World Mycotoxin Journal, August 2008; 1(3): 307-313

Wageningen Academic Publishers

Human exposure to fumonisins from home grown maize in Tanzania

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Received: 13 June 2007 / Accepted: 19 December 2007

Abstract

Fumonisins contaminate maize worldwide resulting in unacceptable fumonisin exposures in people relying on maize as staple food. This study determined fumonisins B_1 (FB_1) and B_2 (FB_2) in maize from 120 rural households: 30 from each of four main maize producing regions of Tabora, Ruvuma, Iringa and Kilimanjaro in Tanzania in order to estimate total fumonisin (FB_1 + FB_2) exposures to adult individuals in the households. The average daily per capita maize consumption of 771 g, recommended by the Tanzania Food and Nutrition Centre (TFNC) for an adult relying on it as a main meal, and also average daily per capita maize consumptions of 129, 308 and 356 g documented for Tanzania, were used in the exposure estimation. The fumonisins were determined by HPLC using fluorescence detection. Total fumonisins exposure (µg/kg body weight (bw)/day) was determined by multiplying average daily per capita maize consumption (kg) by fumonisin level in maize (µg/kg) from a given household and then dividing by an average bw of an adult of 60 kg. Of the 120 samples, 52% were contaminated with fumonisins at levels of up to 11,048 µg/kg (median; 363 µg/kg). Based on the recommended maize consumption of 771 g/person/day, fumonisin exposures to adult individuals in 38% of the households would exceed the provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg bw, recommended by the Joint FAO/WHO Expert Committee on Food Additives. At the least documented maize consumption of 129 g/person/day, fumonisin exposures in 16% of the households were still above the PMTDI. Reduction of the maize consumption level to 40 g/person/day is an impractical, and reduction of the maximum contamination level to 155 µg/kg is a possibly practical, option for effective minimisation of fumonisin exposures in these communities. A relatively larger study is needed in order to generate comprehensive data for the formulation of appropriate strategies to minimise fumonisin exposures in Tanzania.

Keywords: fumonisins, exposure, maize, Tanzania

1. Introduction

In Tanzania, maize is grown and used as a staple food for the majority of populations living in rural areas who are basically resource-poor farmers. According to the food security department (FSD) (1996) of Tanzania, maize is grown on an average of 2 million hectares or about 45% of the cultivated land. Daily maize consumption levels in Tanzania are similar to levels reported for other countries in Africa including a level of 456 g/person reported by Shephard *et al.* (2007) for the former Trankei region of South Africa. On average, the daily per capita maize consumption in Tanzania varies from 129 (FAO, 1992) to 308 g (FSD, 1996) at national level. In the high maize-producing regions, the consumption can rise to 356 g/person/day (Nkonya *et al.*, 1998). However, the Tanzania Food and Nutrition Centre (TFNC, 1997) recommends 771 g as daily per capita maize consumption for adequate energy intake in communities relying on maize as staple food. Unfortunately, maize is known to be highly vulnerable

to contamination with fumonisin mycotoxins (Miller, 2001; Kpodo *et al.*, 2000; Shephard *et al.*, 1996). Fumonisins B_1 (FB_1), B_2 (FB_2) and B_3 (FB_3) are the most abundant forms of fumonisins in naturally contaminated food and feed (WHO, 2002).

Fumonisin B_1 has been linked to widespread outbreaks of leukoencephalomalacia in horses and pulmonary oedema in pigs fed with corn screenings in the USA in 1989/90 (Kellerman *et al.*, 1990). Reports of studies in animals show that FB_1 can cause cancer of the liver (Gelderblom *et al.*, 1991) and of the kidney (Riley *et al.*, 1994) in rats and mice. These toxins have also been linked to high incidences of oesophageal cancer observed in the former Transkei region in South Africa (Rheeder *et al.*, 1992) and China (Chu and Li, 1994). The International Agency for Research on Cancer categorised FB_1 as a group 2B carcinogen; possibly carcinogenic to humans (WHO, 2002). In view of the available data at the time, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended a provisional tolerable daily intake (PMTDI) of 2 µg/kg body weight (bw) for FB_1, FB_2 and FB_3, alone or in combination (WHO, 2002). According to WHO (1997) provisional tolerable daily intake is the reference value established by JECFA, used to indicate the safe level of intake of a contaminant, when chronic exposure is relevant. One of the options for reduction of fumonisin exposure is setting and enforcing a maximum tolerable limit (MTL) for the toxins in food. For instance, in the European Union, an MTL of 1000 µg/kg maize flour is in force (Van Egmond *et al.*, 2007). In view of the high maize consumption among populations in rural South Africa, Marasas *et al.* (1997) suggested an MTL of 100 to 200 µg/kg for fumonisins in maize for human consumption in that country.

