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Mycotoxin exposure in rural residents in northern Nigeria: A pilot study using multi-urinary biomarkers

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ABSTRACT

A pilot, cross-sectional, correlational study was conducted in eight rural communities in northern Nigeria to investigate mycotoxin exposures in 120 volunteers (19 children, 20 adolescents and 81 adults) using a modern LC–MS/MS based multi-biomarker approach. First morning urine samples were analyzed and urinary biomarker levels correlated with mycotoxin levels in foods consumed the day before urine collection. A total of eight analytes were detected in 61/120 (50.8%) of studied urine samples, with ochratoxin A, aflatoxin M_1 and fumonisin B_1 being the most frequently occurring biomarkers of exposure. These mycotoxin biomarkers were present in samples from all age categories, suggestive of chronic (lifetime) exposures. Rough estimates of mycotoxin intake suggested some exposures were higher than the tolerable daily intake. Overall, rural consumer populations from Nasarawa were more exposed to several mixtures of mycotoxins in their diets relative to those from Kaduna as shown by food and urine biomarker data. This study has shown that mycotoxin co-exposure may be a major public health challenge in rural Nigeria; this calls for urgent intervention.

1. Introduction

Mycotoxins, toxic secondary metabolites produced by various fungi on diverse agricultural commodities, induce a range of harmful effects (cancers, immune suppression and target organ toxicities) in many animal species (Bondy and Pestka, 2000; CAST, 2003; IARC, 1993, 2002). Aflatoxin B_1 (AFB_1) is classified as a group 1 human carcinogen IARC (2002) and represents the second leading cause of hepatocellular carcinoma globally. Aflatoxins induce adverse immune system and growth effects in animals (Bondy and Pestka, 2000), and are suggested to have similar effects in chronically exposed populations (Gong et al., 2002, 2003, 2004; Jiang et al., 2005, 2008; Jolly et al., 2011; Obuseh et al., 2011; Shuaib et al., 2010a,b; Turner et al., 2003, 2007). In ecological studies, fumonisin B_1 (FB_1) contamination levels in maize has been associated with the incidence of esophageal and liver cancer (Chu and Li, 1994; Sun et al., 2007, 2011; Yoshizawa et al., 1994) and is classified as a group 2B carcinogen (IARC, 2002). Fumonisin exposure has additionally been associated with the incidence of neural tube defects (Missmer et al., 2006). In animals, deoxynivalenol (DON) has been linked with gastroenteritis, anorexia, reduced weight gain and immune toxicity as well as interference with DNA and RNA synthesis and neurological processes (Pestka, 2010a,b). Ochratoxin A (OTA) and zearalenone (ZEN) are nephrotoxic and estrogenic, respectively (CAST, 2003; Gilbert et al., 2001; O'Brien and Dietrich, 2005). Human exposure to these natural toxins is predominantly through consumption of contaminated foods, though occupational exposures can include inhalation (Kuiper-Goodman, 1999; Oluwafemi et al., 2012). In sub-Saharan African countries like Nigeria, consumption of poor quality grains and other mycotoxin-prone foods as staples predominate and are the major sources of exposure; in part due to a lack of awareness of the problem, but significantly exacerbated by low income status which restricts dietary choice and variety (Bankole and Adebanjo, 2003; Bankole et al., 2006b).

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Several reports are available on the occurrence of mycotoxins in various foodstuffs in Nigeria, including those consumed by individuals with a low income. For example, aflatoxins have been reported in over 70% of stored maize and maize-based snacks, groundnut and groundnut products, rice, millets, sorghum and dried melon seeds (Atehnkeng et al., 2008; Bankole et al., 2006a, 2010; Ezekiel et al., 2012a, 2013; Kayode et al., 2013; Makun et al., 2009, 2010, 2011) while FBs, OTA, DON, ZEN amongst various other mycotoxins have also been found to contaminate agricultural products in Nigeria (Adejumo et al., 2007; Afolabi et al., 2006; Ezekiel et al., 2012b,c; Makun et al., 2013). About 50% of groundnut cake (*kulikuli*) and stored maize in Nigeria had co-occurrence of more than one of these 10 mycotoxins — AFB_1, alternariol, beauvericin, emodin, FBs, DON, moniliformin, nivalenol (NIV), OTA and ZEN (Adetunji et al., submitted manuscript; Ezekiel et al., 2012b). Despite the available data on mycotoxin occurrence in diverse foods in Nigeria, the risks posed to consumers are not clearly defined as exposure and risk assessment are complex, and the toxicology of complex mixtures of mycotoxins remains a major limitation. Few reports are available in Nigeria on the levels of dietary and occupational exposures to aflatoxins (Adejumo et al., 2013; Ibeh et al., 1991; Oluwafemi et al., 2012); whilst individual exposure estimates are lacking for other mycotoxins. Bio-monitoring of mycotoxins in biological fluids such as blood or urine will be useful to generate more reliable information on exposure incidence at the individual level compared to dietary assessments (Turner et al., 2011, 2012a; Warth et al., 2013b). Hence, the present study aimed at assessing mycotoxin exposure in rural dwellers in Nigeria using a recently developed multi-biomarker method which is capable of quantifying up to 15 analytes that represent measures of eight mycotoxins and selected mycotoxin metabolites (Warth et al., 2012a).

