Mycotoxin health risk assessment modelling among maize-subsistence farmers living in Centane, Eastern Cape Province, South Africa.

by

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DECLARATION

I, Hester Maria Burger, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgments indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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DEDICATION

I would like to dedicate this work to two very inspiring and exceptional scientists that I had the privilege to know, to learn from, and to be mentored by. You did not just share your vast knowledge and experience but also opened the world of scientific research to me. I will never forget what you taught me and all that you did for me on my journey as a scientist. My sincere appreciation and gratitude to you all.

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Psalm 119:105 "*Your word is a lamp to my feet. And a light to my path*". Amplified© Bible

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GENERAL ABSTRACT

Harmful mycotoxins such as fumonisin B (FB), deoxynivalenol (DON), and zearalenone (ZEA), produced by ubiquitous food-borne fungal species, are known to contaminate maize crops from subsistence farming areas in Centane, Eastern Cape Province (EC), South Africa. The daily consumption of home-grown maize in these areas has resulted in FB exposure of five to ten times above the recommended provisional maximum tolerable daily intake (PMTDI) of 2 μg kg^{-1} body weight day^{-1}, as set by The Joint FAO/WHO Expert Committee on Food Additives. From a public health perspective, not only can these mycotoxins cause various human diseases but also impact food security. For this purpose, mycotoxin risk assessment among maize-subsistence farmers is critical to ensure evidence-based risk management. Currently, in resources poor settings, a deterministic risk assessment approach remains the easiest and most accessible. This approach, based on epidemiological data, includes using a total mean mycotoxin level (μg kg^{-1}) multiplied by the individual total mean dry/raw maize intake in g day^{-1} divided by body weight (kg) and expressed as a probable daily intake (PDI). The resultant PDI is thereafter compared to the relevant mycotoxin PMTDI to determine the risk. However, this type of assessment remains one-dimensional and can only quantify risk. To address this limitation, the current study was aimed at developing a mycotoxin risk assessment model that not only quantifies the risk of exposure to multi-mycotoxins (FB, DON, and ZEA) but it is also interactive. The new model was based on the Mycotoxin Risk Assessment Model (MYCORAM) that was originally developed for South African commercial maize consumers and referred to as the MYCORAM-II. The overall purpose of the MYCORAM-II is to assess the percentage of maize consumers above the relevant mycotoxins PMTDI. In the current study, a multiphase study design was used to develop and evaluate the model consisting of phase 1) dietary maize intake, body weight data collection, phase 2) the development of dietary dry maize intake per body weight categories, phases 3 and 4) mycotoxin levels data and the development of the MYCORAM-II, phase 5) evaluation of the MYCORAM-II using published mycotoxin levels in home-grown maize from Centane, and phase 6) to assess the appropriateness of the national maximum safety levels as set by the South African Department of Health for FB and DON in dry maize. The development of the model

was limited to the availability of secondary data, its validity, and the type of data that has been collected and published from studies conducted over the past 10 years in rural maize-subsistence farming areas in EC, South Africa. Applying mycotoxin levels in home-grown maize from Centane to the MYCORAM-II indicated that for total FB between 80-90% of maize, consumers were above its PMTDI based on overall higher levels of FB. In comparison, DON and ZEA with their lower levels in home-grown maize resulted in a lower percentage of consumers above the respective PMTDIs. The MYCORAM-II also indicated that the South African national maximum safety levels will not protect subsistence maize consumers between (89% and 90% will be at risk). In addition, the MYCORAM-II indicated differential risk scenarios when using published data from African countries. Overall, the MYCORAM-II highlighted the distinct roles of FB levels in home-grown maize, the number of positive samples, and maize intake practices. Despite the limitation of the MYCORAM-II, its simplicity and interactive function render it useful to describe multi-mycotoxin exposure among subsistence maize farmers not only in Centane, EC, but also in Africa. Its application can also be expanded towards developing population-specific safety levels, facilitating decision-making during risk management, and the evaluation of appropriate public health interventions aimed at reducing mycotoxin exposure.

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ABBREVIATIONS

ASIR	Age Standardised Incidence Rates
CAC	Codex Alimentarius Commission
CAGE	Cut down, Annoyed, Guilty and Eye-opener
CPUT	Cape Peninsula University of Technology
DOH	Department of Health
DON	Deoxynivalenol
EC	Eastern Cape Province
FAO	Food and Agriculture Organisation
FB	Fumonisin B
IARC	International Agency for Research on Cancer
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MSLs	Maximum Safety Levels
MYCORAM	Mycotoxin Risk Assessment Model
OC	Oesophageal Cancer
PDI	Probable Daily Intake
PI	Principle Investigator
PMTDI	Provisional Maximum Tolerable Daily Intake
UCT	University of Cape Town
WHO	World Health Organisation
ZEA	Zearalenone

PART A: STUDY PROTOCOL

1 Background

1.1 Problem statement

Exposure to harmful mycotoxins can be regarded as a silent public health epidemic, especially among subsistence farmers due to their chronic exposure to a high concentration of harmful toxins (Cardwell, 2000; Wild and Gong, 2010). Mycotoxins such as fumonisin B (FB), produced by ubiquitous food-borne fungi, are known to contaminate maize crops produced by subsistence farmers in Centane, Eastern Cape Province (EC) of South Africa. Fumonisin B, an International Agency for Research on Cancer (IARC) type 2B carcinogen, has been associated with several human diseases (e.g. oesophageal and liver cancer, neural tube defects, and childhood growth and developmental problems) (IARC, 2002; Mismer et al., 2006; Sun et al., 2007; Wu et al., 2014). In this regard, maize-subsistence farmers from Centane, EC have consistently shown probable daily fumonisin intake of 5 to 10 times above the corresponding provisional maximum tolerable daily intake (PMTDI) of 2 µg kg^{-1} body weight day^{-1}, as set by The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2012; Shephard et al., 2019). With the recent discovery of the presence of deoxynivalenol (DON) and zearalenone (ZEA) mycotoxins in home-grown maize from Centane, the health impact of multiple mycotoxin exposure has become an important public health consideration (Shephard et al., 2013a). The exposure to unsafe food of vulnerable groups, such as young children and people with existing diseases, and where food is discarded due to contamination, is creating an additional burden of food insecurity in South Africa.

In South Africa, 78-95% of people, depending on the type of dietary assessment method applied (24-hour recall vs. food frequency questionnaire), consume maize daily (Labadarios et al., 2003). The monotonous consumption of staples such as maize is, however, associated with food insecurity and nutrient deficiencies (Burger et al., 2010; Shisana et al., 2013). Despite the mandatory fortification of commercial maize meal (since 2003) of six vitamins and two minerals to address national micronutrient deficiencies, those consuming home-grown maize as part of a traditional daily dietary

pattern are more likely at risk for food insecurity, less diversity in their diet, and a poor nutritional status (Burger et al., 2010; Shisana et al., 2013).

Adults living in maize-subsistence farming areas in Centane are known to consume an average of between 340-630 g of cooked maize daily (Burger et al., 2010; Burger et al., 2014; Lombard et al., 2012; 2013).

These toxins, produced at any stage during the pre-and postharvest periods, are thermally and chemically stable and therefore regarded as highly persistent (Bullerman and Bianchini, 2007; Burger et al., 2013; Munkvold, 2003). Thus, to mitigate the risk of exposure to mycotoxins, appropriate models to characterise risk are warranted especially among maize-subsistence farmers, to prioritise risk management responses and strategies.

1.2 Mycotoxins and their occurrence in Centane, Eastern Cape

Maize crops are susceptible to filamentous fungal species that produce secondary metabolites called mycotoxins (Marasas, 2001; Marasas et al., 2012). The major mycotoxigenic fungal species responsible for the production of the FB mycotoxins are of the *Fusarium* genus such as *F verticillioides* and *F. proliferatum*, whereas *F. graminearum* and other related species are responsible for DON and ZEA. Fumonisins mycotoxins consist of 28 analogues, classified into four main groups: A, B, C, and P series (Marasas, 1996; Rheeder et al., 2002). From a toxicological perspective FB_1, FB_2 and FB_3 are the most relevant based on their high prevalence (Rheeder et al., 2002).

For the past 30 years, the main focus of mycotoxicological research in maize-subsistence areas in EC has mainly included the three FB analogous and their collective total level (Total FB = FB_1+FB_2+FB_3) (reviewed in Marasas, 2001). However, with the development of multi-mycotoxin analytical methods, the presence of two additional mycotoxins in subsistence maize from Centane, that of DON and ZEA, have also been identified (Shephard et al., 2013a, 2013b). These two mycotoxins have been associated with health disorders associated with the gastrointestinal and reproduction system, respectively (Amuzie and Pestka, 2010; Warth et al., 2013).

Mycotoxin contamination levels in maize crops are affected by numerous factors across the pre- and postharvest periods (including storage); consequently, they also

vary greatly across different seasons. The mean level of total FB in subsistence maize from Centane has ranged from 168-4006 (median 17-2870) μg kg^{-1} over 30 years (calculated from total FB data obtained from 12 studies conducted from 1985 to 2015 (Rheeder et al., 2016; Shephard 2007; 2013a; Tshalibe et al., 2020). Maize-subsistence agricultural practices associated with increased risk of fungal infection and mycotoxin production includes i) using a maize crop as a monoculture, ii) use of untreated seed, iii) late planting, iv) failure to remove crop residues, v) leaving crop residue to remain on the soil surface, vi) plant damage, vii) absence of pest control measurements, viii) inadequate drying of crops and ix) improper storage facilities (Fandohan et al., 2003; Munkvold et al., 2003; Ncube, 2008; Ncube et al., 2010; Wilke, 2007).

During the postharvest processing of maize, such as sorting, milling (dry and wet), cleaning and thermal treatment (cooking, roasting, baking, frying, brewing, nixtamalisation, and extrusion) only partially removes or degrades FB (Braun and Wink, 2018; Bullerman and Bianchini, 2007; Humph and Voss, 2004). The chemical conversion of FB within the food matrix during different food processing conditions remains complex and challenging. Currently, modified and/or bound mycotoxins (masked mycotoxins) are undetectable by standard analytical technologies, and their toxicological effect, independent, synergistic or additive effect relative to free mycotoxins, are an emerging concern to food safety specialists and authorities (Braun and Wink, 2018).

In rural maize-subsistence farming areas, postharvest practices and food processing include some form of traditional sorting, shelling, dehulling, winnowing and milling using hands, grinding rocks, wooden mortar and pestle, handle-operated shelling machines, small-scale motorised shelling or milling technologies (Alberts et al., 2019; Burger et al., 2010). Of these methods, sorting and shelling has proven to reduce FB levels the most effectively, between 69-71% and 57-65%, respectively (Desjardins et al., 2000; Fandohan et al., 2006; Kimanya et al., 2008; Matumba et al., 2015; Sydenham et al., 1994; Van der Westhuizen et al., 2010; 2011). In Centane, the traditional cooking of porridge using home-grown maize has shown very little reduction of FB (11.3%) (Shephard et al., 2012). Moreover, visibly mouldy maize is preferred for the preparation of traditional beer (Shephard et al., 2005; Burger et al., 2010). Affordable, culturally sensitive (and acceptable) ways to reduce mycotoxins effectively

remain challenging within the context of poor infrastructure, poverty, food insecurity, and lack of food diversity (Alberts et al., 2017; IARC, 2015).

