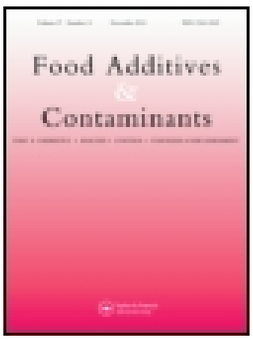

Food Additives & Contaminants: Part A

ISSN: 1944-0049 (Print) 1944-0057 (Online) Journal homepage: http://www.tandfonline.com/loi/tfac20

Urinary aflatoxin exposure monitoring in rural and semi-urban populations in Ogun state, Nigeria

Chibundu N. Ezekiel, Oyetunde T. Oyeyemi, Oluwawapelumi A. Oyedele, Kolawole I. Ayeni, Ifeoluwa T. Oyeyemi, Williams Nabofa, Chinomso U. Nwozichi & Adeyemi Dada

To cite this article: Chibundu N. Ezekiel, Oyetunde T. Oyeyemi, Oluwawapelumi A. Oyedele, Kolawole I. Ayeni, Ifeoluwa T. Oyeyemi, Williams Nabofa, Chinomso U. Nwozichi & Adeyemi Dada (2018): Urinary aflatoxin exposure monitoring in rural and semi-urban populations in Ogun state, Nigeria, Food Additives & Contaminants: Part A, DOI: 10.1080/19440049.2018.1475752

To link to this article: https://doi.org/10.1080/19440049.2018.1475752

Accepted author version posted online: 29 May 2018.
Published online: 19 Jun 2018.

FOOD ADDITIVES & CONTAMINANTS: PART A
https://doi.org/10.1080/19440049.2018.1475752

Taylor & Francis
Taylor & Francis Group

Urinary aflatoxin exposure monitoring in rural and semi-urban populations in Ogun state, Nigeria

Chibundu N. Ezekiel [a], Oyetunde T. Oyeyemi[b], Oluwawapelumi A. Oyedele[a], Kolawole I. Ayeni[a], Ifeoluwa T. Oyeyemi[c], Williams Nabofa[d], Chinomso U. Nwozichi[e] and Adeyemi Dada[f]

[a]Department of Microbiology, Babcock University, Ilishan Remo, Nigeria; [b]Department of Basic Sciences, Babcock University, Ilishan Remo, Nigeria; [c]Department of Biological Sciences, University of Medical Sciences, Ondo, Nigeria; [d]Department of Physiology, Benjamin Carson (Senior) School of Medicine, Babcock University, Ilishan Remo, Nigeria; [e]Department of Adult Health Nursing, School of Nursing, Babcock University, Ilishan Remo, Nigeria; [f]Department of Chemical Pathology, Lagos State University Teaching Hospital, Ikeja, Nigeria

ABSTRACT

Aflatoxins are a major class of fungal toxins that have food safety importance due to their economic and health impacts. This pilot aflatoxin exposure biomonitoring study on 84 individuals was conducted in a rural (Ilumafon) and a semi-urban community (Ilishan Remo) of Ogun state, Nigeria, to compare aflatoxin exposures among the two population cohorts. First morning urine samples were obtained from the participants, and the urinary aflatoxin M_1 (AFM_1) levels were measured by a quantitative Helica Biosystems Inc. ELISA kit assay. About 99% (83 out of 84) of the urine samples had detectable AFM_1 levels in the range of 0.06 to 0.51 ng mL^{-1} (median: 0.27 ng mL^{-1}). The mean urinary AFM_1 levels were significantly ($p = 0.001$) higher in the semi-urban population (0.31 ± 0.09 ng mL^{-1}) compared to the rural population (0.24 ± 0.07 ng mL^{-1}). There were, however, no significant differences in mean urinary AFM_1 levels of males and females, and among children, adolescents and adults. This study indicates high aflatoxin exposure to the extent of public health concerns in the studied populations. Thus, more efforts are required for aflatoxin exposure monitoring and control in high-risk regions.

ARTICLE HISTORY
Received 11 January 2018
Accepted 28 April 2018

KEYWORDS
Aflatoxin B_1; aflatoxin M_1; urinary biomarkers; ELISA; exposure

Introduction

Aflatoxins are toxic secondary metabolic products of *Aspergillus* origin. They are produced in crops during planting, harvesting and post-harvest handling such as drying, storage, transportation and food processing (Ayalew et al. 2016). Contamination of crops and processed foods by aflatoxins is a continuous challenge in developing countries where poor agricultural practices and inadequate postharvest conditions are commonplace (Williams et al. 2004; IARC 2015). In Nigeria, a tropical country with hot and humid climate, aflatoxins have been widely reported in a variety of agricultural crops such as maize, peanuts (groundnuts), rice, sorghum, melon seeds (Makun et al. 2009, 2010; Adetunji et al. 2014; Ezekiel et al. 2016; Oyedele et al. 2017), and their ready-to-eat products such as maize- and peanut-based snacks, *kunu-zaki, ogi, ogiri* and roasted peanuts (Bankole et al. 2005; Ezekiel et al. 2012, 2013, 2015; Kayode et al. 2013; Afolabi et al. 2015; Okeke et al. 2015; Adedeji et al. 2017).