2. Methods

2.1. Study population

This study recruited rural residents of Nasarawa and Kaduna States situated in the northern part of Nigeria. The communities covered were: Agwatashi, Akwanga, Gako and Garaku in Nasarawa State; and Barde, Kurmin Bomo, Maitozo and Mararaban Rido in Kaduna State. The majority of the population in the rural communities depend on agriculture (e.g. large scale cultivation of staples including groundnuts, maize, millet and sorghum) for their income. The choice of States was based on data obtained from a previous survey carried out between July and August 2011 to determine the distribution and levels of mycotoxins in stored maize in Nigeria (Adetunji et al., submitted manuscript). Data obtained suggested populations in Nasarawa State were more exposed to dietary mycotoxins relative to those in Kaduna State, thus warranting further studies with more reliable conclusions based on individual exposure.

2.2. Study design and sampling

A cross-sectional survey which involved purposive selection of five families each from targeted sub-communities within Kaduna and Nasarawa was conducted in September and October 2012. From each of the five families, three participants only which included two adults (one male and one female; age: ≥20 years old) and one younger individual were recruited thereby constituting 120 participants. The younger individuals were categorized as either children (aged ≤8 years) or as adolescents (aged ≤19 years). Eight breastfeeding mothers and one partially breastfed male child were among the participants recruited in Nasarawa State. Individuals with previous medical record of kidney, liver or other metabolic problems were excluded from the study. A well-structured questionnaire was designed and administered by trained interviewers to each participant prior to sample collection in order to obtain basic information relating to demography (age, sex and education), food consumption pattern (frequency of weekly consumption of mycotoxin-prone staples such as groundnut, maize, rice and sorghum, and weight of meal consumed on previous day), socio-economic and general health status. Analyzed questionnaire data are reported in detail elsewhere (Ezekiel et al., manuscript in preparation); however, it was observed that maize, groundnut, sorghum and rice constituted about 39, 29, 12 and 9% of the overall diets consumed by participants.

2.3. Ethical considerations

The Ethics Committee of the Ministry of Health situated in the studied states in Nigeria approved the study. Informed written consent was obtained from all participants prior to inclusion in the study, and analytical measurements were conducted as blind analysis to participant's information. Parents gave informed consent on behalf of their children and adolescents.

2.4. Samples

About 40-ml of first morning urine sample was collected from each of the 120 recruited participants prior to consumption of food or water. A 25 g random portion of the meal consumed by the individuals in each family on the day prior to urine donation was also obtained. All urine and food samples were immediately frozen at −20 °C in Nigeria and sent on dry ice to Austria for analysis.

2.5. Reagents and chemicals

Methanol (LC gradient grade) and glacial acetic acid (p.a.) were purchased from Merck (Darmstadt, Germany), acetonitrile (ACN; LC gradient grade) from VWR (Leuven, Belgium), and ammonium acetate (MS grade) from Sigma-Aldrich (Vienna, Austria). The mycotoxin conjugates deoxynivalenol-3-*O*-glucuronide (DON-3-GlcA) and zearalenone-14-*O*-glucuronide (ZEN-14-GlcA) were synthesized by optimized procedures as described in detail by Fruhmann et al. (2012) and Mikula et al. (2012). Other mycotoxin reference standards were purchased from Romer Labs Diagnostic GmbH (Tulln, Austria) [DON, deepoxy-DON (DOM-1), NIV, T-2 toxin, HT-2 toxin, OTA, AFM_1, FB_1 and FB_2] or Sigma (ZEN, α- and β-zearalenol). Water was purified by an Elga Purelab ultra analytic system from Veolia Water (Bucks, UK). Solid standards were dissolved and combined to a multi-standard working solution for preparation of calibrants and spiking experiments as described in Warth et al. (2012a). Deoxynivalenol-15-*O*-glucuronide (DON-15-GlcA) was obtained from UHPLC separation and subsequent fractionation of a highly contaminated human urine sample which contained both, DON-3-GlcA and DON-15-GlcA. Details of this separation are published elsewhere (Warth et al., 2012b).

