Association between maternal multi-mycotoxin exposure and birth anthropometric growth of residents in rural Eastern Cape, South Africa

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PREFACE

This mini-dissertation was presented in article format. Monique Entres, the Magister Scientiae (MSc) student, wrote the article: “Association between mycotoxin exposure levels of rural Eastern Cape pregnant mothers and their infants’ anthropometric measures and gestational age at birth” in accordance with the authors’ guidelines for Food and Chemical Toxicology to which the article (Chapter 3) will be submitted.

The co-authors of the article in Chapter 3, provided permission that the article be submitted for examination purposes. The article is still to be submitted to the journal; therefore, no permission was obtained from the editor of the journal.

The following signatures and statement confirm that the student and co-author provided permission to include the article (Chapter 3) in this mini-dissertation.

“By submitting this research assignment, I declare that all content of the work contained therein is my own, original work, that I am the sole author thereof and that I have not previously in it’s entirely or in part submitted it for obtaining any qualifications. I hereby provide consent for the article to be published as part of the Magister Scientiae in Dietetics mini-dissertation of Me M Entres.”

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ABSTRACT

Background

The former Transkei region of the Eastern Cape (EC) is a deep rural area characterised by a high prevalence of poverty and underdevelopment. Subsistence farming is a major source of food and maize consumption is part of a culturally distinct dietary pattern and ethnic tradition. It has furthermore been well-documented that the home-grown maize in these rural areas are extremely high in mycotoxins. Mycotoxins are low-molecular-weight metabolites that are produced by fungi that grow on the maize. Aflatoxins (AF), deoxynivalenol (DON), zearalenone (ZEA) and fumonisin (FB) are some of the major mycotoxins that influence human health. The mycotoxins are associated amongst other thing with hepatitis, liver cancer, stunting and immune suppression, gastro-intestinal disorders, anorexia, nausea, emesis, headache, chills, giddiness and convulsions, precocious pubertal changes in children, early menarche and possibly infertility and an increased risk of oesophageal and liver cancer, neural tube defects and stunting.

Very little is known about the association of maternal exposure and anthropometric measures of infants at birth. Although there is some evidence that AF affects human foetal growth, none or very little, human research has been done on the association of other mycotoxins such as DON, ZEA and FB. Therefore, the aim of this study is to determine the association between maternal multi-mycotoxin exposure and anthropometric outcomes at birth of mothers and their infants in rural areas of the EC, South Africa.

Methods

This sub-study was part of a larger prospective study (PhilaSana). The PhilaSana study was a longitudinal study focussing on the factors affecting infant and young child feeding and their growth patterns during the first 1 000 days. The study used systematic and snowball sampling to recruit pregnant women at various villages within the pre-selected area. Women were included if they were pregnant and lived in the area. Ethical approval was obtained from the Health Research Ethics Committee (HREC) at North-West University. Informed consent was obtained in the participants' first language, isiXhosa. Data were collected from mothers and children and included socio-demographic information, maternal and child general health (self-reported), maternal and infant dietary intake, anthropometric measures and maize samples. To determine mycotoxin concentration on maize, 1 kg home-grown maize samples were collected per household. The maize was analysed by LC-MS/MS for DON, ZEA and FB concentrations.

Mycotoxin (DON, ZEA and FB) exposures were measured, expressed as probable daily intake (PDI) in μgkg^{-1} body weight (bw) day^{-1} (the deterministic approach).

Based on the mycotoxin concentrations found on the maize (determined from another sub-study), and the mean raw maize intake, mycotoxin exposure was calculated. Furthermore, infant birth anthropometric measurements including weight, length, head circumference (HC) and gestational age (GA) were recorded based on the information in the Road to Health Booklet (RtHB). Lastly, the association between maternal mycotoxin exposure and infant birth anthropometric measurements and GA were determined.

Pregnant women (n=92), and infants at birth were included. Only women consuming home-grown maize were included. 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 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. Mean daily intake of cooked maize was then converted to raw maize according to recipes obtained during the development of the questionnaire and portion size photographs.

Mycotoxin exposures of pregnant women were determined according to maize contamination levels. The mean total FB (FB_1 + FB_2 +FB_3), DON and ZEA levels were obtained from the analysis of home-grown maize collected from the same households. Mean contamination levels were found to be 24.5 μgkg^{-1} for DON, 31.0 μgkg^{-1} for ZEA and 1 035.0 μgkg^{-1} for FB.

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 and was not normally distributed. Median, and IQR were reported for the mycotoxin exposures (DON, ZEA and FB), as well as for infant weight, length, HC and GA. Maternal age, weight and total raw maize intake was also reported as median and inter quartile range (IQR).

Birth weight, length, HC and GA were divided into tertiles (tertile 1 = lower third, tertile 2 = the middle third and tertile 3 = the upper third). The Krusskal-Wallis test were used to compare the tertiles against mycotoxin exposure levels. Generalized linear regressions were performed to determine the impact of maternal age, weight and maize intake (as confounders). Significant levels were set at $p \leq 0.05$. Data from alcohol consumption, tobacco use, HIV and TB were not included due to large numbers of missing values.

Results and discussion

Maternal exposure levels for DON and ZEA were lower than that of FB. Although the median of the DON exposure was under the probable maximum tolerance daily intake (PMTDI), the IQR indicated that there were participants with levels above the expected safety levels. The median and IQR for ZEA was below PMTDI levels, as opposed to the median for FB, that was much higher than the estimated PMTDI. The IQR indicated exposure levels as high as 52 μgkg^{-1} bw day^{-1} compared to the PMTDI of < 2 μgkg^{-1} bw day^{-1}.

Results indicated that DON exposure might be associated with infant birth weight, with higher DON exposure in the upper tertile, while FB exposures were associated with lower birth weight of the infants. For both DON and FB higher exposures were associated with a smaller HC. There were indications for all three mycotoxins that higher exposures resulted in infants with a lower GA, although no significant associations were found. However, generalized linear regressions indicated that although DON and FB were both associated with infant weight, maternal weight might have influenced the results. The same was found for the association of DON and FB with HC, which might have been influenced by maternal age. After adjustment for confounders no association between ZEA and any of the anthropometric measures were found.

Thus, although data indicate that DON exposure was associated with greater infant birth weight and FB with smaller infant birth weight and that both DON and FB exposures were associated with smaller HCs, associations could have been influenced by confounding factors such as maternal weight and age. More research is required to better understand the associations between DON, ZEA and FB and infant anthropometric measures at birth. Furthermore, more research is needed to determine additional factors that might influence maternal health and the anthropometric measures of infants at birth.

KEYWORDS: Mycotoxins, deoxynivalenol (DON), zearalenone (ZEA), fumonisins (FB), anthropometry at birth, gestational age (GA)

LIST OF ABBREVIATIONS

AF	Aflatoxin
BMI	Body mass index
bw	Body weight
CHO	Carbohydrates
CVD	Cardiovascular disease
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
EC	Eastern Cape
FA	Fatty acid
FAO	Food and Agriculture Organization
FB	Fumonisins
FFQ	Food frequency questionnaire
GA	Gestational age
GLUT5	Fructose transporter, type 5
GWG	Gestational weight gain
HC	Head circumference
HREC	Health Research Ethics Committee
HIV	Human immunodeficiency virus
IGFs	Insulin-like factors
IL-1	Interleukin 1

IL-6	Interleukin 6
IL-8	Interleukin 8
IQR	Interquartile range
IOM	Institute of Medicine
IGFs	Insulin-like growth factors
IUGF	Intrauterine growth failure
IUGR	Intrauterine growth restriction
kg	Kilogram
LBW	Low birth weight
LOA	Loss of appetite
MDI	Mean daily intake
µg	Microgram
NTDs	Neural tube defects
NAFLD	Non-alcoholic fatty liver disease
n	Number
PDI	Probable daily intake
PMTDI	Probable maximum tolerance daily intake
RAPP	Ratio and Portion size plate
REE	Resting energy expenditure
RtHB	Road to health booklet
ROS	Reactive oxygen species
SD	Standard deviation

SGA	Small for gestational age
SGLT-1	Sodium – glucose linked transporter 1
SIR	Systemic inflammatory response
TB	Tuberculosis
USA	United State of America
ZEA	Zearalenone

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CHAPTER 1: INTRODUCTION

1.1 **Introduction**

Food contaminated by mycotoxins is considered to be a global public health priority and pose a threat to humans and animals, as well as world economies regarding industry and international maize exports (Bryden, 2007; Miller, 1998). In this regard, mycotoxins have the capacity to cause numerous adverse health effects. Of the 300-400 known mycotoxins, aflatoxins (AF), deoxynivalenol (DON), zearalenone (ZEA) and fumonisin (FB) are considered the most important regarding their effect on human health (Bennet & Klich, 2003; Marasas *et al*., 2008; Maresca & Fantini, 2010). These mycotoxins are produced by food-borne fungi and include *Aspergillus spp*. (AF), *Penicillium spp.* and *Fusarium spp.* (DON, ZEA and FB) (Miller, 2002).

The name mycotoxin is a combination of the Greek word for fungus 'mykes' and the Latin word 'toxicum' meaning poison. The term 'mycotoxin' is usually reserved for the relatively small, toxic chemical products formed as secondary metabolites by a few fungi that readily colonise crops in the field, after harvest or during storage (Turner *et al*., 2009). Among the thousands of species of fungi, approximately 100 belongs to genera *Aspergillus, Penicillium* and *Fusarium* which are known to produce mycotoxins (Wagacha & Muthomi, 2008). Due to their various toxic effects and thermal stability, the presence of these mycotoxins on food and feeds are potentially hazardous to the health of both humans and animals (Miller, 2002). There is furthermore ample evidence that the inhabitants of sub-Sahara Africa are experiencing chronic high dietary exposure to these mycotoxins (Wagacha & Muthomi, 2008).

Mycotoxin management methods used in commercial farming cannot realistically be used in subsistence farming. This is mostly because of the characteristics of the food systems and the technological infrastructure resulting in uncontrolled mycotoxin levels. The threat is worsened by the fact that staple diets in many African households are grain crops such as maize. The maize is highly susceptible to mycotoxin contamination (Wagacha & Muthomi, 2008).

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. Outbreaks of the related mycotoxicoses are clustered and related to specific geographical areas (Marasas *et al*., 2008). Vulnerable populations with a daily staple diet such as rural maize producing subsistence-farming populations (with poor agricultural practices) are often susceptible to chronic high exposure levels (Marasas *et al*., 2008). Chronic exposure of mycotoxins (even at low levels), in an unvaried diet, may incur adverse health outcomes or possibly worsen other existing disease conditions (Bryden, 2007). Furthermore, the co-occurrence of mycotoxins, their possible

synergistic and/or additive effect is currently poorly understood (Eaton & Klaassen, 2001; Scudamore & Patel, 2009; Waśkiewicz *et al*., 2012).

Unfortunately, very little is known about the measurement, risk assessment, exposure levels and health effects of these mycotoxins (Gelderblom *et al*, 2008; Marasas *et al*., 2008). The determination of mycotoxin exposure forms a vital part of human risk assessment and the processes used. However, a lack of monitoring mycotoxin levels in South African maize used for human consumption further contributes to the uncertainty when determining risks (Gelderblom *et al*, 2008; Marasas *et al*., 2008).

Chronic exposure in animals, such as pigs and mice, changes the immune system, affecting susceptibility to infections, and possibly cause growth faltering (Kumi *et al.,* 2014). The mechanism of growth faltering remains unclear. Higher doses used in some animal models may reflect DON-induced food rejection; though at more moderate exposures, it may reflect poor uptake and retention of nutrients due to DON-induced damage and inflammation of the intestinal mucosa (Kumi *et al.*, 2014). Deoxynivalenol has been shown to transfer to the foetus of pregnant sows (Tiemann *et al*., 2008), and exposure during pregnancy in sows has been linked to restrictions in both growth (Tiemann *et al*., 2008) and immune function. Deoxynivalenol was also present in the liver and kidneys of the foetus after exposure of the sow (Tiemann *et al*., 2008). Deoxynivalenol further restricts the growth of mice at doses lower than what will restrict appetite, and is associated with changes in growth hormone levels controlled by insulin-like growth factors (IGFs) (Voss, 2010).

Aflatoxins are not present in South African commercial and home-grown maize, and were thus excluded from the present study (Burger *et al*., 2013). Various studies on the fungal and mycotoxin contamination of home-grown maize in the Amathole District Municipality in rural Eastern Cape (EC), have established a consistent pattern of *Fusarium verticillioides* infection and FB contamination (Rheeder *et al*., 1992; Shephard *et al*., 2007; Van der Westhuizen *et al.,* 2008; Van der Westhuizen *et al.,* 2010). These studies have shown that large variations in contamination levels of individual maize samples collected in different years can occur. The presence of FB in maize grains has been associated with the risk of oesophageal cancer in inhabitants of rural EC, China and north-eastern Italy (Peraica *et al.,* 1999). Recently high levels of two other mycotoxins, DON and ZEA were also observed in urine of adults living in high-FB exposure areas in the EC (Shephard *et al.,* 2013) (Figure 1-1).

Figure 1-1: Maize samples from the rural Eastern Cape

The former Transkei region (currently part of the Amatole District Municipality) of the EC (Figure 1.2) is a deep rural area characterised by a high prevalence of poverty and underdevelopment.

Figure 1-2: An example of a subsistence farm in rural Eastern Cape

A limited number of studies have been conducted to determine the association of these mycotoxins on infant growth (Smith *et al*., 2012). Furthermore, very little is known about the association of high levels of maternal exposure, and the growth and general health of the foetus or infants (Lombard *et al*., 2014). There is some evidence that AF affects foetal growth, but no research has been done on the association of other mycotoxins such as DON, ZEA and FB (Lombard, 2014).

