GHENT UNIVERSITY

FACULTY OF PHARMACEUTICAL SCIENCES

Nutrition and Dietetics department

Mycotoxins in maize and breast milk, and the role of pre- and post-harvest practices in Ethiopia

Addisalem Mesfin

2023

A doctoral dissertation submitted to Ghent University in fulfillment of the requirements for the degree of Doctor in Pharmaceutical Sciences

The study was conducted in the context of a joint PhD between Ghent University (UGent), Belgium and Jimma University (JU), Ethiopia

Members of the examination committee:

Prof. Katrien Remaut
Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Ghent University, Belgium

Prof. Evelien Wynendaele
Department of Pharmaceutical Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Belgium

Prof. Kris Audenaert
Plant and Crop department, Faculty of Bioscience engineering, Ghent University, Belgium

Prof. Katleen Raes
Department of Food Technology, Food Safety and Health, Faculty of Bioscience engineering, Ghent University, Belgium

Dr. Emmanuel K.Tangni
Physical and Chemical Health Risks, Organic Contaminants and Additives, Sciensano, Tervuren (Belgium)

Dr. Dessalegn Tamiru
Nutrition and Dietetics department, Faculty of public health, Jimma University

Promoters:

Prof. Sarah De Saeger
Centre of Excellence in Mycotoxicology and Public Health, Faculty of Pharmaceutical Sciences, Ghent University, Belgium

Prof. Marthe De Boevre
Centre of Excellence in Mycotoxicology and Public Health, Faculty of Pharmaceutical Sciences, Ghent University, Belgium

Prof. Carl Lachat
Department of Food Technology, Safety and Health, Faculty of Bioscience engineering, Ghent University, Belgium

Prof. Tefera Belachew
Nutrition and Dietetics department, Faculty of public health, Jimma University

Please refer this work as follows

Addisalem Mesfin (2023). Mycotoxins contamination in maize and breast milk, and the role of pre- and post-harvest practices in Ethiopia. Thesis submitted in the fulfilments of the requirements for the degree of Doctor in the Pharmaceutical Sciences, Ghent University.

Acknowledgment

Before anything else, I would like to express my sincere gratitude to my promoters for all of the time and effort you have dedicated to me, especially working from a distance. Thank you for the insightful and timely feedback, and for consistently being there for me with all the support I needed in every step of the PhD journey. Working under your supervision has been a great comfort to me and learned a lot through the process. Thank you for challenging me to think further and work harder. I hope you recognize how much of an impact the different qualities I learned from each of you have on me and my career.

I am profoundly grateful to NUFFIC Netherlands Initiative for Capacity Development in Higher Education (NICHE/ETH/179) for financing the PhD. I also want to thank the support I received from the Ghent University - MYTOX-SOUTH® consortium and VLIR-UOS Network program (ET2017NET039A103). Moreover, I would like to extend my thanks to the PI and co-PIs of BUNMAP thematic research at Addis Ababa University for giving me the opportunity to work together. I would also like to express my sincere thanks to the study participants for consenting and participating in the study. My acknowledgments also go to the data collectors and supervisors. I want to recognize the great support I received from Christ'l Detavernier, Mario VandeVelde, and Frédéric Dumoulin during the laboratory analysis of the samples and also appreciate the assistance I received from Arnau Vidal, Annie De Lobelle, and Marianne Bailleul during my stay in the lab. I would like to offer my special thanks to my comrade Kokeb Tesfamariam, it is a pleasure to have met someone like you in my PhD trajectory with whom I could discuss my PhD activities whenever I wanted. My family, friends, and colleagues at Hawassa University deserve thanks for their encouragement and support during my PhD study. Finally, I would like to express my gratitude to my husband Behailu Deksisa for accompanying me through the highs and lows of this academic journey. My boy, Zeal Behailu, *y*our presence in my life continues to fuel my motivation in this journey, I dedicate this PhD thesis to you.

I prize God, the almighty for everything!

Table of Contents

Abbreviations and acronyms

AFB1	Aflatoxin B1
AFs	Aflatoxins
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
AME	Alternariol Methyl Ether
BUNMAP	Butajira Nutrition, Mental Health, And Pregnancy
3-ADON	3-acetyldeoxynivalenol
15-ADON	15 acetyldeoxynivalenol
DALYs	Disability Adjusted Life Years
DAS	Diacetoxyscirpenol
DDT	Dichloro Diphenyl Trichloroethane
DOM	Deepoxy-deoxynivalenol
DON	Deoxynivalenol
FAIR	Findable, Accessible, Interoperable, and Reusable
FAO	Food Agriculture Organization
FB1	Fumonisin B1
FB2	Fumonisin B2
FB3	Fumonisin B3
FFQ	Food Frequency Questionnaire
ELISA	Enzyme Linked Immunosorbent Assays
ENN B	Enniatin B
FUMs	Fumonisins
FUS-X	Fusarenon X
GAP	Good Agricultural Practices
GEMS	Global Environment Monitoring System
GPS	Global Positioning System
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis and Critical Control Points
HFIAS	Household Food Insecurity Access Scale
HT-2	HT2-toxin
IARC	International Agency for Research on Cancer
IQRs	Interquartile Ranges
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LMICs	Low- and Middle-Income Countries
LC	Liquid Chromatography
LC-MS/MS	Liquid Chromatography tandem Mass Spectrometry
LFIA	Lateral Flow Immunoassay
LOQ	Limit of Quantification
LOD	Limit of Detection
MS	Mass Spectrometry
NEO	Neosolaniol
OTA	Ochratoxin A

ODK	Open Data Kit
PACA	Partnership for Aflatoxin Control in Africa
PC	Principal Components
PCA	Principal Component Analysis
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RASFF	Rapid Alert System for Food and Feed
ROC	Roquefortine C
SPE	Solid Phase Extraction
SNNPR	Southern Nations and Nationalities Peoples region
SPSS	Statistical Package for Social Sciences
SSA	Sub-Saharan Africa
SSD2	Standard Sample Description 2
STE	Sterigmatocystin
T-2	T2-toxin
TDI	Tolerable Daily Intake
UPLC	Ultra Performance Liquid Chromatography
WHO	World Health Organization
ZAN	Zearalanone
ZEN	Zearalenone
STROBE-nut	Strengthening the Reporting of Observational Studies in Epidemiology-Nutritional Epidemiology

Summary

Mycotoxins are toxic secondary metabolites produced by different fungi that contaminate food and feed and eventually cause health threats to humans and animals. People in low- and middle-income countries (LMICs) bear more of the health burden of mycotoxins. In Ethiopia, contamination of agricultural food products, mainly maize, and groundnut, with aflatoxins (AFs) and other mycotoxins was reported. A few biomonitoring studies have also shown the exposure of the Ethiopian population to mycotoxins. The main contributors to mycotoxin occurrence in Africa like Ethiopia are non-optimal pre- and post-harvest practices, climate change, the lack or low enforcement of mycotoxins legislation, lack of awareness about mycotoxins, and undiversified diet. Overall, there is a growing need for quality, well-described, and properly stored mycotoxins data, globally. However, the available mycotoxin contamination data especially data from LMICs are not organized. Besides, there is variability in the data in terms of the sampling, analytical methods, and reporting formats used. Thus, harmonization and standardization of mycotoxins contamination data is key to preserving data uniformity and decreasing uncertainties.

Therefore, this PhD study aimed to investigate multiple mycotoxins in maize crop samples from household stores and in breast milk samples, and further examine the pre- and post-harvest practices, household food processing methods, and dietary practices in Ethiopia. Moreover, this PhD intended to propose the missing mycotoxins contamination data descriptors, and structure and integrate them with the existing Food Contamination Monitoring and Assessment Programme of the WHO Global Environment Monitoring System (GEMS/FOOD) and European Food Safety Authority (EFSA) descriptors. The PhD is organized into 7 chapters.

Chapter 1 gives a general overview of the different types of mycotoxins and their producing fungi, the distinctive features of the different mycotoxins, their overall importance in terms of human and animal health, and their impact on the economy. Moreover, the occurrence and co-occurrence of mycotoxins in food and biological fluids are discussed. A brief description of the current status of the magnitude of the mycotoxins problem in Africa and Ethiopia is included in this chapter. Further, factors that contribute to mycotoxin occurrence in Ethiopia and Africa are briefly discussed. This chapter also introduces the different mycotoxin analytical techniques. In the end, the gaps in the quality, quantity, storage, and harmonization of worldwide mycotoxins data with a special focus on LMICs are discussed as a general crosscutting problem in mycotoxins research.

Chapter 2 demonstrates the information gaps in mycotoxins research in Ethiopia that led to the plan of this PhD study, and subsequently describes the objectives of this PhD study.

In **chapter 3,** the findings from the analysis of multiple mycotoxins in maize crops sampled from household storages and their human exposure assessment in South and West Ethiopia are discussed. Besides, maize-related post-harvest handling practices and household food processing methods are also presented in this chapter. The results showed that the maize samples were contaminated with *Fusarium* toxins i.e. deoxynivalenol (DON), followed by nivalenol (NIV), 3-acetyldeoxynivalenol (3-ADON), zearalenone (ZEN), and fumonisins (FUMs), and they also co-occurred in different samples. The proportion of DON in the samples was significantly higher in the South (77%) than in West (29%) Ethiopia. The exposure assessment further showed that the mean consumption of DON (2.31 µg/kgBW/day) and ZEN (0.47 µg/kgBW/day) by the studied Ethiopian population groups exceeded the tolerable daily intakes (TDIs). Similar magnitudes of fumonisin B1 (FB1) (19% in both South and West), fumonisin B2 (FB2) (19%, South *vs.* 18%, West), and fumonisin B3 (FB3) (12%, South *vs.* 13%, West) were found. On the contrary, *Aspergillus* mycotoxins such as AFs generally did not contaminate the samples. The overall finding in this study shows that multiple fusariotoxins were detected in maize samples from Ethiopia but unexpectedly this was not the case for *Aspergillus* mycotoxins. Similar practices of completely drying maize crops before harvest, application of insecticide, sorting, and use of the sorted out maize for feed were also reported from both South and West Ethiopia.

Chapter 4 presents the analysis of 16 mycotoxins in breast milk samples of lactating women in South Ethiopia. Moreover, maize-related pre- and post-harvest and household processing practices and women's dietary practices were examined. The results revealed no significant exposure to most of the mycotoxins, except for modest exposure to FB2 (15% ranging from undetectable to 24.98 ng/mL) and FB3 (9% ranging from undetectable to 7.52 ng/mL). Mainly, good pre- and post-harvest practices were reported however, suboptimal practices i.e. allowing maize crops to excessively dry on the field before harvesting and the use of porous storage materials, utilizing sorted-out maize for feed and making local beverages were reported by the majority. Besides, we reported higher consumption of maize with low dietary diversity among the women. No association between the pre- and post-harvest practices, household processing methods, and women's dietary practices with FUMs exposure was justified.

Chapter 5 in general, discusses the gaps in the description, arrangement, and documentation of the available mycotoxin contamination data globally, with a special focus on LMICs, and

further particularly discusses the missing items to describe mycotoxin contamination data in the GEMS/FOOD and EFSA food contaminants databases. Thus, mycotoxins experts were involved and proposed missing mycotoxins contamination data descriptors using a consensus-building Delphi process. The proposed descriptors were then integrated with the existing GEMS/FOOD and EFSA descriptors and arranged as study, sample, and assay types. A total of 22 descriptors were proposed and most of the descriptors proposed were related to the pre-harvest practices (crop variety, cultivation method, maturity level of the crop at harvest, previous crop planted, soil management methods, application of fungicide/insecticide on agriculture field, occurrence of any natural disaster), and post-harvest practices (mode of transportation from field to storage, ventilation methods, temperature, and moisture control, application of fungicide/ insecticide in storage, storage period and type). We expect the output from this work to initiate internationally harmonized mycotoxin contamination data descriptors and standardized reporting formats.

Chapter 6 summarizes the key findings from this PhD study and presents the general conclusions made.

Chapter 7 describes the issue of mycotoxins from a global perspective and highlights the contribution of the findings from this PhD study in light of the research gaps observed. Finally, this chapter presents recommendations for possible future research needs on *Fusarium* mycotoxins in Ethiopia, pathways and contributing factors for mycotoxins transfer to breast milk, the role of pre- and post-harvest practices in mycotoxins reduction and control, mycotoxins co-occurrence, mycotoxin biomonitoring and exposome, validation of more mycotoxin biomarkers, *in vitro* and *in vivo* toxicological studies, and climate change and mycotoxin risks. Moreover, the chapter presents measures that should be taken in the future by government and non-government bodies, farmers, food regulators, and consumers for reducing the mycotoxins problem in Ethiopia, and Africa at large.

Samenvatting

Mycotoxines zijn toxische secundaire metabolieten geproduceerd door verschillende schimmels die voedsel en diervoeders besmetten en uiteindelijk gezondheidsklachten bij mens en dier kunnen veroorzaken. Mensen in lage- en middeninkomenslanden (LMIC) worden meer blootgesteld aan mycotoxines en ondervinden bijgevolg een groter gezondheidsrisico. In Ethiopië werd melding gemaakt van besmetting van landbouwproducten, voornamelijk maïs en aardnoten, met aflatoxines (AF's) en andere mycotoxines. Enkele biomonitoringstudies hebben ook de blootstelling van de Ethiopische bevolking aan mycotoxines aangetoond. De belangrijkste factoren die bijdragen aan het voorkomen van mycotoxines in Afrika, zoals Ethiopië, zijn niet-optimale pre- en post-oogstpraktijken, klimaatverandering, het ontbreken of de lage handhaving van mycotoxinewetgeving, gebrek aan bewustzijn over mycotoxines en een niet-gediversifieerd dieet. Er is wereldwijd een groeiende behoefte aan hoogwaardige, goed beschreven en correct opgeslagen gegevens over mycotoxines. De beschikbare gegevens rond mycotoxine besmettingen, met name gegevens van LMIC, zijn echter niet georganiseerd. Bovendien is er variabiliteit in de gegevens van de gebruikte bemonstering, analytische methoden en rapportageformaten. Daarom is harmonisatie en standaardisatie van gegevens over besmetting met mycotoxines essentieel om de uniformiteit van de gegevens te behouden en onzekerheden te verminderen.

Dit doctoraatsonderzoek onderzocht meerdere mycotoxines in stalen van mais en in moedermelk, en onderzocht ook de pre- en post-oogstpraktijken, huishoudelijke voedselverwerkingsmethoden en voedingspraktijken in Ethiopië. Bovendien was dit doctoraat bedoeld om de ontbrekende descriptoren van gegevens over de besmetting met mycotoxines voor te stellen, en deze te structureren en te integreren met de descriptoren van het bestaande programma voor monitoring en beoordeling van voedselcontaminatie van de Wereld Gezondheidsorganisatie (WHO) Global Environment Monitoring System (GEMS/FOOD) en de Europese Autoriteit voor voedselveiligheid (EFSA). Het doctoraat is onderverdeeld in 7 hoofdstukken.

Hoofdstuk 1 geeft een algemeen overzicht van de verschillende soorten mycotoxines en hun producerende schimmels, de onderscheidende kenmerken van de verschillende mycotoxines, hun algemene belang voor de gezondheid van mens en dier, en hun impact op de economie. Bovendien wordt het voorkomen en samen voorkomen van mycotoxines in voeding en biologische vloeistoffen besproken. In dit hoofdstuk wordt een korte beschrijving gegeven van de huidige stand van zaken van de omvang van de mycotoxineproblematiek in Afrika en

Ethiopië. Verder worden factoren die bijdragen aan het voorkomen van mycotoxines in Ethiopië en Afrika kort besproken. Dit hoofdstuk introduceert ook de verschillende analysetechnieken voor mycotoxines. Uiteindelijk worden de hiaten in de kwaliteit, kwantiteit, opslag en harmonisatie van wereldwijde mycotoxinegegevens met een speciale focus op LMIC besproken als een algemeen transversaal probleem in het mycotoxineonderzoek.

Hoofdstuk 2 toont de informatielacunes in het onderzoek naar mycotoxines in Ethiopië die hebben geleid tot het plan van dit doctoraatsonderzoek, en beschrijft vervolgens de doelstellingen van het doctoraat.

In hoofdstuk 3 worden de analyseresultaten van meerdere mycotoxines in maïsgewassen, bemonsterd uit huishoudelijke opslag, besproken en wordt een menselijke blootstellingschatting in Zuid- en West-Ethiopië uitgevoerd. Daarnaast worden in dit hoofdstuk ook maïsgerelateerde verwerkingspraktijken na de oogst en huishoudelijke voedselverwerkingsmethoden beschreven. De resultaten toonden aan dat de maïsmonsters besmet waren met *Fusarium*-toxinen, d.w.z. deoxynivalenol (DON), gevolgd door nivalenol (NIV), 3-acetyldeoxynivalenol (3-ADON), zearalenone (ZEN) en fumonisinen (FUM's), en ze kwamen ook samen voor in verschillende monsters. Het aandeel DON in de monsters was significant hoger in het Zuiden (77%) dan in het Westen (29%) van Ethiopië. Uit de blootstellingsanalyse bleek verder dat de gemiddelde consumptie van DON (2,31µg/kg lichaamgewicht/dag) en ZEN (0,47 µg/lichaamgewicht/dag) door de bestudeerde Ethiopische bevolkingsgroepen de toelaatbare dagelijkse inname (TDI) overschreed. Vergelijkbare hoeveelheden fumonisine B1 (FB1) (19% in zowel Zuid als West), fumonisine B2 (FB2) (19%, Zuid vs. 18%, West) en fumonisine B3 (FB3) (12%, Zuid vs. 13 %, West) werden gevonden. Integendeel, Aspergillus-mycotoxines zoals AF's contamineerden de monsters over het algemeen niet. Dit onderzoek laat zien dat meerdere fusariotoxinen werden gedetecteerd in maïsmonsters uit Ethiopië, maar onverwachts was dit niet het geval voor Aspergillus-mycotoxines. Soortgelijke praktijken van het volledig drogen van maïsgewassen vóór de oogst, toepassing van insecticide, sorteren en gebruik van de uitgesorteerde maïs voor diervoeder werden ook gemeld in zowel Zuid- als West-Ethiopië.

Hoofdstuk 4 presenteert de analyse van 16 mycotoxines in moedermelkmonsters van vrouwen die borstvoeding geven in Zuid-Ethiopië. Bovendien werden maïsgerelateerde pre- en post-oogst- en huishoudelijke verwerkingspraktijken en voedingsgewoonten van vrouwen onderzocht. De resultaten toonden geen significante blootstelling aan de meeste mycotoxines, behalve een bescheiden blootstelling aan FB2 (15% variërend van niet-detecteerbaar tot 24,98

ng/ml) en FB3 (9% variërend van niet-detecteerbaar tot 7,52 ng/ml). Er werden vooral goede pre- en post-oogstpraktijken gerapporteerd, maar er werden ook suboptimale praktijken gerapporteerd, d.w.z. maïsgewassen op het veld extreem laten drogen voordat ze worden geoogst en het gebruik van poreuze opslagmaterialen, het gebruik van uitgesorteerde maïs voor diervoeder en het maken van lokale dranken door de meerderheid. Bovendien rapporteerden we een hogere consumptie van maïs met een lage voedingsdiversiteit onder de vrouwen. Er werd geen verband aangetoond tussen de pre- en post-oogstpraktijken, huishoudelijke verwerkingsmethoden en voedingsgewoonten van vrouwen met blootstelling aan FUMs.

Hoofdstuk 5 bespreekt in het algemeen de lacunes in de beschrijving, rangschikking en documentatie van de wereldwijd beschikbare mycotoxinecontaminatiegegevens, met speciale aandacht voor LMIC, en bespreekt verder in het bijzonder de ontbrekende items om mycotoxinecontaminatiegegevens in de GEMS/FOOD en EFSA te beschrijven. Daarom hebben we experts op het gebied van mycotoxines geconsulteerd en bijkomende descriptoren voorgesteld met behulp van een consensusopbouwend Delphi-proces. De voorgestelde descriptoren werden vervolgens geïntegreerd met de bestaande GEMS/FOOD- en EFSA-descriptoren en gerangschikt als studie-, monster- en testtypes. Er werden in totaal 22 descriptoren voorgesteld en de meeste van de voorgestelde descriptoren hadden betrekking op de pre-oogstpraktijken (gewasvariëteit, teeltmethode, rijpheidsniveau van het gewas bij de oogst, eerder geplant gewas, methoden voor bodembeheer, toepassing van fungicide/insecticide op landbouwveld, optreden van een natuurramp) en praktijken na de oogst (wijze van transport van veld naar opslag, ventilatiemethoden, temperatuur- en vochtbeheersing, toepassing van fungicide/insecticide tijdens opslag, opslagperiode en -type). We verwachten dat de output van dit initiatief internationaal geharmoniseerde descriptoren van mycotoxinecontaminatiegegevens en gestandaardiseerde rapportageformaten zal opleveren.

Hoofdstuk 6 vat de belangrijkste bevindingen van dit doctoraatsonderzoek samen met de algemene conclusies.

Hoofdstuk 7 geeft een korte beschrijving van het probleem van mycotoxines vanuit een mondiaal perspectief en belicht de bijdrage van de bevindingen van dit doctoraatsonderzoek in het licht van de geconstateerde hiaten in het onderzoek. Ten slotte bevat dit hoofdstuk aanbevelingen voor mogelijk toekomstig onderzoek naar *Fusarium*-mycotoxinen in Ethiopië, routes en bijdragende factoren voor de overdracht van mycotoxinen naar de moedermelk, de rol van pre- en post-oogstpraktijken bij het verminderen en beheersen van mycotoxinen, gelijktijdig voorkomen van mycotoxinen, mycotoxinen biomonitoring en exposoom, validatie

van meer mycotoxine-biomarkers, *in vitro* en *in vivo* toxicologische studies, en klimaatverandering en mycotoxinerisico's. Bovendien stelt het hoofdstuk maatregelen voor die in de toekomst zouden moeten worden genomen door overheids- en niet-gouvernementele instanties, boeren, voedselregelgevers en consumenten om het mycotoxineprobleem in Ethiopië en Afrika in het algemeen te verminderen.

Chapter 1. General introduction

1.1 Mycotoxins and their significance

1.1.1 Basics of mycotoxins

Mycotoxins are one of the food hazards besides others such as drug and pesticide residues, microbial pathogens, zoonotic diseases, parasites, and antibiotics that are under policymakers' consideration [1]. The word mycotoxin originated from two combined words: the Greek word "mykes" meaning "fungi" and the Latin word "toxicum" meaning "poison" [2]. Mycotoxins are secondary metabolites produced by fungi. In contrast to the primary metabolites (e.g. sugars, amino acids), mycotoxins are secondary to the producing fungus meaning that they are not needed for the normal metabolic process of the fungus [3], yet give protection for a growing fungus in case of a change in environmental conditions [4]. Mycotoxins also serve as crucial elements in the defensive mechanisms employed by mycotoxigenic fungi to counter resident microbes by functioning as signaling molecules that regulate host reactions and facilitating effective colonization. This provides mycotoxin-producing fungi with a competitive edge within their ecological niche and contributes to the pathogenicity, aggressiveness, and/or virulence of these mycotoxin-producing fungi. Additionally, mycotoxins can confer a competitive advantage to mycotoxin-producing fungi by inducing oxidative stress in plants which this stress can result in cellular damage and weaken the plant's capacity to defend pathogens and fungi [5, 6].

Mycotoxins have a low molecular weight and are highly stable which can resist the high temperatures of boiling and frying under cooking conditions and even sterilization processes [7]. Thereby, mycotoxicology stands for the field of mycotoxins research, while related animal and human diseases are specified as mycotoxicoses [8]. *Aspergillus*, *Fusarium*, and *Penicillium*, and barely *Alternaria* and *Claviceps* are the significant and most investigated fungal genera, globally [9]. Different species of these fungi may produce one or more different types of mycotoxins, and on the other side, these species may produce the same mycotoxins which is one of the reasons for the abundance of mycotoxins in the environment [10].

Pre-harvest and post-harvest are the key moments when mycotoxins can be produced in grains. Pre-harvest fungi e.g., *Fusarium* and *Alternaria* species usually occur while the plant is in a growing field, and post-harvest fungi e.g. *Aspergillus* and *Penicillium* species predominantly occur during storage [11]. There is also a possibility that pre-harvest fungi continue growing during transport and storage [12].

Around four hundred mycotoxins have been identified and more types of mycotoxins are yet to be identified. Only a few of them are largely present in human food and feed and are further

relatable to human and animal health. In particular, aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FUMs), patulin, trichothecenes, and zearalenone (ZEN) are of most significant concern in terms of human health, agriculture, and economy [13, 14]. Historically, acute toxicity occurred in Russia during the period of World War II as food was scarce due to the war that forced people to consume moldy foods [15]. Later, another incidence of the death of 100,000 turkeys was documented in 1962 due to the consumption of AFs-contaminated peanut meals. AFs were then characterized as the first among the mycotoxins as a result of the studies conducted after the deaths of the turkeys [16]. Moreover, in 1974, the consumption of maize highly contaminated by AFs triggered a hepatitis outbreak and became the cause of the death of 100 individuals in India [17]. On the other side, FUMs were first identified in South Africa due to the high occurrence of esophageal cancer which was associated with the consumption of maize contaminated with FUMs [18].

Mycotoxins may be inhaled or dermally absorbed by humans, however, the primary pathway for human exposure to mycotoxins is through the consumption of contaminated plant-derived foods [10, 19]. The secondary route is through the transmission of mycotoxin-contaminated crops into feed and then to animal food products like milk, meat and eggs [20]. A global survey by BIOMIN from 2008–2017 reported that at least one of the mycotoxins was present in 88% of the approximately 74, 821 feed samples collected [21]. Another report from 52 countries also indicated that 79% or more feed samples were contaminated by mycotoxins [22].

Mycotoxins often occur in a variety of agricultural commodities and products, including maize, sorghum, and other cereals, soybeans, and also in different fruits, nuts, and spices [23]. AFs, FUMs, ochratoxins, deoxynivalenol (DON), and ZEN are more common in cereals and foods derived from cereals [24]. Particularly, maize is a major cereal that is usually contaminated with AFs, FUMs, ZEN, and DON [25]. Overall, mycotoxins are estimated to be present in up to 60–80% of world food commodities [26] which is a big danger to global food security, leading to economic problems, and most importantly health hazards for humans.

1.1.2 Significance of mycotoxins

Mycotoxins are toxic to both humans and animals. Numerous *in vivo* investigations and scientific reports show the toxicological consequences of several mycotoxins [27]. Depending on the concentration and length of exposure, mycotoxins may have acute toxicity or chronic health effects [28]. Acute toxicity is marked by hazardous and fatal symptoms as a result of short-term exposure where effects appear immediately after exposure to high doses while long-term mycotoxin exposure at low concentrations can lead to chronic toxicity [29]. Mycotoxins

normally cause an inflammatory reaction at low dosages but at high quantities, they may cause immunosuppression over time possibly as a consequence of leaky gut caused by mycotoxins infecting the intestinal tract [30, 31]. Along the detoxification process of the body, mycotoxins may also chronically accumulate mainly in the liver [8]. Moreover, mycotoxins have an impact on DNA and RNA polymerase, bind to ribosomes, interact with proteins, cause apoptosis and necrosis, interact with mitochondria and ionophores, inhibit important metabolic enzymes, affect hormones, and cause epigenetic modifications [3]. As a consequence, mycotoxins have been known to cause cancer and have neurotoxic, hepatotoxic, teratogenic, mutagenic, and estrogenic effects [3, 23]. There is also a suspicion that children in low- and middle-income countries (LMICs) are experiencing impaired linear growth because of exposure to AFs [32]; however, the findings are inconsistent and the mechanisms are not clear yet [33]. The levels of mycotoxins exposure in humans are mainly affected by the magnitude of contamination and the amount of contaminated food consumed [32, 34]. The level of mycotoxin toxicity may also significantly vary depending on many factors: species sensitivity, individual sensitivity, age, sex, health and nutritional status, the weight of a person, bio-accessibility, mechanisms/modes of action, metabolism, defense mechanisms, exposure to infectious agents, co-occurrence of mycotoxins and presence of pharmacologically active substances [29]. The health implications of mycotoxins differ as we progress through the different human life stages. The mycotoxin hazard index was remarkably higher for children below 3 years of age compared to adults [8]. Children, especially young children, are typically more prone to toxins probably because of their lesser capacity for detoxification due to their immature metabolism. Besides, new-borns relatively consume more food unlike their smaller body weight [35]. Therefore, it is extremely concerning that these toxins are present in infants and young children's foods, as well as in breast milk from mothers exposed to mycotoxins [36]. To achieve a well-targeted intervention, it is thus essential to understand the distinctions between exposure in children and adults to identify vulnerable and high-risk populations [34].

Animals can be exposed to mycotoxins through consumption of contaminated feeds which can lead to reduced growth rates, reduced performance, impaired gut health, suppress the immune system, different illness and may ultimately lead to death [3, 20]. Moreover, mycotoxins can be transferred from exposed animals to animal tissues, eggs and milk [20]. A major concern, however, is the occurrence of aflatoxin M1 (AFM1) residues in milk [37].

Besides the health impacts on humans and animals, mycotoxins may be responsible for post-harvest loss and consecutively may negatively affect food security [38]. Most importantly, the presence of mycotoxins along the food and feed production, processing, and distribution may

also impact the economy in terms of reduced revenues due to rejected export items or lower prices of less quality crops [39]. The cost of reduced human capital due to the adverse health effects and other related health care services are also the indirect impacts of mycotoxins which are usually given less emphasis [39, 40]. For these reasons, mycotoxins were for instance one of the top ten food danger categories identified by the Rapid Alert System for Food and Feed (RASFF) in 2017 [41].

1.2 Commonly occurring mycotoxins

1.2.1 *Aspergillus* mycotoxins

Aspergillus fungal species exist over a large area of the globe with a total of 339 known species. Only a few of them considerably produce mycotoxins with *Aspergillus flavus* and *Aspergillus niger* being major ones [42]. AFs and OTA are the principal mycotoxins produced by *Aspergillus* fungi and the less-prominent ones are patulin, citrinin, aflatrem, aflavinine, cyclopiazonic acid, sterigmatocystin, gliotoxin, paspalinine, and versiconol [43, 44]. Since most common species of *Aspergillus* grow vigorously at temperatures of 37°C or higher, these species are ideally suited for growth in tropical climates. Nuts, coffee, cocoa, dried fruits, grapes, maize, and rice are the major agricultural produces regularly contaminated with *Aspergillus* mycotoxins [45, 46].

Aflatoxins

There are plentiful of AFs in nature due to the wide spreading of the producing fungus i.e. *Aspergillus flavus* and also owing to the fact that AFs can be produced by several other *Aspergillus* species [46]. There are different types of AFs and their derivatives in nature, and four are known to be hazardous to humans and animals, namely aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). Besides, AFM1 which is the hydroxylated form of AFB1 is also considered similarly toxic to its parent form, AFB1. Both humans and lactating animals excrete AFM1 into their milk after hydroxylating AFB1 in the liver [43]. Considerable attention has been paid to AFs among all the mycotoxins, and they are being studied more closely than any other type of mycotoxins [47]. As time has gone on, policymakers have begun to pay attention to the importance of AFs due to their detrimental effects on both human and animal health as well as the trade industry. Aflatoxicosis stands for the negative health effects that are caused by intake of high levels of AFs which ultimately can even lead to death. Chronic AFs exposure may lead to liver damage, gastrointestinal dysfunction, decreased appetite, decreased reproductive function, and decreased growth [48]. AFs are highly toxic and even low amounts can be carcinogenic, hepatotoxic, teratogenic, and

mutagenic to humans [49]. Ample evidence has shown that AFB1 is the most predominant, potent, and hepatocarcinogenic (especially in synergy with hepatitis B infection) of all the aflatoxin groups identified. The International Agency for Research on Cancer (IARC) classified AFB1, AFB2, AFG1, AFG2, and AFM1 as carcinogenic to humans (Group 1). Subsequently, AFG1 is more toxic than AFB2, and AFG2 is the last in order of their carcinogenicity. AFs also contribute to immuno suppression, especially in population groups such as young children and people living with HIV/AIDS whose immunity is known to be weak [47]. Stunting is associated with exposure to AFs in infants but it is unclear how AFs contribute to stunting since no dose-response connection has been established [48, 50] and, also due to the complexity that aflatoxin-exposed populations may experience other risk factors for developmental faltering in infants [51]. Hot and dry climates (+/- 30 to 40 degrees latitude) are more favorable for the production of AFs and the problem may be even worse if accompanied by drought, pests, delayed harvest, insufficient drying, and poor post-harvest handling practices [50]. Maize, wheat, rice, pulses, spices, milk, and milk products are the main food sources of AFs [52]. Besides, AFs may also appear in processed foods as they resist severe roasting, extrusion, baking, and cooking processes [53]. However, except for AFM1 in milk, it is less common that AFs transfer into other edible tissues of animals [37]. The chemical structures of the four major AFs are depicted in **Figure 1.1**.

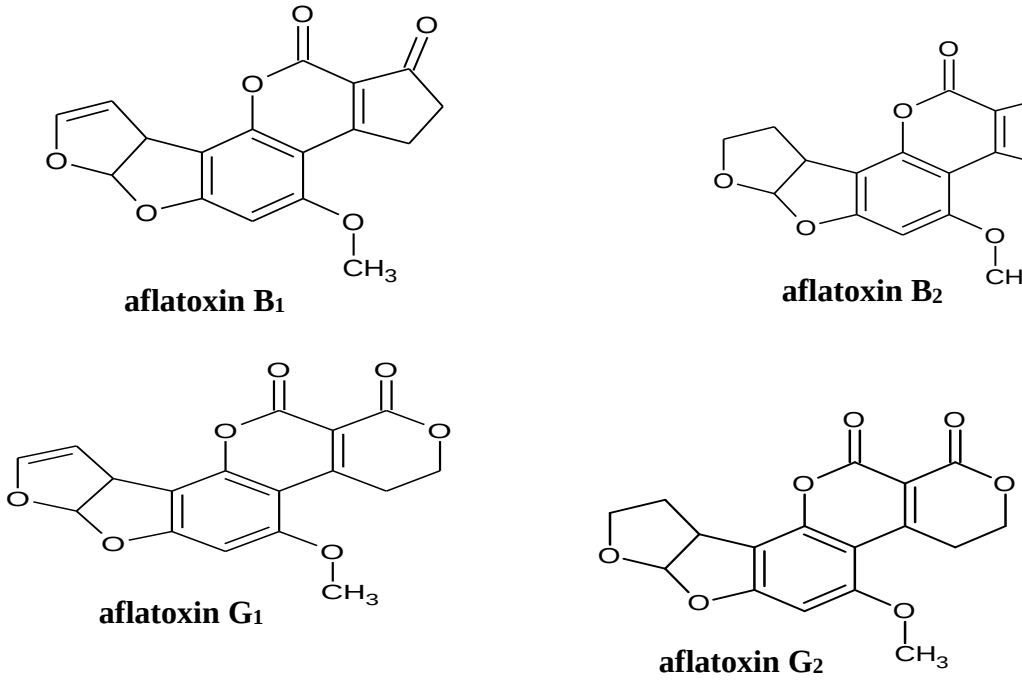


Figure1.1 Chemical structures of aflatoxin B1, aflatoxin B2, aflatoxin G1, and aflatoxin G2

Ochratoxins

Aspergillus ochraceus and *Aspergillus carbonarius* produce a collective of mycotoxins called ochratoxins. Besides, *Penicillium verrucosum* can also produce ochratoxins [54, 55]. Ochratoxins are mainly known to disrupt the activities of the kidney which can be caused by both acute and chronic exposures [56]. Ochratoxins are mainly grouped in three forms: OTA, ochratoxin B, and C which are similar in their fabric and also equally pose hazards (**Figure 1.2**). However, OTA constitutes a major risk to both human and animal health worldwide as it is prevalent in various plant and animal source foods unlike ochratoxin B and C [57]. The main food sources for OTA are cereals. Besides, coffee, pulses, spices, wine, beer, and animal products such as milk and cheese can be contaminated by OTA [58]. It is also observed that persistent exposures to low levels of OTA may prevail more danger than short-term exposures to high levels. OTA has been recognized as nephrotoxic, mutagenic, teratogenic, and immunosuppressive in many animals [8, 58]. Moreover, several animal studies showed the carcinogenicity of OTA, hence, it has been categorized by IARC as possibly carcinogenic (Group 2B) to humans as well [59].

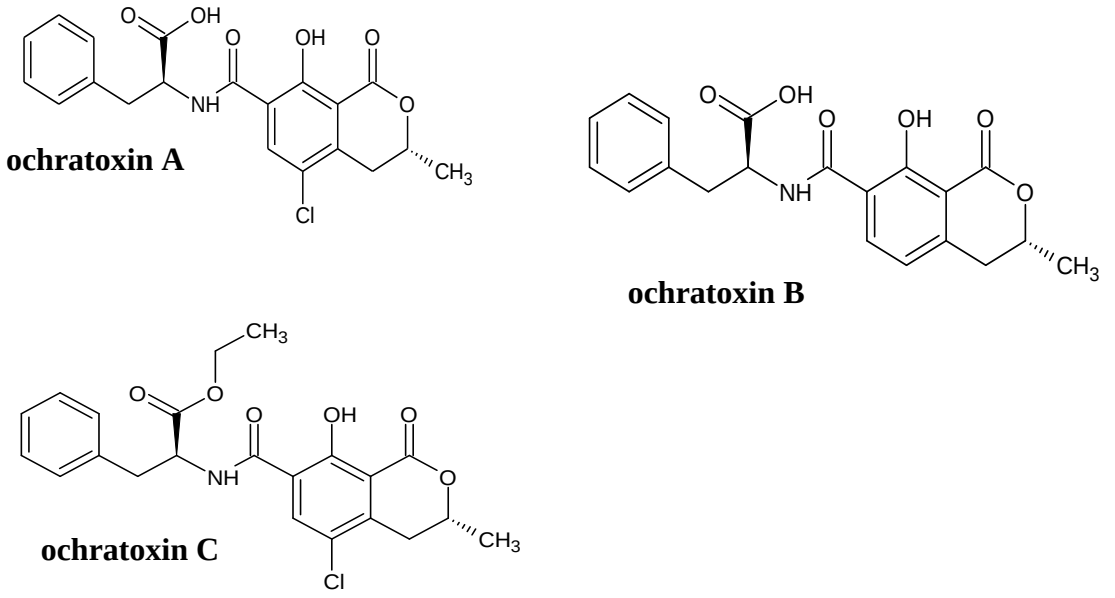


Figure 1.2 Chemical structures of ochratoxin A, ochratoxin B, and ochratoxin C

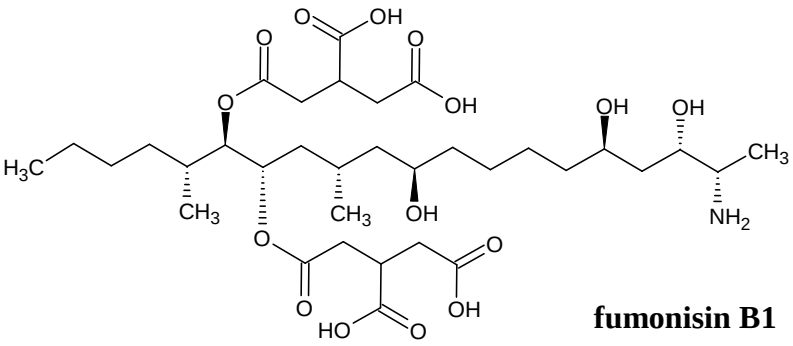
1.2.2 *Fusarium* mycotoxins

Fusarium fungal species are widespread and cause diverse plant diseases and also rot in stored food products [11]. Environmental conditions of warm wet weather which comes after dry weather at the time of flowering of a plant may favor the incidence of Fusarium fungi. Damage to the matured crop by insects also permits Fusarium fungi to enter the ear and kernels [23].

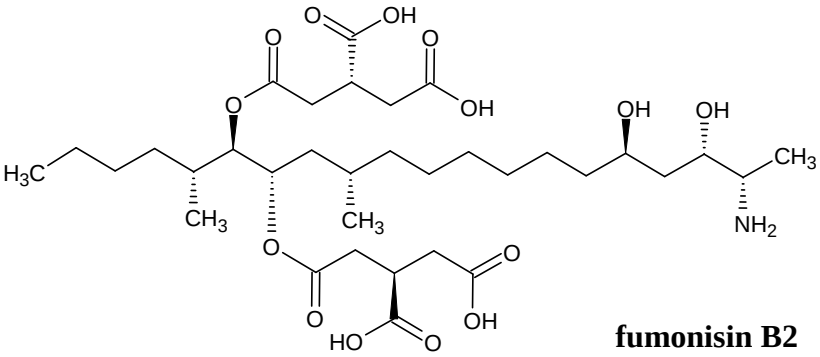
Although Fusarium fungi produce a variety of toxins; ZEN, FUMs, trichothecenes A which include T-2 toxin and HT-2 toxin, and trichothecenes B which include nivalenol and DON are the most frequently occurring, agriculturally relevant, and toxicologically significant ones [11, 60]. Neosolaniol, 4,15-diacetoxyscirpenol, and its unconjugated metabolites 15-monoacetoxyscirpenol and scirpentriol of type-A trichothecenes are some examples of Fusarium mycotoxins not much covered in studies [61]. Fusarium species mainly contaminate crops such as maize, rice, sorghum, barley, wheat, rye, and coffee [23, 62].

Fumonisins

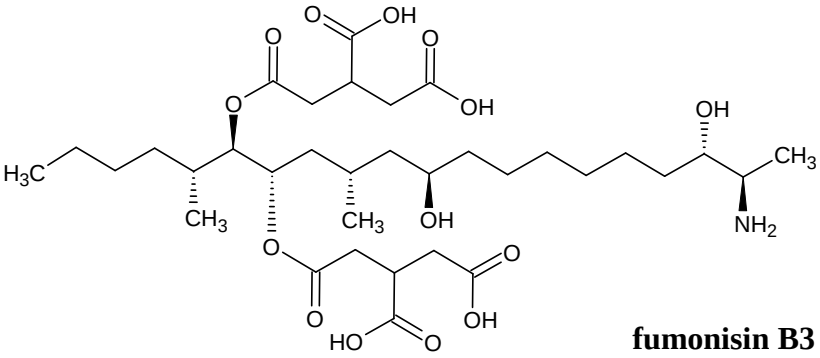
FUMs are a group of mycotoxins produced by different *Fusarium* species mainly *Fusarium verticillioides* and *Fusarium proliferatum* species [63]. Structurally, FUMs share the same basic aminopolyol backbone as sphingoids, but have different hydrolysable side chains [37]. About 12 different forms of FUMs are known, and the three most significant ones are fumonisin B1 (FB1), fumonisin B2 (FB2), and fumonisin B3 (FB3); with (FB1) being the most toxic and frequently studied one [53] **(Figure 1.3)**.



fumonisin B1



fumonisin B2



fumonisin B3

Figure 1.3 Chemical structures of fumonisin B1, fumonisin B2 and fumonisin B3

Acute toxicity from FUMs in humans is not common, and generally speaking, the study reports so far on the health effects of FUMs are not conclusive [64]. IARC categorized FB1 and FB2 as Group 2B meaning that they are possibly carcinogenic to humans mainly causing esophageal and liver cancers [59]. The mechanism of FUMs carcinogenicity is relatable to the disruptions of lipid metabolism, membrane structure, and signal pathways caused by the competitive effect of FUMs resulting in ceramide synthase inhibition. As a result, intracellular accumulation of sphinganine and other sphingoid bases takes place which causes the toxicity and the possible carcinogenicity of FUMs [65, 66]. Though evidence is inconclusive, recently, high levels of stunting and growth impairment in children have also been linked to FUMs [67]. On top, FUMs

exposure has been associated with neural tube defects in a developing fetus as FUMs hinder folate retention by depleting sphingolipids [68, 69].

Generally, FUMs contaminate crops such as maize, wheat, sorghum, and barley, with FB1 as the most prevalent of all FUMs in human food [70]. Contamination of animal-source foods with FUMs is generally low. For instance, even at high dietary FUMs concentrations, it was estimated that the transfer rate of FUMs into milk was only 0.1% [71]. On the other side, studies have revealed contamination of human breast milk with FUMs [72, 73]. FUMs are stable compounds that are not affected by heat unless a very high temperature is applied. Industrial-scale processing methods like roasting, frying, and extrusion cooking at temperatures above 180°C showed higher (90% or more) reduction of FUMs [74].