2.6. Equipment

Samples were analyzed using an ABSciex QTrap® 5500 LC–MS/MS system (Foster City, CA, USA) equipped with a Turbo V electrospray ionization (ESI) source interfaced with an Agilent 1290 series UHPLC system (Waldbronn, Germany). For data evaluation the vendors Analyst software (version 1.5.1) was used.

2.7. Mycotoxin exposure assessment

The concentration of 15 urinary analytes, either the parent mycotoxins or their metabolite(s), were measured simultaneously using a rapid “dilute and shoot” liquid chromatography tandem mass spectrometry-based method as described by Warth et al. (2012a). Several of the analytes are described in the literature as “validated” exposure biomarkers (AFM_1, OTA, FB_1 and total DON i.e. free DON + DON glucuronides), whilst other analytes at this time represent biological

measures waiting for validation in part by demonstration of dose–response relationships, these mycotoxins include FB_2, DOM-1, ZEN, ZEN-14-GlcA, α-zearalenol, β-zearalenol, T-2 toxin, HT-2 toxin and NIV.

Samples above the limit of detection (LOD) were regarded as "positive" for detection. The resulting limits of quantification (LOQs) defined as the lowest reference standard which was reproduced with a RSD below 20% (given in μg analyte/L urine and taking the sample dilution into account) as well as the analytical range are presented in the form (LOQ, LOD — maximum) for each specific analyte as follows: AFM_1 (0.15 μg/L, 0.05–5.0 μg/L), FB_1 (2.0 μg/L, 2.0–200 μg/L), FB_2 (0.7 μg/L, 2.0–200 μg/L), OTA (0.15 μg/L, 0.05–5.0 μg/L), DON (4.0 μg/L, 4.0–400 μg/L), DON-3-GlcA (6.0 μg/L, 4.0–400 μg/L), DOM-1 (30 μg/L, 12–400 μg/L), NIV (4.0 μg/L, 4.0–400 μg/L), T-2 toxin (1.0 μg/L, 1.0–100 μg/L), HT-2 toxin (40 μg/L, 12–400 μg/L), ZEN (0.6 μg/L, 0.4–40 μg/L), ZEN-14-GlcA (1.0 μg/L, 1.0–100 μg/L), α-zearalenol (1.0 μg/L, 1.0–100 μg/L), and β-zearalenol (1.0 μg/L, 1.0–100 μg/L). It is important to note that this method was optimized to monitor high and moderate exposures toward major mycotoxins rather than to detect low background traces.

The food analysis utilized a 5 g subsample of food and was performed by means of a multi-mycotoxin LC–MS/MS method developed by Sulyok et al. (2006, 2007) which covers currently more than 300 different mycotoxins and other microbial metabolites. However, only six major mycotoxins occurring in Nigerian food are reported in this paper, as they relate to urinary measures described here. The complete data set from the food analysis will be described elsewhere, Ezekiel et al. (manuscript in preparation).

2.8. Statistical analysis

The SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA) was used for data analyses. Means were separated by the Duncan's Multiple Range test (DMRT) and tested for significance by analysis of variance (ANOVA) at $\alpha = 0.05$. For the contamination levels of six mycotoxins in food relevant to the urinary assay, mycotoxin concentration data were transformed using the equation $y = \mathrm{Log}_{10}(1 + \mu g/kg \text{ of mycotoxin})$ to create a normal distribution. Correlation analysis was performed to determine the relationship between concentrations of mycotoxin biomarkers in urine and levels of mycotoxins in food consumed on the day prior to urine sample collection.

3. Results

3.1. Overview of demographic data of participants

The participants consisted of 19 children (9 male, 10 female; mean age: 5 years; range 1–8 years), 20 adolescents (12 male, 8 female; mean age: 13 years; range 9–19 years), and 81 adults (38 male, 42 female; mean age 38 years; range 20–80 years). The mean ages of children, adolescents and adults were not statistically ($p > 0.05$) different by region, 4.4, 13.1 and 36.1 years for Nasarawa, and 5.4, 11.8 and 39.5 years for Kaduna, respectively. The average weight (±SD) of meal consumed on previous day by each age group are given: children (277 ± 203 g), adolescents (454 ± 339 g) and adults (672 ± 235 g).