The data presented in this mini-dissertation was a sub-study of the larger PhilaSana study. The aim of the PhilaSana study was to conduct an in-depth investigation into the association between multiple mycotoxin exposures, and other factors associated with infant and young child growth during the first 1 000 days of life. The PhilaSana study was a longitudinal study including pregnant women and their infants. The women and their infants were visited every six months to collect data.

The PhilaSana large study included the following objectives:

1. To develop and validate culturally specific (to the area) infant and young child (0-24 months) dietary assessment tools with the use of food photograph series;

2. To assess and describe a variety of known factors, [including socioeconomic, household food security, demographic, health (mother and infant), diet (mother and infant) and infant feeding practices], contributing to childhood stunting during the first 1 000 days of life;

3. To monitor infant growth-related health indicators and nutritional / dietary factors during the first 1 000 days of life;

4. To estimate multi-mycotoxin [DON, ZEA and total FB (FB_1 + FB_2 + FB_3)] exposure as probable daily intakes among children during the first 1 000 days;

5. To determine the possible relationship between infant growth, and multi-mycotoxin exposure during the first 1 000 days of life.

1.2 **Problem statement**

Home-grown maize from areas in Amathole District Municipality in rural EC are known to be contaminated with high levels FB, and to a lesser extent by DON and ZEA (Shephard *et al.,*2013). This might pose an important health risk to those exposed (Shephard *et al.,* 2013). Commercial maize also consumed in some areas, generally contains far lower levels of mycotoxins, but could still pose a risk as it is consumed daily in large quantities (Burger *et al*., 2013; Lombard *et al*., 2014). There is a possible association between AF and impaired growth in children (Gong *et al.,* 2003; Smith *et al.,* 2012) and therefore the role of other mycotoxins on infant growth and development are also of importance (Smith *et al.,* 2012). However, very little is known about this (Lombard, 2014).

In low- and middle-income countries, many individuals are not only malnourished, but are also chronically exposed to high levels of toxic fungal metabolites in their diet. The heterogeneous distribution of mycotoxins within a given food commodity hinders accurate exposure

measurement and hence associations between exposure and human health have been difficult to establish (Gong *et al*., 2003). Mycotoxin exposure due to maize consumption of the mother during pregnancy and anthropometry of infants at birth are both important in this respect.

The association of multi-mycotoxin exposure during pregnancy and foetal growth is unknown due to limited research available. As reported by Turner and colleagues (2003), growth faltering in Ghanaian children have been associated with dietary exposure to AF, especially during the weaning period. However, with animal studies, exposure to low levels of AF had growth faltering outcomes in utero. This study also showed a strong correlation between maternal AF exposure during pregnancy and infant growth.

Very little is known concerning DON, ZEA and FB in maternal exposure and anthropometry of infants at birth in Africa, due to the lack of effective risk assessment methods (Lombard, 2014).

This sub-study therefore focuses on prenatal maternal mycotoxin exposure assessment and the association between the exposure and infant anthropometric growth at birth and gestational age.

1.3 **Aim of the sub-study**

The aim of this sub-study was to determine the association between mycotoxin exposure of pregnant women and anthropometric measures of infants at birth and gestational age.

1.4 **Objectives of this sub-study**

The following specific objectives have been identified to reach the aim of the sub-study and are based on objectives 2, 3, 4 and 5 of the PhilaSana study:

- To estimate multi-mycotoxin (DON, ZEA and FB) exposure levels of pregnant women;
- To obtain anthropometric measures of infants at birth and their gestational age;
- To determine the association between maternal multi-mycotoxin exposure and anthropometric measures of infants at birth and their gestational age.

Data used from the larger PhilaSana study to reach each objective were obtained during the first (during pregnancy) and second visit of the large study.

1.5 **Layout of the mini-dissertation**

Chapter 1 of the mini-dissertation is an introduction of the large study, as well as the sub-study. It provides a short summary of mycotoxins and the population the study is conducted in. The

problem statement is included in this chapter and discussed. The chapter further discusses the aims and objectives of the larger PhilaSana research project, as well as the aims and objectives of this sub-study that forms part of the large project.

Chapter 2 includes the detailed literature review of the topic, covering the background of the topic, studies that have been done previously and a conclusion on the studies to summarise current literature. For easier reading and a better understanding of the known mechanisms of the different mycotoxins, a flow chart and summary boxes were designed. This was also created to visually show the possible affected pathways, leading to foetus growth faltering, after mycotoxin exposure in the maternal diet.

In Chapter 3 the article is presented. The article will be submitted to Food and Chemical Toxicology. It is written according to the author guidelines of this international peer reviewed journal. The article will include an introduction and methodology, results with a detailed discussion and a summarized conclusion and key messages.

Chapter 4 consists of a discussion regarding the overall findings of the study, as well as the conclusion and recommendations for further studies. Chapter 5 provides all the annexures of the mini-dissertation:

Annexures added

1. Health Research Ethics Committee approval
2. Questionnaires:

- General health questionnaire
- Validated food frequency questionnaire designed for this population
- Anthropometric measurements form
- Consent forms

3. Food and chemical toxicology requirements
4. Mycotoxin analyses procedures

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Mycotoxins are toxic chemical products produced (Abu-Saad & Fraser, 2010) as secondary metabolites by fungi growing on crops in the field or after harvest (Turner *et al.,* 2009). *Fusarium verticilliodies* and *F. proliferatum* fungi, frequently contaminate maize and other cereal grains (Marasas *et al*., 2001; Marin *et al*., 2004), growing best at high temperatures in humid climates (Marin *et al*., 1995). It can also occur during processing and storage and thus it affects quality and food safety levels (Sforza *et al.,* 2006).

Although there are thousands of mycotoxin species, approximately 100 belongs to genera *Aspergillus, Penicillium* and *Fusarium* (Wagacha & Muthomi, 2008). Of the 300 - 400 known mycotoxins, the most important mycotoxins affecting human health, include deoxynivalenol (DON), zearalenone (ZEA), fumonisin (FB) and aflatoxins (AF) (Wagacha & Muthomi, 2008). Deoxynivalenol, ZEA, and FB are produced by fungi of the *genera Fusarium* (Wagacha & Muthomi, 2008). A systematic review by Lombard (2014) indicates that various studies have been conducted regarding the exposure of AF and FB, however, very little is known about DON and ZEA (Lombard, 2014).

2.2 Mycotoxin exposure

There is ample evidence that the inhabitants of sub-Saharan Africa are experiencing dietary exposure to food-borne mycotoxins, particularly AF and FB (Wagacha & Muthomi, 2008). As presented in various studies (Burger *et al.,* 2013; Gelderblom *et al.,* 1988; Lombard *et al.,* 2014; Reeder *et al*., 1992; Shephard *et al.,* 2007; Van der Westhuizen *et al.,* 2008) people from rural areas in the Eastern Cape (EC) are known to be exposed to multiple mycotoxins.

This study area is known to be underdeveloped with a high prevalence of poverty. The majority of food sources come from subsistence farming, with maize consumption as part of the culturally distinct dietary pattern and ethnic tradition in this area (Lombard *et al.,* 2013; Lombard *et al.,* 2014).

Various studies on mycotoxin contamination of home-grown maize in the Amathole District Municipality have established a consistent pattern of Fusarium verticillioides infection and FB contaminations (Rheeder *et al.,* 1992; Shephard *et al.,* 2007; Van der Westhuizen *et al.,* 2008; Van der Westhuizen *et al.,* 2010). These studies have shown that large variations in contamination levels of individual maize samples collected in different years can occur.

Individual samples of maize intended for human consumption in this area have been shown to be contaminated with various types of FB (Shephard *et al.,* 2013).

Commercial maize, that is rarely consumed in the studied area, generally contains far lower levels of mycotoxins, but could still pose a risk as it is consumed in large quantities (Burger *et al.,* 2010; Burger *et al.,* 2013; Lombard *et al*., 2014,). In general, it would appear that the high consumption of maize in this region increased the risk of the population to be exposed to a mixture of mycotoxins such as DON, ZEA and FB (Shephard *et al*., 2013).

Unfortunately, very little is known about the potential impact of multi-mycotoxin exposure (DON, ZEA and FB) on pregnancy. A conceptual framework has been developed to indicate what is currently known, and includes both human and animal studies. From here on summary boxes will provide a link / explanation to various relevant topics and how it fits into the conceptual framework (Figure 2-1). No information on ZEA could be included as too little information is available on the topic.

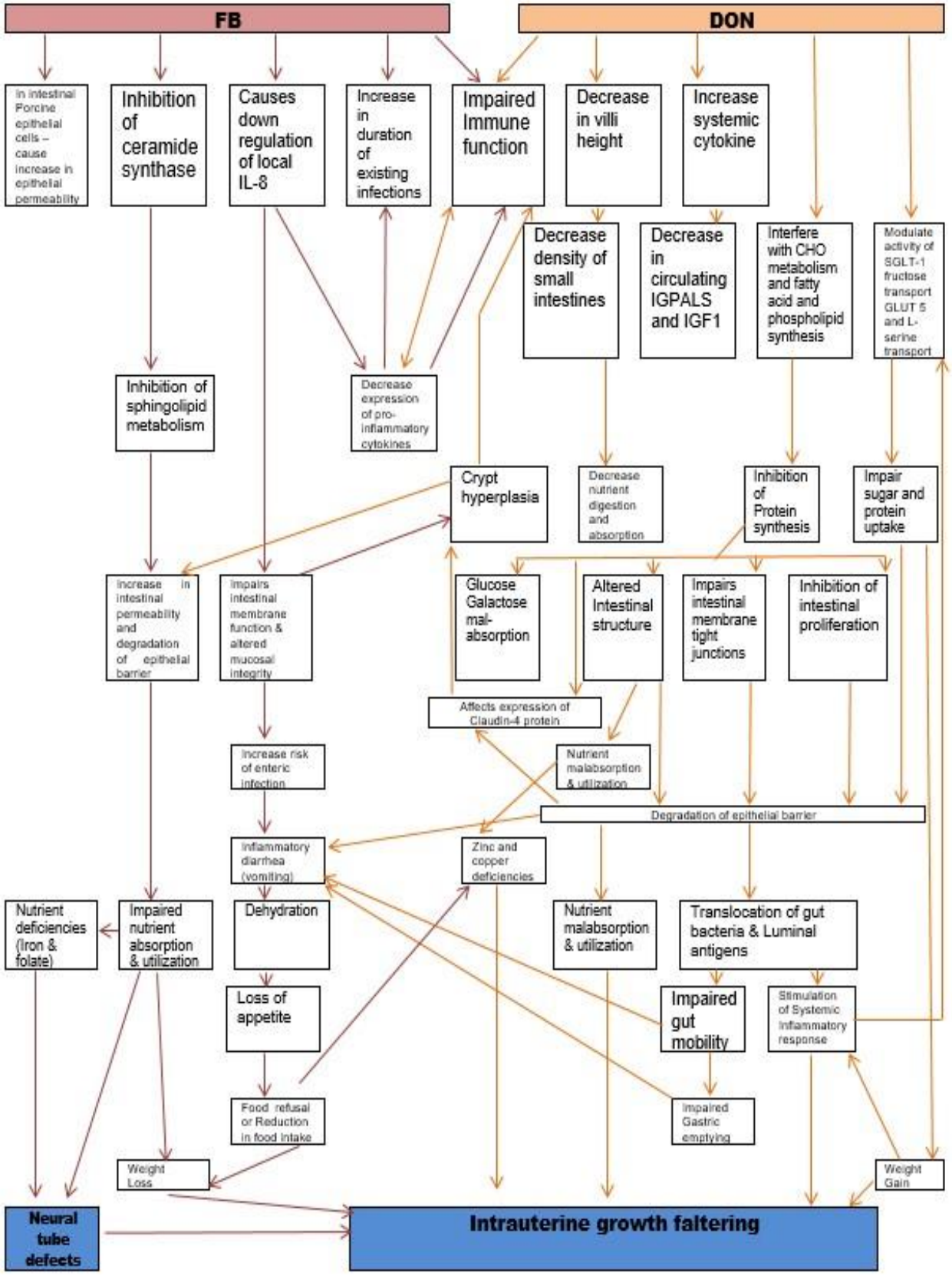

Figure 2-1: Conceptual framework – Elaborated on and adapted from Smith *et al.*, 2012.

2.2.1 Deoxynivalenol

Deoxynivalenol is primarily a pre-harvest problem, as reported by Pestka and Smolinski in 2005, however, it can also occur during storage in areas where moisture content is less strictly controlled. Mycotoxin exposure from grain handling has been suggested (Garon *et al.,* 2006; Hopton *et al.,* 2010), whereby an association between grain farming and perinatal health in Norwegian farmers was reported (Kristensen *et al.,* 1997); with the highest risk in seasons with poor quality harvest.

Although, less toxic than other trichothecenes, DON is more common in the seeds of safflower, barley, rye, and wheat and in feed mixtures (Miller *et al.,* 2001). When ingested in high doses by animals, it causes nausea, vomiting and diarrhoea, while in small doses, it can cause weight loss and food refusal (Miller *et al.,* 2001). Due to the symptoms induced by DON, it is known as vomitoxin (Miller *et al.,* 2001). Deoxynivalenol has also been reported as the causative agent of gastrointestinal poisonings (Luo, 1994) and as a suspected etiologic agent of gastroenteritis in children (CDC, 1999). Summary box 1 provides the basic information on the association with maternal diet and infant growth faltering.

