Food Control 41 (2014) 76–81

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Co-exposures of aflatoxins with deoxynivalenol and fumonisins from maize based complementary foods in Rombo, Northern Tanzania

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ARTICLE INFO

Article history:
Received 1 August 2013
Received in revised form
16 December 2013
Accepted 31 December 2013

Keywords:
Exposure
Aflatoxins
Fumonisins
Deoxynivalenol
Tanzania

ABSTRACT

Children consuming maize based foods in Tanzania may be exposed to multiple mycotoxins. We estimated co-exposures of aflatoxins with Deoxynivalenol (DON) and fumonisins for children in rural Tanzania. Food consumption by the children was estimated by twice administering a 24 h dietary recall questionnaire to mothers of 18–24 months old children in Kikelelwa village. Each mother also, provided a sample of maize based flour used for feeding her child in the previous day. Each child's body weight (bw) was measured by following standard procedures. Aflatoxins, DON and fumonisins were determined in each sample using validated HPLC methods. Exposures for a mycotoxin were estimated by multiplying flour consumption (g/child/kgbw/day) by its contamination (μg/kg). Complete data were obtained for 41 children. Maize flour consumption ranged from 16 to 254 g/child/day. Thirteen (32%) of the 41 children consumed flour with detectable aflatoxin levels (range, 0.11–386 μg/kg), resulting in exposures from 1 to 786 ng/kg bw/day. All these children exceeded the aflatoxins exposure of concern (0.017 ng/kg bw/day). Eighteen (44%) of the children consumed flour with detectable DON levels (57–825 μg/kg) and 34 (83%), detectable fumonisins levels (63–2284 μg/kg), resulting in respective exposure ranges of 0.38–8.87 μg/kg bw/day and 0.19–26.37 μg/kg bw/day. Twelve (66%) of the DON exposed children and 56% of the fumonisins exposed children exceeded the respective provisional tolerable daily intakes of 1 μg/kg bw and 2 μg/kg bw. Co-exposures for aflatoxins with both DON and fumonsins were determined in 10% of the 41 children. Co-exposures of aflatoxins with fumonisins alone were found in 29% and of fumonisins with DON alone in 41% of the children. The study showed that children consuming maize based complementary foods in Northern Tanzania are at a risk of exposure to multiple mycotoxins. We recommend adoption of appropriate measures to minimize exposures of multiple mycotoxins in Tanzania.

1. Introduction

Maize is prone to contamination by multiple mycotoxins such as aflatoxins, deoxynivalenol (DON) and fumonisins. Aflatoxins are secondary metabolites from mainly the fungi of genus *Aspergillus*. These toxins, aflatoxin B1 (AFB1) in particular, are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic (IARC, 1993; Wild & Gong, 2010). Aflatoxins have been associated with impaired growth in young children (Gong et al., 2004).

DON (also called vomitoxin), is a secondary metabolite of the *Fusarium* species (Miller, 1995; WHO, 2011a). DON and its derivatives, 3-acetyldeoxynivalenol (3AcDON), 15-acetyldeoxynivalenol (15AcDON) are members of the Type B trichothecenes (Miller, 1995; WHO, 2001, 2011a). It causes vomiting and feed refusal in pigs and has been associated with acute food-borne illness involving gastrointestinal symptoms in India and China, (IARC, 1993; WHO, 2011a). The DON toxicity was evaluated by the 56th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) which established a provisional maximum tolerable daily intake (PMTDI) of

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http://dx.doi.org/10.1016/j.foodcont.2013.12.034

1 μg/kg body weight (bw) (WHO, 2001). Taking into account the new data generated since 2001, the 72nd meeting of JECFA re-evaluated DON and found that the PMTDI of 1 μg/kg bw was still appropriate (WHO, 2011a). However, the meeting decided to convert the PMTDI to a group PTMDI for DON and its acetylated derivatives (3-Ac-DON and 15-Ac-DON) because the 3-Ac-DON is converted to DON and therefore contributes to the total DON-induced toxicity (WHO, 2011b). In deciding this, the Committee considered the toxicity of the acetylated derivatives equal to that of DON.