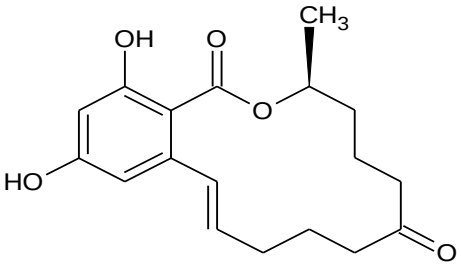
Other relevant Fusarium mycotoxins

ZEN is one of the important mycotoxins produced by *Fusarium* fungi namely *Fusarium graminearum*, *Fusarium crookwellense*, *Fusarium sporotrichioides*, and *Fusarium culmorum*. Cereals and cereal-based foods are commonly contaminated by ZEN [75]. Though the adverse human health risks from ZEN exposure are not conclusive yet [76], it seeks attention that it can cause reproductive problems through binding estrogen receptors in some animals and possibly in humans [77, 78]. Another group of mycotoxins that are also produced by *Fusarium* fungi are trichothecenes. They are collectively non-volatile and contain epoxides that are further classified based on their functional groups. Trichothecenes also mostly occur in cereals such as wheat, rye, barley, oats, and maize. They are divided into two main groups: type A and B. The type A trichothecenes include neosolaniol, T-2 toxin, HT-2 toxin, and diacetoxyscirpenol (DAS). The type B trichothecenes comprise DON, nivalenol, 3-acetyldeoxynivalenol, and 15-acetyl- deoxynivalenol [79-81]. Data on the toxicokinetics of trichothecenes in humans is scarce. Hence, as per IARC's classification, trichothecenes were designated as Group 3 meaning that they are not classifiable as to their carcinogenicity to humans because of a lack of enough evidence. So far, reports from animal clinical studies revealed that T-2 toxin and DAS are the most potent among the trichothecene mycotoxins [62]. HT-2 usually co-occurs with T-2 as it is the main metabolite of T-2. T-2 toxin is predominantly produced by *Fusarium sporotrichioides* and other *Fusarium* species i.e. *Fusarium equiseti* and *Fusarium acuminatum* also produce both T-2 and HT-2 [82]. T-2 toxin mainly affects heart muscle, nerves, and immune system. Cereals and cereal food products are commonly contaminated by T-2 and HT-2 toxin [83]. DAS is produced by *Fusarium* species such as *Fusarium poae, Fusarium semitectum, Fusarium verticillioides, Fusarium sporotrichioides, and Fusarium acuminatum*

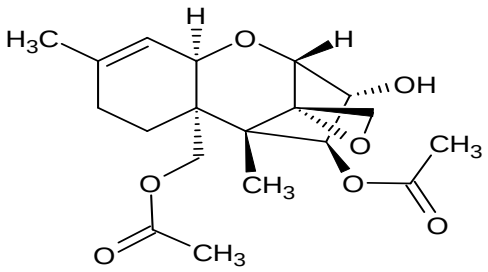
[84]. The toxicity of DAS is manifested as nausea, vomiting, diarrhea, hypotension, neurological symptoms, chills, and fever similarly both in humans and animals. It has been also reported that DAS causes inhibition in the multiplication and production of immunoglobulin in human lymphocytes with little inter-individual variation. DAS contaminates cereals such as maize and barley [62].

DON is mainly produced by *Fusarium graminearum* or by *Fusarium culmorum* in some regions. DON is basically found in small grains, such as oats, wheat, and barley, but also in maize [85]. It shows a lower risk of toxicity compared to type A trichothecenes, such as T-2 toxin [81]. Though the toxicity of DON is not limited to a specific organ, its toxicity is manifested mainly in the intestine and immunity system [86].

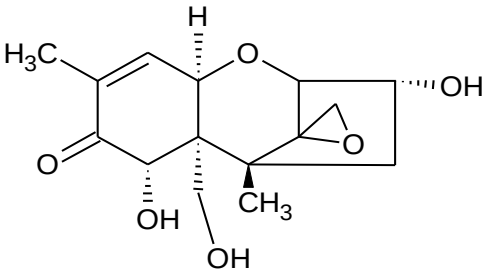
Nivalenol is produced by a *Fusarium* species named *Fusarium nivale*. Wheat, barley, and maize are the main crops contaminated by nivalenol. Nivalenol and DON bear similarities in their chemical structure and toxicological effects like causing nausea, vomiting, diarrhea, and ultimately death [87]. Nivalenol has been identified to cause hepatotoxicity and immunotoxicity in animals [88]. So far, no studies have been published examining the human effects of nivalenol [75]. **Figure 1.4** shows the chemical structures of mycotoxins produced by *Fusarium* fungi.



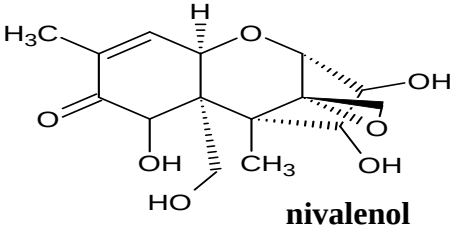
zearalenone



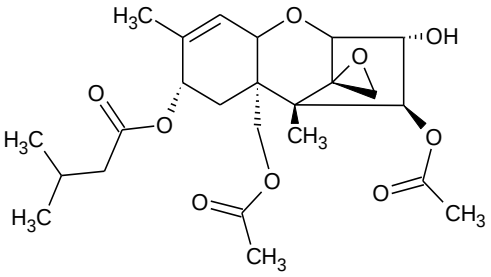
diacetoxyscirpenol



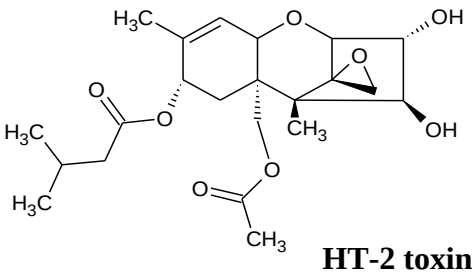
deoxynivalenol



nivalenol



T-2 toxin



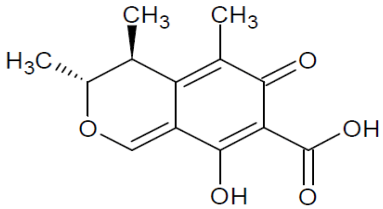
HT-2 toxin

Figure 1.4 Chemical structures of zearalenone, diacetoxyscirpenol, deoxynivalenol, nivalenol, T-2 toxin, and HT-2 toxin

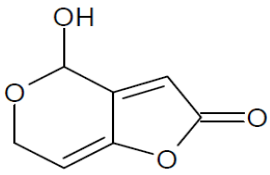
1.2.3 *Penicillium* mycotoxins

Citrinin is produced by *Penicillium* mainly by *Penicillium citrinum* and also *by Penicillium verrucosum* and *Penicillium expansum* [89]. This mycotoxin can also be synthesized by various *Aspergillus* species. The toxicity of citrinin in humans and animals is still poorly understood. Citrinin has been categorized as Group 3 by IARC showing that it is unclassifiable as a carcinogen for humans due to limited information [90]. However, there is a concern that the exposure and toxicity of citrinin may lead to serious kidney failure [91]. Citrinin commonly occurs at post-harvest such as in stored rice, barley, wheat, and also in fruits, vegetables, and beans [92].

Patulin is produced by diverse species of *Penicillium* such as *Penicillium expansum (Penicillium leucopus), Penicillium crustosum, Penicillium patulum (Penicillium urticae & Penicillium griseofulvum),* but is also produced by *Aspergillus* fungi *i.e Aspergillus clavatus* [93]. In recent times, the global patulin food contamination data showed that the levels recorded were concerning as patulin is associated with immunotoxicity and alteration of the gastrointestinal system [8]. However, the carcinogenic potential of patulin is still not clear. Apples and apple juices are the leading food items contaminated by patulin [94]. The structures of citrinin and patulin are shown in **Figure 1.5**



citrinin



patulin

Figure 1.5 Chemical structures of citrinin and patulin

1.2.4 Emerging and modified mycotoxins

"Emerging mycotoxins" denote a variety of mycotoxins whose existence is increasingly recorded in current times. Emerging mycotoxins include enniatins, beauvericin, moniliformin, and fusaproliferin [95].

Enniatins are produced by different *Fusarium* species, such as *Fusarium avenaceum, Fusarium oxysporum, Fusarium poae,* or *Fusarium tricinctum* that mostly contaminate cereals. Twenty-nine types of enniatins have been identified so far [96], however, enniatins A, A1, B, and B1 are quite regularly detected in different food and feed samples. *In vivo* studies have shown a lesser/no toxicity of enniatins as compared to the relatively higher toxicity documented *in vitro* studies [97]. It appears that effective degradation of enniatins in animal intestinal systems has been noticed though more studies are required on the subject. Instead, enniatins are more known to be potent to mitochondria [75].

Beauvericin is produced by *Fusarium* species like *Fusarium proliferatum, Fusarium subglutinans, Fusarium verticillioides,* or *Fusarium oxysporum*. According to EFSA 2014 scientific recommendation, a threat of acute health effects in humans due to beauvericin exposure is not expected, however reliable conclusions regarding the chronic health effects of this toxin cannot be made since supporting evidence, particularly *in vivo* experiments are not available [97, 98].

Among the diverse species of *Fusarium* fungi that produce moniliformin, the common one is *Fusarium proliferatum*. Previously, a Keshan disease outbreak in humans was linked to moniliformin exposure. Moniliformin has been identified to cause lymphocytes, skeletomyocytes, and cardiomyocytes toxicity, though it is considered to be less toxic than enniatins and beauvericin. Moreover, though it is not entirely understood how moniliformin acts, there is an indication that exposure to this toxin can possibly induce toxicity of the mitochondria in a similar way to that of enniatins. The major food sources for moniliformin are cereals such as rice, wheat, oats, barley, rye, and maize [75].

Fusarium proliferatum, *Fusarium subglutinans*, and *Fusarium verticillioides* are known to produce the toxin, fusaproliferin which contaminates cereals. An experiment on the fusaproliferin toxicity in animals particularly in chickens showed that the toxin can pose a danger to human and animals health, but this needs further investigation [97].

Another emerging mycotoxin is sterigmatocystin produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus* section *Nidulantes, subclade Versicolores* that resemble AFs.

Sterigmatocystin is included in the IARC list of possible human carcinogens (Group 2B) as animal-based research outcomes proved that sterigmatocystin can cause tumors [99].

Apart from the emerging mycotoxins, another concern is the occurrence of modified mycotoxins. The name "modified mycotoxins" refers to "any modification of the basic chemical structure of mycotoxins either by chemical or biological modifications" [100]. The modification of mycotoxins can occur due to the response of plants to adverse situations, during food processing [101], and during the gastrointestinal digestion process [61].

Despite the lack of data, the majority of the available studies currently suggest that modified mycotoxins are less toxic than their original forms, though ample study reports have also indicated that microorganisms in the gastrointestinal system can hydrolyze modified mycotoxins back into their original form. In this regard, modified mycotoxins may create additional risks to human health [102]. Furthermore, maximum levels that safeguard consumers from these mycotoxins have not been established so far as data on the exposure and toxicology are lacking [88]. Therefore, protecting populations from health hazards associated with consuming mycotoxins should thoroughly cover the examination of all mycotoxin forms that may have negative effects on humans and animals [103]. The tolerable daily intake (TDI)/ benchmark dose lower confidence limit/ lowest observed adverse effect level/ no observable effect level of commonly occurring mycotoxins [104] are depicted in **Table 1.1.**

Table 1.1. TDI, BMDL, LOAEL, and NOEL of commonly occurring mycotoxins

Mycotoxins	**TDI**[1,2] (µg/kg bw /day)	**BMDL**[2,3] (µg/kg bw /day)	**LOAEL**[2,4] (mg/kg bw/day)	**Updated year**
Aflatoxins (sum of B1, B2, G1, G2, M1)		0.4		2020
Deoxynivalenol, 3-Acetyldeoxynivalenol, 15-Acetyldeoxynivalenol and deoxynivalenol-3-O-glucoside group	1			2017
Enniatins (including Enniatin A, A1, B, B1)			0.09	2014
Fumonisins	0.1			2018
	TDI[1,2] (µg/kg bw)		**NOEL**[2,5] (µg/kg bw /day)	
Ochratoxin A		14.5		2020
Nivalenol	1.2			2017
T-2 toxin, HT-2 toxin and its phase I/II metabolites	0.02			2017
Zearalenone and its phase I/II metabolites	0.25			2016
4,15-Diacetoxyscirpenol	0.65			2018

[1] TDI tolerable daily intake
[2] bw body weight
[3] BMDL benchmark dose lower confidence limit
[4] LOAEL lowest observed adverse effect level
[5] NOEL no observable effect level

1.3 Co-occurrence of mycotoxins

Multiple types of mycotoxins may co-occur in food products as different fungal species can co-infect the commodities, while one single fungus can also produce different types of mycotoxins [3]. As a consequence, co-contamination of multiple mycotoxins in food commodities has attracted attention in recent years [105]. Data on the co-occurrence of mycotoxins in various foods and drinks are growing globally as a result of the improvements made in the performance of analytical technologies like liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) that allows for the simultaneous identification of mycotoxins [106]. Moreover, the concept of multiple mycotoxins exposure has been introduced into risk assessment methods [9]. However, the mechanism through which a single mycotoxin manifests its toxicity might ultimately differ from a mycotoxin mixture [29] due to the complexity of the mechanism of action, the toxicodynamics, and toxicokinetics, etc. This makes the toxicity risk prediction from multiple mycotoxins very challenging [28, 107]. The co-occurrence of two or more different mycotoxins in food or biological samples has been documented as a subject of studies. As an illustration, the *Fusarium* mycotoxins i.e DON, ZEN, and FUMs co-occur in food [25] mainly due to the nature of *Fusarium* fungi which can produce more than one mycotoxin where their

toxicity effects often add up [105]. Specifically, ZEN, DON, or OTA come along with FB1 in maize [28]; and patulin, DON, and T-2 mycotoxins have been found together in food. *In vitro* studies revealed that in synergy they may have cytotoxic effects [108]. Moreover, OTA and AFs co-occur and were frequently examined in plasma [109]. A study also revealed that OTA and AFs have additive effects. This may be attributed to the reason that both OTA and AFs affect DNA pairing and duplication which implies that they may cause cancerous abnormalities [110]. On another side, the combined effects of OTA and FB1 have been extensively researched during the past recent years [28]. Above all, the co-exposure to most of the health significant mycotoxins i.e. AFB1 and FUMs is worrisome as they are thought to be genotoxic and may lead to regenerative cell proliferation. In general, studies conducted so far on the combined toxicity of co-occurring mycotoxins show a huge knowledge gap where the need for *in vivo* studies was particularly emphasized [9].

1.4 Biomonitoring of mycotoxins

The usual trend in mycotoxins exposure assessment is an indirect approach that employs a combination of food contamination with food consumption data. Food consumption data are most often available from surveys done at the country level that are primarily intended to determine energy and/or nutrients. Thus, the available food consumption data from the national databases may not provide complete information for mycotoxins exposure assessment. Specifically, the applicability of data from such surveys has a major pitfall in the foods categories as they are not designed considering their susceptibility to mycotoxins contamination. The lack of large-scale food consumption data in most LMICs is also another challenge [53]. Above all, mycotoxins are present in various food items which makes the quantification of mycotoxins from these diverse food products challenging. In addition, mycotoxins are often heterogeneously distributed in food samples making representative sampling a critical factor in occurrence data generation. Therefore, biomonitoring of mycotoxins in body fluids or tissues using validated biomarkers has the potential for a more accurate estimation of exposure by capturing concentrations of either the original toxin or a metabolite from various sources [53, 111]. The term biomarker refers to a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [112]. Biomonitoring is usually understood as a test done to examine mycotoxins/chemical agents' exposure derived from the intake of contaminated food, but additionally, it encompasses a qualitative and/or

quantitative examination of the possibility of exposure that comes from other sources such as inhalation [27, 113].

Mycotoxin biomarkers can be detected using three approaches: biomarkers of exposure, biomarkers of effect, and non-targeted methods. The biomarkers of exposure approach is a specific method that follows standardized analytical methods for measuring parent mycotoxins and their metabolites. The second method called biomarkers of effect is non-specific and measures the changes in the biological process in the body that arise from exposure to toxins. The non-targeted approach involves the determination of unknown mycotoxin derivatives [27]. Critical identification of biomarkers is then imperative in terms of linking mycotoxin exposure with health effects [29].

Biomarker-based studies are typically challenged by the need to apply validated and relevant biomarkers [114]. A biomarker validation study should focus on choosing the appropriate biological fluid or tissue that better indicates the metabolism and kinetics of the specific biomarker precursors [115].

In this regard, complementary approaches of *in vitro* and *in vivo* experiments are strong tools to predict the bioavailability, metabolism, and health effects of mycotoxins and to further attain a conclusive understanding [114]. However, a lot of uncertainties remain in the quantitative investigation of mycotoxin biomarkers in humans since most of the toxicokinetic studies for mycotoxins have been done in animals and explicit data for humans are usually lacking [113].

Urine, serum, plasma, and breast milk are the common mycotoxin biomonitoring matrices [27]. From these matrices, urine is a non-invasive method with plentiful mycotoxin biomarkers even though the results are not replicable as it vary from one day to another [116]. On the other hand, serum and plasma sample collection demand skilled personnel [117], and yet, mycotoxin assessment from serum and plasma analysis are more reliable as they are relatable with chronic exposure to mycotoxins [118]. In the case of breast milk, the sampling is not demanding rather the mechanism of the absorption and transfer of mycotoxins to breast milk is still unclear [119], and the accuracy of mycotoxins determination in breast milk is complicated by many factors. Among the factors are timing and duration of lactation, birth order, stability of toxins, and the accuracy of the analytical tools [120].

AFB1 was one of the food contaminants that was considered in the first biomarkers-based human exposure studies which demonstrated a statistical relationship between AFB1 intake and urinary AFM1 excretion. Further, albumin-bounded AFB1 and AFB1-DNA adducts in urine

were investigated in exposure assessment research. Urinary AFB1-N7-guanine adduct and primary DNA adduct of AFB1 have been then proven to be reliable indicators of acute exposure to AFB1 [53].

From the commonly known mycotoxins, AFs, DON, and OTA biomarkers have been validated so far while appropriate biomarkers for FUMs and other mycotoxins are not readily available [53, 114]. For AFs, aflatoxin Q1, aflatoxin P1, and AFB-N7-guanine are the recommended urinary biomarkers for acute exposure assessment, whereas AFB1-lysine in plasma is suggested for the assessment of chronic AFs exposure. For exposure assessment of DON in urine, the proposed biomarkers are free DON, DON-15-glucuronide, and DON-3-glucuronide. On the other hand, a reliable biomarker is not available for nivalenol thus further studies are required. The main biomarker of T-2 toxin in urine and plasma is HT-2, however, more work is required to authorize the other available T-2 toxin metabolites for investigation in human urine or feces. For DAS, 15-monoacetoxyscirpenol is considered in urine and feces. In the case of OTA, the major ones are OTA, OTα, and their glucuronides in urine and also in plasma. Though sufficient data are not available on citrinin, dihydro-citrinone can be used as citrinin biomarker in urine. Different metabolites are considered as biomarkers of ZEN in urine i.e. α-ZEL, β-ZEL, 8-OH-ZEN, 15-OH-ZEN, and ZEN-14-glucuronide [114].

FUMs are not readily absorbed, accordingly, they are mainly excreted through the fecal route. The possible biomarkers that have been identified so far are free FUMs and hydrolyzed FUMs [114]. The low absorption of FUMs coupled with quick removal from the body make FUMs have a short half-life. Considering this challenge, an indirect FUMs exposure assessment method is needed in the FUMS biomarkers development process in humans [53]. Experimental studies that examine the effect of FUMs consumption on the sphinganine/sphingosine ratio have also been introduced by different researchers but still, the progress made in the development of FUM biomarkers was complex and challenging compared to AFs [111]. This has also challenged the progress made in maternal and infant FUMs exposure assessment [121].

Despite the lack of validated biomarkers, human breast milk has been reported to be a source of FUMs exposure for children [64]; and also OTA has been reported as the most frequently detected mycotoxin in breast milk, following AFs [122]. In this regard, the current developments made in high-resolution mass spectrometry and the respective advancements in analytical data processing can enhance the accuracy and effectiveness of the biomarker identification procedure [114] in breast milk as well.

1.5 Overview of mycotoxins in Africa and Ethiopia

1.5.1 Mycotoxins in Africa

Typically, the populations in LMICs are affected most by the health effects of mycotoxin exposures [123] where foods are mostly contaminated with mycotoxins. Agricultural produce in LMICs is usually susceptible to mycotoxin contamination due to the dependence on subsistence farming [124]. In general, the principal mycotoxins of economic and health significance in Africa are AFs, FUMs, ochratoxins, trichothecenes, and ZEN including the modified and emerging mycotoxins such as 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, beauvericin, and enniatins [88, 125]. Similarly, in sub-Saharan Africa (SSA), the prevalent mycotoxins were reported to be AFs (43.7%) followed by FUMs (21.9%), ochratoxins (12.5%), ZEN (9.4%), DON (6.2%), and beauvericin (6.2%) [126]. Yet, the trend of mycotoxins from the subsequent years of studies in SSA has shown an increase in FUMs (72.6%), ZEN (52.2%), and DON (49.5%) [21].

In SSA, the majority of people's daily cereal consumption is dominated by maize, and even it is reported to be consumed up to three times a day. Maize is also included in most of the complementary foods prepared for children in many African countries [127]. Besides, the East African Community region produces and consumes a lot of peanuts [25]. An analysis of 2328 commonly consumed foods was performed in the first multi-center SSA Total Diet Study, and peanuts, peanut oil, and maize were found to be crops that are highly contaminated with AFs [128]. Another report from the West and South-East regions of SSA also showed that peanut and maize were the most regularly contaminated staple crops with AFs [129]. Therefore, this is one of the reasons why mycotoxin contamination in Africa has become more of a concern as maize contamination and fatalities have been documented since the late 1960s till recent years [130]. An estimated 4.5 billion people living in LMICs are at risk of dietary exposure to AFs as their staple diets consist of cereals, legumes, and oilseeds [131]. Most importantly, the East African region is probably the most struck by mycotoxicosis in the whole world [132]. Rural Kenya faced a massive onset of aflatoxicosis in 2004 as a result of consuming locally grown maize infected with AFs [133]. The danger of AFs did not seem to reduce in Kenya even years after the outbreak. For instance, in 2010, 83 out of 130 maize samples from Kenya showed aflatoxin levels above the recommended maximum limit of 10 µg/kg [134]. Severe illness and deaths have also been recorded from another aflatoxicosis outbreak in the central part of Tanzania from consumption of high aflatoxin contaminated foods [130]. Moreover, a review of surveys conducted for close to four decades in Uganda has consistently shown high aflatoxin

levels in foods [135]. More mycotoxin contamination findings from different African countries have also been reported.

According to IARC, a significant increase in mortality and morbidity among 500 million poor people who live in SSA, Latin America, and Asia was estimated due to exposure to different mycotoxins [32]; and the problem of mycotoxins continues to affect people living majorly in Africa and other LMICs [67]. A study by Liu and Wu. indicated that an estimated 40% of liver cancer cases in Africa are thought to be caused by dietary AFs exposure [136]. A similar prediction has also been made in Uganda that cancer prevalence documented among the Ugandans population might be associated with the consumption of mycotoxin-contaminated foods [137]. Hepatitis B and, to a lesser extent, hepatitis C virus infections are recognized for their synergistic association with aflatoxins in promoting liver cancer. Consequently, evaluating the impact of aflatoxin on liver cancer should be conducted independently for populations with and without chronic hepatitis B virus infection. The majority of liver cancer instances are concentrated in Sub-Saharan Africa (SSA) and Southeast Asia, where the prevalence of hepatitis B virus is high, and there is limited control over aflatoxin exposure in the food supply [136]. Given the heightened concerns about the human health implications of aflatoxins in Africa especially in the East Africa region, experts have recommended that mitigation strategies incorporate hepatitis B virus vaccination and the regular monitoring of mycotoxin exposure at the hospital level [132].

On another side, the linkage between FUMs exposure and esophageal cancer in Kenya was also reported [138]. Likewise, mycotoxins exposure in children has been reported in different countries in Africa. To mention, data from global comparative exposure studies revealed AF-alb adduct was detected in more than 90% of young children in Gambia and Benin, in contrast to less than 1% in children sampled from developed countries [139]. In Sierra Leone, 88% AFs and 35% OTA were found in breast milk sampled from breastfeeding mothers in under-five clinics [140]. A study that examined the relationship between child growth with AFs and FUMs exposure in Tanzania revealed that solely exposure to FUMs or together with AFs could cause growth faltering in children [141]. Overall, many research in SSA have also verified the fact that children are frequently exposed to and are at risk of mycotoxins effects [142]. This may be attributed to maize-based complementary foods [143] and animal milk [142] that are often introduced to infants. Besides, chronic exposure to AFs through contaminated human breast milk during breast feeding could be another cause as evidenced in Nigeria, Sierra Leone, Ghana, and Sudan [39].

Yet, it is worth highlighting that the limited epidemiological studies conducted in Africa are geared towards two mycotoxins (AFs and FUMs). No epidemiological research on the effect of other mycotoxins such as DON and ZEN among mothers, infants and young children has yet been published [121]. As there is still a sizeable research gap, data on the occurrence and toxicology of mycotoxins is limited which subsequently affected the regulation and control of mycotoxins in SSA [36].

1.5.2 Mycotoxins in Ethiopia

In Ethiopia, mycotoxins have been sparingly studied regardless of some of the observed problems they have caused [144]. Basically, maize is the primary crop produced in Ethiopia followed by *teff* (*Eragrostis tef*), sorghum, wheat, barley, and finger millet which are ranked in decreasing order [145]. The presence of mycotoxins in the above-mentioned commonly produced crops have been documented, and these crops are also known to be used as inputs for making alcoholic and non-alcoholic beverage besides their contribution to Ethiopian meals [146]. Comprehensively, among the mycotoxins, primarily AFs, followed by FUMs, OTA, and DON were found in a variety of agricultural food products i.e. groundnuts, cereals, milk, coffee, and beer [147]; where maize and groundnut were reported as the most mycotoxin contaminated commodities in the country [50]. Even further, other raw or processed foods i.e. 13% of red pepper and 8% of *shiro* powder were found positive with AFs [148]. Besides food commodities, a few mycotoxin studies have also been conducted in biological samples in Ethiopia. For instance, a study conducted in Southern Ethiopia revealed that AFM1 was found in 64.4% (LOD = 0.005 µg/kg) of the breast milk samples [149]. The presence of AFs i.e. AB2, AG2, and AFM1 in urine samples of children was reported in another study [150]. It was also shown that high AFs levels were detected in serum samples of children from rural Ethiopia [151]. Moreover, more than 95% of FUMs and other mycotoxins including ochratoxins, ZEN, nivalenol, and DON in serum samples of pregnant women [152] and 53.3% (LOD= 0.00015 µg/kg) of AFM1 in urine samples of lactating women [153] were reported.

Between 2010 and 2019, the incidence of cancer in Ethiopia saw a 32% rise, with the most prevalent cases in 2019 being leukemia, cervical cancer, breast cancer, colon and rectum cancer, and stomach cancer [154]. While esophageal cancer is not the primary cause of cancer in Ethiopia, it's noteworthy that Africa exhibited the highest age-standardized incidence and mortality rates for esophageal cancer in 2019, often referred to as the "African esophageal cancer corridor," extending from Ethiopia and Kenya to South Africa [155].

Mycotoxins occurrence (2013-2022) from different studies in Ethiopia [147, 149, 150, 152, 153, 156-158] is summarized in **Figure 1.6.**

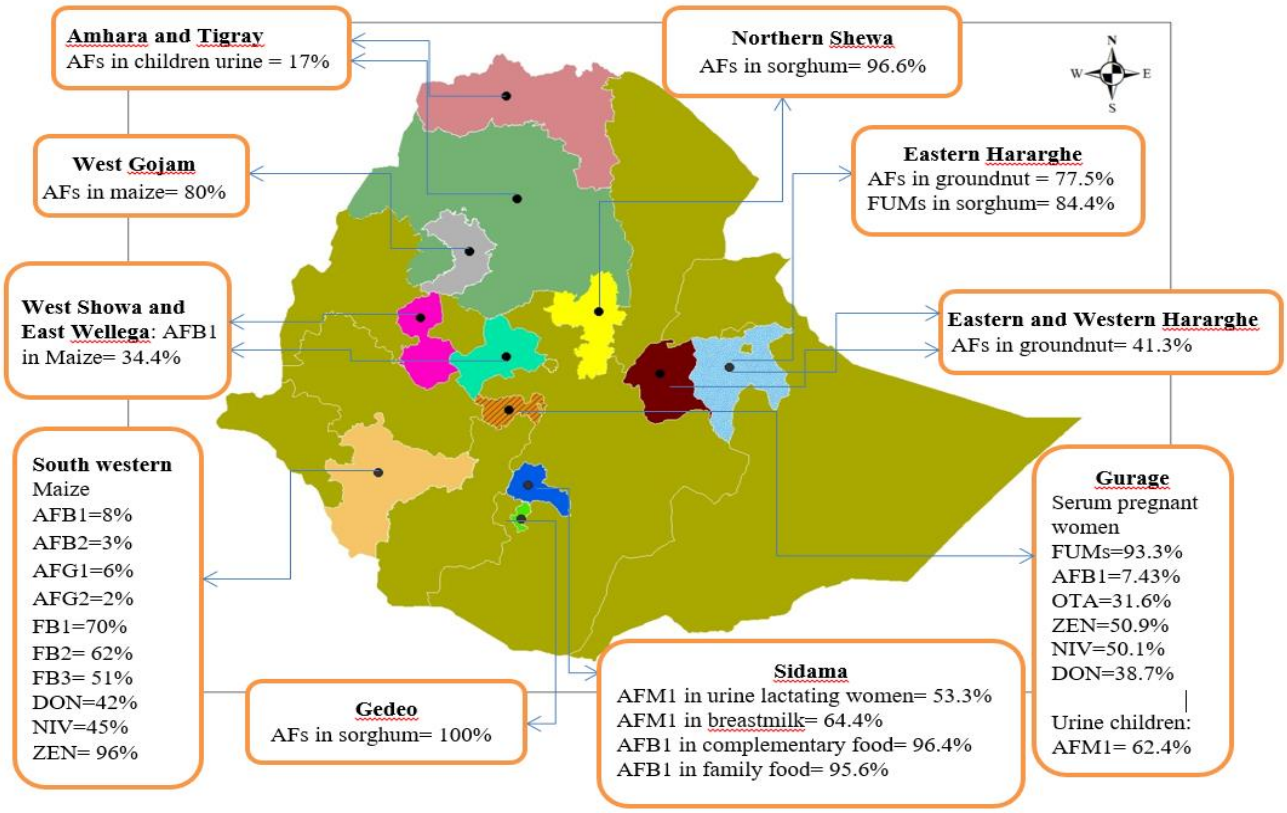


Figure 1.6 Mycotoxins occurrence (percentage of positive samples) in food and biological samples in Ethiopia from 2013-2022.

1.6 Contributors of mycotoxin occurrence in Africa and Ethiopia

1.6.1 Pre- and post-harvest practices

Basically, the main pre-harvest factors that lead to mycotoxin production are drought stress, insect infestation, warm temperature, poor soil fertility, delayed harvesting, tilling and deep plowing, limited implementation of crop rotation, the timing of the production cycle, seed variety, use of fertilizers, irrigation methods, weed, and insect control [159]. For instance, soil could be a significant reservoir for fungi like *Aspergillus flavus* and many *Fusarium* species from residues of previous crops can possibly be transferred to the next crop production. Thus, rotating crops reduces the chance of fungus retained in the soil and the subsequent mycotoxin occurrence during pre-harvest time [9]. As another pre-harvest instance, it was indicated that maize varieties that mature early during production are proved to be less susceptible to FB1 contamination [160]. Moreover, enhancing irrigation systems capable of combating drought stress, enhancing soil nutrient quality and overall health, and employing varieties resistant to

drought, diseases, and insects can aid in minimizing plant stress and the subsequent vulnerability to mycotoxigenic fungi [161]. Mechanical injury, insect infestation, time of harvesting, drying method, types of storage structure and conditions, handling, and processing are the major contributors to mycotoxin occurrence during harvest and post-harvest time [8, 162].

In the case of LMICs, lots of studies confirmed that inadequate pre- and post-harvest crop handling practices were the major factors that are linked with aflatoxin contamination [163]. More particularly, high insect and rodent infestation coupled with poor storage practices is the characteristic of many rural households and still majorly contributing to the high level of mycotoxins occurrence in developing nations [25]. For instance, as the storage period of groundnuts increased (>7 months), an increment in AFB1 levels was documented in a study from Mali [164]. There was also evidence in Tanzania that a storage period of more than 14 days was associated with aflatoxin contamination [165]. Additionally, a study carried out in Chad complemented the fact that harvest time, length of drying, location, and type and storage conditions influence mycotoxin production in groundnut [166]. Similar poor pre- and post-harvest practices have been reported in Ethiopia. Grain storage methods on farms do not offer adequate protection against environmental and biological deterioration agents, and additionally, porous bags are mostly used while storing crops in households [167]. A review has shown that local traders spray water on already-dried food produces like peanuts and spices with the intention of increasing profit on sale by increasing the weight of the commodities. This malpractice of local vendors in Ethiopia is regarded as the most hazardous for fungi development and mycotoxins production [168]. Moreover, a study in Ethiopia confirmed that threshing sorghum grains on bare ground exposed grains to high levels of FUMs contamination which underlined the fact that such non-optimal harvesting practices of farmers meaningfully increase the chance of FUMs contamination [169]. Hence, the reported mycotoxin contamination in different food and feed products in Ethiopia may be explained by the traditional crop production coupled with inadequate harvesting, drying and storage practices [170].

Mycotoxins cannot easily be removed from food and feed once present, which is why preventing and controlling mycotoxins from crops during production is promoted as a better approach [10]. Over the years, the application of good agricultural practices (GAP) and good manufacturing practices (GMP) accompanied by hazard analysis and critical control points

(HACCP) during food processing has been proposed by experts to alleviate the problems of fungi development and mycotoxins production along the food chain [88].

In this regard, some efforts have been observed in Africa to mitigate mycotoxin problems during the pre- and post-harvest period. For instance in Guinea, proper drying and storage conditions were introduced to the community through an intervention study and the result showed a substantial reduction in AFs levels in the study villages [171]. Improved storage bags i.e. hermetic metal silos and hermetic grain bags were introduced in Zimbabwe and their effect on the reduction of AFB1 and FB1 in maize was examined. The hermetic storages showed a promising finding in controlling AFB1 production, though not the case for FB1 [172]. A similar finding was also reported in Kenya [173]. Another intervention study in Zimbabwe also reported that using hermetic technology compared to using conventional storage has brought a remarkable decrease in the level of AFM1 in urine samples collected from children and women [174]. Compared to their counterparts, the application of various post-harvest mitigation strategies among a trial group in Tanzania also showed a decrease in AFs exposure [175].

At a larger scale, attempts to prevent and reduce the problems of mycotoxins with a particular focus on AFs were seen in Africa. In this regard, the Partnership for Aflatoxin Control in Africa (PACA) has taken the lead in collaboration with the African Union [176] and other international collaborative programs such as MYTOX-SOUTH® [130]. However, the adoption of mycotoxin mitigation technologies in Africa is usually not moving so well due to the limited capacity and the costs related to these technologies [125, 177]. A similar situation applies in Ethiopia where agriculture is mostly owned by smallholder farmers (74%), and hence the use of modern pre- and post-harvest techniques is limited which ultimately creates an enabling environment for mycotoxins contamination [178]. Thus, there is still the dilemma that many of the LMICs cannot prevent and control food contamination due to a lack of resources, nor can they afford to reject contaminated foods [179]. As a consequence, a tendency for AFs reduction for instance was not observed in these areas despite the efforts made to reduce AFs contamination in foods and feeds [129].

1.6.2 Climate change

Variability in weather conditions across the different seasons causes variability in the extent of mycotoxins contamination. For instance, occurrence of mycotoxins is exacerbated if rainy seasons come after dry seasons [129]. Due to changes in weather conditions, data from 2013–2017 have shown variation from one year to another in the occurrence and degree of mycotoxin contamination in maize grown in different countries [21]. Overall, mycotoxins are best

produced at temperatures ranging from 24-28°C [13]. Production of more mycotoxins is warranted when fungal metabolism is exposed to unfavorable conditions impacted by global warming such as warmer temperatures, drought, and stress [27]. As a result, droughts and floods caused by climate change can worsen the problem of mycotoxins.

Climate change has also been strongly suggested to affect the distribution of mycotoxins in terms of the geographical areas and the season in which they occur [37]. AFs, which used to merely exist in tropical and sub-tropical climates have now expanded to every continent. On the other side, FUMs which were formerly known only to be found in temperate climates are also becoming prevalent in Africa's tropical climate [22]. Important contributors to the high occurrence of mycotoxins in Africa might thus be the presence of a conducive climatic environment for the growth of mycotoxin-producing fungi and the impact of climate change. Particularly in East African countries, mostly the climate is warm and humid making it ideal for fungi to grow and produce mycotoxins. Scholars are also claiming that mycotoxins prevalence will distinctly increase in East Africa as climate change will affect and make the region more warm and humid [25]. For instance, in several areas of Malawi, it is projected that climate change will subject the main food crop (maize) to increased levels of AFB1 at harvest, in a way that has not previously been recorded [180]. In addition, increment in pest and insect populations, early maturation and ripening of crops, decreased plant resilience, and change in host pathology are the indirect effects of climate change that induce mycotoxins occurrence. These changes along with warm and humid weather are mainly associated with storage problems which are commonly seen in LMICs [159]. In addition, agriculture in Africa is highly reliant on the prevailing weather and climate conditions makes Africa more prone to the undesirable effects of climate change [181]. However, there are still significant gaps in scientific evidence that explore the role of climate change in mycotoxin predictive models and risk assessments [182].

1.6.3 Mycotoxins legislation

Lack of mycotoxin regulations makes a population to be continuously exposed to mycotoxins [10]. Various organizations around the world, including Codex Alimentarius Commission of the World Health Organization (WHO) and the Food Agriculture Organization (FAO), the European Commission, and the United States Food and Drug Administration set regulatory limits on levels of mycotoxins in food and feed [14, 183]. The regulatory limits in food and feed are not similar across different countries as the capacity and development status of a country is taken into consideration while setting the limits. For instance, almost all European

countries have strong regulations on AFs which significantly decreased the chance of AFs exposure by the population [184]. Until 2003, close to 100 countries in the world which account for 87% of the global population, had their maximum limits for mycotoxins; however, by that time, explicit regulation for mycotoxins has been known to exist only in 15 African countries representing approximately 59% of the continent's population [183]. Africans are therefore more predisposed to the undesirable effects of mycotoxins since legal rules and standards are largely absent [162]. The gap in institutionalized regulation of mycotoxins is especially prominent in the majority of the SSA countries as it is observed that maximum limits for AFs (which is the most regulated mycotoxin in the world) have not even been placed for the staple crop i.e. maize [183]. Furthermore, little enforcement measures are taking place in African countries even for the pre-defined regulations. This is due to the challenge that most of the crop productions are undertaken by subsistence farmers at a small scale level [185]; and also due to the inefficient government control of the marketing system as informal trade is prevalent [25].

Similarly in Ethiopia, the Ethiopian Standard Authority states that only five food products have regulations primarily for AFs. Even further, only the regulation on beer is compulsory, and not the case for the rest of the four food items [147]. Besides, most of the agencies working on food safety in Ethiopia do not usually perceive mycotoxins contamination of foods as one of the parameters that should be considered in food inspection and certification criteria [178].

1.6.4 Other contributors

Lack of awareness intensified the problem of mycotoxins in Africa besides the poor pre- and post-harvest management, climate change, and absence of proper legislation. The majority of the populations in Africa have a limited understanding of the negative health implications of consuming mycotoxin-contaminated foods [165, 178, 186]; even if an increase in health problems related to mycotoxins i.e. liver cancer, esophageal cancer, neural tube disorders, stunted growth, and other health problems have been noticed [179]. In Africa, platforms for public information about mycotoxins are inadequate which contributes to poor awareness among consumers [25], and even among government officials [187]. For instance, in Tanzania and South Africa, farmers demonstrated poor knowledge about the animal and human health risks associated with mycotoxins. Another study in South Africa indicated that moldy grain is not discarded but used for making traditional beer which could be also another incident that shows the poor understanding of the African community towards mycotoxins [188, 189]. In the same way, a study found that the risks associated with moldy food consumption are not well

understood by many Ethiopians. This is supported by reports that moldy cereals are quite used either for feed or to make local drinks in Ethiopia [190, 191].

Additionally, food insecurity [192] and an undiversified diet [123] are among the driving factors for the increased incidence of exposure and consequent effects of mycotoxin among the African population. As household food insecurity predisposes to an undiversified diet, the food system in these communities is highly dependent on a few food crops that are usually prone to fungi growth. It is also possible that the problem of mycotoxins in Africa might be aggravated by the widespread undernutrition which impairs the biochemical detoxification capacity of the body [185].

Ethiopia experiences the highest food insecurity in the world [158]. A very recent prediction shows that in 2023 Ethiopia will commence one of the world's worst humanitarian crises [193]. The diet of the Ethiopian population has also known to be monotonous that mostly comprise staple foods that are prone to mycotoxins contamination, such as maize [147, 167, 176]. Malnutrition is also another critical health challenge in Ethiopia. Among children, the recent national report shows 37% are stunted, 7% are wasted and 21% are underweight [194]. The underlying food insecurity and undiversified diets compounded by the prevalent malnutrition might have interlinked with the mycotoxins exposure in Ethiopia.

Low political commitment, shortage of trained personnel, and infrastructure for mycotoxin research might also explain why mycotoxins are disproportionally prevalent in Africa [192]. A similar scenario exists in Ethiopia where mycotoxin research has limitations in terms of a lack of enabling policy environment, laboratory, personnel, and institutional capacity [195], and also the challenge in purchasing mycotoxin standards by researchers as they are not often readily available.

Moreover, in LMICs, there is no marketing system that benefits and encourages farmers/traders to produce/sell quality crops [48]. In many LMICs including East African Community countries, export standard grains are expected to fit quality grades, however, the grains left and consumed in-country are undesirable ones. This has led to higher consumption of contaminated and low-quality foods by the local population [25]. Therefore, the non-vigilant food safety bodies [36] coupled with the poor economy that is not able to capacitate the mycotoxin monitoring and mitigation system [196] might further contribute to the already multi-causal and inter-related mycotoxin problems observed in SSA [36] and other LMICs. **Figure 1.7** summarizes the main contributors to mycotoxin occurrence in Africa and Ethiopia.

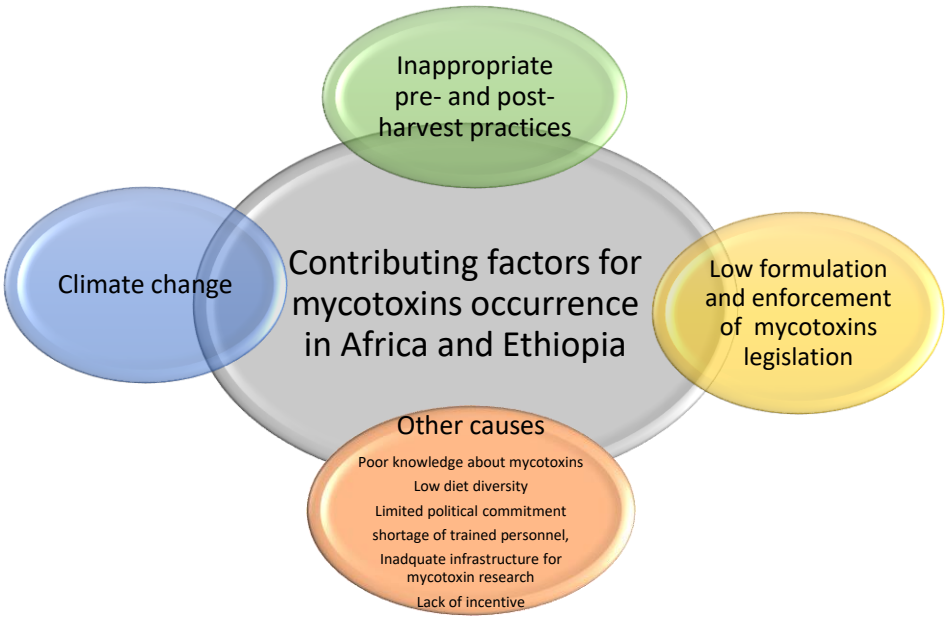


Figure 1.7 Major contributors to mycotoxin occurrence in Africa and Ethiopia

1.7 Multi-mycotoxins analytical methods

To be universally acceptable, mycotoxins contamination data is suggested to come from laboratory analysis methods that are validated along with accreditation and participation in proficiency testing [197]. Basically, conventional and rapid screening methods are the two major categories of analytical methods used for determining mycotoxins. The conventional methods include liquid chromatography (LC), thin-layer chromatography, and gas chromatography coupled to a detection system: mainly fluorescence detection or mass spectrometry (MS). The rapid screening methods comprise immunochemical techniques, such as enzyme-linked immunosorbent assays (ELISA) and lateral flow immunoassay (LFIA) which are more simple than the complex immunosensors/biosensor techniques [198].