Consumption of aflatoxin-contaminated foods has been associated with several health challenges such as growth retardation in children, hepatocellular carcinoma, gastrointestinal dysfunction, immune system toxicity, and deaths across Africa (Gong et al. 2002, 2016; Turner et al. 2003, 2012; Lewis et al. 2005; Probst et al. 2007; Turner 2013; Yard et al. 2013; IARC (International Agency for Research on Cancer) 2015; All Africa 2016; Outbreak News Today 2017). However, the degree of morbidity and mortality depends on several factors including the toxin levels in the food, extent of consumption of the contaminated foods, age and developmental stage of exposed individuals (Williams et al. 2004; IARC (International Agency for Research on Cancer) 2015). Among the known types of aflatoxins, aflatoxin B_1 (AFB_1) is the most toxic and is classified as a category 1 human carcinogen (IARC 2002).

CONTACT Chibundu N. Ezekiel chaugez@gmail.com Department of Microbiology, Babcock University, Ilishan Remo, Ogun State, Nigeria

AFB_1, when ingested by humans via contaminated foods, can be metabolised into several metabolites which serve as biomarkers of exposure (Routledge and Gong 2011; Leong et al. 2012; Warth et al. 2016). The metabolites include aflatoxin albumin adduct (AF-alb) in blood (Wild and Turner 2002; Turner et al. 2007), aflatoxin N7-guanine in urine (Groopman et al. 1993), and aflatoxin M_1 (AFM_1) in urine (Wild and Turner 2002) and breast milk (Degen et al. 2013). In the urine, AFM_1 is more commonly detected than the aflatoxin-guanine complex (Abia et al. 2013; Ezekiel et al. 2014; Ali et al. 2016, 2017; Schwartzbord et al. 2016; Chen et al. 2017). Urine is often preferred to blood for aflatoxin biomonitoring in high-risk regions due to its non-invasiveness, ease of collection and usefulness in providing data on recent (within 48 h) daily exposures (Zhu et al. 1987; Groopman et al. 1992; Chen et al. 2017).

Common analytical platforms for aflatoxin monitoring in human urine include the Enzyme-linked immunosorbent assay (ELISA) (Groopman et al. 1993; Ali et al. 2016), high performance liquid chromatography (Groopman et al. 1993; Ali et al. 2017) and liquid chromatography tandem mass spectrometry (Rubert et al. 2011; Solfrizzo et al. 2011; Warth et al. 2012). The ELISA, however, remains a low-cost, easily accessible, and high throughput technique available for urine biomonitoring in developing countries such as Nigeria where resources for high-end techniques are scarce. Recently, Chen et al. (2017) used the ELISA to prove a strong correlation between aflatoxin levels in blood and urine.

Despite the widespread contamination of aflatoxins in crops and processed foods in Nigeria, only few reports are available for aflatoxin monitoring by biological fluids: blood (Oluwafemi et al. 2012; Mcmillian et al. 2018), breast milk (Adejumo et al. 2013) and urine (Ezekiel et al. 2014). Considering the need for continuous monitoring of daily exposures in high-risk (tropical) regions, and the paucity of data for comparison of aflatoxin exposures among rural dwellers and urban/semi-urban populations, this study aimed to assess aflatoxin exposures in two population cohorts (rural and semi-urban) in Ogun State, Nigeria using the urinary ELISA method.

Materials and methods

Study area

The study was carried out in two communities (Ilumafon and Ilishan Remo) in Ogun State, Nigeria. The study locations were purposely selected for convenience of sampling. Ilumafon is a rural settlement comprising about 200 dwellers who subsist on agriculture, growing own crops for income. Crops grown and consumed by the rural dwellers of Ilumafon include cassava, cocoa, oil palm and maize. Ilishan Remo is a semi-urban community of about 10,000 residents due to the presence of a tertiary education institution within the community. The residents were made up of students, civil servants, farmers and marketers. Farming and production of own food is considered a secondary source of income among Ilishan Remo residents, and foods are usually purchased from local markets and stores within the community.

Study design and study population

A pilot, cross-sectional study made up of 84 participants (38 males and 46 females; age: 2–65 years) was conducted in the selected locations between December 2016 and January 2017. The participants were informed about the purpose of the research, and based on their willingness to participate in the study, 84 individuals were randomly selected. Each participant signed an informed consent; parents gave consent for their children. The age categories reported by Ezekiel et al. (2014) (children: ≤8 years; adolescents: ≤19 years; adults: ≥20 years) were adopted, and the categories had 13, 24 and 47 participants, respectively. Of the 84 participants, 45 were recruited at rural community (Ilumafon), while 39 were from semi-urban area (Ilishan Remo). A well-structured questionnaire was administered to each participant by trained interviewers to obtain their demographic information, socio-economic status and dietary preferences. Translation to Yoruba language was necessary for the dwellers of the rural community. The study was approved by the Babcock University Health Research Ethics Committee (BUHREC154/17).

