 cups of your household’s maize every time we visit. We will give you 2 cups of maize back so that you do not have less maize to eat.*

**Will you benefit from taking part in this research?**

If you decide to take part in the study you will know about your and your baby’s health. We will talk to you about your and your baby’s health and will let you know if there are any problems in terms of your health. Although we are not treating patients ourselves, we will send you to the clinic if we think that you are having any health problems.

This study is important because it will give us more information about why babies are not growing as they should. This will help all the other babies in your area since we will teach the nursing staff and mothers in the area about this. If the babies are growing better, they will not get sick so often and they will be doing better at school.

**Are there risks involved in you taking part in this research?**

As far as we can see there is no risk in taking part of the study. However, there will be a trained emergency health person and a baby nurse on the team of researchers should anything happen. Although it is possible that you might have an allergic reaction to the injection, it is very rare. Also, because you are close to having the baby, if you have the baby while visiting us, we will take you to the nearest hospital or clinic for medical support.

**What will happen in the unlikely event of some form of discomfort occurring as a direct result of you taking part in this research study?**

You might experience some discomfort when you have to give us a urine and breast milk sample since you might be shy. We will not allow anybody (not from your household or from the research team) to be there when you collect the samples. Some of the questions on the questionnaires may also cause some
discomfort, but if you do not want to answer any questions, feel free to say so to the interviewer and that would be fine.

*Should you have the need for further discussions after each visit an opportunity will be arranged for you to speak with the nurse on the study or with the researchers.*

**Who will look at the information?**

*Nobody will know what information you give us. Everybody that is part of the study will have a special number that only you and the researchers will know. We will always write down the number and not your name. The urine, blood and breast milk you will give us will also have only the special number on.*

When we tell other people about what we found in the study we will always tell them about the group and we will never tell them about only your results. Only the researchers will be able to see the information you give us and the papers will be locked in the office of Dr Lombard. When we put the information on the computer we will also put a password on so that only the researchers can get to the information.

*Sometimes the sponsors of the study (the South African Government) and the people from the ethics committee might have a look at the papers to make sure that the researchers are keeping them safe.*

*Data will be stored for 7 years and will be used for research related to the growth of babies and their health.*

**Will you be paid to take part in this study and are there any costs involved?**

No, you will not be paid to take part in the study but we will give you a small food parcel to say thank you for your time after every visit. At the end of the study (after two years) we will give you a little baby blanket, also to say thank you.

There will thus be no costs involved for you, if you take part.

**Is there anything else that you should know or do?**

You can contact Dr Lombard at 018 299-2085 if you have any further queries or encounter any problems.

You can also contact the Health Research Ethics Committee via Mrs Carolien van Zyl at 018 299 2094; carolien.vanzyl@nwu.ac.za if you have any concerns or complaints that have not been adequately addressed by the researcher.

You will receive a copy of this information and consent form for your own records.
How will you know about the findings?

The findings of the research, regarding you and your baby will be shared with both you and your mother after every visit as well as at the end of the visit by Dr Lombard.

Declaration by participant

By signing below, I ...................................................... agree to take part in a research study entitled: The PhilaSana Project: The relationship between mycotoxin exposure and infant and young child growth amongst infants from deep rural areas of the Eastern Cape Province, South Africa – a Pilot Study.

I declare that:

I have read this information and consent form and it is written in a language with which I understand.

I have had a chance to ask questions to both the person asking permission, as well as the researcher and all my questions have been answered.

I understand that taking part in this study is voluntary and I have not been forced to take part.

I may choose to leave the study at any time and will not be judged in any way.

I may be asked to leave the study before it has finished, if the researcher feels it is the best for me, or if I do not follow the study plan, as agreed to.

I agree / not agree that the researcher may take photos of the research project and that they will use it to show other researchers what they have done in the project. ______________(signature)

I agree / not agree that the researcher may test my baby for HIV. ______________(signature)

I want to / don’t want to know the HIV results of my baby. _________________ (signature)

Signed at (place) ....................................................... on (date) .................................... 20....

_________________          ________________          ________________

Signature of participant                      Signature of witness
Declaration by person obtaining consent

I (name) ............................................................. declare that:

I explained the information in this document to ...........................................

I encouraged her to ask questions and took adequate time to answer them.

I am satisfied that she adequately understands all aspects of the research, as discussed above

I did/did not use an interpreter.

Signed at (place) .................................................. on (date) ......................... 20...

______________  _______________  _______________

Signature of person obtaining consent            Signature of witness

Declaration by researcher

I (name) ............................................................. declare that:

I explained the information in this document to ...........................................

I encouraged her to ask questions and took adequate time to answer them.

I am satisfied that she adequately understands all aspects of the research, as discussed above

I did use an interpreter.

Signed at (place) .................................................. on (date) ......................... 20...

______________  _______________  _______________

Signature of researcher            Signature of witness
ISIVUMELWANO SOKUZIBANDAKANYA KUPHANDO: Komama abubudala abangaphantsi kweminyaka eyi 18.

ISIHLOKO SOVAVANYO

Uvavanyo ulibizwa nogukaba NguPhilasana: Kufunwa ukwaziwa ngobudlelwane obukhoyo noluphando phakathi kwe mytoxin nokuchaphazeleka kwamasana, kwabatwana abakhulayo basezilalini zase Empumakoloni, eMzantsi Afrika

REFERENCE NUMBERS: NWU-00207-14-S1

REFERENCE NUMBERS: NWU-00207-14-S1

UMPHANDI OMKHULU

Dr Martani Lombard
IDILESI
Room 149, G16
Igumbi 149, G16
North-West University
Kwi Dyunivesithi yoMntla -Ntshona
Faculty of Health Sciences
Kwicandelo lwempilo nobunzululwazi
Private Bag X6001
Potchefstroom
2522

INOMBOMBOLO MFONOMFONO:
018 299-2085
Molweni

Singabaphandi abasuka kwi Dyunivesithi yaseMtla-Ntshona siphanda ngobume bempilo nesonono somama kunye netsana.

Uyamenywa ukuba uthatbathe ixesha lako ukuba ufunde inkukachaezikule fomu malungu nophando.