Considering that maize is a staple food for the majority of Tanzanians, it is necessary to estimate the magnitude of the fumonisin contamination of home grown maize in Tanzania and use that data to estimate dietary exposure of fumonisins with a view to developing a tolerable limit for fumonisins in maize for consumption by the Tanzanian population. However, data on occurrence and level of contamination of Tanzanian maize with fumonisins are limited. The only data available were reported by Doko *et al.* (1996) for 9 samples of maize from Tanzania examined in a study of natural co-occurrence of fumonisins and zearalenone in cereals and cereal-based foods from Eastern and Southern Africa. Therefore, this study reports data on occurrence, level and estimated human exposure of fumonisins in home-grown maize in rural Tanzania and suggests an MTL for fumonisins in maize for human consumption in Tanzania.

2. Materials and methods

Study areas

The study was conducted in 120 households; 30 from each of the villages of Nyabula (Iringa region), Litapwasi (Ruvuma region), Kikelelwa (Kilimanjaro region) and Kigwa (Tabora region) in Tanzania. The regions were chosen based on their high maize production capacity compared to many other regions of the country (FSD, 1996). For each region, the Regional Administrative Director suggested the leading district in terms of maize production and the District Administrative officer, the village with households relying on maize as staple food and located in an area that was accessible by road. In each of the chosen villages, 30 households who (in accordance with the views of the village executive officers) could have some stock of maize were selected for the survey. Data on agro-ecological location for each region were obtained from the Ministry of Agriculture, Food Security and Cooperatives, Dar es Salaam, Tanzania. Data on mean temperature and relative humidity recorded over 35 years (1971-2005) for Ruvuma and Iringa were obtained from the Tanzania Meteorological Agency (TMA), Dar es Salaam, Tanzania. These climatological data are summarised in Table 1.

Sampling of maize

In December 2005 and January 2006, sampling of home grown maize was carried out in each of the 120 households during the fifth to sixth months after harvest in order to

Table 1. Climatological data for Tabora, Ruvuma, Iringa and Kilimanjaro regions, Tanzania.

Region	Agro-ecological zone	Sub- zone	Altitude (m)	Rainfall (mm/year)	Rainfall season	Mean temperature (°C)	Mean relative humidity (%)
Tabora	Plateau	Western	800-1500	800-1000	November-April	nr	nr
Ruvuma	Plateau	Southern	800-1500	900-1300	November-April	16-27	51-77
Iringa	Southern Highlands	Southern	1200-1500	800-1400	December-April	15-27	47-64
Kilimanjaro	Northern Highlands	Northern	1000-2500	1000-2000	November-January and March-June	nr	nr

nr = no records.

take into account possible production of mycotoxins in maize during storage. For stores where maize was found stored in cob form, the outer surface of the stock of stored maize was divided into four vertical sides. Nine cobs of maize were drawn from each of the 4 sides in such a way that three cobs of maize were drawn from random points on the upper, the middle and the bottom layer of the stock. Most storage structures were constructed with wooden material leaving space between posts through which it was possible to push a hand and draw out cobs of maize. In total, 36 cobs were sampled by hand; each cob comprising an incremental sample. All the cobs were shelled into grains and the grains mixed to obtain a homogeneous (aggregate) sample. In the case of households where maize was found stored as grains (commonly in polypropylene bags) a probe was used to draw samples from various points of each bag. In all cases, at least one kilogramme of a well mixed aggregate sample was packaged in a well-sealed container, which was then transported to the Tanzania Food and Drugs Authority (TFDA) laboratory in Dar es Salaam for analysis.