Just like DON, fumonisins are metabolites from *Fusarium* genus of fungi. These toxins, fumonisin B1 (FB1) in particular, have been linked with toxic and carcinogenic effects in animals and humans (WHO, 2001, 2011b) and were recently associated with poor child growth (Kimanya, De Meulenaer, Roberfroid, Lachat, & Kolsteren, 2010). The 56th meeting of JECFA established a group PMTDI of 2 μg/kg bw for FB1, fumonisin B2 (FB2) and fumonisin B3 (FB3), alone or in combination, (WHO, 2001). The 74th meeting of JECFA re-evaluated fumonisins based onnew data that had been generated from 2001 but retained the PMTDI of 2 μg/kg bw (WHO, 2011a, 2011b).

Maize is staple food in Tanzania and mothers use it as complementary food for their children. Previous studies of mycotoxins contamination in rural Tanzania found high contaminations of aflatoxins and fumonisins in maize (Kimanya et al. 2008, 2009). In the study by Kimanya et al. (2008), aflatoxins were detected in 18% of 120 samples at levels up to 158 μg/kg with 12% of all the samples exceeding the Tanzanian maximum limit (ML) of 10 μg/kg, for total aflatoxins [sum of AFB1, aflatoxin B2(AFB2), aflatoxin G1(AFG1) and aflatoxin G2 (AFG2)]. In the same study it was reported that aflatoxins co-occurred with fumonisins in 10% of the samples analyzed and the maximum total fumonisins (sum of FB1 and FB2) contamination was 11,048 μg/kg.

One of the options to prevent exposure to mycotoxins from a food is enforcement of MLs for the toxins in the food. In order to be able to set a protective ML for a toxin of concern one has to perform exposure assessment for that toxin. In the year 2010 we performed exposure assessment for fumonisins from maize based complementary foods in Rombo, Northen Tanzania and found that about 15% of infants consuming maize based foods were exposed to fumonisin levels above the PMTDI of 2 μg/kg bw (Kimanya et al., 2010). With a probabilistic exposure assessment we were able to recommend an ML of 1000 μg/kg for fumonisins in maize for human consumption in Tanzania (Kimanya, De Meulenaer, Van Camp, & Kolsteren, 2012). We however indicated that when this limit is enforced, daily maize consumption as complementary food should not exceed 20 g for a child under one year of age, implying that the diet should become more diverse and supplemented with other available cereals or legumes.

There are no reports on dietary aflatoxins exposure in Tanzania, neither are there reports on DON contamination or exposure in Tanzania, despite the knowledge that fumonisins co-occur with aflatoxins (Kimanya et al., 2008) or DON (Abia et al., 2013; WHO, 2001) in maize and maize based foods. Thus, one cannot evaluate appropriateness of the existing ML for AFB1 (5ppb) or total aflatoxins (10ppb) for maize and maize based foods in Tanzania. Similarly, one cannot formulate appropriate ML for DON in maize and maize based foods for human consumption in Tanzania. This study investigated co-contamination and exposure of aflatoxins with DON and fumonisins in maize based complementary foods in a maize consuming community in Tanzania.

2. Materials and method

2.1. Study area

The study was conducted in Kikelelwa village, Northern Tanzania. Outcome of a survey of fumonisins contamination in maize from four villages of four main maize producing regions of Tanzania found higher levels of fumonisins in this village (Kimanya et al., 2008).

2.2. Recruitment of infants

A total of 57 children at weaning age in Kikelelwa were randomly selected from the birth register available at the village dispensary. In Tanzania, all infants born in clinics are registered soon after birth and those born at home are registered on the day the child is taken to the clinic for immunization.

2.3. Complementary food survey

The survey was conducted about one month after the maize harvest to ensure most families had enough food to feed their children. Prior to the survey, a training session was conducted for all the field workers. A questionnaire was administered to the mothers by field workers to record the information such as child age and maize based food intake. Each child's body weight (bw) was measured by following the standard procedure, with two repeats, and recorded to the nearest 0.1 kg. Diet information was recorded on two consecutive days using a 24 h dietary recall questionnaire. The mother of each child who consumed maize based food during the 24 h dietary recall, provided information about number of days she fed her child with maize based food in the previous week. During each visit, an amount of complementary flour equivalent to that used in preparation of the complementary food in the previous day was packed in a khaki paper bag, sealed and then transported to the Tanzanian Food and Drugs Authority (TFDA) laboratory in Dar es Salaam for mycotoxins determination.

2.4. Mycotoxins determination

Two packages of complementary flour; collected during the two 24 h dietary recalls, were opened and the contents thoroughly mixed together using a laboratory mixer to constitute a composite sample. Different portions of the composite flour were subjected to aflatoxins, deoxynivalenol and fumonsins analysis as described below.