1.3 Human health impacts and exposure to mycotoxins

The human health impacts associated with exposure to FB include an increased cancer risk (liver and oesophageal), childhood growth and developmental problems (stunting and neural tube defects) (Kimanya et al., 2010; Marasas et al., 2001; 2004). Whereas for DON these include gastrointestinal disorders, immune-suppression, anorexia, nausea, emesis, headache, chills, giddiness, and convulsions (Amuzie and Pestka, 2010), and for ZEA: precocious pubertal changes in children, early menarche, and other infertility related conditions due to its endocrine disruptive effect (Warth et al., 2013).

In the EC, the level of FB contamination and exposure has been associated with the risk of oesophageal cancer (OC) (Marasas, 2001). Subsistence-maize areas such as Bizana (north-eastern part of EC) with lower FB levels in maize has shown lower OC incidences in Centane, whereas high levels of FB contaminated maize and higher OC incidences co-occur (Rheeder et al., 1992; Shephard et al., 2007). Oesophageal cancer age-standardised incidence rates (ASIR) per 100 000 for Bizana and Centane stratified by gender, are summarised in **Table *1*** (Somdyala et al., 2013).

Table 1: Oesophageal cancer incidence rates from the population-based Eastern Cape Province Cancer Registry for two municipality areas

Area	Period	ASIR per 100 000 for males	ASIR per 100 000 for females
Bizana	1998-2002	37.2	19.4
	2003-2007	32.2	14.1
Centane	1998-2002	48.5	40.5
	2003-2007	54.3	38.9

ASIR: Age-standardised incidence rates (Somdyala et al., 2013)

Over the past nine years, OC incidence rates have consistently remained lower in Bizana compared to Centane. While this is also observed in the FB contamination levels of subsistence maize, the mechanistic link between OC and FB remains elusive.

Moreover, high FB levels in maize and OC incidence have also been observed in other countries such as China and Iran (Shephard et al., 2002; Sun et al., 2007; Wu et al., 2014).

1.4 Characteristics of maize-subsistence farming communities in Centane

Many households in Centane, Mnqunma Local Municipality, EC of South Africa produce maize to sustain their daily food staple (Burger et al., 2010; Lombard et al., 2012; 2013). Home-grown maize consumption among adults from these areas is two to four times higher per day (340-630 g) compared to EC commercial maize consumers (153 g) (Burger et al., 2010; Burger et al., 2014; Lombard et al., 2012; 2013). Adult maize consumers usually consume two meals a day that may include either bread and a maize-based dish or two maize-based dishes (Burger et al., 2010; Lombard et al., 2012; 2013). Furthermore, the consumption of traditional maize beer is normally a part of social gatherings where it is served in a communal dish (Burger et al 2010; Shephard et al., 2005; Matsha et al., 2006). Most adults have an estimated beer intake of 2 L per drinking event which may occur once a week. To validate this high intake, the four-question CAGE scoring method was used to measure alcohol dependence and regular use (Bradley et al., 2001; Ewing, 1984; O'Brien, 2008). The CAGE questions consist of "Have you ever felt you needed to **C**ut down on your drinking?"; "Have people **A**nnoyed you by criticising your drinking?"; "Have you ever felt **G**uilty about drinking?"; "Have you ever felt you needed a drink first thing in the morning (**E**ye-opener) to steady your nerves or to get rid of a hangover?" (Bradley et al., 2001; Ewing, 1984). A score of 2 (out of 4) was attributed to this group as an indicator of regular alcohol use (Burger et al., 2010). The consumption of maize beer may exacerbate exposure since "visibly mouldy" home-grown maize is preferred for the preparation to enhance the taste, and therefore may contain higher levels of FB (Shephard et al., 2005). However, even maize perceived as visibly good/healthy may contain high levels of FB and increase the consumption of unsafe food.

With the presence of a traditional monocereal diet, in addition to poverty, unemployment, and food scarcity, the additional loss of maize due to fungal and resultant mycotoxin contamination will also further contribute to food insecurity and possibly to existing micronutrient deficiencies (Alberts et al., 2019; Burger et al., 2010;

Eastern Cape Community Survey, 2018; Labadarios et al., 2011; Mnquma Local Municipality Socio-economic Review and Outlook, 2017; Wenhold and Faber, 2008).

1.5 Risk factors associated with increased mycotoxin exposure

Dietary and agricultural practices are not the only factors associated with an increased risk of exposure to mycotoxins. Other factors such as climate change, food insecurity, level of education, farm size, the non-existence of agricultural extension workers, lack of mycotoxin risk awareness and knowledge, absence of surveillance systems, inadequate regulatory standards and/or enforcement thereof, have also been suggested (Adegoko and Letuma, 2013; Burger et al., 2010; Fernandez et al., 2017; Jolly et al., 2009; Kumar and Popet, 2010; Misihairabgwi et al., 2017; Rheeder et al., 2016; Shephard, 2008). The multiplicity of factors associated with an increased risk of exposure is not fully characterised among susceptible subpopulations such as maize-subsistence farmers from Centane. However, the identification of modifiable risk factors such as those associated with the pre-and postharvest period is critical for reduction and prevention strategies (IARC, 2015).

1.6 Mycotoxin risk assessment and management in South Africa

The regulation of mycotoxins in food crops such as maize, is a global priority for public health, industry, and trade. Notwithstanding, such regulations require a scientific and evidence-based process that is systematic, comprehensive, transparent, and participatory. In this regard, mycotoxin risk analysis consists of risk assessment, risk management, and risk communication (FAO and WHO, 2009). Such risk assessment would comprise four critical steps: i) hazard identification and ii) characterisation (collectively also referred to as hazard assessment), iii) exposure assessment, and iv) risk characterisation. The overarching purpose of all these components is to provide regulatory guidelines and strategies to protect consumers' health against the harmful effects of mycotoxins. South Africa, a producer and user of maize, no national mycotoxin risk analysis process currently exists and has recently adopted the international Codex Alimentarius Commission (CAC) maximum safety levels (MSLs) (CAC, 2014; Department of Health, 2016). These include 4 mg kg^{-1} for FB and 2 mg kg^{-1} for DON in raw maize and whole maize (including maize meal and flour), whereas 2 mg kg^{-1} for FB and 1 mg kg^{-1} for DON in maize products (flour, meal, flakes or semolina) were set (CAC, 2014; Department of Health, 2016).

However, the problem with using international standards is that these are inappropriate within the context of subsistence farming and resource-scarce areas (Shephard et al., 2019). Applying specifically the FB safety levels to the Mycotoxin Risk Assessment Model (MYCORAM) indicated that between 74-92% of South African commercial maize consumers will be exposed above the PMTDI for FB (Burger et al., 2014; Shephard et al., 2019). The MYCORAM, an interactive model based on national raw commercial maize intake, was developed to i) identify specific South African maize-consumer groups at risk, ii) simulate risk scenarios using different contamination levels, and iii) predict maximum level (Burger et al., 2014). The MYCORAM has recently been used to develop national safety limits by a reputable South African grain manufacturing company to safeguard commercial maize consumers (Burger et al., 2014). Currently, the risk assessment approach in maize-subsistence farming areas include a deterministic approach and expressed as the probable daily intake (PDI) using mycotoxin levels (μg kg^{-1}) multiplied by the dry/raw portion of maize consumed in g day^{-1} divided by body weight (kg) (Alberts et al., 2019). The PDI can then be compared to the relevant mycotoxin PMTDI to assess risk. The use of a deterministic approach is based on utilising epidemiological data obtained from surveys and, despite its limitations and methodological challenges, is often the easiest in resources poor settings (Burger et al., 2014; Alberts et al., 2019).

1.7 Mycotoxin exposure as a public health problem

The economic and human health impacts of exposure to multiple mycotoxins such as FB, DON, and ZEA are overall poorly defined (Gbashi et al., 2018; Wild and Gong, 2010; Wu et al., 2014). Human studies investigating related health outcomes are limited and most stem from a few epidemiological studies with overwhelming evidence from *in vivo* and/or *in vitro* mechanist studies. With competing health priorities and poor health systems in a country such as South Africa, the chronic exposure to mycotoxins among subsistence farmers is regarded as of no consequence (Shephard et al, 2008; Shephard et al., 2019). The type of human studies required to address the health impacts of mycotoxins is expensive, labour intensive, time-consuming, complex, and require a participatory multiple stakeholder approach including government, research entities, funders; international agencies, subsistence farming communities, and traditional leadership, private sector and agricultural support systems (Wild and Gong, 2010; IARC, 2015).

However, in maize-subsistence farming areas in EC, the pre-existing burdens of food scarcity, poverty, nutrient deficiencies, OC incidence and other diseases such as HIV/AIDs and TB, the additional chronic intake of toxins may have a contributory effect (Hansotia et al., 2019; Massyn et al., 2020; Shisana et al., 2013; Somdyala et al., 2013; Statistics South Africa, 2018). Nonetheless, FB specifically is carcinogenic to animals, associated with liver and kidney cancers (Wild and Cong, 2010). Emerging human health concerns in this regard are the possible synergetic or additive effects of the simultaneous exposure to mycotoxins. In regards to this, the co-exposure to FB and DON and the activation of pro-inflammatory cytokines, morphological changes, and immune disruption have been observed in porcine intestines, known to be physiologically similar to humans (Bracarense et al., 2012; Pierron et al., 2016). More human studies are required to assess the true causal associations between exposure and health outcomes to guide mycotoxin risk assessment and management.

1.8 Research gap

Risk assessment, the evidence-informed process of guiding risk management and public health responses, remains critical. In the case of exposure to mycotoxins such as FB, DON, and ZEA, the absence of measurable adverse outcomes in humans hinders health risk assessment and the only useful tool remains the quantification of exposure and risk. Moreover, mycotoxin risk assessment (and management) approaches at the rural farm/household level will vastly differ from those in well-developed economic markets (Alberts et al., 2019). This is further impacted by the government's lack of political and economic commitment to mycotoxin exposure being a public health problem in these areas. To fill this methodological gap in the literature, appropriate and novel risk assessment approaches are warranted with what data is available from maize-subsistence farming areas in South Africa. Besides, a risk assessment method capable of validating mycotoxin prevention and reduction strategies is currently the method of choice while biomonitoring techniques remain expensive and inaccessible. For this purpose, the current study will focus on the development of a maize-subsistence farming health risk assessment model and validate this model using different real-time mycotoxin levels.

2 Study Aim

This research aim is to develop a multi-mycotoxin (FB, DON, and ZEA) health risk assessment model to determine the percentage of adult home-grown maize consumers from maize-subsistence farming areas in Centane, EC who are exposed above the PMTDI levels for each respective mycotoxin.

3 Study Objectives

The following objectives are for meeting the study aim:

1. To develop a multi-mycotoxin (total FB, DON, and ZEA) health risk assessment model for maize-subsistence farmers from Centane, EC using relevant secondary data (home-grown-maize intake in g day^{-1} and body weight in kg) from research conducted in these areas over the past 10 years.
2. To evaluate the relevancy and effectiveness of this health risk assessment model as a public health tool using available mycotoxin levels (µg kg^{-1}) in home-grown maize obtained from maize-subsistence areas in Centane, EC.
3. To assess the appropriateness of the South African National Department of Health's current maximum levels using this risk assessment model.