Sample collection

A first morning urine sample (20 mL) was collected from each recruited participants (n = 84). Urine samples were collected prior to consumption of food or water for the day. The urine samples were immediately transferred to the laboratory and then frozen until aflatoxin analysis. Prior to analysis, urine samples were brought to ambient temperature and centrifuged at 5000 rpm for 5 min to obtain a clear supernatant.

Determination of aflatoxin M_1 in urine by ELISA

AFM_1 in the urine samples was assayed by a quantitative ELISA kit assay (Helica Biosystems, Inc. AFLMO1U) according to manufacturer's instructions. Briefly, all reagents and urine samples were allowed to reach ambient temperature. Aliquots of both the AFM_1 standards and samples (urine supernatant) were diluted 1:20 with distilled water. Assay buffer (200 µL) was dispensed into each mixing well, and 100 µL of each diluted standard and sample was added to the appropriate mixing well containing the assay buffer. The mixture (100 µL) in each mixing well was transferred to a corresponding antibody coated microtiter well which was then incubated at ambient temperature for 1 h. After incubation, the content of each microwell was discarded, and the microwells were washed three times with PBS-Tween wash buffer and dried. Aliquots (100 µL) of a conjugate and a substrate were added consecutively with a step of incubation at room temperature for 15 min after each solution was added. A stop solution (100 µL) was then added, and the optical densities (OD) of the reaction solution in the microtitre plates were read at 450 nm using a Rayto (RT-2100C) Microplate reader (Rayto Life and Analytical Sciences Co. Ltd, Shenzhen, China).

The corresponding aflatoxin concentration in each well was estimated from standard curve plotted using AFM_1 standard solutions (0–4 ng mL^{-1}) and taking into account the inverse proportionate level of the absorption intensity to AFM_1 concentration in the samples. Blank urines were spiked at three concentration levels (0.8, 1.5 and 4 ng mL^{-1}) of AFM_1 standard in an assay validation test in order to determine the recovery (range: 84–106%) and limit of detection (0.06 ng mL^{-1}).

Statistical analysis

Data was analysed by SPSS 17.0 for Windows (SPSS Inc., IL, USA). Questionnaire data were analysed by simple descriptive statistics, while one-way ANOVA and Student's t-test were used to test for significance ($\alpha = 0.05$) in aflatoxin data for age groups, sex and location.

Results and discussions

Demographic characteristics and dietary preferences of the participants within the cohorts

The demographic characteristics and dietary preferences of the 84 participants recruited into this

Table 1. Demographic characteristics and dietary preferences of the participants during a urinary biomarker study in Ogun state, Nigeria.

	Number (percentage) of respondents		
Characteristics	Rural	Sub-urban	Overall
Subjects	45	39	84
Male	16(35.6)	22(56.4)	38(45.2)
Female	29(64.4)	17(43.6)	46(54.8)
Age (n (%); mean±SD)			
Children (age range: 2–7 years)	8(17.8); 4.6 ± 1.3	5(12.8); 4.8 ± 2.3	13(15.5); 4.7 ± 1.7
Adolescent (age range: 9–19 years)	11(24.4); 12.1 ± 2.9	13(33.3); 18.5 ± 1.1	24(28.6); 15.8 ± 3.7
Adult (age range: 20–65 years)	26(57.8); 39.6 ± 12.1	21(53.9); 25.6 ± 6.2	47(55.9); 33.3 ± 12.1
Occupation			
Local traders of foodstuffs	18(40.0)	-	18(21.4)
Farmers	21(46.7)	-	21(25.0)
Civil servants	6(13.3)	6(15.4)	12(14.3)
Student	-	33(84.6)	33(39.3)
Dietary preferences per day			
Tuber-based foods	33(73.3)	6 (15.4)	39(46.4)
Cereal-/nut-based foods	12(26.7)	33(84.6)	45(53.6)
Combined/equal preference	7(15.6)	8(20.5)	15(17.9)

aflatoxin biomonitoring study are presented in Table 1. About 45% of the participants were males, while 55% were females. In the rural setting (Ilumafon, n = 45), a higher percentage of participants 64.4% were females while the male participants had a higher percentage 56.4% in the semi-urban community (Ilishan-Remo). The mean ages (years) of participants within the rural setting (Ilumafon) were 4.6, 12.1 and 39.6 for children, adolescents and adults, while children, adolescents and adults in the semi-urban community (Ilishan Remo) had mean ages of 4.8, 18.5 and 25.6, respectively. In the two locations, adults were the most recruited, followed by adolescents and children. Participants from the rural community (Ilumafon) were farmers (47%), small-scale traders (40%) and civil servants (13%), whilst the participants from the semi-urban community (Ilishan Remo) were students (85%) and civil servants (15%). With respect to dietary preferences, participants from the rural setting (Ilumafon) consumed more tuber-based foods (73%), while those from the semi-urban community (Ilishan Remo) preferred cereal-based foods 33(85%). Overall, dietary preferences of the 84 participants were 54%, 46% and 18% for cereal-based foods, tuber-based foods, and a combination of both types of foods, respectively.