2.4.1. Determination of aflatoxins and fumonisins

Aflatoxin B_1, B_2, G_1 and G_2 were determined using the method described by Stroka, Anklam, Joissen, and Gilbert (2000) and as reported in Kimanya et al. (2008). The limit of detection (LOD) was 0.53 for AFB1, 0.15 for AFB2, 0.24 for AFG1 and 0.01 for AFG2.

To assess the suitability of the method, AFB1 and AFG1 were spiked into blank maize lour samples, each at 0.76, 3.81 and 6.85 μg/kg. On average AFB1 was recovered by 89% (three samples; relative stanrd deviation (RSD) of 15.7%) and AFG1 by 107% (three samples; RSD of 19.8%). The blank maize flour samples were also spiked with AFB2 and AFG2, each at 0.56, 1.31 and 1.675 μg/kg. On average the toxins were recovered by 87%. The RSD value for three maize flour samples spiked with the AFB2 was 14.3% and AFG2, 22.9%. The results were corrected for recovery.

FB1 and FB2 were determined as rescribed in Kimanya et al. (2008), using a liquid chromatographic method based on Sydenham, Shephard, and Thiel (1992) and slight modifications made by Samapundo, De Meulenaer, De Muer, Debevere, and Devlieghere (2006). The limit of detection for FB1, was 53 μg/kg and for FB2, 47 μg/kg. The limits of detection for the methods were based on the mean value of the blank readings plus three standard deviations.

To evaluate the suitability of the method, blank maize flour samples were spiked with FB1 and FB2 each at 100, 200, 300, 400

and 500 μg/kg. Average recovery for FB1 was 106% – five samples, RSD of 16.6% and for FB2, 92% – five samples; RSD of 15.3%. The results were corrected for recovery.

2.4.2. Determination of deoxynivalenol

Deoxynivalenol in maize flour was extracted by using DonStar™ R-Immunoaffinity Columns (IAC) (Romer Labs, Inc. America) as per manufacturer's instructions with slight modification of the extraction procedure. Deoxynivalenol was extracted from the sample by mixing 20 ml of distilled water with 5 g maize flour followed by gentle shaking for 15 min. The slight modification of the method was made in order to minimize interference with matrix whereby the supernatant was separated by centrifuging the mixture in a centrifuge set at 4000 g, for 20 min. For cleanup, first the IAC was fitted onto a manifold allowing the content in it to drip out. Then 1 ml of the supernatant was directly applied to the column at flow rate of approximately 1 ml per minute until all the content had passed over the column and the column was then washed with 5 ml of PBS. Four ml of methanol, applied at two installments of 2 ml, was used to elute the DON. The eluate was dried under a stream of nitrogen at 45 °C to evaporate the methanol. DON was reconstituted with 250 μL of mobile phase composed of 10% acetonitrile in water, ready for HPLC quantification.

Fifty μL of the DON solution was injected into HPLC (Shimadzu, Tokyo, Japan). Separation was done in a Waters Spherisorb ODS-1, 5 μm column at a flow rate of 0.6 ml/min. Detection was achieved with a UV detector set at wavelength of 218 nm.

DON concentration in each sample was estimated using a calibration curve prepared with DON reference standard solutions. DON standard was supplied by Sigma–Aldrich Co Ltd Dorset, UK in powder form.

Method verification was carried out by evaluating the linearity, accuracy (as recovery) and detection limit. Blank samples were spiked with DON reference standard at five concentrations of 100, 120, 130, 150 and 180 μg/kg. These were extracted using the procedure described in the method and final solutions were injected into the HPLC. The method was found to be linear at the studied concentration levels and the average recovery value was 87% which was used to correct sample results. The limit of detection was found to be 52 μg/kg.

2.5. Exposure estimation

Exposures were estimated for children who consumed flours with detectable mycotoxins. The procedure used is described in Kimanya et al. (2010). Briefly, for each child exposure was calculated by multiplying the mycotoxin (total aflatoxins, total fumonisins or DON) levels in the flour consumed and maize based flour consumption in per kg body weight as estimated in this study. Maize based flour consumption was estimated from weight of stiff or thin porridge consumed. Fifteen samples of porridges were oven dried to constant weight, followed by adjustment of the dried matter to the moisture content of maize flour (13%). Drying and adjustment established that average weight proportion of flour in thin and stiff porridge is 17% and 36%, respectively. So flour intake for those who consumed thin porridge was calculated as 17% of weight of the thin porridge intake (g) and for those who consumed stiff porridge as 36% of weight of the stiff porridge intake (g).