The performance of the mycotoxin analytical methods is determined essentially by the ability to detect very low concentrations which are expressed in terms of the limit of quantification (LOQ) and limit of detection (LOD). LOQ is “the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy”. LOD is “the lowest quantity of a substance that can be distinguished from the absence of that substance (blank value) within a stated confidence limit” [199]. In addition, due to the possibility of mycotoxins co-occurring in a single sample, there is a need for analytical techniques that are able to detect different mycotoxins at a time [177]. In light of this, HPLC linked with MS or multiple detectors has got better acceptance as it is a confirmatory method that can identify various types of mycotoxins

in a single analysis [177, 200]. The recent version i.e. ultra performance liquid chromatography (UPLC) is becoming a preferable method for the determination of multiple mycotoxins than the traditional HPLC due to its enhanced chromatographic resolution and peaks, better sensitivity, and capacity of analyzing multiple mycotoxins in a single run within a short time. Coupling LC with MS/MS can also highly increase the analytical specificity, reliability, and ultimately the detection capacity as analytes are identified at the minimum by two attributes: their precursor and product ion mass. As a result, the LC-MS/MS approach has been widely in use nowadays [201, 202]. **(Figure 1.8)**

A wide range of methods exist for mycotoxin analysis using LC-MS/MS in food, feed, and biological samples. Extraction and clean-up are the major steps followed in sample preparation for mycotoxin analysis. These steps are very crucial as they greatly affect the recovery of specific compounds which ultimately define the overall accuracy of the chromatographic method applied [203]. Mycotoxins are extracted using polar solvents such as methanol, acetone, acetonitrile, ethyl acetate, diethyl ether, dichloromethane, chloroform, or a combination of these solvents with the addition of only a small quantity of water and acid solution [204]. As a result, different ratios of methanol-water and acetonitrile-water mixtures are the most widely used polar solvents [202]. Depending on the lipid profile of the samples, non-polar solvents such as hexane can also be applied either before or after extraction to eliminate the lipid and decrease their matrix effects [204]. Besides the solvents, solid phase extraction (SPE) is most commonly used to extract and clean up mycotoxins. There are different types of SPE columns. The conventional SPE columns include anionic exchange (SAX) column, NH2-SPE column, and C18-SPE column. There are also special SPE columns such as TC-M160 column, mycosep 228 Aflapat multifunctional column, Oasis HLBTM SPE cartridges and, TC-M160 column. This approach also functions using antibody-containing immune affinity columns that can retain and purify target mycotoxins. Since the immune affinity columns are expensive, an alternative cheap, simple, and quick cleaning-up method that has been in use for the past several years is the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method [202, 205].

While LC methods are highly sensitive to detect mycotoxins, the equipment is very expensive. Aside from the cost of the equipment, there are also costs associated with sample preparation, analytical standards, and training of staff performing the analysis. As a result, these methods are most frequently used in the developed world and make them rarely accessible to developing nations [9]. Another challenge with regard to LC methods is the possibility of analytical problems that may arise from the multiple mycotoxins analysis. Results from multi-mycotoxins

analysis may be impacted by the possibility of matrix carry-over that arises from the simultaneous extraction of different analytes that are known to have varying polarities by their nature [206]. Moreover, a constant challenge in the LC-MS/MS analysis of modified mycotoxins exists since these mycotoxins are chemically diverse with a lot of isomeric forms in which specific analytical standards for the determination of these mycotoxins usually lack [200]. Application of a variety of mycotoxin analysis techniques including thin layer chromatography, enzyme-linked immunosorbent assays, lateral flow immunoassay, HPLC, LC-MS, LC-MS/MS, and UPLC were reported from Ethiopia. Nevertheless, it should be noted that most of the latest mycotoxin analyses reported by different scholars had not been conducted by laboratories existing in Ethiopia, but instead carried out through partnerships with researchers and institutions from developed nations. This indicates that there is a huge demand for accredited and qualified laboratories in Ethiopia which can positively contribute to the overall food safety monitoring system, and improve the export system and the country's economic status at large [178].

Figure 1.8 Waters Premier (left) and Xevo (right) liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) instruments

1.8 Current status of mycotoxin data harmonization and standardization

Data on mycotoxin contamination from various food sources and geographical areas are available. Meanwhile, there is a growing interest in quality mycotoxin data to get a clear insight into mycotoxins exposure [207].

The mycotoxin contamination data reported so far show a major gap in terms of sampling. Sampling is the most underestimated yet one of the most crucial components of different

interconnected food-related activities [208]. At large, incorrect and unrepresentative sampling makes the estimation of the mycotoxins risk distribution at regional, national, and global levels doubtful [209]. Sampling errors appear to outweigh errors that derive during sample preparation and laboratory analysis. This is usually the case in sampling from lots for inspection purposes which leads to much doubt about the mycotoxin level reported from lots. If samples are inadequate, the mean concentration of positive samples can be greatly influenced by the analytical detection capacity (LOD) which eventually leads to an overestimation of the risk from the foods consumed [210]. A reliable sampling technique is more essential specifically in the case of AFs as their distribution is highly heterogeneous especially if the sampling is done from food items with larger sizes [135]. Similarly, a more cautious way of sampling is expected in case of stored food items due to the heterogeneous distribution of mycotoxins in storage [211]. Essentially, bigger sample sizes are essential for commodities with larger particles or samples with heterogeneous nature [212]. Critical elements which go hand in hand with sampling for obtaining trustworthy analytical data are method performance and measurement uncertainty [213].

Organizations such as EFSA and WHO pool food contaminants data. Food safety authorities within the European Union and research organizations are the major entities that submit mycotoxins occurrence data in food and feed to EFSA [214]. The Global Environment Monitoring System/Food Contamination Monitoring and Assessment Program, also known as GEMS/Food, collects information on food contamination from various countries of the world and makes it available for synthesis and review by the global community. However, most of the generated mycotoxin data are scattered, especially data from LMICs are limited and highly fragmented. Besides, substantial data that are generated every time by the privately owned food and feed companies were either not published nor submitted to international organizations such as WHO and EFSA [209, 214]. A great variation in terms of the types of mycotoxins analyzed, the analytical methods employed, and the approach used to report the findings were also seen in the available data. Moreover, the use of the existing data may sometimes be challenging as some of the information included in the datasets might not be complete [26]. This is attributable to the non-harmonized sampling or analytical techniques employed by the majority of mycotoxin surveys which makes the comparison between databases and trustworthy mapping of mycotoxin risks very challenging [209].

For instance, uncertainties have been reflected by Eskola *et al*. on the FAO world estimation of mycotoxin contamination prevalence in crops. The uncertainties in the prevalence estimation

originated from not considering the various mycotoxin maximum limits set in different counties, the variability in the LODs/LOQs reported, or due to lack of clarity on how the mycotoxin incident was reported (>LOD or >LOQ). Besides, mycotoxins contamination data collected by WHO from developing nations are scarce as seen in the Joint FAO/WHO Expert Committee on Food Additives (JECFA), IARC, or GEMS/Food databases, where the bulk of the data came from Europe, America, and the Western Pacific. As a result, different geographic regions were not adequately represented in the global mycotoxins estimation [26]. Similarly, uncertainties were also noted in food and human biomonitoring data with regard to mycotoxin exposure assessment. The gaps mentioned were lack of harmonized food consumption data [215]; uneven distribution of mycotoxins in foods; managing mycotoxins data below the LOD; inaccuracy around the mycotoxin analysis method used and differences within and between individuals in human biomonitoring studies [216]. Therefore, harmonization and validation of the sampling, and also analytical parameters are required for performance requirements to be fulfilled [13, 213], to enhance the suitability of a method for the intended purpose, and to balance differences across methods [213].

When files are not well formatted and archived, searching and extracting data would become problematic for both humans and electronic devices which causes inadequate access to metadata and databases. This creates difficulty in the evaluation, dissemination, and sharing of data by researchers [217]. An explicit description of each piece of data is essential for the proper usage of stored data that originate from many sources. Formulating proper data descriptors is thus one of the important steps that enhance machine readability and usability of data by researchers [218]. Above all, standardization of mycotoxins contamination data reporting is very crucial for keeping the uniformity of data, reducing data uncertainties, and enhancing data interoperability [219]. The applicability of standardized data can further permit comparability across different datasets, and large-scale analysis and gives the opportunity to create partnerships with other researchers [210]. In response, Findable, Accessible, Interoperable, and Reusable (FAIR), which is a set of community-approved principles, was developed and released in 2016 by a collaborative team of experts. Its main aim was to make data easy to explore, access, integrate, cite, and reuse. The significance of FAIR principles is much higher if applied at the very early stage of a research implementation process, resulting in enhanced external collaboration, optimize the value of data for publications, and increase the significance of research outcomes among the scientific community [217].

1.9 References

1. Unnevehr L.J. Food safety in food security and food trade. 10th edition". International Food Policy Research Institute Washington DC. 2003.

2. Pittet A. Natural occurrence of mycotoxins in foods and feeds. An update review. Rev. Med. Vet. 1998; 149 (6): 479–492.

3. Stein R.A. and Bulboacă A.E. Mycotoxins, in Foodborne diseases. 2017. 407-446.

4. Wu F. and Mitchell N.J. How climate change and regulations can affect the economics of mycotoxins. Special issue: Mycotoxins in a changing world. World Mycotoxin Journal. 2016; 9 (5): 653-663.

5. Venkatesh N. and Keller N.P. Mycotoxins in Conversation With Bacteria and Fungi. Front Microbiol. 2019; 10 403.

6. da Silva E.O., Bracarense A.P.F.L. and Oswald I.P. Mycotoxins and oxidative stress: where are we? World Mycotoxin Journal. 2018; 11 (1): 113-134.

7. Milićević D.R., Škrinjar M. and Baltić T. Real and perceived risks for mycotoxin contamination in foods and feeds: Challenges for food safety control. Toxins. 2010; 2 572-592.

8. Raduly Z., Szabo L., Madar A., Pocsi I. and Csernoch L. Toxicological and medical aspects of Aspergillus-derived mycotoxins entering the feed and food chain. Front Microbiol. 2019; 10 2908.

9. Battilani P., Palumbo R., Giorni P., Dall'Asta C., Dellafiora L., Gkrillas A., Toscano P., Crisci A., Brera C., De Santis B., Rosanna-Cammarano R., Della-Seta M., Campbell K., Elliot C., Venancio A., Lima N., Gonçalves A., Terciolo C. and Oswald I.P. Mycotoxin mixtures in food and feed: holistic, innovative, flexible risk assessment modelling approach. EFSA Supporting Publications. 2020; 17 (1).

10. Omotayo O.P., Omotayo A.O., Mwanza M. and Babalola O.O. Prevalence of mycotoxins and their consequences on human health. Toxicol Res. 2019; 35 (1): 1-7.

11. Logrieco A., Bottalico A., Mul´e G., Moretti A. and Perrone G. Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. European Journal of Plant Pathology. 2003; 109 645–667.

12. Dawlal P., Barros E. and Marais G.J. Evaluation of maize cultivars for their susceptibility towards mycotoxigenic fungi under storage conditions. Journal of Stored Products Research. 2012; 48 114-119.

13. Elkenany R.M. and Awad A. Types of mycotoxins and different approaches used for their detection in foodstuffs. Mansoura Veterinary Medical Journal. 2020; 21 (4): 25-32.

14. Bennett J.W. and Klich M. Mycotoxins. Clinical Microbiology Reviews. 2003; 497–516.

15. Mayer C.F. Endemic panmyelotoxicosis in the Russian grain belt. I. The clinical aspects of alimentary toxic aleukia (ATA); a comprehensive review Military Surgeon. 1953; 113 (3): 173-189.

16. Sargeant K., Sheridan A., O'kelly J. and Carnaghan R.B.A. Toxicity associated with certain samples of groundnuts. Nature. 1961; 192 1096–1097.

17. Wilson D.M. and Payne G.A. Factors affecting Aspergillus flavus group infection and aflatoxin contamination of crops, ed. L E.D. and D. G.J. 1994: Academic Press, San Diego, Cali.

18. Sydenham E.W., Shephard G.S., Thiel P.G., Marasas W.F. and Stockenstrom S. Fumonisin contamination of commercial corn-based human foodstuffs. J. Agric. Food Chem. 1991; 39 2014-2018.

19. Marroquin-Cardona A.G., Johnson N.M., Phillips T.D. and Hayes A.W. Mycotoxins in a changing global environment--a review. Food Chem Toxicol. 2014; 69 220-30.

20. Reddy K., Salleh B., Saad B., Abbas H.K., Abel C.A. and Shier W.T. An overview of mycotoxin contamination in foods and its implications for human health. Toxin Reviews. 2010; 29 (1): 3-26.

21. Gruber-Dorninger C., Jenkins T. and Schatzmayr G. Global mycotoxin occurrence in feed: A ten-year survey. Toxins (Basel). 2019; 11 (7).

22. Kovalsky P., Kos G., Nahrer K., Schwab C., Jenkins T., Schatzmayr G., Sulyok M. and Krska R. Co-occurrence of regulated, masked and emerging mycotoxins and secondary metabolites in finished feed and maize-an extensive survey. Toxins (Basel). 2016; 8 (12).

23. El-Sayed R., A., Jebur A., B., Kang W. and El-Demerdash F., M. An overview on the major mycotoxins in food products: characteristics, toxicity, and analysis. Journal of Future Foods. 2022; 2 (2): 91-102.

24. Wan J., Chen B. and Rao J. Occurrence and preventive strategies to control mycotoxins in cereal-based food. Compr Rev Food Sci Food Saf. 2020; 19 (3): 928-953.

25. Ankwasa E.M., Francis I. and Ahmad T. Update on mycotoxin contamination of maize and peanuts in East African Community Countries. Journal of Food Science and Nutrition Therapy. 2021; 7 (1).

26. Eskola M., Kos G., Elliott C.T., Hajslova J., Mayar S. and Krska R. Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25. Crit Rev Food Sci Nutr. 2020; 60 (16): 2773-2789.

27. Habschied K., Kanizai Saric G., Krstanovic V. and Mastanjevic K. Mycotoxins-biomonitoring and human exposure. Toxins (Basel). 2021; 13 (2).

28. Klaric S.M. Adverse effects of combined mycotoxins. Arh Hig Rada Toksikol. 2012; 63 (4): 519-30.

29. Cheli F., Giromini C. and Baldi A. Mycotoxin mechanisms of action and health impact: 'in vitro' or 'in vivo' tests, that is the question. World Mycotoxin Journal. 2015; 8 (5): 573-589.

30. Proietti M., Del Buono A., Pagliaro G., Del Buono R. and Di Rienzo C. The intestinal permeability syndrome, celiac disease, gluten sensitivity, autistic spectrum, mycotoxins and immunological tolerance. Mediterranean Journal of Nutrition and Metabolism. 2013; 6 (2): 99-104.

31. Sun Y., Huang K., Long M., Yang S. and Zhang Y. An update on immunotoxicity and mechanisms of action of six environmental mycotoxins. Food Chem Toxicol. 2022; 163 112895.

32. IARC. Mycotoxin control in low- and middle income countries, Wild C.P. ,Miller J.D. and Groopman J.D., Editors. 2015, IARC working group report no. 9.

33. Tesfamariam K., De Boevre M., Kolsteren P., Belachew T., Mesfin A., De Saeger S. and Lachat C. Dietary mycotoxins exposure and child growth, immune system, morbidity, and mortality: a systematic literature review. Crit Rev Food Sci Nutr. 2019; 60 (19): 3321-3341.

34. Gong Y.Y., Shirima C.P., Srey C., Kimanya M.E. and Routledge M.N. Deoxynivalenol and fumonisin exposure in children and adults in a family study in rural Tanzania. World Mycotoxin Journal. 2015; 8 (5): 553-560.

35. Cohen-Hubal E.A., Sheldon L.S., Burke J.M., McCurdy T.R., Berry M.R., Rigas M.L., Zartarian V.G. and Freema N.C.G. Children's exposure assessment: A review of factors influencing children's exposure, and the data available to characterize and assess that exposure. Environmental Health Perspectives. 2000; 1081 (6).

36. Chilaka C., A. and Mally A. Mycotoxin occurrence, exposure and health implications in infants and young children in Sub-Saharan Africa: A review. Foods. 2020; 9 (11).

37. Fink-Gremmels J. and Van der Merwe D. Mycotoxins in the food chain: contamination of foods of animal origin, in Chemical hazards in foods of animal origin. 2019. 241-261.

38. Samuel A.O.A. Fungal mycotoxins in foods: A review. Cogent Food & Agriculture. 2016; 2.

39. Mogopodi D., Mbisana M., Raditloko S., Chibua I. and Paphane B. Toward safe food systems: Analyses of mycotoxin contaminants in food and preventive strategies thereof for their formation and toxicity. IntechOpen. 2022.

40. Degraeve S., Madege R.R., Audenaert K., Kamala A., Ortiz J., Kimanya M., Tiisekwa B., De Meulenaer B. and Haesaert G. Impact of local pre-harvest management practices in maize on the occurrence of Fusarium species and associated mycotoxins in two agro-ecosystems in Tanzania. Food Control. 2016; 59 225-233.

41. Font G. and Ruiz M.J. Mechanism of mycotoxins. Food Chem Toxicol. 2019; 123 520-521.

42. Perrone G. and Gallo A. Aspergillus species and their associated mycotoxins, in Mycotoxigenic Fungi. 2017. 33-49.

43. Navale V., Vamkudoth K.R., Ajmera S. and Dhuri V. Aspergillus derived mycotoxins in food and the environment: Prevalence, detection, and toxicity. Toxicology Reports. 2021; 8 1008-1030.

44. Kagot V., De Boevre M., Landschoot S., Obiero G., Okoth S. and De Saeger S. Comprehensive analysis of multiple mycotoxins and Aspergillus flavus metabolites in maize from Kenyan households. International Journal of Food Microbiology. 2022.

45. Taniwaki M.H., Pitt J.I. and Magan N. Aspergillus species and mycotoxins: occurrence and importance in major food commodities. Current Opinion in Food Science. May 2018; 23 38-43.

46. Pitt I.J. and Hocking D.A. Fungi and food spoilage. 3rd ed. 2009: Springer.

47. Guchi E. Aflatoxin contamination in groundnut (Arachis hypogaea L.) caused by Aspergillus species in Ethiopia. Journal of Applied & Environmental Microbiology. 2015; 3 (1): 11-19.

48. IFPRI. Aflatoxins: Finding solutions for improved food safety, Grace L.U.a.D., Editor. 2013.

49. Peraica M., Radic B., Lucic A. and Pavlovic M. Diseases caused by molds in humans. Bulletins of the World Health Organization. 1999.

50. Negash D. A review of aflatoxin: occurrence, prevention, and gaps in both food and feed safety. Journal of Nutritional Health & Food Engineering. 2018; 8 (2).

51. Mitchell N.J., Hsu H.H., Chandyo R.K., Shrestha B., Bodhidatta L., Tu Y.K., Gong Y.Y., Egner P.A., Ulak M., Groopman J.D. and Wu F. Aflatoxin exposure during the first 36 months of life was not associated with impaired growth in Nepalese children: An extension of the MAL-ED study. PLoS One. 2017; 12 (2): e0172124.

52. Puri S., Shingh S. and Tiwari P. Mycotoxins: A threat to food security and health. International Journal of Applied Sciences and Biotechnology. 2019; 7 (3): 298-303.

53. Marin S., Ramos A.J., Cano-Sancho G. and Sanchis V. Mycotoxins: occurrence, toxicology, and exposure assessment. Food Chem Toxicol. 2013; 60 218-37.

54. Tola M., Kebede B. and Yildiz F. Occurrence, importance and control of mycotoxins: A review. Cogent Food & Agriculture. 2016; 2 (1).

55. Ostry V., Malir F. and Ruprich J. Producers and important dietary sources of ochratoxin A and citrinin. Toxins (Basel). 2013; 5 (9): 1574-86.

56. Harwig J., Kuiper-Goodman T. and Scott P.M. Microbial food toxicants: ochratoxins. In M. Reichcigl (Ed.), Handbook of foodborne diseases of biological origin 1983: Boca Raton, FL: CRC Press.

57. Zahra N., Jamil N., Ahmad S.R., Saeed M.K., Kalim I. and Sheikh A. A Review of mycotoxin types, occurrence, toxicity, Detection methods and control. Biological Sciences - PJSIR. 2019; 62 (3): 206-218.

58. El Khoury A. and Atoui A. Ochratoxin A: general overview and actual molecular status. Toxins (Basel). 2010; 2 (4): 461-93.

59. IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Some naturally occuring subsitances: food items and consitituties, hetrocyclic, aromatic amines and mycotoxins 1993; 56.

60. De Saeger S., Audenaert K. and Croubels S. Report from the 5th International symposium on mycotoxins and toxigenic moulds: Challenges and perspectives (MYTOX) held in Ghent, Belgium, May 2016. Toxins (Basel). 2016; 8 (5).

61. Schaarschmidt S. and Fauhl-Hassek C. The fate of mycotoxins during the primary food processing of maize. Food Control. 2021; 121.

62. Yazar S. and Omurtag G.Z. Fumonisins, trichothecenes and zearalenone in cereals. Int J Mol Sci. 2008; 9 (11): 2062-2090.

63. Weidenbörner M. Foods and fumonisins. Eur Food Res Technol 2001; 212 262–273.

64. JECFA. Mixed committee FAO/OMS experts in food additives. Fumonisins, in Report TRS 1002-JECFA 83/55. Tox Monograph FAS74-JECFA 83.WHO. 2016.

65. IARC Improving public health through mycotoxin control IARC Scientific Publication No 158. 2012.

66. IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC press. 2002.

67. Palumbo R., Crisci A., Venancio A., Cortinas-Abrahantes J., Dorne J.L., Battilani P. and Toscano P. Occurrence and co-occurrence of mycotoxins in cereal-based feed and food. Microorganisms. 2020; 8 (1).

68. Missmer S.A., Suarez L., Felkner M., Wang E., Merrill A.H., Rothman K.J. and KaHendricks K.A. Exposure to fumonisins and the occurrence of neural tube defects along the Texas–Mexico border. Environmental Health Perspectives. 2006; 114 (2).

69. Stevens V.L. and Tang J. Fumonisin B1-induced sphingolipid depletion inhibits vitamin uptake via the glycosylphosphatidylinositol-anchored folate receptor. Biological Chemistry. 1997; 272 (29): 18020–18025.

70. Shephard G.D., Westhuizen L.V.D., Gatyeni P.M., Katerere D.R. and Marasas W.F.O. Do fumonisin mycotoxins occur in wheat? Agric. Food Chem. 2005; 53 9293-9296.

71. EFSA. Opinion of the scientific panel on contaminants in food chain on a request from the commission related to fumonisins as undesirable substances in animal feed. The EFSA Journal. 2005.; 235 1 - 32.

72. Coppa C.F.S.C., Cirelli A.C., Gonçalves B.L., Barnab E.M.B., Petta T., Franco L.T., Javanmardi F., Khaneghah A.M., Lee S.H.I., Corassin C.H. and Oliveira C.A.F. Mycotoxin occurrence in breast milk and exposure estimation of lactating mothers using urinary biomarkers in Sao Paulo, Brazil. Environmental Pollution. 2021; 279.

73. Magoha H., Meulenaer B.D., Kimanya M., Hipolite D., Lachat C. and Kolsteren P. Fumonisin B1 contamination in breast milk and its exposure in infants under 6 months of age in Rombo, Northern Tanzania. Food and Chemical Toxicology. 2014; 74 112–116.

74. DeVries J.W., Trucksess M.W. and Jackson L.S., eds. Mycotoxins and food safety: Advances in experimental medicine and biology. Advances in Experimental Medicine and Biology Vol. 504. 2002. Pages.

75. Ekwomadu T.I., Akinola S.A. and Mwanza M. Fusarium mycotoxins, their metabolites (free, emerging, and masked), food safety concerns, and health impacts. Int J Environ Res Public Health. 2021; 18 (22).

76. EFSA. Risks for animal health related to the presence of zearalenone and its modified forms in feed. EFSA Journal. 2017; 15 (7): 4851.

77. Bulgaru C.V., Marin D.E., Pistol G.C. and Taranu I. Zearalenone and the Immune Response. Toxins (Basel). 2021; 13 (4).

78. Hussein H.S. and Brasel J.M. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology Reports. 2001; 167 (101–134).

79. EFSA. Risk to human and animal health related to the presence of 4,15-diacetoxyscirpenol in food and feed. EFSA journal. 2018; 16 (8): 5367.

80. Sudakin D.L. Trichothecenes in the environment: relevance to human health. Toxicology Letters 2003; 143 97-107.

81. Wu F., Groopman J.D. and Pestka J.J. Public health impacts of foodborne mycotoxins. Annu Rev Food Sci Technol. 2014; 5 351-72.

82. Creppy E.E. Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicology Letters. 2002; 127 19–28.

83. Wu Q., Qin Z., Kuca K., You L., Zhao Y., Liu A., Musilek K., Chrienova Z., Nepovimova E., Oleksak P., Wu W. and Wang X. An update on T-2 toxin and its modified forms: metabolism, immunotoxicity mechanism, and human exposure assessment. Arch Toxicol. 2020; 94 (11): 3645-3669.

84. Omurtag G.Z., Tozan A., Sirkecioflu O., KumbaracÂ V. and Rollas S. Occurrence of diacetoxyscirpenol (anguidine) in processed cereals and pulses in Turkey by HPLC. Food Control 2007; 18 970–974.

85. Technology. C.f.A.S.a. Mycotoxins: Risks in plant, animal, and human systems: task force report No. 139, Bruegel P., Editor. 2003.

86. Liao Y., Peng Z., Chen L., Nussler A.K., Liu L. and Yang W. Deoxynivalenol, gut microbiota and immunotoxicity: A potential approach? Food Chem Toxicol. 2018; 112 342-354.

87. Fang J., Dan H., Olaniran A.O., Mokoena M.P., Jianhong X. and Jianrong S. Occurrence, toxicity, production and detection of Fusarium mycotoxin: a review. Food Production, Processing and Nutrition. 2019; 1 (1).

88. Chilaka C.A., De Boevre M., Atanda O.O. and De Saeger S. The status of Fusarium mycotoxins in Sub-Saharan Africa: A review of emerging trends and post-harvest mitigation strategies towards food control. Toxins (Basel). 2017; 9 (1).

89. Frisvad J.C., Thrane U., Samson R.A. and Pitt I.J. Important mycotoxins and the fungi which produce them. Adv Exp Med Biol. 2006; 571 3–31.

90. IARC. IARC working group on the evaluation of the carcinogenic risk of chemicals to humans: some naturally occurring and synthetic food components. International Agency for Research on Cancer. 1986; 421.

91. WHO. Environmental Health Criteria 11: Mycotoxins. International Programme on Chemical Safety. 1979.

92. WHO/IARC IARC monographs on th evaluation of the carcinogenic risk of chemicals to humans: some naturally occuring and synthetic food components furocoumarins and ultraviolet radiation 1986; 40.

93. Samson R.A., Houbraken J., Varga J. and Frisvad J.C. Polyphasic taxonomy of the heat resistant ascomycete genus Byssochlamys and its Paecilomyces anamorphs. Persoonia. 2009; 22 14–27.

94. WHO. Mycotoxins. 2018. Available from: https://www.who.int/es/news-room/fact-sheets/detail/mycotoxins.

95. Vaclavikova M., Malachova A., Veprikova Z., Dzuman Z., Zachariasova M. and Hajslova J. Emerging' mycotoxins in cereals processing chains: Changes of enniatins during beer and bread making. Food Chemistry 2013; 136 750–757.

96. Sy-Cordero A.A., Pearce C.J. and Oberlies N.H. Revisiting the enniatins: a review of their isolation, biosynthesis, structure determination and biological activities. The Journal of Antibiotics 2012; 65 (541–549).

97. Gruber-Dorninger C., Novak B., Nagl V. and Berthiller F. Emerging mycotoxins: Beyond traditionally determined food contaminants. Journal of Agricultural and Food Chemistry. 2016; 65 7052−7070.

98. EFSA. Scientific opinion on the risks to human and animal health related to the presence of beauvericin and enniatins in food and feed: EFSA Panel on Contaminants in the Food Chain (CONTAM). EFSA Journal 2014; 12 (8): 3802.

99. EFSA. Scientific opinion on the risk for public and animal health related to the presence of sterigmatocystin in food and feed. EFSA Journal. 2013; 11 (6).

100. Rychlik M., Humpf H.U., Marko D., Danicke S., Mally A., Berthiller F., Klaffke H. and Lorenz N. Proposal of a comprehensive definition of modified and other forms of mycotoxins including "masked" mycotoxins. Mycotoxin Res. 2014; 30 (4): 197-205.

101. Rai M. and Varma A., eds. Mycotoxins in food, feed and bioweapons. 2010, Springer. Pages.

102. Marcin-Bryła M., Wa´skiewicz A., Ksieniewicz-Wo´zniak E., Szymczyk K. and J¸edrzejczak R. Modified Fusarium mycotoxins in cereals and their products—metabolism, occurrence, and toxicity: An updated review. Molecules. 2018; 23 963.

103. Lorenz N., Danicke S., Edler L., Gottschalk C., Lassek E., Marko D., Rychlik M. and Mally A. A critical evaluation of health risk assessment of modified mycotoxins with a special focus on zearalenone. Mycotoxin Res. 2019; 35 (1): 27-46.

104. EFSA OpenFoodTox: EFSA's chemical hazards database. Version 5, Kovarich S. ,Ciacci A. ,Baldin R. ,Roncaglioni A. ,Mostrag A. ,Tarkhov A. ,Carnesecchi E. ,Gibin D. ,Di Piazza G. ,Pasinato L. ,Sartori L. ,Benfenati E. ,Yang C. ,Richardson J. and Dorne J.L., Editors. 2022.

105. Grenier B. and Oswald I. Mycotoxin co-contamination of food and feed: meta-analysis of publications describing toxicological interactions. World Mycotoxin Journal. 2011; 4 (3): 285-313.

106. Shephard G.S., Berthiller F., Burdaspal P.A., Crews C., Jonker M.A., Krska R., Lattanzio V.M.T., MacDonald S., Malone R.J., Maragos C., Sabino M., Solfrizzo M., van Egmond H.P. and Whitaker T.B. Developments in mycotoxin analysis: an update for 2011-2012. World Mycotoxin Journal. 2013; 6 (1): 3-30.

107. Steinkellner H., Binaglia M., Dall'Asta C., Gutleb A.C., Metzler M., Oswald I.P., Parent-Massin D. and Alexander J. Combined hazard assessment of mycotoxins and their modified forms applying relative potency factors: Zearalenone and T2/HT2 toxin. Food Chem Toxicol. 2019; 131 110599.

108. Fernández-Blanco C., Elmo L., Waldner T. and Ruiz M. Cytotoxic effects induced by patulin, deoxynivalenol and toxin T2 individually and in combination in hepatic cells (HepG2). Food and Chemical Toxicology 2018; 120 12-23.

109. Arce-Lopez B., Lizarraga E., Vettorazzi A. and Gonzalez-Penas E. Human biomonitoring of mycotoxins in blood, plasma and serum in recent years: A review. Toxins (Basel). 2020; 12 (3).

110. Klarić M.S., Rašić D. and Peraica M. Deleterious effects of mycotoxin combinations involving ochratoxin A. Toxins. 2013; 5 1965-1987.

111. Turner P.C. and Snyder J.A. Development and limitations of exposure biomarkers to dietary contaminants mycotoxins. Toxins (Basel). 2021; 13 (5).

112. Atkinson A.J. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clinical Pharmacology Therapeutics. 2001; 69 (3): 89-95.

113. De Nijs M., Mengelers M.J.B., Boon P.E., Heyndrickx E., Hoogenboom L.A.P., Lopez P. and Mol H.G.J. Strategies for estimating human exposure to mycotoxins via food. World Mycotoxin Journal. 2016; 9 (5): 831-845.

114. Vidal A., Mengelers M., Yang S., De Saeger S. and De Boevre M. Mycotoxin biomarkers of exposure: A comprehensive review. Comprehensive Reviews in Food Science and Food Safety. 2018; 0.

115. Dragsted L.O., Gao Q., Scalbert A., Vergères G., Kolehmainen M., Manach C., Brennan L., Afman L.A., Wishart D.S., Andres L.C., Garcia-Aloy M., Verhagen H., Feskens E.J.

and Praticò G. Validation of biomarkers of food intake: critical assessment of candidate biomarkers. Genes & Nutrition. 2018; 13 (14).

116. Solfrizzo M., Gambacorta L., Lattanzio V.M., Powers S. and Visconti A. Simultaneous LC-MS/MS determination of aflatoxin M1, ochratoxin A, deoxynivalenol, de-epoxydeoxynivalenol, alpha and beta-zearalenols and fumonisin B1 in urine as a multi-biomarker method to assess exposure to mycotoxins. Anal Bioanal Chem. 2011; 401 (9): 2831-41.

117. Slobodchikova I. and Vuckovic D. Liquid chromatography – high resolution mass spectrometry methodfor monitoring of 17 mycotoxins in human plasma for exposurestudies. Journal of Chromatography A. 2018; 51–63.

118. Duarte S.C., Pena A. and Lino C.M. Human ochratoxin A biomarkers: From exposure to effect. Critical Reviews in Toxicology. 2011; 41 (3): 187-212.

119. Warth B., Braun D., Ezekiel C.N., Turner P.C., Degen G.H. and Marko D. Biomonitoring of Mycotoxins in Human Breast Milk: Current State and Future Perspectives. Chem Res Toxicol. 2016; 29 (7): 1087-97.

120. Renfrewa M.J., Hayb A.M.W., Sheltonf N., Lawc G., Wallise S., Maddene S., Shiresb S., Sutcliffeb A. and Woolridged M.W. Assessing levels of contaminants in breast milk: methodological issues and a framework for future research. Paediatric and Perinatal Epidemiology. 2008; 22 72–86.

121. Lombard M.J. Mycotoxin exposure and infant and young child growth in Africa: what do we know? Ann Nutr Metab. 2014; 64 Suppl 2 42-52.

122. Ropejko K. and Twarużek M. The occurrence of ochratoxin A in human body fluids – review. Toxin Reviews. 2019; 40 (3): 347-360.

123. Wu F., Mitchell N.J., Male D. and Kensler T.W. Reduced foodborne toxin exposure is a benefit of improving dietary diversity. Toxicol Sci. 2014; 141 (2): 329-34.

124. Wild C.P. Aflatoxin exposure in developing countries: The critical interface of agriculture and health. Food and Nutrition Bulletin. 2007; 28 (2).

125. Gbashi S., Edwin Madala N., De Saeger S., De Boevre M., Adekoya I., Ayodeji Adebo O. and Berka Njobeh P. The socio-economic impact of mycotoxin contamination in Africa, in Mycotoxins - Impact and Management Strategies. 2018.

126. Darwish W.S., Ikenaka Y., Nakayama S.M. and Ishizuka M. An overview on mycotoxin contamination of foods in Africa. J Vet Med Sci. 2014; 76 (6): 789-97.

127. IARC. Mycotoxin control in low- and middle-income countries, in IARC Working Group Report No. 9, Wild C.P. ,David M.J. and Groopman J.D., Editors. 2016, World Health Organization/International Agency for Research on Cancer, Geneva, Switzerland.

128. Ingenbleek L., Sulyok M., Adegboye A., Hossou S.E., Kone A.Z., Oyedele A.D., Kisito C., Dembele Y.K., Eyangoh S., Verger P., Leblanc J.C., Le Bizec B. and Krska R. Regional Sub-Saharan Africa Total Diet Study in Benin, Cameroon, Mali and Nigeria reveals the presence of 164 mycotoxins and other secondary metabolites in foods. Toxins (Basel). 2019; 11 (1).

129. Benkerroum N. Aflatoxins: Producing-molds, structure, health issues and incidence in Southeast Asian and Sub-Saharan African Countries. Int J Environ Res Public Health. 2020; 17 (4).

130. Gong Y.Y., Van-Rensburg B.J., Kimanya M. and Van-Egmond H.P. Foreword WMJ special issue 'Mycotoxins in Africa'. World Mycotoxin Journal. 2018; 11 (3): 305-309.

131. Williams J.H., Phillips T.D., Jolly P.E., Stiles J.K., Jolly C.M. and Aggarwal D. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am J Clin Nutr. 2004; 80 (5): 1106-22.

132. Kimanya M.E. The health impacts of mycotoxins in the eastern Africa region. Current Opinion in Food Science. 2015; 6 7-11.

133. Lewis L., Onsongo M., Njapau H., Schurz-Rogers H., Luber G., Kieszak S., Nyamongo J., Backer L., Dahiye A.M., Misore A., DeCock K. and Rubin C. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. Environ Health Perspect. 2005; 113 (12): 1763-7.

134. Kang'ethe E. Situation analysis: improving food safety in the maize value chain in Kenya, Kang'ethe E., Editor. September 2011, FAO.

135. Gong Y.Y., Routledge M., Kimanya M.E., Musoke G., Nelson F., Sonoiya S. and Manyong V. Building an aflatoxin safe East African Community: Aflatoxin standards for food: Technical Policy Paper 8, in Knowledge Platform 2015 Situational Analysis East Africa Region, USAID, Editor. 2015.

136. Liu Y. and Wu F. Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA. 2010; 118 (6).

137. Lukwago F.B., Mukisa I.M., Atukwase A., Kaaya A.N. and Tumwebaze S. Mycotoxins contamination in foods consumed in Uganda: A 12-year review (2006–18). Scientific African. 2019; 3.

138. Kedera C.J., Plattner R.D. and Desjardins A.E. Incidence of Fusarium spp. and levels of fumonisin B1 in maize in Western Kenya. Applied And Environmental Microbiology. 1999; 65 41-44.

139. Gong Y.Y., Turner P.C., Hall A.J. and Wild C.P. Aflatoxin exposure and impaired child growth in West Africa: An unexplored international public health burden?, in Mycotoxins

Detection Methods, Management, Public Health and Agricultural Trade. , Leslie J.F., Editor. 2008. 53–66.

140. Jonsyn F.E., Maxwell S.M. and Hendrickse R.G. Ochratoxin A and aflatoxins in breast milk samples from Sierra Leone. Mycopathologia. 1995; 131 (121-126).

141. Shirima C.P., Kimanya M.E., Routledge M.N., Srey C., Kinabo J.L., Humpf H.U., Wild C.P., Tu Y.K. and Gong Y.Y. A prospective study of growth and biomarkers of exposure to aflatoxin and fumonisin during early childhood in Tanzania. Environ Health Perspect. 2015; 123 (2): 173-8.

142. James A. and Zikankuba V.L. Mycotoxins contamination in maize alarms food safety in sub-Sahara Africa. Food Control. 2018; 90 372-381.

143. Turner P.C., Collinson A.C., Cheung Y.B., Gong Y.Y., Hall A.J., Prentice A.M. and Wild C.P. Aflatoxin exposure in utero causes growth faltering in Gambian infants. International Journal of Epidemiology. 2007; 36 1119–1125.

144. Kebede H., Liu X., Jin J. and Xing F. Current status of major mycotoxins contamination in food and feed in Africa. Food Control. 2020; 110.

145. CSA. Agricultural sample survey in 2017/2018. Report on area and production of major crops. Addis Ababa, Ethiopia. 2018.

146. Ezekiel C.N., Ayeni K.I., Misihairabgwi J.M., Somorin Y.M., Chibuzor-Onyema I.E., Oyedele O.A., Abia W.A., Sulyok M., Shephard G.S. and Krska R. Traditionally processed beverages in Africa: A review of the mycotoxin occurrence patterns and exposure assessment. Compr Rev Food Sci Food Saf. 2018; 17 (2): 334-351.

147. Ayelign A. and De Saeger S. Mycotoxins in Ethiopia: Current status, implications to food safety and mitigation strategies. Food Control. 2020; 113.

148. Fufa H. and Urga K. Screening of aflatoxins in shiro and ground red pepper in Addis Ababa. Ethiopian Medical Journal. 1996; 34 (4): 243-249.

149. Eshete M., Gebremedhin S., Alemayehu F.R., Taye M., Boshe B. and Stoecker B.J. Aflatoxin contamination of human breast milk and complementary foods in southern Ethiopia. Maternal & Child Nutrition. 2020; 17 (1).

150. Ayelign A., Woldegiorgis A.Z., Adish A., De Boevre M., Heyndrickx E. and De Saeger S. Assessment of aflatoxin exposure among young children in Ethiopia using urinary biomarkers. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2017; 34 (9): 1606-1616.

151. Tessema M., De Groote H., Brouwer I.D., De Boevre M., Corominas A.V., Stoecker B.J., Feskens E.J., Belachew T., Karakitsou A. and Gunaratna N.S. Exposure to aflatoxins and

fumonisins and linear growth of children in rural Ethiopia: a longitudinal study. Public Health Nutr. 2021; 24 (12): 3662-3673.

152. Tesfamariam K., Argaw A., Hanley-Cook G.T., Gebreyesus S.H., Kolsteren P., Belachew T., De Velde M.V., De Saeger S., De Boevre M. and Lachat C. Multiple mycotoxin exposure during pregnancy and risks of adverse birth outcomes: a prospective cohort study in rural Ethiopia. environment international. 2022; 160.

153. Boshe B., Gebremedhin S., Alemayehu F., Eshete M., aye M. and Stoecker B.J. Aflatoxin exposure among lactating women in southern Ethiopia. Food Science & Nutrition. 2020; 8 (12).

154. Awedew A.F., Asefa Z. and Belay W.B. National Burden and Trend of Cancer in Ethiopia, 2010-2019: a systemic analysis for Global burden of disease study. Sci Rep. 2022; 12 (1): 12736.

155. Jiang Y., Lin Y., Wen Y., Fu W., Wang R., He J., Zhang J., Wang Z., Ge F., Huo Z., Wang R., Peng H., Wu X., He J. and Li S. Global trends in the burden of esophageal cancer, 1990-2019: results from the Global Burden of Disease Study 2019. J Thorac Dis. 2023; 15 (2): 348-364.

156. Yilma S., Sadessa K. and Kebede D. Fungal Infections and Aflatoxin Contamination in Maize Grains Collected from West Showa and East Wallega Zones, Ethiopia. International Journal of Current Research and Review. 2019; 11 (21): 16-22.

157. Mohammed A., Chala A., Dejene M., Fininsa C., Hoisington D.A., Sobolev V.S. and Arias R.S. Aspergillus and aflatoxin in groundnut (Arachis hypogaea L.) and groundnut cake in Eastern Ethiopia. Food Addit Contam Part B Surveill. 2016; 9 (4): 290-298.

158. Ayele T. A review of the trends and causes of food insecurity in Ethiopia. Food Science and Quality Management. 2020; 99.

159. Daou R., Joubrane K., Maroun R.G., Khabbaz L.R., Ali Ismail A. and Khoury A.E. Mycotoxins: Factors influencing production and control strategies. AIMS Agriculture and Food. 2021; 6 (1): 416–447.

160. Ndemera M., Landschoot S., De Boevre M., Nyanga L.K. and De Saeger S. Effect of agronomic practices and weather conditions on mycotoxins in maize: a case study of subsistence farming households in Zimbabwe. World Mycotoxin Journal. 2018; 11 (3): 421-436.

161. Xu F., Baker R.C., Whitaker T.B., Luo H., Zhao Y., Stevenson A., Boesch C.J. and Zhang G. Review of good agricultural practices for smallholder maize farmers to minimise aflatoxin contamination. World Mycotoxin Journal. 2022; 15 (2): 171-186.

162. Neme K. and Mohammed A. Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategies. A review. Food Control. 2017; 78 412-425.

163. Kamala A., Kimanya M., Haesaert G., Tiisekwa B., Madege R., Degraeve S., Cyprian C. and De Meulenaer B. Local post-harvest practices associated with aflatoxin and fumonisin contamination of maize in three agro ecological zones of Tanzania. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2016; 33 (3): 551-9.

164. Waliyar F., Osiru M., Ntare B.R., Kumar K.V.K., Sudini H., Traore A. and Diarra B. Post-harvest management of aflatoxin contamination in groundnut. World Mycotoxin Journal. 2015; 8 (2): 245-252.

165. Fundikira S.S., De Saeger S., Kimanya M.E. and Mugula J.K. Awareness, handling and storage factors associated with aflatoxin contamination in spices marketed in Dar es Salaam, Tanzania. World Mycotoxin Journal. 2021; 14 (2): 191-200.

166. Signaboubo S., Christelle M. and Naoura G. Identification ofpost-Hharvest operations related to the contamination of Arachis hypogaea L. (Groundnut) by Mycotoxins in the Province of Mayo Kebbi Ouest in the Republic of Chad. Agricultural Sciences. 2021; 12 (04): 406-413.