3.2. Occurrence of mycotoxin biomarkers of exposure and main metabolites in human urine

The urine samples from the participants were analyzed for a total of 15 urinary analytes of either the parent mycotoxin or mycotoxin metabolite(s). Of the eight analytes detected, those described in the literature as "validated" exposure biomarkers (AFM_1, OTA, FB_1 and total DON) were observed in addition to FB_2, ZEN and ZEN-14-GlcA (Table 1). One or more of the analytes was detected in 61/120 (50.8%) urine samples, and were detected significantly ($p = 0.03$) more frequently in Nasarawa [39/60 (65%)] compared to Kaduna [22/60 (37%)]. However, there were no statistically significant differences in the frequency of "at least one analyte" being detected by age group; children 9/19 (47.3%), adolescents 11/20 (55.0%), and adults 41/81 (50.6%) (Table 2). About 75% (46/61) of the urines positive for mycotoxins showed the presence of single mycotoxin species while 25% (15/61) of the urines were found to contain biomarkers for more than one mycotoxin family. Of these 15, two different mycotoxin species were observed in eight urines, three species in five urines and four species in two urines (Table 2). A further seven samples had more than one mycotoxin or metabolite from the same family e.g., DON and DON-15-GlcA. By region, for those individuals with at least one detectable analyte multiple species were observed slightly more frequently in Nasarawa 10/39 (32.3%) compared to Kaduna 5/22 (22.7%). There were no differences in the frequency of >1 analyte by age, though >2 analytes was more common in adults compared to children, however the low numbers restrict the statistical strength of this observation, see Table 2. Urine from seven out of the eight breastfeeding mothers, all from Nasarawa, contained at least one mycotoxin species, Fig. 1 is a representative chromatogram from one of these in which FB_1 (3.6 μg/L) and AFM_1 (1.5 μg/L) was detected. Mycotoxins were more frequently observed in urine from Nasarawa compared ($p < 0.05$) to Kaduna, Table 2.

Overall, OTA, AFM_1 and FB_1 were the most frequently detected mycotoxins, see Table 1. OTA was the most prevalent [34/120 (28.3%): mean of positives (SD; max) was 0.2 μg/L (0.1; 0.6)], followed by AFM_1 [17/120 (14.2%): mean = 0.3 μg/L (0.4; 1.5)] and FB_1 [16/120 (13.3%): mean = 4.6 μg/L (2.8; 12.8)]. DON, ZEN and their metabolites DON-15-GlcA and ZEN-14-GlcA, respectively, and FB_2 were detected less frequently. DON, FB_2 and ZEN were only observed in individuals who also had detectable DON-15-GlcA, FB_1 or ZEN-14-GlcA, respectively. Only four analytes (AFM_1, DON-15-GlcA, FB_1 and OTA) were detectable in the samples from children, FB_2 and ZEN-14-GlcA were additionally detected in urine from adolescents and all eight analytes were detected in adults (Table 3).

3.3. Mycotoxin levels in food consumed by participants

For six of the eight mycotoxins species found in the urine of participants at least one was also found in 74% (29/39) of the food samples consumed by these individuals on the day before urine sample collection. The frequencies of detection, range and mean concentrations of the food mycotoxins were: AFB_1 ($n = 15/39$, 38.5%; mean = 2.5 μg/kg; range = nd–8.3 μg/kg), FB_1 ($n = 29/39$, 74.4%; mean = 209 μg/kg; range = nd–1590 μg/kg), FB_2 ($n = 27/39$,

Table 1
Occurrence and concentrations of urinary mycotoxin biomarkers and main metabolites in human urine from northern Nigeria.

Mycotoxins	Number (%) of positive samples[a]	Concentration (μg/L)		
		Max	Mean[b]	SD
Aflatoxin M_1	17 (14.2)	1.5	0.3	0.4
Deoxynivalenol (DON)	1 (0.8)	2.0[c]	2.0	–
DON-15-*O*-glucuronide	6 (5.0)	8.0	3.5	2.6
Total DON (DON-15-GlcA + DON)	6 (5.0)	10.0	3.9	3.3
Fumonisin B_1	16 (13.3)	12.8	4.6	2.8
Fumonisin B_2	2 (1.7)	1.0[c]	1.0	0.0
Ochratoxin A	34 (28.3)	0.6	0.2	0.1
Zearalenone (ZEN)	1 (0.8)	0.3[c]	0.3	-
ZEN-14-*O*-glucuronide	8 (6.7)	44.5	9.5	14.4
Total ZEN (ZEN-14-GlcA + ZEN)	8 (6.7)	44.5	9.5	14.4
All participants ($n = 120$)	61 (50.8)	–	–	–

[a] Urine samples with analyte concentrations above the LOQ and those less than the LOQ but higher than the LOD.
[b] The mean values reported herein were calculated for positive samples by considering half LOQ (LOQ / 2) for less than LOQ values.
[c] Maximum values are half LOQ (LOQ / 2).

Table 2
Co-occurrence of urinary mycotoxin biomarkers of exposure and main metabolites in different age groups of rural dwellers in northern Nigeria.