4 Study Methodology

4.1 Study design

A multi-phase study design will be used to develop and evaluate the maize-subsistence mycotoxin risk model aimed at quantifying the risk of exposure to multi-mycotoxins (FB, DON, and ZEA). The development of the model will be limited to the availability of a subset of already published data, its validity, and the type of data that has been collected in studies conducted over the past 10 years in rural maize-subsistence farming areas in EC, South Africa. The current model will be based on the Mycotoxin Risk Assessment Model (MYCORAM) and will utilise home-grown maize intakes and body weight obtained from adults living in maize-subsistence farming areas of Centane, EC. In contrast, the MYCORAM used commercial maize intake (and body weight) obtained from a South African national consumer survey (Burger et al., 2014). The purpose of using this existing model is its simplicity, noted usefulness, and relevancy (Burger et al., 2014; Alberts et al., 2019; Shephard et al., 2019). Furthermore, its effectiveness as an important public health tool will also be assessed by evaluating whether the current safety levels as set by the South African DOH are protective within the context of home-grown maize consumers from maize-farming areas in Centane, EC.

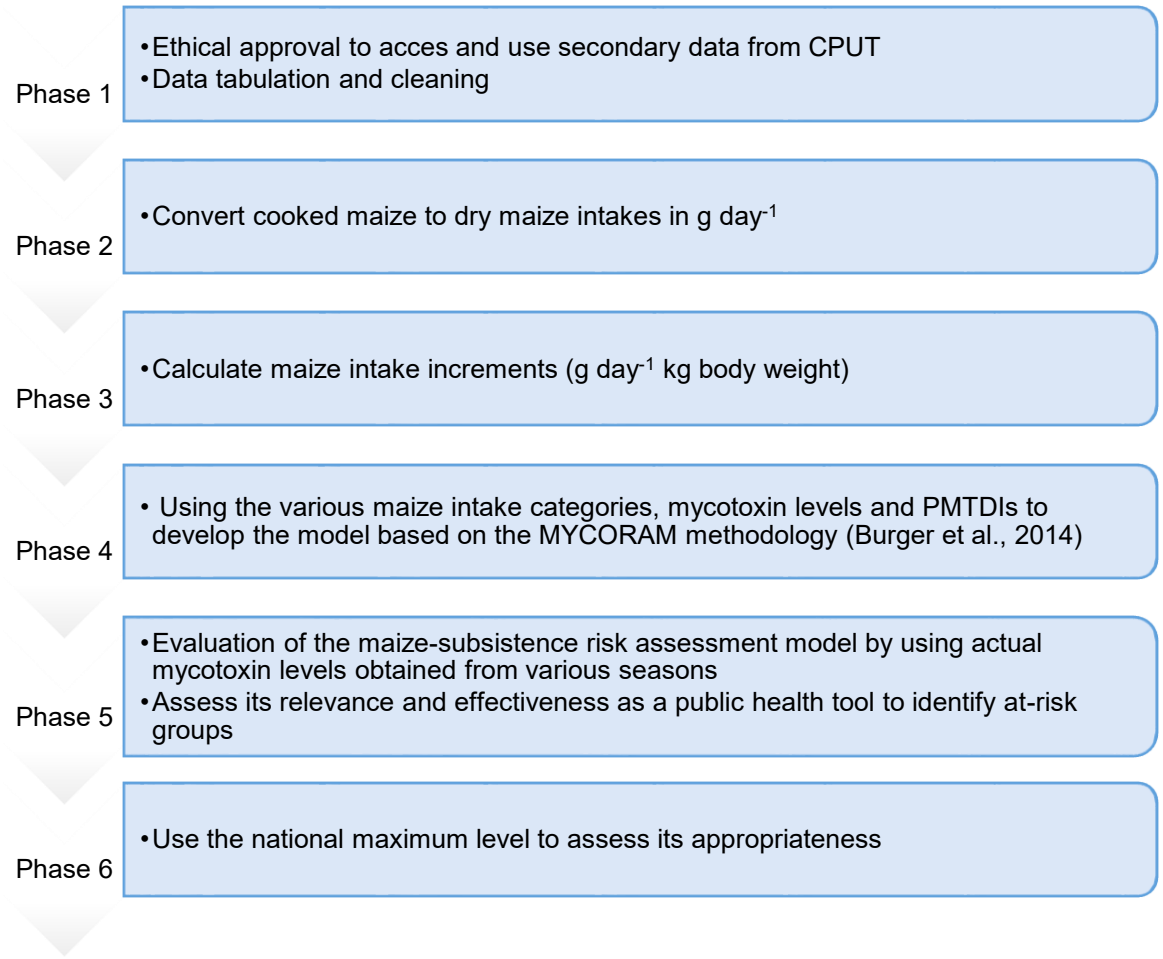

Figure 1: Multi-phase approach to develop and evaluate the maize-subsistence mycotoxin risk assessment model as a public health tool

Phase 1:

Obtaining ethical approval from both the Health Research Ethics Committee (the University of Cape Town) and the Faculty of Applied Science Researchers Ethics Committee, Cape Peninsula University of Technology. Once approval has been obtained, secondary data from all the studies done in the past 10 years among maize-subsistence farmers in EC, related to assessing maize intake, body weight, and mycotoxin level in home-grown maize, will be accessed. All data will be tabled in an Excel spreadsheet to clean the data and ensure there are no duplicates or other inconsistencies.

Phase 2:

Normally, dietary maize intake assessed via food frequency questionnaires (FFQ), 24-hour recalls or food records, are expressed as the intake of the cooked and prepared food item. For modelling exposure assessment, however, the dry/raw maize portion as an ingredient in certain traditional maize-based dishes consumed by maize consumers from subsistence farming areas in Centane, are of relevance (Burger et al., 2010, 2014; Alberts et al, 2019). With the development and validation of a culturally appropriate maize dietary assessment tool (FFQ and picture book), all the usual and traditional maize-based dish recipes were obtained and the ratio of maize to other ingredients were estimated and expressed as a maize-factor (Lombard et al., 2012, 2013). This provides a useful way of calculating individuals' dry maize intake per day and increases the accuracy of exposure assessment. The traditional maize-based dishes or food items consumed by maize-subsistence farmers are summarised in Table 2 and provide the specific ratios of maize when combined with other ingredients as well as the maize-factor. Secondary maize intake data spanning 10 years will be collated and the dry maize intake will be calculated using the relevant maize-factor according to the ratios of maize to other ingredients.

Table 2: The maize-factor for each traditional dish or food item normally consumed by maize–subsistence farmers (Adapted from Lombard et al., 2012; 2013)

Maize Dish or Maize Item	**Factor**
Bread Group	
Baked bread	1.65
Steamed bread	1.98
Cornbread	3.63
Dumplings	2.18
Vetkoek	1.63
Cereal Group	
Whole kernels	1.90
Soft porridge	7.31
Stiff pap	3.02
Crumbly porridge	1.75
Samp	3.2

Maize Dish or Maize Item	**Factor**
Mixed dishes Group (+ ratio)	
Maize meal + imifino *1:1*	5.33
Maize meal + imifino *1:2*	6.85
Maize meal + imifino *1:5*	17.46
Maize meal + imifino *2:1*	3.92
Maize meal + spinach *1:1*	4.62
Maize meal + spinach *1:2*	7.32
Maize meal + spinach *2:1*	3.66
Maize meal + spinach *1:5*	11.97
Maize meal + pumpkin *1:3*	12.79
Maize meal + pumpkin *3:1*	3.22
Maize meal + pumpkin *1:2*	6.00
Maize meal + pumpkin *2:1*	3.31
Maize meal + dried *beans 1:2*	6.67
Maize meal + dried *beans 2:1*	2.67
Maize meal + dried *beans 1:3*	8.71
Maize meal + dried *beans 3:1*	2.38
Samp + beans 2:1	5.29
Samp + beans 3:1	4.66
Samp + beans 5:1	3.87
Samp + beans 1:2	8.84
Soup 1:1	5.40
Soup 2:1	0.48
Soup 1:2	10.0
Soup 1:3	11.0
Mealie rice + imifino *1:3*	8.97
Mealie rice + imifino *3:1*	2.96
Mealie rice + imifino *1:2*	7.99
Mealie rice + imifino *2:1*	2.30
Mealie rice + spinach *1:3*	7.86
Mealie rice + spinach *3:1*	3.21

Maize Dish or Maize Item	**Factor**
Mealie rice + spinach *1:2*	20.10
Mealie rice + pumpkin *1:3*	7.98
Mealie rice + pumpkin *3:1*	2.72
Mealie rice + pumpkin *1:2*	5.78
Mealie rice + pumpkin *2:1*	3.88
Maize-based Beverage Group	
Amarewu	3.2
Traditional beer	3.2

Imifino: Type of wild green spinach-like leafy vegetable (Lombard et al., 2012)
Amarewu: Traditional sour fermented beverage (Lombard et al., 2012)

Phases 3 and 4

The first step in creating the risk model according to the MYCORAM methodology (Burger et al., 2014) is to create maize intake categories in g kg^{-1} body weight day $^{-1}$. Similar to the maize intake data, secondary data on body weight from previous research among maize-subsistence farmers will also be obtained and the data transferred to an Excel spreadsheet for cleaning. The various maize categories and corresponding mycotoxin levels will be chosen to reflect probable daily intake (PDI) scenarios below, equal to, and above the PMTDI of each respective mycotoxin. For example, an intake of 10 g kg^{-1} body weight day^{-1} (within the 10 to 20 maize intake category) and FB contamination level of 100 µg kg^{-1} will provide a PDI of 1.0 µg kg^{-1} body weight day^{-1}, which is below the PMTDI of FB (2 µg kg^{-1} body weight day^{-1}). This intake and levels are deemed safe for maize consumers, whereas a maize intake of 20 g kg^{-1} body weight day $^{-1}$ with the same level of FB (100 µg kg^{-1}) will render a PDI of 2 µg kg^{-1} body weight day^{-1}which is equal to the PMTDI of FB and an indication of risk or probability of harm.

Table 3: Development of the maize-subsistence risk assessment model using six steps (adapted from Burger et al., 2014)

Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
Dry maize intake categories in g kg^{-1} body weight day^{-1}	**Calculate the percentage (%) individuals per maize category**	**FB levels in μg kg^{-1}**	**DON levels in μg kg^{-1}**	**ZEA levels in μg kg^{-1}**	**Plot percentage (%) of each maize category (y-axis) against the mycotoxin levels (x-axis)**
20 and above		100	50	25	
10 to 19.9		200	100	50	
4 to 9.9		500	250	125	
2 to 3.9		1000	500	250	
1 to 1.9		2000	1000	500	
Up to 0.9					

Phase 5

This phase will be the obtaining of relevant mycotoxin levels of FB, DON, and ZEA in home-grown maize from studies conducted in the past 10 years in rural maize-subsistence farming areas in EC, South Africa. Using the maize-subsistence graph developed in phases 3 and 4, and applying actual mycotoxin levels, the health risk assessment model's relevance and effectiveness as a public health tool will be evaluated by determining the percentage of home-grown maize consumers at risk (above the PMTDI) for each mycotoxin. These phases will also identify whether this risk assessment model will be useful and practical in identifying and predicting at-risk groups and MSLs.

Phase 6

The MSLs for public health as set by the South African DOH (2016) for FB (FB_1 +FB_2) include that of 4 000 µg kg^{-1} in dry maize and 20 000 µg kg^{-1} for maize flour and maize meal. Whereas for DON it is 2 000 µg kg^{-1} in maize with ZEA not included (DOH, 2016). These MSLs will be evaluated by applying them to the model and to evaluate whether maize consumers from maize-subsistence areas in Centane are vulnerable to multi-mycotoxin exposure (as identified by the percentage above the PMTDI) or protected by these MSLs (very low or zero percentage above the PMTDI).