167. Worku A.F., Merkuz A., Kalsa K.K., Tenagashaw M.W. and Habtu N.G. Occurrence of mycotoxins in farm-stored wheat in Ethiopia. African Journal of Food Agriculture Nutrition and Development. 2019; 19 (4): 14829-14847.

168. Fanta A. and Tesafa F. Aflatoxin related challenges and mitigation strategies of Ethiopia in spices, herbs and pulses' domestic and international markets. August 2018.

169. Taye W., Ayalew A., Chala A. and Dejene M. Aflatoxin B(1) and total fumonisin contamination and their producing fungi in fresh and stored sorghum grain in East Hararghe, Ethiopia. Food Addit Contam Part B Surveill. 2016; 9 (4): 237-245.

170. Wolde M. Effects of aflatoxin contamination of grains in Ethiopia. International Journal of Agricultural Sciences. 2017; 7 (4): 1298-1308.

171. Turner P.C., Sylla A., Gong Y.Y., Diallo M.S., Sutcliffe A.E., Hall A.J. and Wild C.P. Reduction in exposure to carcinogenic aflatoxins by postharvest intervention measures in west Africa: a community-based intervention study. Lancet. 2005; 365 1950–56.

172. Murashiki T.C., Chidewe C., Benhura M.A., Manema L.R., Mvumi B.M. and Nyanga L.K. Effectiveness of hermetic technologies in limiting aflatoxin B1 and fumonisin B1 contamination of stored maize grain under smallholder conditions in Zimbabwe. World Mycotoxin Journal. 2018; 11 (3): 459 - 469.

173. Walker S., Jaime R., Kagot V. and Probst C. Comparative effects of hermetic and traditional storage devices on maize grain: Mycotoxin development, insect infestation and grain quality. Journal of Stored Products Research. 2018; 77 34-44.

174. Dembedza M.P., Chidewe C., Benhura M.A., Mvumi B.M., Manema L.R. and Nyanga L.K. Effectiveness of hermetic maize grain storage technology in limiting aflatoxin

exposure in women and children from smallholder farming areas. World Mycotoxin Journal. 2019; 12 (3): 233-243.

175. Kamala A., Kimanya M., De Meulenaer B., Kolsteren P., Jacxsens L., Haesaert G., Kilango K., Magoha H., Tiisekwa B. and Lachat C. Post-harvest interventions decrease aflatoxin and fumonisin contamination in maize and subsequent dietary exposure in Tanzanian infants: a cluster randomised-controlled trial. World Mycotoxin Journal. 2018; 11 (3): 447-458.

176. Macauley H. Cereal crops: rice, maize, millet, sorghum, wheat: Background Paper. 2015.

177. Temesgen A. and Teshome G. Major mycotoxins occurrence, prevention and control approaches. Biotechnology and Molecular Biology Reviews. 2018; 12 (1): 1-11.

178. Mamo F.T., Abate B.A., Tesfaye K., Nie C., Wang G. and Liu Y. Mycotoxins in Ethiopia: A review on prevalence, economic and health impacts. Toxins (Basel). 2020; 12 (10).

179. Ladeira C., Frazzoli C. and Orisakwe O.E. Engaging one health for non-communicable diseases in Africa: Perspective for mycotoxins. Front Public Health. 2017; 5 266.

180. Warnatzsch E.A., Reay D.S., Camardo L.M. and Battilani P. Climate change impact on aflatoxin contamination risk in Malawi's maize crops. Frontiers in Sustainable Food Systems. 2020; 4.

181. Nji Q.N., Babalola O.O., Ekwomadu T.I., Nleya N. and Mwanza M. Six main contributing factors to high levels of mycotoxin contamination in African foods. Toxins (Basel). 2022; 14 (5).

182. Chhaya R.S., O'Brien J. and Cummins E. Feed to fork risk assessment of mycotoxins under climate change influences - recent developments. Trends in Food Science & Technology. 2022; 126 126-141.

183. FAO. Worldwide regulations for mycotoxins in food and feed in 2003, in Food and Nutrition Paper 81. 2004. 165.

184. Egmond H.V. and Jonker M. Worldwide and European regulations for mycotoxins & analytical methods expected from BioCop. Research for man and environment.National Institute for Public Health and the Environment. Brussels. 2004; 1-65.

185. Shephard G.S. Impact of mycotoxins on human health in developing countries. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2008; 25 (2): 146-51.

186. Bell V., Ferrão J. and Fernandes T.H. Nutrition, food safety and quality in sub-Saharan Africa. EC NUTRITION Review Article. July 11, 2017.

187. Wild C.P. and Gong Y.Y. Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis 2010; 31 (1): 71–82.

188. Suleiman R.A., Rosentrater K.A. and Chove B. Understanding postharvest practices, knowledge, and actual mycotoxin levels in maize in three agroecological zones in Tanzania. Journal of Stored Products and Postharvest Research. 2017; 8 (7).

189. Shephard G.S., Burger H.M., Gambacorta L., Krska R., Powers S.P., Rheeder J.P., Solfrizzo M., Sulyok M., Visconti A., Warth B. and Westhuizen L.V.D. Mycological analysis and multi-mycotoxins in maize from rural subsistence farmers in the former Transkei, South Africa. Agricultural and Food Chemistry. 2013.

190. Beyene A.A., Woldegiorgis A.Z., Adish A.A., De Saeger S. and Tolossa A.L. Assessment of mothers' knowledge and practice towards aflatoxin contamination in complementary foods in Ethiopia: from pre-harvest to household. World Mycotoxin Journal. 2016; 9 (4): 535-544.

191. Mesfin A., Tesfamariam K., Belachew T., De Saeger S., Lachat C. and De Boevre M. Multi-mycotoxin profiling in maize reveals prevalence of Fusarium mycotoxins in South and West Ethiopia. World Mycotoxin Journal. 2022; 15 (1): 73-83.

192. Hell K. and Mutegi C. Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. African Journal of Microbiology Research. 2011; 5 (5): 459-466.

193. WFP. Ethiopia food security outlook: June 2022 to January 2023. 2023.

194. EPHI and ICF. Ethiopia mini demographic and health survey 2019: Final report. 2021: Rockville, Maryland, USA.

195. PACA. Aflatoxin Challenge in Eastern and Southern Africa,. in improving health, trade and food security through regional efforts to mitigate aflatoxin contamination. 2014. Golden Peacock Hotel, Lilongwe, Malawi.

196. Kimatu J.N., McConchie R., Xie X. and Nguluu S.N. The significant role of post-harvest management in farm management, aflatoxin mitigation and food security in sub-Saharan Africa. Greener Journal of Agricultural Sciences. 2012; 2 (6): 279-288.

197. Gilbert J. Quality assurance in mycotoxin analysis. Food, nutrition and agriculture 1999.

198. Turner N.W., Bramhmbhatt H., Szabo-Vezse M., Poma A., Coker R. and Piletsky S.A. Analytical methods for determination of mycotoxins: An update (2009-2014). Analytica Chimica Acta. 2015; 901 12-33.

199. Neogen. Mycotoxin handbook. 2017 [cited 2020 July 01].

200. Lagana A. Introduction to the toxins special issue on LC-MS/MS methods for mycotoxin analysis. Toxins (Basel). 2017; 9 (10).

201. Patil U.S., King S., Holleran S., White K., Stephenson C. and Reuther J. Identifying challenges and risks associated with the analysis of major mycotoxins in feed and botanicals. AOAC International. 2019; 102 (6).

202. Zhang L., Dou X.W., Zhang C., Logrieco A.F. and Yang M.H. A review of current methods for analysis of mycotoxins in herbal medicines. Toxins (Basel). 2018; 10 (2).

203. Razzazi-Fazeli E. and Reiter E.V. Sample preparation and clean up in mycotoxin analysis: principles, applications and recent developments, in Determining Mycotoxins and Mycotoxigenic Fungi in Food and Feed. 2011. 37-70.

204. Rahmani A., Jinap S. and Soleimany F. Qualitative and quantitative analysis of mycotoxins. Comprehensive Reviews in Food Science and Food Safety. 2009; 8.

205. Arroyo-Manzanares N., Huertas-Pérez J.F., García-Campaña A.M. and Gámiz-Gracia L. Mycotoxin analysis: new proposals for sample treatment. Advances in Chemistry. 2014; 2014 1-12.

206. Stroka J. and Maragos C.M. Challenges in the analysis of multiple mycotoxins. World Mycotoxin Journal. 2016; 9 (5): 847-861.

207. Martins C., Assunção R., Nunes C., Torres D. and Alvito P. Are data from mycotoxins' urinary biomarkers and food surveys linked? A review underneath risk assessment. Food Reviews International. 2020; 37 (4): 373-398.

208. Miraglia M., De Santis B., Minardi V., Debegnach F. and Brera C. The role of sampling in mycotoxin contamination: an holistic view. Food Addit Contam. 2005; 22 Suppl 1 31-6.

209. Perrone G., Ferrara M., Medina A., Pascale M. and Magan N. Toxigenic fungi and mycotoxins in a climate change scenario: Ecology, genomics, distribution, prediction and prevention of the risk. Microorganisms. 2020; 8 (10).

210. Van-Egmond H.P., Schothorst R.C. and Jonker M.A. Regulations relating to mycotoxins in food: perspectives in a global and European context. Anal Bioanal Chem. 2007; 389 (1): 147-57.

211. Whitaker T. Sampling for mycotoxins. mycotoxins in food: Detection and control. 2004; 69-81.

212. Betina V. Chapter 1: sampling, sample preparation, extraction and clean-up, in Chromatography of mycotoxins - techniques and applications. 1993. 3-11.

213. Alldrick A.J., van Egmond H.P., Solfrizzo M., Ozer H., Gofflot S., Angelov A. and Gotcheva V. Towards harmonized approaches for mycotoxin analyses: an assessment. Quality Assurance and Safety of Crops & Foods. 2009; 1 (2): 76-85.

214. Eskola M., Altieri A. and Galobart J. Overview of the activities of the European Food Safety Authority on mycotoxins in food and feed. World Mycotoxin Journal. 2018; 11 (2): 277-289.

215. Assunção R., Silva M.J. and Alvito P. Challenges in risk assessment of multiple mycotoxins in food. World Mycotoxin Journal. 2016; 9 (5): 791-811.

216. Routledge M.N. and Gong Y.Y. Developing biomarkers of human exposure to mycotoxins, in Determining Mycotoxins and Mycotoxigenic Fungi in Food and Feed, Saeger S.D., Editor. 2011, Woodhead Publishing Series in Food Science, Technology and Nutrition. 415-427.

217. ZONTAL. FAIR Data: How data increase the value of Biotechs. 2020.

218. Wilkinson M.D., Dumontier M., Aalbersberg I.J., Appleton G., Axton M., Baak A., Blomberg N., Boiten J.W., da Silva Santos L.B., Bourne P.E., Bouwman J., Brookes A.J., Clark T., Crosas M., Dillo I., Dumon O., Edmunds S., Evelo C.T., Finkers R., Gonzalez-Beltran A., Gray A.J., Groth P., Goble C., Grethe J.S., Heringa J., t Hoen P.A., Hooft R., Kuhn T., Kok R., Kok J., Lusher S.J., Martone M.E., Mons A., Packer A.L., Persson B., Rocca-Serra P., Roos M., van Schaik R., Sansone S.A., Schultes E., Sengstag T., Slater T., Strawn G., Swertz M.A., Thompson M., van der Lei J., van Mulligen E., Velterop J., Waagmeester A., Wittenburg P., Wolstencroft K., Zhao J. and Mons B. The FAIR guiding principles for scientific data management and stewardship. Sci Data. 2016; 3 160018.

219. Gal M. and Rubinfeld D.L. Data Standardization. SSRN Electronic Journal. 2018.

Chapter 2. Rationale and objectives of the study

2. Rationale and objectives of the study

The need to build a system for ensuring the safety and quality of food throughout the food value chain is one part of the Ethiopian food and nutrition policy [1]. Furthermore, ensuring food safety besides increasing diet quality was also one of the objectives of the recent Ethiopian food-based dietary guideline [2]. However, as the majority of the staple cereals and other food crops that are consumed locally by the Ethiopian population are produced by local farmers through subsistence farming, there is no well-established system for ensuring the safety of the produces. Maize is a fundamental dietary staple in Ethiopian households especially in rural areas. It is an integral source of calorie and other nutrients such as vitamins, minerals and fiber for the rural poor [3]. However, because of the susceptibility of maize to various fungal species, this pose a threat to the crop with the capacity to generate mycotoxins [4].

Understanding the sources and health risks of mycotoxin contamination and exposure are crucial to prioritize and implement effective interventions in resource limited settings like Ethiopia. Yet, the burden of mycotoxins has been largely overlooked in Ethiopia as more attention is given to microbial and other food safety hazards that usually cause acute problems. Therefore, foods continue to be sources of exposure for mycotoxins and other contaminants for the general population including pregnant and lactating mothers, and children in Ethiopia.

Breast milk is an ideal food in start of an infant life due to its unique composition and immunological benefits. Breastfeeding is a culturally established significant practice and serves as an economical infant food in Ethiopia. However, mycotoxins from contaminated foods can be transferred to breast milk during lactation period [5].

The available studies in Ethiopia seem to focus on AFs in staple crops. The lack of data is much more prominent in mycotoxins biomonitoring such as breastmilk. Studies that cover the occurrence of various mycotoxins in different settings using reliable analytical methods were rarely done. As a consequence, mycotoxin risk assessments and formulation of regulations lags significantly in Ethiopia. To date, Ethiopia has regulations in place for only a limited number of mycotoxins, and even most of these regulations are non-binding. Therefore, this underscores the necessity for more mycotoxins data from different settings in Ethiopia giving more focus to biomarkers-based studies among vulnerable groups of the population.

Furthermore, the role of agricultural practices, storage conditions, and dietary practices on mycotoxins contamination and exposure should be well documented as the importance of

optimal agricultural and post-harvest practices was repeatedly indicated in previous studies from Ethiopia.

In a broader context, it is prominent that the universally existing mycotoxin data are not well managed to map the global trends due to irregularity in data generation procedures, reporting formats, and data storage. Lately, the significance of mycotoxins data harmonization and standardization is getting interest.

Given these gaps, the first objective of this PhD **(chapter 3)** was to determine and compare multiple mycotoxins occurrence in maize crops sampled randomly from household storages in three agro-ecological zones of South (Sidama zone) and West (Jimma zone) Ethiopia, and to conduct an exposure assessment of different mycotoxins for the study population based on the maize contamination and consumption data. This part of the PhD was also intended to assess maize-related post-harvest practices and household food processing methods implemented by the study households.

The second objective **(chapter 4)** was to determine multiple mycotoxins in breast milk samples of lactating women and examine the pre- and post-harvest practices and household processing methods with regard to maize in Meskan and Mareko districts in southern Ethiopia. Besides, the role of dietary practices and food insecurity for mycotoxin contamination in breast milk samples were examined. This objective was part of the multi-disciplinary Butajira Nutrition, Mental Health, and Pregnancy (BUNMAP) mother-child cohort study of Addis Ababa University, Ethiopia. Specifically this objective is an extension of a study within the BUNMAP cohort study that examined multi-mycotoxins in the serum of a cohort of the same mothers (the lactating women) during their pregnancy period.

The third objective **(chapter 5)** was to propose missing mycotoxins contamination data descriptors, especially with the aim to increase mycotoxin data applicability to LMICs integrate the newly proposed descriptors with the existing GEMS/food and EFSA descriptors and organize them as study, sample and assay types.

2.1 References

1. FDRE. Food and nutrition policy. 2018.

2. FDRE, MoH and EPHI. Ethiopia: food based dietary guidelines. 2022: Addis Ababa, Ethiopia.

3. CSA W.a. Comprehensive food security and vulnerability anaysis (CFSVA). 2019, WFP.

4. Logrieco A., Battilani P., Leggieri M.C., Jiang Y., Haesaert G., Lanubile A., Mahuku G., Mesterhazy A., Ortega-Beltran A., Pasti M., Smeu I., Torres A., Xu J. and Munkvold G. Perspectives on Global Mycotoxin Issues and Management From the MycoKey Maize Working Group. Plant Dis. 2021; 105 (3): 525-537.

5. Warth B., Braun D., Ezekiel C.N., Turner P.C., Degen G.H. and Marko D. Biomonitoring of Mycotoxins in Human Breast Milk: Current State and Future Perspectives. Chem Res Toxicol. 2016; 29 (7): 1087-97.

Chapter 3. Multi-mycotoxin profiling in maize reveals prevalence of *Fusarium* mycotoxins in South and West Ethiopia

Redrafted from: Mesfin A., Tesfamariam K., Belachew T., De Saeger S., Lachat C. and De Boevre M. Multi-mycotoxin profiling in maize reveals prevalence of *Fusarium* mycotoxins in South and West Ethiopia. World Mycotoxin Journal. 2022; 15 (1): p. 73-83. doi: 10.3920/WMJ2020.2645

3.1 Abstract

Multi-mycotoxin exposure data are missing to guide risk assessment and legislation in Ethiopia. This study, therefore, aimed to determine mycotoxin contamination levels in maize samples from 176 randomly selected household storages in three agro-ecological zones of South (Sidama zone) and West (Jimma zone) Ethiopia, and to examine the post-harvest practices and household-processing methods. Liquid chromatography coupled with tandem mass spectrometry was used to quantify 23 mycotoxins. All of the study families consume maize each day, or 3-6 a week or at least once or twice a week. More (77%) samples in Sidama were contaminated with *Fusarium* mycotoxin DON than in Jimma (29%) ($P<0.001$). Similar contamination of fumonisin B1 (19%, Sidama vs. 19%, Jimma), fumonisin B2 (19%, Sidama vs. 18%, Jimma) and fumonisin B3 (12%, Sidama vs.13%, Jimma) contamination were observed ($P>0.05$). In Sidama, only one sample was contaminated with the *Aspergillus* mycotoxins aflatoxin B2 and another sample with aflatoxin B1. Of all samples, 40% were contaminated with 3-5 types of *Fusarium* mycotoxins and only 4% of the samples were contaminated with 6-8 types of mycotoxins. After the harvested maize was dried on the field, the majority (87%) of respondents in Jimma reported that they removed the maize within one day, which was less practiced (17%) in Sidama. The majority (95%) of the households in Sidama, and some (28%) in Jimma, reported that they dried maize before storage, mainly using the sun. Close to two third of the study participants in the two zones reported that they applied the chemical dichloro-diphenyl-trichloroethane (DDT) during maize storage. All (100%) households in both zones reported that they sorted visible moldy maize grains before the preparation of maize flour while most (79%, Sidama vs 72%, Jimma) of them reported that they keep the moldy maize for feed. Protective pre- and post-harvest strategies of *Fusarium* mycotoxins contamination, with a special focus on deoxynivalenol and zearalenone, should be well promoted in the study areas by the agricultural, health and food safety programs as they are possible human and animal health threats.

Keywords: post-harvest, household-processing, storage, agro-ecological zones, deoxynivalenol

3.2 Introduction

*Aspergillu*s mycotoxins like AFs have received substantial attention when compared to *Fusarium* mycotoxins as they frequently contaminate food and feed. AFs are potent human carcinogens, while *Fusarium* mycotoxins are attributed as probable to non-carcinogenic to humans [1, 2]. Contamination of maize and groundnuts with AFs is widespread, leading to possibly acute as well as chronic exposure to these toxins in Africa [3]. Recently, more research has been directed towards *Fusarium* fungi which are common plant pathogens and produce, among others, FUMs, ZEN, and DON. In the context of SSA, FUMs are more studied than other *Fusarium* mycotoxins [4].

The effect of mycotoxins on human health has been a subject of many uncertainties. Mycotoxins have been investigated in relation to a wide range of acute and chronic adverse human health effects, but the evidence for all is limited [5]. Thus, continued research on understanding the effects and modes of mycotoxins action in various species is imperative [6, 7].

Maize is a major staple food crop grown in diverse agro-ecological zones and farming systems, and consumed by people with varying food preferences and socio-economic backgrounds in SSA [8]. A report from Ethiopia indicated that the average annual consumption of maize was 66.7 kg per equivalent adult, which constitutes nearly 20 percent of the total calorie intake in the country [9]. In Ethiopia, major agricultural food commodities such as groundnut, cereals, milk, coffee, and beer are contaminated by mycotoxins, predominantly by AFs, FUMs, OTA, and DON (**Chapter 1)** [10].

Estimates regarding mycotoxins' exposure from food, awareness of mycotoxins, and the availability of analytical methodologies in Ethiopia are lacking to provide a national mycotoxin legislation [10]. Studies on mycotoxin contamination of maize and other crops in Ethiopia mainly focused on a single mycotoxin (or group) such as AFs or/and FUMs [11-18]. To date, there is insufficient data regarding the presence of multiple mycotoxins in Ethiopian staple crops. In addition, the studies did not document the post-harvest and storage practices of smallholder farmers that may contribute to multiple mycotoxin contamination.

In order to make a significant contribution to mycotoxin risk assessments in Ethiopia, the aim of this study was to determine multi-mycotoxin in maize crops among households in three agro-ecological zones of South and West Ethiopia. Additionally, this study examined post-harvest handling practices and household food processing with regard to maize.

3.3 Materials and Methods

3.3.1 Study location

The study was conducted in Sidama and Jimma zones of South and West Ethiopia, respectively **(Figure 3.1**). Sidama zone comprises 23 districts with an agro-ecological setting of almost half (46%) relatively lower altitude, while only (0.6%) are high land and 41% is covered with middle altitudes. The zone mainly depends on production activities of *enset* (*Ensete ventricosum*) for subsistence consumption, and coffee for local markets and exports. Similarly, maize is the main staple crop produced and consumed in the area. Most areas of the Sidama lowland are favorable for mycotoxin production if complemented with poor storage conditions. From this zone, *Boricha* (low land), *Dale* (mid land), and *Aribegona* (high land) districts were purposively selected for the study. Jimma zone is divided into 13 districts with an agro-ecological setting of high lands (15%), mid lands (67%), and low lands (18%). This zone is one of the major coffee-growing areas and contributes significantly to the national economy of the country. Major crops grown other than coffee are maize, teff, sorghum, barley, beans and peas, root crops (*enset* (*Ensete ventricosum*) and potato), and fruits. From this zone, Kersa (mid land) and Mana (low land) districts were included in this study.

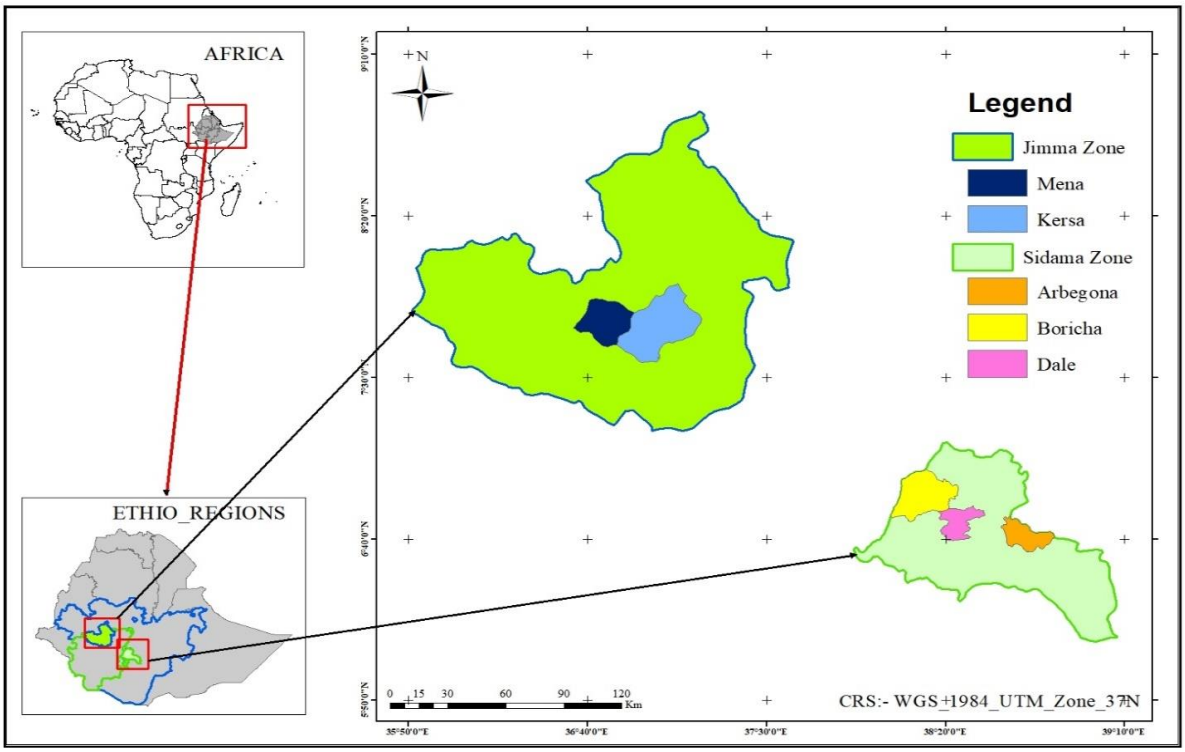


Figure 3.1 Map of the study areas

3.3.2 Study design and sampling

The single population proportion formula [19] was used to calculate the household sample size from Sidama zone. The average proportion of AFs (p=5.5%) contamination in maize in smallholder farmers' stocks from a previous study in South and South-West Ethiopia was used to calculate the sample size [20]. A 95% confidence level and 0.05 precision was considered and the sample size calculated was 79. A design effect of 1.5 was considered to account the heterogeneity within clusters, thus the final sample size calculated for Sidama zone was 79x1.5=118. However, the same sample size calculation procedure was not followed for Jimma zone because Jimma zone was included later to compare the difference in maize mycotoxin contamination levels in South and West Ethiopia so that interventions would be prioritized. Thus, a half sub-sample (n=60) of the 118 was considered for the Jimma zone. A total of 178 samples were considered from the two zones but due to two non-responses from Sidama zone, finally, 176 samples were taken. Multi-stage cluster sampling was implemented. Initially, districts that are known with better maize production and also which represent the lowland, midland and highland agro-ecological zones were purposefully selected based on the consultation of agricultural offices of the Sidama and Jimma zones.

From Sidama zone, 9 out of 113 villages i.e. three districts from each of the lowland, midland, and highland agro ecological zones were selected using a probability that was proportional to the population size of the villages [19]. Further, the sample size (n=118) was allocated to the villages/kebeles proportionally according to their population size. The sample households from each village were selected by simple random sampling technique using lottery method from the list of households available in each village. Only two districts were considered from Jimma zone.

Households with recent experience of producing maize and with maize stock that stored for at least two months at the time of data collection were included in the study. Households without maize stock and respondents with medical or non-medical problem which limit them from being interviewed were excluded from the study. Maize grains that were available in selected households (n=176) were sampled from March-May 2018, which is the second cropping season (short-rainy season). The main cropping season is usually from mid-June till September and the harvesting time is around October. November till January or February is a time of surplus crops, but then after March till May mostly the lean-time for rural households is covered.

3.3.3 Data and maize sample collection

Data collection

From each of the 176 households, both women and men were involved in the interview. Men responded to questions related to the maize postharvest handling and storage practices, and women were interviewed on the maize-based flour preparation techniques. Data collectors were diploma and 1st degree graduates who can speak the local language. Prior to the data collection, the data collectors were trained for two days. On the first day, the study objectives, general principles of data collection, ethical issues, and the contents of the questionnaire were discussed in detail with the data collectors followed by demonstration sessions. On the second day, they practiced the questionnaire, did role plays, and received feedback, accordingly. A food frequency questionnaire by Gibson (2005) [21] was adapted to collect information related to the consumption pattern of food items (maize, barley, groundnut, and milk) which are commonly known to be contaminated with mycotoxins. Study respondents were asked to recall their families' usual consumption frequency of the four selected mycotoxin-prone foods (maize, barley, groundnut, and milk). The respondents qualitatively recalled the frequency of consumption of these selected food items within a month period [21].

Maize sample collection

Three handfuls of unprocessed maize grains each from the top, middle and lower parts of the maize stocks of 2-4 months were collected from each study household (n=176) using polythene plastic bags. The samples were labeled, identified, and kept frozen at -20°C prior to analysis.

3.3.4 Laboratory analysis procedure for maize grains

A validated multi-mycotoxin LC-MS/MS method was used to analyze and quantify multiple mycotoxins in the maize samples [22]. Briefly, on a well-ground and homogenized five gram of each sample (Ika Werke® milling machine), 20 mL acetonitrile/water/acetic acid (79/20/1, v/v/v) was added, tumbled for 60 minutes on an end-over-end tumbler, and centrifuged for 15 min at 3,300 g followed by defatting with 10 mL hexane. Further purification was done by separating the samples into two parts. One part (10 mL) of the defatted extract was filtered through a glass filter (for all mycotoxins) and the second part (10 mL) was mixed with 20 mL acetonitrile/acetic acid (90/10, v/v). The mixed second extract (30 mL) was purified using a Multisep®226-column (for AFs and ZEN, specifically). Then, 2 mL extract from the glass filtered was added to the purified extract. This was followed by evaporation to dryness under a

gentle nitrogen flow. The samples were then re-dissolved with 150 µL of mobile phase A/mobile phase B (60/40, v/v) injection solvent, and the re-dissolved extract was transferred into an LC-MS/MS vial prior to analysis. A Waters Acquity Ultra Performance Liquid Chromatography (UPLC) system coupled to a Quattro Premier MS (Waters, Milford, MA) was used to analyze the samples for 23 mycotoxins, equipped with Masslynx® software for data acquisition and processing. An injection volume of 20 µL was considered for analyses. The column used was a Symmetry C18 (5 µm, 150 x 2.1 mm) with a similar guard column (Symmetry C18, 3.5 µm, 10 x 2.1 mm). The mobile phase flow was 0.3 mL/min. The analysis run time was 28 min. The limits of detection (LOD) and the limits of quantification (LOQ) are reported in Table 1. Details on the analysis protocol of this quantitative LC-MS/MS methodology are detailed in Monbaliu et al. (2010). The samples were considered positive with any of the mycotoxins if the samples were above the LOD. First line control for the quantified results was done using the control spike (repeated injection of the spike) from the calibration curve. In cases where deviations occur for the results of the first line control, a reference was made on the working document. Besides, in instances where the blank sample used for the calibration curve was determined to be not truly blank or contaminated, a correction working template was employed for the calibration curves.

3.3.5 Mycotoxin exposure assessment from maize consumption

Weighed food record data of the 2016 Ethiopian National Household Consumption-Expenditure Survey was used to estimate maize consumption. The survey included representative samples from all regions of Ethiopia. The Sidama zone lies in Southern Nations and Nationalities Peoples Region (SNNPR) and Jimma zone in Oromia region. The maize consumption data of the two study zones were thus extracted from their respective regions. It is noteworthy that actual maize consumption data were not collected from the study villages rather, the regional maize consumption data were extrapolated for the study villages. The reported adult equivalent consumption of maize (Kg) were 113.06 in SNNPR and 81.08 in Oromia, which thus extrapolated as the maize consumption data for Sidama and Jimma zones, respectively. The maize consumption was then executed as 5.1 g/kg body weight (BW)/day for Sidama and 3.6 g/kg BW/day for Jimma. Using Microsoft Excel 2013, calculations of exposure assessment were performed using three scenarios: a lower (LOD as zero), medium (1/2 LOD) and upper bound (LOD). Finally, the maize consumption of each zone was multiplied with the mean, maximum and 95 percentiles of mycotoxin contamination data to get their respective estimated mean, maximum and 95 percentiles intakes of mycotoxins. TDI of Europe was used

to compare the estimated intakes of the mycotoxins (DON, 3-ADON, 15-ADON, HT-2, T-2, AFG2, AFG1, AFB2, AFB1, FB1, FB2, FB3, and ZEN).

3.3.6 Data analysis

Data entry, screening and analyses were carried out using Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc. Chicago IL, USA). Prior to analysis, data were screened for missing values and outliers. Variables were tested for normality using the Shapiro-Wilk test. Descriptive analysis was done using frequency, percentage, mean,/median appropriate for categorical and continuous variables. A Chi-square test for categorical data and a Mann-Whitney U-test for continuous variables were used to test a statistically significant difference between the two study zones. All the continuous data of the mycotoxin results were not normally distributed, therefore, the Mann-Whitney U-test was used and the results were presented as median and interquartile range (1^{st} and 3^{rd}). P-values <0.05 were considered statistically significant.

3.3.7 Ethical considerations

A written informed consent was obtained from all participants. Confidentiality of the information was maintained.

3.4 Results and Discussion

3.4.1 Results

Socio-demographic characteristics of the respondents in Sidama and Jimma, Ethiopia

Including the two parents, the average family size was six in both zones. Almost half (43.1%, Sidama vs. 48.3%, Jimma) of the mothers in both zones, but only 19% and 33% of the fathers in Sidama and Jimma zones, respectively did not have a formal education. In both zones, the main source of income for the majority (93.1%, Sidama vs. 88.3%, Jimma) of the households was agriculture. All households grew maize in Sidama zone, and 97% of the households in Jimma zone. Likewise, '*enset*' was almost grown in all (98%) households of Sidama zone; on the other hand, teff (70%) and sorghum (64%) are grown in Jimma zone.

Mycotoxin contamination of maize crops from household stocks in Sidama and Jimma

From the 23 mycotoxins tested in the maize samples (**Table 3.1**), a significantly higher number of samples in Sidama (77%) were contaminated with DON than in Jimma (29%), ($P<0.001$). The nivalenol (NIV) contamination was 42% in Sidama and 31% in Jimma ($P=0.122$). The ZEN contamination was 23% in Sidama and 15% in Jimma ($P=0.228$). Subsequently, even at the lower scenario, the estimated mean intakes of DON (2.31) and ZEN (0.47) in Sidama were almost twice higher than the 1 and 0.25 TDIs, respectively. A comparable contamination with FB1 (19%), FB2 (19%, Sidama *vs* 18%, Jimma), and FB3 (12%, Sidama *vs* 13%, Jimma) were obtained ($P>0.05$). Nevertheless, significantly higher levels of contamination were observed for 3-acetyldeoxynivalenol (3-ADON) ($P<0.001$) and roquefortine C (ROQ C) ($P=0.022$) in Sidama zone. The mean intakes for FUMs and 3-ADON in the three scenarios were below the TDI, however, the maximum intakes were 18x for FB1 and 7x for FB2 higher compared with the respective TDIs.

Only few samples were contaminated with 15-acetyldeoxynivalenol (15-ADON) (6%), diacetoxyscirpenol (DAS) (5%), alternariol methyl ether (AME) (2%), enniatin B (ENN B) (4%), and only one sample was contaminated with neosolaniol (NEO), T-2, OTA, AFB2 and AFB1 in Sidama. None of the samples were contaminated with fusarenon X (FUS-X), HT-2, AFG2, AFG1, and alternariol (ALT) in both study areas. However, at the high scenario, the mean intake for both HT-2 and T-2 were found to be 0.09 which was higher than the TDI (0.06). As TDI for AFs does not exist, the benchmark dose lower confidence limit was used. The estimated mean intakes of AFs were thus found to be lower than the benchmark dose lower confidence limit (0.4) both in Sidama and Jimma.

Moreover, 36% of the samples were contaminated with one or two types of mycotoxins, 40% with 3-5 types, and only 4% of the samples were contaminated with 6-8 types of mycotoxins in which NIV, DON, 3-ADON, ZEN appeared together in most of the samples.

Table 3.1. Mycotoxin contamination of maize crops from household stocks in Sidama (n=111) and Jimma (n=55)

Mycotoxin[1]	LOD (µg/kg)	LOQ (µg/kg)	% of positive samples		Median (25th, 75th)[2] (µg/kg)		Maximum (µg/kg)		P value[3]
			Sidama	Jimma	Sidama	Jimma	Sidama	Jimma	
NIV	66	132	42	31	0(0,108)	0(0,62)	1052	228	0.122
DON	111	222	77	29	239(42,594)	0(0,71)	2158	468	<0.001*
NEO	16	31	1	0	0(0,0)	0(0 0)	47	<LOD	0.481
FUS-X	30	61	0	0	0(0,0)	0(0,0)	<LOD	<LOD	1
3-ADON	9	18	38	4	0(0,0)	0(0,0)	110	12	<0.001*
15-ADON	6	11	6	0	0(0,0)	0(0,0)	173	<LOD	0.058
DAS	1	2	5	2	0(0,0)	0(0,0)	6	21	0.399
HT-2	17	34	0	0	0(0,0)	0(0,0)	<LOD	<LOD	1
FB1	58	116	19	19	0(0,0)	0(0,83)	7069	1402	0.213
T-2	17	34	1	2	0(0,0)	0(0,0)	24	24	0.22
FB3	42	85	12	13	0(0,0)	0(0,0)	470	99	0.94
FB2	45	89	19	18	0(0,0)	0(0,0)	2712	627	0.874
ZEN	33	65	23	15	0(0,0)	0(0,0)	2447	1066	0.228
ENN	0	0	4	0	0(0,0)	0(0,0)	19	<LOD	0.155
AFG2	4	9	0	0	0(0,0)	0(0,0)	<LOD	<LOD	1
AFG1	3	7	0	0	0(0,0)	0(0,0)	<LOD	<LOD	1
AFB2	3	6	1	0	0(0,0)	0(0,0)	5	<LOD	0.481
AFB1	3	6	1	0	0(0,0)	0(0,0)	4	<LOD	0.481
STE	9	17	6	2	0(0,0)	0(0,0)	28	18	0.204
ALT	22	44	0	0	0(0,0)	0(0,0)	<LOD	<LOD	1
AME	32	65	2	0	0(0,0)	0(0,0)	33	<LOD	0.318
OTA	6	13	1	0	0(0,0)	0(0,0)	605	<LOD	0.48
ROC	2	4	9	0	0(0,0)	0(0,0)	10	<LOD	0.022*

No of co-occurring mycotoxins	*n* (%)
0	33 (20)
1-2	59 (36)
3-5	67 (40)
6-8	7 (4)

[1] 3-ADON (3-acetyldeoxynivalenol), 15-ADON (15acetyldeoxynivalenol), AFB1 (aflatoxin B1), AFB2 (aflatoxin B2), AFG1 (aflatoxin G1), AFG2 (aflatoxin G2), ALT (alternariol), AME (alternariol methyl ether), DAS (diacetoxyscirpenol), DON (deoxynivalenol), ENN (enniatin B), FB1 (fumonisin B1), FB2 (fumonisin B2), FB3 (fumonisin B3), FUS-X (fusarenon X), HT-2 (HT2-toxin), NEO (neosolaniol), NIV (nivalenol), OTA (ochratoxin A)), ROC (roquefortine C), STE (sterigmatocystin), T-2 (T2-toxin), ZEN (zearalenone)

[2] Continuous data are presented as median and interquartile range (1st and 3rd) using Mann-Whitney U-test

[3]*Significance at p<0.05 shows the statistically significant difference between the median (µg/kg) values from Sidama and Jimma

Consumption frequency of common mycotoxin-prone foods

A similar maize consumption pattern in the two zones was observed (**Table 3.2**). Most (49.1%, Sidama vs. 40.0%, Jimma) households reported that they consume maize more than once per day. Some households consume maize once per day or 3 to 6 times per week. None of the participants reported never consumed maize. Contrarily, most of the respondents in both zones reported that they never consumed barley or groundnuts. Milk consumption showed difference between the two zones.

Table 3.2. Consumption frequency of common mycotoxin-prone foods in Sidama and Jimma zones, Ethiopia, 2018

Food item	More than once per day		Once per day		3-6 times per week		Once or twice per week		Twice or less per month		Never		P-value[1]
	Sidama	Jimma	Sidama	Jimma	Sidama	Jimma	Sidama	Jimma	Sidama	Jimma	Sidama	Jimma	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
Maize	57(49)	24(40)	21(18)	16(27)	34(29)	15(25)	4(3)	5(8)	0	0	0	0	0.24
Barley	0	0	3(3)	2(3)	7(6)	4(7)	11(9)	5(8)	18(15)	16(27)	77(66)	33(55)	0.44
Ground nut	0	0	0	3(5)	0	1(2)	1(1)	2(3)	3(3)	4(7)	112(97)	50(83)	0.008*
Milk	36(32)	1(2)	30(26)	4(7)	25(22)	2(3)	16(14)	5(8)	3(3)	10(17)	4(3)	37(63)	<0.001*

[1] *Significance at $p<0.05$

Post-harvest handling and storage of maize crops

Most (80%) households in Jimma and almost half (57%) in Sidama harvested the maize four months before the current survey (**Table 3.3**). The majority (85%, Sidama vs 90%, Jimma) reported that they usually harvest maize when it gets completely dry. The majority (87%) in Jimma removed the harvested maize from the field within one day, but only 17% in Sidama (P<0.001). Contrarily, 75% in Jimma reported that they stacked up the maize in a storage room and 38% in Sidama reported that they spread out the maize, while 25% in Jimma used a combination of the practices (P<0.001).

Furthermore, 95% *vs.* 28% of the respondents in Sidama and Jimma reported that they dry maize before storage, mostly by sun-drying. Additionally, close to two thirds of the study participants in the two zones reported that they used grain protection chemicals before storing maize, mainly the insecticide dichloro-diphenyl-trichloroethane (DDT) (P=0.094).

Table 3.3. Post-harvest handling and storage practices of maize crops in Sidama and Jimma zones, Ethiopia, 2018

Variables	**Sidama**[1,2]	**Jimma**[1,2]	**χ2**
	***n* (%)**	***n* (%)**	**P-value**[3]
Usual maize harvest timing	*n*=115	*n*=60	0.041*
When still green	0	2 (3)	
When begun to dry	17 (15)	4 (7)	
When completely dry	98 (85)	54 (90)	
Maize removal from the field after harvest	*n*=112	*n*=53	<0.001*
Immediately after one day	19 (17)	46 (87)	
After a few days	22 (20)	6 (11)	
After a week	44 (39)	1 (2)	
After two weeks	27 (24)	0	
Maize storage facility	*n*=116	*n*=60	<0.001*
Indoor earth floor	41 (35)	20 (33)	
On the roof	4 (3)	0	
Concrete floor	8 (7)	0	
Wooden floor	38 (33)	21 (35)	
Outdoor	6 (5)	1 (2)	
On the roof and outdoor	11 (10)	17 (28)	
On the roof and wooden floor	6 (5)	0	
Others	2 (2)	1 (2)	
How the maize stored	*n*=116	*n*=60	<0.001*
Cobs in maize bags	8 (7)	7 (12)	
Cobs without maize bags	43 (37)	38 (63)	
Grain in maize bags	26 (2)	12 (20)	
Grain without bags	33 (28)	0	
Other (specify)	6 (5)	3 (5)	
Arrangement in the storage place	*n*=115	*n*=60	<0.001*
Stacked up in storage room	72 (62)	45 (75)	
Spread out in storage room	43 (38)	3 (5)	
Other (specify)	0	12 (20)	
Use of grain protection chemical in storage	*n*=115	*n*=60	
Yes	69 (60)	39 (65)	
No	46 (40)	21 (35)	
Type of chemical	*n*= 67	*n*=29	0.057
Dichloro Diphenyl Trichloroethane (DDT)	64 (96)	24 (83)	
Pill	2 (3)	1 (3)	
Other	1 (1)	4 (14)	

[1] categorical data are presented as proportion and compared using chi-square test (χ2).
[2] n number of responses for a variable
[3]* significant at p<0.05

Household maize flour processing techniques in Sidama and Jimma

All households (100%) reported that they sorted visible moldy maize grains before the preparation of maize flour either by hand or both by hand and winnowing (**Table 3.4**). The practice of dehulling was significantly higher in Sidama (22%) than in Jimma (2%) (P<0.001). Conversely, soaking/washing maize grains was significantly lower in Sidama (2%) than in Jimma (23%) (P<0.001). The majority (79% Sidama *vs.* 72% Jimma) of households reported that they conserved removals and undesirable parts of sorted and dehulled maize for feed.

Table 3.4. Household maize flour processing techniques in Sidama and Jimma zones, Ethiopia, 2018

Variables	**Sidama**[1,2]	**Jimma**[1,2]	**χ2**
	***n* (%)**	***n* (%)**	**P-value**[3]
Sort visibly moldy maize grains before preparation of maize flour	116 (100)	60 (100)	0.003*
If yes, how?			
By hand	72 (62)	28 (47)	
Winnowing	9 (8)	0	
By hand and winnowing	35 (30)	32 (53)	
Dehull maize grains before preparation of maize flour	25 (22)	1 (2)	<0.001*
Soak/wash maize grains before preparation of maize flour	2 (2)	13 (23)	<0.001*
Roast maize grains before preparation of maize flour	4 (4)	2 (4)	1.000
Fate of undesirable parts of maize grains			0.246
Burned	0	2 (3)	
Making beverages	1 (1)	1 (2)	
Stock for feed	90 (79)	43 (72)	
Throw away	19 (17)	12 (20)	
Other	4 (3)	2 (3)	

[1]Categorical data are presented as proportion and compared using chi-square test (χ2).
[2] n number of responses for a variable
[3]*Significant at p<0.05

3.4.2 Discussion

So far, most of the mycotoxin contamination studies documented in Ethiopia focused either on AFs or/and FUMs than multiple mycotoxin profile. This study, therefore, reports the multiple mycotoxin in Ethiopia to support risk assessments and legislation. From the 23 mycotoxins analyzed, the most prevalent contaminants were the *Fusarium* toxins DON, followed by NIV, 3-ADON and ZEN. Much more in Sidama, the mean intake for DON and ZEN were twice higher than the TDI even when the LOD is considered zero. The maximum intakes as well as the intakes at the middle and low scenarios were far higher than the TDIs.