Uyacelwa ukuba uthatbathe ukuphila ukhwayo ukuphila futhi ukucelwe ukuphila.

Kubalulekile gqitha intoyukuba ukephalaza ukubayinxalenyi yophando kwane ukubalulekile ukuphila kwakhele ukuze akucacile. Ukuthabatha inxaxheba koluphando akusosinyanzelokwaye unelungelo lokwala xa ungenamqweni. Xa usala akusayikubakho ngxaki nangaluphina uhlobo phakathi kwakho. Unalo ilungelo lokuyeka naninina xa ufuna ukwenza njalo nangona ubuyukhulu ukuba yinxalenyi yophando

Oluphando lugqitha liqoqo lwemithetho lophonke lweZobinzulu-Iwazi Kwicandelo Lwezempilo kwi Dyunivesithi yoMntla–Ntshona(NWU-00207-14S1) kwi Campus yasePotschefstroom kwaye lizakakakhulu ngokomqago nqubo nesukuqo kunye nqubo nomqabiso kwezisimiso zase Helsinki kunye nqobo lwemithetho lozophando. Kuyakuthi ke amalunye eqoqo lwemithetho lophonke lweZobinzulu-Iwazi akhe aziphengulule incwadi zophando akhangele ukuba umlapho lusahamba ngokomqago.

**Lungantoni oluphando?**

Oluphando luzakakakhulu kwilali ezikufutshane Mazeppa Bay nase Qolora kufuphi nolwandle. Sifuna ukuba umbona ukhunda kyingakatunzi, kwase usana lithi liwutye kuze chaphazele njani ukukhulu kosana lwakhe. Siyakuthi sibandwendwele omama netsana abathe bathabatha inxaxheba kasithandathu eminyakeni emibini. (Owokuqalq iyakuba phambi kukoza layesana nosana & lwazi)

Xa uva ukubona inxaxheba koluphando sizakubuza imibuzo embalwa malungu nobume bempiloyakho kunye nesana futhi nangutya enikutyayo. Sakuthi siphinde sikathathu abantu abafakazi abafakazi abakhele abathe bathubaza amalungana abababenze endlo. Abantu abazakuthabatha inxaxheba koluphando ngamama abiyi 120 kunye namba abo.

80
Isizathu sokuba senze oluphando:

• Kufuna ulwazi ukuba wena nosana lwakho nitya ukutya okunjani nokungakani kwinyangang eggithileyo sakuthi sikubonise ifanekiso ukwenza oko

• Siqonde neme ko yakhaya nendlela utyiwa ngayo ukuthi ichapahezela kanjani usana.

• Sakuthi silandle indlela usana olukhula ngayo ndlela yokutya kwiminyaka emibini yokuqala kubom besana.

• Sakukhangela umbona lowo niwutyawo ukuba unokukhunta na, kungangakani okokukhunta enithe nakutya eminyakeni emibini. Ukuze sibone oku siyakunyanzeleka ukuba sithabathe isampile zomchamo, ubusi lwebele kunye nokutya okutiyiwa.

• Sakukhangela ukuba ukukhunta kumbona okanye ezinye izizathu ezenza ukuba isana lingakhuli ngendlela efaneleyo kunezinye intsanaezingaphantsi kweminyaka emibini.

Kungani ukuba wena uthathathe inxaxheba?

Wena uyamenywa ukuba uthathathe inxaxheba kuba ukhulelwe uzakufuna usanakungekudala. Kwaye kuba ungumhlali wase Mazeppa Bay okanye eQolora

Wena kolu uhlulo ninelungelo lokuthathatha inxaxheba:

Xa uneminyaka eyi 16-17 ukulungele uphando

Akusayi kuthathatha inxaxheba:

Xa uneminyaka engaphansi kwe 16, xa undwendwele indawo, okanye utwana wakho ezelwe kudala.

Iyintoni inxaxheba yakho?

Ulindelwe ukuba ukwazi ukuba ukuthetha nabaphandi kathathu nogyaka (kwiminyaka emibini) malunga nalembandela elandelayo:

ngobume bakho bempilo yakho (izinto ezinjenge HIV, isifo sephephakunye notywala)

ngendlela otya ngayo nentlobo yombona uwutyayo,

Ulindeleke usinike imvume yokuba sithabathe ubungakanani bomzimba wakho maxawonke sikundwendwele(kathathu) ngonyaka
Ulindeleke usinike usine umchamo owakho (kathathu ngonyaka)

Ulindeleke ukuba usiphe isiphe ikomityi/imagi ezibini zombona elele sindwendwele ikhaya lakho. Siphinde sikunike imbona wakho ukuze ungashotelwa ngumbona wokutya

Yintoni enizoyifumana wena ngoluphando?


Oluphando lubalulekile kakhuulo ngoba liyakuthi lusinike lwazu olunzulu malunga namasana angakhuli ngedlela ebefanele akhula ngayo. Olulwazi ke lakuthi luncede amasana nokufundisa abongikazi nomama bengingqi. Xa amasana ekhula kakhule akasayikugula kaninzi kodwa bakufunda kakakhu le esikolweni.

Bukhona na ubungozi xa uthabatha inxaxheba koluphando?


Kuyakwenzenka ntoni xa ufumanisa ukungaphathheki kakhule kuba kungenxa yoluphando?

Mhlambe kungakho ukungaphathheki kakhule xa ufanele isiphe umchamo wakho kunye nebisile lebele ungaphathwa zintloni. Asisayi kuvumela nabanina ukuba abesegumbini elinye newe xawuzasinika isample zomchamo nebisi lebele. Eminye yemibu noy ingenza into yokuba ungaphathethi

kakhule kodwa ke xa ungathandi ukuyiphendula wamkeleli kile ukxelala umuntu ozakube ekubuza imibuzo ukuba uwuzuwwuphendula lowombuzo ongakuphathhi kakhule, kwaye kwamkelekile.

Xa ufuna ukubanengxozo nabaphandxi emva kokubonana nabo malungano kucaciselwa kwezinto ongaziqondyo wamkelekile ukidibana nomongikazi okanye umphandi
**Ngubani ozakubona olwazi othe wasipha lona?**

Akekho uzakuba nolwazi lokuba wena ungubani ngoba wonke umuntu othathatha inxaxheba uzakunikwa inombolo azakubizwa ngayo ngumphandhi kuthetha ozakuba nolwazi. Maxesha onke kuzakusetyenziswa inolombolo leyo ozakube iyi iphiwe hayi igama lako. Nesample zakho zegazi, zomchamo nobisi nazo zizakuba nenombolo kuthetha.