	Number (%) of exposed individuals in age groups			Distribution across location		
Number of mycotoxins in positive[a] samples	Children (n = 19)	Adolescents (n = 20)	Adults (n = 81)	Kaduna (n = 60)	Nasarawa (n = 60)	Total (n = 120)
1	7 (36.8)	9 (45.0)	30 (37.0)	17 (28.3)	29 (48.3)	46 (38.3)
2	2 (10.5)	0 (0.0)	6 (7.4)	3 (5.0)	5 (8.3)	8 (6.7)
3	0 (0.0)	1 (5.0)	4 (4.9)	2 (3.3)	3 (5.0)	5 (4.2)
4	0 (0.0)	1 (5.0)	1 (1.2)	0 (0.0)	2 (3.3)	2 (1.7)
Total	9 (47.3)	11 (55.0)	41 (50.6)	22 (36.7)	39 (65.0)	61 (50.8)
Distribution of more than one mycotoxin in urines across targeted state in Nigeria						
Kaduna	2/13 (15.4)	1/6 (16.7)	2/41 (4.9)			
Nasarawa	0/6 (0.0)	1/14 (7.1)	9/40 (22.5)			

[a] Urine samples with analyte concentrations above the LOD.

69.2%; mean = 78.0 μg/kg; range = nd–610 μg/kg), DON (n = 5/39, 12.8%; mean = 3.6 μg/kg; range = nd–8.0 μg/kg), ZEN (n = 5/39, 12.8%; mean = 46 μg/kg; range = nd–222 μg/kg) and OTA (n = 1/39, 2.6%; mean = 1.5 μg/kg) (Fig. 2).

3.4. Correlation of mycotoxin levels in food and urine samples

For each individual mycotoxin correlation analysis of the mycotoxin concentration in the food consumed by each individual on the day prior to urine collection and concentrations excreted in urine were conducted, using all data points. AFB_1 levels in the food correlated significantly, albeit modestly (r = 0.31; p = 0.02) with the excreted AFM_1, and FB_1 levels in food and urine also correlated modestly though significantly (r = 0.28; p = 0.02). When data points from positive samples only were used, relationships of similar strength and significance were observed for both analytes.

3.5. Family exposure pattern

Family exposure patterns based on urinary concentrations were observed for AFB_1, FB_1, OTA and ZEN-14-GlcA. In most cases where these analytes occurred, at least two individuals per family were exposed. Specifically, exposure of at least an adult and a younger individual per family to AFB_1, FB_1, OTA and ZEN-14-GlcA was observed in 3/13 (23.1%), 5/10 (50.0%), 11/19 (57.9%) and 3/5 (60%) positive family cases, respectively.

4. Discussion

About 4.5 billion people live in regions of the world that are at risk of aflatoxin exposure, most reside in developing countries where in some cases exposures due to dietary intakes are frequent and at high levels (Williams et al., 2004). Studies on exposure assessment to mycotoxins in utero, in children, adults and pregnant mothers using urinary or blood biomarkers have been reported in several African countries including Benin and Togo, Cameroon, Egypt, Ghana, Guinea, Kenya, Nigeria, South Africa and The Gambia (Abia et al., 2013b; Gong et al., 2002, 2003, 2004, 2012; Nikiema et al., 2008; Obuseh et al., 2011; Oluwafemi et al., 2012; Piekkola et al., 2012; Polychronaki et al., 2008; Scholl et al., 2006; Shephard et al., 2013; Turner et al., 2000, 2002, 2003, 2005a,b, 2007, 2008c; van der Westhuizen et al., 1999; Wild et al., 2000; Wojnowski et al., 2004). However, reports are rare for mixtures and are not reported for exposures involving urine and multi-mycotoxin biomarkers in Nigeria. This study is therefore the first report on multi-mycotoxin exposure assessment in Nigeria using urinary biomarkers and also correlating urinary exposure levels to levels of mycotoxins in foods. In addition, this study provides data in children, adolescents and adults. All five classes (aflatoxins, DON, fumonisins, ochratoxins and zearalenone) of mycotoxins detected in this study from the direct exposure assessment of volunteers' urine have also been recently reported in urine of exposed individuals from Cameroon (Abia et al., 2013b), and four of these classes were additionally observed in South African urines (Shephard et al., 2013). This suggests widespread exposure across the African population.