Distribution of aflatoxin M_1 levels in urine of the two cohort groups

The levels of AFM_1 found in urines of rural dwellers and semi-urban residents in Ogun state, Nigeria, are presented in Table 2. AFM_1 was detected in 98% (range: 0.11–0.39 ng mL^{-1}) of the urine samples collected from the rural setting (Ilumafon), whilst all of the urine samples from the semi-urban community (Ilishan Remo) contained detectable levels (range: 0.06–0.51 ng mL^{-1}) of AFM_1. The mean AFM_1 level (0.31 ± 0.07 ng mL^{-1}) in the urines from the semi-urban community (Ilishan Remo) was significantly ($p = 0.001$) higher than the mean levels (0.24 ± 0.07 ng mL^{-1}) quantified in urines from participants living in the rural environment (Ilumafon). On the overall, 83 (99%) out of the 84 urine samples contained detectable AFM_1 levels, with mean of 0.27 ± 0.08 ng mL^{-1} (range: 0.06–0.51 ng mL^{-1}; median: 0.27 ng mL^{-1}).

Table 2. Aflatoxin M_1 levels in urines of rural and semi-urban dwellers in Ogun state, Nigeria.

	Aflatoxin levels (ng mL^{-1}) by location		
	Rural (n = 45)	Semi-urban (n = 39)	Overall (n = 84)
Percentage positive	97.7	100	98.8
Range	0.11–0.39	0.06–0.51	0.06–0.51
Mean ± standard dev.	0.24^{b}±0.07	0.31^{a}±0.09	0.27 ± 0.08
Median	0.24	0.32	0.27

Means with different superscript alphabets are significantly different (α = 0.05).

Aflatoxin biomonitoring studies, especially those that utilise the urinary AFM_1 biomarkers, are increasing in number and span different continents due to the need to provide exposure data from all food sources (Abia et al. 2013; Polychronaki et al. 2008; Warth et al. 2012, 2014; Ezekiel et al. 2014; Kouadio et al. 2014; Ali et al. 2016, 2017; Schwartzbord et al. 2016; Ayelign et al. 2017; Chen et al. 2017). Our study adds to the growing body of evidence on this subject. In this study, the urinary AFM_1 mean values reported for both cohorts were higher than the mean values of 54 ± 15 pg mL^{-1} and 99 ± 71 pg mL^{-1} reported by Ali et al. (2016) in Bangladeshi adults from urban and rural settings, respectively. Our study further presents a higher overall mean value for AFM_1 in urines compared to the overall means reported in the urines of individuals in Egypt (5.5 pg mL^{-1}, Polychronaki et al. 2008), Guinea (97 pg mL^{-1}, Polychronaki et al. 2008) and Ethiopia (0.0064 ng mL^{-1}, Ayelign et al. 2017). The overall mean value in our present paper is, however, relatively similar to the means reported in urine of individuals from northern Nigeria (0.3 μg L^{-1}, Ezekiel et al. 2014), Cameroon (0.33 ng mL^{-1}, Ediage et al. 2013) and Thailand (0.33 μg L^{-1}, Warth et al. 2014). Furthermore, the prevalence of aflatoxin exposure found in our study is higher than the prevalence (<50%) of aflatoxin exposure via urinary AFM_1 reported for any population; although more recently, a re-analysis of 120 urine samples from Nigeria, by a more sensitive method, which earlier was reported to have 14.2% prevalence for urinary AFM_1 (Ezekiel et al. 2014), has now been shown to increase to 70% prevalence (Šarkanj et al. 2018). This confirms that aflatoxins exposure is very high in Nigeria.