Xasichaza imiphumela yophando sakuthetha siquke bonke abantu asisayikuchaza ubume bakho wedwa. Ngalaphandhi kuthetha abenelungelo lokuba nolwazi lophandayo kwaye namaphepha ayakuxixelwa endaweni ekhuselelo kuyapho ke ofisi Ka Dr Lombard. Naxa sifaka olulwazi ekhombutheni kuqhubakho inolombolo esetyenziswayo ukuvula inkcuchacha zophando, ngabaphandhi kuthetha abenelungelo lokuvula iyi yeke ezo.

Ngamanye amaxesha iziponsas zophando (uRhulumente womZantsi Afrika) kunye namalungu eqoqo lwemithetho lophando ayakwazi ukuwafuna amaphepha kuba befuna ukuqinisekisa ukuba abekwe eligcinweni olulungileyo. Amaphepha ophando akugcinwa ininyaka eyi7.

**Uwusayi kubhatalwa ngokuthathatha inxaxhebe kuthetha?**

Akusayikubhatalwa ngokuthathatha inxaxheba kodwa akhona amqithiqlithana oyakuthi uwafuna eleke sikundwemdwelwe. Kwakuthi ke xasesifikelele ukupheleni kophando siyakuthi siniphe esincinci sengubo njengombulelo kodwa kuyakwenzeka oko emva kweminyaka emibini.

Akesayikubhataliswa mali ngoku thatha inxaxheba.

**Kukhona ofuna ukukwazi okungokunye?**

Xa kukho into ongayiqondiyo nazi inombolo zomxeba zika Dr Lombard.

Ungaphinde unxibelelwano neliqoqo lwemithetho lophando IweZobinzulu-lwazi nonkosikazi Carolien van Zyl at 018 299 2094; carolien.vanzyl@nwu.ac.za ingakumbi xa unsesikalazo okanye okungakwenelisiyo malunganabaphandi.

**Uyakuthi ufumane zonke inkcukhacha zakho zophando.**

**Uzakukwazi njani ngenkcukhacha zophando**

Inkcukhacha uyakuthi uzifumane roqo emva kokubabesikkhe sakundwendwela nasekupheleni kophando uyuzifuna Ku Dr Lombard.
Isiqinisekiso salowo uthabatha inxaheba

Uyakuthi usayine ngezantsi Mna __________________________ ndiyavuma ukuzibandakanya koluphando lePhilaSana. lobudlelwane phakathi kwe mytoxin nokuchaphazeleka kwamasana, kwabatwana abakhulayo basezilalini zase Empumakoloni, eMzantsi Afrika

Ndiiyaqisekisa ukuba:

Ndilufundile olulwazi ngophando nefomu ebhalwe ngolwimi lwam endilivayo kwaye ndacacelwa konke

Ithuba lokubuza imibubozo kubaphandi ngabendingakuqondi ndilinikiwe, nomphandi uyiphendule yonke imibuzo yam.

Ndikuqonda ukuthabatha inxaxheba kwam koluphando kukuthanda kwam ngaphandle kokunyanzelwa

Ndiiyaqonda ukuthabatha inxaxheba kwam koluphando kungokuthanda kwam akusosinyanzelo

Ndingayeka nanini ndithanda akuzuba nasiphene kum.

Ndingacelwa ukuba ndiyeye phambi kwexesha ukuba umphandi ubona kundilungele oko, okanye andilandeli imigaqo yophando njengoko besivu melene.

Ndiiyavuma/andivumi ukuba umphandi athabathe ifoto azisebenzise kuphando azibonise abanye abaphandi abakwenzileyo kuphando __________________________ (sayina)

________________________ __________________________ __________________________

Isayinwe e ngosukulwesi 20

________________________ __________________________ __________________________

Isityikityo somthathinxaxheba Isityikityo sengqina

Isiqinisekiso salowo ofumene inkcukhacha zophando

________________________ __________________________ __________________________

Mna(Igama) Ndiyaqinisekisa ukaba
Ndimchazele lonke ulwazi olusemqulwini malunga nophando u ....................................................

Ndimxelele ukuba abuze imibuzo, uthabathe ixesha lakhe phambi kokuphendula

Ndiqinisekile ukuba uyakuqonda konke okumalunga nophando njengoko be ndichazele

Ndiyisebenzisile/kangendisebenzise itoliki

_________________  __________________  __________________

Isayinwe e ngosukulwesi 20...

Isityikityo somthathinxaxheba __________________

Isityikityo sengqina __________________

Isiqinisekiso somphandi __________________

____________________________

Mna(Igama) Ndiyaqinisekisa ukaba

Ndimchazele lonke ulwazi olusemqulwini.

Ndimxelele ukuba abuze imibuzo, uthabathe ixesha lakhe phambi kokuphendula

Ndiqinisekile ukuba uyakuqonda konke okumalunga nophando njengoko be ndichazele

Ndiyisebenzisile/kangendisebenzise itoliki

_________________  __________________  __________________

Isayinwe e ngosukulwesi 20

____________________________

Isityikityo somthathinxaxheba Isityikityo sengqina
PARTICIPANT INFORMATION LEAFLET AND WRITTEN CONSENT FORM:

Mothers older than 18 years

TITLE OF THE RESEARCH PROJECT:

The PhilaSana Project: The relationship between mycotoxin exposure and infant and young child growth amongst infants from deep rural areas of the Eastern Cape Province, South Africa – a Pilot Study

REFERENCE NUMBERS: NWU-00207-14-S1

PRINCIPAL INVESTIGATOR:

Dr Martani Lombard

ADDRESS:

Room 149, G16
North-West University
Faculty of Health Sciences
Private Bag X6001
Potchefstroom
2522

CONTACT NUMBER:

018 299-2085
Good day

We are researchers from the North-West University and we look at the health and nutrition of mothers and their babies.