We also adjusted each child's average maize based flour consumption by multiplying it with his/her weekly frequency (number of days in a week) of maize based food consumption divided by seven. This adjustment was done to obtain a better estimate of the habitual maize based food intake of the child than what would be obtained from the repeat 24-h dietary recall alone.

In general, we determined mycotoxin exposure for each child using the formula:

$$Y_i = C_i \cdot D_i \cdot X_i, \text{ where:}$$

$\mathbf{Y_i}$ = The daily intake by a child "i" of total aflatoxins (ng/kg bw), total fumonisins μg/kg bw or DON (μg/kg bw);
$\mathbf{C_i}$ = The aflatoxins (ng/kg), total fumonisins (μg/kg) or DON (μg/kg) in the maize based flour sample from the family of child "i".
$\mathbf{D_i}$ = The number of days the child received maize based complementary food in the previous week divided by seven (number of days in a week).
$\mathbf{X_i}$ = The average daily consumption of maize based food (kg/kg bw) by the Child "i"; as estimated from the daily consumption of complementary food.

2.6. Risk characterization

According to EFSA (2007) and Benford et al. (2010), risk characterization for compounds that are both genotoxic and carcinogenic, such as aflatoxins, should be based on margins of exposures (MOEs). The MOE for aflatoxins was determined by dividing the benchmark dose lower limit (BMDL) of 170 ng/kg bw/day (EFSA, 2007) by the toxin exposure for each child. The BMDL of 170 ng/kg bw/day represents the lower limit of the bench mark dose (BMD) at 95% confidence estimated as the dose required to produce a small response (10% extra cancer risk) above the control for rodents. MOE lower than 10,000 indicates a public health concern, implying that aflatoxin exposure above 0.017 ng/kg bw/day (as obtained by dividing 170 ng/kg bw/day by 10,000) represents the risk of public health concern. Thus, in this study all exposures above 0.017 ng/kg bw/day were considered of public health concern.

Risk characterization for fumonisins or DON exposure was done by comparing the exposure by each child with the PMTDI of 2 μg/kg bw or 1 μg/kg bw, set for fumonisins or DON, respectively. Exposures above the PMTDIs were considered of public health concern.

2.7. Statistical analysis of data

The statistical package used was Stata version 11.1 of 2009 (Stata 11.1; Statacorp, Texas, USA). The error was set at 5% for all the tests done.

3. Results

3.1. Study subjects and food consumption

Mothers of 53 children consented for their children to participate in the study. Complete data in terms of two 24 h dietary recalls, weight at the time of survey and the mycotoxin (aflatoxins, DON and fumonisins) levels were obtained for 41 children only. Data were missing for children who did not eat maize based food or whose mothers had exhausted flour stocks from which samples could be taken, 24 h prior to interviews. Of the 41 children whose complete data were available, 20 were males and 21 females. The children were 18–25 months old and their body weight ranged from 7 to 14.2 kg. The average weight for these children was 10.51 kg.

At the time of the survey, all the children had already been introduced to complementary foods. Maize based flour consumption ranged from 16 to 254 g/child/day (mean, 100 g/child/day), with 83% of these flours being of pure maize. The rest of the flours were made of different cereals or legumes, in addition to maize. The other cereals and legumes used to prepare complementary flours are finger millet, wheat, rice, soy beans and groundnuts.

3.2. Occurrence of mycotoxins

The complementary flours were contaminated by mycotoxins at varying occurrences and levels. Table 1 shows the distribution of aflatoxins, DON and fumonisins in the flours.

The table shows further that some flours were contaminated at levels that exceed the maximum limits (MLs) set in Tanzania (Kimanya et al., 2008) for aflatoxins (5 μg/kg for AFB1, 10 μg/kg for total aflatoxins) or in European Union (EU) (EU, 2006) for fumonisins (1000 μg/kg) or DON (750 μg/kg) in maize for direct human consumption. As shown in Table 2 none of seven flour samples containing other cereals or legumes in addition to maize, were contaminated at levels that exceed the MLs. Table 3 shows that aflatoxins, DON and fumonisins co-occurred at different combinations and proportions.

