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EXPOSURE OF CHILDREN 4 TO 6 MONTHS OF AGE TO AFLATOXIN IN KISUMU COUNTY, KENYA

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ABSTRACT

Contamination of foods by aflatoxins is a global health problem in both developed and developing countries. Exposure to the toxins is associated with a range of effects on health including stunting in children. Commodities at high risk of aflatoxin contamination include cereals, legumes, milk, fish and meats. Children are more vulnerable to effects of aflatoxin exposure compared to adults. Being genotoxic, levels of aflatoxins in foods should be kept as low as possible, given that there is no known threshold at which they may pose a health risk. This study investigated the potential exposure of young children to aflatoxin contamination in Kisumu County, Kenya. Kisumu County may have the potential for low to high levels of aflatoxin contamination due to prevailing weather conditions as well as reliance on maize, sorghum, cassava and rice as the main staple foods, groundnuts as snack and *omena* (*Rastrienobola argentea*) and milk as cheap sources of protein. These foods are also used as weaning foods in the County. Samples of *omena*, rice, groundnuts, cassava, maize, and sorghum were collected from Kibuye wholesale market, Kibuye open air market, Ahero market, Oile market and Mamboleo market in Kisumu County using a combination of cluster and systematic sampling. Processed cow's milk samples were collected from supermarkets and raw cow's milk samples from 3 market milk bazaars in the County. Analysis of solid foods was done using HELICA Total Aflatoxin Assay, intended for quantitative detection of aflatoxin B_1, B_2, G_1 and G_2. Milk sampling was done using the European model outlined in the Codex Alimentarius. Aflatoxin M_1 levels in milk were analyzed using HELICA Aflatoxin M_1 Assay. Aflatoxin levels in the foods ranged from 0 to 34.5 ppb aflatoxin B_1, 0.012 to 0.127 ppb aflatoxin M_1 in processed milk and 0.0002 to 0.013 ppb aflatoxin M_1 in raw milk. All the food products, except cassava, had samples with detectable aflatoxin levels. Daily aflatoxin consumption ranged from 35 ng (4.43/kgBw/day) to as high as 872 ng (110.4 ng/kgBW). These findings indicate that weaning children in Kisumu County are potentially exposed to levels of aflatoxins above the permissible amounts, given that the food stuffs that were analyzed are the commonly used weaning food items. Its effects on their health should be assessed and efforts taken to reduce potential exposure both from the commonly suspected sources as well as from milk.

Key words: Aflatoxin, exposure, infant, weight, height

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INTRODUCTION

Aflatoxins are a naturally occurring group of mycotoxins that are produced by *Aspergillus flavus* and *Aspergillus parasiticus* [1]. The impact of the toxins affects both developing and developed worlds and are of public health importance because of their effects on human health and food safety [2]. It is estimated that 4.5 billion people in developing countries have chronic exposure to large amounts of aflatoxins in their diets [3]. Food contamination by aflatoxins starts in the field and is worsened by erratic weather conditions and poor agronomic practices [4]. The toxins contaminate a wide range of agricultural produce and products before harvest and under post harvest conditions [5]. Some of the foods prone to aflatoxin contamination include maize, wheat, rice, millet, sorghum, beans, soya beans, peanuts, and spices. The toxins are also found in milk, eggs, and meat products due to consumption of contaminated feeds by animals [6]. Consumers in developing countries feed on contaminated grain, despite the consequences due perennial scarcity of food [7].

Studies carried out in Kenya reveal that more than 40% of diets in both rural and urban communities are comprised of maize and maize products and are likely to be contaminated by aflatoxins [8]. Maize flour is one of the main ingredients in weaning porridge in most Kenyan communities including Kisumu County. Aflatoxin poisoning resulting from consumption of contaminated maize has been reported in Eastern Kenya [5], the worst having occurred in 2004 [5, 9]. Contamination levels of 133 to 376 times above the regulatory limit have also been reported in peanut-growing areas in South Nyanza, Kenya and 40% of food samples from farmers had aflatoxin levels above the regulatory limit of 10 ppb [10]. Recent research findings revealed mean aflatoxin levels ranging from 37 ppb to 54 ppb in maize in Nyanza Region compared to 21 ppb to 44 ppb in Eastern Region [11]. These findings reveal the prevalence of high levels of aflatoxin in Nyanza region and Kisumu County forms part of the region.