You and your baby are being invited to take part in a research study that forms part of the PhilaSana project. Please take some time to read the information in this form, which will explain the details of this project. Please ask the researcher any questions about any part of this project that you do not fully understand. It is very important that you are happy and that you clearly understand what this research is about and how you can be part of it. Also, taking part in the study is completely your choice and you are free to say no. If you say no, this will not affect you negatively in any way. You are also free to stop being part of the study at any point, even if you do agree to take part.

This study has been approved by the Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (NWU-00207-14-S1) (Potchefstroom Campus) and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki ad the ethical guidelines of the National Health Research Ethics Council. It might be necessary for the research ethics committee members or relevant authorities to inspect the research records.

What is this research study all about?

This study will be done in villages around Mazeppa Bay and Qolora by Sea. We want to see if there is any mold on the maize you and your baby eat and if this mould causes your baby to grow less than he/she should. We will visit the mothers and their babies that take part in the study six times in two years (of which the first one will be before your baby is born).

If you agree to take part in the study, we will ask you questions about your and your baby’s health and the food you eat. We will also look at you and your baby’s body measurements (what you and your baby weigh, how tall you and your baby are as well the size of your waist and hips and the size of your baby’s head and his/her upper arm). We will also get some urine from you and your baby and do some small blood tests for your baby. The blood tests will include different things to see if your baby is healthy and growing as he/she should and will include a HIV test. If you decide to breast feed, we will ask you to express some of your breast milk (just once). The people who will do the study will be experienced health researchers trained in nutrition and health. 120 mothers and their babies will be part of the study.
The purpose of this study is to:

look at ways to know what and how much you and your baby have eaten during the last month with food photographs;

understand the different reasons (such as your household circumstances, health and diet) that influence the growth of your baby during the first two years;

follow your baby’s growth related health reasons and food patterns during the first two years of your baby’s life;

see if the maize you and your baby eat has any mold on, and how much maize you and your baby take in during the two years. To see this, we will take some urine, breast milk and food samples.

see if any health reasons cause your baby to grow less than other babies in the first two years.

Why have you and your baby been invited to participate?

You and your baby have been invited to participate because you are now pregnant and will have a baby soon. Also because you live in either Mazeppa Bay or Qolora by Sea.

You have also complied with the following inclusion criteria:

Are in the right age group for the study (18 – 45 years).

You will not be part of the study if:

You are younger than 16 years old or older than 45 years; only visiting the area; your baby is born too early.

What will your responsibilities be?

You will be expected to talk to us three times a year (for two years) about:

you and your baby’s health (such as HIV, TB and alcohol),

you and your baby’s eating habits,

the maize you and your baby eat,

You will be expected to allow us to measure you and your baby’s body every time we visit;
your height, your weight and the area around your waist will be measured,

your baby’s height, his/her weight, the area around his/her head and the area around his/her arm will be measured,

You will be expected to give us some of you and your baby’s urine every time we visit you (three times a year);

If you decide to breastfeed you will be expected to give us a little bit of your breast milk (once);

You will be expected to give us about 2 teaspoons of your baby’s blood twice during the study of which one is a test for HIV;

You will also have to tell us if you want us to do an HIV test on your baby or not, and if you want to know the results or not;

You will be expected to give us 2 cups of your household’s maize every time we visit. We will give you 2 cups of maize back so that you do not have less maize to eat.

Will you and your baby benefit from taking part in this research?

If you decide to take part in the study you will know about you and your baby’s health. We will talk to you about you and your baby’s health and will let you know if there are any problems in terms of your health. We will also let you know if we think your baby is not growing as he should. Although we are not treating patients ourselves, we will send you to the clinic if we think that you or your baby is having any health problems.

This study is important because it will give us more information about why babies are not growing as they should. This will help all the other babies in your area since we will teach the nursing staff and mothers in the area about this. If the babies are growing better, they will not get sick so often and they will be doing better at school.

Are there risks involved in your taking part in this research?

As far as we can see there is no risk in taking part of the study. However, there will be a trained emergency health person and a baby nurse on the team of researchers should anything happen. Although it is possible that you and your baby might have an allergic reaction to the injection, it is very rare. Also, because you are close to having your baby, if you have your baby when visit us, we will take you to the nearest hospital or clinic for medical support.
What will happen in the unlikely event of some form of discomfort occurring as a direct result of your taking part in this research study?

The only discomfort your baby will experience might be when we take blood from him/her. However, this will only be a small amount of blood and will feel like a pin prick. Having the special nappy on while we try to collect the urine for the baby may also be uncomfortable for him/her but will not hurt.

You might experience some discomfort when you have to give us a urine and breast milk sample since you might be shy. However, we will not allow anybody (not from your household or from the research team) to be there when you collect the sample. Some of the questions on the questionnaires may also cause some discomfort, but if you do not want to answer any questions, feel free to say so to the interviewer and that would be fine.

Should you have the need for further discussions after each visit an opportunity will be arranged for you to speak with the nurse on the study or with the researchers.

Who will look at the information?

Nobody will know what information you give us. Everybody that is part of the study will have a special number that only you and the researchers will know. We will always write down the number and not your name. The urine, blood and breast milk you will give us will also have only the special number on.

When we tell other people about what we found in the study we will always tell them about the group and we will never tell them about only your results. Only the researchers will be able to see the information you give us and the papers will be locked in the office of Dr Lombard. When we put the information on the computer we will also put a password on so that only the researchers can get to the information.

Sometimes the sponsors of the study (the South African Government) and the people from the ethics committee might have a look at the papers to make sure that the researchers are keeping them safe.

Data will be stored for 7 years and will be used for research related to the growth of babies and their health.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in the study but we will give you a small food parcel to say thank you for your time after every visit. At the end of the study (after two years) we will give you a little baby blanket, also to say thank you.
There will thus be no costs involved for you, if you do take part.

**Is there anything else that you should know or do?**

You can contact Dr Lombard at 018 299-2085 if you have any further queries or encounter any problems.

You can also contact the Health Research Ethics Committee via Mrs Carolien van Zyl at 018 299 2094; carolien.vanzyl@nwu.ac.za if you have any concerns or complaints that have not been adequately addressed by the researcher.

You will receive a copy of this information and consent form for your own records.