The current methodology involves no sample clean-up, and is thus both more rapid and significantly cheaper than other methods (Shephard et al., 2013; Solfrizzo et al., 2014). However, this imparts a significant reduction in analytical sensitivity, and thus higher frequencies of detection would be predicted with more sensitive methods. Thus our survey is demonstrating occurrence and co-

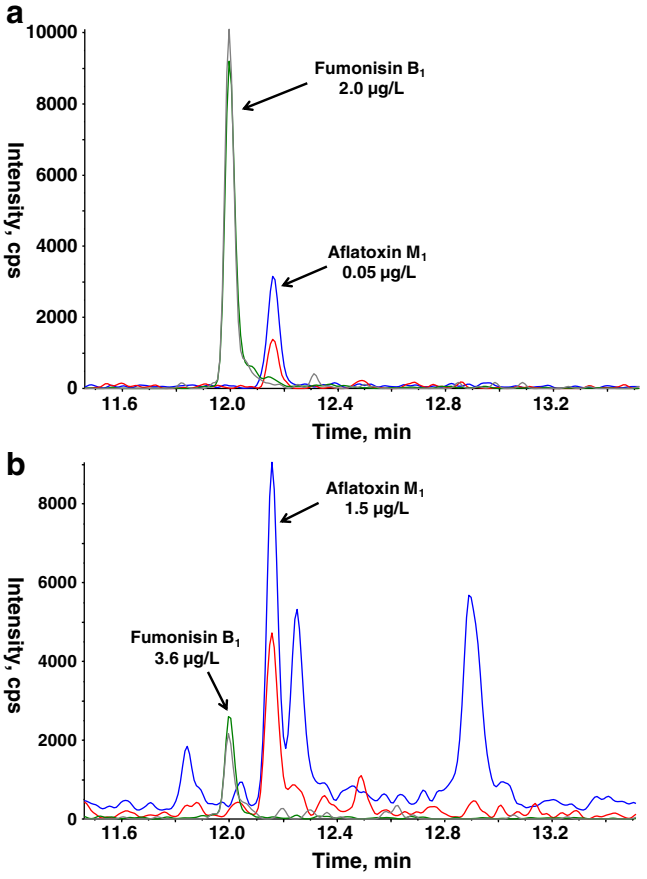

Fig. 1. SRM chromatogram of the biomarkers aflatoxin M_1 and fumonisin B_1 for (a) a reference standard, and (b) a naturally contaminated urine sample from a breastfeeding mother in Nasarawa State, Nigeria. For each biomarker two individual mass transitions are illustrated for unambiguous identification according to the EC (2002). Note that for the standard the injected concentration is given and that the naturally contaminated sample was diluted 1:10 before measuring (resulting in an injected concentration of 0.15 μg/L AFM_1 and 0.36 μg/L FB_1).

Table 3
Levels of urinary mycotoxin biomarkers and main metabolites in different age groups of rural dwellers in northern Nigeria.

Mycotoxins	Occurrence[a] (*n*) and mean[b] ± SD (μg/L) of mycotoxins in age groups					
	Children		Adolescents		Adults	
	n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD
Aflatoxin M_1	2	0.1 ± 0.0	4	0.3 ± 0.4	11	0.4 ± 0.5
Deoxynivalenol	–	–	–	–	1	2.0
Deoxynivalenol-15-*O*-glucuronide	1	1.5	1	1.5	4	4.6 ± 2.6
Fumonisin B_1	4	3.7 ± 0.7	3	6.9 ± 5.4	9	4.2 ± 2.2
Fumonisin B_2	–	–	1	1.0	1	1.0
Ochratoxin A	4	0.1 ± 0.1	6	0.2 ± 0.1	24	0.2 ± 0.1
Zearalenone	–	–	–	–	1	0.3
Zearalenone-14-*O*-glucuronide	–	–	1	2.0	7	10.5 ± 15.2

[a] Number of urine samples with analyte concentrations above the LOD.
[b] The mean values reported herein were calculated for positive samples by considering half LOQ (LOQ / 2) for less than LOQ values.

occurrences of mycotoxins at the higher end of the exposure spectrum. Despite this limitation, there was a high frequency in the detection of urinary mycotoxins, data suggestive that exposure was frequent in Nigeria.

Several of the mycotoxin measures in urine have been suggested as "validated" exposure biomarkers, including urinary AFM_1, total DON, OTA, and FB_1, though the strengths of reported dose responses relationships between assessed mycotoxin intake and the urinary measure vary by mycotoxin (Turner and Pasturel, 2013; Turner et al., 2012, 2013a). Using estimated transfers of the toxin to urine, estimated urine volumes (1.5 L per day) and a mean body weight of 60 kg, rough estimates of intake for adults only are reported, see below. The percent transfers reported were 1.5% (Zhu et al., 1987), 72% (Turner et al., 2010a), 50% (Schlatter et al., 1996) and 0.3% (van der Westhuizen et al., 2011), for AFM_1, DON, OTA and FB_1, respectively. In our study, the prevalence of mycotoxins in urine was OTA > AFM_1 > FB_1/FB_2 > ZEN/ZEN-14-GlcA > DON/DON-15-GlcA, data in good agreement with Sri Lankan adults (Desalegn et al., 2011).