A study carried out on animal feeds in Nairobi revealed that only 5% of the total 72 samples collected were below the regulatory limit of 10ppb; aflatoxin levels ranged from 5.13-1123 ppb, with the largest proportion being between 11-99 ppb [12]. Codex Committee on Food Contaminants and additives reported a concentration of < 0. 002 (µg/kg) (0.1 ng/person/day) aflatoxin M_1 for African region, which is far below the international regulatory limit of 0.5 µg/kg [13]. However, intake of both high and low aflatoxin levels has been associated with negative health outcomes [12].

Exposure to aflatoxins may be a causative factor for child stunting and underweight, neurological impairment, immunosuppression and child mortality, according WHO Expert Group Meeting in July 2005 [13]. Studies have also demonstrated a possible association between aflatoxin exposure and growth faltering, particularly stunting in young children and in children with kwashiorkor [14, 15]. Aflatoxin exposure may affect child growth through immune suppression resulting in increased susceptibility to infectious disease, inhibition of protein synthesis caused by aflatoxin-induced disruption to RNA, or intestinal malabsorption [2, 14]. Stunting during childhood has been associated with a reduction in adult size, reduced work capacity and adverse reproductive outcomes.

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Aflatoxin B_1, found mainly in cereals and groundnuts is considered the most toxic and most potent [16]. Aflatoxin AFM_1 is a marker of AFB_1 intake, being detectable in milk 12-24 hours after ingestion and poses a health risk to breast-feeding infants, as well infants being weaned on cow's milk [17]. Both AFM_1 and AFB_1 have been found in breast milk samples from Ghana and Nigeria [18]. Studies carried out in The Gambia, Guinea, Kenya, Benin, Togo, and Senegal, revealed that about 85% to 100% of children had either detectable levels of serum aflatoxin albumin adducts or urinary aflatoxins [19]. These findings indicate that high proportions of infants in tropical Africa may have prenatal and postnatal exposure to aflatoxins. However, methodological constraints have inhibited extensive investigations to assess exposure of infants to AFM_1 through breast milk [20].

Direct ingestion of high concentrations of aflatoxins is fatal and chronic exposure is associated with liver cancer, spontaneous abortion, immunosuppression, cirrhosis and other liver diseases. Chronic exposure to aflatoxin may also interfere with micronutrient metabolism, resulting in malnutrition deficiencies in children < 5 years of age. Stunting rate among children < 5 years of age in Nyanza Province was 26.9% according to Kenya Demographic and Health Survey (KDHS), 2008/2009 [21]. The figures were 35.4% based on data from Kenya Multiple Indicator Cluster Survey 2002 and 31.1% based on data from KDHS, 2003 [22]. High stunting figures have been recorded in all the counties in Nyanza Region; Kisii 35.3%, Homa Bay 37.0%, Kisumu 33.1%, Migori 46.2%, and Siaya 38.4% [23]. Infants have a higher vulnerability to aflatoxins and their effects compared to adults [2]. This is because infants have a lower capacity for biotransformation of aflatoxins than adults, resulting in a longer circulation time of the toxins.

Forty percent of deaths in children < 5 years (4.5 million children) worldwide occur in Sub Sahara Africa and a big proportion of these deaths could be attributed to growth faltering. A study in West Africa reveals that young children who were chronically exposed to aflatoxin in foods were stunted and underweight, as measured by World Health Organization (WHO) Z-scores [24]. A positive correlation has also been established between wasting in children above six months and consumption of aflatoxin contaminated weaning flours in a cross sectional study in Kisumu District, Kenya [25]. However, this study only focused on flours from households excluding other common weaning foods like omena and milk and market foods. Given the potential adverse health effects of this dietary toxin on children, it is important that appropriate interventional strategies are instituted especially in developing countries. Most children in Kenya are breastfed until at least the latter part of the second year and begin to receive cereal-based gruel before the age of 3 months [26], which could increase their exposure to the toxin.