**How will you know about the findings?**

The findings of the research will be shared with you after every visit as well as at the end of the visit by Dr Lombard.

**Declaration by participant**

By signing below, I …………………………………..…………. agree for myself and my baby to take part in a research study entitled: The PhilaSana Project: The relationship between mycotoxin exposure and infant and young child growth amongst infants from deep rural areas of the Eastern Cape Province, South Africa – a Pilot Study.

I declare that:

I have read this information and consent form and it is written in a language with which I understand.

I have had a chance to ask questions to both the person asking permission, as well as the researcher and all my questions have been answered.

I understand that taking part in this study is voluntary and I have not been forced to take part.

I may choose to leave the study at any time and will not be judged in any way.

I may be asked to leave the study before it has finished, if the researcher feels it is the best for me, or if I do not follow the study plan, as agreed to.

I agree / not agree that the researcher may test my baby for HIV. ____________(signature)

I want to / don’t want to know the HIV results of my baby. ________________ (signature)
I agree / not agree that the researcher may take photos of the research project and that they will use it (without my/my baby’s name) to show other researchers what they have done in the project.

________________ (signature)

Signed at (place) ........................................ on (date) ...................... 20...

_________________   _________________   __________________
Signature of participant     Signature of witness

Declaration by person obtaining consent

I (name) .......................................................... declare that:

I explained the information in this document to ........................................

I encouraged her to ask questions and took adequate time to answer them.

I am satisfied that she adequately understands all aspects of the research, as discussed above

I did/did not use an interpreter.

Signed at (place) ........................................ on (date) ...................... 20...

_________________   _________________   __________________
Signature of person obtaining consent     Signature of witness

Declaration by researcher

I (name) .......................................................... declare that:

I explained the information in this document to ........................................

I encouraged her to ask questions and took adequate time to answer them.
I am satisfied that she adequately understands all aspects of the research, as discussed above.

I did use an interpreter.

Signed at (place) ........................................ on (date) .................................. 20....

_________________  ___________________  ___________________
Signature of researcher  Signature of witness
PARTICIPANT INFORMATION LEAFLET AND WRITTEN PERMISSION FORM:

 Mothers of minor mothers

TITLE OF THE RESEARCH PROJECT:

The PhilaSana Project: The relationship between mycotoxin exposure and infant and young child growth amongst infants from deep rural areas of the Eastern Cape Province, South Africa – a Pilot Study

REFERENCE NUMBERS: NWU-00207-14-S1

PRINCIPAL INVESTIGATOR:

Dr Martani Lombard

ADDRESS:

Room 149, G16
North-West University
Faculty of Health Sciences
Private Bag X6001
Potchefstroom
2522

CONTACT NUMBER:

018 299-2085
Good day

We are researchers from the North-West University and we look at the health and nutrition of mothers and their babies.

Your child and her baby (your grandchild) are being invited to take part in a research study that forms part of the PhilaSana project. Please take some time to read the information in this form, which will explain the details of this project. Please ask the researcher any questions about any part of this project that you do not fully understand. It is very important that you are happy and that you clearly understand what this research is about and how your child and her baby can be part of it. Also, giving permission for your child and her baby to take part in the study is completely your choice and you are free to say no. If you say no, this will not affect you, your child or her baby negatively in any way. You are also free to stop your child and her baby from being part of the study at any point, even if you do agree that they may take part.

This study has been approved by the Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (NWU-00207-14-S1) (Potchefstroom campus) and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki ad the ethical guidelines of the National Health Research Ethics Council. It might be necessary for the research ethics committee members or relevant authorities to inspect the research records.

What is this research study all about?

This study will be done in villages around Mazeppa Bay and Qolora by Sea. We want to see if there is any mold on the maize your child and her baby eat and if this mould causes her baby to grow less than he/she should. We will visit the mothers and their babies that take part in the study six times in two years (of which the first one will be before the baby is born).

If you give permission for your child and her baby to take part in the study, we will ask your child questions about her and her baby’s health and the food they eat. We will also look at your child and her baby’s body measurements (what she and the baby weigh, how tall she and the baby are as well the size of her waist and hips and the size of the baby’s head and his/her upper arm). We will also get some urine from your child and her baby and do some small blood tests for the baby. The blood tests will include different things to see if your child’s baby is healthy and growing as he/she should and will include a test for HIV. If your daughter decides to breast feed, we will ask her to express some of her breast milk (just once). The people who will do the study will be experienced health researchers trained in nutrition and health. 120 mothers and their babies will be part of the study.
The purpose of this study is to:

look at ways to know what and how much your daughter and her baby have eaten during the last month with food photographs;

understand the different reasons (such as your daughter’s household circumstances, health and diet) that influence the growth of the baby during the first two years;

follow the baby’s growth related health reasons and food patterns during the first two years of the baby’s life;

see if the maize your daughter and her baby eat has any mold on, and how much maize they eat during the two years. To see this, we will take some urine, breast milk and food samples from your daughter and her child.

see if any health reasons cause the baby to grow less than other babies in the first two years.

Why have your child and her baby been invited to participate?

Your child and her baby have been invited to participate because she is now pregnant and will have a baby soon. Also because your child and her baby live in either Mazeppa Bay or Qolora by Sea.

Your child and the baby have also complied with the following inclusion criteria:

Are in the right age group for the study (16 – 45 years).

Your child and the baby will not be part of the study if:

your child is younger than 16 years’ old

or older than 45 years;

only visiting the area;

her baby is born too early.

What will your child’s responsibilities be?

Your child will be expected to talk to us three times a year (for two years) about:

her and the baby’s health (such as HIV, TB and alcohol),
her and the baby’s eating habits,

the maize they eat,

Your child will be expected to allow us to measure her and the baby’s body every time we visit;

Your child will be expected to give us some of her and the baby’s urine every time we visit (three times a year);

If your child decides to breastfeed she will be expected to give us a little bit of her breast milk (once);

Your child will be expected to give us about 2 teaspoons of her baby’s blood twice during the study of which one is for an HIV test;

You will have to tell us if you want us to do an HIV test on the baby or not, and if you want to know the results or not;

Your child will be expected to give us 2 cups of her household’s maize every time we visit. We will give her 2 cups of maize back so that they do not have less maize to eat.