Comparing the urinary AFM_1 levels in the two cohorts, we found significantly higher mean values from the semi-urban community (Ilishan Remo) than from the rural setting (Ilumafon). This finding, however, does not agree with Ali et al. (2016), who reported higher AFM_1 levels in urines of adult rural dwellers than in urines from adult urban dwellers in Bangladesh. It should, however, be noted that the samples size of 39 individuals from a population of about 10,000 resident in the semi-urban community (Ilishan Remo) is not representative unlike the sampled rural village (Ilumafon). The disparity in the reported AFM_1 levels from the two cohorts could be attributed to the occupation and dietary preferences of the participants in the groups that we studied. There are evidences that suggest higher aflatoxin exposures in populations that depend on cereal- and nut-based foods, which are more prone to aflatoxin contamination, than in those with diversified diets (IARC (International Agency for Research on Cancer) 2015; Watson et al. 2015). In our study, participants from the rural setting (Ilumafon) were primarily farmers and traders of locally produced foodstuffs, whilst the semi-urban (Ilishan Remo) participants made up of students and civil servants. This implies that most of the rural (Ilumafon) dwellers consume their own crops, mainly tuber-based foods, which are less prone to aflatoxin contamination (Abass et al. 2017), whilst the semi-urban (Ilishan Remo) residents who were predominantly students and civil servants tend to consume more cereal (e.g. maize)- and nut (peanut)-based foods of local and industrial origin. This may have contributed to a lower exposure level within this cohort compared to the semi-urban cohort. Furthermore, handling (e.g. transportation lag from farm to markets, storage duration and conditions) of crops used to make the cereals which the participants from the semi-urban community depended on may also have been a contributor to the higher exposure levels observed in this cohort compared to the rural dwellers who did not need to store their crops for months before consumption. Aflatoxin contamination of crops is mostly a storage problem requiring urgent interventions (Shephard et al. 2003; Ezekiel et al. 2017; Misihairabgwi et al. 2017).

With respect to age and sex (Table 3), no significant differences were recorded for AFM_1 levels in the urine samples of male *versus* female participants, and among children, adolescents and adults, although the male participants had higher mean urinary AFM_1 levels than the females, and the urines from children and adolescent age groups recorded higher AFM_1 levels than the adult group. The non-significant findings for variations in AFM_1 levels of male and female participants recorded in our study agree with the reports of Ediage et al. (2013) and Ali et al. (2017). On the other hand, the dietary requirements (e.g. higher food consumption per body weight) and rapid metabolism of the children and adolescents (Kostelanska et al. 2010; Lombard 2014) compared to adults may have contributed to the higher urinary AFM_1 levels detected for the children and adolescents groups than in the adults, although statistical analysis did not reveal significant differences between the groups.

Conclusion

Aflatoxin contamination of foods and subsequent human exposure remains a food safety and public health challenge in Nigeria. For the first time, we have compared aflatoxin exposure in two cohorts distinguished by type of residential location (rural and semi-urban) which influenced their dietary preferences. Exposure was higher among the semi-urban (ILishan Remo) population than within the rural (Ilumafon) dwellers. More efforts

Table 3. Distribution of aflatoxin M_1 levels in the urines of rural and semi-urban dwellers in Ogun state, Nigeria, grouped by age and sex.

	Aflatoxin levels (ng mL^{-1}) by sex		Aflatoxin levels (ng mL^{-1}) by age		
	Male (n = 38)	Female (n = 46)	Children (n = 13)	Adolescents (n = 24)	Adults (n = 47)
Percentage positive	100	97.8	100	95.8	100
Range	0.11–0.47	0.06–0.51	0.11–0.51	0.15–0.38	0.06–0.45
Mean ± standard dev.	0.28 ± 0.09	0.27 ± 0.08	0.28 ± 0.12	0.28 ± 0.07	0.27 ± 0.08
Median	0.29	0.27	0.28	0.27	0.27

should, however, be put in place to control the quality of foods consumed in Nigeria, especially among the younger age groups in order to minimise chronic health effects from toxin exposure over a long period. For the semi-urban residents, dietary diversity is highly recommended coupled with sourcing of high-quality grains. Educational modules need to be developed in partnership with farmers, agricultural extension workers, traditional leaders, and other significant members of the community on proper storage, processing and handling of agricultural products. In view of the limited sample size per cohort, further surveillance and intervention studies with larger sample sizes and across multiple seasons are required.

Acknowledgements

Authors thank Chinonso P. Ahuchaogu and Victoria C. Ezekiel for their support during sampling. We thank Babcock University Teaching Hospital for providing the ELISA reader for this study.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Chibundu N. Ezekiel http://orcid.org/0000-0002-2113-2948

References

Abass AB, Awoyale W, Sulyok M, Alamu EO. 2017. Occurrence of regulated mycotoxin and other microbial metabolites in dried cassava products from Nigeria. Toxins. 9:207.

Abia WA, Warth B, Sulyok M, Krska R, Tchana A, Njobeh PB, Turner PC, Kouanfack C, Eyongetah M, Dutton M, et al. 2013. Biomonitoring of mycotoxin exposure in Cameroon using a urinary multi-biomarker approach. Food Chem Toxicol. 62:927–934.

Adedeji BS, Ezeokoli OT, Ezekiel CN, Obadina AO, Somorin YM, Sulyok M, Adeleke RA, Warth B, Nwangburuka CC, Omemu AM, et al. 2017. Bacterial species and mycotoxin contamination associated with locust bean, melon and their fermented products in south-western Nigeria. Int J Food Microbiol. 258:73–80.