4.2 Study timeline 2020-2021

Task	August	September	October	December	January	February
Finalise protocol	X	X				
Ethics submission and review		X				
Data management			X			
Model development			X			
Evaluation of the model			X			
Draft article				X	X	
Submission of mini-thesis						X

5 Ethical Considerations

In the study proposed in this document, the principal researcher (Dr. H-M Burger, MPH student) will uphold all research integrity and ethical principles as stated in the Declaration of Helsinki and relevant institutional policies.

The intended study will be submitted for ethical approval at the Health Research Ethics Committee, UCT, as well as the Faculty of Applied Sciences Research Ethics Committee, CPUT to access secondary data. The data to develop the health risk assessment model was collected over a 10-year period by researchers from CPUT (including the principal researcher Dr. H-M Burger). Since all the secondary data required has already been published in various peer-reviewed scientific journals, only information on maize intake and body weight from adults (men and women), and FB, DON, and ZEA levels in home-grown maize from maize-subsistence areas in Centane, EC, South Africa will be used. The raw data is currently in Excel spreadsheet format and stored on a password-protected computer only accessible by the principal investigator and researcher (Dr. H-M Burger). No personal information or identifiable indicators are evident in this Excel database; only the unique study number that each voluntary participant received during the original studies are listed. Therefore, none of the data can be linked to a specific participant and his/her personal information.

5.1 Study risks and benefits

The current study poses minimal risk since it will utilise secondary data already published in peer-reviewed scientific journals and has already received ethical approval. Most of the studies conducted were descriptive and entailed assessing maize intake and practices, measuring body weight and food sampling, and multi-mycotoxin analyses of home-grown maize from maize-subsistence farmers from Centane, EC. Although the current research will not pose a direct benefit to the participants, it will however have a broader positive public health impact.

Since maize-subsistence farmers living in Centane are known to be exposed to harmful mycotoxins produced by food-borne fungi that infect home-grown maize, the current study is aimed at the development of a multi-mycotoxin health risk assessment model to quantify this risk. From a public health perspective, the consumption of unsafe food is a major concern, therefore public health tools to assist with decision-

making and the development of appropriate responses and strategies to reduce exposure are critical.

5.2 Informed consent

The current study will not require additional informed consent since this is a secondary data study using data that has already been obtained via an informed consent process and has received ethical approval before peer-reviewed publication. Although the various published research studies conducted in the area had different aims and objectives, their overarching purpose has always been that of i) increasing our understanding of and ii) reducing the exposure to harmful mycotoxins among maize-subsistence farmers. The main purposes have been explained both orally and in writing (in their home language of isiXhosa) to each participant during the informed consent process. The current study's aim is no different and falls within these purposes and therefore it is anticipated that no additional consent will be necessary to use the data.

5.3 Privacy and confidentiality

All data will be collated in a new Excel spreadsheet with only the unique study number, maize intake, body weight, and mycotoxin levels visible. No other data will be accessed, and the data obtained will only be used for the intended purposes described in this protocol. Each participant normally receives a unique study number to ensure anonymity and confidentiality. The current study poses no additional risks or harm to any of the participants who participated in the original studies. Since the data has already been published in various articles over the past 10 years, ethical approval would have been obtained. All databases compiled as results of this study will be password protected with only the principal investigator and student (Dr. H-M Burger) allowed access to its contents. It is therefore anticipated that participants' identity and privacy will be protected at all stages of the current study.

5.4 Data analysis and monitoring

Data analysis will be done in Excel by the student and the resultant model will also be evaluated using published mycotoxin levels and other relevant scientific publications on mycotoxin risk assessment. The researcher will remain the custodian of the data and will be responsible for managing the databases and safeguarding access.

5.5 Resources

All resources required to conduct the study, develop the risk assessment model, evaluate the model, and complete the study are available to the researcher and principal investigator.

5.6 Study outcome and authorship

The results from this study will be used for the completion of a mini-thesis as part of the requirement for the degree Master of Public Health, Environmental Health Track, UCT. It is anticipated that the study will be published in a peer-reviewed, high-impact journal and presented at conferences relevant to the field of mycotoxicology and food safety. In recognition of the work of fellow mycotoxins researcher collaborators of 10 years and more, who have contributed to the data used in this study, authorship will be made available (Dr. MJ Lombard, Northwest University; Dr. HA Alberts, CPUT and Dr. JP Rheeder, CPUT).

5.7 Conflicts of interest

The principal investigator and researcher have no perceived, real or potential conflicts of interest to declare.

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PART B: JOURNAL READY MANUSCRIPT

Mycotoxin health risk assessment among maize-subsistence farmers: Introducing the Mycotoxin Risk Assessment Model (MYCORAM-II) and its applications

Journal: Food Control (Appendix 1)

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Abstract

Harmful mycotoxins such as fumonisin B (FB), deoxynivalenol (DON), and zearalenone (ZEA), produced by ubiquitous food-borne fungal species, are known to contaminate maize crops from subsistence farming areas in Centane, Eastern Cape Province (EC), South Africa. The daily consumption of home-grown maize in these areas has resulted in FB exposure of five to ten times above the recommended provisional maximum tolerable daily intake (PMTDI) of 2 μg kg^{-1} body weight day^{-1}, as set by The Joint FAO/WHO Expert Committee on Food Additives. From a public health perspective, not only can these mycotoxins cause various human diseases but also impact food security. For this purpose, mycotoxin risk assessment among maize-subsistence farmers is critical to ensure evidence-based risk management. The aim of the current study was to develop a unique subsistence maize Mycotoxin Risk Assessment Model (MYCORAM-II) for Total FB (FB_1 + FB_2 + FB_3), DON, and ZEA using secondary data from published studies. The overall purpose of the MYCORAM-II is to assess the percentage of subsistence maize consumers equal to or above the relevant mycotoxins PMTDI. The newly constructed MYCORAM-II was further evaluated using mycotoxin levels in home-grown maize from previous studies conducted in Centane. Thereafter it was used to assess the appropriateness of the South African maximum safety levels for FB and DON in dry maize, as established by the Department of Health. Various selected studies conducted in Africa relevant to FB were also used to assess the MYCORAM-II usefulness in different FB risk scenarios. Applying mycotoxin levels in home-grown maize from Centane indicated that for total FB, between 80-90% of maize consumers were above the respective PMTDI; whereas applying the South African national maximum safety levels, that of 4 000 μg kg^{-1} for FB_1 + FB_2 and 2 000 μg kg^{-1} for DON, showed that 90% and 89% of home-grown maize consumers will be at risk of exceeding the PMTDI, respectively. From a public health perspective, this may have a severe impact on the health status and the existing burden of disease of people living in subsistence farming areas in the EC. Four FB risk scenarios were constructed from studies conducted in Africa (Tanzania, Nigeria, Zimbabwe, and Algeria) and highlighted the distinct roles of FB levels in home-grown maize, the number of positive samples, and maize intake practices. The MYCORAM-II indicated that between 36 to 100% of these consumers were at risk of exposure to harmful toxins. Despite the limitation of the MYCORAM-II, its simplicity and interactive

function render it useful to describe multi-mycotoxin exposure among subsistence maize farmers not only in Centane, EC but also in Africa. Its application can also be expanded towards developing population-specific safety levels, facilitating decision-making during risk management, and the evaluation of appropriate public health interventions aimed at reducing mycotoxin exposure.

1 Introduction

Equitable access to safe maize remains a challenge in especially maize-subsistence farming areas within South Africa. In this regard, the first target of the United Nations' Sustainable Development Goals under goal number 2 (Zero Hunger) reads: "By 2030, end hunger and ensure access by all people, in particular the poor and people in vulnerable situations, including infants, to **safe**, nutritious and sufficient food all year round" (UN 2015). This projection, aimed at low- and middle-income countries, has yet to have an impact on vulnerable populations consuming unsafe food daily and more so in times when food is scarce. In South Africa, food security has been the main focus of the Department of Health and an increase in household food security from 2002 to 2017 has been achieved (Statistics South Africa, 2019). This narrow focus, however, has neglected food safety as a critical function of both nutrition and public health (Misselhorn and Hendriks, 2017; Ngumbela et al., 2020).

In subsistence farming areas in the Eastern Cape Province (EC), South Africa, harmful mycotoxins produced by filamentous food-borne fungal species are known to contaminate maize crops. The most predominant mycotoxins, fumonisin B_1, (FB_1) an International Agency for Research on Cancer (IARC) type 2B carcinogen, are associated with several human diseases namely oesophageal and liver cancer, neural tube defects, and childhood growth and developmental problems (IARC, 2002; Mismer et al., 2006, Sun et al., 2007; Wu et al., 2014). The maize-based traditional staple diet of farmers from Centane, EC has increased their daily exposure to fumonisin B (FB) five to ten times above the recommended provisional maximum tolerable daily intake (PMTDI) of 2 µg kg^{-1} body weight day^{-1}, as set by The Joint Food and Agricultural Organisation/World Health Organisation (FAO/WHO) Expert Committee on Food Additives (Shephard et al., 2019*;* JECFA, 2012). Apart from FB, the presence of two other mycotoxins, that of deoxynivalenol (DON) and zearalenone (ZEA) in home-grown maize from Centane, highlights the importance of the health impact of chronic

multiple mycotoxin exposure (Marasas, 1996; Rheeder et al., 2002; Shephard et al., 2013a). Deoxynivalenol is cytotoxic and immunosuppressive, resulting in gastrointestinal dysfunction, reduced nutrient absorption, risk of infectious diseases as well as decreased growth in especially animals, whereas various gastrointestinal problems have been observed in humans (Etzel, 2014; Payros et al., 2016; Pinton and Oswald, 2014; Waśkiewicz et al., 2014). Dietary exposure to ZEA, a non-steroidal estrogenic mycotoxin, has been linked to precocious puberty and other reproductive problems (Asci et al., 2014; Massart et al 2008; Warth et al., 2013).

Addressing public health issues requires a systematic, transparent, and action-based process known as mycotoxin risk analysis, consisting of risk assessment, and management, and communication is critical to ensure safe food (FAO and WHO, 2009). The process consists of risk assessment, a science-based approach that includes hazard identification and characterisation, collectively also referred to as hazard assessment, exposure assessment, and risk characterisation (FAO and WHO, 2009). In advanced economic markets, however, the regulation of mycotoxins is often an interplay between economics, politics, preservation of trade, and public health considerations (Wu and Guclu, 2012). In South Africa, both a large producer and user of maize grain with an approximate production of over 10 million tons each year, commercial maize is known to contain much lower levels of mycotoxins and is regulated, monitored, and controlled by specific structures, legislation, policy, and procedures (Burger et al., 2013; Locke et al 2013; Shephard et al., 2019). In 2016, South Africa adopted the international Codex Alimentarius Commission (CAC) maximum safety levels (MSLs) of 4 and 2 mg kg^{-1} for raw maize and whole maize (including maize meal and flour) respectively, for FB and DON: 2 mg kg^{-1} for maize and 1 mg kg^{-1} for maize product (flour, meal, flakes or semolina) (CAC, 2014; Department of Health, 2016). While these strategies protect commercial maize consumers from harmful mycotoxins, maize-subsistence farmers in resource-poor areas remain vulnerable (Shephard et al., 2019).