Most techniques used to measure aflatoxin in food samples follow three main steps: extraction to remove the aflatoxin from complex mixtures of materials in which it is found; purification to remove interferents and finally detection and quantification using either traditional techniques or immunochemical techniques [27]. The traditional techniques include, Thin Layer Chromatography (TLC), Gas Chromatography (GC), and High Pressure Liquid Chromatography (HPLC). These methods are well proven and widely

accepted; however, they are often viewed as laborious, time intensive and expensive. There are also immunochemical based assays that are used for detecting aflatoxin, among them; Radioimmunoassay (RIA), Enzyme-Linked Immunosorbent Assay (ELISA), and Immunoaffinity Column Assay (ICA). For purposes of this study, ELISA technique was used to determine aflatoxin contamination levels in food samples.

This study aimed to investigate the potential exposure of weaning children 4 to 6 months of age to aflatoxin contamination in Kisumu County, Kenya. Certain characteristics make Kisumu County prone to aflatoxin contamination: 40% of households in Nyanza region, and by extension Kisumu County, are exposed to aflatoxin contamination; higher levels of aflatoxin contamination have been recorded in foods in some areas of Nyanza compared to areas known for frequent outbreak of aflatoxicosis [11]; some of the foods grown and consumed in the County, including maize, sorghum and groundnuts are high risk commodities for aflatoxin contamination. Results of KDHS 2008/2009 reveal stunting figures of 26.9% among children < 5 years of age in Nyanza Province [21] and a possible association has been reported between aflatoxin exposure and stunting in young children [14]. Prevailing climatic conditions in Kisumu County including drought, erratic rainfall (1200mm and 1300mm per annum), high temperatures ranging between 20^{o}C and 35^{o}C and high humidity (40-89%), provide a favorable environment for growth of mould and production of aflatoxins [26]

MATERIAL AND METHODS

This was a cross sectional survey study conducted in Kisumu East and Nyando Districts, Kisumu County, Kenya. Markets were selected for inclusion in the survey based on geographic location of the population served by the market, having enough vendors with a variety of food products of interest to the study; and the availability of maize, sorghum, cassava, rice, omena (*Rastrienobola argentea*) and milk. The markets selected were: Kibuye wholesale market, Kibuye open market, Oile Market, Mamboleo market and Ahero market.

Two hundred and nine dried food samples (500g each) including: 50 omena, 31 rice, 22 groundnuts, 37 cassava, 41 maize, and 28 sorghum, were collected from Kibuye wholesale market, Kibuye open air market, Oile market, Ahero market and Mamboleo market. Sampling of dry foods was done using a combination of cluster and systematic sampling. Five hundred grams each of available maize, sorghum, polished rice, cassava, groundnuts and omena was collected from each bag of consenting sellers. Rice and Cassava were sampled because they form part of the staple food and are also used as weaning flours in the County. The foods were sampled based on the number of bags available in the market. If there were ≤10 bags, sampling was done on all the bags and if there were >10 bags, the square root of the extra bags was calculated and added to the 10 bags. The products were scooped from selling bags at different points of the bags to ensure uniformity, using respective vendor tools such as tins, and double packaged in paper envelopes to avoid cross contamination and moisture entry [28]. The packages were labeled and the sources recorded and stored for two days before being analyzed for aflatoxins at KARI Kitale, Kenya.

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A total of 80 milk samples were collected as follows: 50 processed milk samples from the 5 most common milk brands from the major supermarkets in Kisumu City; and 30 raw milk samples were collected from Ahero, Mamboleo, and Guba market milk bazaars. The milk samples were analyzed for aflatoxin M_1. The European model, which recommends that a 500g sample composed of five 100 g portions of milk is taken from a batch, be used for the minimum sample size and sample selection method was applied [29]. The milk samples were transported in cooler boxes and immediately frozen and stored at -20^{0}C before being transported in coolers to KARI Kitale for analysis.