Adejumo O, Atanda O, Raiola A, Bandyopadhyay R, Somorin Y, Ritieni A. 2013. Correlation between aflatoxin M_1 content of breast milk, dietary exposure to aflatoxin B1 and socio-economic status of lactating mothers in Ogun State, Nigeria. Food Chem Toxicol. 56:171–177.

Adetunji M, Atanda O, Ezekiel CN, Sulyok M, Warth B, Beltran E, Krska R, Obadina O, Bakare A, Chilaka CA. 2014. Fungal and bacterial metabolites of stored maize (*Zea mays* L.) from five agroecological zones of Nigeria. Mycotoxin Res. 30:89–102.

Afolabi CG, Ezekiel CN, Kehinde IA, Olaolu AW, Ogunsanya OM. 2015. Contamination of groundnut in South-Western Nigeria by aflatoxigenic fungi and aflatoxins in relation to processing. J Phytopathol. 163:279–286.

Ali N, Blaszkewicz M, Hossain K, Degen GH. 2017. Determination of aflatoxin M_1 in urine samples indicates frequent dietary exposure to aflatoxin B_1 in the Bangladeshi population. Int J Hyg Environ Health. 220:271–281.

Ali N, Hossain K, Blaszkewiez M, Rahman M, Mohanto NC, Alim A, Degen GH. 2016. Occurrence of aflatoxin M_1 in urines from rural and urban adult cohorts in Bangladesh. Arch Toxicol. 90:1749–1755.

All Africa. 2016. Tanzania: food poisoning linked to 14 deaths in 2 regions. [Accessed 2017 Aug 21]. http://allafrica.com/stories/201607290685.html.

Ayalew A, Hoffmann V, Lindahl J, Ezekiel CN 2016. The role of mycotoxin contamination in nutrition: the aflatoxin story. In: Covic N, Hendriks SL, editors. Achieving a nutrition revolution for Africa: The road to healthier diets and optimal nutrition, ReSAKSS Annual Trends and Outlook Report 2015. Washington DC: International Food Policy Research Institute; p. 98–114.

Ayelign A, Woldegiorgis AZ, Adish A, De Boevre M, Heyndrickx E, De Saeger S. 2017. Assessment of aflatoxin exposure among young children in Ethiopia using urinary biomarkers. Food Addit Contam A. 34:1606–1616.

Bankole SA, Ogunsanwo BM, Eseigbe DA. 2005. Aflatoxins in Nigerian dry-roasted groundnuts. Food Chem. 89:503–506.

Chen G, Gong YY, Kimanya ME, Shirima CP, Routledge MN. 2017. Comparison of urinary aflatoxin M1 and aflatoxin albumin adducts as biomarkers for assessing aflatoxin exposure in Tanzanian children. Biomarkers. doi:10.1080/1354750X.2017.1285960

Degen GH, Munoz K, Hengstler JG. 2013. Occurrence of mycotoxins in breast milk. In: S. Zibadi, R.R. Watson, V. R. Preedy, Editors. Handbook of dietary and nutritional aspects of human breast milk. Wageningen Academic Publishers; 813–831. doi:10.3920/978-90-8686-76448 [[ISSN: 2212-375-X]].

Ediage EN, Di Mavungu JD, Song S, Sioen I, Da Saeger S. 2013. Multi-mycotoxin analysis in urines to assess infant exposure: a case study in Cameroon. Environ Int. 57–58:50–59.

Ezekiel CN, Abia WA, Ogara IM, Sulyok M, Warth B, Krska R. 2015. Fate of mycotoxins in two popular traditional cereal-based beverages (*kunu-zaki* and *pito*) from rural Nigeria. LWT Food Sci Technol. 60:137–141.

Ezekiel CN, Ayeni KI, Misihairabgwi JM, Somorin Y, Chibuzor-Onyema I, Oyedele OA, Abia WA, Sulyok M,

Shephard GS, Krska R. 2017. Compr Rev Food Sci Food Saf. doi:10.1111/1541-4337.12329.

Ezekiel CN, Sulyok M, Babalola DA, Warth B, Ezekiel VC, Krska R. 2013. Incidence and consumer awareness of toxigenic *Aspergillus* section *Flavi* and aflatoxin B_1 in peanut cake from Nigeria. Food Control. 30:596–601.

Ezekiel CN, Sulyok M, Somorin Y, Odutayo FI, Nwabekee SU, Balogun AF, Krska R. 2016. Mould and mycotoxin exposure assessment of melon and bush mango seeds, two common soup thickeners consumed in Nigeria. Int J Food Microbiol. 237:83–91.

Ezekiel CN, Sulyok M, Warth B, Odebode AC, Krska R. 2012. Natural occurrence of mycotoxins in peanut cake from Nigeria. Food Control. 27:338–342.

Ezekiel CN, Warth B, Ogara IM, Abia WA, Ezekiel VC, Atehnkeng J, Sulyok M, Turner PC, Tayo GO, Krska R, et al. 2014. Mycotoxin exposure in rural residents in northern Nigeria: A pilot study using multi-urinary biomarkers. Environ Int. 66:138–145.