In resource-poor settings, mycotoxin risk assessment is commonly estimated using a deterministic approach whereby the individual risk is expressed as a probable daily intake (PDI) (Alberts et al., 2019). This approach includes using quantitative epidemiological data. Despite the known limitations and methodological challenges of collecting such data, this remains the most accessible, affordable and easiest, and

currently also allows for the comparison of similar studies across Africa (Burger et al., 2014; Alberts et al., 2019). For health risk assessment, the PDI can then be compared to the relevant mycotoxin PMTDI and thus evaluate and describe the probability and seriousness of the exposure to harmful mycotoxins.

Mycotoxin research conducted in Centane, EC over the past 20 years have provided valuable quantitative data on dietary maize intake patterns, mycotoxin levels, and anthropometrical measurements using validated methods. In addition, a South African Mycotoxin Risk Assessment Model (MYCORAM) was developed to i) identify specific South African maize-consumer groups at risk, ii) simulate risk scenarios using different contamination levels, and iii) predict a maximum level (Burger et al., 2014). Thereafter, the MYCORAM was used to develop safety limits for a South African grain manufacturing company to safeguard commercial maize consumers (Burger et al., 2014). This unique model provides an opportunity to develop a similar tool to be used to assess a highly at-risk population. Therefore, the current study is aimed at the development of a maize-subsistence farming health risk assessment model building on MYCORAM. This model is the first health risk assessment tool that focuses on the consumption of home-grown maize as a staple and is called MYCORAM-II. Moreover, the MYCORAM-II was validated utilising different real-time mycotoxin levels in home-grown maize and applied to different mycotoxin scenarios across Africa. Illustrated in this article is that, despite the limitations of the model, it is used for public health policy decision-making to reduce vulnerable populations' exposure to harmful levels of mycotoxins.

2 Material and Methods

2.1 Study design

To develop the MYCORAM-II, a subset of secondary published data from a large research project conducted in 2010 in Centane, EC among maize-subsistence farming households was used including dry maize intakes and body weights (Burger et al., 2010; Lombard et al., 2012; 2013). The parent project used a multiphased approach to develop and evaluate the mycotoxin risk assessment model, referred to as the MYCORAM-II, and included: 1) calculating various dry maize intake per body weight categories in g kg^{-1} body $weight^{-}$ day^{-1}; 2) determining the percentage (%) of individuals within a specific maize intake category; 3) use of different contamination levels of FB, DON, and ZEA in μg kg^{-1} relevant to their PMTDIs; 4) plotting the percentage (%) of individuals within each maize category (y-axis) against the mycotoxin levels (x-axis) with a linear association (y= mx + b); 5) evaluation of actual mean mycotoxin levels in home-grown maize from Centane; and 6) establishing the appropriateness of the South African national regulatory maximum levels for mycotoxin contamination. This health risk model was based on the development of the first South Africa nation-wide MYCORAM by Burger et al. (2014) with the difference that it is aimed at estimating risk among a focused high-risk subpopulation from the EC exposed to FB, DON, and ZEA. Data required for the MYCORAM-II included dietary dry and raw maize intake, body weight, and the various PMTDIs relevant to FB, DON, and ZEA. To evaluate the model, actual levels of these mycotoxins from published data were used as well as the regulatory level of the South African National Department of Health.

2.2 Study area

This study used data obtained from published studies conducted among maize-subsistence farming households in Centane, EC. Centane, together with the Butterworth and Ngqamakhwe Traditional Councils, forms the Mnqunma Local Municipality (Figure 1 and Figure 2) and is situated in the South-Eastern part of the EC Province and is under the authority of the Amathole District Municipality in South Africa. Land tenure, access, and management are mostly governed by local traditional leaders such as chiefs, headmen, and their respective committees. The Mnqunma Local Municipality, like many other areas within the EC, are often referred to as rural,

characterised by lack of access to resources, underdeveloped infrastructure, poverty, household food insecurity, poor diet diversity, unemployment, and lower levels of education (Statistics South Africa, 2019). The total population is approximately 252 390 people living across 3 270 km² (Mnquma Local Municipality website, 2021). Of the 75 410 households in this local municipality, 57% are involved in agricultural activities that mostly involve maize production (Mnquma Local Municipality website, 2021). Governmental agricultural support, such as providing cultivation subsidies, and supply supported fertilizers, pesticides, and seeds, to small farming households across the EC, is about 25.1% (Mkhonto and Musundire, 2019; Statistics South Africa, 2018). Sixty-one percent of the population are between the ages of 15 and 64 years with 99% isiXhosa speaking Africans, of which 54% are female (Mnquma Local Municipality website, 2021; Municipalities of South Africa, 2021; Statistics South Africa, 2018; Labadarios et al., 2011; Lahiff, 2003; Ntsebeza, 2005).

Figure 2: Map of the Amathole District Municipality, with the arrow indicating the study site near Kentani

Source: https://municipalities.co.za/map/1007/mnquma-local-municipality

Figure 3: Typical scenery in Centane with traditional farmsteads spread across the area with no formal addresses or available listings thereof and connected with gravel roads.

(Source: HM Burger)

2.3 Study phases

2.3.1 Phases 1 and 2: Dietary maize intake, body weight data, and the development of dietary dry maize intake per body weight category

The individual daily intake (in g day^{-1}) of home-grown maize, including cooked kernels or cobs, maize-based dishes such as maize porridge, traditional maize-based beverages, and any combined maize-based dishes, obtained from published studies conducted in Centane, EC, was used (Burger et al., 2010; Lombard et al., 2012; 2013). A database with all the cooked maize-based dishes was created and each maize-based item was converted to a dry maize portion followed by the calculation of a mean in g day^{-1} for each individual as well as a study group total mean intake. Similarly, corresponding individual body weight data in kg were also added to the database (Burger et al., 2010; Lombard et al., 2012; 2013).

The dietary home-grown maize intake data was originally obtained using a culturally appropriate and validated maize dietary assessment method consisting of a food frequency questionnaire (FFQ) and picture book (Burger et al., 2010; Lombard et al., 2012; 2013). The FFQ contained traditional maize-based dishes commonly consumed

and all the various recipes, including the various ratios of maize to other ingredients, were available, allowing for the conversion of individual cooked maize-based dishes (including combined or mixed maize dishes for example spinach and maize meal) into an individual raw/dry maize portion (Burger et al., 2010; Lombard et al., 2012; 2013). Body weight measurements were also determined according to standardised practices such as using a scientific scale to measure the mean of two readings in kg, after the removal of shoes and any heavy clothing, in a private setting by a trained fieldworker in anthropometry (Burger et al., 2010; Lombard et al., 2012; 2013).

To develop the MYCORAM-II, various dry maize intake per body weight categories in g kg^{-1} body weight day^{-1} were created using the secondary data which includes the individual dry maize intake per day and body weight (kg), transferred to a new database. The dry maize intake categories were created by dividing the dry maize intake per person per day by the body weight, similar to those developed for the first MYCORAM (Burger et al., 2014). In each of these dry maize intake categories, the number of individuals was calculated and expressed as a percentage (%).

2.3.2 Phases 3 and 4: Mycotoxin levels and the development of the MYCORAM-II

Various mycotoxin levels (in µg kg^{-1}) for FB, DON, and ZEA were selected specificially so that, if multiplied by the lower limit of a dietary maize category, it will equal the respective PMTDI of the mycotoxin. For this model, the total FB was used which includes combining the individual levels of the three most predominant FB analogues (FB_1, FB_2, and FB_3) known to be present in home-grown maize (Marasas, 2001; Burger et al., 2014). The respective PMTDIs were 2.0 µg kg^{-1} body weight day^{-1} for FB; 1.0 µg kg^{-1} body weight day^{-1} for DON and 0.5 µg kg^{-1} body weight day^{-1} for ZEA (JECFA, 2000; 2001; 2012). Finally, the percentage of individuals within a dietary maize category was plotted on a line graph with the maize intake per body weight category, reflecting those above the PMTDI of a specific mycotoxin on the y-axis, and the selected mycotoxin levels for FB, DON, and ZEA respectively on the x-axis. The graph, therefore, provides a way of estimating those at risk of exposure to harmful mycotoxins if the level of mycotoxin is known.

2.3.3 Phases 5 and 6: Evaluation of the MYCORAM-II

To evaluate the MYCORAM-II, individual mean mycotoxin levels in home-grown maize from Centane, obtained from published data across 20 years, was used. This data originated from studies conducted by the Mycotoxicology Unit, Cape Peninsula University of Technology (former PROMEC Unit, South African Medical Research Council). These included six studies conducted during 2000, 2003, 2011, 2013, 2015, 2016 and are summarised in Table 1. The 2000 and 2003 studies provided data on FB whereas from 2011 and onwards DON and ZEA were included using multi-mycotoxin analyses. Fumonisin B remained the most prominent mycotoxin with consistently high levels in all collected maize samples. Similarly, the maximum safety level for FB_1 and FB_2 (combined) as well as DON in dry maize, maize meal, and maize flour, adopted by the South African National Department of Health in 2016, was applied to the MYCORAM-II for their appropriateness to protect people consuming home-grown maize as a daily staple.

Table 1: Mycotoxin level used to evaluate the MYCORAM-II

Period	Description of maize collected and mycotoxin analysis	Mycotoxin analysis	n	Mycotoxin	Level in µg kg^{-1}	Reference
2000	Visibly healthy home-grown maize was collected during July and August, 4-6 weeks postharvest from four areas within Centane. Random samples of either 2 kg grain or 10-12 cob were collected and analysed. Mycotoxin levels were log-transformed and the mean with the minimum and maximum for the whole area (Centane) was used. Thirty-seven of the forty samples tested positive for the presence of FB.	Liquid Chromatography (LC)	40	FB_T	973 (nd-7965)	Rheeder et al., 2016
2003	Collection and expression of mycotoxin levels are similar to that presented above. Of the 24 samples collected, 23 were positive for FB.	LC	24	FB_T	2150 (nd-8385)	Rheeder et al., 2016
2011	Random 1 kg visibly healthy maze samples were collected from four areas within Centane during July 2011. Mean, medians and ranges were provided for two analytical methods.	LC-Mass Spectrometry (MS) /MS	54	FB_T	4006 (32-26113)*	Shephard et al., 2013a
				DON	4.7 (2.2-14)	

Period	Description of maize collected and mycotoxin analysis	Mycotoxin analysis	n	Mycotoxin	Level in µg kg^{-1}	Reference
	Only the means of the dilute and shoot method is presented here since it includes FB1, FB2, and FB3 as well as DON and ZEA. Samples were 93%, 100%, and 39% positive for all the FB analogous, DON, and ZEA respectively.			ZEA	44 (0.6-329)	
2013 - 2015	Home-grown maize was collected postharvest and during the storage period in 2013, 2014, and 2015. Samples were analysed and a combined mean was calculated. Samples were 100% positive for total FB, 18% for DON, and 14% for ZEA.	LC-MS/MS	52	FB_T	1035 (1.-15590)**	Tshabile et al., 2020
				DON	25 (4-403)**	
				ZEA	31 (1-1523)**	
2016	Maximum safety level in dry maize.	Adopted from the Codex Alimentarius Committee (CAC)	-	FB_1 + FB_2 in dry maize	4000	CAC, 2014 Department of Health, 2016***
				DON in dry maize	2000	

n: sample sizes, Total FB (FB_T)= FB_1+FB_2+FB_3; n/d: not detected, FB: Fumonisins B; DON: deoxynivalenol; ZEA or ZON: zearalone.