3.3. Exposure and risk of exceeding exposure levels of concern

Mycotoxins exposures were estimated for children who consumed food with detectable contaminations. Contamination was found in 13 samples for aflatoxins, 18 for DON and 34 for fumonisins. Aflatoxins exposures for all the 13 children exceeded the limit of concern of 0.017 ng/kg bw/day and ranged from 1 to 786 ng/kg bw/day (median, 12 ng/kg bw/day), with MOEs from 0.22 to 169 (median, 12). DON exposures for the 18 children ranged from 0.38 to 8.87 μg/kg bw/day (median, 1.34 μg/kg bw/day) with 12 (66%) of them exceeding the PMTDI of 1 μg/kg bw. Fumonisins exposures for the 34 children ranged from 0.19–26.37 μg/kg bw/day (median, 2.3 μg/kg bw/day) with 19 (56%) of them exceeding the PMTDI of 2 μg/kg bw. The numbers, 13 for aflatoxins, 12 for DON and 19 for fumonisins, of children exceeding the tolerable intakes, represent 32%, 29% and 46%, respectively, of all (41) children studied.

4. Discussion

This is the first report of deoxynivalenol contamination and exposure in maize based foods consumed in Tanzania. We have established that children consuming maize based complementary foods in Tanzania are exposed to multiple mycotoxins, DON in addition to fumonisins and aflatoxins.

The levels of DON contamination (range, 57–825 μg/kg) found in the maize flour are similar to levels of 43–435 μg/kg reported for maize in Cameroon (Abia et al., 2013) and levels of 1–930 μg/kg reported for marketed processed foodstuffs in Italy (Girillo et al., 2003). Slightly higher levels (up to 1250 μg/kg) were reported for processed foods in other parts of the world (WHO, 2011a). The variations in contamination levels are dependent on factors such as weather difference, extent of processing and type of food (WHO, 2011a, 2011b; Miller, 1995).

Table 1
Contamination levels and occurrences of mycotoxins in maize based flour.

Mycotoxin	Level (μg/kg)		Occurrence (%) at different ranges (μg/kg)	
	Median	Range	>LoD	>ML
Aflatoxin B1	1.27	0.53–364	24	5
Total aflatoxins	0.91	0.11–386	32	5
Deoxynivalenol	119	57–825	44	2
Fumonisins B1	329	57–1672	80	5
Total fumonisins	367	63–2284	83	12

LoD, Limit of detection, 0.53 for AFB1, 0.15 for AFB2, 0.24 for AFG1, 0.01 for AFG2, 52 μg/kg for DON, 53 μg/kg for FB1 and 47 μg/kg for FB2; Total aflatoxins, AFB1 + AFB2 + AFG1 + AFG2; Total fumonisins, FB1 + FB2; ML, Maximum limit, aflatoxins (5 μg/kg for AFB1, 10 μg/kg for total aflatoxins) or fumonisins (1000 μg/kg) or DON (750 μg/kg) in maize.

Table 2
Level of total aflatoxins, deoxynivalenol or total fumonisins in individual mixed cereal and legume flours.

Mix	Number of samples	Total aflatoxins (μg/kg)	Deoxynivalenol (μg/kg)	Total fumonisins (μg/kg)
Maize with finger millet	4	ND	248	954
		ND	144	229
		ND	82	ND
		1.46	ND	91.8
Maize with rice and finger millet	1	ND	ND	68.8
Maize with finger millet, soy bean and groundnuts	1	2.87	ND	ND
Maize with finger millet, wheat and groundnuts	1	0.12	389	702

ND, Not detected.

The highest exposure of 8.87 μg/kg in this community is higher than highest exposure reported by JECFA (WHO, 2001, 2011a) for national dietary exposures to DON. JECFA (WHO, 2011a) reported national dietary exposures for DON for 11 countries whereby the highest exposure was 3 μg/kg bw/day for Czech Republic. As reported by other researchers (Shephard, 2008; Sherphard et al., 2007) the risk of mycotoxins exposures in Africa is higher than in other parts of the world because of the high maize/cereal based food consumption in this region. Maize flour consumption estimated in this study ranged from 7 to 254 g/child/day with average consumption of 100 g/child/day. The average maize flour consumption of 100 g/day for these children (as estimated by this study) is similar to the maize flour consumption of 106 g/day for adult individuals in Africa as recorded by WHO GEMS/Food Regional Diets (WHO, 2003). This average consumption (100 g/child/day) is by far higher than the average maize flour consumption recorded by WHO GEMS/Food Regional Diets of 8.8 g/day (for Europe), 31.2 g/day (for Far East), 31.8 g/day (for Middle East), and 40 g/day (for Latin America). Indeed, Shephard, Kimanya, Kpodo, Gnonlonfin, and Gelderblom (2013) demonstrated that maize consumption can vary by as much as 100-fold between developed countries and developing countries, particularly in Africa.