Summary box 1

With maternal exposure to DON, there are two main pathways that directs in to nausea, vomiting and / or diarrhoea, causing loss of appetite refusal of food intake, leading to involuntary weight loss:

1. DON exposure leads to an increase release of systemic cytokines, causing protein synthesis to be inhibited, resulting in intestinal proliferation to be inhibited and / or the tight junctions in the gut epithelial to be impaired (crypt hyperplasia). Crypt hyperplasia causes the epithelial barrier to degrade and the density of the small intestine to decrease, resulting in bacterial translocation and a decrease in gut mobility (impaired gastric emptying)

2. Inflammatory diarrhoea, nausea and vomiting are the result of impaired gastric emptying. Nausea and vomiting may result in altered food intake or complete food refusal, leading to weight loss. Diarrhoea and vomiting leads to dehydration (due to high volumes of fluid losses), causing lethargy and loss of appetite, decreased food intake and weight loss.

Trichothecenes (such as DON) cause protein synthesis inhibition (McLaughlin *et al.,* 1977) and are known to cause neurotoxicity, immunosuppression and renal toxicity (Richard, 2007). Deoxynivalenol, is non-classifiable as carcinogenic to humans (WHO/IARC, 1993), however, it

can cause harmful health effects like anorexia, weight loss, malnutrition, endocrine dysfunction and immune alterations (Pestka, 2010).

The estimated daily intake of DON ranges from 0.77 to 2.4 ug/kg body weight/day (FAO/WHO, 2001). Because of its effects in humans along with its resistance to food processing, DON has driven the attention of food security efforts to control its presence in food (Amuzie & Pestka, 2010).

In animal studies, DON induces a spectrum of effects in farm and laboratory animals including emesis, immunotoxic effects, and suppression of appetite and growth (Voss, 2010). Understanding the biochemical mechanisms for DON's growth effects is of paramount importance for accurately assessing risks of this common mycotoxin as well as establishing appropriate management and regulatory strategies (Amuzie & Pestka, 2010). Following oral exposure, DON is rapidly absorbed into the tissues of monogastric animals and can reach peak plasma concentrations within 15 - 30 min after dosing (Amuzie *et al*., 2008; Prelusky *et al.,* 1988).

Deoxynivalenol is detoxified by deep oxidation via gut microflora (He *et al.,* 1992; Swanson *et al.,* 1988) and by glucuronidation in the liver (Obol'skii *et al*., 1998). Upregulation of proinflammatory cytokines such as interleukin 6 (IL-6), tumour necrosis alpha, and interleukin 1 (IL-1) is a central outcome of DON exposure to macrophages *in vitro* and in spleen, liver, and lung *in vivo* (Amuzie *et al*., 2008; Azcona-Olivera *et al.,* 1995; Dong *et al*., 1994; Zhou *et al.,* 1997).

In other studies, done by Rotter *et al*. (1996), as well as by Pestka and Smolinski (2005), chronic exposure in animals modulated the immune system (affecting susceptibility to infections), and also caused growth faltering. Deoxynivalenol has been shown to transfer to the foetus of pregnant sows (Tiemann *et al*., 2008), and exposure during pregnancy in sows has been linked to restrictions in both growth (Tiemann *et al.,* 2008) and immune function. Deoxynivalenol was subsequently observed in the liver and kidney of the foetus following exposure (Tiemann *et al.,* 2008). Deoxynivalenol further restricts the growth of mice at doses lower than those restricting appetite, an effect associated with changes in growth hormone levels controlled by insulin-like growth factors (IGFs) (Voss, 2010).

In addition, *Fusarium* toxins from grains have been proposed to induce labour at an early stage of pregnancy (reviewed by Pestka & Smolinski, 2005). Given that DON can cross the placenta of animals (Goyarts *et al.,* 2007; Tiemann *et al.,* 2008) it is likely that in-utero exposure to DON will occur in humans. The detoxification capacity of the foetus will not be fully developed, at a

time of rapid growth and cell turnover (Myllynen *et al.,* 2009); thus, pregnancy may represent a critical window for DON exposure.

Growth faltering was highlighted as a likely consequence of DON exposure (Pestka & Smolinski,

2005), and thus exposure during pregnancy may be of particular importance.

The possible health effects of DON in humans are still being investigated and this description of intake in a population that may be particularly vulnerable provides considerable motivation to conduct epidemiological studies (Hepworth *et al.,* 2012).

All animals are highly sensitive to DON, and evidence has been reported by Pestka and Smolinski (2005) that exposure to DON in animals leads to systematic absorption in plasma, tissue and body fluids (including blood, milk, urine and faeces).

2.2.2 Zearalenone

Among the cereals and grains in which zearalenone (ZEA) occurs, maize has been shown to have the highest contamination levels. The growth of ZEA-producing fungi mainly occurs in temperate conditions and high levels of ZEA in cereals are mainly associated with wet mild weather and improper storage in high moisture environments (Gareis, 2003; Goertz *et al*., 2010; Marques *et al.,* 2008).

Exposure to this mycotoxin has been linked to some cases of precocious puberty in girls (Massart *et al*., 2008). Besides estrogenic effects, ZEA can also cause toxicity by production of reactive oxygen species (El GolliBennour *et al.,* 2009). Although this mycotoxin is also classified as non-carcinogenic to humans (WHO/IARC, 1993) it is still of interest due to estrogenic activity along with its anabolic effects.

The association between the consumption of mouldy grains and hyperestrogenism in pigs has been observed since 1920. High concentrations of ZEA in pig feed may cause disturbances related to conception, absorption and other problems. Reproductive problems have also been observed in cows and other species (El-Nezami *et al.,* 2002).

Zearalenone is unfortunately the most understudied mycotoxin, with very limited human studies in growth faltering available to date and thus very little can be discussed about it. It does however emphasize the importance of more research regarding ZEA and its health effects.

2.2.3 Fumonisin

Three groups of FBs exist, including FB_1, FB_2 and FB_3. Due to favourable fungal growth conditions, FB often co-occur with AF, especially in maize (Kpodo *et al.,* 2000; Kimanya *et al.,* 2008; Sun *et al.,* 2011). Fumonisin contamination has been associated with the growing of maize but not so much during harvesting and storage (Kimanya *et al.,* 2008; Sun *et al.,* 2011).

The determination of FB exposure with validating biomarkers is very important in the human risk assessment process. However, a lack of mycotoxin surveillance in rural subsistence farming areas in South African further contributes to the uncertainty when determining risk (Gelderblom *et al.,* 2008; Marasas *et al.,* 2008).

Neural tube defects (NTDs) are embryonic defects of the brain and spinal cord resulting from failure of the neural tube to close. Spina bifida and anencephaly (failure of anterior tube closure) are the most common forms of NTD (Voss *et al*., 2009). Fumonisins have been implicated as a risk factor for NTDs (Gelineau-van Waes *et al*., 2009; Missmer *et al.,* 2006; Suarez *et al.,* 2012). Hendricks (1999) proposed that FB were involved in a cluster of NTDs that affected babies born to Mexican-American women living in the Texas counties bordering Mexico in 1990 - 1991 (Missemir *et al.,* 2006; Voss *et al*., 2009).

Multiple observations suggested that the NTDs outbreak and the epizootics shared a common etiology. Maize meal samples collected in the USA during the NTDs outbreak had relatively high average FB levels (Hendricks, 1999). Other regions with high maize-based food consumption and documented FB contamination (Dombrink - Kurtzman & Dvorak 1999; Yoshizawa *et al.,* 1994) also had high prevalence of NTDs (Mutchinick *et al.,* 1999). The findings by Missemir *et al.* (2006) suggest that FB exposure increases the risk of NTDs, proportionate to dose, up to a threshold level, at which point foetal death may be more likely to occur.

The association between FB with NTDs became of interest as these mycotoxins disrupt the folate receptor in cells. The role of sphingolipids and cholesterol, major constituents of lipid rafts associated with the folate receptor, is critical for the early embryonic development. The induction of NTDs was only partly prevented by folate supplementation (Missemir *et al.,* 2006).

Recent in vitro and animal studies provide further support for the hypothesis that NTDs occur with exposure to FBs (Flynn *et al*., 1997; Gelineau-van Waes *et al.,* 2009; Sadler & Tam, 2002; Stevens & Tang 1997; Wang & Herron, 1991). Fumonisins disrupt sphingolipid metabolism in the gastrointestinal tract of mice (Enongene *et al.,* 2000), damages intestine permeability (Lallès *et al.,* 2009), and has been associated with decreased food consumption, growth retardation and body weight in piglets (Dilkin *et al.,* 2003). Summary box 2 summarizes the mechanism.

Summary box 2

Maternal FB exposure inhibits the enzyme ceramide synthase leading to inhibition of sphingolipid biosynthesis, resulting in 2 pathways that can get interrupted:

1. Altered sphingolipid metabolism causes an increase in free sphingoid bases that disturbs the signalling cascade involved in embryonic morphogenesis by functioning as ligands for S1P receptors leading to the deregulation in cell proliferation, cell differentiation and cell migration.

2. Inhibited sphingolipid metabolism causes a depletion of downstream glycosphingolipids which impair the expression and function of GP1-anchored folate receptors causing blocking in absorption of folate follows resulting in folate deficiency in the pregnant woman, increasing the risk and prevalence of NTDs at birth of the infant.

In India, a foodborne disease outbreak in 1995 characterized by diarrhoea and abdominal pain was reported to be associated with consumption of maize and sorghum (Bhat *et al.,* 1997). Samples collected from patients' households were all positive for FB and contained higher levels of FB_1 than those of non-patients. FB_1 was therefore considered to contribute to the outbreak. These findings have raised concern that FB may induce intestinal enteropathy, a subclinical condition of the small intestine, characterized by reduced absorptive capacity and increased intestinal permeability, therefore mediating stunting (Smith *et al.,* 2012). Summary box 3 links these findings to the mechanisms in the conceptual framework.

Summary box 3

FB exposure will affect the intestinal porcine epithelial cells leading to an increase in the epithelial permeability and a decreased density of the small intestine, flattening villi and crypt hyperplasia will follow.

Exposure to FB during pregnancy interrupts the sphingolipid metabolism leading to intestinal epithelial permeability and translocation of gut bacteria; resulting in altered gut mobility and gastric emptying that will have inflammatory diarrhoea and even vomiting as a result leading to dehydration and loss of appetite, causing reduction or complete refusal of food. Weight loss follows, that increases the risk of and development of growth faltering or IUGR.

Fumonisin B_1, the most predominant and well-studied isoform, is nephrotoxic and hepatotoxic in several species, and a possible human carcinogen (WHO/IARC, 2002; JECFA, 2012). FB_1, has further been associated with liver and oesophageal cancers in high-exposure populations (Alizadeh *et al*., 2012; Chu & Li, 1994; Persson *et al*., 2012; Rheeder *et al*., 1992). The presence of FB in maize has been associated with cases of oesophageal cancer in inhabitants of the rural areas in the Eastern Cape (South Africa), in China and in north-eastern Italy (Peraica *et al.,* 1999).

The mechanism of action of FB_1 induced NTDs is the inhibition of uptake and metabolism of folic acid (Stevens & Tang, 1997) while its carcinogenic effects are related to the overall disruption of lipid metabolism, membrane structure and cellular signal pathways (WHO/IARC, 2002; JECFA, 2012). The carcinogenic character of FB does not seem to involve interaction with DNA (Coulombe, 1993).

2.3 **Subsistence farming**

In most low- and middle-income countries, farming remains important for rural households as their primary food source. According to the latest Food and Agriculture Organization (FAO) estimates, 805 million are exposed to food insecurity or hunger, with 50% of the global population exposed to hunger living in smallholder subsistence farming communities (Shisana *et al.,* 2014).

Many of these countries are politically unstable and due to poor socio-economic status, underdeveloped agricultural practices and weather changes, control of mycotoxins is difficult or in some cases totally absent (Gbashi *et al.,* 2018). The interaction of politics, economy and technology eventually determine the impact on health, which will differ between countries. Specific and simple measures should therefore be devised and introduced to reduce the levels of mycotoxin exposure in maize, by targeting populations at risk (Gbashi *et al.,* 2018).

Household food security is defined as year-round access to adequate, nutritious and safe food to meet the nutritional needs of all household members. Although South Africa produce an adequate food supply at national level, it does not necessarily translate into food security at household level (Shisana *et al.,* 2014). The recent statistics show that 28.3% households are at

risk of hunger and another 26% are actually food insecure (Shisana *et al.,* 2014). It is also expected that child- or female-headed households, in rural areas, are most at risk (Govender *et al.,* 2016).

Subsistence farming is usually an important way to improve food security and nutrition. Unfortunately, even if agricultural interventions can improve economic growth and reduce poverty, there is little evidence that this positively affects nutrition security (Sibhatu & Qaim, 2017). Despite various interventions to reduce food insecurity and global hunger, food insecurity and undernutrition remain highly prevalent in many countries. It is currently estimated that approximately 11% of the world's population is chronically undernourished, indicating a deficiency of total calories (Sibhatu & Qaim, 2017).

Furthermore, one-third of the global population suffers from micronutrient malnutrition, especially in countries in Asia and Africa, with an increase in incidence in sub-Saharan Africa. The people living here are usually more negatively affected by food insecurity (Sibhatu & Qaim, 2017).

In low- and middle-income countries, many individuals are not only malnourished but also chronically exposed to high levels of mycotoxins via their diet. The heterogeneous distribution of mycotoxins within a given food commodity, hinders accurate exposure measurement and hence associations between exposure, acute or chronic, and human health have been difficult to establish (Gong *et al*., 2003).