Gong YY, Cardwell K, Hounsa A, Egal S, Turner PC, Hall AJ, Wild CP. 2002. Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. Br Med J. 325:20–21.

Gong YY, Watson S, Routledge MN. 2016. Aflatoxin exposure and associated human health effects, a review of epidemiological studies. Food Saf. 4:14–27.

Groopman JD, Hall AJ, Whittle H, Hudson GJ, Wogan GN, Montesano R, Wild CP. 1992. Molecular dosimetry of aflatoxin-N7-guanine in human urine obtained in the Gambia, West Africa. Cancer Epidemiol Biomarkers Prev. 1:221–227.

Groopman JD, Wild CP, Hasler J, Junshi C, Wogan GN, Kensler TW. 1993. Molecular Epidemiology of aflatoxin exposures: validation of aflatoxin-N7-guanine levels in urine as a biomarker in experimental rat models and humans. Environ Health Perspect. 99:107–113.

[IARC] International Agency for Research on Cancer. 2002. Some traditional herbal medicines, some mycotoxins, naphthalene, and styrene. IARC Monogr Eval Carcinogen Risk Chem Hum. 82:171–300.

[IARC] International Agency for Research on Cancer. 2015. Mycotoxin control in low- and middle-income countries. In: Wild CP, Miller JD, Groopman JD, editors. IARC Working Group Report No. 9. Lyon.

Kayode OF, Sulyok M, Fapohunda SO, Ezekiel CN, Krska R, Oguntona CRB. 2013. Mycotoxins and fungal metabolites in groundnut- and maize-based snacks from Nigeria. Food Addit Contam Part B. 6:294–300.

Kostelanska M, Sosnovcova I, Lacina O, Hajslova J 2010. Determination of mycotoxins in infant and baby food using UPLC-MS/MS analytical method. Proceedings of 6th ISC Modern Analytical Chemistry, Prague, p. 1–5.

Kouadio JH, Lattanzio VM, Ouattara D, Kouakou B, Visconti A. 2014. Assessment of mycotoxin exposure in Côte d'Ivoire (Ivory Coast) through multi-biomarker analysis and possible correlation with food consumption patterns. Toxicol Int. 21:248–257.

Leong Y, Latiff AA, Ahmad NI, Rosma A. 2012. Exposure measurement of aflatoxins and aflatoxin metabolites in human body fluids. Short Review Mycotoxin Res. 28:79–87.

Lewis L, Onsongo M, Njapau H, Shurz-Rogers H, Luber G, Kieszak S, Nyamongo J, Backer L, Dahiye AM, Misore A, et al. 2005. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and Central Kenya. Environ Health Perspect. 113:1763–1767.

Lombard MJ. 2014. Mycotoxin exposure and infant and young child growth in Africa. What do we know? . Ann Nutr Metab. 64:42–52.

Makun HA, Anjorin ST, Moronfoye B, Adejo FO, Afolabi OA, Fagbayibo G, Balogun BO, Surajudeen AA. 2010. Fungal and aflatoxin contaminations of some human food commodities in Nigeria. Afr J Food Sci. 4:127–135.

Makun HA, Gbodi TA, Akanya HO, Salako EA, Ogbadu GH. 2009. Fungi and some mycotoxins found in mouldy Sorghum in Niger State, Nigeria. World J Agric Sci. 5:5–17.

Mcmillian A, Renaud JB, Burgess KMN, Orimadegun AE, Akinyinka OO, Allen SJ, Miller JD, Ried G, Sumarah MW. 2018. Aflatoxin exposure in Nigerian children with severe acute malnutrition. Food Chem Toxicol. 111:356–362.

Misihairabgwi JM, Ezekiel CN, Sulyok M, Shephard GS, Krska R. 2017. Mycotoxin contamination of foods in Southern Africa: a 10-year review (2007–2016). Crit Rev Food Sci Nutr. doi:10.1080/10408398.2017.1357003

Okeke CA, Ezekiel CN, Nwangburuka CC, Sulyok M, Ezeamagu CO, Adeleke RA, Dike SK, Krska R. 2015. Bacterial diversity and mycotoxin reduction during maize fermentation (steeping) for *ogi* production. Front Microbiol. 6:1402.

Oluwafemi F, Odebiyi T, Kolapo A. 2012. Occupational aflatoxin exposure among feed mill workers in Nigeria. World Mycotoxin J. 4:385–389.

Outbreak News Today. 2017 July 24. Aflatoxin kills 4 children in Tanzania, linked to consumption of maize. [Accessed 2017 Aug 21]. http://outbreaknewstoday.com/aflatoxin-kills-4-children-in-tanzania-linked-to-consumption-of-maize-54210/.

Oyedele OA, Ezekiel CN, Sulyok M, Adetunji MC, Warth B, Atanda OO, Krska R. 2017. Mycotoxin risk assessment for consumers of groundnut in domestic markets in Nigeria. Int J Food Microbiol. 215:24–32.