Will your child and the baby benefit from taking part in this research?

If you decide to give permission for your child and her baby to take part in the study your child will know about her and the baby’s health. We will talk to your child about her and the baby’s health and will let her know if there are any problems in terms of their health. We will also let your child know if we think her baby is not growing as he/she should. Although we are not treating patients ourselves, we will send your child or the baby to the clinic if we think that she or the baby is having any health problems.

This study is important because it will give us more information about why babies are not growing as they should. This will help all the other babies in your area since we will teach the nursing staff and mothers in the area about this. If the babies are growing better, they will not get sick so often and they will be doing better at school.

Are there risks involved in your child and her baby in taking part in this research?

As far as we can see there is no risk in taking part of the study. However, there will be a trained emergency health person and a baby nurse on the team of researchers should anything happen. Although it is possible that your child or her baby might have an allergic reaction to the injection, it is very rare. Also, because
your child is close to having the baby, if she has the baby when visiting us, we will take her to the nearest hospital or clinic for medical support.

**What will happen in the unlikely event of some form of discomfort occurring as a direct result of your child and her baby taking part in this research study?**

The only discomfort her baby will experience might be when we take blood from him/her. However, this will only be a small amount of blood and will feel like a pin prick. Having the special nappy on while we try to collect the urine for the baby may also be uncomfortable but will not hurt.

Your child might experience some discomfort when she has to give us a urine and breast milk sample since she might be shy. We will not allow anybody (not from your household or from the research team) to be there when she collect the sample. Some of the questions on the questionnaires may also cause some discomfort, but if your child do not want to answer any questions, she can feel free to say so to the interviewer and that would be fine.

*Should you or your child have the need for further discussions after each visit an opportunity will be arranged for you or her to speak with the nurse on the study or with the researchers.*

**Who will look at the information?**

*Nobody will know what information your or your child give us. Everybody that is part of the study will have a special number that only you, your child and the researchers will know. We will always write down the number and not you or her or the baby’s name. The urine, blood and breast milk your child will give us will also have only the special number on.*

*When we tell other people about what we found in the study we will always tell them about the group and we will never tell them about only your child and her baby’s results. Only the researchers will be able to see the information your child gives us and the papers will be locked in the office of Dr Lombard. When we put the information on the computer we will also put a password on there so that only the researchers can get to the information.*

*Sometimes the sponsors of the study (the South African Government) and the people from the ethics committee might have a look at the papers to make sure that the researchers are keeping them safe.*

*Data will be stored for 7 years and will be used for research related to the growth of babies and their health.*
Will your child be paid to take part in this study and are there any costs involved?

As grandmother, you will not get paid for your daughter or her baby to participate in the study. Your child will also not be paid to take part in the study but we will give her a small food parcel to say thank you for her time after every visit. At the end of the study (after two years) we will give her a little baby blanket, also to say thank you.

There will thus be no costs involved for you or your child, if you give permission for her and her baby to take part.

Is there anything else that you should know or do?

You can contact Dr Lombard at 018 299-2085 if you have any further queries or encounter any problems.

You can also contact the Health Research Ethics Committee via Mrs Carolien van Zyl at 018 299 2094; carolien.vanzyl@nwu.ac.za if you have any concerns or complaints that have not been adequately addressed by the researcher.

You will receive a copy of this information and consent form for your own records.

How will you know about the findings?

The findings of the research will be shared with your child after every visit as well as at the end of the visit by Dr Lombard. It is important that you also understand that even though you give permission for your child and her baby to be part of the study, we are not allowed to give you any information or results from your child or her baby. We are only allowed to give it to your child.

Declaration by participant

By signing below, I .................................................. agree to let my child and her baby take part in a research study entitled: The PhilaSana Project: The relationship between mycotoxin exposure and infant and young child growth amongst infants from deep rural areas of the Eastern Cape Province, South Africa – a Pilot Study.

I declare that:

I have read this information and consent form and it is written in a language with which I understand.

I have had a chance to ask questions to both the person asking permission, as well as the researcher and all my questions have been answered.
I understand that taking part in this study is voluntary and my child has not been forced to take part.

My child may choose to leave the study at any time and will not be judged in any way.

My child may be asked to leave the study before it has finished, if the researcher feels it is the best for her, or if she does not follow the study plan, as agreed to.

I AGREE / NOT AGREE that the researcher may test the baby for HIV. ____________(signature)

I WANT TO / DON’T WANT TO know the HIV results of the baby. ______________________ (signature)

I agree / not agree that the researcher may take photos of the research project and that they will use it to show other researchers what they have done in the project. ______________(signature)

Signed at (place) .................................................. on (date) ......................... 20....

_________________                     ________________                     ________________

Signature of participant  Signature of witness

Declaration by person obtaining consent

I (name) ................................................................. declare that:

I explained the information in this document to ..............................................

I encouraged her to ask questions and took adequate time to answer them.

I am satisfied that she adequately understands all aspects of the research, as discussed above

I did use an interpreter.

Signed at (place) .................................................. on (date) ......................... 20....

_________________                     ________________                     ________________

Signature of person obtaining consent  Signature of witness
Declaration by researcher

I (name) .................................................................... declare that:

I explained the information in this document to ..............................................

I encouraged her to ask questions and took adequate time to answer them.

I am satisfied that she adequately understands all aspects of the research, as discussed above

I did use an interpreter.

Signed at (place) ...................................................... on (date) ......................... 20....

_________________    ___________________    ___________________
Signature of researcher    Signature of witness
ISIVUMELWANO SEMVUNE SOKUZIBANDAKANYA KUPHANDO: Komama nentombiyakhe abubudala obungaphantsi kweminyaka eyi 18.