Subsequently, almost a quarter of the samples from Sidama and Jimma were contaminated with FB1, FB2, and FB3 in which some households were found with more times higher intake than the TDI. Basically, most of the samples were contaminated by 1-5 types of *Fusarium* mycotoxins. Temperature plays an important role in *Fusarium* species, in particular, DON may occur in warmer climates [23]. Thus, DON and other *Fusarium* mycotoxins may occur in Ethiopian climates, but so far they have not received significant attention.

A study in Ethiopia observed that 29%, 18%, and 18% of the maize grain samples collected from small-scale farmers contained DON, NIV and FUMs, respectively [12]. Since LODs and LOQs were not mentioned the incidence level of this study cannot be compared with our findings. A study of small-holder farmers in different agro-ecological areas stocks in South and South-West Ethiopia revealed that ZEN was the most prevalent mycotoxin (96%) detected, followed by FB1 (70%), where the reported LODs were 0.12 μg kg^{-1} and 3.2 μg kg^{-1}, respectively [20]. These estimates are significantly higher than the prevalence of contaminations reported in the present manuscript, however, the DON (42%) and 3-ADON (6%) contaminations reported were lower than our findings even with a lower reported LODs of 1.2 μg kg^{-1}. Another study, covering a wider agro-ecology of Ethiopian maize-growing areas analyzed samples of 6 to 7 months of storage and reported 77% of FUMs contaminations [18]. We cannot compare the result with the current study once more as the LOD and LOQ was not mentioned.

In Sidama, ROC, STE, DAS, 15-ADON, and ENN B were also found in few samples. AFB2, AFB1, OTA, and NEO were only found in one sample, and AME in two samples but none of the samples were contaminated with these mycotoxins in Jimma. ALT, HT-2, FUS-X, AFG2, and AFG1 were not found in any of the samples in the two study areas. Comparably, a previous study showed that only few samples in Ethiopia were contaminated with the *Aspergillus* toxins AFB1, AFB2, AFG1, AFG2, and AFM1 [20].

Despite this, however, AFs are a concern in Ethiopia. Various other studies indicated that crops such as maize, groundnut, and sorghum were highly contaminated with AFs. For instance, a study in different Gedeo-zones of South Ethiopia indicates that AFs were found in 100% of maize samples, even exceeding the Food and Drug Administration (FDA) and European Union regulatory levels [24]. Another study also found an 88% AF contamination of maize crops [12]. In one study, 78% of groundnut samples were AF-positive [25], and in another study, 41% of groundnut seed samples from farmers' stocks were contaminated [15]. Sorghum samples collected from different agro-ecological zones of producers, marketplace, and final consumers in Ethiopia, Mali, Burkinafaso, and Sudan showed that the Ethiopian grain sorghum had relatively high mean concentrations of AFs [26]. AFB1 contamination in underground pit-stored sorghum [27], and AFs in sorghum and finger millet [14] were also reported in other studies. Besides, AFs were detected in 96% of complementary foods collected from four regions of Ethiopia, though only 2.5% of the samples exceeded the maximum limit [13]. Furthermore, AFs were found in 11 out of 12 domestic beer brands in Ethiopia [16].

Aflatoxin producers are favored by warm conditions; and, wounding by insects, mammals, birds, and mechanical processes all result in significant infections during the pre-harvest period. Delayed harvest, late irrigation, and rain during warm periods are also associated with increased AF levels [23]. Although studies suggest that AFs occur in both pre- and post-harvest conditions, the present study did not find notable AFs contamination.

Harvesting maize when completely dry is a common practice in the study households. Removing the maize from the field right away after harvest and sun drying the maize before storage were also commonly practiced in Jimma and Sidama, respectively. These practices partly may explain the non-presence of significant AFs contaminations in the present study since damp crops coupled with high temperature are known causes of AFs development in storage. The majority of the study participants in the two zones also reported that they applied grain protection chemicals, mainly DDT, before storing maize. It is an internationally-banned chemical, including in Ethiopia, except for its use in disease vector control [28]. We doubt that farmers actually use DDT, rather they may tend to report similar insecticides/chemicals with the name DDT as it is very well known in rural areas of Ethiopia for malaria control. A review indicated that an insecticide such as DDT, potentially reduces the physical damage on stored grains caused by insect invasion which can further reduce infestation, fungal infection, and therefore, mycotoxin contamination [29], the same may work for other insecticides also. Thus, the abundant use of this reported chemical in combination with the protective post-harvest and

storage practices by the studied households possibly are the reasons for the non-occurrence of AFs in almost all the maize samples.

Other probable reasons for the discrepancies in different mycotoxin contaminations reported between the current and previous studies could be related to the sampling protocol. The current study took samples from randomly-surveyed household maize stocks, but most of the previous studies in Ethiopia focused on maize producing lots or cooperatives. The mycotoxin contamination may show difference as the storage conditions and facilities may differ between cooperatives and households.

All of the participants in the two zones reported that they sorted visible moldy maize grains before preparation of maize flour, but dehulling and soaking were not practiced. The fact that mycotoxin contamination is likely to happen along the chain from production up to storage, traditional household processing of crops is a vital contact point to reduce the level of mycotoxins. Studies have also been documented on the effect of sorting, dehulling, and soaking in reducing mycotoxin infestation [30-32]. Indeed, this emphasizes the need for these traditional maize processing techniques to be well articulated and promoted in the agricultural and health extension programs of Ethiopia.

Though most households have a good practice of sorting moldy maize, the majority of the households also reported that they reserved the sorted moldy maize for feed. This may pose a risk with possible mycotoxin residues from the feeds in edible animal products like milk, meat, and offal. Especially, following ingestion of aflatoxin-contaminated feeds, a part of the ingested AFB1 is degraded in the rumen, and hydroxylase in the liver to an equally potent AFM1 [33]. This may have some detrimental effects on human health particularly on children, who are high milk consumers. This implies that such undesirable practices in the community need to be given due attention.

Fusarium mycotoxins which are produced by *Fusarium spp* [34], were highly prevalent in the maize samples. This is concerning as DON and other trichothecenes' exposure has been linked to acute poisoning outbreaks [1]. Even lower doses of chronic exposure to these mycotoxins cause growth retardation and immunotoxicity whereas higher doses hinder reproduction and development [35].

For FUMs, studies indicate a possible role in esophageal cancer and neural tube defects (NTD), although no conclusive remarks could be drawn to date [5]. A retrospective document review from ten referral hospitals in different regions of Ethiopia revealed there were 777 diagnosed

esophageal cancer patients from 2012–2017 [36]. Likewise, a study in three hospitals in Addis Ababa, Ethiopia pinpointed an incidence rate of NTD as 63.4 per 10,000 births among live and stillbirths [37]. Another hospital-based study in northern Ethiopia as well showed an incidence rate of 131 per 10,000 births [38]. Above all, compared to data from 75 countries worldwide, the NTD report from Ethiopia is comparable to African (5.2–75.4; 11.7 per 10,000 births), South-East Asian (1.9–66.2; 15.8 per 10,000 births), Western Pacific (0.3–199.4; 6.9 per 10,000 births) and Eastern Mediterranean (2.1– 124.1; 21.9 per 10,000 births), but much higher than Europe (1.3–35.9; 9.0 per 10,000 births) and America (3.3– 27.9; 11.5 per 10,000 births) [39]. Notably, consumption of mycotoxins and less diversified foods were characterized in LMICs especially. The above reported esophageal cancer rates and NTD incidences in Ethiopia could be carefully explained by the consumption of FUMs-contaminated foods.

A review paper summarized the oxidative stress induced by multiple mycotoxins, however, the mechanisms involved in the activation of the signaling pathways that result in cell death or increased permeability for the different mycotoxins remain uncertain [40]. In addition, although studies have reported an association between mycotoxins intake with child malnutrition, the evidence to date is too weak to draw firm conclusions [41].

Eventually, although this study documented the contamination level of 23 mycotoxins in household stock maize samples of Ethiopia, it did not compare the mycotoxin levels with the legislations available in developed countries. A study shows it is impractical to achieve the same legal framework having differences in food consumption patterns across the world and also without performing risk assessments. Standardization of regulatory limits for mycotoxins may work for countries with similar food consumption patterns and food security status such as the European Union countries [42]. Thus, it is convincing that the available legislations from developed countries are less applicable for cases like Ethiopia. Rather, exposure assessments were conducted though the exposure estimates were based on raw maize mycotoxin contamination data which in fact the household processing may reduce the contamination level.

3.5 Conclusions

Uncommonly, the prevalence of multiple fusariotoxins in maize was reported in Ethiopia. A higher contamination grade of DON and ZEN led to the fact that TDIs were exceeded. The estimated maximum intakes of FB1 and FB2 by some households were also much higher than the reported TDIs. Fusariotoxins, mainly DON and ZEN, should be considered as a possible human health threat in terms of chronic toxicity in the study areas. In addition, most households reported the use of the highly-toxic insecticide DDT in maize storage. The post-harvest handling practices and traditional maize processing techniques of sorting, dehulling, and soaking which are protective of maize mycotoxin contamination should be well promoted in the study areas.

3.6 References

1. IARC. Improving public health through mycotoxin control, John I.P., Christopher, P. W., Robert, A., Baan, W., Gelderblom, C. A., David, M., Ronald, T. R. and Felicia, W, Editor. 2012, IARC: Albert Thomas, 69372 Lyon Cedex 08, France.

2. Makun H. Mycotoxin and food safety in developing countries 2013: InTech Open.

3. Bankole S., Schollenberger M. and Drochner W. Mycotoxins in food systems in Sub-Saharan Africa: A review. Mycotoxin research. 2006; 22 (3 PG - 163-9): 163-169.

4. Ifeoluwa A., Patrick N., Adewale O., Cynthia C., Okoth S., De Boevre M. and De Saeger S. Awareness and Prevalence of Mycotoxin Contamination in Selected Nigerian Fermented Foods. Toxins. 2017; 9 1-16.

5. Ostry V., Malir F., Toman J. and Grosse Y. Mycotoxins as human carcinogens-the IARC Monographs classification. Mycotoxin Res. 2017; 33 (1): 65-73.

6. Wild C.P. and Gong Y.Y. Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis 2010; 31 (1): 71–82.

7. Zain M.E. Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society. 2011; 15 (2): 129-144.

8. Macauley H. Cereal crops: rice, maize, millet, sorghum, wheat: Background paper. October 21-23, 2015.

9. WFP and CSA. Comprehensive food security and vulnerability anaysis (CFSVA). 2019, WFP.

10. Ayelign A. and De Saeger S. Mycotoxins in Ethiopia: Current status, implications to food safety and mitigation strategies. Food Control. 2020; 113.

11. Assaye M.A., Gemeda N. and Weledesemayat G.T. Aspergillus species and aflatoxin contamination of pre and post-harvest maize grain in West Gojam , Ethiopia. Food Science and Nutrition 2016; 2 (13).

12. Ayalew A. Mycotoxins and surface and internal fungi of maize from ethiopia. AJFAND. 2010; 10 (9): 4109-4123.

13. Ayelign A., Zewdu Woldegiorgis A., Adish A. and De Saeger S. Total aflatoxins in complementary foods produced at community levels using locally available ingredients in Ethiopia. Food Additives & Contaminants: Part B. 2018; 11 (2): 111-118.

14. Chala A., Taye W., Ayalew A., Krska R., Sulyok M. and Logrieco A. Multimycotoxin analysis of sorghum (Sorghum bicolor L. Moench) and finger millet (Eleusine coracana L. Garten) from Ethiopia. Food Control. 2014; 45 29-35.

15. Mohammed A., Chala A., Dejene M., Fininsa C., Hoisington A., Sobolev S. and Arias S. Aspergillus and aflatoxin in groundnut (Arachis hypogaea L.) and groundnut cake in Eastern Ethiopia. Food Additives & Contaminants: Part B. 2016; 9 (4): 290-298.

16. Nigussie A., Bekele T., Fekadu Gemede H. and Zewdu Woldegiorgis A. Level of aflatoxins in industrially brewed local and imported beers collected from Ethiopia market. Cogent Food & Agriculture. 2018; 4 (1): 1-13.

17. Taye W., Ayalew A., Chala A. and Dejene M. Aflatoxin B1 and total fumonisin contamination and their producing fungi in fresh and stored sorghum grain in East Hararghe , Ethiopia. Food Additives & Contaminants: Part B. 2016; 9 (4): 237-245.

18. Tsehaye H., Bente Brurberg M., Sundheim L., Assefa D., Tronsmo A. and Marte Tronsmo A. Natural occurrence of Fusarium species and fumonisin on maize grains in Natural occurrence of Fusarium species and fumonisin on maize grains in Ethiopia. European Journal of Plant Pathology. 2016; 147 141-155.

19. Magnani R. Sampling guide 1997: FANTA.

20. Getachew A., Chala A., Skow Hofgaard I., Bente Brurberg M., Michael S. and Marte Tronsm A. Multimycotoxin and fungal analysis of maize grains from south and southwestern Ethiopia. Food Additives & Contaminants: Part B. 2018; 11 (1): 64-74.

21. Gibson R. Principles of nutritional assessment. 2005: Oxford University Press.

22. Monbaliu S., Van Poucke C., Detavernier C., Dumoulin F., Van De Velde M., Schoeters E., Van Dyck S., Averkieva O., Van Peteghem C. and De Saeger S. Occurrence of mycotoxins in feed as analyzed by a multi-mycotoxin LC-MS / MS method. Agriculture and food chemistry 2010; 58 66-71.

23. Milani J.M. Ecological conditions affecting mycotoxin production in cereals: a review. Veterinarni Medicina. 2013; 58 (8): 405-411.

24. M. Chauhan N., P. Washe A. and Minota T. Fungal infection and aflatoxin contamination in maize collected from Gedeo zone , Ethiopia. SpringerPlus. 2016; 5 (753): 1-8.

25. Chala A., Mohammed A., Ayalew A. and Skinnes H. Natural occurrence of aflatoxins in groundnut (Arachis hypogaea L.) from eastern Ethiopia. Food Control. 2013; 30 (2): 602-605.

26. Ssepuuya G., Van Poucke C., Njumbe Ediage E., Mulholland C., Tritscher A., Verger P., Kenny M., Bessy C. and De Saeger S. Mycotoxin contamination of sorghum and its contribution to human dietary exposure in four sub-Saharan countries. Food Additives and Contaminants Part A 2018; 35 (7): 1384-1393.

27. Taye W., Ayalew A., Chala A. and Dejene M. Aflatoxin B(1) and total fumonisin contamination and their producing fungi in fresh and stored sorghum grain in East Hararghe, Ethiopia. Food Addit Contam Part B Surveill. 2016; 9 (4): 237-245.

28. UNEP. Stockholm convention on persistent organic pollutants (POPs). 2009: Geneva, Switzerland.

29. Felix D'Mello J., Macdonald A., Wilko D., Dijksma T., Dujardin A. and Placinta C. Pesticide use and mycotoxin production in Fusarium and Aspergillus phytopathogens. BioMed Research International. 1998; 104 741-751.

30. Hove M., De Boevre M., Lachat C., Jacxsens L., Nyanga L.K. and De Saeger S. Occurrence and risk assessment of mycotoxins in subsistence farmed maize from Zimbabwe. Food Control. 2016; 69 36-44.

31. Stasiewicz M.J., Falade T.D.O., Mutuma M., Mutiga S.K., Harvey J.W., Fox G., Pearso T.C., Muthomi J.W. and Nelson R.J. Multi-spectral kernel sorting to reduce a fl atoxins and fumonisins in Kenyan maize. Food Control. 2017; 78 203-214.

32. Matumba L., Monjerezi M., Chirwa E., Lakudzala D. and Mumba P. Natural occurrence of AFB 1 in maize and effect of traditional maize flour production on AFB 1 reduction in Malawi. African Journal of Food Science. 2009; 3 (12): 413-425.

33. Johanna F. Mycotoxins in cattle feeds and carry-over to dairy milk: a review. Food Additives and Contaminants: Part A 2008; 25 (2): 172-80.

34. Ismaiel A.A. and Papenbrock J. Mycotoxins: Producing fungi and mechanisms of phytotoxicity. Agriculture. 2015; 5 492-537.

35. Pestka J. Toxicological mechanisms and potential health effects of deoxynivalenol and nivalenol. World Mycotoxin Journal. 2010; 3 (4): 323-347.

36. Wondimagegnehu A., Hirpa S., Abaya S.W., Gizaw M., Getachew S., Ayele W., Yirgu R., Demeke T., Dessalegn B., Diribi J., Kaba M., Assefa M., Jemal A., Kantelhardt E.J. and Addissie A. Oesophageal cancer magnitude and presentation in Ethiopia 2012-2017. PLoS One. 2020; 15 (12): e0242807.

37. Gedefaw A., Teklu S. and Tadesse B.T. Magnitude of neural tube defects and associated risk factors at three teaching hospitals in Addis Ababa, Ethiopia. BioMed Research International. 2018; 3 1-10.

38. Berihu A.l., Welderufael L.A., Berhe Y., Magana T., Mulugeta A., Asfaw S. and Gebreselassie k. High burden of neural tube defects in Tigray, Northern Ethiopia: Hospital-based study. PLoS One. 2018; 13 (11).

39. Zaganjor I., Sekkarie A., L. Tsang B., Williams J., Razzaghi H., Mulinare J., E. Sniezek J., J. Cannon M. and Rosenthal J. Describing the prevalence of neural tube defects worldwide: A systematic literature review. PLoS One. 2016; 11 (4): e0151586.

40. da Silva E.O., Bracarense A.P.F.L. and Oswald I.P. Mycotoxins and oxidative stress: where are we? World Mycotoxin Journal. 2018; 11 (1): 113-134.

41. Tesfamariam K., De Boevre M., Kolsteren P., Belachew T., Mesfin A., De Saeger S. and Lachat C. Dietary mycotoxins exposure and child growth, immune system, morbidity, and mortality: a systematic literature review. Crit Rev Food Sci Nutr. 2019; 60 (19): 3321-3341.

42. Matumba L., Poucke C.V., Ediage E.N. and De Saeger S. Keeping mycotoxins away from the food: Does the existence of regulations have any impact in Africa? Crit Rev Food Sci Nutr. 2017; 57 (8): 1584-1592.

Chapter 4. Mycotoxins exposure of lactating women and its relationship with dietary and pre- and post-harvest practices in rural Ethiopia

Redrafted from: Mesfin A., Lachat C., Gebreyesus S.H., Roro M., Tesfamariam K., Belachew T., De Boevre M. and De Saeger S. Mycotoxins exposure of lactating women and its relationship with dietary and pre/post-harvest practices in rural Ethiopia. Toxins journal. 2023; 15 (4): p. 285. doi: 10.3390/toxins15040285

4.1 Abstract

Mycotoxins can be transferred to breast milk during lactation. Hence, the presence of multiple mycotoxins (aflatoxins B1, B2, G1, G2, and M1, alpha and beta zearalanol, deoxynivalenol, fumonisins (FUMs) B1, B2, B3, and hydrolyzed B1, nivalenol, OTA, ochratoxin alpha, and zearalenone) in breast milk samples was assessed in our study. Furthermore, the relationship between total FUMs and pre- and post-harvest and the women's dietary practices was examined. Liquid chromatography coupled with tandem mass spectrometry was used to analyze the 16 mycotoxins. An adjusted censored regression model was fitted to identify predictors of mycotoxins, i.e., total FUMs. Only fumonisin B2 (15% of the samples) and fumonisin B3 (9% of the samples) were detected while fumonisin B1 and nivalenol were detected only in a single breast milk sample. No association between total FUMs and pre- and post-harvest and dietary practices was found ($p < 0.05$). The overall exposure to mycotoxins was low in the studied women, although FUMs contamination was not negligible. Moreover, the recorded total FUMs was not associated with any of the pre- and post-harvest and dietary practices. Therefore, to better identify predictors of FUMs contamination in breast milk, longitudinal studies with food samples in addition to breast milk samples and with larger sample sizes are needed for the future.

Keywords: breast milk; multiple mycotoxins; FUMs; pre- and post-harvest

4.2 Introduction

As described in **Chapter 1**, mycotoxins significantly affect both human and animal health, and the economy of the agriculture food system [1].

AFB1 undergoes hydroxylation to form AFM1 metabolites, which are excreted in the milk of lactating mammals due to the consumption of AFB1 contaminated food or feed [2]. Though poorly understood, other mycotoxins can also potentially be excreted from the dietary intake of mothers to breast milk during lactation [2]. AFM1 and OTA are the most studied mycotoxins in breast milk [3]. AFB1 and its metabolite AFM1 seem to be the main ones found in breast milk in SSA [4]. Although *Fusarium* mycotoxins are reported to be highly abundant in food crops in Africa (DON (81%), FUMs (51%) and ZEN (44%)) [5], they are less studied in breast milk [6]. A study in Tanzania reported FB1 (44.3%) in breast milk samples [7].

A comprehensive and easy actionable guide of pre-harvest and post-harvest factors that focus on the prevention and control of mycotoxins in grains has been framed by experts in five key areas: (1) sustain plants' vigor and health; (2) reduce toxigenic fungal populations in growing plants and storage; (3) rapidly reduce the moisture content of grains and avoid rehydration; (4) safeguard husks/hulls or pericarp/testa; and (5) clean and remove mycotoxin high-risk components [8]. Food processing can further reduce mycotoxin levels through physical removal and decontamination [9]. Moreover, diversifying diet lowers the risk of mycotoxin exposure by decreasing the intake of common mycotoxin-contaminated staple foods such as maize **Chapter 1** [10].

The dependence of African diets on monotonous cereals increases mycotoxin exposure [11]. The risk is even higher in SSA due to frequent and multiple contaminations of mycotoxins in staple diets [4]. Similarly, data on the prevalence of mycotoxins in different food crops has been generated so far. This indicate a possible public health risk for Ethiopian population, although national data on the human health implications of mycotoxins exposure are missing in Ethiopia [12, 13].

In Ethiopia, knowledge of fungi and mycotoxins is generally minimal. Poor household storage and processing practices of staple foods and intentional adulteration by traders are common [14]. Moreover, little effort has been exerted in the control of mycotoxins except for the regulation of very few commodities by the Ethiopian Standard Authority **Chapter 1** [15].

To date, studies conducted in Ethiopia have focused on food crops and mainly on AFs while not covering other mycotoxins such as FUMs in a multi-mycotoxin approach. Hence, studies

on bio-monitoring of mycotoxins such as in breast milk are lacking except a study reported by Mesfin et al. (2020) on AFs exposure among lactating women in the Sidama region [16]. Moreover, though the importance of good agricultural practices has been indicated in previous studies from Ethiopia [13, 15-17], the effects of maize agronomic practices, storage conditions, and dietary practices on mycotoxins exposure among lactating women have not been reported.

The present study examined the exposure to multiple mycotoxins in the breast milk samples of lactating women in Meskan and Mareko districts of southern Ethiopia. Moreover, the study examined the relationship between total FUMs and maize pre- and post-harvest and the women's dietary practices.

4.3 Materials and Methods

4.3.1 Study design and location

A community-based cross-sectional survey nested in the BUNMAP was conducted. BUNMAP is a mother–child cohort study of Addis Ababa University, Ethiopia. The study site was the Butajira Health and Demographic Surveillance Site of Meskan and Mareko districts, Gurage zone, Southern Nations and Nationalities, and Peoples Regional State, Ethiopia **(Figure 4.1)**. The Butajira Health and Demographic Surveillance Site is one of the oldest surveillance sites in Africa established in 1986 which consists of nine rural villages and one urban village of different ecological zones. A cohort study within the BUNMAP examined multi-mycotoxins in serum samples of mothers during their pregnancy period. The study reported that the pregnant mothers were co-exposed to at least five mycotoxins, of which FUMs and tenuazonic acid were the most frequently detected [18]. Our current study extended and assessed multi-mycotoxins among the same mothers during their lactation period.

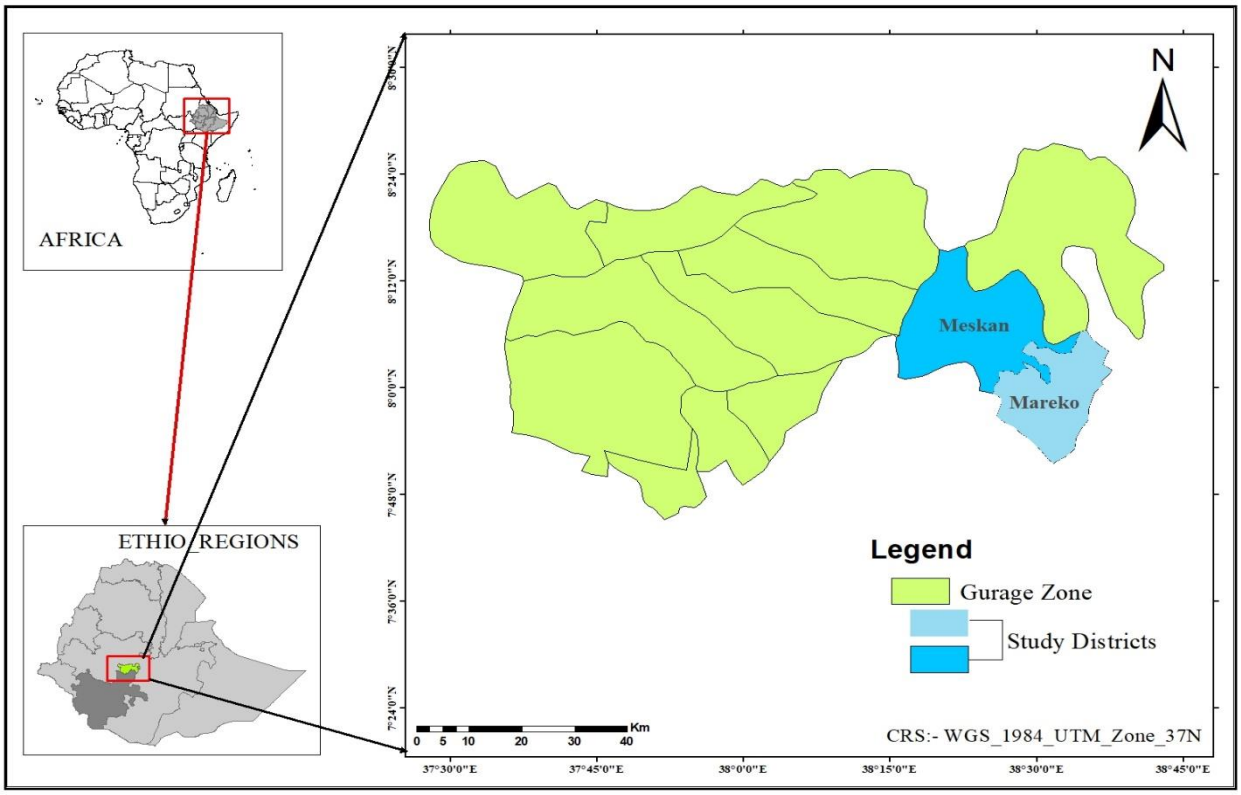


Figure 4.1 Map of the study area

4.3.2 Study subjects and sampling

The study included all lactating women who had infants between three and five months during the study period. A total of 138 mother–infant dyads using the BUNMAP open cohort were recruited.

4.3.3 Data and sample collection

Measurements

The data were collected and supervised by the Butajira Health and Demographic Surveillance Site experienced data collectors and supervisors. Training was given to data collectors and the questionnaire was also pre-tested before use. The understandability of some questions was rectified as per the feedback received from the pretest. The questionnaire-based data were electronically collected using Open Data Kit (ODK). Completeness of the data was checked by the supervisors at the end of each interview and a final checkup was ensured by the principal investigator before submission to the central database.

The five sections of the questionnaire were: socio-demographic and economic information; dietary diversity and food frequency of the lactating women; household food insecurity; pre- and post-harvest practices; and household food processing methods with regard to maize. The socio-demographic and economic questionnaire was adapted from the previous questionnaire developed by the BUNMAP study group during the baseline survey. The Minimum Dietary Diversity for Women developed by the Food and Agricultural Organization was used to assess the food groups consumed by the lactating women 24 hours before the survey time [19]. The number of food groups consumed by the women out of 10 food groups was recorded. The frequency of consumption of mycotoxin-prone foods, i.e., maize, sorghum, and millet, were qualitatively assessed using adapted food frequency questionnaires (FFQ) over a reference period of one month [20].

The Household Food Insecurity Access Scale (HFIAS) was used to assess the food security status of the respondents' households [21]. The questionnaire contains nine occurrence questions, and each of the nine questions were followed by frequency-of-occurrence questions with a recall period of one month. A higher cumulative score of HFIAS indicates an increased possibility of food insecurity. The HFIAS guideline summarizes the cumulative HFIAS score into four levels of household food insecurity: food secured, mild, moderate, and severe food insecurity.

Questions on pre- and post-harvest practices related to maize, knowledge about mycotoxins, and household maize processing techniques were adapted from questionnaires used by previous similar studies [22]. The women were interviewed about the maize pre- and post-harvest practices employed by their families as women in Ethiopia engage in the majority of the agricultural activities.

Breast milk sample collection

The women themselves hand-expressed ≥25 mL of breast milk into sterile 50 mL falcon tubes. Each woman was instructed on how to manually hand express and provide a sample depending on the availability of breast milk. The samples were immediately kept in ice boxes, and, at the end of each data collection day, they were transferred to −20 °C at the local health center. The samples were transported from the study site to the Ethiopian Public Health Institute in Addis Ababa, Ethiopia, and kept at −40 °C until they were shipped to Belgium. The samples were then shipped in dry ice to the Centre of Excellence in Mycotoxicology and Public Health, Faculty of Pharmaceutical Sciences, Ghent University, Belgium, and stored at -80 °C until analysis. All breast milk samples were thawed prior to extraction for the determination of mycotoxins.

4.3.4 LC-MS/MS analysis of the breast milk samples

The breast milk samples were quantitatively analyzed for 16 different mycotoxins. An extraction and the LC-MS/MS method with the XEVO TQ-S (Waters®, Manchester, UK) that has been validated and published previously by a research team in our laboratory (LOD (0.20–3.40 ng/mL), relative standard deviation intra-day (2.25–14.09%) and inter-day (1.60–17.46%) precisions, apparent recovery (86.67–118.43%), and linearity (>0.991 R^2) was used [23].

One mL of milk was added to a fifty mL centrifuge tube. Internal standards, i.e., 40 µL of a 1 µg/mL deepoxy-deoxynivalenol (DOM) solution (50 µg/mL stock diluted 50 times in MeOH) and 40 µL of a 1 µg/mL zearalanone (ZAN) solution (1 mg/mL stock diluted 1000 times in ACN) were added. For the control spike, 50 µL of multi-mycotoxin standard mixture was added. The samples were then incubated in the dark for 15 min. Subsequently, 4 mL ACN/formic acid (99/1 v/v) was added and vortexed for 30 s. Next, the samples were shaken on the overhead shaker for 10 min, followed by centrifugation at 3000 mg for 10 min. Afterwards, an OASIS® PRiME HLB column (6 cc (200 mg) extraction cartridges, part no WAT106202, Waters TM, Ireland) was conditioned with 3 mL ACN/formic acid (99/1, v/v). The supernatant was then transferred to the Oasis PRiME HLB cartridge (60 mg/3 cc) and the eluate was collected in a glass test tube. The samples were evaporated at 40 °C under a gentle nitrogen flow. An injection solvent was prepared by adding 50 mL MeOH LC-MS grade to a 100 mL volumetric flask, and then the volumetric flask was filled with water up to the mark and homogenized. The dry residue was then dissolved in 200 µL of the water/MeOH (50/50 v/v) injection solvent and vortexed. Afterwards, 200 µL n-hexane was added, vortexted, and then centrifuged at 3000× g for 2 min. The samples were then passed through a Millipore filter

(0.22 μm) and ultra-centrifuged (10,000 rpm for 10 min). Subsequently, 100 μL of the lower phase was transferred into a vial, and any air bubbles were removed. Finally, the samples were analyzed using the Waters® Acquity UPLC (column HSS T3 1.8 μm) system coupled to a Quattro XEVO TQ-S mass spectrometer.

Mobile phase A: water/methanol/acetic acid (94/5/1, *v*/*v*/*v*) + 5 mM ammonium acetate, and mobile phase B: water/methanol/acetic acid (2/97/1, *v*/*v*/*v*) + 5 mM ammonium acetate were used. The gradient elution program started at 95% mobile phase A, and mobile phase B increased gradually to 65% in 7 min. Then, mobile phase B increased gradually to 99% in 13 min. The total run time was 18 min. The mass spectrometer was operated in the positive (ES+) electrospray ionization mode. The presence of mycotoxins in a sample was confirmed according to the European Commission Decision (EC) No. 2002/657 recommendations: having 3 or more identification points; a signal to noise ratio of >3; the use of relative ion intensity and relative retention time [24]. MassLynx version 4.1 and TargetLynx version 4.1 (Waters®, Manchester, UK) software were used for data acquisition and processing. The limits of detection (LOD) and the limits of quantification (LOQ) are reported in Table 1. The samples were considered positive with any of the mycotoxins if the concentrations were above the LOD.

4.3.5 Data management and analysis

Questionnaire-based data collected using ODK was transferred from a server to be analyzed in R version 4.0.5 (31 March 2021) [25]. The data were cleaned by identifying missing values and outliers. A Shapiro–Wilk test of normality was carried out to check if the data were normally distributed using a *p*-value > 0.05 implying that the distribution is normal. A descriptive analysis of frequency and percentage for categorical variables was used. The mean and standard deviation for the normally distributed data and the median and interquartile ranges (IQRs) for the non-normally distributed data were used to describe the continuous variables.

Tabulation of Minimum Dietary Diversity for Women was carried out by adding one point if any food in the 10 food groups was consumed and summed into a score ranging from 0 to 10. Each woman was then coded yes or no for scoring at least ≥5, followed by a calculation of the proportion of women who score at least ≥5 [19].

The HFIAS was scored first by coding the frequency-of-occurrence as 0 for all cases where the answer to the corresponding occurrence question was “no” and then summing the codes for each frequency-of-occurrence question. The household response to the nine frequency-of-occurrence questions was coded into three levels rarely, sometimes, and often. The maximum score for a household was 27. Finally, the households were categorized into food secure, mildly

food insecure, moderately food insecure, and severely food insecure as described in Coates et al. (2007) [21].

We computed total FUMs by summing up FB1, FB2, and FB3 as these are the major FUMs grouped together. The Shapiro–Wilk test showed that the total FUMs data were not normally distributed (p-value < 0.05) due to left censoring of 85% of the data. A censored regression model was hence used [26].

Four major categories of variables (pre-harvest practices, post-harvest practices, mycotoxin knowledge, and maize household processing methods) were considered as predicting variables. To avoid over-adjustment and multi-collinearity across the pre- and post-harvest practices, a data reduction approach was applied to summarize these agricultural practices. For this purpose, a principal component analysis (PCA) with varimax rotation was applied to generate latent factors explaining the variance within each pre- and post-harvest practice [27]. The amount of variance carried in eigenvalues and scree plots was used to decide the number of principal components (PC) to be retained. A PC with an eigenvalue < 1 was not retained. After selecting the PCs, the contribution of the original variables in a PC was inspected using values of varimax rotated loadings. Loadings < 0.1 were blanked and any variable that showed a larger loading was regarded as a contributor to that PC. Those variables which did not appear in the retained PCs were not represented in the subsequent regression analysis. Finally, scores from the newly constructed PCs were generated for the 138 samples and used for the regression analysis. Univariate regression model between each PC and the total FUMs was first fitted, and further multivariate regression analysis was executed including all of the PCs and confounders, i.e., age, educational level of the women, and households' food security status. A p-value of 0.05 was taken as the significance level. The fitness of the models in terms of log-likelihood was compared and the multivariate model showed better model fitness (log-likelihood −117) than the univariate models (log-likelihood −124 to −126).

4.3.6 Ethical considerations

The study was approved by the Institutional Review Board of Addis Ababa University, College of Health Sciences (099/17/SPH). A written informed consent was taken from all women. Confidentiality of the data was maintained.

4.4 Results and Discussion

4.4.1 Results

Socio-demographic characteristics of the lactating women

A total of 138 lactating women participated in the study, of whom 99.3% were married, with a mean age of 27.4 ± 5.2 years. Just under half (48.6%) of the participants had a formal education, whereas 21.7% were able to read and write and 29.7% had no formal education. The median family size was 5 ± 4.6, and agriculture was the major source of income (68.1%), followed by small personal businesses (18.1%). The type of the household harvested crops were reported to be maize (93.4%) followed by teff (37.3%) and wheat (13.1%).

Mycotoxin exposure in the lactating women

Except for FUMs and nivalenol, the other mycotoxins, i.e., AFB1 (LOD = 0.20, LOQ = 0.39), AFB2 (LOD = 0.37, LOQ = 0.75), AFG1 (LOD = 0.31, LOQ = 0.62), AFG2 (LOD = 0.23, LOQ = 0.46), and AFM1 (LOD = 0.38, LOQ = 0.77); alpha (LOD = 3.00, LOQ = 6.00) and beta (LOD = 2.70, LOQ = 5.40) zearalanol; DON (LOD = 3.01, LOQ = 6.02), hydrolyzed FB1 (LOD = 2.42, LOQ = 4.85), OTA (LOD = 0.66, LOQ = 1.32), OT alfa (LOD = 0.47, LOQ = 0.95), and ZEN (LOD = 2.19, LOQ = 4.38), were not detected (<LOD). The FUMs contaminated 16% of the total samples; of which the most prevalent contamination was due to FB2 (15%), followed by FB3 (9%). The contamination by FB1 and nivalenol was negligible with only 1% of the samples (**Table 4.1**).

Table 4.1. Mycotoxins in the breast milk samples of the lactating women (*n* = 138)

Mycotoxin [1]	**LOD** [2] **(ng/mL)**	**LOQ** [3] **(ng/mL)**	**Positive Samples *n* (%)**	**Maximum (ng/mL)**
NIV	2.78	5.57	1 (1)	2.81
FB1	3.40	6.80	1 (1)	8.26
FB2	1.99	3.98	21 (15)	24.98
FB3	2.38	4.76	12 (9)	7.52
Total FUMs	-	-	22 (16)	32.51

[1] FB1 (fumonisin B1), FB2 (fumonisin B2), FB3 (fumonisin B3), NIV (nivalenol), and total FUMs (total fumonisins).
[2] LOD limit of detection: the samples were considered positive if values were equal to or above the LOD.
[3] LOQ limit of quantification.

Dietary practices and food security status

Maize was found to be the most frequently consumed crop by all (100%) of the women. The majority (79.7%) consumed maize more than once per day, and the rest consumed it once per day (5.8%) or 3–6 times per week (10.9%). Just over half (52.2%) of the study women reported that they never consumed sorghum. Of the other half who consumed sorghum, only a few of them reported that they consumed it more than once per day (7.2%) and once per day (1.4%). Instead, 21.7% reported that they consumed sorghum twice or less per month, and 10.9% reported this figure as being 3–6 times per week. The majority (84.8%) of the study women reported that they never consumed millet. Few of the women (8.7%) consumed millet twice or less per month. None of the women reported that they consumed millet on a daily basis.

Regarding the food security status of the households, only 23.2% of the households of the study women were food secure but the rest (76.8%) were experiencing different levels of food insecurity: mild (20.3%), moderate (37.7%) and severe (18.8%).

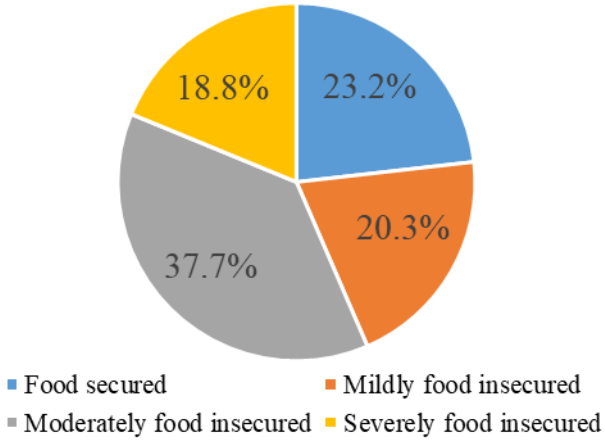


Figure 4.2 Food security status of the study households

Food groups consumed by the lactating women

The food groups consumed by the lactating women are shown in **Table 4.2**. Higher consumption of grains, white roots, and tubers were observed (98%) in contrast to approximately non-consumption (1%) of nuts and seeds. Higher consumption of non-green vegetables (80%) and green vegetables (70%) was also reported compared to relatively low consumption of eggs (8%) and meat, poultry, and fish (7%). Nearly half (46%) of the women consumed dairy products. Accordingly, most (77%) of the study women demonstrated a lower dietary diversity score (<5 food groups).

Table 4.2. Food groups consumed by the lactating women (*n* = 138)

Food Groups [1]	***n* (%)**
Grains, white roots and tubers, and plantains	135 (98)
Pulses	40 (29)
Nuts and seeds	1 (1)
Milk and milk products	63 (46)
Meat, poultry, and fish	10 (7)
Eggs	11 (8)
Dark green leafy vegetables	97 (70)
Vitamin A-rich fruits and vegetables	26 (19)
Other vegetables	110 (80)
Other fruits	21 (15)
MDD-W [2]	
<5 food groups	106 (77)
≥5 food groups	32 (23)

[1] The FAO food groups of minimum dietary diversity for women were used.
[2] MDD-W: stands for minimum dietary diversity for women

Knowledge of the lactating women about mycotoxins

Most (66.7%) of the women had the knowledge that high temperatures lead to mold formation, whereas just over half (52.9%) mentioned moisture as a cause and some (11.5%) of them also mentioned the use of expired insecticides as a possible cause.

Moreover, the knowledge of the women about the health effects of consuming mycotoxin contaminated foods/crops was investigated whereby approximately half (55.1%) reported that they did not know the health effects. The other half of the women gave varied responses that mycotoxins induce diarrhea (35.8%), vomiting (16.4%), and growth impairment (8.2%).

Buying moldy grains of maize or other crops was not common as the majority (95%) of the women demonstrated good knowledge that they do not buy such crops from the market. Additionally, the respondents were brought to their attention which crops they think are more

susceptible to mold spoilage. The majority (90.6%) of the women reported cereals as the most susceptible crops to mold spoilage compared to other crops. Furthermore, they recalled the types of cereals that they think are more prone to mold formation, and majorly, they mentioned maize (90.5%), followed by sorghum (37.7%) and wheat (29%).

Pre- and post-harvest practices concerning maize

The maize-related pre- and post-harvest practices of the study households are presented in **Table 4.3**. From the survey, no other watering method for maize fields other than rainwater (100%) was mentioned. Weeding the maize field (97%)**,** cleaning residues from the previous harvest (91%), and harvesting maize when it becomes completely dry (89%) were also reported by the majority of the women**.** Moreover**,** more than half (56%) of the women revealed that they rotate crops to avoid growing the same crop. The application of insecticides/fungicides in the maize growing field was the least (23%) reported pre-harvest practice.

For the post-harvest practices, sorting out undesirable maize grains before storage was largely (98%) practiced, followed by decontamination of moldy maize grains (81.1%), disinfecting the storage area before storing maize (71%), and the use of insecticides/fungicides in storage (66.7%). However, fumigation as one of the storage cleaning methods was less practiced (10.8%).

Predominantly (60.1%), a woven polypropylene bag locally called “madaberia” was mentioned as maize storage material used, and only 16.6% reported that they use local storage made from wood or bamboo. Most importantly, the majority (86%) of the families of the study women store maize for four months up to one year.

Table 4.3. Pre- and post-harvest practices of maize among households of lactating women (n = 138)

Variables	Categories	*n* (%)
n = 100		
Residues cleared from field	Yes	91 (91)
	No	9 (9)
Maize variety used	"Shone-pioneer"	35 (35.3)
	"extension"	26 (25.8)
	"mirit zer" [1]	10 (10)
	"BH660"	14 (13.9)
	Others	15 (15)
Field watering method	Rainwater	100 (100)
Insecticides/fungicides on field	Yes	23 (23)
	No	77 (77)
n = 97		
Sort spoiled grains before storage	Yes	95 (98)
	No	2 (2)
n = 138		
Storage material	"gotera" [2] made from teff straw and mud	5 (3.6)
	"gotera" [2] made from wood or bamboo	23 (16.6)
	Woven polypropylene bag	83 (60.1)
	Others	27 (19.5)
Insecticides/fungicides in storage	Yes	92 (66.7)
	No	46 (33.3)
Decontamination of spoiled grain	Yes	112 (81.1)
	No	26 (18.9)
n = 100		
Duration of storage	1-3 months	14 (14.1)
	4-6 months	50 (49.9)
	7-9 months	12 (11.6)
	10-12 months	24 (24.4)

[1] mirit zer: is the Amharic version of "improved variety".
[2] gotera: stands for a local name for storage made from local materials such as wood and mud.