*Mean total FB calculated from adding FB_1, FB_2 and FB_3 values.

** Values rounded to nearest 1.

***file:///E:/Laptop%20Jan%202021/MPH%20for%20ethics/No-987-Sept-05-2016-Foodstuffs-Cosmetic-and-Disinfectants-Act-54-1972.pdf

To investigate the usefulness of the MYCORAM-II, mycotoxin levels from home-grown maize were applied. These levels were obtained from published studies conducted in African countries known for maize-subsistence farming and mycotoxin contamination. For this purpose published data was used where FB levels in home-grown maize (µg kg^{-1}) were available, as well as risk assessment expressed as a probably daily intake (PDI: µg kg^{-1} body weight day^{-1}) using relevant mean dietary maize intakes (g per person per day) and body weight (kg).The assumption is that the MYCORAM-II will reflect the percentage of home-grown maize consumers from these African studies that are above the PMTDI for FB and consequently at risk.

2.4 Statistical analysis

STRATA®14, Statistics/Data Analysis, Copyright 1985-2015, StataCorp LP, United States was used to test for normality as well as to determine the numerical means, ± standard deviation, and the median with the minimum and maximum ranges for quantitative data such as age, raw maize intake, and body weight. Microsoft Excel (Microsoft Office, 2016) was used for the development and evaluation of the MYCORAM-II.

2.5 Ethical considerations

Ethical approval for this study was obtained from the Human Research Ethics Committee (HREC), University of Cape Town (HREC reference: 060/2021, Appendix 2), and the Faculty of Applied Science Research Ethics Committee, Cape Peninsula University of Technology (Reference no. BRGHES001/03/2021, Appendix 3) and CPUT site permission dated: 29 March 2021, Appendix 4).

3 Results

3.1 Dry maize intake per body weight categories

Four hundred and ninety-five adult men and women home-grown maize consumers' maize intake (g day^{-1}) and body weight (kg) data were used to develop the various dietary maize categories for the MYCORAM-II (Table 2). Since the data for maize intake and body weight data were not normally distributed, the median, minimum, and maximum were also provided. The mean age ± standard deviation (median, minimum and maximum) of the total study population (N = 495) was 40 ± 15 years (39, 18-82), whereas the means for dry maize intake and body weight for the total population were 515 ± 467 g day^{-1} (414, 0-3785) and 72 ± 18 kg (69, 41-150), respectively.

Table 2: Dietary dry maize per body weight categories for the MYCORAM-II (N = 495)

Dry maize intake categories in g kg^{-1} body weight day^{-1}	n	Percentage (%) individuals per dietary maize category
20 and above	29	5.9
10 to 19.9'	109	22
4 to 9.9'	129	38.8
2 to 3.9'	69	13.9
1 to 1.9'	33	6.7
Up to 0.9	63	12.7
Total	495	100

n: sample size, number of individuals within a dietary maize category.

In each of the maize categories, the percentage of individuals was calculated similarly to the original MYCORAM (Burger et al., 2013).

3.2 The constructed maize-subsistence MYCORAM-II

The minimum level of each maize intake category was used to identify a specific mycotoxin level that will provide a probable daily intake above the respective PMTDI for each mycotoxin. To construct the MYCORAM, the specific level for each mycotoxin was plotted on the x-axis whereas the percentage of individuals above or equal to the PMTDI (Table 3) was plotted on the y-axis (Figure 3). The completed MYCORAM-II provides the percentage of individuals equal to or above the respective PMTDI depending on the mycotoxin level applied to the model.

Table 3: Mycotoxin level relevant to their PMTDI

Dietary maize intake minimum level in g kg^{-1} body $weight^{-1}$ day^{-1}	FB_T levels in μg kg^{-1}	DON levels in μg kg^{-1}	ZEA levels in μg kg^{-1}	PMTDI in μg kg^{-1} body $weight^{-1}$		
				FB_T	DON	ZEA
20	100	50	25	2	1	0.5
10	200	100	50			
4	500	250	125			
2	1000	500	250			
1	2000	1000	500			

FB_T = Total Fumonisin B = FB_1 + FB_2 + FB_3; DON: Deoxynivalenol, ZEA: Zearalenone;
PMTDI: Provisional Maximum Tolerable Daily Intake

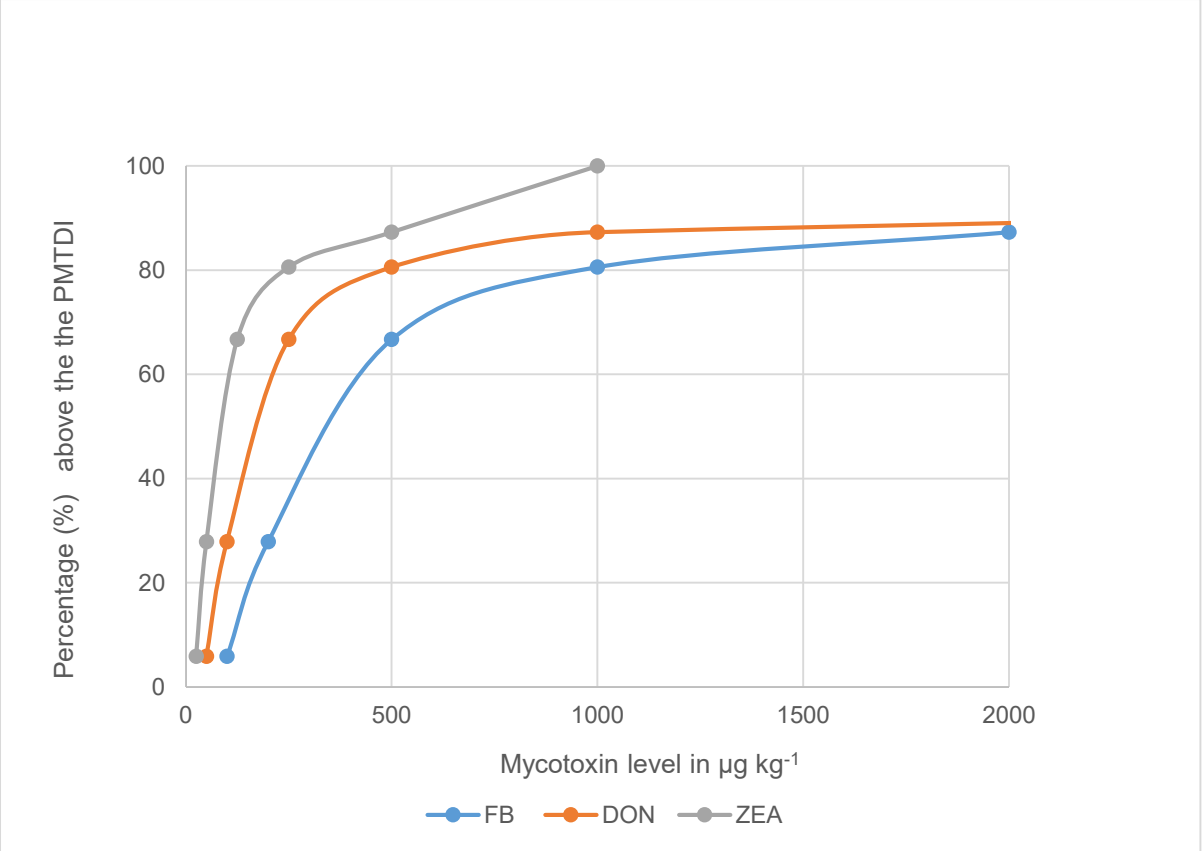

Figure 3: The constructed MYCORAM-II showing a specific level for each mycotoxin plotted against the percentage of individuals above or equal to the PMTDI

3.3 Evaluation of the MYCORAM-II

Applying the various mycotoxin levels to the MYCORAM-II indicated that 80- 90% of individuals consuming FB-contaminated home-grown maize are above the relevant PMTDI.

Table 4 Evaluation of the MYCORAM-II using various mycotoxin levels in home-grown maize from Centane

Period	Mycotoxin	Level in µg kg^{-1}	Percentage (%) of individuals above the respective PMTDI
2000	FB_T	973	80

Period	Mycotoxin	Level in µg kg^{-1}	Percentage (%) of individuals above the respective PMTDI
2003	FB_T	2150	88
2011		4006	90
	DON	4.7	1
	ZEA	44	23
2013 - 2015	FB_T	1035	82
	DON	25	3
	ZEA	31	11

FB_T = Total Fumonisin B = FB_1 + FB_2 + FB_3; DON: Deoxynivalenol, ZEA: Zearalenone; PMTDI: Provisional Maximum Tolerable Daily Intake. See Table 3 for how the mycotoxin levels were obtained and/or determined.

The safety levels set by the South African Department of Health showed that almost 90% of individuals from maize-subsistence farming areas in Centane will be at risk for both FB and DON mycotoxins (Table 5).

Table 5 Evaluation of the South African National maximum safety levels in dry maize using the MYCORAM-II

Period	Mycotoxin	Level in µg kg^{-1}	Percentage (%) of individuals above the respective PMTDI
2016	FB_1 + FB_2*	4000	90
	DON*	2000	89

FB_T = Total Fumonisin B = FB_1 + FB_2 + FB_3; DON: Deoxynivalenol, ZEA: Zearalenone; PMTDI: Provisional Maximum Tolerable Daily Intake. * Safety levels in dry maize according to the Department of Health (2016).

Only four African countries were identified that adhered to the inclusion criteria of having data on FB levels in home-grown maize and mycotoxin exposure (Table 6). For Nigeria, only three districts were included and the levels from the Derived Savanna and Humid Forest districts were excluded since their levels were quite similar (2154 and 2414 µg kg^{-1}, respectively) and within range of what was observed in maize from the Southern Guinea Savanna (2675 µg kg^{-1}). Using FB levels in maize grain obtained from selective maize-subsistence areas in Africa and applying these levels to the MYCORAM-II indicated the role of the amount of maize consumed in respect to the level of mycotoxins. It was noted that the percentage of positive samples as observed in Tanzania was much lower in comparison to Zimbabwe and Algeria. Regarding reported PDIs, Kimanya et al (2009) showed a PDI of 2.9 µg kg^{-1} body weight day^{-1} (range 0.1-10.4) among 4 (6%, n = 67) subsistence households consuming stored maize in the Rombo district, Tanzania. Specific selective agro-ecological zones in Nigeria including Sudan Savanna, Northern Guinea Savanna, and Southern Guinea Savanna indicated PDIs of 0.7, 2.8, and 2.5 µg kg^{-1} body weight day^{-1}, respectively (Adetuniji et al., 2014). The PDIs from two districts in Zimbabwe: Shamva (n = 166 households) and Makoni (n = 222 households) expressed as ranges, were 0.1-5.8 and 0.2-8.1 µg kg^{-1} body weight day^{-1}, respectively (Murashiki et al., 2017). In Algeria, a collective PDI from the sum of FB_1+FB_2 in maze purchased from various markets, 12.9 µg kg^{-1} body weight day^{-1} was observed by Mahdjoubi et al (2020).