OTA was detected in only one of the 39 food samples in this study. Thus it is possible that the urinary OTA originated from alternative food sources not collected in this survey or that exposure occurred in items of food consumed ahead of the collection, perhaps a reflection of the slow toxicokinetics of OTA (Duarte et al., 2011). It will therefore be important to conduct longer term biomonitoring surveys, especially with reference to understanding correlations between OTA intake and the urinary measure. Additional risk foods will include cocoa products, groundnut and sorghum (CAST, 2003). For the 24 adults with detectable urinary OTA, the mean estimated OTA intake was 0.01 μg/kg bw/day (max = 0.03), a value close to the suggested tolerably daily intake (TDI) of 0.017 μg/kg bw/day (Schlatter et al., 1996) derived from the tolerable weekly intake of 0.12 ng/kg bw/day recommended by EFSA (2006).

Urinary AFM_1 has been well established as a biomarker of exposure for the recent ingestion of AFB_1 (Groopman et al., 1992a,b), and AFM_1 was the second most frequently observed urinary mycotoxin in this survey. Urinary AFM_1 in these Nigerian samples was modestly associated with levels of aflatoxin in food. The mean urinary AFM_1 levels in our study were higher than levels reported for some Egyptian populations [children with kwashiorkor (range: 10–30 pg/mL), children with marasmus (range: 44–70 pg/mL) (Hatem et al., 2005) and healthy

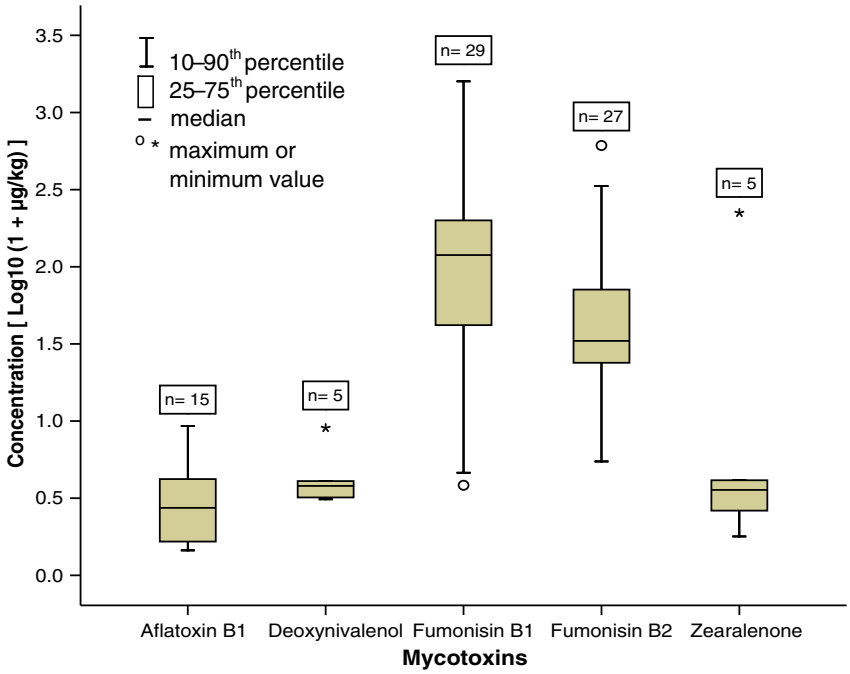

Fig. 2. Box plots showing contamination levels of six urinary relevant mycotoxins in 39 food samples from participants' homes in northern Nigeria. The sixth mycotoxin (ochratoxin A) was found in just one food sample. *n* = number of food samples positive for mycotoxin.

children (range: 5.0–6.2 pg/mL; Polychronaki et al., 2008)] but similar to levels reported for HIV infected individuals in Cameroon (range: <LOQ–1.38 μg/L; Abia et al., 2013b), pregnant women in Egypt (range: 4–409 pg/mg; Piekkola et al., 2012), Guinean children (range: 8–801 pg/mL; Polychronaki et al., 2008) and adult populations in Ghana (range: nd–115 pg/mL; Obuseh et al., 2010). The levels of AFM_1 in urines of Sierra Leone's children were however higher than levels from our study (Jonsyn-Ellis, 2001). AFM_1 is a specific biomarker of AFB_1 exposure, a Class 1 carcinogen (IARC, 2002); there is no level of exposure that is regarded as safe. In those urines with detectable AFM_1 we roughly estimate a mean intake of AFB_1 of 0.67 μg/kg bw/day (max = 2.5 μg/kg bw/day).