Traditional household maize processing practices in flour preparation

Sorting out visibly moldy maize grains in preparation for maize flour was a common practice reported by almost all (97.8%) of the women. In contrast, only 10.9% practice dehulling, similarly only 10.9% practice soaking/washing, and almost none **(**0.7%**)** of the women reported practicing roasting maize grains in flour preparation. Even more, the women were asked what they do with the undesirable grains removed after the sorting and dehulling of maize grains. Unfortunately, most (71.7%) of them reported that they use it for feed, others (31.1%) reported it for making beverages, and only a few (3.6%) of them reported that they burn it.

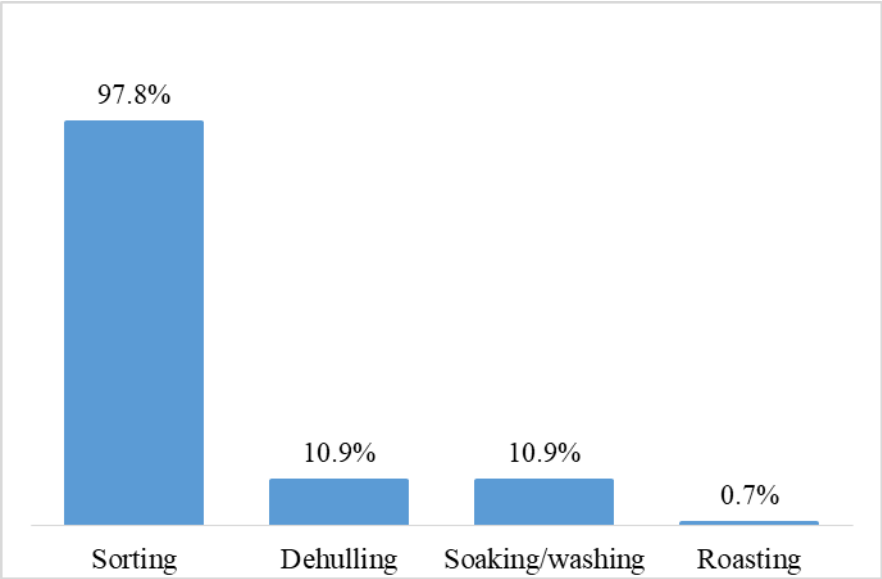


Figure 4.3. Traditional household maize processing practices

Predictors of total FUMs exposure in breast milk samples of the lactating women

Only FUMs were considered for further statistical analysis since the rest of the mycotoxins were not found (<LODs), except a single sample was contaminated with nivalenol. Moreover, as only one sample was contaminated by FB1, the total FUMs for the remaining 137 samples was computed as a summation of FB2 and FB3 only.

The results of the multivariate regression between each PC and total FUMs are depicted in **Table 4.4**. Three PCs were retained from the PCA of the pre-harvest practices which represented 54% of the total variance. Frequency of weeding and use of insecticide in the field were the variables embodied in PC1 (20%); variety common name were used in PC2 (19%) and removing residues from previous harvest were used in PC3 (15%). Crop rotation, sensory methods used for harvesting maize, and special drying method applied were the variables not represented in the first three selected PCs of the pre-harvest group.

Similarly, the PCA for post-harvest practices was represented by the first three PCs explaining 59% of the total variance. The variable storage condition was denoted in PC1 (24%), the use of pesticides in storage contributed to PC2 (19%), and decontamination of molds was represented in PC3 (1%). The variables, i.e., disinfecting storage, use of fumigation in storage, type of storage material, and duration of storage were not retained in the first three PCs.

The PCA of mycotoxin knowledge was represented in five PCs. The total variance explained by the PCs was 67%. PC1 (22%) contained two variables i.e., crops prone to mold spoilage and maize prone to mold spoilage. Mycotoxin causes diarrhea was the variable that contributed to

PC2 (17%). Other health effects of mycotoxin were encompassed in PC3 (11%). Wheat prone to mold spoilage is represented in PC4 (10%) and moisture causes mold in storage is represented in PC5 (7%). Do not know the health effect of mycotoxins, use of expired insecticides causes mold in storage, high temperature causes mold in storage, other causes of mold in storage, have heard about fungus affected maize, mycotoxin causes vomit, mycotoxin causes growth impairment, buy moldy crops, and sorghum prone to mold spoilage were the variables not represented in the five PCs.

Two PCs were retained from the PCA of the maize household processing method which explained 57% of the total variance. Making beverages from maize removals and making other things from maize removals were the variables represented in PC1 (34%) and PC2 (23%), respectively. Variables: maize removals use as feed, dehull and soak maize before making flour were not retained.

In the multivariate model, the age of the women showed a statistically significant association with total FUMs ($p < 0.034$). A one-year increase in age showed on average a 1.21 ng/mL decrease in FUMs level.

Table 4.4. Predictors of total FUMs exposure in breast milk samples of the lactating women (*n* = 138)

Predictors	**Univariate Analysis**		**Multivariate Analysis**	
	Coefficients	*P*-Values	Coefficients	*P*-Values
Pre-harvest practices				
PC1	-0.31	0.889	0.81	0.736
PC2	0.55	0.818	-1.11	0.699
PC3	-1.87	0.493	-2.06	0.496
Post-harvest practices				
PC1	-0.04	0.984	-0.77	0.744
PC2	5.07	0.078	-6.02	0.068
PC3	0.78	0.745	2.09	0.385
Mycotoxin knowledge				
PC1	-0.12	0.921	0.40	0.762
PC2	0.62	0.672	0.75	0.631
PC3	0.33	0.847	-0.35	0.850
PC4	-1.55	0.427	-2.01	0.382
PC5	2.33	0.283	2.36	0.323
Household maize processing methods				
PC1	1.96	0.227	1.08	0.522
PC2	1.77	0.386	2.43	0.303
Consumption frequency of maize	-1.58	0.589	-1.88	0.540
Dietary diversity	-0.01	0.998	0.10	0.961
Age			-1.21	0.034 *
Education level			1.50	0.544
Household food security			0.28	0.907

Censored regression analysis was executed. Total number of observations = 138; left-censored = 117; uncensored = 21. Principal component analysis was used to summarize variables. PC: principal component. FFQ: food frequency questionnaire. Dietary diversity for the women was recorded using the FAO MDD_W. Pre-harvest (PC1: frequency of weeding and use of insecticide in field. PC2: variety common name. PC3: removing residues from previous harvest). Post-harvest (PC1: storage condition. PC2: use of pesticides in storage. PC3: decontamination of molds). Knowledge (PC1: crops prone to mold spoilage and maize prone to mold spoilage. PC2: mycotoxin causes diarrhea. PC3: other health effects of mycotoxin. PC4: wheat prone to mold spoilage. PC5: moisture causes mold in storage). Processing (PC1: make beverages from maize removals. PC2: make other things from maize removals).

The asterisk * show a statistically significant p-value < 0.05.

4.4.2 Discussion

As far as we are aware, this is the first study in Ethiopia which assessed multiple mycotoxins in breast milk. Our finding indicated that FUMs contaminated 16% of the entire sample set, with contamination levels ranging from <LOD to 32.5 ng/mL. Therefore, this finding suggests that the parent mycotoxins present in food have the potential to be transferred without alteration and can be measured in breastmilk.

Although the synergy or antagonism interaction of mycotoxin mixtures is still not fully understood, synergy among *Fusarium* toxins has been highlighted in studies [28, 29]. AFM1 is the most studied mycotoxin in breast milk, while there are limited studies on FUMs and other mycotoxins. Therefore, we were unable to find studies reporting FUMs' contamination in breast milk samples from Ethiopia. However, *Fusarium* mycotoxins such as FUMs were documented in Ethiopian agricultural products and in blood samples from two studies. Compared to our finding, one of the studies in serum was conducted in the same study area as ours' by another BUNMAP cohort study research team and reported a higher prevalence of FUMs exposure (FB2 (98.8%), FB3 (95.3%), and FB1 (93.3%)) [17]. The other study in serum samples of 6–35 months children in Ethiopia reported a comparable prevalence of FB3 (11%) to our finding but in contrast to the prevalence of FB2 (0%) and FB1 (2%) [30].

Additionally, studies from Tanzania and Brazil analyzed FB1 contamination in samples of breast milk. The Tanzanian investigation found 44.3% of FB1 at concentrations ranging between 6.57 to 471.05 ng/mL (LOD = 5.5 ng/mL) [7]. The relatively higher LOD reported in that study may further indicate that the concentrations in the positive samples were higher. On the other hand, the study from Brazil found FB1 (LOD = 2.1 ng/mL) contamination only in two samples (3%) with concentrations of 2.2 and 3.4 ng/mL [31], which is comparable to our finding.

The most likely explanation for the occurrence of FUMs in our study might be related to the women's reported high consumption of maize (100%) and low dietary diversity (77%). It has been determined that maize crops are more susceptible to FUMs contamination [32]. Moreover, a less diversified diet increases the risk of mycotoxin exposure, particularly in low-income settings [33, 34].

As late harvesting favors the occurrence of FB1 and FB2 [35], the other reason for the observed FUMs in our study might also be related to the majority (89%) of the study households allowing maize crops to over dry on the field before harvesting.

However, the level of FUMs' contamination recorded in our study was not immense. The abundant good pre- and post-harvest practices reported by the women might explain the low magnitude of FUMs observed in our study compared to the other studies reported elsewhere. Moreover, the variation in breast milk sampling might also be another reason for the observed difference [7, 31]. Numerous studies have indicated that good pre- and post-harvest practices play a significant role in reducing mycotoxins contamination [36, 37].

To date, evidence indicates that FUMs are not acutely toxic but there are several studies covering their chronic toxicity and carcinogenicity in animals; yet, their health impact on humans is unclear [38, 39]. Biomarker-based epidemiological studies of FUMs [38] with robust experimental research and adequate sample size are still required. Even though the threat of FUMs is not explicitly evidenced in humans, studies have shown that their exposure increases the risk of cancer, neural tube defects, and other birth defects [40, 41]. Therefore, the consumption of FUMs by infants through contaminated human breast milk should not be ignored [41].

Unexpectedly, none of the breast milk samples in our study were contaminated with AFs, hydrolyzed FB1, DON, ZEN, OTA, ochratoxin alpha, alpha, and beta zearalanol. Regarding AFM1, our result is consistent with studies conducted in Angola (LOD = 0.005 ng/mL) [42] and Brazil (LOD = 0.02 ng/mL) [31] in which AFM1 was not detected in breast milk samples. In Ethiopia, we found only one study which assessed AFM1 in 360 breast milk samples collected in two seasons. AFM1 was detected in 64.4% (LOD = 0.005 ng/mL) of the samples that ranged from undetectable to 0.143 ng/mL [16]. Though no other published studies on mycotoxins in breast milk from Ethiopia exist, another survey in the Sidama zone, Ethiopia assessed urinary AFM1 levels in lactating women. The study revealed that AFM1 (LOD = 0.00015 ng/mL) exists in 53.3% of the samples (undetectable to 2.6 ng/mL) [43]. Moreover, from the same study area as ours, 62.4% AFM1 at concentrations ranging from 0.15 to 0.4 ng/mL (LOD = 0.00015 ng/mL) in urine samples among children 12–59 months of age was reported [44]. Moreover, urinary AFM1 among school-age children (93%) [45], pregnant women (72.5%) [46], and young children (7%) [47] has also been reported in Ethiopia. Furthermore, studies conducted in Lebanon (93.8%, range: 0.0002–0.0079 ng/mL, LOQ = 0.0002 ng/mL) [48], India (41%, range: 0.00391.2 ng/mL, LOD = 0.0078 ng/mL) [49], Ecuador (13%, range: 0.053-0.458 ng/mL, LOD = 0.033 ng/mL) [2], and Guatemala (5%, range: 0.004–0.333 ng/mL, no LOD) [50] also consistently reported AFM1 in breast milk samples. This discrepancy might be due to the fact that the above-mentioned studies reported a much lower

LOD and LOQ compared to our study. This may also imply that the concentrations reported in the studies might not be high.

In addition, contrary to our findings, other mycotoxins such as beauvericin, alternariol monomethyl ether, ennaintins, OTA and B, sterigmatocystin, ZEN, AFB2, AFG2, and aflatoxin M2 have been intermittently reported in breast milk by several studies [49, 51-53].

Differences in mycotoxin concentrations between our study and others might be attributable to differences in analytical equipment performance including the LOD and LOQ, biological matrix analyzed, breast milk sampling method, sample size, and pre- and post-harvest methods.

The different analytical approaches used in various studies might explain the differences in the mycotoxins detected. The specificity of analytical equipment used in detecting mycotoxins in human milk has been questioned [6]. LC-MS/MS offers great promise in terms of specificity and accuracy of analyte identification, but ELISA methods are less specific [6, 54].

Some of the studies compared with our findings monitored mycotoxins in urine samples and others in serum samples. Urinary mycotoxin exposure shows recent exposure depending on the type of food consumed. Instead, breast milk is a complex matrix, and transfer rates from diet appear to be lower. For instance, the FB1 consumed may be retained and excreted in biological fluids before getting to breast milk [7]. Determining mycotoxin levels both in maternal blood plasma and in milk is the prime approach to estimate lactational transfer in humans [55]. Except for AFs and OTA, data on the lactational transfer of most mycotoxins including FUMs are insufficient [6, 51, 55, 56].

Moreover, the difference between our findings and others could be due to variations among the studies in breast milk sampling. Some of the studies focused on early lactation, while others targeted lactating mothers of 6 months and onwards. Other factors such as the timing of the day, hind or fore milk, and the order that an infant is born in a family may introduce a difference in the stability of toxins and may affect the mycotoxins excretion in breast milk [6, 55, 57].

On top of the sampling, the variation in the sample sizes accounted for and their respective power between our study and others might affect the proportion of the mycotoxins detection. Though all lactating women found in the study areas during our survey time were included, the limited number of lactating women found may under power our study.

On the other hand, the good pre- and post-harvest practices reported by the women might have played a significant role in controlling mycotoxin production or decontaminating the food crops [37]. This might imply that the chance for fungi development and production of their respective

mycotoxins in the staple food, i.e., maize, consumed by the women has been minimized. On top of this, the reported practice of sorting moldy maize grains (97.8%) might have also meaningfully reduced the chance of consuming mycotoxin-contaminated maize crops by the women [34]. The sorted-out maize grains were mainly used for feed (71.7%). However, the chance of the women consuming mycotoxins transferred from contaminated feed [58] might have decreased due to the low consumption of milk and milk products (46%) and other animal products, i.e., meat (7%) and eggs (8%). On top of that, weather variability and the subsequent agricultural practices observed between and within seasons in Ethiopia might make mycotoxins prediction difficult [59].

The regression analysis provided no evidence for a statistically significant association between total FUMs and women's pre- and post-harvest and dietary practices. Instead, it did identify the age of the women as a predisposing factor for the occurrence of FUMs in breast milk samples. The few studies on FUMs occurrence in breast milk provided no information on the contributing factors. Thus, a comparison between our findings with these studies cannot be sought. We suggest further similar studies to perform multiple regression analysis considering more confounding variables.

Lastly, despite the absence of occurrence of the common mycotoxins in our study, the knowledge gap observed among the lactating women on some of the basic understandings about mycotoxins was not negligible. For instance, approximately half of the mothers did not consider the health implications of mycotoxin consumption. Thus, disseminating information to communities on ways how to manage moldy foods and feeds and preventing mycotoxin exposure is important [14].

Strengths and weaknesses of the study

We analyzed 16 mycotoxins in breast milk samples. Moreover, we used LC-MS/MS which is a highly specific analytical technique in detecting mycotoxins at low concentrations. However, as a multi-mycotoxin method was used, LODs varied from one mycotoxin to another and reduced the sensitivity. Data from longitudinal studies are suitable to capture temporal sequences and generate cause and effect relations [60]. Thus, the cross-sectional survey we employed was unable to examine the stage of lactation and seasons as possible factors for mycotoxins occurrence in the breast milk samples. It is likely that the relatively small sample size used in our study might have also influenced the statistical power and the subsequent statistical inferences, i.e., the coefficients and *p*-values of the regression result [61-63]. Moreover, we sampled only breast milk and tried to associate the mycotoxin result with the

dietary and pre- and post-harvest practices recall data. Incorporating data on mycotoxin food contamination, which is an important factor linking biological samples to pre- and post-harvest practices, could offer a partial explanation for the regression findings of our study. This is because some mycotoxins in breast milk may represent metabolized forms of the original mycotoxins found in food. Due to these limitations, the findings of our study should be interpreted with caution.

4.5 Conclusions

No significant exposure to most of the mycotoxins was recorded, with the exception of modest exposure to FB2 and FB3. Irrespective of the absence of common mycotoxins, however, limited knowledge among the study participants about mycotoxins is warranted. This stresses the need for mycotoxin sensitization of the households in the study areas. A possible association between the ages of the women with FUMs exposure was ruled out; however, this was not the case for any of the pre- and post-harvest and dietary practices. Hence, to understand mycotoxin presence in breast milk, there is a need robust epidemiological studies, such as case-control studies, that consider food contamination data, involve larger sample sizes, and cover various lactation stages.

4.6 References

1. Agriopoulou S., Stamatelopoulou E. and Varzakas T. Advances in occurrence, importance, and mycotoxin control strategies: prevention and detoxification in foods. Foods. 2020; 9 (2).

2. Ortiza J., Jacxsensa L., Astudillob G., Ballesterosb A., Donosob S., Huybregtsa L. and De Meulenaera B. Multiple mycotoxin exposure of infants and young children via breastfeeding and complementary/weaning foods consumption in Ecuadorian highlands. 2018; 118 541-548.

3. Alvito P. and Pereira-da-Silva L. Mycotoxin exposure during the first 1000 days of life and its impact on children's health: A clinical overview. Toxins. 2022; 14 (189).

4. Chilaka C., A. and Mally A. Mycotoxin occurrence, exposure and health implications in infants and young children in Sub-Saharan Africa: A review. Foods. 2020; 9 (11).

5. BIOMIN. World mycotoxin survey. The global threat, January – September 2021. 2021, BIOMIN: Pottenbrunn, Austria.

6. Warth B., Braun D., Ezekiel C., Turner P., Degen G. and Marko D. Biomonitoring of mycotoxins in human breast milk: current state and future perspectives. Chem Res Toxicol. 2016; 29 (7): 1087-97.

7. Magoha H., Kimanya M., De Meulenaer B., Roberfroid D., Lachat C. and Kolsteren P. Risk of dietary exposure to aflatoxins and fumonisins in infants less than 6 months of age in Rombo, Northern Tanzania. Matern Child Nutr. 2016; 12 (3): 516-27.

8. Matumba L., Namaumbo S., Ngoma T., Meleke N., De Boevre M., Logrieco A. and De Saeger S. Five keys to prevention and control of mycotoxins in grains: A proposal. Global Food Security. 2021; 30.

9. Karlovsky P., Suman M., Berthiller F., De Meester J., Eisenbrand G., Perrin I., Oswald I., Speijers G., Chiodini A., Recker T. and Dussort P. Impact of food processing and detoxification treatments on mycotoxin contamination. Mycotoxin Res. 2016; 32 (4): 179-205.

10. IARC. Mycotoxin control in low- and middle income countries, Wild C.P. ,Miller J.D. and Groopman J.D., Editors. 2015, IARC working group report no. 9.

11. Logrieco A., Miller J., Eskola M., Krska R., Ayalew A., Bandyopadhyay R., Battilani P., Bhatnagar D., Chulze S., De Saeger S., Li P., Perrone G., Poapolathep A., Rahayu E., Shephard G., Stepman F., Zhang H. and Leslie J. The Mycotox Charter: increasing awareness of, and concerted action for minimizing mycotoxin exposure worldwide. Toxins (Basel). 2018; 10 (4).

12. Muhie O. and Bayisa A. Is aflatoxin a threat to human-health in Ethiopia? a systematic review. International Journal of Collaborative Research on Internal Medicine & Public Health. 2020; 12 (4).

13. Mamo F., Abate B., Tesfaye K., Nie C., Wang G. and Liu Y. Mycotoxins in Ethiopia: A review on prevalence, economic and health impacts. Toxins (Basel). 2020; 12 (10).

14. Beyene A.A., Woldegiorgis A.Z., Adish A.A., De Saeger S. and Tolossa A.L. Assessment of mothers' knowledge and practice towards aflatoxin contamination in complementary foods in Ethiopia: from pre-harvest to household. World Mycotoxin Journal. 2016; 9 (4): 535-544.

15. Ayelign A. and De Saeger S. Mycotoxins in Ethiopia: Current status, implications to food safety and mitigation strategies. Food Control. 2020; 113.

16. Eshete M., Gebremedhin S., Alemayehu F., Taye M., Boshe B. and Stoecker B. Aflatoxin contamination of human breast milk and complementary foods in southern Ethiopia. Matern Child Nutr. 2021; 17 (1): e13081.

17. Mesfin A., Tesfamariam K., Belachew T., De Saeger S., Lachat C. and De Boevre M. Multi-mycotoxin profiling in maize reveals prevalence of Fusarium mycotoxins in South and West Ethiopia. World Mycotoxin Journal. 2022; 15 (1): 73-83.

18. Tesfamariam K., Argaw A., Hanley-Cook G., Gebreyesus S., Kolsteren P., Belachew T., Van de Velde M., De Saeger S., De Boevre M. and Lachat C. Multiple mycotoxin exposure during pregnancy and risks of adverse birth outcomes: a prospective cohort study in rural Ethiopia. Environ Int. 2022; 160 107052.

19. FAO. Minimum dietary diversity for women. 2021, FAO: Rome, Italy.

20. Gibson R. Principles of nutritional assessment. 2005: Oxford University Press.

21. Coates J., Swindale A. and Bilinsky P. Household Food Insecurity Access Scale (HFIAS) for measurement of food access: indicator guide ver 3. 2007, FANTA Academy for Educational Development.

22. Ndemera M., Landschoot S., De Boevre M., Nyanga L.K. and De Saeger S. Effect of agronomic practices and weather conditions on mycotoxins in maize: a case study of subsistence farming households in Zimbabwe. World Mycotoxin Journal. 2018; 11 (3): 421-436.

23. Martins C., Assuncao R., Costa A., Serrano D., Visintin L., De Boevre M., Lachat C., Vidal A., De Saeger S., Namorado S., Vidigal C., Almeida E., Alvito P. and Nunes C. earlyMYCO: A pilot mother-child cohort study to assess early-life exposure to mycotoxins-challenges and lessons learned. Int J Environ Res Public Health. 2022; 19 (13).

24. communities. T.c.o.t.E. Commision decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, in 2002/657/EC), Union E., Editor. 2002, Official Journal of the European Communities.

25. RCoreTeam. R: A language and environment for statistical computing. 2021.

26. Henningsen A. censReg: censored regression (tobit) models R package version 0.5. 2017.

27. Jolliffe I. Principal component analysis 2ed ed. Springer Series in Statistics 2002, New York USA: Springer.

28. Alassane-Kpembi I., Schatzmayr G., Taranu I., Marin D., Puel O. and Oswald I. Mycotoxins co-contamination: Methodological aspects and biological relevance of combined toxicity studies. Crit Rev Food Sci Nutr. 2017; 57 (16): 3489-3507.

29. Mueller A., Schlink U., Wichmann G., Bauer M., Graebsch C., Schuurmann G. and Herbarth O. Individual and combined effects of mycotoxins from typical indoor moulds. Toxicol In Vitro. 2013; 27 (6): 1970-8.

30. Tessema M., De Groote H., Brouwer I.D., De Boevre M., Corominas A.V., Stoecker B.J., Feskens E.J., Belachew T., Karakitsou A. and Gunaratna N.S. Exposure to aflatoxins and fumonisins and linear growth of children in rural Ethiopia: a longitudinal study. Public Health Nutr. 2021; 24 (12): 3662-3673.

31. Coppa C.F.S.C., Cirelli A.C., Gonçalves B.L., Barnab E.M.B., Petta T., Franco L.T., Javanmardi F., Khaneghah A.M., Lee S.H.I., Corassin C.H. and Oliveira C.A.F. Mycotoxin occurrence in breast milk and exposure estimation of lactating mothers using urinary biomarkers in Sao Paulo, Brazil. Environmental Pollution. 2021; 279.

32. Lee H. and Ryu D. Worldwide occurrence of mycotoxins in cereals and cereal-derived food products: Public health perspectives of their co-occurrence. Agric. Food Chem. 2017; 65 7034–7051.

33. Wu F., Groopman J.D. and Pestka J.J. Public health impacts of foodborne mycotoxins. Annu Rev Food Sci Technol. 2014; 5 351-72.

34. Ezekiel C., Ortega-Beltran A. and Bandyopadhyay R. The need for integrated approaches to address food safety risk: the case of mycotoxins in Africa, in The Future of Food Safety, First FAO/WHO/AU International Food Safety Conference. 2019: Addis Ababa, Ethiopia.

35. Carbas B., Soares A., Freitas A., Silva A., Pinto T., Andrade E. and Brites C. Mycotoxin incidence in pre-harvest maize grains. 2020.

36. Smith L., Prendergast A., Turner P., Mbuya M., Mutasa K., Kembo G. and Stoltzfus R. The potential role of mycotoxins as a contributor to stunting in the SHINE trial; for the

Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial team. Clin Infect Dis. 2015; 61 Suppl 7 S733-7.

37. Rose L.J., Okoth S., Flett B.C., Van Rensburg B.J. and Viljoen A. Preharvest management strategies and their impact on mycotoxigenic fungi and associated mycotoxins, in Mycotoxins - Impact and Management Strategies. 2019, IntechOpen: London, UK.

38. Gelineau-van Waes J. Fumonisins, in Reproductive and Developmental Toxicology. 2022, Elsevier: Amsterdam, The Netherlands. 955-981.

39. Tesfamariam K., De Boevre M., Kolsteren P., Belachew T., Mesfin A., De Saeger S. and Lachat C. Dietary mycotoxins exposure and child growth, immune system, morbidity, and mortality: a systematic literature review. Crit Rev Food Sci Nutr. 2020; 60 (19): 3321-3341.

40. IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC press. 2002.

41. Kamle M., Mahato D.K., Devi S., Lee K.E., Kang S.G. and Kumar P. Fumonisins: Impact on agriculture, food, and human health and their management strategies. Toxins (Basel). 2019; 11 (6).

42. Duarte S., Silva L., Pereira A., Gimbi M., Cesar C., Vidal V., Basilio R., Almeida A., Lino C. and Pena A. Mycotoxins exposure in Cabinda, Angola-a pilot biomonitoring survey of breastmilk. Toxins (Basel). 2022; 14 (3).

43. Boshe B., Gebremedhin S., Alemayehu F., Eshete M., aye M. and Stoecker B.J. Aflatoxin exposure among lactating women in southern Ethiopia. Food Science & Nutrition. 2020; 8 (12).

44. Ayele M., Haile D., Alonso S., Sime H., Abera A., Balcha K., Roba K., Guma G. and Endris B. Aflatoxin exposure among children of age 12–59 months in Butajira district, South-Central Ethiopia: a community based cross-sectional study. BMC Pediatrics. 2022; 22.

45. Gebreegziabher T., Dean M., Elias E., Tsegaye W. and Stoecker B. Urinary aflatoxin M1 concentration and its determinants in school-age children in Southern Ethiopia. Nutrients. 2022; 14 (13).

46. Tsegaye W., Fereja M., Gebreegziabher T. and J. Stoecker B. Urinary aflatoxin M1 concentrations among Pregnant momen in Bishoftu, Ethiopia. 10.1096/fasebj.30.1_supplement.1149.28FASEB. 2016.

47. Ayelign A., Woldegiorgis A.Z., Adish A., De Boevre M., Heyndrickx E. and De Saeger S. Assessment of aflatoxin exposure among young children in Ethiopia using urinary

biomarkers. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2017; 34 (9): 1606-1616.

48. Elaridi J., Bassil M., Kharma J., Daou F. and Hassan H. Analysis of aflatoxin M1 in breast milk and its association with nutritional and socioeconomic status of lactating mothers in Lebanon. J Food Prot. 2017; 80 (10): 1737-1741.

49. Mehta R., Wenndt A., Girard A., Taneja S., Ranjan S., Ramakrishnan U., Martorell R., Ryan P., Rangiah K. and Young M. Risk of dietary and breastmilk exposure to mycotoxins among lactating women and infants 2-4 months in northern India. Matern Child Nutr. 2021; 17 (2): e13100.

50. Jolly E.P., Mazariegos M., Contreras H., Balas N., Junkins A., Aina I., Minott S., Wang M. and Phillips T. Aflatoxin exposure among mothers and their infants from the western highlands of Guatemala. Matern Child Health. 2021; 25 (8): 1316–1325.

51. Braun D., Abia W., Sarkanj B., Sulyok M., Waldhoer T., Erber A., Krska R., Turner P.C., Marko D., Ezekiel C. and Warth B. Mycotoxin-mixture assessment in mother-infant pairs in Nigeria: From mothers' meal to infants' urine. Chemosphere. 2022.

52. Ferrufino-Guardia E., Chavez-Rico V. and Larondelle Y. Ochratoxin a in human breast milk, maternal and placental blood from Cochabamba-Bolivia. Asociación Española de Toxicología. 2019; 36 (2): 116-125.

53. Ezekiel C., Abia W., Braun D., Sarkanj B., Ayeni K., Oyedele O., Michael-Chikezie E., Ezekiel V., Mark B., Ahuchaogu C., Krska R., Sulyok M., Turner P. and Warth B. Mycotoxin exposure biomonitoring in breastfed and non-exclusively breastfed Nigerian children. Environment International. 2022; 158.

54. Cebin Coppa C., Mousavi Khaneghah A.M., Alvito P., Assunção R., Martins C., Eş I., Gonçalves B., De Neeff D.V., Sant'Ana A., Corassin C. and Oliveira C. The occurrence of mycotoxins in breast milk, fruit products and cereal-based infant formula: A review. Trends in Food Science & Technology. 2019; 92 81-93.

55. Degen G., Muñoz K. and Hengstler J. Occurrence of mycotoxins in breast milk, in Handbook of dietary and nutritional aspects of human breast milk. 2013, Wageningen Academic Publishers: Wageningen, The Netherlands. 813-832.

56. Tonon M., R. Reiter G. and Scussel M. Mycotoxins levels in human milk: a menace to infants and children health. Current Nutrition & Food Science,. 2013; 9 33-42.

57. Renfrew M., Hay A., Shelton N., Law G., Wallis S., Madden S., Shires S., Sutcliffe A. and Woolridge M. Assessing levels of contaminants in breast milk: methodological issues and a framework for future research. Paediatr Perinat Epidemiol. 2008; 22 (1): 72-86.

58. Tolosa J., Rodriguez-Carrasco Y., Ruiz M.J. and Vila-Donat P. Multi-mycotoxin occurrence in feed, metabolism and carry-over to animal-derived food products: A review. Food Chem Toxicol. 2021; 158 112661.

59. Zereyesus Y., Birkelo C. and van-der Merwe D. Mycotoxin prevalence and mitigation measures in Ethiopia. 2020, Feed the Future Innovation Lab for Livestock Systems: Gainesville, FL, USA.

60. Ramji S. Study design: observational studies. Indian pediatrics. 2022; 59.

61. Dochtermann N. and Jenkins S. Multivariate methods and small sample sizes. Ethology. 2011; 117 (2): 95-101.

62. Faber J. and Fonseca L. How sample size influences research outcomes. Dental Press J Orthod. 2014; 19 (4): 27-9.

63. Serdar C., Cihan M., Yucel D. and Serdar M. Sample size, power and effect size revisited: simplified and practical approaches in pre-clinical, clinical and laboratory studies. Biochem Med (Zagreb). 2021; 31 (1): 010502.

Chapter 5. Essential descriptors for mycotoxin contamination data in food and feed

Redrafted from: Mesfin A., Lachat C., Vidal A., Croubels S., Haesaert G., Ndemera M., Okoth S., Belachew T., Boevre M., De Saeger S. and Matumba L. Essential descriptors for mycotoxin contamination data in food and feed. Food Res Int. 2022; 152 (1). doi: 10.1016/j.foodres.2021.110883

5.1 Abstract

Mycotoxin food contamination data is scattered, isolated, and poorly described. Reporting mycotoxin contamination data in a standardized manner is essential for collaborative research and integrated large-scale data analysis. The present study aimed to complement the existing European Food Safety Authority (EFSA) and Global Environment Monitoring System (GEMS) mycotoxin contamination data descriptors for application in LMICs in particular. A three-round Delphi process was followed to establish a consensus on the missing descriptors among the MYTOX-SOUTH® mycotoxin experts. Most (75%) of the MYTOX-SOUTH® study participants were professors from 6 universities. Twenty-two descriptors (17 study levels, 1 sample level, and 4 assay levels) were proposed by the participants which were mainly related to pre- and post-harvest periods of a food/feed sample. The existing descriptors from EFSA (33) and GEMS (25) with the new proposed MYTOX-SOUTH® (22) descriptors, a total of 80 descriptors, were arranged as study, sample, and assay categories and organized as a data submission template. The current format helps mycotoxin contamination data to become more informative, reusable, and applicable especially to data from LMICs. A standardized global reporting format for mycotoxin contamination data will enable national authorities to perform mycotoxins exposure and risk assessments and share data for international benchmarking. Standardized reporting and sharing of mycotoxin contamination data should be further advocated in ongoing research and become common practice in authorities, companies, academia, and other entities working on mycotoxin in food and feed.

Keywords: MYTOX-SOUTH®, EFSA, GEMS, Delphi, Consensus

5.2 Introduction

Continuous monitoring of mycotoxins in food and feed products is key to prevent significant problems in marketing, distribution, and consumption [1]. To date, mycotoxins contamination data are generated from a variety of settings, crops, and analytical methods. They are typically generated from a limited number of mostly unrepresentative samples and some are analyzed with uncertified laboratory reference procedures [2-4]. Furthermore, the existing data are scattered, isolated, and poorly described, which challenges a comprehensive assessment of mycotoxins exposure.

Given the myriad of challenges associated with varying analytical methodologies, different mycotoxins contamination data may be stored in different formats using different database systems and information models. Reporting mycotoxins contamination data in a standardized manner is essential for internal consistency, collaborative research, and large-scale analysis, including and not limited to comparability between datasets [5]. A long-term vision on the curation of mycotoxins contamination data and the development of specific tools (*e.g.* database, study descriptors, and data reporting templates) is necessary to conduct mycotoxins research at scale, assess temporal trends, or conduct foresight modeling. The first step is to ensure a harmonized and structured description of mycotoxins contamination data [6]. A data descriptor provides information needed for interpretation and (re)use of data. Consensus on data descriptors facilitates data harmonization and contributes to making data more discoverable, interpretable, and reusable [7].

The WHO, in partnership with the FAO, collects food contamination data from nationally recognized institutions through the Food Contamination Monitoring and Assessment Programme of the WHO Global Environment Monitoring System, commonly known as GEMS/FOOD. This template contains twenty-two data fields/descriptors for food contamination [8]. Likewise, the EFSA is mandated by the European Commission to collect all available data on the occurrence of different chemical contaminants in food and feed. The EFSA data collection template contains 94 elements/descriptors for data collection of all chemical food contaminants and 7 specific elements for mycotoxins [9].

To date, however, there is no common set of data descriptors for mycotoxin contamination data. Here, we propose a minimal set of study, sample, and assay level variables to describe food/feed mycotoxin contamination data. The ultimate aim is to contribute to a comprehensive set of data descriptors that are globally applicable without any distinction of the sources.

The proposed descriptors collate the existing GEMS/FOOD and EFSA variables and propose an additional set of optimized descriptors to increase applicability, especially to data from LMICs. To ensure transparency in information provenance, descriptors are organized by source (EFSA, GEMS, and newly added "MYTOX-SOUTH®" descriptors). The EFSA reporting document for contaminants and food additives contains more extensive descriptors. For this research work, the EFSA simplified Standard Sample Description 2 (SSD2) data submission format was used.

The study presents a consensus process within the MYTOX-SOUTH® consortium, a global partnership of over 25 institutions from 15 countries. It involved experts with different professional backgrounds like agronomy, mycology, plant sciences, animal sciences, pharmaceutical sciences, toxicology, nutrition and food sciences, and analytical chemistry to address mycotoxins in one health food systems (https://mytoxsouth.org/).

5.3 Materials and Methods

A Delphi method was implemented using a similar approach used to develop essential items for Strengthening the Reporting of Observational Studies in Epidemiology-Nutritional Epidemiology (STROBE-nut) [10]. A Delphi method is a structured group process that is used to collect and assess the opinions of a group of experts. It is a practical way to collect information and to reach a consensus between experts that are unable to convene physically. The Delphi technique allows disagreements between participants and the collection of diverse opinions without causing conflicts. Participants are not influenced by the ideas of others as they are not exposed to individual responses [11]. A three-stage Delphi procedure usually suffices to reach a consensus and collect the needed information [12, 13].

A team composed of researchers from MYTOX-SOUTH® research institutions coordinated the study using online and physical meetings. There were no predefined criteria for the number of participants in a Delphi study. Yet, most studies suggested ten to fifteen subjects could be sufficient if the background of the Delphi subjects is homogeneous [12]. Hence for the present study, an invitation email was first sent to 34 researchers from a variety of academic and research institutions and disciplines. Snowballing was encouraged to recruit as many participants as possible. An invitation letter together with informed consent was sent and participants were given three weeks to reply. One reminder email was sent after two weeks. The participants provided essential input for the list of descriptors through email. They agreed to propose only a minimal number of new items to reduce the workload of data providers as much as possible.

A three-stage Delphi procedure was conducted with three meetings of the coordinating team following each Delphi round (**Figure 5.1**). The coordinating team processed the comments from each Delphi round and sent back documents for feedback. Space for a free-text response was provided in the templates for participants to elaborate on responses in each round.

5.3.1 Delphi I

In the Delphi round I, open-ended questions were sent to the participants and ten of them provided lists of mycotoxin contamination data descriptors (Supplementary Table I). The first face-to-face meeting of the coordinating team was conducted on the proposed lists of descriptors. The data descriptors followed a modular structure as proposed by the ISA-tab (Investigation/Study/Assay) protocol [14], similar to previous data descriptors for nutrition research [15]. By merging items into themes, and removing unclear items or those with lower priority, the number of descriptors was kept at a minimum. The descriptors used by EFSA and

GEMS were then added and all re-arranged together with newly obtained descriptors as study, sample, and assay level descriptors.

5.3.2 Delphi II

The combined list of descriptors from the Delphi round I was sent to the participants for review in the Delphi II (Supplementary Table II). The list of descriptors was then prepared as dichotomous questions, to assess the level of agreement on the items included. The consultation also contained the possibility to suggest a new number of items. The inputs of the participants were discussed in the second face-to-face meeting of the coordinating team and items with a consensus of >70% were retained.

5.3.3 Delphi III

In the Delphi Round III, the questions were similar to those asked during the Delphi II round and considered the refined items obtained from the Delphi Round II and meeting II (Supplementary Table III). This round allowed the participants to reach an acceptable consensus and stability of answers within the group. The consensus was set to be achieved as an agreement of >80% in this round. After the three Delphi rounds were completed, the arranged descriptors were then adapted to the EFSA simplified SSD2 online data submission template with its instruction document. This template was pre-tested with a sample of three mycotoxin researchers from the Center of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium to collect feedback on the clarity of wording and time needed to fill out the information.

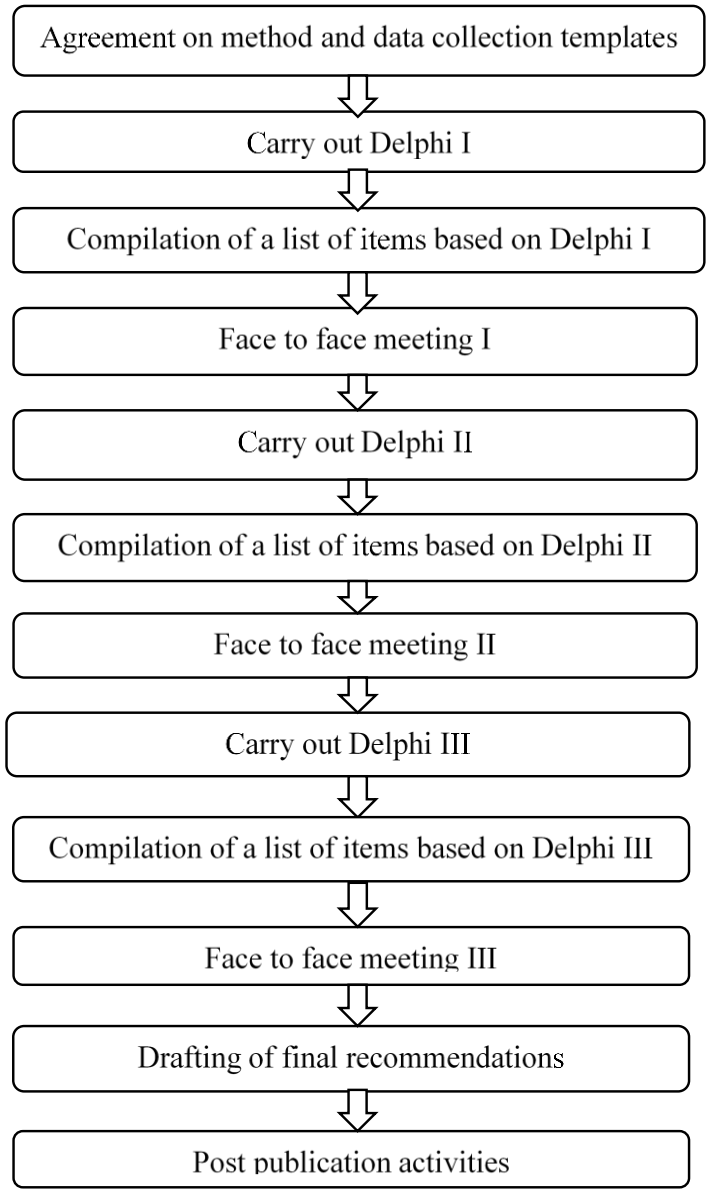


Figure 5.1 Steps followed for the development of study descriptors for mycotoxin contamination data

5.3.4 Ethical considerations

An information letter and informed consent were distributed first. Consent was obtained by email. Participants' confidentiality was ensured since only the researcher and the principal investigator had access to aspects like the origin of the feedback opinions and responses during the Delphi rounds.

5.4 Results and Discussion

5.4.1 Results

Demographic characteristics of the study participants

First, an invitation email was sent to 34 researchers from different MYTOX-SOUTH® institutions and disciplines, and 14 of them replied to the email. However, only 10 gave their consent and participated in the first Delphi round and 9 (7 participants from the first round and 2 additional experts) participated in the second Delphi round and 10 in the third Delphi round. The response rate was 29.4% (10/34) in the Delphi I, 75% (9/12) in the Delphi II, and 83.3% (10/12) in the Delphi III rounds. Half (50%) of the participants were from Belgium, and the rest were from Tanzania, Kenya, Argentina, Spain, and Zimbabwe. The majority (83%) were female. By profession, 25% were from pharmaceutical sciences and others were from different disciplines such as food safety, agronomy, veterinary, nutrition, food science, and analytical chemistry. All reported working in the university as academic staff and most (75%) were full professors.

Participants' consensus on the summarized descriptors

In the Delphi I round, 62 descriptors were suggested by participants, of which 35 were already found to be part of either the EFSA or GEMS mycotoxin contamination data descriptors. Seven unclear and less-priority descriptors were removed. The remaining 20 descriptors were then rated by the participants in the Delphi II. Two additional descriptors were suggested at this stage. In the Delphi III round, a total of 22 descriptors (17 study levels, 1 sample level, and 4 assay levels) were considered. In the Delphi II, >70% agreement was achieved for the 20 descriptors, and in the Delphi III, >80% agreement was achieved for the 22 descriptors. In the Delphi III round, almost all of the study and sample-level descriptors achieved the highest (100%) consensus among participants.

The specific MYTOX-SOUTH® descriptors retained in the final round are listed in Tables 1, 2 & 3 side by side with the EFSA and GEMS descriptors. An empty space in the MYTOX-SOUTH® descriptors column indicates that the original EFSA or GEMS descriptor alone was considered adequate and no additional descriptor was proposed by the MYTOX-SOUTH® consortium.