2.4 **Maternal health**

Women of child-bearing age (especially pregnant and lactating women), infants and young children are amongst the most nutritionally vulnerable population groups for malnutrition (Adu-Afarwuah *et al.,* 2017). The primary causes include factors such as inadequate food intake, poor nutritional quality of diets, frequent infections and short inter-pregnancy intervals (Adu-Afarwuah *et al.,* 2017). In Africa for instance, 20% of women have a low body mass index (BMI) because of chronic hunger, especially seen in areas with a high prevalence of human immuno-deficiency virus (HIV). Anaemia, vitamin A and zinc deficiencies are highly prevalent. Unfortunately, the results of the above factors are often reflected in low pregnancy weight gain and high infant and maternal morbidity and mortality (Adu-Afarwuah *et al.,* 2017). In maternal pregnancy dietary intake, mycotoxin exposure may occur in-utero and through breastfeeding, predisposing children to the risk of chronic exposure from a very early stage of life (Sherif *et al*., 2009). Unfortunately, validated exposure biomarkers for mycotoxin exposure are limited and are avital part of the risk assessment process.

2.4.1 Pregnancy weight gain and birth outcomes

According to Morrison and colleagues (2016), maternal malnutrition primarily results from a diet with inadequate calorie intake. This then results in undernutrition. However, maternal malnutrition can also follow from a diet with reduced micronutrient intake due to for instance poor diversity. Either way, these occurrences will have an impact on the pregnancy as well as infant anthropometric outcomes at birth (Morrison & Regnault, 2016).

Unfortunately, very little data is available on the link between habitual diet and neonatal anthropometric measurements and gestational age at birth. Previous studies mostly focussed on investigating specific nutrients or food items, and the effect of overall diet was often overlooked. To date, dietary patterns have mostly been associated with complex conditions or diseases such as cardiovascular diseases (CVD) (Cetin *et al.,* 2010).

A study conducted by Budree *et al.* (2017) indicated that a high-quality diet during pregnancy, was positively associated with a greater birth weight and birth length, especially during the first trimester. There is a wide range of nutrient interactions and because of this it is crucial that maternal dietary patterns should be investigated in more depth, to determine the association between maternal nutrition and foetal growth (Budree *et al*., 2017). Unfortunately, cultural, geographical, and regional influences in areas, affect the maternal dietary patterns, and thus different anthropometric health outcomes among infants should be expected at birth (Budree *et al.*, 2017).

2.4.2 Maternal diet and its effect on intra-uterine growth

Intrauterine growth restriction (IUGR) refers to poor growth of a foetus while in the mother's womb during pregnancy (Budree *et al.,* 2017). Outcomes at birth are measured by anthropometry. Anthropometric measurements usually include body weight, length and head circumference (HC) of new-borns. It is also seen as the primary determinants of impaired foetal growth, intrauterine environment, and maternal nutrition (Budree *et al.,* 2017).

Intrauterine growth restriction affects annually up to 30 million new-borns in developed countries. Unfortunately, this figure can increase up to six times in low- and middle-income countries (Black *et al.,* 2013) with the highest prevalence mostly in South East Asia, Africa and Latin America (Ashworth, 1998; Imdad & Bhutta, 2011). It is strongly associated with the prevalence of perinatal mortality and morbidities, such as premature births, hypoglycaemia and hypothermia. These conditions have a cascading effect on the infant. It could further impact on the infant in later life, in the form of poor cognitive development leading eventually to neurologic impairment in adulthood, as well as an increased risk of cardiac, renal and pulmonary diseases,

(Imdad & Bhutta, 2011) obesity, diabetes mellitus (DM), endothelial dysfunction, non-alcoholic fatty liver diseases (NAFLD) and kidney disease (Budree *et al.,* 2017).

Furthermore, impaired foetal growth, especially HC, is also associated with non-optimal neuro-developmental outcome (Budree *et al.,* 2017). Summary box 4 link DON and FB exposure to possible IUGR.

Summary box 4

DON exposure causes endothelial cell dysfunction, resulting in the inhibition of protein synthesis. Inhibited protein synthesis will cause altered intestinal structures, thus a decrease in nutrient absorption and utilization. Micronutrient deficiencies will occur due to the decreased absorption and utilization of nutrients.

FB exposure leads to the inhibition of ceramide synthase and the inhibition of the sphingolipid metabolism. Inhibitions will increase intestinal permeability and cause epithelial barrier degradation resulting in impaired nutrient absorption and utilization, as well as micronutrient deficiencies; focusing on iron and folate.

Intrauterine growth restriction at birth is amongst other things because of poor maternal nutritional status before conception, during conception and during pregnancy (Ferro-Luzzi *et al*., 1998; Imdad & Bhutta, 2011; Martorell *et al*., 1998). The primary focus of preventing IUGR during pregnancy is to focus on maternal and foetal nutrition. Ideally energy and macronutrient intake as well as micronutrient intake should be optimal in relation to their respective needs (Ferro-Luzzi *et al.,* 1998; Imdad & Bhutta, 2011; Martorell *et al.,* 1998). Maternal nutrition includes every aspect in the supply of the infant, thus maternal food consumption, circulating concentrations, uteroplacental blood flow, and nutrient transfer across the placenta (Stephenson & Symonds, 2002).

Low birth weight (LBW) is officially defined as a birth weight less than 2 500 g (NIH, 2013). It most often results from premature delivery, intrauterine growth failure (IUGF) or disruption, or a combination thereof (Abu-Saad & Fraser, 2010). Small for gestational age (SGA) does not necessarily indicate IUGF (Black *et al.,* 2013). Even with birth weight in normal ranges, it may hide low birth weight that is below the genetic expectations. This could also be due to poor maternal and thus foetal nutrition (Stephenson & Symonds, 2002).

Low birth weight is one of the most important secondary factors for neonatal deaths. This is especially true in low- and middle-income countries (Abu-Saad & Fraser, 2010). However, regardless of the above, LBW is strongly associated with perinatal morbidity and increased risk of long-term disability (Abu-Saad & Fraser, 2010). Low birth weight infants are also at greater risk of developing iron deficiency anaemia. This could lead to impaired development and eventually influence neurodevelopment (Abu-Saad & Fraser, 2010).

Preterm birth, defined as gestational age less than 37 weeks (NIH, 2013), contributes substantially to the incidence of low birth weight. It is also the leading underlying cause of infant mortality (Abu-Saad & Fraser, 2010). Infants with birth weights below the 10th percentile for their gestational age (GA) are classified as SGA, and even if born to term, they are at an increased risk of neonatal mortality (Abu-Saad & Fraser, 2010; Black *et al.,* 2013).

Western dietary patterns with primarily unhealthy food choices are not able to provide the increased requirements for the pregnant mother and the infant (Hajianfar *et al.,* 2018). The Mediterranean diet also proved to have a positive outcome during pregnancy, with decreased risk of IUGR. Another dietary pattern result indicated that women that consume more wheat and cereal related dietary patterns, had an increased risk of IUGR (Hajianfar *et al.,* 2018).

It is clearly stated that there is an association between dietary intake, either restricted (availability and variety) or sufficient (wide variety and availability) and infant anthropometric outcomes at birth (Hajianfar *et al.,* 2018). In contrast, high maternal intake of CHO was negatively associated with infant length and abdominal circumference (Hajianfar *et al.,* 2018). In a study done by Northstone and Emmett (2008), it was shown that the women with prior problematic pregnancies or higher socioeconomic status or education level were more prone to have a healthy dietary pattern. This study also showed that “health conscious” dietary patterns were negatively associated with decreasing educational level, age, and socioeconomic status. Thus, risk factors of IUGR are poor educational level and socioeconomic status as well as age (Hajianfar *et al.,* 2018). So much so that it was suggested by researchers that women with low socioeconomic status needs to be provided specific dietary intervention programs to improve their nutritional intake to be sufficient for the demands of pregnancy (Hajianfar *et al.,* 2018).

Macronutrient deficiencies later in the pregnancy is less severe than in the early pregnancy, since catch-up growth still occurs. However, the earlier in the pregnancy undernutrition occurs, the bigger the chances that it will be permanent (Stephenson & Symonds, 2002). In this regard mycotoxins also play a role (Summary box 5).

Micronutrient imbalances before and during pregnancy can have a negative influence on mother and the foetus (Abu-Saad & Fraser, 2010; Morrison & Regnault, 2016). This can lead to significantly high reproductive risks, ranging from infertility to foetal structural defects, abnormal foetal development and growth, and long-term diseases (Morrison & Regnault, 2016).

Micronutrients supplementation during the peri-conceptional period are related to improved birth outcomes (Abu-Saad & Fraser, 2010). This may be due to alterations in maternal and foetal metabolism (Morrison & Regnault, 2016). In addition to maternal nutrient supply, the effectiveness of the placenta in transporting nutrients and oxygen to the foetus is important in determining foetal growth (Morrison & Regnault, 2016). Undernourished women, especially those in developing countries (and often subsistence farmers) are at particular risk of micronutrient deficiency (Berti *et al*., 2012; Catov *et al.,* 2011; Cetin *et al*., 2010).

Summary box 5

There is a direct link between nutrient absorption and mycotoxin exposure to both DON and FB:

Maternal DON exposure will inhibit protein synthesis that will lead to the inhibition of intestinal proliferation and crypt hyperplasia causing villi to flatten, as well as the degradation of the epithelial barrier. Inhibition of protein synthesis and flattened villi is will result in the translocation of gut bacteria and impaired gut mobility and delayed gastric emptying that will increase the prevalence of inflammatory diarrhoea. Diarrhoea contributes to impaired nutrient availability, decreased absorption and utilization of nutrients due to the altered intestinal architecture. Micronutrient deficiencies (especially iron, zinc and copper) will be the end result of poor nutrient availability, absorption and utilization.

DON exposure during pregnancy will also modulate the activity of SGLT-1, fructose transport GLUT5 and L-serine transport, thus resulting in altered carbohydrate metabolism, as well as altered fatty acid and phospholipid synthesis.

FB exposure will inhibit ceramide synthase which interrupts the sphingolipid metabolism and an increased in intestinal permeability. This leads to inflammatory diarrhoea that causes a decrease in nutrient uptake and utilization, leading to either weight loss or weight gain.

2.5 Diseases and conditions associated with maternal malnutrition

Exposure to diseases or pre-existing co-morbidities and conditions, such as substance abuse (Cogswell *et al.,* 2003), HIV, diarrheal diseases or infections, malaria (Landis *et al.,* 2009) and

tuberculosis (TB) reduce the mother's ability to sufficiently consume and absorb the required macro- and micronutrients to meet the increased needs of the foetus for optimal development (Goldenberg *et al.,* 2009). With maternal HIV for instance, the resting energy expenditure (REE) increases, dietary intake may decrease due to loss of appetite, side-effects from treatment or advanced symptoms of the disease, and thus results in lower nutrient absorption due to impaired and altered gastro-intestinal complications. This finally ends up in increased progression of HIV and a worse outcome for the infant (Morrison & Regnault, 2016; Ramachandran, 2002; Ramlal *et al.,* 2015).

According to Cetin and Laoreti (2015), the risk for foetal and maternal complications decrease with a birth weight between 3 100 g and 3 600 g of the infant.

Currently, there is not a consensus on which anthropometric measurement should be used to identify malnutrition or acute malnutrition during pregnancy, nor which cut-off value should be used. Some programs use the normal BMI cut-off value of 18.5 kg/m² for adult women, assuming it is applicable for pregnant women. Because of the interaction between pre-pregnancy BMI and gestational weight gain (GWG) on pregnancy and infant outcomes, the Institute of Medicine (IOM) in 1990 recommended different ranges of GWG for women with low, normal and high BMI (IOM, 1990).

According to a systematic review by Siega-Riz and colleagues (2009), gestational weight gain is negatively associated with adverse pregnancy and foetal outcomes. This is so even in women with a normal pre-pregnancy BMI. The evidence further indicates a negative association between excessive GWG and increased birth weight, caesarean delivery rate and postpartum weight retention (IOM, 1990; O'Tierney-Ginn *et al.,* 2014; Siega-Riz *et al.,* 2009). Foetal development is also influenced by physiological factors such as i) maternal body composition, ii) maternal nutrition status, iii) poor maternal health, iv) metabolism, v) placental nutrient supply, vi) socioeconomic and demographic as well as vii) environmental factors (Budree *et al.*, 2017; Cetin & Laoreti, 2015; Dean *et al*., 2014; Stephenson & Symonds, 2002; , ,).

Furthermore, according to Cetin and Laoreti (2015) and Siega-Riz *et al.* (2009), various studies indicated a linear, direct relationship between lower GWG and a decreased birth weight. The data is particularly evident for risks regarding GWG and SGA amongst women with lower pre-pregnancy weight. Based on basic human and animal research, DON and FB might be associated with a smaller GA (IOM, 1990).

Maternal malnutrition in late gestation is also associated with reduced placental and foetal weights according to Stephenson & Symonds (2002). They concluded that these findings were

evident in both human and animal studies. Furthermore, animal experiments indicate that maternal environment predominantly influences later foetal growth (Stephenson & Symonds, 2002). Once again, high FB and DON exposures might be exacerbating the process.

The study done by Cetin and Laoreti (2015), reported that in male infants, maternal height, BMI and weight gain during pregnancy were significant predictors of both lean and fat mass. This suggest that maternal body composition and maternal inflammatory environment might modulate metabolic fitness of male neonates, as predicted by fat and lean mass. Furthermore, it seems that maternal weight gain and pre-pregnancy body composition were equally predictive of male foetal growth when placental weight and GA were accounted for (Cetin & Laoreti, 2015). In terms of female infants, the only measured maternal factors predicting female body composition were inflammation markers including plasma C-reactive protein and IL-6. Maternal weight gain and pre-pregnancy anthropometrics were equally predictive of male foetal growth when placental weight and GA were accounted for (Cetin & Laoreti, 2015, O'Tierney-Ginn *et al.,* 2014). Summary box 6 indicates the possible role of DON and FB in this process.