Aflatoxin B_1 levels in staple food samples were analyzed using HELICA Total Aflatoxin Assay. An aflatoxin specific antibody optimized to cross react with Aflatoxin B_1, B_2, G_1 and G_2, was coated by a polystyrene microwell. The toxins were extracted from a ground sample of 20g portion and 100 ml of 70% methanol. The extracted samples and hydrogen peroxidase preservative conjugate (HRP) Aflatoxin B_1 were mixed and added to the antibody coated microwell. Microwell contents were decanted and nonspecific reactants were removed by washing. An enzyme substrate TMB was added and the colour blue developed. The intensity of the color was inversely proportional to the concentration of Aflatoxin in the sample or standard (14). The sample had been diluted at a ratio of 5 to 1 with 70% methanol, and so the aflatoxin shown by the standard was multiplied by 5 in order to indicate the ng of aflatoxin per gram of commodity.

Aflatoxin M_1 levels were analyzed using Helica Aflatoxin M_1 Assay (Aflatoxin M_1 ELISA Quantitative), with high affinity for aflatoxin M_1. Helica Aflatoxin Assay is a solid phase competitive enzyme immunoassay used for detection of aflatoxin M_1 in milk and milk products. In this procedure, an antibody with a high affinity for aflatoxin M_1 was coated onto polystyrene microwells. 200µL of milk sample was added to the appropriate microwell and aflatoxin M_1 was bound to the coated antibody. Subsequently, aflatoxin bound to horseradish peroxidase (HRP) was added and it bound to the antibody not already occupied by aflatoxin M_1 present in the sample or standard. After 15 minutes of incubation, the contents of the wells were decanted, washed and an HRP substrate was added which developed a blue color in the presence of an enzyme. The intensity of the color was directly proportional to the amount of bound conjugate and inversely proportional to the amount of aflatoxin M_1 in the sample. An acidic stop solution was added which changed the chromogen colour from blue to yellow. The microwells were measured optically by a microplate reader with an absorbance filter of 450nm (OD450). Results were determined by comparing the optical density of the sample to the optical density of the kit standards.

Aflatoxin contamination of food samples was calculated as average aflatoxin levels in all samples of a specified food. Potential exposure of children to aflatoxin was determined by calculating the absolute amount of aflatoxin a weaning child would consume assuming intake of 60g of solid food and 500 ml of milk and the amount of aflatoxin consumed in ng/kg body weight/day using the formula:

Exposure (ng/ kg body weight/day)
= (Contamination level) (amount consumed)/ body weight [3].

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In a similar approach, aflatoxin intake through milk was determined by assessing median aflatoxin M_1 concentration in milk samples, then multiplying by milk consumption of the population [13]. These formulae have been applied to arrive at data in Table 2. Data was analyzed using Statistical Package for Social Sciences (SPSS) software (IBM SPPS Statistics®) [21]

RESULTS

Aflatoxin levels in the dried foods ranged between 0 to 34.5 ppb aflatoxin B_1 and 0.0002 to 0. 13 ppb aflatoxin M_1 in milk samples (Table 1). All the solid food products, except cassava, had aflatoxin levels above the Kenyan regulatory limit of 10 ppb for aflatoxin B_1.

Dietary exposure to aflatoxin (ng/ kgBW/day) of a child 6 months (7.9 kg) of age with a daily consumption of 60g of mixed cereal flour and 500mls of milk per day in Kisumu County was calculated using the formula: Exposure (ng/kgBW/day) = (Contamination level) (amount consumed)/ body weight (Table 2) [3] .

DISCUSSION

This study showed that the potential for aflatoxin exposure in Kisumu County, depending on the combination of contaminated foods consumed, could range from none or minimal consumption of aflatoxin to consumption of levels as high as 34.5 ng/g of food. The findings of the study reveal that most of food stuffs that were sampled, except cassava, had relatively low to high levels of aflatoxin contamination. At these levels, if the food items were used as weaning foods for infants, assuming consumption of cereal and tubers, and milk, infants could have aflatoxin intakes as high as 115 ng/kg body weight per day, which could translate to 0.115µg/kg body weight/day.

Concern on aflatoxin mainly focuses on exposure to very high levels of aflatoxin as occurs during aflatoxicosis outbreaks, and reflected by most studies on aflatoxin contamination having been carried out in Eastern Province, an area that is assumed to have highest levels of exposure. However, chronic exposure to low levels is associated with liver cancer and malnutrition including micronutrient deficiencies and stunting [2]. Neurological impairment, immunosuppression and child mortality have also been associated with aflatoxin exposure

High stunting rates have been reported in Nyanza region from different studies [21]. An assessment of a potential association between stunting and reported aflatoxin levels in 7 areas in Kenya support a potential association corroborating a likely influence of aflatoxin exposure on stunting (Table 3). Stunting figures in Nyanza region, as well as Kisumu County are high and a potential role of aflatoxin exposure should be explored.