A total of 17 MYTOX-SOUTH® mycotoxin data descriptors were added at the study level namely: sampling location; sampling from lot; pre-harvest condition of the crop (7 descriptors); post-harvest condition of the crop (7 descriptors); and any treatment to prevent mycotoxin

contamination (**Table 5.1**). Further information about the 17 data descriptors at a study level is provided in the subsequent sections.

MYTOX-SOUTH® -5.1. *Sampling location*

If the crop or commodity is sampled from a farm or storage, specific information should be provided about the specific region/s, district/s, village/s of sampling with additional GPS coordinates information if any.

MYTOX-SOUTH® -10.1**.** *Sampling from lot*

Lot weight should preferably be expressed in tones. If the lot or sub-lot consists of individual packages or units, however, then the number of packages or unit weight or the number of sub-lots should be expressed. To apply the sampling method, the weight and number of separate and identifiable parts of a large lot need to be described. Subsequently, the combined total weight of all the incremental samples taken from the lot or sub-lot (kg) should be reported as well.

MYTOX-SOUTH® -12.1**.** *Pre-harvest condition of the crop*

The type (e.g. hybrid, synthetic variety…) of the crop variety used; type of cultivation method employed while growing the crop; maturity level of the crop at harvest (e.g. premature, mature, dry); previous crop; the soil management method applied (e.g., plowing, minimum tillage, zero tillage); application of fungicide/insecticide, and any natural disaster such as drought or locust occurred during the growing of the crop are considered essential pieces of information in this regard.

MYTOX-SOUTH® -12.2. *Post-harvest condition of the crop*

Related to the post-harvest period of the sampled crop, mode of transportation used from field to storage, mechanism/s implemented for ventilation; mechanism/s implemented to control temperature and moisture; application of fungicide/insecticide in storage; duration of the crop stored before the sampling date and the type of the storage used(e.g. metal silo bins, hermetic bags, insecticide-treated bags, underground pits, plastic bags, storage made from wood/straw and mud) are important information.

MYTOX-SOUTH® -13.1. *Any treatment to prevent mycotoxin contamination*.

Information related to any special treatment (e.g. biocontrol, genetically modified variety) applied to prevent mycotoxin contamination of the sampled food/feed should be provided. Such information supports a better interpretation of the data.

Table 5.1. Study level descriptors for mycotoxin contamination data suggested by the MYTOX-SOUTH® consortium and integrated with EFSA and GEMS descriptors

Descriptor number	EFSA descriptor	GEMS descriptor	MYTOX SOUTH® descriptor
1	Local organization identification code	Country providing the record	
	Country	Country of food origin	
2	Sampling program identification code		
	Legal reference		
	Program type		
3	Sampling method		
	Sampler		
	Sampling point		
4	Sampling event identification code		
	Sampling unit type and size		
	Other sampling unit identifications		
5	Sample taken identification code		MYTOX-SOUTH® -5.1. Sampling location
	Country providing therecord		
	Area of sampling		
6	Date of food sampling	Time period of food sampling	
7	Sampling strategy: describe how the sample was selected from the population being monitored or surveyed	Representativeness of the samples	
8	Sample taken size and unit		
	Additional sample taken information		
9	Sampling method: define the way the samples have been collected for analysis		
10	Sampling point: define the point of the food chain where the sample was taken		MYTOX-SOUTH® -10.1. Sampling from lot
11	Country and area of processing of the sample taken		

Descriptor number	EFSA descriptor	GEMS descriptor	MYTOX SOUTH® descriptor
12	Method of production: recommended to report weather the sample was obtained from the produce of traditional farming		MYTOX-SOUTH® -12.1. Pre-harvest condition of the crop 12.1.1. type of the crop variety used 12.1.2. type of cultivation method employed while growing the crop 12.1.3. maturity level of the crop at harvest 12.1.4. previous crop 12.1.5. soil management method applied 12.1.6. application of fungicide/insecticide 12.1.7. any natural disaster such as drought or locust occurred MYTOX-SOUTH® - 12.2. Post-harvest condition of the crop 12.2.1. mode of transportation used from field to storage 12.2.2. mechanism/s implemented for ventilation 12.2.3. mechanism/s implemented to control temperature 12.2.4. mechanism/s implemented to control moisture 12.2.5. application of fungicide/insecticide in storage 12.2.6 duration of the crop stored before the sampling date 12.2.7. type of the storage used
13	Additional information on the matrix sampled		MYTOX-SOUTH® -13.1. Any treatment to prevent mycotoxin contamination

At the sample level, one MYTOX-SOUTH® mycotoxin contamination data descriptor was added and this was related to commodity intended use (**Table 5.2**).

MYTOX-SOUTH® -13.1. *Commodity intended use*

The commodity type as food or feed should be first stated and then the intended use of the food or feed sampled should be further described. If for feed, specific information about the type of animal species intended for the feed is necessary. On the other hand, if the sample is food for human consumption, the last consumer (e.g. infant, adult, therapeutic/supplementary food) should be specified.

Table 5.2. Sample level descriptors for mycotoxin contamination data suggested by the MYTOX-SOUTH® consortium and integrated with EFSA and GEMS descriptors

Descriptor number	EFSA descriptor	GEMS descriptor	MYTOX-SOUTH® descriptor
1		Serial number of the record	
2	Laboratory sample code		
3		Date of record creation	
4	Date of sample analysis		
5	Sample analyzed identification code		
6	Sample analysis reference time		
7	Language		
8	Product code	Food identifier	
9	Type and description of matrix Type of sample taken (e.g. food, feed) Identifying the sub-domain of the matrix catalogue to be used		
10		Number of samples analysed	
11	Sample analyzed portion sequence and size with unit		
12	Additional information on the sample analyzed portion		
13	Product full text description		MYTOX-SOUTH® -13.1. Commodity intended use: the intended consumer in case of food and the intended animal species in case of feed
14	Product treatment: clearly indicate if the original sample is treated or not		
15	Product comment		
16	Additional information on the sample analyzed		

At the assay level, four MYTOX-SOUTH® mycotoxin contamination data descriptors were included (**Table 5.3**) and these include the number of samples with concentrations below the limit of detection; mycotoxins legislation available for the country; description of the reference for mycotoxins legislation, and; mycotoxins maximum limits of the country.

MYTOX-SOUTH® -16.1. *Number of samples with concentrations below the LOD*

The number and percentage of samples whose concentrations are below the LOD of a specific mycotoxin should be reported.

MYTOX-SOUTH® -30.1. *Mycotoxins legislation available for the country*

The data provider should specify whether specific mycotoxins legislation is available in the country.

MYTOX-SOUTH® -30.2. *Reference for mycotoxins legislation*

If the country from where the data is provided has mycotoxins legislation, the reference for the legislation should be described.

MYTOX-SOUTH® -30.3. *Mycotoxins maximum limits of the country*

The percentage of samples that have mycotoxin concentrations above the maximum limit established by the legal authority of the country should be reported.

Table 5.3. Assay level descriptors for mycotoxin contamination data suggested by the MYTOX-SOUTH® consortium and integrated with EFSA and GEMS descriptors

Descriptor number	EFSA descriptor	GEMS descriptor	MYTOX-SOUTH® descriptor
1	Laboratory identification code		
2		Number of laboratories participating in sample analyses	
3	Laboratory accreditation	Indicator of analytical quality assurance	
4	Additional information on the laboratory		
5	Type and description of the parameter	Contaminant identifier	
6	Analytical method type reference code		
7	Additional information on the analytical method		
8	Description of the analyzed matrix		
9	Additional information on the analyzed matrix		
10	Result identification code		
11	Accreditation procedure for the analytical method		
12		Basis for the analytical values	
13	Result unit	Unit of reporting for contaminant levels	
14		Range of quantified analytical concentrations	
15	Result lower and upper limit of the working range CC alpha and beta	Range of analytical limits in the data set. E.g. LOQ, LOD	
16		Number of samples with concentrations below LOQ	MYTOX-SOUTH® - 16.1. Number of samples with concentrations below LOD
17	Type of result		
18		Mean/median concentration	
19		90th Percentile Concentration	
20		Standard Deviation (Optional)	
21	Result value recovery		

Descriptor number	EFSA descriptor	GEMS descriptor	MYTOX-SOUTH® descriptor
22	Result value corrected for recovery		
23	Result value uncertainty		
24	Expression of result percentage/ comment of the result: e.g. in case the sample was reconstituted before analysis		
25	Type of limit for the result evaluation		
26	Evaluation of the result		
27	Action taken		
28	Additional information on the evaluation		
29	Clarification note on result value Result value recovery rate Result value corrected for recovery		
30	Expression of result: express the results in the same units as the maximum levels laid down in Regulation (EC) 1881/2006 and Directive 2002/32/EC		MYTOX-SOUTH® - 30.1. Mycotoxins legislation available for the country MYTOX-SOUTH® - 30.2. Reference for mycotoxins legislation MYTOX-SOUTH® - 30.3. Mycotoxins maximum limits of the country
31		Confidentiality of data	
32		Remarks/References	

Pre-test feedback

Feedback from the pre-test was received from three participants. The average time reported for filling out data for a single sample was 42 minutes. The most time-consuming part as noted by one of the participants was going between files to know what to complete in each column. In actual data submission, we assume that the data filling time per sample may be lower than 42 minutes, as a person gets accustomed to the template while the data filling is moving forward from sample to sample. Another feedback given was to include not applicable (N/A) option in the selection menus, for cases where some descriptors might not be applicable for some data providers. The other participant reported that the contents included in the data-filling template were understandable and doable.

5.4.2 Discussion

Different food safety initiatives deal with mycotoxin contamination in food and feed. Specifically, GEMS and EFSA assemble food contaminants and additives reports from different countries. These reporting templates however lack some important items to describe mycotoxin contamination data, in particular data from LMICs. Improving reporting quality and completeness facilitates the applicability of results [6]. To our knowledge, the present study is the first to address the issue of standardizing the reporting of mycotoxin contamination data. The present effort complements the GEMS and EFSA reporting templates. Adding MYTOX-SOUTH® descriptors to the GEMS and EFSA templates enables mycotoxin contamination data to become more informative and reusable for intended purposes, particularly where harmonization of data is of concern.

We propose 22 additional mycotoxin contamination data descriptors. Out of these, most were related to the pre- and post-harvest period of a sampled food/feed as mostly mycotoxin contamination occurs during these times. Battilani et al. argued previously for the need to add more pre- and post-harvest management information of mycotoxin contamination data to support risk assessment modeling [16].

The present effort also draws attention to the need for mycotoxin contamination data from LMICs which are characterized by a higher degree of subsistence food production and consumption patterns. Under such settings, poor pre- and post-harvest handling practices are often observed and these might have been taken into consideration during the proposal of such important missing descriptors.

Providing detailed information about the sampling location of the crop/commodity is important as more accurate geographical information is a prerequisite to better understand, map, and

manage mycotoxins contamination [17]. GPS coordinates can also be used for modeling the occurrence of toxigenic fungi and their mycotoxins and well modeling future occurrence [18]. Data from such technologies can also be used to identify associations between location and other remotely sensed parameters like diseases, vectors, and climatic information.

Describing the way the samples have been collected is useful as it helps to review the validity of the applied sampling technique and enables the correct interpretation of the identified mycotoxin contamination spots. Appropriate sampling is also important to make rational decisions on the fate of lots that might be contaminated with mycotoxins [5].

Reporting the intended use of the sampled crop/commodity was also suggested as a descriptor. As the mycotoxin consumption thresholds established for humans and animals are different, knowing whether the commodity is intended for animal or human consumption may guide authorized bodies in enforcing mycotoxin regulations. Moreover, knowledge of the type of animal species is important considering specific species differences in toxic kinetics and toxic effects.

From the analytical report's perspective, besides the LOQ, the number of samples with concentrations below the LOD should be reported. LOD, as an actual determined value, has applications in areas such as food analysis [19]. For different purposes like dietary exposure assessments, quantifying data between the LOD and LOQ promote the best use of available data especially if a great percentage of it lies below the LOQ [20]. Hence, it is important information as laboratories/researchers may either consider samples positive with any of the mycotoxins if the samples are above the LOD/LOQ.

The FAO showed that in 2003, at least 99 countries out of 119 had mycotoxin regulations on the existence or absence of specific mycotoxin limits and regulations in food and feed. This guided further efforts towards standardization and harmonization of mycotoxin regulations, particularly for countries where trade relations exist [5]. Primarily, such information assists data users to identify gaps in the existence of mycotoxin legislation across different countries and continents. If mycotoxin legislations exist, identifying references to the existing legislations helps data users to access different mycotoxin legislation across the globe so that it allows comparing mycotoxin limits established by different countries and continents. Furthermore, if the report provider is from a country/locality where mycotoxin legislation did not exist, such information may extend to a wider network working on mycotoxin regulations and trigger initiation of mycotoxin legislation in the country/locality.

Thus, providing detailed context about mycotoxin contamination of sampled crops/commodities using a standardized reporting template helps to suggest subsequent mycotoxin mitigation strategies. The strategies can potentially be absorbed by different initiatives working on contributing factors of mycotoxin contamination and are of utmost importance for the locality/country providing the report.

The above-proposed descriptors will help GEMS to provide technical cooperation with countries wishing to initiate and strengthen food contaminant monitoring programs. Similarly, the descriptors from the current study will be useful for EFSA as it regularly updates the Standard Sample Description based on EFSA Scientific Opinions [9]. In establishing a standardized process for the submission of specific requirements by contaminants and food additives, EFSA intends to enable an efficient and effective collection and submission process for chemical occurrence data in food and feed.

To date, many private sectors and researchers in LMICs do not submit mycotoxin contamination data to the organizations like WHO or EFSA, which lead to limited information on mycotoxin contamination of food and feed in these areas. As such, the proposed mycotoxin contamination data descriptors from this study expand the scope of the EFSA and GEMS descriptors to other food safety authorities in LMICs. Even this initiative may extend to LMICs without food safety authorities to initiate a new one.

A lack of data harmonization and descriptors requires a continuous and costly investment to help researchers easily find, access, integrate, cite, and reuse data [21]. There is a need to make the current reporting format follow findable, accessible, interoperable, reusable (FAIR) principles, which enable data discovery, archiving of digital assets to ensure high-quality data and metadata and support third-party reuse [21]. Satisfying the FAIR principles also requires seeking a closer alliance between data archivists and researchers [22].

5.5 Conclusions

The descriptors and standardized reporting format from this study should be advocated for ongoing and upcoming food safety-related initiatives. This enables organizations, academia, and other entities to use it for pooling and sharing mycotoxin contamination data in food and feed. The study followed the Delphi method which helped to identify descriptors that experts considered important in relation to mycotoxins contamination data. The present approach, however, did not allow discussion and consensus among the participants that provided data. Although the invited participants represent a sample of international researchers on mycotoxin researchers, the consensus process does not necessarily represent an international consensus on

these data descriptors. It is anticipated, however, that the present study report is a useful first step towards an internationally agreed set of items that are widely used to describe mycotoxin data. Although further discussion are welcome, it is hoped that the present descriptors provide an incentive towards a standardized global reporting format for mycotoxin contamination data, which in turn, will enable national authorities to perform mycotoxins exposure and risk assessments.

5.6 References

1. Agriopoulou S., Stamatelopoulou E. and Varzakas T. Advances in occurrence, importance, and mycotoxin control strategies: prevention and detoxification in foods. Foods. 2020; 9 (2).

2. IARC. Mycotoxin control in low- and middle income countries, Wild C.P. ,Miller J.D. and Groopman J.D., Editors. 2015, IARC working group report no. 9.

3. Milićević D.R., Škrinjar M. and Baltić T. Real and perceived risks for mycotoxin contamination in foods and feeds: Challenges for food safety control. Toxins. 2010; 2 572-592.

4. Misihairabgwi J.M., Ezekiel C.N., Sulyok M., Shephard G.S. and Krska R. Mycotoxin contamination of foods in Southern Africa: A 10-year review (2007-2016). Crit Rev Food Sci Nutr. 2019; 59 (1): 43-58.

5. Van-Egmond H.P., Schothorst R.C. and Jonker M.A. Regulations relating to mycotoxins in food: perspectives in a global and European context. Anal Bioanal Chem. 2007; 389 (1): 147-57.

6. Banno M., Tsujimoto Y. and Kataoka Y. The majority of reporting guidelines are not developed with the Delphi method: a systematic review of reporting guidelines. J Clin Epidemiol. 2020; 124 50-57.

7. Iain H. Data descriptors: Providing the necessary information to make data open, discoverable and reusable. 2014 October 22nd]. Available from: https://blogs.lse.ac.uk/impactofsocialsciences/2014/10/22/data-descriptors-open-discoverable-nature/.

8. WHO. Instructions for electronic submission of data on chemical contaminants in food. January 2002: Geneva.

9. EFSA. Risks for animal health related to the presence of zearalenone and its modified forms in feed. EFSA Journal. 2017; 15 (7): 4851.

10. Lachat C., Hawwash D., Ocké M., Berg C., Forsum E., Hörnell A., Larsson C., Sonestedt E., Wirfält E., Åkesson A., Kolsteren P., Byrnes G., Keyzer W., Cam p.J., Cade J., Slimani N., Cevallos M., Egger M. and Huybrechts I. Strengthening the reporting of observational studies in epidemiology-nutritional epidemiology (STROBE-nut): An extension of the STROBE statement. PLoS Med. 2016; 13 (6): e1002036.

11. Cross V. The same but different: A Delphi study of clinicians' and academics' perceptions of physiotherapy undergraduates. Physiotherapy. 1999; 85 (1): 28-39.

12. Hsu C. and Sandford B. The Delphi technique_ making sense of consensus. Practical Assessment, Research, and Evaluation. 2007; 12 (10).

13. Yousuf M. Using experts' opinions through Delphi technique. Practical Assessment, Research & Evaluation. 2007; 12 (4): 1-8.

14. Sansone S., Serra P., Field D., Taylor C., Tong W., Brandizi M., Maguire E. and Sklyar N. Towards interoperable reporting standards for omics data: Hopes and hurdles. PubMed PMID. 2009; 112-115.

15. Yang C., Pinart M., Kolsteren P., Camp J., De Cock N., Nimptsch K., Pischon T., Laird E., Perozzi G., Canali R., Hoge A., Stelmach-Mardas M., Ove Dragsted L., Maria Palombi S., Dobre I., Bouwman J., Clarys P., Minervini F., De Angelis M., Gobbetti M., Tafforeau J., Coltell O., Corella D., De Ruyck H., Walton J., Kehoe L., Matthys C., De Baets B., De Tré G., Bronselaer A., Giacco R., Lombardo R., De Clercq S., Hulstaert N. and Lachat C. Perspective: essential study quality descriptors for data from nutritional epidemiologic research. Adv Nutr. 2017; 8 (5): 639-651.

16. Battilani P., Palumbo R., Giorni P., Dall'Asta C., Dellafiora L., Gkrillas A., Toscano P., Crisci A., Brera C., De Santis B., Rosanna-Cammarano R., Della-Seta M., Campbell K., Elliot C., Venancio A., Lima N., Gonçalves A., Terciolo C. and Oswald I.P. Mycotoxin mixtures in food and feed: holistic, innovative, flexible risk assessment modelling approach. EFSA Supporting Publications. 2020; 17 (1).

17. Palumbo R., Crisci A., Venancio A., Cortiñas-Abrahantes J., Dorne J., Battilani P. and Toscano P. Occurrence and co-occurrence of mycotoxins in cereal-based feed and food. Microorganisms. 2020; 8 (1).

18. Scholten O., Ruckenbauer P., Visconti A., Osenbruggen V. and Den-Nijs A. Food safety of cereals: a chain wide approach to reduce Fusarium mycotoxins 2002, Wageningen University.

19. Bernal E. Limit of detection and limit of quantification determination in gas chromatography, in Advances in Gas Chromatography, Guo X., Editor. 2014, InTech.

20. EFSA. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA journal. 2010; 8 (3).

21. Rachel. FAIR data: how data increases the value of biotechs. 2020 September 10]. Available from: https://www.zontal.io/resources/whitepaper-fair-data.

22. Dunning A., De Smaele M. and Böhmer J. Are the FAIR data principles fair? International Journal of Digital Curation. 2017; 12 (2).

Chapter 6. General conclusions

This PhD study was intended to determine multiple mycotoxins in maize and breast milk samples in rural Ethiopia using the highly sensitive and specific LC-MS/MS method **(Chapter 3&4)**. The analyzed maize samples were found to be mainly contaminated with different *Fusarium* toxins but this was not the case for *Aspergillus* mycotoxins. DON was the *Fusarium* mycotoxin that contaminated most of the samples followed by NIV, 3-ADON, ZEN, and FUMs in a decreasing order. Co-occurrence of these *Fusarium* mycotoxins was also recorded in the samples. The exposure assessment analysis further showed that the mean intakes of DON and ZEN exceeded the TDIs, and some of the FUMs' intakes were also higher than the TDI. Regarding post-harvest handling practices and household maize processing techniques reported in our study, variations were observed between South and West Ethiopia. However, similar practices i.e. completely drying maize crops before harvest, application of insecticide, sorting, and use of the sorted-out maize for feed were reported in the two study areas (**chapter 3**). Similarly, our finding from the breast milk analysis showed that only *Fusarium* mycotoxins i.e. FUMs were detected. We also found out that the diet of the lactating women was less diversified which their diets largely comprised of cereals, mainly maize. Good pre- and post-harvest practices were reported by the majority of the women, however, undesirable practices of allowing maize crops to over-dry on the field before harvesting, storing maize in absorbent materials, and utilizing sorted-out maize for feed and making local beverages were reported. In our study, no association between FUMs in breast milk with pre- and post-harvest practices, dietary practices, and knowledge about mycotoxin was warranted (**chapter 4**).

In general, we report that AFs and other *Aspergillus* mycotoxins were unusually not detected but rather fusariotoxins were present in food and biological samples from Ethiopia. This is a concern as *Fusarium* mycotoxins were not repeatedly covered and given attention in Ethiopia, thus the findings from this study can contribute to guide risk assessment and legislation of *Fusarium* mycotoxins in Ethiopia. Though we did not report an association between FUMs in breast milk with the pre- and post-harvest practices, dietary practices, and knowledge about mycotoxin, it is worrisome that some poor pre- and post-harvest and dietary practices, and gaps in knowledge about mycotoxins have been observed in the studied communities. Thus, this shows the need to give more attention to optimal agricultural practices as the best prevention mechanism since previous evidence also supports the fact that *Fusarium* mycotoxins usually arise from the agriculture field and continue to grow at the post-harvest time and in storage. Moreover, given the fact that a single *Fusarium* species can produce different *Fusarium* mycotoxins, the need for multi-mycotoxins analysis that allows to capture the occurrence of

multiple mycotoxins should be emphasized in Ethiopia. The Ethiopian government should also work on enhancing food and dietary diversity as one mechanism of mycotoxin mitigation strategies.

The variations in the sensitivity and specificity of analytical methods, the LODs applied, sample sizes and sampling techniques, pre- and post-harvest practices reported, and weather conditions of the data collected year between our study and previous studies in Ethiopia should be taken into consideration for the gaps observed in the reporting of *Aspergillus* mycotoxins. Despite our findings, the issue of *Aspergillus* mycotoxins cannot be underestimated since previous studies repeatedly reported the occurrence of *Aspergillus* mycotoxins in Ethiopia.

In a broader global context, the available mycotoxins data are not well described, structured, and stored. Particularly, the mycotoxins contamination data from LMICs is more doubtful as the sample collection, sample preparation, and analytical methods applied have many uncertainties. Thus, the study (**chapter 5**) was planned to propose additional mycotoxins contamination data descriptors that complement the existing GEMS/FOOD and EFSA mycotoxin contamination data descriptors and enhance the applicability of mycotoxins contamination data especially to LMIC. The new proposed descriptors from our study were combined with the GEMS/FOOD and EFSA descriptors and eventually structured into different categories. We perceive that the proposed descriptors and the structured data format will make mycotoxin contamination data more informative and applicable, possibly as input for the scientific committee of EFSA and GEMS/Food, and for predictive modeling initiatives. Moreover, it will increase scalability and encourage more initiatives on the standardization and harmonization of mycotoxin contamination data and its reporting formats, globally.

Chapter 7. Broader international context, relevance, and future perspectives

7.1 Broader international context and relevance

It is challenging to provide adequate and quality food that is both nutritious and free from environmental contaminants, especially in areas with dense populations [1]. Mycotoxin occurrence is still a global phenomenon and is one of the leading human and animal health hazards. Mycotoxins such as AFB_1, AFB_2, AFG_1, and AFG_2, DON, ZEN, FB_1, T-2 toxin, and OTA are of global concern which contaminates the food chain [2]. Elimination of mycotoxin-contaminated foods due to the danger they cause to human health must be balanced with the possibility of diminished food security as it compromises the quantity of food that can be supplied to the increasing global population [3].

According to a study report by Liu et al. (2012), AFs exposure may be the cause of 23% of liver cancer cases each year, worldwide [4]. The health effects of exposure to other dietary mycotoxins have not been well studied, which might be due to uncertainty in establishing the link between exposure to these mycotoxins and specific human diseases [3]. As per the Lancet nutrition series estimation, maximum efforts by nutrition-specific interventions can possibly reduce the magnitude of stunting by only about 20%, which clearly shows there are more uncovered contributors to stunting. Thus, it was indicated that one of the exposing factors that lead to stunted growth might be exposure to potentially harmful agents such as mycotoxins [5].

Cereals are the most important food groups that are widely consumed and form an important source of dietary energy, minerals, fiber, and vitamins globally. However, cereals are prone to fungi growth on the agricultural field or after harvest and during storage [6]. In a global context, *Fusarium* fungi have been recognized as one of the ten most important contaminants of plant-based foods, especially cereals [7]. Moreover, except for variations in particular regions and sub-regions, the occurrence of mycotoxins such as AFs, FUMs, DON, ZEN, T2 toxin, and OTA seems to be common across all the processed or unprocessed feeds, worldwide [8]. Maize is produced abundantly and is one of the most significant agricultural commodities in the world. However, maize is also known to be one of the most susceptible cereals to fungi attack and contamination by the respective mycotoxins [9]. Successive years (50 years) global data indicated that higher consumption of maize has been observed whereas the consumption of crops such as sorghum, millets, and cassava has deteriorated over the years [10]. The weight of evidence from global level systematic review and meta-analysis studies on the occurrence of FUMs in cereals and cereal food products has shown that the highest FUMs concentration was observed in foods prepared from maize followed by wheat and then other cereals, barley, rice, and oat in decreasing order [11].

Changes in stratospheric ozone depletion and climate change are the major environmental changes happening in the world [12]. Extreme weather conditions in the course of the continually changing climate have become a concern as it has caused changes in mycotoxin occurrence in crops [13]. Climate change brings changes in temperature, wind, and rainfall which disrupt the distribution of mycotoxins in a complicated manner. Entirely new kinds of health hazards might not appear due to these changes. Instead, climate change intensifies and broads the health hazards that are already caused by the existing environmental threats. In Europe, as a result of the continued change in climate, there is a prediction that distinct patterns of mycotoxins exposure may be seen in the near future, or it may have already begun to occur [12]. The agriculture and food industry sectors deprive of billions of dollars yearly as a consequence of mycotoxin-contaminated crops. Increased development of *Aspergillus* in grains has been observed in Asia due to climate change-induced rises in temperature and humidity. Consequently, 95 % of feed samples collected in South Asia and three-quarters in China, Indonesia, Philippines, and Vietnam revealed the occurrence of AFs contamination. FUMs occurrence predominated in Mexico, Central, and South America in which the prevalence is close to 90% with mean levels that are very harmful to swine and dairy animals. Trichothecenes such as DON and T2 contamination in the United States is a typical situation. *Fusarium* mycotoxins like FUMs, DON, and T2 are found in the region of Egypt, Jordan, and South Africa [14].

A mycotoxin working group reported that there is a lot more information about the levels of mycotoxins exposure in countries where the problems are rare compared to those where the problems are common. Thus, the need to do mycotoxin contamination and exposure assessments in different food items and populations of developing countries has been indicated [15]; especially in SSA where mycotoxin information is more scanty [16]. In this regard, this PhD study makes some important contributions in determining the occurrence of mycotoxins in one of the important staple crops i.e. maize in Ethiopia **(chapter 3).** Therefore, the results from our study substantiated the existing pieces of evidence and deepen the current understanding of mycotoxins in food crops in Ethiopia and SSA at large.

Despite the major knowledge gaps in the understanding around the toxicokinetic of most mycotoxins in humans due to critical ethical issues in conducting such studies [17], various mycotoxin biomonitoring studies from different countries have been reported in recent times. From studies that focused on a single mycotoxin, the most frequently studied mycotoxin was AFB1 through AFM1 analysis in urine samples. As the LOD of the methods varies across different studies, variability in the amount (moderate to high) of AFM1 was seen across different

countries in the world. A higher level of AFM1 was reported in Africa [18]. Another review reported that the most often found mycotoxin biomarkers in plasma and serum samples were AFB1-lys and OTA. Overall, this shows that exposure to AFB1 and OTA is widespread across the world [17]. The occurrence of DON in urine samples was largely documented in European countries [19]. As part of the European human biomonitoring initiative research, DON and fumonisin B1 were prioritized among other mycotoxins based on the consultation of the EU Policy Board, EFSA, and the directorate-general for health and food safety [20]. Concerning multi-mycotoxin studies, DON, OTA, and AFs were the more frequently co-occurring mycotoxins in most biological samples [18].

Breast milk as one of the human bio specimen has demonstrated potential for introducing mycotoxin residues from lactating mothers to infants. However, little is known to date about the pattern of mycotoxins and their metabolites in breast milk as well as lactational transfer rates or potential added effects [21]. Therefore, we studied and contributed to the profiling of mycotoxins in breast milk samples from Ethiopia **(chapter 4**) as there is very limited published data currently available about mycotoxins in breast milk **(chapter 1**).

The development of multi-mycotoxin analytical methods has been a priority in recent years to better capture potential mixtures of parent, modified, and emerging forms of mycotoxins in a single assay [22]. In this sense, this PhD study has elucidated the particular significance of multi-mycotoxin studies. Emerging mycotoxins such as enniatins, fusarenon X, and alternaria toxins besides other major mycotoxins have been characterized in maize samples **(chapter 3)**. Mycotoxins which have received little or no attention in breast milk so far such as nivalenol have been also examined in our study. Furthermore, this is the first study in Ethiopia that characterized multi-mycotoxins and reported FUMs in breast milk **(chapter 4**). It is hoped that such research activities will give additional information to the existing shreds of evidence that helps to set up interventions for the most susceptible community groups (infants and mothers) and protect them from the undesirable effects of mycotoxins and other food contaminants [21]. The implementation of well-validated multi-mycotoxin analysis using robust analytical methods like LC-MS/MS in our research underscores the necessity for creating accredited national quality control laboratories. These laboratories would be responsible for certifying the quality of both local food and export products at their source, while also developing safety regulations in a manner consistent with developed countries.

Nowadays, curiosity is raising in the investigation of the potential aggregated effects of mycotoxins exposure with other hazardous environmental exposures that may show synergetic

action in the internal biological system. This has led to exposome approach with the application of omics technologies [18]. The exposome concept refers to 'the cumulative measure of environmental influences and associated biological responses throughout the life span including exposures from the environment, diet, behavior, and endogenous processes [23]. This helps to understand additional factors, aside from the genome, that may involve human phenotypes that alter biological processes [24]. Toxic compounds such as mycotoxins are a vital element of exposome-based studies [25]. However, only the main mycotoxins have been examined as part of human exposome leaving little information on the rest of the mycotoxins, the modified and the emerging ones [18]. This gap has been shown in the literatures search that the number of available studies on mycotoxins of exposome are quite few compared to studies done on other exposome categories. As mycotoxins constitute a significant part of the human exposome, the progression in high resolution mass spectrometers and exposome research consortium should be viewed as an opening to ally mycotoxins in the advancing exposome studies [26].

There is an interrelation between the series of crop value chain processes, thus every step of the way in these processes has a significant influence on one another and should not be viewed separately. Better awareness about plant resistance to disease, good agronomic practices, better fungicide management, and better storage methods have been employed globally. There are major pre- and post-harvest approaches for limiting mycotoxin production that are currently in use. The most significant pre-harvest practices in a global context comprise resistant varieties, the application of fungicides and/or biocontrol application (eg. Aflasafe), and multiple cultural practices. In the case of post-harvest, drying, storage, cleaning and sorting, and certain food processing techniques were the most important ones [27]. Basically, key areas of mycotoxin research and management have been guided by an expert group i.e. the MycoKey Maize Working Group. The Working Group suggestions place a strong emphasis on the value of genetic resistance, insect control, grain drying and cleaning methods, and the invention of tools that are applicable for assessing new mycotoxin hazards in maize which takes into consideration the impact of the changing climate as well [28]. As an illustration, mycotoxin prevention and control activities in temperate regions of the world seem to gear towards choosing transgenic insect-resistant varieties that are resistant to insect attack [29]. Also in Europe, studies indicate that a key part of the mycotoxin management approach is controlling insect attacks. Specifically, it has been demonstrated that controlling the European corn borer with insecticide application or transgenic Bt maize hybrids reduces FUMs and other *Fusarium* toxins [30, 31]. Scholars have indicated the need to give more emphasis to scaling up pre- and post-harvest intervention studies in Africa. The

outputs of the studies should ultimately intend to reach policymakers and strive to bring positive outcomes on the issue of food security, food safety, and the health of the population [32].

Certain processing methods such as sorting and milling significantly decrease mycotoxin levels in the end food product. Furthermore, a diversified diet decreases the chance to toxins exposure. A wonderful experience from Qidong, China has shown that a change in free trade policy across borders has brought transformation from the supply of AF-risk foods to that of less risky food sources which has resulted in better health outcomes in the community. The free trade policy increased the import of rice and substituted the main cereal consumed in the area i.e., maize. As a result, lower levels of AFs exposure and a sharp decline in liver cancer cases were documented, since AFs contamination is substantially lower in rice than in maize [33]. The overall food consumption in LMICs is usually subjected to few food groups thus, characterized as less diversified and liable to contamination by hazardous toxins. Unlike developed countries where infrastructure and established monitoring systems for food contaminants exist, farming societies in rural parts of developing countries frequently lack the resources to restrict contaminated foods consumption by the community [34]. Yet, there is limited evidence on the role of pre-post-harvest practices, household food processing methods, and dietary diversity in mycotoxin contamination and exposure in developing countries like Africa. To this end, this PhD study partially tried to document these as contributors to mycotoxin occurrence in maize and breast milk samples. Moreover, we believe that our research provides valuable insights into the community's pre-post-harvest practices and also food processing techniques that help to guide and inform practitioners and planners **(chapter 3 and 4)**.

Countries without or with low implementation of mycotoxin regulations have a higher chance to import foods contaminated with mycotoxins even from developed countries [35]. Close to 15% of the food supply in the United States is imported from foreign producers which is prone to food safety hazards including contamination by mycotoxins. Foreign supplier verification programs for importers of food and the accreditation of third-party auditors/certification have been the newly suggested rules. The rules were designed to purchase foods that are safe and safeguard food security in the U.S. through strengthened import verifications [36]. Within European Union, there is also an established RASFF which is an information exchange platform between food safety authorities of European countries that intends to protect the European population from food safety risks along the food chain. The RASFF is highly engaged in the inspection of imported foods at borders within European Economic Area [37]. Another best experience has been observed in the harmonization of European Union limits for patulin in different food products in

2003 which raised the adoption of the regulatory limit by different countries. This makes patulin the second-most regulated mycotoxin in the world, after AFs which indicates that harmonization is very crucial [38]. An encouraging initiative was observed in establishing the East African Bureau of Standards by the East African Community to set common standards for goods and services in general and, set limits for mycotoxins in food and feeds. This has brought substantial progress in enhancing the quality and safety of foods in the region [39].

Dietary exposure to mycotoxins is usually assessed either from food consumption data collected at country-level dietary studies or data obtained from pocket studies done by researchers on mycotoxin occurrence in different food commodities. The scientific committee of EFSA gathers detailed data on large-scale food consumption over 5 years. A "Concise European Food Consumption Database" was established by EFSA in 2008 to improve the Comprehensive European Food Consumption Database which was intended to do thorough exposure calculations in 22 different European countries [40]. Limited mycotoxin data has been generated so far from developing countries even where the quality of the existing data is doubtful. Thus, there is a huge difference in the volume and quality of data generated by laboratories in industrialized countries compared with LMICs [35].

The lack of unified mycotoxin assessment and risk analysis methods among countries resulted in heterogeneity in mycotoxins guidelines and regulations. Harmonization of mycotoxin assessment and risk analysis methods has not been attained so far though the significance of having uniform methods and data descriptors has been quite acknowledged [41]. In the case of Africa, PACA has developed a pilot project i.e. Africa Aflatoxin Information Management System in Senegal, Gambia, Malawi, Nigeria, Tanzania, and Uganda which aimed to assemble and harmonize AFs data. Such initiatives contribute to countries in the continent joining forces and bringing a larger scale of AFs surveillance and management [42]. Our study offered the opportunity to merge and arrange the existing GEMS/FOOD and EFSA mycotoxin contamination data descriptors and reporting templates and also contributed a new set of mycotoxin contamination data descriptors that are globally applicable **(chapter 5)**. The current proposed descriptors and template from our study assist to have a structured and consistent set of mycotoxin contamination data, worldwide. This further initiates standardization and harmonization of mycotoxin contamination data and the reporting templates, and further stimulates different researchers to work together and conduct extensive studies.

7.2 Future perspectives

Depending on the findings and gaps observed in this PhD research, and based on the extensive literature search, the following recommendations for future research and developments are proposed.

7.2.1 Promoting mono and interdisciplinary mycotoxin research

In general, food consumption and contamination data are mostly used for human mycotoxin exposure and risk assessment in which most developing countries lack well-documented food consumption databases. Besides, exposure assessment from food cannot capture all sources of contaminations since the distribution of mycotoxins is heterogeneous in foods across geographic regions, production years, and due to application of food processing and preparation practices [43]. In this regard, biomonitoring and exposome studies are the promising approaches as they inform the internal dose of exposure (bioavailability of mycotoxins) from aggregated sources [44]. However, only very few mycotoxin biomarkers have been validated so far. In addition, data on biomonitoring for most of the mycotoxins are lacking especially in LMICs.

Thus, researchers and funding organizations in the future need to focus on biomonitoring and exposome studies in different biological samples for a comprehensive exposure assessment of multiple mycotoxins. Complementing mycotoxin biomonitoring data with the distinction of the major exposure sources i.e. mycotoxin contamination data in commonly consumed foods should also not be overlooked. Furthermore, efforts should be exerted to validate biomarkers for more mycotoxins and emerging ones. Further initiatives are also required to integrate biomonitoring data in mycotoxin risk assessments.

Considerable gaps in the available pieces of evidence have been shown on the synergetic and antagonistic effects of multiple mycotoxins co-occurrence, and also on the chemical structures and toxicology of the emerging and modified mycotoxins. Thus, more experimental studies with complementary approaches of *in vitro* and *in vivo* need to be conducted in the future to make a definite comprehension and prediction on the bioavailability, metabolism, and health effects of mycotoxins. Epidemiological investigations of vulnerable community groups are also further required to better understand the link between the levels of mycotoxins exposure with health outcomes.

This doctoral dissertation filled important data gaps on the examination of multiple mycotoxins and revealed the occurrence of *Fusarium* mycotoxins in maize and breast milk in Ethiopia however, there are still major information gaps in these areas. Except for FUMs, in Africa, the focus given for the examination, prevention, and mitigation of *Fusarium* mycotoxins such as

DON and ZEN is less compared to *Aspergillus* mycotoxins. Therefore, future studies should mark the profiling of *Fusarium* mycotoxins in different staple foods and biological samples, and their mitigation strategies accordingly.

As indicated by different researchers, the sampling of breast milk, stage of lactation, the timing of the day, hind or fore milk, the order in which an infant is born in a family, variation in the sample sizes, and consideration of key confounding variables in a regression model are important factors that should be considered in the study of mycotoxins in breast milk [21]. Thus, more research efforts using advanced analytical approaches and rigorous sampling and laboratory extraction procedures are required to uncover the pathways and contributing factors for mycotoxins transfer to breast milk. As AFs and OTA are the most covered mycotoxins in breast milk, future studies should also emphasize the examination of other mycotoxins in breast milk.

In this PhD dissertation, pre- and post-harvest practices and household-level processing methods in relation to maize have been documented. Previously, a maize working group under the MycoKey project identified the most important pre- and post-harvest management for FUMs, AFs, DON, and ZEN. Maize genotype selection, followed by insect management and knowledge of good agricultural practices were regarded as the most significant practices at the pre-harvest time and rapid grain drying followed by grain cleaning or sorting for the post-harvest time [28]. Thus, priority should be given to conducting more intervention studies on the effectiveness and promotion of the different pre- and post-harvest practices and also food processing approaches recommended for mycotoxin reduction and control. Besides the pre- and post-harvest issues, further extensive experimental studies that focus on the potential effects that climate change can bring on toxigenic fungi and mycotoxin risks should be conducted.

The need to do operational research on food safety issues is crudely indicated in the Ethiopian National Nutrition Program document. Overall, the effective reduction of mycotoxin contamination in the food supply would require a multifaceted approach. For instance, social sciences have a key role in devising ways how to increase farmers' awareness of mycotoxins contamination, what motivates them to adopt solutions to reduce contamination, how to make these innovations affordable, and how to make sure that they are accessible quickly and easily [45]. Economists can support this with an examination of the costs, feasibility, and sustainability of mycotoxin mitigation strategies [46]. Thus, research organizations, research projects, and universities should encourage and leverage resources for multidisciplinary mycotoxin research among different sectors and institutions.

7.2.2 Sensitizing and raising awareness among farmers, consumers, and policy makers

Different scholars reported that farmers and consumers in Africa have a poor understanding about the negative effects of mycotoxins. It is therefore vital that community groups (farmers, traders, and consumers) should be sensitized and instructed about the risks of mycotoxin contamination in food, and also their prevention and potential management measures. The role of Good Agricultural Practices (GAP) as a first line of mycotoxin prevention mechanism coupled with Good Manufacturing Practices (GMP) should be well integrated into the agriculture and manufacturing/trade sectors. This has support in the current Food and Nutrition Policy of Ethiopia that appropriate food safety practices and technologies should be applied throughout the agriculture value chain [47]. Additionally, it is crucial to identify and promote economically feasible endogenous household processing techniques that serve as protective measures against mycotoxins.

A mycotoxin education package should also be oriented toward the enhancement of food security and ultimately increasing dietary diversity of families. Diverse diets are double-barrelled for both food toxicologists and nutritionists by minimizing the risk of human exposure to toxins from contaminated food and also increasing the chance to get diverse micronutrients. The dual advantage of promoting diet diversity helps to increase the efficient use of resources in intervention programs and should be accounted in agricultural and public health-related activities [48]. This is in line with the recent food-based dietary guidelines of Ethiopia where the need to increase consumption of a diversified diet is included as one of the key messages [49].

The agriculture and health extension workers should collaborate in creating awareness about mycotoxins prevention and mitigation strategies for farmers and rural consumers using the already established platforms. For instance in Ethiopia, farmer training centers, child health day, pre- and post-natal care visits can be used as contact points to raise awareness about mycotoxins. It is also valuable to educate traders on how the action of commercializing moldy foods can negatively influence the food environment and eventually affect the consumers' health. Mainstreaming media such as radio and television can be utilized to reach out to traders, and also farmers, and consumers at a larger scale. Furthermore, government officials must be informed with evidence-based knowledge about mycotoxins to engage them in regulation, control, and policy action.

7.2.3 Incentives for farmers and traders

In developing nations, it is frequently difficult to enforce mycotoxins regulations for small-scale farmers because there is often little price difference in the market between contaminated and non-

contaminated food crops and food products [34, 50]. In such settings, it is worth devising a rewarding mechanism for farmers and traders who produce and sell quality food crops by stakeholders, such as government agriculture and food safety centers, and also by non-governmental and private organizations working on agriculture and food safety. One approach can be empowering farmers who produce quality food crops by linking them with farmers' cooperatives that help farmers bargain for better market price linkage. The experience from the Ethiopian Commodity Exchange can be taken as another exemplary approach to empowering farmers and traders with quality crops. The Ethiopian Commodity Exchange follows a modern agricultural commodity trading system that safeguards the rights and benefits of sellers, buyers, intermediaries, and society while keeping crop quality at its core. Another previous Ethiopian experience that might be applied as a tool to reward farmers is giving recognition to farmers who perform well in agricultural production as "model farmers." Establishing incentive systems for farmers and traders should thus be considered one of the future mycotoxin reduction and control strategies.