Summary box 6

DON exposure during pregnancy will have an interference with the carbohydrate metabolism, and fatty acid and phospholipid synthesis that will contribute to excessive weight gain and obesity. Obesity causes stimulation of systemic inflammatory response.

Maternal DON and FB exposure during pregnancy will increase some systemic cytokines releases, leading to the stimulation of systemic inflammatory immune response and altered immune function.

2.6 Problem statement

Chronic exposure to mycotoxins, even at low levels in an unvaried diet, may incur adverse health outcomes or possibly exacerbate other existing disease conditions (Bryden, 2007). Because of this it has been speculated that mycotoxins might have a negative impact on infant growth and development (Smith *et al*., 2012). However, very little is known about this topic (Lombard *et al.,* 2014). Home-grown maize from subsistence farming in the Amatole District Municipality is known to be contaminated with DON, ZEA and FB, and can thus pose important health risks to the exposed population (Shephard *et al.*, 2013), especially regarding the more vulnerable part of the population (pregnant mothers, infants and young children).

Various factors influence infant anthropometric measures at birth, including maternal health, age, weight, health and lifestyle factors such as smoking tobacco and alcohol consumption. Unfortunately, mycotoxin exposure can not only exacerbate the impact of these factors, it could add additional health related factors which can worsen infant anthropometric measures at birth. From the little research done (human studies, animal studies and basic research) it is clear that mycotoxins exposure may also decrease maternal appetite (and thus nutrient intake), diarrhoea and vomiting (and thus decrease nutrient uptake), lower gastrointestinal function (and thus lower nutrient absorption) and increased inflammatory reactions. It is however unclear which of these are directly or indirectly associated with infant birth anthropometry.

Therefore, the aim of this study was to determine the association between mycotoxin exposure of pregnant women and infant birth anthropometric measures and GA at birth. The objectives of this study were to estimate multi-mycotoxin exposure levels of pregnant women and to determine the anthropometric measures of pregnant women and their infants at birth, as well as the association between maternal multi-mycotoxin exposure and infant birth anthropometric measures and GA at birth.

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CHAPTER 3: ARTICLE

Chapter 3 provides the article written for publication in Food and Chemical Toxicology. The paper has not yet been submitted but is already written in the style specified in the author guidelines.

Association between mycotoxin exposure levels of rural Eastern Cape pregnant woman and their infants' anthropometric measures and gestational age at birth

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Running title: Association between maternal mycotoxin exposure and anthropometric measures of infants at birth in rural South Africa

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3.1 **Abstract**

Background: In rural areas of the Eastern Cape (EC), South Africa, the majority of people are maize-subsistence farmers. However, it is well-documented that this maize has high mycotoxin concentrations of fumonisin (FB), deoxynivalenol (DON) and zearalenone (ZEA) (secondary metabolites produced by fungi growing on the maize).

Aim: To determine the association of maternal pregnancy exposure of three mycotoxins (DON, ZEA and FB) with anthropometric measures and gestational age (GA) of infants at birth.

Methods: It was part of a larger prospective study conducted in the Amatole District, in the EC of South Africa with a longitudinal analysis of data collected during pregnancy of the mother and infant birth anthropometric measures and GA (two visits). Systematic and snowball sampling was used because random sampling was impossible due to poor infrastructure. Pregnant women staying permanently in the area and consuming only home-grown maize were included. To determine mycotoxin concentration, 1 kg maize samples were collected from participating household. Maize was analysed by LC-MS/MS for DON, ZEA and FB concentrations. Maternal maize consumption was determined with a validated culturally specific quantitative food frequency questionnaire (QFFQ) and portion size photographs. Exposure were calculated based on maize mycotoxin levels, raw maize consumption and maternal weight. Anthropometric outcomes of infants at birth included: weight (kg), length (cm), head circumference (HC) (cm) and gestational age (GA) (weeks). Generalized linear regression modelling were conducted to determine associations of mycotoxin exposure with anthropometric outcomes in crude models and with adjustment for specific confounders (maternal weight, age, and maize intake).

Results: Median maternal daily raw maize intake (n = 92) was 785.0 g/day (± 513.8 g). Median maternal exposure levels for DON were 0.276 μgkg^{-1} bw day^{-1}, ZEA 0.003 μgkg^{-1} bw day^{-1} and for FB 9.676 μgkg^{-1} bw day^{-1}. For DON and ZEA these levels were lower than the relevant provisional maximum total daily intake (PMTDI) of 1.0 and 0.5 μgkg^{-1} bw day^{-1} respectively. For FB these medians were much higher than the recommended PMTDI of 2 μgkg^{-1} bw day^{-1}.

Unadjusted data of infant's anthropometric measures divided into tertiles indicated that infants with a higher birth weight (third tertile) were significantly exposed to higher DON exposures ($p = 0.047$) while those with a lower birth weight (lower tertile) were significantly exposed to higher FB exposures ($p = 0.05$). Infants in the lower tertile of HC (smallest HC) were significantly associated with higher exposure of DON and FB ($p = 0.01$ for both). Lastly, infants in the lower tertiles of GA were also significantly associated with higher exposures of DON ($p = 0.030$), ZEA ($p = 0.045$) and FB ($p = 0.040$). When data was distributed according to exposure levels and

adjusted for confounders (maternal age, weight and raw maize intake) it indicated only significant associations for DON and FB exposures with birth weight ($p = 0.047$ and $p = 0.05$ respectively). Borderline associations were found for DON and FB when adjusted for maternal weight ($p = 0.06$ for both). However, all differences were found in the middle tertile indicating a no dose-response association but rather a U-shaped association. Adjusted data of head circumferences associated with exposures indicated that maternal age might influence outcome, especially for exposures of DON and FB ($p = 0.01$ and $p = 0.03$ respectively). However, the differences were once again indicated a U-shaped association and thus not a dose-response association.

Conclusion: A positive association was found between DON exposure and infant birth weight and an inverse association between FB with infant birth weight. DON and FB exposure levels were also associated with smaller HC. DON, ZEA and FB were associated with smaller GA. However, results could have been due to confounding factors such as maternal weight and age. More research is needed to understand the association between mycotoxin exposure and anthropometric measures of infants at birth as well as the role of additional confounding factors.

Key words: Mycotoxins, pregnant women, birth anthropometry, deoxynivalenol (DON), zearalenone (ZEA), fumonisin (FB).

3.2 **Introduction**

The term ‘mycotoxin’ is reserved for small, toxic chemical products formed by secondary metabolites by fungi that readily colonise crops in the field or after harvest (Turner *et al*., 2009). Mycotoxins frequently contaminate maize and other grains (Marasas *et al*., 2001; Marin *et al*., 2004), growing optimally at high temperatures in humid areas (Marin *et al*., 1995). It can also occur during processing and storage when done incorrectly, affecting food quality and safety levels (Sforza *et al.,* 2006). It is well studied that the inhabitants of sub-Sahara Africa are experiencing high amounts of dietary exposure to food-borne mycotoxins, including deoxynivalenol (DON), zearalenone (ZEA) and fumonisin (FB) due to subsistence farming (Wagacha & Muthomi, 2008).

In most low- and middle-income countries (LMIC), farming remains the primary food source. Subsistence farming is an important part of improving food security and nutrition. Unfortunately, there is little evidence that this practice can positively affect nutrition security (Sibhatu & Qaim, 2017). Further-more, it is well-documented that the maize produced by subsistence farmers contains high levels of mycotoxins. In LMIC settings, many individuals chronically exposed to mycotoxins are also malnourished (Gong *et al*., 2003). High maternal mycotoxin exposure may affect infants in-utero and through breastfeeding, predisposing infants to the risk of chronic exposure from a very early stage of life (Sherif *et al*., 2009).

Women of child-bearing age, especially pregnant and lactating women, infants and young children are amongst the most nutritionally-vulnerable population groups for malnutrition (Adu-Afarwuah *et al.,* 2017). Maternal health can be compromised by diseases or conditions such as substance abuse (Cogswell *et al.,* 2003), human immunodeficiency virus (HIV), diarrheal diseases or infections, malaria (Landis *et al.,* 2009) and tuberculosis (TB). This reduces the mother’s ability to sufficiently consume and absorb the required macro- and micronutrients to meet the increased demands of the foetus (Goldenberg *et al.,* 2009).

Micronutrient imbalances before and during pregnancy can have a negative influence on mother and the foetus (Abu-Saad & Fraser, 2010; Morrison & Regnault, 2016). This can lead to significantly high reproductive risks, ranging from infertility to foetal structural defects, abnormal foetal development, growth and long-term diseases (Morrison % Regnault, 2016).

When ingested in high doses by animals, DON causes nausea, vomiting and diarrhoea, while in small doses, it can cause weight loss and decreased food intake (Miller *et al.,* 2001). Deoxynivalenol has also been reported as the causative agent of gastrointestinal poisonings (Luo, 1994) and as a suspected etiologic agent of gastroenteritis in children (CDC, 1999).

Deoxynivalenol causes protein synthesis inhibition (McLaughlin *et al.,* 1977, Smith *et al.,* 2012) and are known to cause neurotoxicity, immunosuppression and renal toxicity (Richard, 2007), as well as anorexia, weight loss, malnutrition, endocrine dysfunction and immune alterations (Pestka, 2010). With maternal high exposure rates or lower chronic exposure rates, DON may also cause infant growth faltering (Pestka & Smolinski 2005).

On the other hand, exposure to ZEA has been linked to some cases of early puberty in girls (Massart *et al*., 2008). Besides estrogenic effects, ZEA can also cause toxicity by production of reactive oxygen species (ROS) (El GolliBennour *et al.,* 2009). Zearalenone is unfortunately the most understudied mycotoxin, with very limited human studies on growth faltering available to date.

Lastly, FB have been implicated as a risk factor for neural tube defects (Gelineau-van Waes *et al*., 2009; Missmer *et al.,* 2006; Suarez *et al.,* 2012). Fumonisins disrupt sphingolipid metabolism in the gastrointestinal tract (Enongene *et al.,* 2000), affect intestinal permeability (Lallès *et al.,* 2009), and is associated with a loss of appetite and anorexia, growth faltering and loss of body weight in piglets (Dilkin *et al.,* 2003).

The rural Eastern Cape (EC) is known to have poor infrastructure, with a high prevalence of poverty. Majority of food sources come from subsistence farming, with maize consumption as part of the culturally distinct dietary pattern and ethnic tradition in this area (Lombard *et al.,* 2013, Lombard *et al.,* 2014). Home-grown maize coming from this area, is known to be contaminated by multiple mycotoxins (Burger *et al.,* 2010). Through high consumption of maize in this region, the population has an increased risk of high and / or chronic multi-mycotoxins exposure to DON, ZEA and FB (Burger *et al.,* 2013).

Thus, this study aims to determine the association between maternal DON, ZEA and FB exposure during pregnancy and infant anthropometric measures at birth.

3.3 **Methods**

The study was part of a prospective study (PhilaSana) with a longitudinal analysis (two visits). Data were collected during pregnancy of the mother and infant anthropometric measures at birth. Systematic and snowball sampling was used to recruit participants. The area included villages within a deep rural section of Centane, in the Amathole District Municipality, EC in South Africa. The areas are characterised by almost no infrastructure (poor roads, no electricity, formal addressing system or community areas) causing poor accessibility to households.

Pregnant women (n=92), age 16-45 were recruited. Women were excluded if they were born or raised outside the area, visiting the area, not consuming only home-grown maize and / or not permanently residing in the area.

3.4 **Study procedures**

Before the onset of the study, goodwill permission was obtained from the local community leaders such as the chiefs and headmen. They were informed of the study and were provided with information such as the inclusion and exclusion criteria as well as the study procedures and risks and benefits of the study. Once the local leaders provided goodwill permission, various little boys were sent to households in the area that may have potential participants. The following day a community meeting was held at a pre-determined area, usually the home of a key woman (the host) in the community (depending on the chief or headman). On this day potential participants were informed of the study. They were given one day to consider participation and were asked to inform other pregnant women about the study. On the third day informed consent was signed in the participants' first language, isiXhosa. Assessment were conducted in various little huts as provided by the host.

Recruitment and the first visit were conducted during pregnancy to determine the mother's current health status and dietary patterns. The second visit was conducted after birth. The anthropometric measures at birth were obtained from the Road to Health booklet (RtHB) (clinic card). Data was collected in the form of questionnaires and anthropometric measurements.

To determine mycotoxin concentration on maize, 1 kg home-grown maize samples were collected per participating household. The maize was analysed by LC-MS/MS for DON, ZEA and FB concentrations (Cawood *et al.,* 1991; Gelderblom *et al.,* 1993).

Dietary information was obtained from the mother of each infant. Food intake was assessed with a validated and culturally specific dietary assessment tool (Lombard *et al.,* 2013, Lombard *et al.,* 2014). The Ratio and Portion Size Photo (RAPP) tool was previously developed and validated among the study population. The validated questionnaire was based on adult habitual diet in the form of a quantitative food frequency questionnaire (QFFQ) and portion size photographs (four portion size photographs for each dish represented in the QFFQ) (Lombard *et al.,* 2014).

To determine raw home-grown maize intake, amount of daily cooked maize consumed (grams) was multiplied by the number of portions consumed. Monthly cooked maize consumption was

determined by multiplying the weekly consumption by four to provide a monthly intake. Monthly cooked maize consumption was then used to determined mean daily intake by dividing by 28 days. Raw maize consumption was calculated from the mean daily cooked maize consumed using a predetermined formula obtained during the development of the food portion size photographs (Lombard *et al*., 2014).