FB_1 was the third most frequently observed mycotoxin in this survey, despite a modest level of sensitivity compared to the method of Gong et al. (2008). As with the aflatoxins, FB_1 in urine was modestly associated with levels in food. In line with reports from Cameroon (Abia et al., 2013b) and Southern Africa (Shephard et al., 2013; van der Westhuizen et al., 2011), rough estimates of the intake for adults where FB_1 was detected in urine raised some concern. For adults with detectable urinary FB_1 the mean estimated intake was 35 μg/kg bw/day (max = 76 μg/kg bw/day); a level significantly greater than the recommended TDI of 2 μg/kg bw/day (SCF, 2003). The high variability in the human toxicokinetics of FB_1 reported by Riley et al. (2012) and the modest association reported by van der Westhuizen et al. (2011) suggest some caution in the absolute values in these data, but clearly indicate that the TDI is being exceeded.

The lower frequencies of the other toxins makes deriving mean estimates of intake less reliable, hence only the maximum concentration of total DON (i.e. DON + DON-glucuronide) and total ZEN (i.e. ZEN + ZEN-glucuronide) were used to evaluate if single persons might be at risk of exceeding the established TDI values. This was not the case for total DON with an estimated maximum exposure of 0.27 μg/kg bw/day (TDI: 1 μg/kg bw/day; FAO/WHO, 2010). However, when using the preliminary urinary excretion rate of total ZEN (9.4%; Warth et al., 2013a) the estimated exposure corresponds to 11.8 μg/kg bw/day, a value which is clearly above the TDI of 0.2 μg/kg bw/day (SCF, 2000).

Previous studies from Nigeria have generally reported high occurrence frequencies/levels of AFB_1, fumonisins and several other mycotoxins in diets (Adejumo et al., 2007, 2013; Ezekiel et al., 2012a, b,c, 2013; Makun et al., 2011, 2013; Oluwafemi et al., 2012). In these rural populations in northern Nigeria, residents cultivate and eat their own maize as staple foods throughout the year. More health risks are likely in children in terms of levels exceeding TDIs when we take into consideration the estimated mean exposures for AFB_1 and FB_1, two potent natural carcinogens. However, as the transfer rates of these toxins remain poorly examined, these estimates were not calculated here.

DON-15-GlcA and ZEN-14-GlcA were the only detected metabolites of DON and ZEN, respectively. A glucuronide of DON was originally suggested by Meky et al. (2003) and Turner et al. (2010a) and the specific DON-15-GlcA was characterized and measured in urine samples recently by Warth et al. (2012a,b). Several studies have now reported DON and DON glucuronides in urine from Chinese (Meky et al., 2003; Turner et al., 2011), Europeans (Hepworth et al., 2011; Šarkanj et al., 2013; Solfrizzo et al., 2011; Turner et al., 2008a,b, 2010a, b; Wallin et al., 2013; Warth et al., 2011,b), Egyptians (Piekkola et al., 2012), Iranians (Turner et al., 2012b), South Africans (Shephard et al., 2013) and Cameroonians (Abia et al., 2013b). The recently characterized DON-3-GlcA (Warth et al., 2011, 2012b) and putative DON-7-GlcA (Šarkanj et al., 2013) were not observed in this present study. For ZEN only the glucuronide was observed in all ZEN positive individuals here, as it was predicted by Warth et al. (2013a).

The observed co-existence of more than one urinary mycotoxin, irrespective of age, agreed with data from our previous reports on multiple mycotoxin contamination of food commodities from Nigeria (Ezekiel et al., 2012a,b,c) and other African countries with a similar climate (Abia et al., 2013a; Warth et al., 2012c). From a risk assessment point of view, the combined effects from multiple unrelated mycotoxins is poorly understood, though recent in vitro data highlights potential additive or synergistic interactions (Wan et al., 2013a,b,c, 2014).

For the first time in Nigeria, this study quantified eight mycotoxin analytes in urine samples from children, adolescent and adult subpopulations in the rural northern parts. For several mycotoxins (AFB_1 and FB_1), estimated intake indicates that the TDI was exceeded in some of the participants within this study population. Given that many samples were below the LOD it is worth emphasizing that the estimated mean intakes reported here, do not reflect the entire sample set, rather the positives only, and the overall means would be somewhat lower. This study provides strong evidence that some individuals in all age groups living in the rural parts of northern Nigeria may be exposed to high levels of toxic and carcinogenic substances produced by fungi; family exposures further strengthen this fact and point to the potential risk from mycotoxin burden in the affected regions. Children who are partially breastfed are not left out as we found a high level of FB_1 (3.8 μg/kg) in the urine sample of a partially breastfed child; most likely coming from the wean food because urine from the breastfeeding mother had no detectable mycotoxin. The child in question, in addition to the mother's milk, consumed 25 g of corn meal on the day before collection [corn contaminated with FB_1 at 161 μg/kg; total fumonisins ($FB_1 + FB_2 + FB_3$) = 218 μg/kg]. The effects of co-exposures, particularly in the more susceptible young (Makri et al., 2004), requires further examination.

Conflict of interest

The authors declare they have no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2014.02.003.

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