The highest level of aflatoxin contamination in the food samples analyzed in our study was 35.4 ppb. Recent research findings revealed mean aflatoxin levels of 37ppb and 54ppb in maize in Homa Bay and Rongo compared to 21ppb, 25ppb and 44ppb in Makueni, Mbeere North and Mbooni East respectively [11]. These findings reveal the

prevalence of high levels of aflatoxin in Nyanza region compared to areas previously known to be prone to aflatoxin contamination. It is also documented that 40% of food samples from farmers in Nyanza Province had aflatoxin levels above the regulatory limit of 10 ppb [10]. Although the levels in our study are not as high as those reported in earlier studies, they pose a health risk to consumers.

Maize has previously been reported as the major source of aflatoxin exposure in Kenya. However, our findings reveal higher aflatoxin levels in sorghum (14.77 ppb) compared to maize (4.19 ppb), although maize had the samples with the highest aflatoxin levels (34.5 ppb). Furthermore, the proportion of sorghum samples with detectable aflatoxin contamination was higher than that of maize, indicating more widespread contamination in sorghum. Others foods analyzed in this study which show potential for aflatoxins exposure include omena and rice. Cassava had the lowest range of aflatoxin levels among the dried foods. Maize, sorghum, dried cassava, rice, groundnuts, fish and milk are among the major foods produced and consequently, consumed in Kisumu County.

Aflatoxin B_1 ($AFLB_1$) is known to occur in most foods, especially in cereals and groundnuts, which are among the common staples foods in Kisumu County. The findings from our study reveal that Nyanza region is not self sufficient in food production. Most of the food consumed in the County comes from the neighboring countries and counties. It is, therefore, possible for aflatoxin contamination to occur through cross border trade. Sorghum, maize and groundnuts had the highest levels of aflatoxin B_1 based on the findings of this study. These foods are also likely to be used as weaning dishes by most households in Africa, Kenya and in Kisumu County [14, 25].

Low levels of aflatoxin were found in both processed and raw milk samples in Kisumu County (Table 3). The findings concurred with levels indicated in the Codex Alimentarius on concentration of aflatoxin M_1 in milk (µg/kg) by regional diet giving a concentration of 0.0018 µg/kg for the African region [13]. Based on the findings of our study, milk may not be a major source of aflatoxin exposure to children in Kisumu County. However, it is important to note that in attempting to keep aflatoxin contamination at levels as low as possible, feeds consumed by dairy animals should be taken into account. This is because aflatoxin B_1 in such feeds is converted to aflatoxin M_1 in the animals and excreted in milk. Additionally, aflatoxin is found in other animal products. A study carried out on animal feeds in Nairobi Province, showed aflatoxins levels ranging from 5.13 to 1123ppb, with the largest proportion being between 11-99ppb [12].

Exposure of weaning children to aflatoxins

Observations from our study confirm that weaning children onto family foods represents a period of increasing aflatoxin exposure. As indicated earlier, more than 40% of diets in both rural and urban communities in Kenya are comprised of maize and maize products [8]. Flour from maize is one of the main ingredients commonly used in preparing weaning porridge in most Kenyan communities including Kisumu County. However, other foods like sorghum, cassava, rice, groundnuts and omena are also used separately or mixed with other cereal flours to prepare weaning gruel. We found potential aflatoxin exposure in children ranging from 4.43 ng/kg body weight/day in a combination of maize

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flour and raw milk to 110.38 ng/kgBW in a combination of sorghum and raw milk. Data from this study record a higher aflatoxin consumption of 0.6 ng/kg body weight/day for a child at 6 months weighing 7.9 kg compared to that indicated by the Codex Alimentarius Committee of 0.1 ng/person/day aflatoxin M_1 through milk for the Africa region. Weighted mean concentration of 0.05 µg aflatoxin M_1 in milk and a consumption of 0.25ng/kgBW have been associated with a prevalence of between 3.2 to 20 cancer cases/year/10^6 [13]. Therefore, even low levels of aflatoxin exposure may have negative health effects on consumers.