Determination of fumonisins in maize

FB_1 and FB_2 in the maize were determined by a liquid chromatographic method based on Sydenham *et al.* (1992) and the slight modifications made by Samapundo *et al.* (2006). Fumonisins were extracted overnight from 15 g of a finely ground portion of the maize with 40 ml of methanol: water (3:1) in 100 ml glass bottles. The slurry was filtered through Whatman No 1 filter paper and the bottle rinsed with 10 ml of the same mix of methanol and water. A 10 ml aliquot of the filtered extract was applied to a strong anion exchange (SAX) cartridge (Varian, Bond-Elut LRC, 500 mg) fitted to a solid phase exchange manifold (Alltech, 24-Port SPE Vacuum Manifold System, ALLTECH Associates Inc, Lokeren, Belgium). Before applying the extract, the SAX cartridge was conditioned with 5 ml of methanol, followed by 5 ml of methanol and water mix (3:1, v:v). After application of the extract, the SAX cartridge was then washed with 8 ml of methanol and water mix (3:1, v:v), followed by 3 ml of methanol. The fumonisins were eluted from the cartridge with 10 ml of 1% glacial acetic acid in methanol. The eluate was collected and evaporated to dryness at 60 °C under a light stream of nitrogen using a Nitrogen Evaporator (PIERCE model 18780, Reacti-Vap coupled with a dry bath; PIERCE Reacti-Therm). The residue was dissolved in 200 µl of methanol. The solution was thoroughly mixed with 200 µl of a derivatising reagent prepared by dissolving 40 mg of ortho-phthaldehyde (OPA) in 1 ml of methanol, followed by 5 ml of 0.1 M sodium tetraborate and 50 µl of β-mercaptoethanol and 20 µl of the mixture injected to the HPLC for analysis using a reversed-phase HPLC fluorescence detection system within 4 min. A Waters HPLC system consisting of a Waters 600 pump and its controller was used. The system was connected to a Shimadzu SIL-10AD*vp* auto injector. Chromatographic separations were performed on a Discovery C8 column (100 X 4.6 mm, 5 µm), (SUPELCO, Supelco Park, Bellefonte, PA, USA). Methanol – 0.1 M sodium dihydrogen phosphate (75:25) mixture adjusted to pH 3.35 with orthophosphoric acid was used as mobile phase. The flow rate of the mobile phase was 1 ml/min. Fluorescence of the fumonisin OPA derivatives was detected at wavelengths of 335 (excitation) and 400 (emission) using a Shimadzu RF-10AXL fluorescence detector and recorded with a Shimadzu C-R3A Chromatopac integrator.

The limit of detection of the analytical method was 53 µg/kg for FB_1 and 47 µg/kg for FB_2. The accuracy and precision of the method were verified by spiking blank samples of maize with FB_1 and FB_2, each at 100, 200, 300, 400 and 500 µg/kg and evaluating their recovery. Average recovery values were 106% (five samples, RSD 16.6%) and 92% (five samples, RSD 15.3%) for FB_1 and FB_2, respectively.

Estimation of fumonisin exposure

Fumonisin exposure was estimated for an adult of 60 kg bw using the following formula:

$$Y_h = XC_h$$

where:

- Y_h = the total fumonisins (FB_1+FB_2) exposure (in µg/kg bw/day) to an adult individual in household 'h',
- X = the average per capita maize consumption (in kg/day), recommended or estimated for an adult individual in Tanzania, and
- C_h = the mass fraction of fumonisins (in µg/kg), determined in the sample of maize from the household 'h.'

Statistical analysis of data

Descriptive statistics for the data were carried out by use of stata 9.0 statistical package (Stata 9.0; STATA).

3. Results

Out of the 120 samples, 62 (52%) were contaminated with FB_1 and FB_2 at levels of up to 6,125 µg/kg (median; 194 µg/kg) and 4,923 µg/kg (median; 203µg/kg), respectively. On average, FB_2 formed 31% of the total fumonisins which ranged from 61-11,048 µg/kg. Fumonisin B_3 was not determined. Fumonisins in the four studied regions varied from region to region in occurrence, median and range as shown in Table 2. This detectable spread of fumonisins was high in Tabora when compared to Ruvuma, Kilimanjaro and Iringa regions. Twenty one (70%) of the samples from Tabora were contaminated with detectable levels of fumonisins. However, the highest fumonisin contamination

Table 2. Occurrence and level of total fumonisins in home-grown maize in Tanzania.