Table 6 MYCORAM-II health risk scenarios in maize-subsistence farming areas in Africa relevant to Fumonisin B exposure

Country	N	Mycotoxin	% positive samples	Mean level (µg kg^{-1})	Mean maize intake (g) Mean body weight (kg) (MYCORAM-II maize category) [A]	MYCORAM-II % of individuals above the PMTDI	Reference
Tanzania	55	FB_T	15	485[B]	356 60 (4 to 9.9)	65	Kimanya et al., 2009
Nigeria SS NGS SGS	70	FB_T	No data	694[B] 29 800[B] 2675[B]	57 60 (1 to 1.9)	72 100 88	Adetuniji et al., 2014
Zimbabwe Shamva Makoni	14 61	FB_1	100 100	265[C] 383[C]	800 60 (10 to 19.9)	36 49	Murashiki et al., 2017
Algeria	30	FB_1	97	14 812[D]	44 60 (Up to 0.9)	100	Mahdjoubi et al., 2020

N = number of maize samples collected for analysis.

% = percentage; PMTDI = Provisional Maximum Tolerable Daily Intake.

Nigerian sampling sites: SS = Sudan Savanna, NGS = Northern Guinea Savanna, SGS = Southern Guinea Savanna.

FB_T = Total Fumonisin B = FB_1 + FB_2 + FB_3. [A] in g kg^{-1} body weight day^{-1} [B] level in stored maize samples; [C] Median levels in maize grain from two districts in Zimbabwe; [D] maize collected from random markets in three areas of Western Algeria: Aint Temouchent, Oran, and Tiaret.

4 Discussion

Maize-subsistence farmers living in Centane, EC are known to be exposed to harmful mycotoxins produced by food-borne fungi that infect home-grown maize daily. From a public health perspective, the consumption of unsafe food is a major concern and therefore requires adequate tools or models to assist with decision-making and the development of appropriate responses and strategies to reduce exposure.

The daily consumption of monotonous staple foods such as maize, has been associated with food insecurity, less dietary diversity**,** and nutrient deficiencies (Burger et al., 2014; Shisana et al., 2013). Maize contaminated with mycotoxins further exacerbates food insecurity with the loss of crops due to visible plant diseases and mould, while during periods food scarcity, contaminated maize is often consumed (Alberts et al., 2019; Burger et al., 2010; Misihairabgwi et al., 2019; Shephard et al., 2008a; Udomkun et al., 2017). Chronic dietary exposure to multiple mycotoxins, which are mostly unavoidable and persistent, may have antagonistic, additive, or synergistic health impacts based on various factors including individual characteristics, bioavailability, and contamination levels (Bracarense et al., 2012). Evidence from mechanistic and animal studies suggested that ingesting both FB and DON, even at low doses, affects the intestinal tract by altering its barrier function and integrity, immune response as well as nutrient absorption (Bracarense et al., 2012; Pierron et al., 2016). Many of these studies have been conducted using *in vivo* and *in vitro* porcine intestinal models known to be anatomically and physiologically similar to that of humans (Ziegler et al., 2016; Zhang et al., 2013). The dual effects of FB and DON in addition to FB's ability to disrupt key lipid parameters have been suggested to contribute to the association between dietary mycotoxin exposure, and childhood developmental and growth problems such as stunting (Burger et al., 2018; Lombard et al., 2014; Smith et al., 2012). This emphasises the importance of considering the concomitant exposure to multiple mycotoxins such as total FB, DON, and ZEA when assessing risk and the potential health impacts.

In this study, the MYCORAM-II, structured similarly to the original MYCORAM (Burger et al., 2014) showed a higher percentage of consumers in the upper dietary dry maize intake categories as a result of a daily diet consisting of home-grown maize. In comparison, in the original MYCORAM stratified by Province, EC presented more

consumers in the lower categories (Burger et al., 2014). The original MYCORAM was based on the dietary intake of commercial maize consumers that are expected to have more diverse diets and access to a variety of other foods. This is also reflected in the total mean dry maize intake of 153 g day^{-1} (range 16-1037, n = 384) for the EC study population (Burger et al., 2014) compared to the current study and that of 515 g day^{-1} (range 0-3785, n = 495). Interestingly, the total mean body weights were quite comparable, with 75 kg (range 36-170) and 72 kg (range 41-150) for the EC and Centane study populations, respectively. These parameters are critical for assessing the risk of exposure to harmful mycotoxins. The various dietary dry maize intake categories applied to the MYCORAM-II can be accepted as reliable data since it was estimated using field-friendly, validated, standardised, and culturally appropriate assessment methods (Lombard et al., 2012; 2013).

Applying actual mycotoxin levels from home-grown maize collected in Centane across different sampling periods to the new MYCORAM-II highlighted the association between the concentration levels of FB_T and the percentage of the consumer at risk. Comparability of the percentage of consumers at risk using mycotoxin levels from four different studies was strengthened by their similar sampling strategies such as the selection of visible good or healthy maize during the harvest and storage stage, between June and August, and the use of liquid chromatography techniques for analysis (Rheeder et al., 2016; Shephard et al., 2013; Tshabile et al., 2020). The MYCORAM-II, similar to the original is, therefore, a useful model to assess risk especially among maize-subsistence farming populations.

The drivers of fungal infection and consequential mycotoxin production in home-grown maize in Centane are complex and related to numerous biophysical and socioeconomic indicators. Environmental factors including i) non-living stressors for example low rainfall, and high average daily maximum temperatures or prolonged drought; ii) living stressors such as an increase in insect pests (e.g. stalk borers); and iii) the availability and access to resources for instance inadequate maize storage facilities, are known to affect fungal infection and mycotoxin production favourably (Alberts et al., 2019; Rheeder et al., 2016). The interaction of socioeconomic drivers, that of financial, human, natural, social, and physical capital, may impact agricultural pre- and postharvest practices and in return maize safety (Alberts et al., 2019; Adegoko and Letuma, 2013; Fernandez et al., 2017, Burger et al., 2010, Jolly et al.,

2009; Kumar and Popet, 2010; Misihairabgwi et al., 2017; Rheeder et al., 2016; Shephard, 2008b). The Eastern Cape Province is known to have the highest Multidimensional Poverty Index in South Africa (Statistics South Africa, 2019). This index consists of various measurables such as i) poverty, ii) health (nutrition and childhood mortality), iii) education (number of school years and school attendance), iv) living conditions (type of fuel used for cooking, lighting, and heating, sanitation, water access, type of dwelling and assets), and v) economic activity (adult unemployment levels) (Statistic South Africa, 2019). These multiple factors may impact negatively on maize-subsistence agricultural practices and in return affect maize fungal infections and mycotoxin production.

Using the MYCORAM-II to investigate the feasibility of the South African national maximum safety levels for FB and DON to protect home-grown maize consumers living in subsistence areas indicated a high percentage (90-89% respectively) of maize consumers at risk. Even from a national perspective, when applying the FB safety level to the original MYCORAM indicated that 74-92% of South African commercial maize consumers are above the PMTDI and at-risk (Burger et al., 2014; Shephard et al., 2019). Within the context of South Africa, with maize being critical not just for consumption and food security but also to the economy, a food safety approach using internationally-based safety levels is not sufficient to protect local maize consumers (Shephard et al., 2019). Therefore, a more prioritised, refined, and evidence-based approach to mycotoxin regulations is critical. In this regard, the MYCORAM-II provides substantiated evidence for the need to urgently address maize food safety among subsistence farmers in the EC.

In the current study, the MYCORAM-II was used to investigate different FB scenarios in Africa using the relevant data from published human FB exposure studies. Although FB is considered as one of the “big five mycotoxins” relevant to agriculture, such studies are limited and challenged by economics, infrastructure, resources, and politics (Collins et al., 2021). Only four such studies were identified and showed an overall high percentage of consumers above the PMTDI (range from 36-100%) depending on the mycotoxin level, percentage of positive samples, dietary intake, and body weight. Notably, the production of traditional staple crops, agricultural practices, dietary patterns, and environmental and climatic factors differ vastly across Africa, and therefore so does the susceptibility to various types of fungal species, mycotoxin

production, and health risks. The reliability of the MYCORAM-II results was impacted by the overall relatively small number of maize samples that were analysed, the analytical methodology used, sources and assessment methods of dietary maize intake estimations, and body weight assumptions. However, applying these parameters to the MYCORAM-II showed different risk outcomes than that of the reported PDIs. The relevance of the MYCORAM-II, although based on data from maize consumers in Centane, South Africa, lies in its using broad-ranged dietary maize intake categories. This, in addition to maize being the main staple crop produced and consumed in Tanzania, Nigeria, and Zimbabwe, also adds to the potential applicability and reliability of the MYCORAM-II when applying the relevant parameters (Babajide et al., 2021; Luzi-Kihupi et al., 2015; Ranum et al., 2014). However, in contrast, in Algeria, being mainly a wheat-producing and consuming country where maize is mostly imported, the risk may have been overestimated when using the MYCORAM-II (Mahdjoubi et al., 2020). However, those consuming maize with contamination levels of 14 812 μg kg^{-1} (range 289-42 143), even at a low mean dietary intake of 44 g day^{-1} will be 100% at risk (Mahdjoubi et al., 2020). A possible limitation to using the data from these studies is that they did not distinguish between cooked or dry maize dietary intake, which is important to estimate and describe the risk more accurately when using the MYCORAM-II.

The public health application of the MYCORAM-II in regards to maize-subsistence populations for risk assessment and ultimately risk analysis, could be of value to all stakeholders involved in mycotoxin regulation, food safety, and public health. In addition to its usefulness in monitoring human mycotoxin-reduction strategies where biomonitoring is not available or unaffordable, the MYCORAM-II could be used to estimate evidence-based safety levels. The MYCORAM-II can also be utilised in future studies in Centane involving community-participatory interventions aimed at increasing the awareness of maize plant disease, mycotoxins, good agricultural practices, and safe maize processing practices. The findings from this study showed that mycotoxin levels in home-grown maize, before and after the community-based interventions, can be applied to assess and describe mycotoxin reduction and the levels of success of the various strategies.

4.1 Limitation of the MYCORAM-II

The current MYCORAM-II, just like the original risk model, is based on data obtained from epidemiological observation studies. Observational descriptive studies using cross-sectional designs, although relatively easy and cost-effective, are not without weaknesses. More so are the challenges of obtaining a representative sample size that is large enough when working in areas such as maize-substance areas that are underdeveloped and infrastructure poor. Centane, EC is known for areas that consist of gravel roads with farmsteads spread far apart, across a mountainous landscape, and that are often unreachable. Most farms have no formal address and are not officially listed anywhere to ensure an adequate sampling strategy. Often during field trips, a central functional road is selected and surrounding farming households are selected based on accessibility as well as where people are available and have consented to participate (Burger et al., 2010). This may result in selection bias and affect the external validity as well as the precision of the findings. In addition, although various dietary assessment tools are available, each type has its own unique methodological and analytical challenges that can lead to specific biases and errors, inevitably impacting the reproducibility and accuracy of the dietary intake data (Gibson et al., 2017). The MYCORAM-II is based on dietary data obtained from a special maize quantitative FFQ that was developed and validated among home-grown maize consumers from Centane to increase the validity and reproducibility of the intakes (Lombard et al., 2012; 2013). However, measurement errors such as recall bias remain a challenge (Lombard et al., 2012, 2013).

The most important limitation of the MYCORAM-II is the unavoidable effects of urbanisation and acculturation, known for changes in diet, lifestyle, demographics, and socio-economics (the so-called nutritional transition), including traditional agricultural practices (Nnyepi, et al., 2015). In this regard, the lack of governmental agricultural support, access to, and availability of social grants and other foods will inevitably affect the validity and reproducibility of the MYCORAM-II practices (Hendriks et al., 2020; Misselhorn and Hendriks, 2017). Moreover, climate and weather considerations, the aging population in EC where the younger generation is becoming more indifferent to agriculture, and other traditional practices may also play a role (Hendriks et al., 2020; Misselhorn and Hendriks, 2017).