ISIHLOKO SOVAVANYO

Uvavanyo ulibizwa nogukaba NguPhilasana: Kufunwa ukwaziwa ngobudlelwane obukhoyo noluphando phakathi kwe mytoxin nokuchaphazeleka kwamasana, kwabatwana abakhulayo basezilalini zase Empumakoloni, eMzantsi Afrika

REFERENCE NUMBERS: NWU-00207-14-S1

UMPHANDI OMKHULU

Dr Martani Lombard

IDILESI
Room 149, G16
Igumbi 149, G16
North-West University
Kwi Dyunivesithi yoMntla -Ntshona
Faculty of Health Sciences
Kwicandelo lwempilo nobunzululwazi
Private Bag X6001
Potchefstroom
2522

INOMBOMBO MFONOMFONO:
018 299-2085
**Molweni**

Singabaphandla abasuka kwi Dyunivesithi yaseMtla-Ntshona siphanda ngobume bempilo nesondlo somama kunye netsana.

Uyamenywa umtwana wakho nosana lwakhe ukuba nithabathe inxaxheba kwivavanyo lophando iPhilasana. Uyacelwa ukuba uthatbathe ixesha lakho ukuba ufunde inkcukacha ezikule fomu malunga nophando.


Oluphando lwapho lapho kwilali ezikufutshane Mazeppa Bay nase Qolora kufuphi nolwandle. Sifuna ukubona ukuba umbona ukhunta kakhakananina, kwaye umtwana nosana bathi bawutye umbona kuthi kuchaphazele njani ukukhula kosana lwakhe. Siyakuthi sibandwendwele omama netsana abathe bathabatha inxaxheba kasithandathu eminyakeni emibini. (Okokuqala iyakuba phambi kukoza losha kosana

Xa unika ivume ukuba intombi yakho nosana bathabathe inxaxheba koluphando sizakubuja intombi yakho imibuzo embalwa malunga nobume bempiloyakhe kunye nesana futhi nangutya enikutyayo. Sakuthi siphinde sikhangele ubukhulu bakhe kunye nobesana lakhe kulamalunye alendelayo: esinqeni, ubude babo, ubunzima bomzimba, intloko yosana kunye nengalo. Sakuthi sicele nomchamo wahke kunye nowosana, negazi elincinane esaneni. Xa ethe waqgiba ekubeni amncancise usana sakucela ukuba asi phu ubisi lwakhe kubekanye kuphela. Abantu abaqhuba oluphando ngabantu abaqeshwe kwicandelo
lezempi nezokutya. Abantu abazakuthabatha inxaxheba koluphando ngomama abayi 120 kunye namasana abo.

Isizathu sokuba senze oluphando:

- Kufuna ulwazi ukuba yena nosasana lwakhe batya ukutya okunjani nobungakani kwinyanganga eeggithileyo sakuthi simbonise ifanekiso ukwenza oko
- Siqonde nemeko yekhaya nendlela utyiwa ngayo ukuthi ichapahezela kanjani usana.
- Sakuthi silandele indlela usana olukhula ngayo ndlela yokutya kwiminyaka emibni yokuqala kubom besana.
- Sakukhangela umbera lowo awutyayo ngumtwana nosana ukuba unokuhunta na, kungangakani okokuhunta enithe nakutya eminyakeni emibini. Ukuze sibone oku siyakunyaneleka ukuba sithabathe isampile zakhe nezosana zomchamo, ubusi iwebele kunye nokutya okutyiwawo.
- Sakukhangela ukuba ukukhunta kumbona okanye ezinye izizathu ezenza ukuba isana lingakhuli ngendlela efaneleyo kunezinye intsanaezingaphantsi kweminyaka emibini.

Kungani utmwana wakho nosana lwakhe bemenywe ukuba bathabathe inxaxheba?

Umthwana wakho nosana lwakhe bamenywa ukuba bathabathe inxaxheba kuba ukhulelewe uzakufuna usanakungekudala. Kwaye kuba ungumhlali wase Mazeppa Bay okanye eQolora

Yena nosana lwakhe bakulungele uthabatha inxaxheba kuba:

Ukiwinganaba elilungileyo lophando kuba uminyaka eyi 16-17

Yena nosana lwakhe abasayi kuthabatha inxaxheba xa:

Xa uneminyaka engaphansi kwe 16, xa undwendwele indawo, okanye utmwana wakho ezelwe kudala.

Iyintoni inxaxheba yakhe utmwana wakho?

- Ulindelwe ukuba ukwazi ukuba ukuthetha nabaphandi kathathu nognyaka (kwiminyaka emibini) malunga nalembandelala elandelayo:

ngobume bakho bempilo kunye nosana lwakho (izinto ezinjenge HIV, isifo sephephakunye notywala) ngendlela enitya ngowo wena nosana lwakhonentlobo yombona enuwutuyayo,
Ulindeleke ukuba usinike imvume yokuba sithabathe ubungakanani bakhe bomzimba yena nosana lwakhe maxawonke sikundwendwele

Ulindeleke usinike usine umchamo wakhe kanye nowosana lwakhe(kathathu ngonyaka)

Uyancancisa uyacelwa ukuba isiphe igcuntswana lobisi lebele (kubekanye)

Ulindeleke ukuba isiphe amatisipuni ambini engazi osana lwakhe kabini uphando lusaqhubeka

Uyukusixelela xa efuna ukuba usana lwakhe livavanyelwe inthsologgwane kagawulayo okanye lumgenziwa uvavanyo kwaye nokuba uayizifuna iziphumo okanye awuzifuni.

Yintoni azokuyifumana yena nosana lwakhe ngoluphando?

Xa uthe wabanika invume yokuthabatha inxaxheba koluphando bayakuthi bafumane ulwazi uluphanglelelo ngempilo yakhe kunye neyosana. Sakuthetha naye ngebume bempilo yakhe kunye neyosana lwakhe kwaye sakumazisa xa kukho ingxaki malunga nomube bempilo yabo. Sakumazisa xasibona ukuba usana lwakhe alikhuli kakhule nakaleniokwengoko kufanelele. Nangona singabanyangi ngokwethu abantu sakukuthumela ekiniki xasibona ukubawena okanye usana lunengxaki yezempilo.

Oluphando lubalulekile kakhulu ngoba liyakuthi lusinike lwazu ulunzulu malunga namasana angakhuli ngedlela ebefanele akhula ngayo. Oluwazi ke lakuthi lusincede ukufundisa abongikazi nomama bengi. Xa amasana ekhula kakhule akasayikugula kaninzi kodwa bakufunda kakakhule esikolweni.

Bukhona na ubungozi xa umtwana nosana bethabatha inxaxheba koluphando?