Region	Occurrence (%)	Median (µg/kg)	Range (µg/kg)
Tabora	70	321	71-2,763
Ruvuma	47	155	62-3,560
Kilimanjaro	47	501	65-11,048
Iringa	43	441	61-3,353

of 11,048 µg/kg was determined in a sample of maize from Kilimanjaro.

Exposure of fumonisins for an adult of 60 kg bw was estimated for each household by multiplying the fumonisin content in maize from that household with the daily per capita maize intake of 771 g recommended by TFNC (1997) for an adult relying on it as a main meal, and also with the average daily per capita maize consumptions of 129, 308 and 356 g documented for Tanzania by FAO (1992), FSD (1996) and Nkonya *et al.* (1998), respectively. Table 3 shows the relationship between the daily per capita intake of maize and exposure to fumonisins, fumonisin concentration in maize above which human exposure would exceed the PMTDI and percentage households with individuals that would exceed the PMTDI at the respective maize intake level. On the basis of the recommended consumption level of 771 g/persn/day, total fumonisins exposure levels for individuals in the studied households ranged from 0.78-141.97 µg/kg bw/day. Individuals in 38% of the 120 households would be exposed to fumonisin levels exceeding the PMTDI of 2 µg/kg bw. The individuals with fumonisin exposure above 2 µg/kg bw/day were from households in which total fumonisin contamination in maize exceeded 155 µg/kg. Individuals in 14% of the 120 households would be exposed to more than 10 µg/kg bw/day. Adults in two of the households in Kilimanjaro would be exposed to more than 70 µg/kg bw/day. The percentage of households in which individuals would be exposed to more than the PMTDI dropped from 38 (in the case of maize intake of 771 g/person/day), through 27 (in the case of maize intake of 356 g/person/day) to 16% (in the case of maize intake of 129 g/person/day).

Table 3. Influence of daily per capita maize intake on exposure to fumonisins.

Daily per capita maize intake (g)	Range of exposure (µg/kg bw/day)	Highest concentration in maize (µg/kg)[1]	Percent households exceeding the PMTDI
771[a]	0.78-141.97	155	38
356[b]	0.36-65.55	314	27
308[c]	0.31-56.72	382	25
129[d]	0.13-23.75	870	16

[1] Concentration above which the maize intake results in exposure above the PMTDI.
[a] The value of consumption level recommended by the TFNC.
[b] The value of consumption level reported by Nkonya *et al.* (1998).
[c] The value of consumption level reported by FSD (1996).
[d] The value of consumption level reported by FAO (1992).

4. Discussion

The study demonstrated that Tanzanian home-grown maize is contaminated with fumonisins. The levels of FB_1, FB_2 and total fumonisins determined by this study are similar to levels of up to 7,900 µg/kg (FB_1), 3,770 µg/kg (FB_2) and 10,140 µg/kg (total fumonisins) determined in maize from the former Transkei region in South Africa (Shephard *et al.*, 1996, 2007). However, the highest level of FB_1 determined in the maize was higher than the highest contaminations reported earlier by Doko *et al.* (1996) for maize from Tanzania (165 µg/kg) and Doko *et al.* (1995) for maize from Benin (3,310 µg/kg), Zambia (1,710 µg/kg), Italy (2,850 µg/kg) and Portugal (4,450 µg/kg).