7.2.4 Strengthening institutional and laboratory capacities

The African Union has a plan to set up an African Food Safety Authority and a RASFF using the European Union model [51]. The establishment and strengthening of national food safety agencies in African countries is also a key issue. Thus, mycotoxicology research as part of food safety research centers and laboratories in Africa should be capacitated. This should anticipate generating quality data and establishing databases that can be used as inputs for initiatives working on reducing and controlling mycotoxins. However, mycotoxin reduction and control is a resource-intensive long-term process that requires joint contributions from different stakeholders. Commitment of political leaders in Africa is an important first step in supporting the establishment and strengthening of infrastructures for mycotoxin research and the training of more mycotoxin experts. Furthermore, previous experiences show that creating partnerships between developed and developing countries is an important approach to building the capacities of institutions and personnel. On another side, mycotoxin professionals in developing countries like Africa in collaboration with different stakeholders should strive to look for and apply to calls for proposals announced by national and international funding agencies. Besides, establishing a rapid alert system can be also a complementary approach to mycotoxin reduction and control actions. Rapid, economical, and easy-to-operate mycotoxin assessment tools especially those that can be applicable for screening mycotoxins in the small-scale agriculture field should then be considered as part of the institutional capacity-building process in Africa.

7.2.5 Establishing and strengthening mycotoxin legislation and regulation

Poor families are known to have limited access to choose a variety of foods due to a lack of resources and may even be pushed to consume already spoiled foods in the worst cases. As regulatory limits differ across countries based on wealth status (developed vs. developing countries), a stricter globally networked mycotoxin regulation may thus negatively impact the economy and health of most poor families in developing countries like Africa [52]. Though such strict regulations are challenging to apply, however, the overall progress made on the formulation and enforcement of mycotoxin legislation and regulation is still a major drawback in Africa. In most African countries, mycotoxin legislation and regulations may lack, or only a few mycotoxins and few food items have legislation, or the enforcement of the already existing mycotoxin legislation and regulations is very loose. Hence, there is a need to formulate regulations for major mycotoxins in staple crops and further strive to extend the regulations to address more mycotoxins and food items in Africa. One of the ways to realize this can be through providing evidence-based policy advice to policymakers. Mycotoxin risk assessment should be also conducted regularly by competent authorities. This can be aligned with programs such as the National Nutrition Program of Ethiopia which highlights the need to do food safety and quality surveillance [53]. Enhancing the commitment of higher government officials and strengthening regulatory bodies is also very vital for the effective implementation of mycotoxins regulation. Besides the need to enhance commitment, standard offices/regulatory bodies must be held accountable for enforcing mycotoxins regulation. Integrating and encouraging compliance to mycotoxin standards and maximum limits by farmers and traders as part of quality grading parameters can be another mechanism to boost the implementation of mycotoxins regulation by standard offices.

7.2.6 Harmonization and standardization of mycotoxin data

For large-scale estimation of mycotoxin occurrence and for establishing an early warning system using predictive modeling of mycotoxins occurrence, the available data that can be used as input should be of high quality and relevant for statistical analysis. This shows the need to harmonize and standardize mycotoxin assessment methods in food and biological samples and also methods used for *in vitro* and *in vivo* toxicokinetics studies. This has been also suggested before by different scholars [22, 54]. In this respect, this PhD tried to fill this gap by proposing a comprehensive set of descriptors related to sampling, sample preparation for laboratory analysis, and analytical methods that apply to mycotoxin assessment in food and feed. In the future, similar work should also be widened to harmonize and standardize the sampling and analytical techniques used in mycotoxin biomonitoring and *in vitro* and *in vivo* toxicokinetics studies. On

top of that, long-term initiatives should be taken by international and regional level organizations such as WHO and EFSA to establish databases and pool mycotoxin occurrence data in biological samples.

On the other side, to increase the applicability of the available mycotoxin data, aggregated mycotoxin data at the global or continent level are needed and GEMS/Food and EFSA are the well-known mandated entities for this task. Therefore, research organizations, governments, and private companies working on mycotoxin food contamination should also be informed and encouraged to submit their data to GEMS/Food and EFSA. It is also very crucial that organizations and researchers follow a standardized data storage and reporting format to increase the applicability of mycotoxins data. Thus, organizations and researchers who are engaged in generating mycotoxin data should be informed that the data collection, storage, and reporting should follow the FAIR principles, especially the application of FAIR principles at the very early stage of research planning should be well promoted. In general, further discussion and communication on the topic of mycotoxin data harmonization and standardization at the global level are needed.

7.3 References

1. Bhat R.V. and Miller J.D. Mycotoxins and food supply. 1991, Agirculture and Consumer Protection.

2. Fokunang C.N., Tembe-Fokunang E.A., Tomkins P. and Barkwan S. Global impact of mycotoxins on human and animal health management. Outlook on Agriculture. 2006; 35 (4).

3. Wu F., Groopman J.D. and Pestka J.J. Public health impacts of foodborne mycotoxins. Annu Rev Food Sci Technol. 2014; 5 351-72.

4. Liu Y., Chang C., Marsh G. and Wu F. Population attributable risk of aflatoxin-related liver cancer: systematic review and meta-analysis. Eur J Cancer. 2012; 48 (14): 2125-36.

5. Bhutta Z., Das J., Rizvi A., Gaffey M., Walker N., Horton S., Webb P., Lartey A. and Black R. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? Lancet. 2013; 382 (9890): 452-477.

6. Pereira V., Fernandes J. and Cunha S. Mycotoxins in cereals and related foodstuffs: A review on occurrence and recent methods of analysis. Trends in Food Science & Technology. 2014; 36 (2): 96-136.

7. Dean R., Van Kan J., Pretorius Z., Hammond-Kosack K., Di Pietro A., Spanu P., Rudd J., Dickman M., Kahmann R., Ellis J. and Foster G. The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol. 2012; 13 (4): 414-30.

8. DSM. DSM world mycotoxin survey. The global threat January-December 2022. 2022.

9. Bryla M., Pierzgalski A., Zapasnik A., Uwineza P., Ksieniewicz-Wozniak E., Modrzewska M. and Waskiewicz A. Recent research on Fusarium mycotoxins in maize-A review. Foods. 2022; 11 (21).

10. Khoury C., Bjorkman A., Dempewolf H., Ramirez-Villegas J., Guarino L., Jarvis A., Rieseberg L. and Struik P. Increasing homogeneity in global food supplies and the implications for food security. Proc Natl Acad Sci U S A. 2014; 111 (11): 4001-6.

11. Farhadi A., Fakhri Y., Kachuei R., Vasseghian Y., Huseyn E. and Mousavi-Khaneghah A. Prevalence and concentration of fumonisins in cereal-based foods: a global systematic review and meta-analysis study. Environ Sci Pollut Res Int. 2021; 28 (17): 20998-21008.

12. WHO. Mycotoxins: children's health and the environment. WHO training package for the health sector. 2011.

13. Janic-Hajnal E., Kos J., Radic B., Anic M., Radovic R., Kudumija N., Vulic A., Dekic S. and Pleadin J. Impact of climate changes on the natural prevalence of Fusarium mycotoxins in maize harvested in Serbia and Croatia. Foods. 2023; 12 (5).

14. Ew. Global mycotoxin challenges: Q1-Q4/2021. 2021.

15. IARC and WHO. Dr Christopher Wild, director of IARC discusses the working group report., in IARC newsletter 2016.

16. Bessy C. Third joint FAO/WHO/UNEP international conference on mycotoxins. 1999.

17. Arce-López B., Lizarraga E., Vettorazzi A. and González-Peñas E. Human biomonitoring of mycotoxins in blood, plasma and serum in recent years: A review. toxins. 2020; 12 147;.

18. Marin S., Cano-Sancho G., Sanchis V. and Ramos A.J. The role of mycotoxins in the human exposome: Application of mycotoxin biomarkers in exposome-health studies. Food Chem Toxicol. 2018; 121 504-518.

19. Wells L., Hardie L., Williams C., White K., Liu Y., De Santis B., Debegnach F., Moretti G., Greetham S., Brera C., Rigby A., Atkin S. and Sathyapalan T. Determination of deoxynivalenol in the urine of pregnant women in the UK. Toxins (Basel). 2016; 8 (11).

20. Schoeters G. and Lange R. Scoping documents for 2021 for the first and second round HBM4EU priority substances. November 2020.

21. Warth B., Braun D., Ezekiel C.N., Turner P.C., Degen G.H. and Marko D. Biomonitoring of Mycotoxins in Human Breast Milk: Current State and Future Perspectives. Chem Res Toxicol. 2016; 29 (7): 1087-97.

22. Battilani P., Palumbo R., Giorni P., Dall'Asta C., Dellafiora L., Gkrillas A., Toscano P., Crisci A., Brera C., De Santis B., Rosanna-Cammarano R., Della-Seta M., Campbell K., Elliot C., Venancio A., Lima N., Gonçalves A., Terciolo C. and Oswald I.P. Mycotoxin mixtures in food and feed: holistic, innovative, flexible risk assessment modelling approach. EFSA Supporting Publications. 2020; 17 (1).

23. Miller G. Chapter 1: The exposome: purpose, definition, and scope, in The Exposome: A Primer. 2014, Acadamic press. 1-12.

24. Turner P.C., Sylla A., Gong Y.Y., Diallo M.S., Sutcliffe A.E., Hall A.J. and Wild C.P. Reduction in exposure to carcinogenic aflatoxins by postharvest intervention measures in west Africa: a community-based intervention study. Lancet. 2005; 365 1950–56.

25. Miller G. Chapter 4: The exposome in environmental health sciences and related disciplines, in The Exposome: A Primer. 2014, Academic Press. 39-53.

26. Marín S., Ramos A., Sanchis V. and Cano-Sancho G. An overview of mycotoxin biomarker application in exposome-health studies. Current Opinion in Food Science. 2021; 39 31-35.

27. Leslie J., Moretti A., Mesterhazy A., Ameye M., Audenaert K., Singh P., Richard-Forget F., Chulze S., Ponte E., Chala A., Battilani P. and Logrieco A. Key global actions for mycotoxin management in wheat and other small grains. Toxins (Basel). 2021; 13 (10).

28. Logrieco A., Battilani P., Leggieri M., Jiang Y., Haesaert G., Lanubile A., Mahuku G., Mesterhazy A., Ortega-Beltran A., Pasti M., Smeu I., Torres A., Xu J. and Munkvold G. Perspectives on global mycotoxin issues and management from the mycoKey maize working group. Plant Dis. 2021; 105 (3): 525-537.

29. Munkvold G. Crop management practices to minimize the risk of mycotoxins contamination in temperate-zone maize, in Mycotoxin Reduction in Grain Chains, Leslie J. and Logrieco A., Editors. 2014, John Wiley & Sons, Ltd. 59-77.

30. Blandino M., Saladini M., Alma A. and Reyneri A. Pyrethroid application timing to control European corn borer (Lepidoptera: Crambidae) and minimize fumonisin contamination in maize kernels. Cereal Research Communications. 2010; 38 (1): 75-82.

31. Folcher L., Delos M., Marengue E., Jarry M., Weissenberger A., Eychenne N. and Regnault-Roger C. Lower mycotoxin levels in Bt maize grain. Agronomy for Sustainable Development. 2010; 30 (4): 711-719.

32. Hell K. and Mutegi C. Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. African Journal of Microbiology Research. 2011; 5 (5): 459-466.

33. Chen J.G., Egner P.A., Ng D., Jacobson L.P., Munoz A., Zhu Y.R., Qian G.S., Wu F., Yuan J.M., Groopman J.D. and Kensler T.W. Reduced aflatoxin exposure presages decline in liver cancer mortality in an endemic region of China. Cancer Prev Res (Phila). 2013; 6 (10): 1038-45.

34. USAID. Aflatoxin: a synthesis of the research in health, agriculture and trade. February 2012, USAID. Mission to East Africa Danya International Kenya.

35. IARC Improving public health through mycotoxin control IARC Scientific Publication No 158. 2012.

36. FDA. Foreign supplier verification programs for importers of food for humans and animals. FDA–2011–N–0143]. 2013; 78 (145).

37. Commission E. Protecting EU consumers from unsafe food: EU's Rapid Alert System for Food and Feed. 12 December 2019.

38. Magan N. and Olsen M. Mycotoxins in food: detection and control. 2004, CRC Press Boca Raton Boston New York Washington, DC.

39. Ankwasa E.M., Francis I. and Ahmad T. Update on mycotoxin contamination of maize and peanuts in East African Community Countries. Journal of Food Science and Nutrition Therapy. 2021; 7 (1).

40. Kumar A., Renuka R.M., Bodaiah B., Mangamu U.K., Lakshmi V. and Poda S. Mycotoxin strategies: impact on global health and wealth. Pharmaceutica Analytica Acta. 2016; 7 (7).

41. Kuiper-Goodman T. Approaches to the risk analysis of mycotoxins in the food supply. 1999.

42. PACA. PACA News in Quarterly newsletter of the partnership for aflatoxin control in Africa-African Union. October-December 2017.

43. De Nijs M., Mengelers M.J.B., Boon P.E., Heyndrickx E., Hoogenboom L.A.P., Lopez P. and Mol H.G.J. Strategies for estimating human exposure to mycotoxins via food. World Mycotoxin Journal. 2016; 9 (5): 831-845.

44. Zhang P., Carlsten C., Chaleckis R., Hanhineva K., Huang M., Isobe T., Koistinen V., Meister I., Papazian S., Sdougkou K., Xie H., Martin J., Rappaport S., Tsugawa H., Walker D., Woodruff T., Wright R. and Wheelock C. Defining the scope of exposome studies and research needs from a multidisciplinary perspective. Environ Sci Technol Lett. 2021; 8 (10): 839-852.

45. Falade T. Aflatoxin management strategies in Sub-Saharan Africa, in Mycotoxins - Impact and Management Strategies. 2019.

46. IFPRI. Aflatoxins: finding solutions for improved food safety, Unnevehr L. and Grace D., Editors. November 2013.

47. FDRE. Food and nutrition policy. November 2018.

48. Wu F., Mitchell N.J., Male D. and Kensler T.W. Reduced foodborne toxin exposure is a benefit of improving dietary diversity. Toxicol Sci. 2014; 141 (2): 329-34.

49. FDRE, MoH and EPHI. Ethiopia: food-based dietary guidelines–2022. Addis Ababa, Ethiopia. 2022.

50. IFPRI. Demand for aflatoxin-safe maize in kenya. Dynamic response to price and advertising, Hoffmann V. ,Moser C. and Herrman T., Editors. December 2018.

51. Times T.N. AU to establish food safety body. 2012.

52. Marroquin-Cardona A.G., Johnson N.M., Phillips T.D. and Hayes A.W. Mycotoxins in a changing global environment--a review. Food Chem Toxicol. 2014; 69 220-30.

53. FDRE. National nutrition program 2016–2020. 2016.

54. Eskola M., Kos G., Elliott C.T., Hajslova J., Mayar S. and Krska R. Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25. Crit Rev Food Sci Nutr. 2020; 60 (16): 2773-2789.

Resume

Addisalem Mesfin
Tel: +251-911919730
P.O Box: 05
E mail: addisemesfin@yahoo.com
Hawassa, Ethiopia

University education	Year attended
MSc in Applied Human Nutrition from Hawassa University, Ethiopia	2009-2011
BSc in Rural Development and Family Sciences from Hawassa University, Ethiopia	2005-2007

Publications

Addisalem Mesfin, Carl Lachat, Seifu Hagos Gebreyesus, Meselech Roro, Kokeb Tesfamariam, Tefera Belachew, Marthe De Boevre, Sarah De Saeger. Mycotoxins Exposure of Lactating Women and Its Relationship with Dietary and Pre/Post-Harvest Practices in Rural Ethiopia. Toxins. 2023, 15: 285

Addisalem Mesfin, Carl Lachat, Arnau Vidal, Siska Croubels, Geert Haesaert, Melody Ndemera, Sheila Okoth, Tefera Belachew, Marthe De Boevre, Sarah De Saeger, Limbikani Matumba. Essential descriptors for mycotoxin contamination data in food and feed. Food Research International. 2022, 152: 110883

Addisalem Mesfin, Kokeb Tesfamariam, Tefera Belachew, Sarah De Saeger, Carl Lachat, Martha De Boevre. Multi-mycotoxin profiling in maize reveals prevalence of *Fusarium* mycotoxins in South and West Ethiopia. World Mycotoxin Journal. 2022, 15(1): 73-83

Kokeb Tesfamariam, Marthe De Boevre, Patrick Kolsteren, Tefera Belachew, **Addisalem Mesfin**, Sarah De Saeger, Carl Lachat. Dietary mycotoxins exposure and child growth, immune system, morbidity, and mortality: a systematic literature review. Critical Reviews in Food Science and Nutrition. 2019, 60:3321–41

Teshale Darebo, **Addisalem Mesfin**, Samson Gebremedhin. Prevalence and factors associated with overweight and obesity among adults in Hawassa city, southern Ethiopia: a community based cross-sectional study. BMC Obesity. 2019, 6:8

Hadiya Hassen, Susan J Whiting, **Addisalem Mesfin**, Samson Gebremedhin. Prevalence and Factors Associated with Undernutrition among Exclusively Breastfeeding Women in Arba Minch Zuriya district, southern Ethiopia: a cross-sectional community-based study. 2019, 29(1): 913-922

Getenesh Birhanu, **Addisalem Mesfin**, Afework Kebebu, Susan J Whiting, Carol Henry. Household food processing methods to enhance the bioavailability of iron and zinc in formulated haricot bean and maize complementary food at Hawassa Zuria, South Ethiopia. African journal of food science. 2014, 8(4): 190-195

Yirgu Fekadu, **Addisalem Mesfin**. Demoze Haile, Barbara J Stoecker. Factors associated with nutritional status of infants and young children in Somali region, Ethiopia: a cross- sectional study. Journal of BMC public health. 2015, 15:846

Addisalem Mesfin, Carol Henry, Susan J Whiting. Use of Pulse Crops in Complementary feeding of 6-23 months infants in Taba Kebele, Southern Ethiopia. Journal of public health in Africa. 2015, 6:357

Trainings and presentations	
Title	**Year attended**
The International Society of Mycotoxicoloy (ISM) best oral presentation award at the 3rd African Society of Mycotoxicoloy symposium, South Africa	4 – 7 September 2022
Missing data, Ghent university, Belgium	14-15 March, 2022
Basic data wrangling in R environment, Hawassa university, Ethiopia	16-21 August, 2021
Multilevel Analysis for Grouped and Longitudinal Data, , Ghent university, Belgium	9-11 April, 2021
Doing research in Africa: funding, ethics, and integrity: organized by Ghent University, Belgium	2019
Mycotoxin extraction, Analysis using LCMS/MS, Data integration using masslynx and quantification, Ghent University, Belgium	15-26 September, 2018
Dietetics, Hawassa university, Ethiopia	June 30-July 04, 2014
Quantitative and Qualitative Research Analysis, Hawassa university, Ethiopia	2014
Operational research, Hawassa university, Ethiopia	2014
Effective Teaching Skills, Jhpiego, Ethiopia	September 3-8, 2012
Sample Analysis and Utilization of Atomic Absorption Spectroscopy, Hawassa university, Ethiopia	Feb 26-28, 2013
Scientific Paper Writing, Hawassa university, Ethiopia	January 28-February 1, 2013
Application of software for Data Analysis and Interpretation, Hawassa university, Ethiopia	February 29-March 2, 2012
Instructional Skill Training, Hawassa university, Ethiopia	November 12-17, 2007

Professional experiences		
Description of core responsibility	**Responsible organization**	**Year**
Assistant professor at the department of Human nutrition, Hawassa university, Ethiopia	Hawassa university, Ethiopia	Current status
Trainer of Nutrition Sensitive Agriculture for subject matter specialists (SMS) and Development Agents (DAs), Ethiopia	SARI (South Agricultural Research Institute), Ethiopia	3 different times
Trainer of ETS (Effective Teaching Skills) for instructors from different Ethiopian universities	Jhpiego, Ethiopia	May 22-27, 2017
Trainer of sanitization of mothers and teachers about the basics of nutrition for adults and children	Anchor and Hawassa University	May 06, 2017
Co-writer of Customizing the ETS (Effective Teaching Skills) training manual to make it up to date and gender responsive	Jhpiego	March 20-24, 2017
Exhibitor of pulse crops based food products at Integrated Nutrition Conference held in Nairobi, Kenya	CIFSRF project at Hawassa University and CRS, Ethiopia	Nov 14-16, 2016
Coordinator of the nutrition and food science component of the CIFSRF project at Hawassa University	CIFSRF project at Hawassa University	2015-2018
Lecturing nutrition related courses, advising undergraduate and graduate students in their thesis work and conducting research	Hawassa University, Ethiopia	2011-2019

Trainer of 'Basics of Nutrition for core care coworkers, mothers and aunts.'	SOS children's Village Program, Hawassa	May 2-4, 2015
Trainer of 'Nutrition Technical Update training' for agriculture instructors at Hawassa University	Jhpiego, Ethiopia	March 02-05, 2015
Co-writer of Nutrition during Emergency e-learning module	eLEFANS project, Ethiopia	September 01, 2014 to July 31, 2016
Trainer of effective teaching skills training for Agriculture and Nutrition instructors at Hawassa University	Jhpiego, Ethiopia	December 15-20, 2014
Co-trainer of content integration training manual	Jhpiego, Ethiopia	2013
Visiting scholar at the College of Pharmacy and Nutrition, University of Saskatchewan, Canada.	CIFSRF project	July 06-September 06, 2013
Coordinator of the department of Human Nutrition, Hawassa university, Ethiopia	Hawassa University	December 24 2012- December 04, 2013
Trainer of 'Farmers training on the nutritional importance of pulses' at Butajira, SNNPRS, Ethiopia	CIFSRF project, Hawassa University	August 18, 2012
Trainer of 'Farmers training on the nutritional importance of pulses' at Halaba, SNNPRS, Ethiopia	CIFSRF project, Hawassa University	May 23, 2012
Internship on Nutrition Assessment, Counseling, and Support for People Living with HIV/AIDS at Adama Hospital, Adama, Ethiopia	Save the Children USA, Ethiopia branch	November 18, 2010- January 03, 2011
Assistant lecturer: assist practical sessions in nutrition related courses and advise students in senior researches	Hawassa University, Ethiopia	September 2009-2011
Graduate assistant: Assist practical sessions in nutrition related courses and advise students in senior researches	Hawassa University, Ethiopia	September 2007-2009
Membership		
International Society of Mycotoxicoloy (ISM) African Society of Mycotoxicoloy (ASM) Ethiopia Nutrition Leaders Network (ENLN) Food and Nutrition Society of Ethiopia Ethiopian Public Health Association Ethiopian Family Guidance Association		

Annexes

Chapter 3 and 4

Table 1: Household characteristics of the study participants'

No	Questions	Response (s)
1	In what month and year were you born?	Month……..…. 1 Year..... 2
2	How old are you now? Compare and correct and /or if inconsistent	_____years
3	What is your family size?	
4	What is your marital status?	Married……...1 Single……..2 Divorced……3 Widowed……4 Other
5	What is the highest level of school you attended?	Illiterate……1 Read and write only…….2 Formal education (highest grade completed _____)……3
6	What is the main source of income for the family?	Farming……1 Government employment……2 Small scale business……....3 No job……..4 Other (specify)_____5
9	Do the family grow crops?	Yes…….1 No……..2
10	If yes to Q#9, list the crops you grow from highest to lowest	1st 2nd 3rd 4th 5th 6th 7th

Table 2: FAO minimum dietary diversity for women

No	Questionnaire rows	Food groups
1	Foods made from grains White roots and tubers and plantains	Grains, white roots and tubers, and Plantains
2	Pulses (beans, peas and lentils)	Pulses (beans, peas and lentils)
3	Nuts and seeds	Nuts and seeds
4	Milk Milk products	Milk and milk products
5	Organ meats Red flesh mammal's meat Processed meat Poultry and other white meats Fish and seafood	Meat, poultry and fish

6	Eggs	Eggs
7	Dark green leafy vegetables	Dark green leafy vegetables
8	Vitamin A-rich vegetables, roots and tubers Vitamin A-rich fruits	Other vitamin A-rich fruits and vegetables
9	Other vegetables	Other vegetables
10	Other fruits	Other fruits

Table 3: Food frequency questionnaire for common mycotoxin prone foods

Type of crop/food	> 1 per day	Once per day	3-6 times per week	Once or twice per week	Two or less per month	Never eaten
Maize						
Sorghum						
Finger millet						
Barley						
Ground nut						
Milk						

Table 4: Household food insecurity

No	Question	Response
1	In the past four weeks, did you worry that your household would not have enough food?	☐ 1=Yes ☐ 0=No ☐ 98=Don't Know
1a	If yes, how often did this happen?	1 = Rarely (once or twice in the past four weeks) 2 = Sometimes (3 to 10 times in the past four weeks) 3 = Often (more than 10 times in the past four weeks)
2	In the past four weeks, were you or any household member not able to eat the kinds of foods you preferred because of a lack of resources?	☐ 1=Yes ☐ 0=No ☐ 98=Don't Know
2a	If yes, how often did this happen?	1 = Rarely (once or twice in the past four weeks) 2 = Sometimes (3 to 10 times in the past four weeks) 3 = Often (more than 10 times in the past four weeks)
3	In the past four weeks, did you or any household member have to eat a limited variety of foods due to a lack of resources?	☐ 1=Yes ☐ 0=No ☐ 98=Don't Know

3a	If yes, how often did this happen?	1 = Rarely (once or twice in the past four weeks) 2 = Sometimes (3 to 10 times in the past four weeks) 3 = Often (more than 10 times in the past four weeks)
4	In the past four weeks, did you or any household member have to eat some foods that you really did not want to eat because of a lack of resources to obtain other types of food?	☐ 1=Yes ☐ 0=No ☐ 98=Don't Know
4a	If yes, how often did this happen?	1 = Rarely (once or twice in the past four weeks) 2 = Sometimes (3 to 10 times in the past four weeks) 3 = Often (more than 10 times in the past four weeks)
5	In the past four weeks, did you or any household member have to eat a smaller meal than you felt you needed because there was not enough food?	☐ 1=Yes ☐ 0=No ☐ 98=Don't Know
5a	If yes, how often did this happen?	1 = Rarely (once or twice in the past four weeks) 2 = Sometimes (3 to 10 times in the past four weeks) 3 = Often (more than 10 times in the past four weeks)
6	In the past four weeks, did you or any household member have to eat fewer meals in a day because there was not enough food?	☐ 1=Yes ☐ 0=No ☐ 98=Don't Know
6a	If yes, how often did this happen?	1 = Rarely (once or twice in the past four weeks) 2 = Sometimes (3 to 10 times in the past four weeks) 3 = Often (more than 10 times in the past four weeks)
7	In the past four weeks, was there ever no food to eat of any kind in your household because of lack of resources to get food?	☐ 1=Yes ☐ 0=No ☐ 98=Don't Know
7a	If yes, how often did this happen?	1 = Rarely (once or twice in the past four weeks) 2 = Sometimes (3 to 10 times in the past four weeks) 3 = Often (more than 10 times in the past four weeks)
8	In the past four weeks, did you or any household member go to sleep at night hungry because there was not enough food?	☐ 1=Yes ☐ 0=No ☐ 98=Don't Know

8a	If yes, how often did this happen?	1 = Rarely (once or twice in the past four weeks) 2 = Sometimes (3 to 10 times in the past four weeks) 3 = Often (more than 10 times in the past four weeks)
9	In the past four weeks, did you or any household member go a whole day and night without eating anything because there was no enough food?	☐ 1=Yes ☐ 0=No ☐ 98=Don't Know
9a	If yes, how often did this happen?	1 = Rarely (once or twice in the past four weeks) 2 = Sometimes (3 to 10 times in the past four weeks) 3 = Often (more than 10 times in the past four weeks)

Table 5: Maize related pre- and post-harvest practices

No	Questions	Response (s)
1	Do you grow maize?	Yes……1 No……..2
2	Before sowing maize, do you clean crop residues (seed heads, stalks, other debris) from the previous harvest?	Yes……1 No……..2
3	Which maize variety do you sow usually?	1. Extension…….1 2. Shone……...2 3. Mirit zer……...3 4. 660……...4 5. Limo……..….5 6. Finer……..….6 7. BH……...7 8. Other_____8
4	How do you usually water the maize growing field?	Rain water……....1 Irrigation water…….2 Both rain and irrigation water…….3 other_____4
5	How often do you weed the maize on the growing field?	Daily……1 Weekly……2 Every two week…...3 Monthly…….……..4 Other_____5
6	Do you use crop rotation?	Yes……1 No……..2
7	Do you usually use pesticide/insecticides on maize growing field?	Yes……1 No……...2
8	What is your usual timing of maize harvesting?	When maize is still green……1 When maize has begun to dry…….2 When maize is completely dry……3 Other_____4

9	Do you have especial drying mechanism of premature harvested or rain attacked maize?	Yes……1 No……...2
10	If your answer for Q9 is yes, how do you dry?	Air drying at sheds……...1 Solar drying …….....2 keep as it is until trashing….3 1 & 2……....…4 Other_____5
11	Do you sort out undesirable crops after drying and before storage?	Yes……1 No……..2
12	Did you treat (disinfect) the storage area before storing maize?	Yes……1 No……..2
13	Where do you store maize?	In "gotara" made from teff straw and mud…….....1 In "gotera" made from wood or bamboo…….2 In underground pits……..….3 In plastic bags……...…….4 In madaberia…….....…5 other_____6
14	How do you store maize in the storage place?	Cobs in maize bags…….1 Cobs without maize bags…2 Grain in maize bags.……3 Grain without bags…….....4 Other_____5
15	Did you use pesticides/insecticide in storage?	Yes……1 No……...2
16	Did you use fumigation during storage?	Yes……1 No……..2
17	Do you use any decontamination methods for mold infected crops?	Yes……1 No……..2
18	If your answer for Q17 is yes, what types of decontamination methods you use? More than one answer is possible	Roasting……....1 Washing……....2 Sorting…….……..3 Using chemicals…....4 Sun drying……..…...5 Other_____6
19	For how long do you usually store maize?	_____months
20	What do you think is the cause for maize mold formation in a storage More than one answer is possible	Moisture…..…….1 Use of expired insecticide…….2 High tempreture……3 Other_____4
21	Have you ever heard about fungus affected maize?	Yes……1 No……..2
22	Do you think consuming moldy food/crop have effect on health?	Yes……1 No……..2
23	If your answer for Q22 is yes, what do you think are the health effects of consuming moldy food/crop? More than one answer is possible	Vomit…….....1 Diarrhea……..…..2 Growth impairment…..3 Cancer……..……...4 Other_____5 I don't know……....6

24	Do you buy any moldy crop from market?	Yes……1 No……...2
25	If your answer for Q24 is yes, what is your reason to buy? More than one answer is possible	Cheap……..1 If dehulled, no problem…...2 No problem……..3 Sort and discard…….....4 Other_____5
26	Which crop type do you think is more susceptible to mold spoilage?	Cereals……1 Legumes…….2 Other_____3
27	If your answer for Q23 is cereals, which cereal do you think is more susceptible to mold spoilage?	maize…….....1 sorghum……...2 wheat…….....3 barley……...4 teff……..….5 other_____6

Table 6: Household maize processing methods

No	Questions	Response (s)
1	Do you sort visibly moldy maize crops in the preparation of maize flour	Yes…….....1 No…….…….....2
2	Do you dehull maize crops in the preparation of maize flour	Yes…….…1 No……..2
3	Do you soak/wash maize crops in the preparation of maize flour	Yes……..……1 No…….……..2
4	Do you roast maize crops in the preparation of maize flour	Yes……..…1 No……....2
5	What do you do with removals/undesirable parts after sorting/dehulling/soaking of maize crops **More than one answer is possible**	Burn…….....1 Making beverages……....2 Stock for feed…….…….....3 Bury…….4 Other_____5

Chapter 5

Supplementary Table I: **Round I Delphi questionnaire**

Direction: please describe, according to your opinion, which variables should be present in a dataset that contains mycotoxin contamination data.

Study level descriptors	
Variable	Properties
E.g. Country	text
Sample level descriptors	
Variable	Properties
E.g. Date of analysis	date
Assay level descriptors	
Variable	Properties
E.g. Samples < LOD	number

Supplementary Table II: **Round II Delphi questionnaire**

Direction: this Delphi round mainly assess whether the participant agree on each item on the list, organized in 3 levels as study, sample and assay, therefore indicate your (dis)agreement with motivation where necessary

No	**Item/descriptors**	**Description**	**Proposed by**	**Do you agree?**	**If not, explain**
1	Country providing the Record	3-character code (AAA) assigned by WHO identifying the country of origin of the food	GEMS and EFSA		
2	Country of origin of the product	the country where the commodity originates from	EFSA and GEMS		
3	Sample location	First, the provider should specify whether the commodity was sampled from farm/storage/industry/retailer. If the commodity was sampled from farm or storage, provide more information about the specific region/s, district/s, village/s of sampling and also GPS coordinate if any	MYTOX-SOUTH®		
4	Time period of food sampling	beginning and ending dates of sample collections	GEMS		

No	Item/descriptors	Description	Proposed by	Do you agree?	If not, explain
	Year of sampling	In case the sampling has been performed over a period of time the start date of sampling should be reported.	EFSA		
5	Program type	Indicate the type of control program or other type of source to which the sample belongs. Eg. Survey	EFSA		
6	Representativeness of the Samples	Statistically/not statistically based and representative for the whole	GEMS		
7	Sampling strategy	It is mandatory to describe how the sample was selected from the population being monitored or surveyed.	EFSA		
8	Sampling method	It is mandatory to define the way the samples have been collected for analysis. In case of pooled samples, the number of the sample also should be provided in 'Number of samples (S.36). (The default value of the number of the sample is "1").	EFSA		
9	Sampling from lot	Describe the way the samples have been collected as per the below protocol • Lot weight in tonnes otherwise if the lot or sublot consists of individual packages or units, then the number of packages or units weight or number of sublots _____tonnes /_____ no • Weight and number of separate and identifiable parts of a large lot designated in order to apply the sampling method _____kg and _____no • Number of incremental samples taken from the lot or sublot. If the lot or sublot consists of individual packages or units, then the number of packages or units should be reported _____no • Combined total weight of all the incremental samples taken from the lot or sublot (kg) _____kg	MYTOX-SOUTH®		
10	Sampling point	It is obligatory to define the point of the food chain where the sample was taken	EFSA		

No	Item/descriptors	Description	Proposed by	Do you agree?	If not, explain
11	Method of production	Recommended to report weather the sample was obtained from the produce of traditional (non-organic) or organic farming.	EFSA		

No	Item/descriptor	Description	Proposed by	Do you agree?	If not, explain
1	Serial Number of the Record	Eg. The entry "01001023" represents record number 1023 created by source 01 in the year 2000	GEMS		
	Laboratory sample code	a unique sample identification number, not longer than 20 characters	EFSA		
2	Date of Record Creation	DD-MMM-YYYY	GEMS		
3	Date of sample analysis	It is mandatory to report the year of analysis. If the analysis has been performed over a period of time the completion date of analysis should be stated	EFSA		
4	Language	language used to complete the free text fields	EFSA		
5	Food identifier	up to an 6-digit code designated by the Codex Alimentarius Commission and a partial list of raw commodities and semi-processed foods	GEMS		
	EFSA Product code	Food and feed products should be described according to the "FoodEx" catalogue of the SSD. It is envisaged that detailed information on the different food groups will be needed to perform the exposure assessment. It is mandatory to report at least the level 2 of the FoodEx code. It is strongly encouraged to classify the food samples at the most detailed hierarchical level available (FoodEx level 3 and 4). This is particularly needed for food groups like "Food for infants and small children" and "Products for special nutritional use", where any available additional descriptions shall be provided. Specific attention needs to be given to the reporting of data on cereal grains. It is very important to make a clear distinction between grains as harvested (unprocessed grains of undefined use, not for human exposure assessment), grains for human consumption and grains as feed. For this reason three distinct groups are available within the FoodEx catalogue.	EFSA		

		Please be aware that the Feed codes starting with "F" are not in use any longer'; "G" codes should be used instead.			
6	Commodity intended use	The intended use of the food or feed sampled should be further described. • If for feed, provide information about the type of animal intended for the feed 1) Immature animals and poultry ☐ 2) Dairy animals ☐ 3) Breeding cattle and swine☐ 4) Mature poultry ☐ 5) Finishing swine ☐ 6) Finishing beef cattle ☐ 7) Beef cattle, swine or poultry ☐ 8) Horses ☐ 9) Other animals (specify)_____ 10 Unknown ☐ • If food for human consumption, state whether the food is intended for 1. Infant ☐ 2. Adult ☐ 3. Special nutritional use☐ 4. Other (specify)_____ 5. Unknown ☐	MYTOX-SOUTH®		
7	Product full text description	Product full text description is essential to check if the EFSA product code (FoodEx code) given by the data provider is consistent with the text description. This will avoid any possible mistakes in coding and additional clarification requests. In addition, as this is a free text element the information could be provided even in the national language. Original description of the sample from the national database can be copied here. It should be avoided to repeat just the FoodEx description. Moreover, any additional information that does not belong to any of the other SSD fields should be reported in here	EFSA		
8	Cropping history	Provide information about some of the important parameters related with growing Ask application of crop rotation and especially previous crop harvested!	MYTOX-SOUTH®		

		• Write the type/name of the crop variety used _____ • Type of cultivation method • Maturity level of the crop at harvest 1. Green ☐ 2. Began to dry ☐ 3. Completely dry ☐ 4. Other (specify)_____ 5. Unknown ☐ • Application of fungicide 1. Yes ☐ 2. No ☐ 3. I don't know ☐			
9	Storage facility/condition	Information related to the facility and condition of storage from where the crop is sampled should be provided • Mechanism/s implemented to control temperature 1. Aerated by circulation of air ☐ 2. Temperature measured regularly ☐ 3. Other (specify)_____ 4. Unknown ☐ • Mechanism/s implemented to control moisture 1. Dry area of storage ☐ 2. Ventilated structures for natural drying ☐ 3. Water impermeable layer from floor ☐ 4. Other (specify)_____ 5. Unknown ☐ • Application of chemical in the storage 1. Yes ☐ 2. No ☐ 3. I don't know ☐ • Duration of storage before the sampling date _____days/_____months • Type of the storage used 1. Metal silo bins ☐ 2. Hermetic bags ☐ 3. Insecticide-treated bags ☐	MYTOX-SOUTH®		

		4. Underground pits ☐ 5. Plastic bags ☐ 6. Storage made from wood or bamboo ☐ 7. Storage made from straw and mud ☐ 8. Other (specify)_____ 9. Unknown ☐			
10	Product treatment	It is mandatory to clearly indicate if the original sample is treated or not, especially if it is a dehydrated product (select: "Dehydration", T131A); in the absence of this information the status “as consumed” will be assumed.	EFSA		
11	Product comment		EFSA		
12	Percentage of moisture in the original sample	It is recommended to report the percentage of moisture in the original sample of feed and for processed cereals based foods for infant and young children	EFSA		

No	**Item/descriptor**	**Description**	**Proposed by**	**Do you agree?**	**If not, explain**
1	Number of laboratories participating in sample analyses	for large countries, data from different laboratories	GEMS		
	Laboratory	It is highly recommended to report here a unique code to identify each laboratory providing laboratory results	EFSA		
2	Indicator of Analytical Quality Assurance	Internal quality assurance/proficiency tests or accredited	GEMS		
	Laboratory accreditation	Yes/No	EFSA		
3	Analytical method code (Text preview)	It is mandatory to specify the analytical instrument used Eg. LCMS	EFSA		
4	Contaminant identifier	Eg. code for mercury	GEMS		
	Parameter code (Text preview)	code the contaminants using the code from the SSD PARAM catalogue. It is mandatory to indicate whether parameter reported is an individual parameter or parameter sum.	EFSA		

5	Parameter type	It is mandatory to indicate whether parameter reported is an individual parameter or parameter sum.	EFSA		
6	Unit of reporting for contaminant levels	Eg. mg/kg	GEMS		
	Result unit	unit of measurement for the values reported	EFSA		
7	Range of Analytical Limits in the Data Set	LOD, LOQ, LOD minimum, LOD maximum, LOQ minimum, LOQ maximum	GEMS		
	Result LOQ and Result LOD	highly recommended to report at least the LOQ of the analytical method	EFSA		
8	Basis for the analytical Values	Eg. Codes for fat content/dry weight as (raw, fresh)	GEMS		
9	Number of samples analysed	number of sample analyses on which the analytical values (mean, median and 90th percentile) for this record are based	GEMS		
10	Sample weight at analysis	Weight in gram of the sample considered for analysis	MYTOX-SOUTH®		
11	Number of samples with concentrations below LOQ	number of sample analyses for which results were below LOQ	GEMS		
12	Range of quantified analytical concentrations	Minimum and maximum concentration	GEMS		
13	Mean concentrations	Mean with lowest and upper bound	GEMS		
	Mean (µg/kg) + standard deviation		EFSA		
14	Result value recovery	It is recommended to provide the recovery value associated with the concentration measurement (as a percentage) when an extraction step is applied in the analytical method.	EFSA		
15	Result value corrected for recovery	Yes/no	EFSA		
16	Result value uncertainty	It is recommended to provide the expanded uncertainty (95% confidence interval) associated with the concentration measurement	EFSA		
17	Expression of result	It is mandatory to express the results in the same units as the maximum levels laid down in Regulation (EC) 1881/200613 and Directive	EFSA		

		2002/32/EC14: • 88% dry matter for feed, • Fat weight for products of terrestrial animal origin, marine oils and vegetable oils and fats, • Whole weight of the products ready to use (marketed as such or after reconstitution as instructed by the manufacturer) for foods for infants and young children		[redacted]	
18	Type of result	Numeric	EFSA		
19	Median Concentration		GEMS		
20	90th Percentile Concentration		GEMS		
21	Standard Deviation (Optional)		GEMS		
22	Mycotoxins maximum limits of the country	Percentage (%) of samples that have mycotoxin concentrations above the concentration established by the local authority	MYTOX-SOUTH®		
23	Confidentiality of Data	Confidential/non confidential	GEMS	[redacted]	
24	Remarks/References		GEMS		
25	Comment of the result (R32)	In case the sample was reconstituted before analysis, e.g. infant formulae and follow-on formulae, it would be relevant to provide information on the exact reconstitution protocol (ratio dry product: added fluid (description of the fluid used) Examples: "14:86 (water)".	EFSA		

Supplementary Table III: **Round III Delphi questionnaire**

Direction: this Delphi round mainly assess whether the participant agree on each item on the list, organized in 3 levels as study, sample and assay, therefore indicate your (dis)agreement with motivation where necessary

y

Study level descriptors					
No	**Item/descriptors**	**Description**	**Proposed by**	**Do you agree?**	**If not, explain**
1	Country providing the Record	3-character code (AAA) assigned by WHO identifying the country of origin of the food	GEMS and EFSA	[redacted]	
2	Country of origin of the product	the country where the commodity originates from	EFSA and GEMS		

Study level descriptors					
No	**Item/descriptors**	**Description**	**Proposed by**	**Do you agree?**	**If not, explain**
3	Sample location	First, the provider should specify whether the commodity was sampled from farm/storage/industry/retailer. If the commodity was sampled from farm or storage, provide more information about the specific region/s, district/s, village/s of sampling and also GPS coordinate if any	MYTOX-SOUTH®		
4	Time period of food sampling	beginning and ending dates of sample collections	GEMS	[redacted]	
	Year of sampling	In case the sampling has been performed over a period of time the start date of sampling should be reported.	EFSA		
5	Program type	Indicate the type of control program or other type of source to which the sample belongs. Eg. Survey	EFSA		
6	Representativeness of the Samples	Statistically/not statistically based and representative for the whole	GEMS		
7	Sampling strategy	It is mandatory to describe how the sample was selected from the population being monitored or surveyed.	EFSA		
8	Sampling method	It is mandatory to define the way the samples have been collected for analysis. In case of pooled samples, the number of the sample also should be provided in 'Number of samples (S.36). (The default value of the number of the sample is "1").	EFSA		
9	Sampling from lot	Describe the way the samples have been collected as per the below protocol • Lot weight in tonnes otherwise if the lot or sublot consists of individual packages or units, then the number of packages or units weight or number of sublots _____tonnes /_____ no • Weight and number of separate and identifiable parts of a large lot designated in order to apply the sampling method _____kg and _____no	MYTOX-SOUTH®		

Study level descriptors					
No	**Item/descriptors**	**Description**	**Proposed by**	**Do you agree?**	**If not, explain**
		• Number of incremental samples taken from the lot or sublot. If the lot or sublot consists of individual packages or units, then the number of packages or units should be reported _____no • Combined total weight of all the incremental samples taken from the lot or sublot (kg) _____kg			
10	Sampling point	It is obligatory to define the point of the food chain where the sample was taken	EFSA	[redacted]	
11	Method of production	Recommended to report weather the sample was obtained from the produce of traditional (non-organic) or organic farming.	EFSA		

Additionally, link for the mycotoxin/s reporting template with the instruction document (chapter 5) can be found online at https://doi. org/10.1016/j.foodres.2021.110883.