Once raw maize intake was calculated, a deterministic analysis method was used to determine the mycotoxin exposure. Mycotoxin exposure was measured in probable daily intake (PDI). Probable daily intake (PDI) (μgkg^{-1} body weight day^{-1}) was calculated by multiplying mean total daily raw maize intake (g/day) with mycotoxin concentration (μg/kg) divided by body weight (kg). The mycotoxin concentration was calculated as the amount of mycotoxin on 1 kg of raw home-grown maize from the area. Concentrations were as follow: DON (24.5 μg/kg), ZEA (31 μg/kg), and total FB = FB_1 + FB_2 +FB_3 (1035 μg/kg). The provisional maximum tolerable daily intakes (PMTDIs) for each DON (1.0 μgkg^{-1} bw day^{-1}), ZEA (0.5 μgkg^{-1} bw day^{-1}) and FB (2.0 μgkg^{-1} bw day^{-1}) was used as cut-off values to indicate high exposure (JECFA, 2002; JECFA, 2012).

The study was conducted according to the Helsinki declaration and the ICH & MRC guidelines. The protocol was approved by the Health Research Ethics Committee of the Faculty of Health Sciences, North-West University (NWU-00207-14-S1). Study information and consent was communicated to participants in their home language; each participant received a participation number, remaining anonymous during processing of data and results interpretation. Pregnant women younger than 18 years of age were provided with information and an assent form. Upon signing the form her mother signed consent while she herself signed assent. If, for any reason, the pregnant potential participant refuse assent (regardless of obtaining consent from the mother), she was not included in the study.

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. Median, and IQR were reported for the mycotoxins (DON, ZEA and FB), as well as for infant weight, length, HC and GA. Maternal age, weight and total raw maize intake was also reported as median and IQR.

Birth weight, length, HC and GA were divided into tertiles (tertile 1 = lower third, tertile 2 = the middle third and tertile 3 = the upper third). The Krusskal-Wallis test was used to compare the mycotoxin exposure levels across tertiles of anthropometric measures. Generalized linear regressions were performed to determine the impact of maternal age, maternal weight and maternal maize intake (as confounders) on infant birth anthropometry (continuous variables) on the mycotoxin exposures (tertiles). Regressions were conducted for each individual confounder

against the growth parameters to determine their association with infant anthropometry as well as in combination with the mycotoxins. Significant levels were set at $p \leq 0.05$. None of the participants' smoked or consumed any alcohol and data was thus not adjusted for these. Lastly, data was also not adjusted for HIV or TB as most mothers declined to answer those questions.

3.5 **Results**

The mean age of the participants were 31.4 years (± 11), with a mean raw maize intake of 785.0 g/day (SD 514.0). A total of 92 pregnant mothers were included. At least 64% (n = 59) 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 = 62) received a child grant or old age grant. In terms of water and sanitation 44% (n = 41) make use of the river / dam while the rest have water tanks. Lastly, 66% (n = 63) used pit toilets while the rest used the bush.

Participants were exposed to all three mycotoxins, however, the exposure levels for DON and ZEA were lower than FB (Table 3.1). Although the median of the DON exposure was under the PMTDI, the IQR indicates that there were participants with levels above the expected safety levels. The median and the IQR for ZEA was below the PMTDI levels. However, the median for FB was much higher than the estimated PMTDI, up to a maximum of 52 μgkg^{-1} bw day^{-1}.

Table 3-1 Mycotoxin exposures of pregnant mothers (n = 92)

Mycotoxin exposure (PDI)	PMTDI	Median	IQR
DON (μgkg^{-1} bw day^{-1})	≥ 1.000	0.276	0.11 - 0.329
ZEA (μgkg^{-1} bw day^{-1})	≥ 0.500	0.003	0.002 - 0.005
Total FB (μgkg^{-1} bw day^{-1})	≥ 2.000	9.676	6.800 - 13.900

PDI = Probable daily intake
PMTDI = Provisional maximum tolerable daily intake
IQR = Interquartile range
DON = deoxynivalenol
ZEA = zearalenone
FB = fumonisin

Table 3.2 reports the basic descriptive information of the participants (infants and pregnant mothers). The median and IQR of birth outcomes were mostly within acceptable ranges, although based on the minimum values there are a number of infants either born with low birth weight / length, small head circumference and small gestational age.

Table 3-2 Basic descriptive information of the participants (infants and pregnant mothers) (n = 92)

	Median	IQR
Birth weight (kg)	3.28	2.92 - 3.59
Birth length (cm)	49.00	48.00 - 51.00
Head circumference (cm)	34.00	34.00 - 35.00
Gestational age (weeks)	40.00	38.00 - 40.00
Maternal age (years)	29.00	23.00 - 35.75
Maternal weight (kg)	72.60	61.00 - 79.35
Maternal maize intake (g)	700.86	466.59 - 934.23

IQR = Inter quartile range

Unadjusted data of infants' anthropometric measures were divided into tertiles and compared against the mycotoxin exposures (as continuous data) (Table 3.3). Results indicated that infants with a higher birth weight (third tertile) were significantly exposed to higher DON exposures ($p = 0.047$) while those with a lower birth weight (lower tertile) were significantly exposed to higher FB exposures ($p = 0.05$). Infants in the lower tertile of HC (smallest HC) were significantly associated with higher exposure of DON and FB ($p = 0.01$ for both). Lastly, infants in the lower tertiles of GA were also significantly associated with higher exposures of DON ($p = 0.030$), ZEA ($p = 0.045$) and FB ($p = 0.040$). No significant associations were found between length and any of the mycotoxin exposures.

Table 3.4 present the Wald chi-square test conducted to test the model effects of the generalized linear modelling. This was to determine the impact of different possible confounding factors on the association found between the maternal DON, FB and ZEA exposure (each subdivided into tertiles) and the infants' birth anthropometry (as continuous data). Data were adjusted for maternal age, maternal weight and maternal maize consumption to determine the impact each have an association between anthropometry and exposure levels. No data was available to add HIV and TB as confounders.

Table 3-3 Comparison of unadjusted mycotoxin exposure levels divided into three tertiles against anthropometric measurements at birth (n = 92)

	DON			**ZEA**			**Total FB**		
Tertiles of birth outcomes	**Lower third**	**Middle third**	**Upper third**	**Lower third**	**Middle third**	**Upper third**	**Lower third**	**Middle third**	**Upper third**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Weight(kg)	0.1	0.2	**0.3*** **	0.1	0.1	0.1	**11.7*** **	9.7288	0.2
	(0.2)	(0.1)	(0.2)	(0.1)	(0.1)	(0.1)	(9.7)	(4.6)	(9.3)
Length(cm)	0.3	0.3	0.3	0.1	0.1	0.1	11.4	11.1	12.5
	(0.2)	(0.2)	(0.2)	(0.1)	(0.1)	(0.1)	(7.3)	(9.0)	(9.2)
Head	**0.4***	0.001	0.3	0.1	0.001	0.1	**16.3***	0.001	10.5
Circumference	(0.3)	(0.1)	(0.2)	(0.1)	(0.01)	(0.1)	(11.9)	(0.1)	(7.0)
(cm)									
Gestational age	**0.7*** **	0.3	0.3	**0.1***	0.1	0.1	**29.7** **	11.4	10.9
(weeks)	(0.4)	(0.2)	(0.3)	(0.1)	(0.1)	(0.1)	(17.3)	(6.9)	(10.4)

DON = deoxynivalenol

ZEA = zearalenone

FB = fumonisin

* p< 0.05, ** p< 0.01, *** p< 0.001

Table 3-4 Association between mycotoxin exposure and birth outcomes using generalized linear models

	DON exposure		ZEA exposure		FB exposure	
	Wald Chi-square test	**P value**	**Wald Chi-square test**	**P value**	**Wald Chi-square test**	**P value**
Birth weight						
Unadjusted birth weight	5.61	0.047	0.63	0.73	5.66	0.05
Adjusted Maternal Age	1.84	0.18	1.23	0.27	1.84	1.18
Adjusted Maternal weight	3.43	0.06	2.59	0.11	3.43	0.06
Adjusted Maternal raw maize intake	1.32	0.25	0.93	0.34	1.32	0.25
Birth Length						
Unadjusted birth length	1.51	0.47	1.08	0.58	1.51	0.47
Adjusted maternal Age	1.35	0.25	1.30	0.25	1.35	0.25
Adjusted maternal weight	0.35	0.56	0.53	0.77	1.10	0.58
Adjusted maternal raw maize intake	0.21	0.89	0.04	0.85	0.02	0.89
Birth Head circumference						
Unadjusted birth HC	9.23	0.01	2.09	0.35	9.30	0.01
Adjusted Maternal Age	4.91	0.01	3.23	0.07	4.91	0.03
Adjusted Maternal weight	0.40	0.53	0.91	0.34	0.40	0.53
Adjusted Maternal raw maize intake	0.73	0.39	1.33	0.25	0.73	0.39
Birth Gestational age						
Unadjusted birth GA	2.10	0.35	0.53	0.77	2.10	0.35
Adjusted Maternal Age	2.60	0.11	2.23	0.14	2.57	0.11
Adjusted Maternal weight	0.15	0.70	0.008	0.93	0.15	0.70
Adjusted Maternal raw maize intake	0.21	0.65	0.07	0.80	0.21	0.65
Wald Chi-Square test results were reported.						

When data was distributed according to exposure levels and adjusted for confounders (maternal age, weight and raw maize intake) it indicated only significant associations for DON and FB exposures with birth weight ($p = 0.047$ and $p = 0.05$ respectively) (Table 3.4). Borderline associations were found for DON and FB when adjusted for maternal weight ($p = 0.06$ for both). Table 3.5 provides more detailed information based on results from Table 3.4. Mycotoxin levels were divided into tertiles and the estimated marginal means of the tertiles were determined. In both cases of birth weight and HC it is clear that the significant differences indicated in Table 4 were mostly at the middle tertile. This indicates a no dose-response association but rather a U-

shaped association (Table 3.5). Adjusted data of head circumferences associated with exposures indicated that maternal age might influence outcome, especially for exposures of DON and FB ($p = 0.01$ and $p = 0.03$ respectively). However, the differences were once again indicated a U-shaped association and thus not a dose-response association.

Discussion and conclusion

The population included in this study is subsistence maize farmers with an extremely homogenous diet (Lombard *et al.,* 2014). The high levels of mycotoxins (DON, ZEA and FB) has been described numerous times in the literature (Burger *et al*., 2010; Marasas *et al.,* 2001; Shephard *et al.,* 2013; Turner *et al.,* 2009). Furthermore, previous research indicates that chronic high exposure to these mycotoxins may have a negative effect on health (Smith *et al.,* 2012). Aflatoxins for example are found in the umbilical cord and thus expose infants *in utero.* However, very little is known about the association between maternal exposure of DON, ZEA and FB during pregnancy and how it affects the anthropometric measures of infants at birth (Lombard, 2014).

Since the diet diversity is extremely low in this community, the high consumption of maize increased the risk of exposure to a mixture of mycotoxins such as DON, ZEA and FB (Shephard *et al.,* 2013). The aim of this study was thus to determine association between maternal exposure levels for DON, ZEA and FB and the exposure in relation to infants' birth anthropometry.

Raw maize intake levels were high, with a median of 700 g. This is mostly due to the low dietary diversity, cultural preferences and availability. Similar intake levels were reported for adults in 2010 (Burger *et al.,* 2010). The maize consumed in the area are not commercial maize and thus not fortified with micronutrients. Undernourished women, especially those in LMIC are often subsistence farmers, with a particular risk of micronutrient deficiency due to the low diversity (Berti *et al*., 2012; Catov *et al.,* 2011; Cetin *et al*., 2010).

Table 3-5 Tertile distribution of adjusted mycotoxin exposures and birth outcomes

	DON exposure			ZEA exposure			FB exposure		
	Tertile 1	**Tertile 2**	**Tertile 3**	**Tertile 1**	**Tertile 2**	**Tertile 3**	**Tertile 1**	**Tertile 2**	**Tertile 3**
	Mean (95% CI)	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**
Birth weight									
Adjusted Maternal Age	3.33 (3.16-3.51)	3.06 (2.89-3.24)	3.33 (3.15-3.51)	3.28 (3.10-3.46)	3.18 (3.00-3.36)	3.26 (3.08-3.45)	3.33 (3.16-3.51)	3.07 (2.89-3.24)	3.33 (3.15-3.51)
Adjusted Maternal weight	3.30 (3.13-3.48)	3.06 (2.89-3.24)	3.36 (3.18-3.55)	3.34 (3.12-3.49)	3.19 (3.00-3.36)	3.24 (3.05-3.37)	3.30 (3.12-3.48)	3.06 (2.90-3.24)	3.36 (3.18-3.55)
Adjusted Maternal raw maize intake	3.40 (3.19-3.60)	3.08 (2.91-3.26)	3.24 (3.02-3.46)	3.29 (3.11-3.47)	3.18 (2.99-3.36)	3.26 (3.07-3.44)	3.40 (3.19-3.60)	3.09 (2.91-3.26)	3.24 (3.03-3.46)
Birth length									
Adjusted Maternal Age	49.52 (48.11-50.92)	48.50 (47.10-49.91)	49.63 (48.23-51.04)	48.83 (47.42-50.25)	49.02 (47.65-50.40)	49.85 (48.39-51.31)	49.52 (48.11-50.92)	48.50 (47.10-49.91)	49.63 (48.23-51.04)
Adjusted Maternal weight	49.40 (47.95-50.85)	48.68 (47.33-50.03)	49.66 (48.29-51.04)	48.95 (47.56-50.34)	49.12 (47.77-50.47)	49.65 (48.27-51.04)	49.39 (47.95-50.85)	48.68 (47.33-50.03)	49.66 (48.29-51.04)
Adjusted Maternal raw maize intake	49.45 (47.76-51.13)	48.65 (47.30-50.00)	49.63 (48.07-51.21)	48.90 (47.53-50.27)	49.12 (47.76-50.47)	49.71 (48.33-51.09)	49.45 (47.80-51.13)	48.65 (47.30-50.0)	49.64 (48.07-51.21)
Birth head circumference									
Adjusted Maternal Age	34.45 (33.79-35.10)	33.44 (32.79-34.10)	34.88 (34.20-35.60)	34.09 (33.40-34.79)	34.64 (33.96-35.32)	33.97 (33.28-34.67)	34.45 (33.79-35.10)	33.44 (32.8-34.1)	34.88 (34.20-35.56)
Adjusted Maternal weight	34.50 (33.81-35.18)	33.52 (32.84-34.19)	34.75 (34.02-35.47)	34.09 (33.34-34.79)	34.64 (33.95-35.33)	33.98 (33.28-34.69)	34.50 (33.81-35.18)	33.52 (32.84-35.50)	34.74 (34.02-35.47)
Adjusted Maternal raw maize intake	34.60 (33.76-35.44)	33.52 (32.85-34.19)	34.62 (33.85-35.40)	34.17 (33.46-34.87)	34.61 (33.92-35.30)	33.93 (33.23-34.63)	34.62 (33.85-35.40)	33.52 (32.85-34.19)	34.60 (33.76-35.44)
Gestational age									
Adjusted Maternal Age	38.58	38.34	39.08	38.45	38.74	38.80	38.58	38.34	39.08