Kuyakwenzeke ntoni xa ufumanisa ukungaphathekiki kakhule kwakhe ngenxa yoluphando?

Mhlambe kungakho ukungaphatheki kahule xa ufanele asiphe umchamo wakhe kunye nebisi lebele angaphathwa zintloni. Asisayi kuvumela nabanina ukuba abesegumbini elinye naye xa esezasini ka isample zomchamo nebisi lebele. Eminye yemibuzo nayo ingenza into yokuba angaphathethi kahule kodwa ke xa engathandi ukuyiphendula wamkelelikile ukuxelala umntu ozakube embuza imibuzo ukuba ukazuyiphendula lowombozo ongampathale kahule, kwaye kwamkelelikile.

Xa efuna ukubanengxoxo nabaphandi emva kokubonana nabo malungano kucaciselwa kwezinto angaziqondiyo wamkelelikile ukidibana nomongikazi okanye umphandi.

**Ngubani ozakubona olwazi othe wasipha lona?**


Xasichaza imiphumela yophando sakuthetha siquke bonke abantu asisayikuchaza ubume bakhe yedwa. Ngabaphandi kuphela abenelungelo lokubona ulwazi lophando kwaye napaphepha ayakutshixelwa endaweni ekhuseelekeleyo kwi ofisi Ka Dr Lombard. Naxa sifaka olulwazi ekhomputheni kuyakubakho inolombolo esetyenziswayo ukuvula inkcuchacha zophando, ngabaphandi kuphela abenelungela lokuvula ifiyile ezo.

Ngamanye amaxesha iziponsas zophando (uRhulumemente womZantsi Afrika) kunye namalungu eqoqo lwemithetho lophando ayakwazi ukuwafuna amaphepha kuba befuna ukuqinisekisa ukuba abekwe eligcinweni olulungileyo. Amaphepha ophando akucinwa iminyaka eyi7.

**Ukasayi kubhatalwa ngokuthabatha inxaxheba kuphando?**

Akasayikubhatalwa ngokuthabatha inxaxheba kodwa akhona amqithiqithana oyakuthi awafumane eleke bemndwemwedele. Kwakuthi ke xasesifikelele ukupheleni kophando siyakuthi simpe isipho esincinci sengubo njengombolelo kodwa kuyakwenzeka oko emva kweminyaka emibini.

**Akasayikubhataliswa mali ngoku thatha inxaxheba.**

**Kukhona ofuna ukukwazi okungokunye?**

Xa kukho into ongayiqondiyo nazi inombolo zomxela zika Dr Lombard.
Ungaphinde unxibelelwano neliqoqo lwemithetho lophando lweZobinzulu - lwazi nonkosikazi Carolien van Zyl at 018 299 2094; carolien.vanzyl@nwu.ac.za ingakumbi xa unsesikhalazo okanye okungakwenelisiyo malunganabaphandi.

**Uyakuthi ufumane zonke inkcukhacha zakhe zophando.**

Inkcukhacha uyakuthi uzifumane roqo emva kokubabesikkhe sakundwendwela nasekupheleni kophando uyuzifuna Ku Dr Lombard.

**Isiqinisekiso salowo uthabatha inxaheba**

Uyakuthi usayine ngezantsi Mna __________________________ ndiyavuma ukubandakanya umtwana wam kunyenosana lwakhe koluphando lePhilaSana. lobudlelwane phakathi kwe mytoxin nokuchaphazeleka kwamasana, kwabatwana abakhulayo basezilalini zase Empumakoloni, eMzantsi Afrika

Ndiyaqisekisa ukuba:

Ndilufundile olulwazi ngophando nefomu ebhalwe ngolwimi lwam endilivayo kwaye ndacacelwa konke

Ithuba lokuza imibubozo kobaphandi ngabendingakuphondi ndilinikiwe, nomphandi uyiphendule yonke imibuso yam.

Ndikuphondo ukuthabatha inxaxheba kwakhe koluphando kuthanda kwakhe ngaphandle akusosinyanzelo

Angayeka nanini ethanda akuzubasiphene kuye.

Angacelwa ukuba ayeke phambi kwexesha ukuba umphandi ubona engalungele oko, okanye engalandelini imigaqo yophando njengoko besivumelene.

Ndiyavuma/andivumi ukuba umphandi angavavanya usana lwakhe ukuba linayo intshologwane ka gawulayo __________________________ (Sayina)_

Ndiyavuma/andivumi ukwazi ngeziphumo zikagawulayo malunga nosana: ______________ (sayina)

Ndiyavuma/andivumi ukuba umphandi athabathe ifoto azisebenzise kuphando ezinosana lwakhe okanye igama lakhe azibonise abanye abaphandi abakwenzileyo kuphando _______________________________ (sayina)

__________________________ __________________________ __________________________

Isayinwe e ngosophukwesi 20
Isityikityo somthathinxaxheba ___________________________ Isityikityo sengqina

Isiqinisekiso salowo ofumene inkukhacha zophando

_________________________ ________________

Mna(Igama) 

Ndinyaqinisekisa ukaba

Ndimchazele lonke ulwazi olusemququlwini malunga nophando u ..............................................................

Ndimxelele ukuba abuze imibuzo, uthabathe ixesha lakhe phambi kokuphendula

Ndiqinisekile ukuba uyuquqonda konke okumalungama nophando njengoko be ndichazele

Ndiyisebenzisile/kangendisebenzise itoliki

_________________________ ________________

Isayinwe e 

ngosukulwesi 

20...

_________________________ ________________

Isityikityo somthathinxaxheba 

Isityikityo sengqina

Isiqinisekiso somphandi

_________________________ ________________

Mna(Igama) 

Ndinyaqinisekisa ukaba

Ndimchazele lonke ulwazi olusemququlwini

Ndimxelele ukuba abuze imibuzo, uthabathe ixesha lakhe phambi kokuphendula

Ndiqinisekile ukuba uyuquqonda konke okumalungama nophando njengoko be ndichazele

Ndiyisebenzisile/kangendisebenzise itoliki
Isayinwe e 

ngosukulwesi

20

Isityikityo somthathinxaxheba ____________________________ Isityikityo sengqina