Occurrence and level of fumonisins in maize varied among the four regions (Table 2). Contamination in Tabora was relatively widespread with 70% of samples from the region contaminated with fumonisins. During the year 2005, Tanzania experienced drought which might have influenced contamination of maize with fumonisins. Tabora might have been specially affected by the drought because when compared with the other studied regions, in normal conditions, it experiences the least maximum annual rainfall (1000 mm/year) (Table 1). Drought-stressed crops are more susceptible to fungal attack and possible fumonisin contamination (Miller, 2001). However, the highest contamination of 6,125 µg/kg (FB_1), 4,923 µg/kg (FB_2) and 11,048 µg/kg (total fumonisins) were determined in samples from Kilimanjaro region. During sampling it was noted that farmers in Kilimanjaro region harvest maize from May to July, a period which coincides with the rain period (Table 1). Maize that matures and is ready for harvest in a rain period is likely to experience high water content over a long period of time, a condition that favours fungal infestation. Gamanya and Sibanda (2001) reported that FB_1 concentration in food decreased from regions with high rainfall and annual moderate temperature to low rainfall regions. Farmers in Kilimanjaro stated that they were compelled to harvest improperly dried maize in order to avoid the previous year's experience of losing maize to thieves who steal from the farms. Early harvested maize is normally kept in the homestead in heaps that take a long time to dry. Maize subjected to delayed drying has been associated with increasing mould and fumonisin

contamination (Bhat *et al.*, 2000). It is, therefore, not surprising to note that the highest FB_1, FB_2 and total fumonisin contaminations were determined in samples from the Kilimanjaro region.

This study demonstrated that Tanzanians consuming maize as staple food are at risk of exposure to unacceptable levels of fumonisins (Table 3). Based on the maize consumption level of 771 g/person/day, fumonisin exposures to individuals in 38% of the households exceeded the PMTDI value of 2 µg/kg bw/day. Total fumonisin levels in maize from those households were above 155 µg/kg. These results suggest that the MTL for fumonisins in maize for human consumption in Tanzania should not be set above 155 µg/kg. This MTL is within the range of 100 to 200 µg/kg suggested by Marasas *et al.* (1997) for maize for human consumption in South Africa. However, the limit is lower (more stringent) than the MTL of 1000 µg/kg set for fumonisins in maize flour for human consumption in the European Union (van Egmond *et al.*, 2007). The difference in MTLs between Africa and Europe is caused by the difference in maize consumption rates between the two regions. Maize consumption in Western Europe is very low; for instance, the daily per capita maize consumption in Germany and United Kingdom is about 7 g (Shephard *et al.*, 1996).

Reducing maize consumption level reduces the percentage of households with exposures above the PMTDI value. However, at the lowest documented maize consumption of 129 g/person/day, 16% of the households were still exposed to fumonisin levels above the PMTDI. It appears that, in order to minimise exposure in these communities, an adult in the household from which the highest contaminated sample (11,048 µg/kg) was taken has to consume a maximum of 40 g of maize per day. Considering, the limited range of available foods for diversification and the low socio-economic capacity among the populations in rural Tanzanians it would be unrealistic to advocate such a low rate of maize intake. Of the populations in the rural areas from which the samples were taken, 31% in Iringa Rural (Iringa), 37% in Rombo (Kilimanjaro), 41% in Ruvuma Rural (Ruvuma) and 48% in Uyui (Tabora) live below the poverty line (URT, 2005). The practical option, therefore, would be appropriate application of a variety of preventative measures, both before and after harvest, in order to reduce fumonisin contamination of maize to as low level as possible. The measures include, for example, selection and use of disease resistant varieties of maize, appropriate use of fertilisers and pesticides; and application of good harvesting, drying and storage practices (Codex Alimentarius Commission, 2002). Once fumonisins occur in maize, their levels can be reduced by the application of a variety of other measures including processing, detoxification, and segregation (Soriano and Dragacci, 2004). Available data indicate that deliberate efforts are needed to improve the pre- and post-harvest maize handling practices in Tanzania in order to minimise fumonisin contamination in the crop. Most of the farmers in Tanzania use recycled seeds, cultivate maize in the same field year after year without fertilisers, and practice intercropping of maize with other crops (Nkonya *et al.*, 1998). These practices influence fungal invasion and subsequent fumonisin production in the crops (Kabak *et al.*, 2006; Soriano and Dragacci, 2004). Although, in the course of this study, farmers admitted that they sort maize to remove mouldy and damaged fractions before storage in order to avoid further deterioration in stores, it was observed that the content of visibly mouldy and damaged kernels in the samples were at levels of up to 56% and correlated positively with the fumonisin levels (data not shown). Thus, sorting as practiced by the farmers is not effective enough to reduce contamination in maize and subsequently minimise exposures in these communities.