	DON exposure			ZEA exposure			FB exposure		
	Tertile 1	**Tertile 2**	**Tertile 3**	**Tertile 1**	**Tertile 2**	**Tertile 3**	**Tertile 1**	**Tertile 2**	**Tertile 3**
	Mean (95% CI)	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**
	(37.86-39.29)	(37.63-39.06)	(38.36-39.81)	(37.73-39.17)	(38.02-39.46)	(38.07-39.53)	(37.86-39.29)	(37.63-39.06)	(38.36-39.81)
Adjusted Maternal weight	38.55 (37.82-39.29)	38.38 (37.65-39.10)	39.07 (38.31-39.83)	38.48 (37.43-39.21)	38.74 (38.01-39.47)	38.77 (38.02-39.52)	38.55 (37.82-39.29)	38.38 (37.65-39.10)	39.07 (38.31-39.84)
Adjusted Maternal raw maize intake	38.48 (37.64-39.32)	38.37 (37.65-39.10)	39.15 (38.26-40.04)	38.49 (37.76-39.22)	38.74 (38.01-39.46)	38.77 (38.03-39.22)	38.48 (37.63-39.32)	38.37 (37.65-39.10)	39.15 (38.26-40.04)

Furthermore, maternal mycotoxin exposure levels of DON and ZEA were in general below the relevant PMTDI. Total FB exposure levels on the other hand was much higher than the PMTDI. Similar results for the maize in this area were reported by Shephard *et al.* (2013) and Burger *et al.* (2010). It is however important to mention the fact that the exposure levels were determined with a deterministic method and not with biomarkers. Biomarkers for DON, ZEA and FB are still in the validation process and could thus not be used in this study. Maternal dietary exposure was conducted with a quantitative food frequency questionnaire (QFFQ) that was specifically designed for this population (Lombard *et al.,* 2014). Food frequency questionnaires tend to lead to over-reporting of intakes and should thus be acknowledge when interpreting raw maize intake and mycotoxin exposure.

Regardless of the possible micronutrient deficiencies, the basic descriptive information of the infants and pregnant mothers reported anthropometric measures at birth within acceptable ranges, however, this information should also be interpreted with caution (especially length) since data was collected from the RTH booklet and quality control was thus not possible.

Maternal DON exposure was significantly associated with infant birth weight and HC. However, since this is mostly those in the middle tertile of exposures. The majority of participants had exposures below the PMTDI and thus it can be concluded that the association found is although statistically significant, not clinically significant (since exposures are below the PMTDI). It is thus possible that the association found were more associated with maternal weight than with DON exposure. Similarly, maternal FB exposures were associated with birth weight and HC. However, unlike the DON levels, the FB levels of the whole group were above the PMTDI. Thus, even if the association is at the middle exposure tertile it might indicate an association that even out at extreme high levels. Yet, data still indicates that maternal weight is significantly associated and might thus contribute to this association.

The same pattern can be seen for the HC. Although significant associations were found between a smaller HC and higher exposures (above PMTDI) it is possible that maternal age influence the results. Once again, the middle tertile were significantly different from the lower and higher tertiles indicating that the difference is probably more due to maternal age.

In general, maternal DON exposure inhibits protein synthesis in pregnant women, leading to impairment of the intestinal membranes and causing the inhibition of intestinal proliferation. This results in impaired gut mobility, causing inflammatory diarrhoea, dehydration and loss of appetite (Smith *et al.,* 2012). However, although it is known that aflatoxins cross the placenta of humans, and DON cross the placenta of animals (Goyarts *et al.,* 2007; Tiemann *et al.,* 2008) results from this study indicate that it is more likely that the lower birth weight was due to

maternal weight rather than DON exposure. More research needs to be conducted on this to officially determine the role of DON in birth weight.

Very little is known about ZEA and maternal exposure. Results from this study however indicated very low exposure levels and thus it was not possible to really conclude anything regarding the role of ZEA in maternal exposure and infant anthropometric measures at birth.

Extremely high levels of maternal FB were identified. Maternal FB exposure had a significant direct association with anthropometric measures at birth regarding weight and HC. However, it remains unclear to what extent maternal age influenced the results.

No other studies in the past measured maternal intake and the association of DON, ZEA and FB with anthropometric measures of infants at birth. It is thus not possible to compare the results against any other human studies. Although significant findings were observed in this study, the data should be interpreted with caution. Due to logistical problems confounding factors such as maternal health (especially HIV, TB and infectious diarrhoea), total energy and protein intake and commercial maize consumption could not be measured. It is entirely possible that these confounders could influence the results reported in this study. Furthermore, the sample size was very small and a deterministic approach was used rather than biomarkers. Most available studies in this specific field were animal studies, with limited available data on humans; thus can this also be seen as a limitation. Furthermore, the quality of measuring and reporting anthropometric measures of infants at birth was not clear as this was not conducted by the research team and thus not standardised.

Key messages

- The population in the rural EC area have a chronic, high FB (> PMTDI) exposures and low DON and ZEA (< PMTDI) exposures.
- Although results from both DON and FB exposure during pregnancy had a significant association with birth weight and HC, no linear association were found, thus the association did not increase with increased exposure.
- The results could have been influenced by confounders such as maternal weight and age.
- Further research and investigation is needed to conclude on maternal exposure and foetal growth faltering.
- It is essential to follow these infants to determine the long-term association of chronic mycotoxin exposure and infants and young children feeding practices and growth monitoring.

- Nutrition education and other interventions are needed to reduce mycotoxin exposure and improve infant and young child feeding.
- Prenatal diet education is very imported in this specific studied area.

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Conflict of interest

The researchers declare that they had no actual or potential conflict of interest.

Contributor statement

Me Entres contributed towards study design and data collection, statistical analysis and quality control of questionnaires. She was primarily responsible for the writing of the paper. Dr Burger was involved in study design, funding sourcing and data collection. Mrs Neethling was involved in article compilation. Prof Gelderblom and Dr Shephard contributed to funding, 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.

Recommendations

The following recommendations can be made to improve the researched field.

- The impact of education and practical methods to lower mycotoxin exposure in diet.
- Prenatal dietary intake, weight and general health.
- Studies to determine if DON, ZEA and FB if transferred to the foetus via the placenta, and through breast milk after birth.
- Infant and young child chronic exposure levels of DON, ZEA and FB and thus feeding practices (breastfeeding, weaning and complimentary feeding) and the long-term association with growth.

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CHAPTER 4: DISCUSSION AND CONCLUSION

4.1 **Discussion**

In South Africa, at least 58% of rural population resides in economically unstable areas which limit their access to nutrient dense foods (Govender *et al.,* 2016).

Subsistence farming can improve food security and nutrition in these rural, underdeveloped areas. Unfortunately, even if farming interventions may improve economic growth and reduce poverty, there is little evidence that nutrition security can be positively affected (Sibhatu and Qaim, 2017). It is currently estimated that approximately 11% of the world's population is chronically undernourished, indicating a deficiency of total calories (Sibhatu and Qaim, 2017). The association between mycotoxin exposure and intra-uterine and infant growth are very limited and understudied (Smith *et al.,* 2012), but is now known that DON, ZEA and FB exposure are occurring during pregnancy, and it has an association with growth of the foetus intra-uterine as well as on anthropometric measures at birth.

The area studied is very rural, underdeveloped, with a high prevalence of poverty. Subsistence farming delivers the majority of food. Due to the studied population's distinct cultural and traditional diet, the consumption of maize is very high (Lombard *et al.,* 2013, Lombard *et al*., 2014). It is well known that multi–mycotoxin occurrence is present in the studied area (Burger *et al.,* 2010, Shephard *et al.,* 2013).

The aim of the study was to determine the association between mycotoxin exposure of pregnant women and anthropometric measures of infants at birth and gestational age.

4.2 **Objectives of this sub-study**

The following specific objectives have been identified to reach the aim in the sub-study and are based on objectives 2, 3, 4 and 5 of the PhilaSana study:

- To estimate multi-mycotoxin (DON, ZEA and FB) exposure levels of pregnant women;
- To obtain anthropometric measures of infants at birth and their gestational age;
- To determine the association between maternal multi-mycotoxin exposure and anthropometric measures of infants at birth and their gestational age.

All the pregnant women in the study were exposed to all three mycotoxins. Home-grown maize coming from this area, is known to be very high in multi-mycotoxins (Burger *et al.,* 2010), and therefore poses a high health risk (Shephard *et al.*, 2013). Multi-mycotoxin exposure increases the health risks of the population in this area (Shephard *et al.*, 2013). Commercial maize is also consumed in some areas, and even though it generally contains far lower levels of mycotoxins, intake could still pose a risk to the population, as it is consumed in large quantities (Lombard *et al*., 2014, Burger *et al.,* 2013).

The mean raw maize intake of the pregnant women was 785.0 g/day. Because of the population's limited, monotonous diet, maternal dietary exposure was conducted with a culturally specific, validated quantitative food frequency questionnaire (QFFQ). Mean exposure levels of mycotoxins in the pregnant women were 0.276ug/kg for DON which is below the PMTDI, for ZEA 0.004ug/kg, also below the PMTDI. Fumonisin exposure levels were high, exceeded the PMTDI more than 5-fold.

There was a significant difference seen in birth weight and head circumference (HC), as well as gestational age (GA). These significant results for maternal DON exposure and the association with birth weight, head circumference (HC) and gestational age (GA) can be explained by the conceptual framework pathways. However, adjusted data may indicate that maternal age and weight might also be influencing the results.

For the unadjusted ZEA data, a significant outcome was seen on smaller GA. Even though a significant association was found, the overall exposure levels were very small and thus it is possible that this association found were rather associated with a confounder such as maternal age / weight. Therefore, more studies need to be done to explain this evident mechanism as there is currently no data available to conclude this result outcome.

With maternal FB exposure, there was a significant association on birth weight, birth HC, as well as GA. It is clear that maternal FB exposure has a significant association with anthropometric measures at birth, and can be explained by the conceptual framework. However, further research needs to be done to explore the role of confounding factors.

4.3 **Recommendations**

- Education and practical methods to lower mycotoxin intake and exposure in diet, with correct harvesting, sorting, sorting and preparation of maize.
- Prenatal education on diet – emphasizing dietary diversity, variation, increase micronutrient intake, amount of macronutrient distribution with meals.

- More research to determine if mycotoxins (DON, ZEA and FB) are transferred to the foetus intra-uterine via the placenta, as evidence is available that aflatoxins has placental transfer to the foetus
- Breast feeding practices – exclusive breastfeeding awareness. More studies are also needed to determine if breast milk transfers mycotoxins to the infant when feeding.
- Chronic exposure of mycotoxins when weaning and complementary feeding are introduced after breastfeeding period in infants needs to be investigated.
- Investigation of infant and young child feeding practices – mycotoxin exposure from 0 – 24 months, how exposure of multi–mycotoxins affects growth and development (according to the conceptual framework of exposure to DON and FB, it is clear that infants are already effected in–utero, increasing the risks for intra-uterine growth restrictions, poor anthropometric measures at birth, altered development and growth faltering tremendously).
- Studies are needed to investigate the consumption of animal products (meat, eggs and milk) that are exposed to mycotoxins, and if consumption of exposed products will increase the overall mycotoxin exposure of humans.
- If infection markers are present in blood due to mycotoxin exposure, as both DON and FB affect the immune system, increase the duration of existing infections, increase systemic cytokines and decrease the expression of local proinflammatory cytokines.
- Investigation on sphingolipid enzymes, to determine if these enzymes are presenting in blood due to mycotoxin exposure; as it is known that both DON and FB interrupt the sphingolipid metabolism. Also, what can be done to reduce the disruption of this metabolism if enzymes are affected?

To conclude the results of the study done, it is evident that when pregnant women are exposed to DON, ZEA and FB during pregnancy, the mycotoxins, together with the adjusted confounding factors, will certainly affect the infant's anthropometric measures at birth, as well as gestational age.