The co-occurrence of DON with the other *Fusarium* toxin, fumonisins, confirms our hypothesis that children complemented with maize based complementary food are exposed to both DON and fumonisins. Co-occurrence and exposures of these *Fusarium* toxins have also been reported by others (Abia et al., 2013; Cirillo, Ritieni, Galvano, & Cocchieri, 2003).

The maximum fumonisins contamination of 2283 μg/kg determined in this study is slightly lower than the maximum contamination of 3201 μg/kg reported in 2010 for the same community

Table 3
Co-occurrence and levels of aflatoxins, deoxynivalenol and fumonsins in the co-contaminated flour.

Mycotoxins	Co-occurrence (%)	Range (μg/kg)		
		Total aflatoxins	Total fumonisins	Deoxynivalenol
Afaltoxins with both deoxynivalenol and fumonisins	10	0.12–0.63	94–702	57–459
Aflatoxins with fumonisins	29	0.12–0.63	85–1672	
Fumonisins with deoxynivalenol	41		94–2284	57–825

Aflatoxins, Total aflatoxins (AFB1 + AFB2 + AFG1 + AFG2); and Fumonisins, Total fumonisins (FB1 + FB2).

(Kimanya et al., 2010). This is possibly due to seasonal variations (Miller, 1995). However, the percentage (46%) of children exceeding PMTDI of 2 μg/kg bw/day is higher than the 15% reported by Kimanya et al. (2010). The higher exposure for the children we studied is logical because they are older (18–25 months old) than the previously studied children (6–8 months old). Other researchers have demonstrated that mycotoxins exposure, after introduction of complementary foods, increase with age (Gong et al., 2004; Shirima et al., 2013).

In addition to fumonisins, DON co-occurred with aflatoxins. The percentage (29%) of samples co-contaminated with aflatoxins and fumonsins in this study is higher than the percentage (10%) of co-contaminated samples reported in Kimanya et al. (2008). The present high aflatoxins co-occurrence may be due to nature of the flours tested. Whereas some of the flours tested in this study contained other cereals and legumes such as groundnuts in addition to maize, the previous study (Kimanya et al., 2008) used maize kernels only.

Thirty two percent, 29% and 46% of all (41) children studied were exposed at levels of health concern (0.017 ng/kg bw/day for aflatoxins, 1 μg/kg bw/day for DON and, 2 μg/kg bw/day for fumonisins). The main cause of the high exposures is the high intake of the maize based foods in this community (Shephard et al., 2013). This study found that none of the flours containing, in addition to maize, other cereals and legumes exceeded limits set for DON, fumonisins or aflatoxins (Table 2). This finding is in support of the suggestion made previously in Kimanya et al. (2012) that partial replacement of maize with other cereals can be used as an approach to reduce fumonisins contamination in complementary flours. It is therefore prudent to suggest development of nutritionally adequate and safe proportions of these other ingredients to promote for complementary foods in Tanzania.

Kimanya et al. (2012) showed further that if the maximum fumonisins level in complementary flour is regulated at 1000 μg/kg, a child 6–8 months oldconsuming a maximum of 20 g of maize flour per day would not exceed the PMTDI of 2 μg/kg bw. Using the data obtained by this study, simple calculations indicate that a child with an average body weight of 10.51 kg (as determined by this study) would have fumonisins exposure of 1.9 μg/kg bw/day in case she or he consumes 20 g of food containing the ML of 1000 μg/kg. This confirms our previous suggestion in Kimanya et al. (2012) that maize intake in children should not exceed 20 g per day when the maximum limit of 1000 μg/kg is enforced. Using the same approach or calculations and considering the PMTDI for DON of 1 μg/kg bw, the appropriate limit for DON contamination in maize or maize based flour should be set at 500 μg/kg or below. This limit is feasible because only one of the samples analyzed in this study exceeded 500 μg/kg. However, this contamination is higher than the ML set in the EU for cereal based foods for infants and young children, of 200 μg/kg for DON (EU, 2006). Suggesting a limit of 200 μg/kg for Tanzania may be impractical because children in this country consume foods made from home grown maize flour which cannot be expected to contain this low contamination as the agricultural practices in Tanzania are not that advanced (Kimanya et al., 2009).