It is worth noting that people living in rural Tanzania have additional fumonisin exposure through consumption of beer brewed from contaminated maize. These people are also at risk of exposure to other forms of mycotoxins such as aflatoxins which have been reported to co-occur with fumonisins (Kpodo *et al.*, 2000). According to the URT (1989), 11% of 472 samples of home-grown maize collected from market places in Tanzania contained total aflatoxin levels at varying levels up to 69.5 µg/kg. The MTL for total aflatoxins in cereals for human consumption in Tanzania is set at 10 µg/kg. This observation shows the importance of determining aflatoxin contamination in maize for human consumption in Tanzania so as to be able to determine the implications of their co-exposure with fumonisins.

The fumonisin exposure data generated by this study might have been over-estimated because the exposure assessment used contamination data for samples of un-milled maize. In the course of milling, maize undergoes a decortication step that is known to reduce fumonisin contamination in the consumed fraction; the flour (Fandohan *et al.* 2005, Soriano and Dragacci, 2004). In this regard, future exposure assessments for fumonisins in maize in Tanzania should employ contamination data determined in ready-to-cook, or if analytical technology permits, ready-to-eat maize. It is important, however, to note that since cooking does not bring about a considerable reduction in the fumonisin content of food (Bhat, 2000, Shephard *et al.*, 1996), people who prefer whole to decorticated maize will be exposed to fumonisins at more or less the levels determined by this study. Children might be at an even higher risk of exposure given that complementary foods are maize-based gruels and that their relative energy needs per kilogram body weight are higher than in adults. Further exposure assessment studies that target infants and children are therefore worth considering.

This paper reports for the first time on the extent of fumonisins contamination in maize and human exposures to these toxins in Tanzania. The study used a convenient sample of 120 households from four out of the 26 regions of Tanzania and was conducted for maize from one season. The study aimed at identifying regions in Tanzania where the contamination is likely to be high and urgent intervention needed. Additionally, this exposure assessment used point estimates of the daily per capita maize consumption for an adult individual in Tanzania. Point estimates are generally used for screening purposes in deterministic exposure assessments to provide evidence for or against carrying out a probabilistic exposure assessment (Parmar *et al.*, 1997). The former are relatively simpler, quicker and less expensive to carry out. Based on the data generated by this study, more studies are needed on fumonisin contamination in maize, involving a relatively larger sample size of households or farmers from more regions in all the agro-ecological zones of Tanzania and conducted for maize from more than one seasons. Likewise, a careful designed and conducted survey of maize consumption in Tanzania is needed in order to generate more comprehensive data for combining with the fumonisin contamination data in a probabilistic exposure assessment. Compared to the data generated by this study, the data to be generated by the probabilistic exposure assessments would provide a better scientific basis for formulation of a MTL for fumonisins in maize for human consumption in Tanzania. However, before those comprehensive data are obtained, a risk management strategy for fumonisins in maize in Tanzania is needed in order to evaluate the findings of this exposure assessment, and if necessary take precautionary measures to protect the public from the high fumonisin exposure and to promote trade in maize. Such measures can easily be regulated within the existing food regulation infrastructure supervised by TFDA. The measures can also serve as guidance for agricultural or health extension officers working with populations living in the rural places who may need to advise farmers and householders on methods for preventing fumonisins contamination in maize. Training of farmers in good agricultural and management practices for the reduction of mycotoxins in foods can also contribute to a reduction in mycotoxin exposure in the rural areas. A study of the traditional maize management practices is necessary to establish practices that minimise mycotoxins in foods. Traditional practices for maize management would be more readily acceptable than imported practices.

Acknowledgements

The authors would like to thank the International Foundation for Science (IFS), the Nutrition Third World (NTW) and the Belgium Technical Cooperation (BTC) for funding this study. The authors are also grateful to the food consumption and mycotoxin research clusters of the NutriFoodChem research group (Ghent University) for their help in designing the study. The authors would also like to thank the management of TFDA and TFNC; and the administrative authorities of Iringa, Tabora, Ruvuma and Kilimanjaro regions for their support during the field work. Our appreciation also goes to Juma Amiri, Anita Bitegeko, Gladness Kanza and other staff who helped analyse the samples.

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