Polychronaki N, Wild CP, Mykkänen H, Amra H, Abdel-Wahhab M, Sylla A, Diallo M, El-Nezami H, Turner PC. 2008. Urinary biomarkers of aflatoxin exposure in young children from Egypt and Guinea. Food Chem Toxicol. 46:519–526.

Probst C, Njapau H, Cotty PJ. 2007. Outbreak of an acute aflatoxicosis in Kenya in 2004: identification of the causal agent. Appl Environ Microbiol. 73:2762–2764.

Routledge MN, Gong YY. 2011. Developing biomarkers of human exposure to mycotoxins. In: De Saeger S, Editor. Determining mycotoxins and mycotoxigenic fungi in food

and feed. Cambridge (UK): Woodhead Publishing; p. 225–244.

Rubert J, Soriano JM, Manes J, Soler C. 2011. Rapid mycotoxin analysis in human urine: a pilot study. Food Chem Toxicol. 49:2299–2304.

Šarkanj B, Ezekiel CN, Turner PC, Abia WA, Rychlik M, Krska R, Sulyok MWarth B. 2018. Ultra-sensitive, stable isotope assisted quantification of multiple urinary mycotoxin exposure biomarkers. Anal Chim Acta. 1019:84–92.

Schwartzbord JR, Leroy JF, Severe L, Brown DL. 2016. Urinary aflatoxin M1 in Port-au-Prince and a rural community in north-east Haiti. Food Addit Contam A. 33:1036–1042.

Shepard GS. 2003. Aflatoxin and food safety: recent African perspectives. J Toxicol Toxin Rev. 22:267–286.

Solfrizzo M, Gambacorta L, Lattanzio VMT, Powers S, Visconti A. 2011. Simultaneous LC–MS/MS determination of aflatoxin M_1, ochratoxin A, deoxynivalenol, de-epoxy-deoxynivalenol, α- and β-zearalenols and fumonisin B_1 in urine as a multi-biomarker method to assess exposure to mycotoxins. Anal Bioanal Chem. 401:2831–2841.

Turner PC. 2013. The molecular epidemiology of chronic aflatoxin driven impaired child growth. Scientifica. doi:10.1155/2013/152879

Turner PC, Collinson AC, Cheung YB, Gong YY, Hall AJ, Prentice AM, Wild CP. 2007. Aflatoxin exposure in utero causes growth faltering in Gambian infants. Int J Epidemiol. 36:1119–1125.

Turner PC, Flannery B, Isitt C, Ali M, Pestka J. 2012. The role of biomarkers in evaluating human health concerns from fungal contaminants in food. Nutr Res Rev. 25:162–179.

Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP. 2003. Modification of immune function through exposure to dietary aflatoxin in Gambian children. Environ Health Perspect. 111:217–220.

Warth B, Braun D, Ezekiel CN, Turner PC, Degen GH, Marko D. 2016. Biomonitoring of mycotoxins in human breast milk: current state and future perspectives. Chem Res Toxicol. 29:1087–1097.

Warth B, Petchkongkaew A, Sulyok M, Krska R. 2014. Utilising an LCMS/MS-based multi-biomarker approach to assess mycotoxin exposure in the Bangkok metropolitan area and surrounding provinces. Food Addit Contam Part A. 31:2040–2046.

Warth B, Sulyok M, Fruhmann P, Mikula H, Berthiller F, Schuhmacher R, Hametner C, Abia WA, Adam G, Frohlich J, et al. 2012. Development and validation of a rapid multi-biomarker liquid chromatography/tandem mass spectrometry method to assess human exposure to mycotoxins. Rapid Commun Mass Spectrom. 26:1533–1540.

Watson S, Diedhiou PM, Atehnkeng J, Dem A, Bandyopadhyay R, Srey C, Routledge MN, Gong YY. 2015. Seasonal and geographical differences in aflatoxin exposures in Senegal. World Mycotoxin J. 8:525–531.

Wild CP, Turner PC. 2002. The toxicology of aflatoxins as a basis for public health decisions. Mutagenesis. 17:471–481.

Williams JH, Philips TD, Jolly PE, Stiles JK, Jolly C, Aggarwal D. 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences and interventions. Am J Clin Nutr. 80:1106–1122.

Yard EE, Daniel JH, Lewis LS, Rybak ME, Paliakov EM, Kim AA, Montgomery JM, Bunnell R, Abudo MU, Akhwale W, et al. 2013. Human aflatoxin exposure in Kenya, 2007: a cross-sectional study. Food Addit Contam. 30:1322–1331.

Zhu JQ, Zhang LS, Hu X, Xiao Y, Chen JS, Xu YC, Fremy J, Chu FC. 1987. Correlation of dietary aflatoxin B1 levels with excretion of aflatoxin M1 in human urine. Cancer Res. 47:1848–1852.