The greatest challenge is estimation of an appropriate ML for aflatoxins. Considering that aflatoxins are both genotoxic and carcinogenic, guidance for management of these toxins is based on evaluation of MOEs. The aflatoxins MOEs for the children ranged from 0.22 to 169. These MOEs are similar to MOEs ranging from 0.2 to 121 estimated by Shephard (2008) for exposures from different types of food in Kenya, Tanzania, Gambia, Benin, Ghana and Botswana. EFSA. (2007) suggested that governments should be concerned of aflatoxin exposure when one's margin of exposure falls below 10,000 or (as afore shown by calculation) when one's exposure exceeds 0.017 ng/kg bw/day. Using the proposed maize based flour consumption of 20 g/day, a child of 10.5 kg would exceed the exposure of 0.017 ng/kg bw/day when aflatoxins contamination in his or her food exceeds 0.009 μg/kg. This calculation suggests that the ML for aflatoxins in maize for complementary foods in Tanzania should not exceed 0.009 μg/kg. We cannot recommend this limit because it is not practical both in terms of laboratory capacity to determine it (the lowest concentration of aflatoxins family we could detect in this study is 0.01 μg/kg) and not technologically and reasonably achievable. If this limit was implemented all (32%) of the flour samples which had detectable aflatoxins, would have to be discarded. An important message from this interpretation is that the existing limits for AFB1 and total aflatoxins in Tanzania of 5 μg/kg and 10 μg/kg, respectively, are not protective to these children. Even the most stringent limits of 2 μg/kg and 4 μg/kg for AFB1 and total aflatoxins, respectively, set for Europe (EU, 2006) are not protective to these children. Possibly, this explains why Europe implements aflatoxins free requirement for foods for infants and an ML of 0.1 μg/kg for processed cereal-based foods and baby foods for infants and young children (EU, 2006).

This study has highlighted the extent of co-exposures to multiple mycotoxins in rural subsistence communities of Tanzania. The effects of interactions of multiple mycotoxins in human health have not been well studied. A review by Grenier and Oswald (2011) indicates that the interactions can be additive, synergistic and can vary with dose, exposure time, animal species involved and toxicological endpoint. Nonetheless, the findings of this study emphasize the need for formulation of appropriate preventive measures that can target more than one form of mycotoxins in Tanzania.

Although, enforcement of regulations in rural communities such as the community we studied (where people rely on home grown crops) is not feasible, the findings of this study can be used to guide review or formulation of mycotoxins MLs to facilitate maize trade in Tanzania. These findings are particularly relevant for DON and fumonisins MLs setting as these two forms of mycotoxins are not yet formally regulated in Tanzania. However, regulation of multiple mycotoxins in foods requires availability of laboratory capacity to determine them simultaneously. Recent developments in mycotoxins analysis using LC-MS/MS have made it possible to determine multiple mycotoixns in foods (Abia et al., 2013; WHO, 2011a, 2011b). This equipment (LC-MS/MS) is available in developed countries, but the high cost of this equipment makes it unavailable for most developing countries including Tanzania. Until resources allow or innovation makes multimycotoxin analytical capacity affordable, developing countries will continue to rely on separate analysis of different forms of mycotoxins, using the available equipment.

5. Conclusion

Children consuming maize based complementary foods in rural Tanzanians are exposed to unacceptably high levels of multiple mycotoxins; aflatoxins, fumonsins and DON. We recommend adoption of the ML of 500 μg/kg for DON in Tanzania. However, implementation of this limit should be accompanied by reduction of maize content in complementary foods to a maximum of level of 20 g/child/day recommended by Kimanya et al. (2012).

Although the MLs of 5 μg/kg for AFB1 and 10 μg/kg for total aflatoxins set for maize in Tanzania are not fully protective against these toxins, we cannot recommend more stringent limits as those limits would most likely not be reasonably achievable.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors are very grateful to the managements of the Tanzania Food and Drugs Authority, Rombo district and Tarakea division for their support and guidance during the implementation of the study. The authors thank the International Foundation for Science (IFS) and the Royal Society of the UK for funding this study.

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