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ANNEXURES

1. Health Research Ethics Committee approval

2. Questionnaires:
- General health questionnaire
- Validated food frequency questionnaire designed for this population
- Anthropometric measurements form
- Consent forms

3. Food and chemical toxicology requirements

4. Mycotoxin analyses procedures

ANNEXURE 1: Health Research Ethics Committee approval

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Dr MJ Lombard
Nutrition
CEN

29 October 2018

Dear Dr Lombard

APPROVAL OF YOUR APPLICATION BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00207-14-A1-02

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

Study title: Association between maternal multi-mycotoxin exposure and birth anthropometric growth of residents in rural Eastern Cape, South Africa

Study leader: Dr MJ Lombard

Student: M Entree-21774862

Application type: Sub-study

Risk level: Minimal (monitoring report required annually)

Expiry date: 31 October 2019 (monitoring report due at the end of October annually until completion)

You are kindly informed that after review by the HREC, Faculty of Health Sciences, North-West University, your ethics approval application has been successful and was determined to fulfil all requirements for approval. Your study is approved for a year and may commence from 29/10/2018. Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation. A monitoring report should be submitted two months prior to the reporting dates as indicated i.e. annually for minimal risk studies, six-monthly for medium risk studies and three-monthly for high risk studies, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC, Faculty of Health Sciences must be notified if the study is temporarily suspended or terminated. The monitoring report template is obtainable from the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECMonitoring@nwu.ac.za. Annually, a number of studies may be randomly selected for an internal audit.

The HREC, Faculty of Health Sciences requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the proposal or other associated documentation must be submitted to the HREC, Faculty of Health Sciences prior to implementing these changes. These requests should be submitted to Ethics-HRECApply@nwu.ac.za with a cover letter with a specific subject title indicating, "Amendment request: NWU-XXXXX-XX-XX". The letter should include the title of the approved study, the names of the researchers involved, the nature of the amendment/s being made (indicating what changes have been made as well as where they have been made), which documents have been attached and any further explanation to clarify the amendment request being submitted. The amendments made should be indicated in yellow highlight in the amended documents. The e-mail, to which you attach the documents that you send, should have a *specific subject line* indicating that it is an amendment request e.g. "Amendment request: NWU-XXXXX-XX-XX". This e-mail should indicate the nature of the amendment. This submission will be handled via the expedited process.

Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form to Ethics-HRECIncident-SAE@nwu.ac.za. The e-mail, to which you attach the documents that you send, should have a specific subject line indicating that it is a notification of a serious adverse event or incident in a specific project e.g. "SAE/Incident notification: NWU-XXXXX-XX-XX". Please note that the HREC, Faculty of Health Sciences has the prerogative and authority to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.

The HREC, Faculty of Health Sciences complies with the South African National Health Act 61 (2003), the Regulations on Research with Human Participants (2014), the Ethics in Health Research: Principles, Structures and Processes (2015), the Belmont Report and the Declaration of Helsinki (2013).

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECApply@nwu.ac.za.

Yours sincerely

Prof Wayne Towers

HREC Chairperson

Prof Minrie Greeff

Ethics Office Head

Current details: [3323952(2)] G:\My Drive\4. Research and Postgraduate Education\4.1.5 Ethics\NWU-00207-14-A1-02\9.1.5.4.1_A1_NWU-00207-14-A1-02_29-10-2018.docm 29 October 2018

File reference: 9.1.5.4.1

ANNEXURE 2: Questionnaires

MEDICAL QUESTIONNAIRE

Participant No. _____ **Date of interview _____**

Interviewer._____ **Date of birth _____**

Age			
Do you have home-grown maize at the moment?		Yes	No
Do you prepare food from your own home-grown maize at the moment?		Yes	No
Do you use home-grown maize to prepare weaning food?		Yes	No
Are you using any medication at the moment?		Yes	No
If yes what for:			
Do you suffer from any chronic disease? (TB, HIV etc)		Yes	No
Do you have any pain or burning when you pass urine?		Yes	No
Do you suffer from high blood sugar?		Yes	No
Have you been ill? Last two weeks: Past month:		Yes	No
If Yes, what was the illness:			
Did you see a doctor or visited the clinic?		Yes	No
Have you had any operations?		Yes	No
If yes what operation:			
Are you pregnant at the moment?		Yes	No

If Yes, how many months?		
Have lost any noticeable weight? Past month: Past three months:	Yes	No
Have you gained weight recently? Past month: Past three month:	Yes	No
Do you have any problems with swallowing food?	Yes	No
Do you experience any stomach discomfort or pain when eating?	Yes	No
Have you suffered from vomiting? At the moment: Past two weeks: Past month:	Yes	No
Have you had any diarrhoea? At the moment: Past two weeks: Past month:	Yes	No

Food frequency questionnaire
Study No. _____ **Date of interview _____**
Interviewer _____ **Date of birth _____**

Type of food BREAD	Yes / No	Home (H) / bought (B)	Portion size	Portions at a time	Less than a month	Amount per month	Amount per week	Amount per day	Total per month	Total per day
White bread										
Brown bread										
Baked bread										
Steamed bread										
Dumplings										
Vetkoek										
Other										

Type of food CEREALS	Yes/No	Home (H) / bought (B)	Portion size	Portions at a time	Less than a month	Amount per month	Amount per week	Amount per day	Total per month	Total per day
Corn on the Cob			Small Medium Large							
Whole Kernels										
Soft porridge			Mini 250g Small 360g Medium 557g Large 630g							
Stiff *pap*			Mini 200g Small 308g							

			Medium 478g Large 640g							
Crumbly *pap*			Mini 300g Small 462g Medium 592g Large 794g							
Samp			Mini 150g Small 207g Medium 414g Large 622g							
Other										

Type of food: MIXED DISHES	**Yes / No**	**Home (H) / bought (B)**	**Ratio**	**Portion size**	**Portions at a time**	**Less than a month**	**Amount per month**	**Amount per week**	**Amount per day**	**Total per month**	**Total per day**
Maize meal + *Imifino*			1:1:1 1:2:2 1:5:5 2:1:1	Mini 300g Small 426g Medium 512g Large 622g							
Maize meal + Spinach			1:1 1:2 1:5 2:1	Mini 250g Small 328g Medium 426g Large 530g							
Maize meal + Pumpkin			1:2 1:3 2:1 3:1	Mini 300g Small 426g Medium 592g Large 794g							
Maize meal + Beans			1:2 1:3 2:1 3:1	Mini 300g Small 445g Medium 544g Large 765g							

Samp + beans			1:2 2:1 3:1 5:1	Mini 350g Small 592g Medium 744g Large 896g							
Soup (Kernels + beans)			1:1 1:2 2:1 3:1	Mini 300g Small 462g Medium 592g Large 794g							

Type of food: MIXED DISHES	**Yes / No**	**Home (H) / bought (B)**	**Ratio**	**Portion size**	**Portion at a time**	**Less than a month**	**Amount per month**	**Amount per week**	**Amount per day**	**Total per month**	**Total per day**
Mealie rice + *Imifino*			1:2:2 1:3:3 2:1:1 3:1:1	Mini 300g Small 466g Medium 592g Large 794g							
Mealie rice + Spinach			1:2 1:3 2:1 3:1	Mini 300g Small 462g Medium 592g Large 794g							
Mealie rice + Pumpkin			1:2 1:3 2:1 3:1	Mini 300g Small 462g Medium 592g Large 794g							
Other											

Type of food MEAT	Yes / No	Home (H) / bought (B)	Portion size	Portions at a time	Less than a month	Amount per month	Amount per week	Amount per day	Total per month	Total per day
Chicken										
Eggs										
Other										

Type of food CONDIMENTS	Yes / No	Home (H) / bought (B)	Portion size	Portion at a time	Less than a month	Amount per month	Amount per week	Amount per day	Total per month	Total per day
Sugar										

Type of food: BEVERAGES	Yes / No	Home (H) / bought (B)	Portion size	Portion at a time	Less than a month	Amount per month	Amount per week	Amount per day	Total per month	Total per day
Tea			Cup Mug							
Coffee			Cup Mug							
Amagewu			Cup Mug							
Amasi			Cup Mug							
Other foods consumed										
Fruit										
Vegetables (not included										

above)										
Sweets										
Cold drink										

Study No. _____ Date of interview _____ Interviewer _____

MOTHER/ CAREGIVER	**HEIGHT 1**	**HEIGHT 2**	**WEIGHT 1**	**WEIGHT 2**	**WAIST CIRCUMFERENCE 1**	**WAIST CIRCUMFERENCE 2**	**COMMENTS**
MEASURE							

CHILD	**HEIGHT 1*** **LENGTH 1***	**HEIGHT 2*** **LENGTH 2***	**WEIGHT 1**	**WEIGHT 2**	**MID UPPER ARM CIRCUMFERENCE 1**	**MID UPPER ARM CIRCUMFERENCE 2**	**HEAD CIRCUMFERENCE 1**	**HEAD CIRCUMFERENCE 2**
Boy **Girl**	**HEIGHT 1*** **LENGTH 1***	**HEIGHT 2*** **LENGTH 2***						

* Standing height is about 0.7 cm less than recumbent length (don't subtract this amount, just indicate measured height / length)

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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

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ISIHLOKO SOVAVANYO

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

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REFERENCE NUMBERS: NWU-00207-14-S1

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Kwi Dyunivesithi yoMntla -Ntshona

Faculty of Health Sciences

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Potchefstroom

2522

INOMBOMBOLO MFONOMFONO:

018 299-2085

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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

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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

HREC Stamp

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

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Potchefstroom

2522

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018 299-2085

ANNEXURE 3: **Food and chemical toxicology requirements**

FOOD AND CHEMICAL TOXICOLOGY

5 AUTHOR INFORMATION PACK

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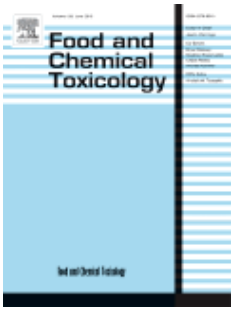

ISSN: 0278-6915

6 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,includingagriculturalandindustrialsafety**,and**consumerproductsafety**.Areas suchassafetyevaluationof**novelfoodsandingredients,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 **3Rs**.

The principal aim of the journal is to publish high impact, scholarly work and to serve as a multidisciplinary forum for research in toxicology. Papers submitted will be judged on the basis of scientificoriginalityandcontributiontothefield,qualityandsubjectmatter.**Studiesshouldaddress atleastoneofthefollowing**:Adversephysiological/biochemical,orpathologicalchangesinduced by**specificdefined**substancesNewtechniquesforassessingpotentialtoxicity,includingmolecular 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 scientificacceptability

Authorsmust**clearlyandbrieflyidentifywhatnoveltoxiceffect(s)ortoxicmechanism(s)**of thechemicalarebeingreportedandwhattheir**significance**isintheabstract.Furthermore,sufficient doses should be included in order to provide information on NOAEL/LOAELvalues.

Manuscripts describing research involving the following areas will not be considered: materials/substancesofonlylocalinterestmaterials/substancesforwhichthechemicalcompositionis notclearlydefinedonlypharmacologicalproperties,orpotentiallybeneficialeffectsusinginvitroorin vivosystemschemicalanalysesoftoxinsinfoodswithoutaddressingthetoxicimplicationtohumans [riskassessmentshouldbeincluded]unrealistichumandoses,inappropriaterouteofexposure,orin vitro experiments that do not reflect serum levels inhumans

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INTRODUCTION

Food and Chemical Toxicology (FCT), an internationally renowned journal, aspires to publish original researcharticlesandreviewson**toxiceffects**,inanimalsorhumans,ofnaturalorsyntheticchemicals 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-relationshipsbetweennutritionandtoxicology**andon*invitro*techniques,particularly those fostering the **3Rs**.

The principal aim of the journal is to publish high impact, scholarly work and to serve as a multidisciplinary forum for research in toxicology. Papers submitted will be judged on the basis of scientific originality and contribution to the field, quality and subject matter. Studies should address at least one of the following: Physiological, biochemical, or pathological changes induced by specific substances Techniques for assessing potential 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

Manuscriptsconcerningmaterials/substancesofonlylocalinterestforwhichthechemicalcomposition ofthematerial/substanceis**notclearlydefined**will**not**beconsidered.Manuscriptsaddressingonly pharmacological properties, or only potentially beneficial effects using in *in vitro* or *in vivo* systems, are not within the scope of thejournal.

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13 Types of paper

TheJournal'smainpurposeisthepublicationofpapersreportingandinterpretingoriginalunpublished toxicologicalresearch,particularlystudiespromotinganunderstandingofthemechanismsunderlying 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 willconciseinterpretativeReviewsoftoxicologicaltopicsofcontemporarysignificance.Letterstothe 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 theJournal.

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ANNEXURE 4: Multi-mycotoxin analysis procedures

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_2O (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_2O (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^{o}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 Precursors and products ions eased

Analyte	Cone Voltage (V)	Precursor Ion	Quantifier Ion (Collision Energy) (V)	Qualifier Ion (Collision Energy) (V)
Fumonisin B_1	50	722.3	334.3 (40)	352.3 (38)
Fumonisin B_2 and B_3	50	706.3	318.3 (40)	336.3 (40)
Deoxynivalenol	35	397.1	203.2 (15)	231.2 (12)
Zearalenone	20	319.1	185.0 (23)	187.0 (19)

Table 2 Calibration curves for each mycotoxin to compensate for matrix effects in the analysis.

Analyte	LOD[1] (µg/kg)	Spike level (µg/kg)	Recovery (%)	RSDr[2] (%)
Fumonisin B_1	0.6	1060	84	2
Fumonisin B_2	0.6	925	66	4
Fumonisin B_3	0.6	520	79	1
Deoxynivalenol	3.7	684	78	4
Zearalenone	1.0	232	95	3

[1] LOD: Limit of detection; [2] RSDr: Relative standard deviation for repeatability.

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.

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/child growth/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).