In conclusion, children in Kisumu County are potentially exposed to low and high levels of aflatoxin contamination through weaning diets from a variety of sources. Appropriate measures should be instituted to reduce exposure to aflatoxins, especially in young children who are highly vulnerable.

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Table 1: Descriptive statistics: Aflatoxin B_1/M_1 levels (Ppb) in market foods

Food Item	**Maximum**	**Median(IQR)**
Omena	2.76	0.6 (0, 2.08)
Rice	11.70	0.5(0,1.2)
Groundnuts	27.6	1.5(1.5,2.0)
Cassava	3.5	0.5(0.5,1.0)
Maize	34.50	0.5(0.5,1.0)
Sorghum	24.50	14.2 (8.5, 19)
Processed milk *	0.127	0.04 (0.03, 0.07)
Raw milk *	0.013	0.008 (0.005,0.01)

*AFM_1

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Table 2: Dietary exposure to aflatoxin (ng kg^{-} body weight/day) of a child 6 months (7.9 kg) of age with a daily consumption of 60g of solid food stuff and 500mls of milk per day in Kisumu County

Food Item	Median Aflatoxin levels (ng/g)	Aflatoxin intake (ng) from food*											
		Meal 1	Meal 2	Meal 3	Meal 4	Meal 5	Meal 6	Meal 7	Meal 8	Meal 9	Meal 10	Meal 11	Meal 12
Rice (g)	0.5					(30g) 18.00	(30g) 18.00	(30g) 18.00	(30g) 18.00		(30g) 18.00	(30g) 18.00	
Cassava (g)	0.5			(5g) 2.50	(5g) 2.50	(5g) 2.50	(5g) 2.50			(10g) 5.00			
Maize (g)	0.5	(60g) 30.0	(60g) 30.0	(35g) 17.50	(35g) 17.50	(15g) 7.50	(15g) 7.50	(20g) 10.0	(10g) 5.0		(30g) 15.0		
Sorghum (g)	14.2			(20g) 284.0	(20g) 284.0	(10g) 142.00	(10g) 142.00	(40) 568.00	(30g) 284.00	(50g) 710.00		(30g) 426.00	(60g) 852.00
Processed milk (ml)	0.04		(500ml) 20.00	(500ml) 20.00		(500ml) 20.00		(500ml) 20.00	(500mls) 20.00	(500mls) 20.00	(500ml) 20.00	(500ml) 20.00	(500ml) 20.00
Raw Milk (ml)	0.01	(500ml) 5.00			(500ml) 5.00		(500g) 5.00						
Total aflatoxin intake (ng)		35.00	50.00	324.00	309.00	190.00	175.00	598.00	327.00	735.00	53.00	464.00	872.00
Exposure to aflatoxin in ng/kg body weight/day**		4.33	6.33	41.01	39.11	24.05	22.15	75.70	41.39	93.04	6.71	58.73	110.38

*For individual foods the value indicated refers to the amount in the g of food consumed, indicated in the brackets above the aflatoxin level
** Exposure (ng kg^{-} body weight/day) = (Contamination level) (amount consumed)/ body weight [3].

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Table 3: A Comparison between Aflatoxin Levels and Stunting in Selected Counties in Kenya

County	Stunting (%) (FAO, 2013)	Highest reported aflatoxin Level & Source	
		Aflatoxin Level	Source
Nairobi	22.7	100 ppb	[12]
Kisumu	33.1	82 ppb	[25]
Homa Bay	37.0	1000 ppb	[10]
Makueni	33.5	46,400 ppb	[9]
Kitui	47.4	46,400 ppb	[9]
Machakos	31.3	160 ppb	[5]
Embu	23.7	21 ppb	[11]
Kakamega (Malava)	34.2	5000 ppb (Rotten maize) 1348 ppb (Clean maize)	[30]
Tongaren (Bungoma)	52.1	5000 ppb (Rotten maize) 1348 (Clean maize)	[30]
Kisii South	35.3	3442ppb	[11]

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