5 Conclusions

To adequately understand and manage mycotoxin exposure among maize-subsistence farmers consuming their own grown maize, a unique risk-based approach is required. Therefore, the current study was aimed at developing a unique maize-subsistence farming health risk assessment model and to validate the model using relevant mycotoxin levels in home-grown maize and applied to different mycotoxin scenarios across Africa. The MYCORAM-II a simple, inexpensive, and interactive model was able to quantify and describe maize mycotoxin health risks among at-risk populations. More importantly, this model assists with developing population-specific safety levels, promotes evidence-based decision-making during risk management strategy and policy development, and aids in the evaluation of appropriate public health interventions aimed at reducing mycotoxin exposure. Despite its limitations and challenges, the MYCORAM-II model may also be useful in other African countries where maize is still consumed as a staple. This model can also be used during community-based collaborative information sessions to explain the impact and scope of exposure to harmful toxins.

6 Conflict of interest

The authors declare that there is no conflict of interest.

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Part C: Appendixes

FOOD CONTROL

An official scientific journal of the European Federation of Food Science and Technology (EFFoST) and the International Union of Food Science and Technology (IUFoST).

AUTHOR INFORMATION PACK

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DESCRIPTION

Food Control is an international journal that provides essential information for those involved in food safety and process control.

Food Control covers the below areas that relate to food process control or to food safety of human foods:

- Microbial **food safety** and **antimicrobial** systems
- **Mycotoxins**
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- **Risk assessment**, including microbial and chemical hazards
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- Good **manufacturing** practices
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- Food **Packaging** technology and materials in contact with foods
- Rapid methods of **analysis** and **detection**, including sensor technology
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Examples:

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Van der Geer, J., Hanraads, J. A. J., & Lupton, R. A. (2010). The art of writing a scientific article. *Journal of Scientific Communications, 163*, 51–59. https://doi.org/10.1016/j.sc.2010.00372.
Reference to a journal publication with an article number:
Van der Geer, J., Hanraads, J. A. J., & Lupton, R. A. (2018). The art of writing a scientific article. *Heliyon*, *19*, Article e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
Strunk, W., Jr., & White, E. B. (2000). *The elements of style* (4th ed.). Longman (Chapter 4).
Reference to a chapter in an edited book:
Mettam, G. R., & Adams, L. B. (2009). How to prepare an electronic version of your article. In B. S. Jones, & R. Z. Smith (Eds.), *Introduction to the electronic age* (pp. 281–304). E-Publishing Inc.
Reference to a website:
Powertech Systems. (2015). *Lithium-ion vs lead-acid cost analysis*. Retrieved from http://www.powertechsystems.eu/home/tech-corner/lithium-ion-vs-lead-acid-cost-analysis/. Accessed January 6, 2016
Reference to a dataset:
[dataset] Oguro, M., Imahiro, S., Saito, S., & Nakashizuka, T. (2015). *Mortality data for Japanese oak wilt disease and surrounding forest compositions*. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.
Reference to a conference paper or poster presentation:
Engle, E.K., Cash, T.F., & Jarry, J.L. (2009, November). *The Body Image Behaviours Inventory-3: Development and validation of the Body Image Compulsive Actions and Body Image Avoidance Scales*. Poster session presentation at the meeting of the Association for Behavioural and Cognitive Therapies, New York, NY.
Reference to software:
Coon, E., Berndt, M., Jan, A., Svyatsky, D., Atchley, A., Kikinzon, E., Harp, D., Manzini, G., Shelef, E., Lipnikov, K., Garimella, R., Xu, C., Moulton, D., Karra, S., Painter, S., Jafarov, E., & Molins, S. (2020, March 25). *Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88)*. Zenodo. https://doi.org/10.5281/zenodo.3727209.

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UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

03 March 2021

HREC REF: 060/2021

Prof H Rother
Division of Environmental Health
School of Public Health & Family Medicine
FHS
Email: Andrea.rother@uct.ac.za
Student: burgerh@cput.ac.za

Dear Prof Rother

PROJECT TITLE: MYCOTOXIN HEALTH RISK ASSESSMENT MODELLING AMONG MAIZE-SUBSISTENCE FARMERS LIVING IN CENTANE, EASTERN CAPE PROVINCE, SOUTH AFRICA. (MASTER'S DEGREE – DR H-M BURGER)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020 & 06 July 2020.

Approval is granted for one year until the 30 March 2022.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Dr H Burger will also be involved in this study.

Please quote the HREC REF 060/2021 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCE HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (!RB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the !CH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Cape Peninsula University of Technology

Faculty of Applied Sciences

Research ethics review: self-evaluation and checklist

The purpose of this self-evaluation and checklist document is to increase ethical awareness and compliance based on well-established national and international protocols and norms governing the ethics of research on human and animal subjects, environment and communities.

Surname and Name	Burger, Hester-Mari
Student/Staff no.	UCT Student no: BRGHES001 / CPUT staff no: 30092228
Degree	Master of Public Health (MPH)
Supervisor	Prof Andrea Rother (UCT)
Faculty	Environmental Health Division School of Public Health and Family Medicine University of Cape Town / Unit of Research Integrity Research Directorate Cape Peninsula University of Technology

Purpose of ethics application (e.g. degree, staff research, Research associates, external funding, CONFCOM, URF)	To obtain CPUT ethical approval and site permission to use CPUT data for a MPH mini-thesis, University of Cape Town.

Categories	**Description**	**Mark appropriate box(s) with an X**
High risk studies:	-These are studies dealing with human subjects, animal subjects (higher invertebrates and vertebrates), endangered plant species, and hazardous materials (poisons, radioactive materials, infectious agents). -These studies must obtain ethics approval.	
Moderate risk studies:	-These studies are carried out in protected areas, private land, external institutions, and communal land. -Researchers are required to obtain acquisition letter or access permit from external partners or relevant regulatory bodies. -Proposals under this category may be granted ethics exemption or ethical approval letter.	

Low risk studies:	-It is believed that these studies will cause minimal/negligible or no harm to people, animals and the environment. -Proposals in this category may be granted ethics exemption approval letter.	X (Study using secondary data already published)

High risk study	**Subjects**	**Documents needed by FREC or documents that must be supplied by the applicant**	**Mark appropriate box(s) with an X**
	Human	-Information and informed consent letter (See guideline). -When primary school pupils and high school students are involved, submit letter from the Department of Basic Education. -Questionnaire/interview questions -The complete research proposal -Turnitin similarity report of the proposal (plagiarism check)	
	Animal vertebrates and higher invertebrates	-CPUT animal ethics application form (AE1) must be completed and submitted to FREC. -A detailed protocol under research methodology section of proposal must be provided. -Indicate in the proposal why other alternatives cannot be used -Research proposal -Turnitin similarity report (plagiarism check)	
	Hazardous materials	-Indicate how these materials will be acquired, contained and disposed of in the research methodology section of proposal. -Research proposal -Turnitin similarity report (plagiarism check)	
	Endangered plants	-Letter/permit from Cape Nature, SANParks or other relevant regulatory body -Research proposal -Turnitin similarity report (plagiarism check)	

Moderate risk study	**External research partners**	**Documents needed**	**Mark appropriate box(s) with an X**

	Private property/supplier	-Data acquisition letter or permit to access premises -Letter from supplier indicating voluntary donation of research materials -Submit research proposal -Turnitin similarity report	
	External Research collaborator/institution	-A letter recognising partnership or an invitation letter from external partner. The letter should indicate that the external partner has the requisite resources to handle the study. -Submit research proposal -Turnitin plagiarism check report	
	Movement or importation/exportation of research materials	-Evidence that there is no risk of respect to exotic and invasive species. -Provide detailed information on how organisms will be handled. -Movement of ticks or other arthropod vectors and may need approval from the Department of Agriculture. -Letter from quarantine department may be needed. -Submit research proposal -Turnitin similarity report (plagiarism check)	

Low risk study	Research will be carried out within CPUT, and will not include human, animals, endangered plants or invasive organisms. All materials will be sourced commercially.	**Documents needed**	**Mark with an X if appropriate**
		-You may be asked to produce evidence that the materials were purchased and not donated. -Submit Research proposal. -Submit Turnitin similarity report (plagiarism check)	-N/A -Research Proposal Attached -The Protocol will be submitted as part of the requirement of an MPH mini-thesis and for submission

			at UCT, a Turnitin similarity report will be required. If I submit this section of the mini-thesis then it will show a 100% similarity in the next Turnitin submission.

Please confirm that you have checked the appropriate boxes in all sections and have all required documents. By my signature below I declare that I am not aware of any potential conflicts of interest, other than those declared on this form, which may influence the ethical conduct of this study.	Yes X	No
Applicant's Surname and Name: Dr Hester-Mari Burger	Applicant's Signature:	Date : 10 March 2021

Enquiries on Research Ethics should be sent to:

Prof Felix Nchu

Chairperson, Faculty Research Ethics Committee, Faculty of Applied Sciences, CPUT

Email: nchuf@cput.ac.za

Tel: 0219596473

Resources and forms

CPUT Research Ethics Online Resources: http://www.cput.ac.za/research-technology-and-innovation/ethics

Cape Nature: https://www.capenature.co.za/wp-content/uploads/2014/01/CAPENATURE-research-Application-Form-2-1-1.pdf

SANParks: https://www.sanparks.org/assets/docs/parks_table_mountain/groups/permit-procedures.pdf

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https://sancor.nrf.ac.za/SiteAssets/News/DAFF%20and%20DEA%20Information%20for%20Research%20Permits%20-%202014.pdf

Animal import and Export Permit, South Africa: https://www.daff.gov.za/daffweb3/DAFF-Services/Imports

Plant import permit into South Africa: https://www.gov.za/services/export-permits-import/phytosanitary-permit

Research Permit Cape Town Municipality: http://resource.capetown.gov.za/documentcentre/Documents/Procedures,%20guidelines%20and%20regulations/Submit%20_Research_Request_Guidelines.pdf

Office of the Deputy Vice Chancellor:
Research, Technology Innovation & Partnerships
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P O Box 1906
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Email: dvcresearch@cput.ac.za

29 March 2021

Dr H-M Burger (student/ staff number: BRGHES001 and 30092228)
Master of Public Health (University of Cape Town)
Unit of Research Integrity
Research Directorate
Cape Peninsula University of Technology

Dear Dr Burger

RE: PERMISSION TO CONDUCT RESEARCH AT CPUT

The Institutional Ethics Committee received your application entitled: "*Mycotoxin health risk assessment modelling among maize-subsistence farmers living in Centane, Eastern Cape Province, South Africa*" together with the dossier of supporting documents.

Faculty Ethics Committee Approval Date: 23 March 2021

Faculty Ethics Committee Approval Reference No: BRGHES001/03/2021

Permission is herewith granted for you to do research at the Cape Peninsula University of Technology.

Wishing you the best in your study.

Sincerely,

Dr David Phaho
Deputy Vice-Chancellor: Research, Technology, Innovation & Partnerships
Cape Peninsula University of Technology | #WeAreCPUT
t: +27 (0) 21 959 6242 | e: dvcresearch@cput.ac.za w: www.cput.ac.za
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