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Risk Assessment of Aflatoxins in Maize-Groundnuts Complementary Foods Consumed by Ghanaian Infants

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HIGHLIGHTS

- Aflatoxins levels of 18.8% of the complementary food samples were above the maximum permitted limit of 10 µg/kg.
- Infants fed on Weanimix from rural households and urban shops could have minimum aflatoxins daily exposure of 0.044 and 0.014 µg/kg bw/d, respectively.
- Increased effort by national regulators and all food producers is necessary to reduce aflatoxins in complementary foods.

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Acronyms and abbreviations
AF=Aflatoxin
MOE=Margin of exposure

ABSTRACT

Background: Complementary foods are given to infants when breast milk alone becomes insufficient in meeting their nutritional needs. The major objective of this study was to assess the prevalence of Aflatoxins (AFs) in Weanimix complementary foods purchased from shops in Accra (Ghana), and to estimate risk of liver cancer development in infants.
Methods: In total, 32 samples of Weanimix were purchased from shops in Accra, an urban centre and analyzed for AFs by Reverse-Phase High Performance Liquid Chromatography (RP-HPLC). Previously published data on levels of AFs in Weanimix prepared in rural households were also collected. The data was analyzed to estimate infants' daily exposure to AFs as well as the risk of liver cancer development.
Results: AFs levels of 18.8% of samples were above the maximum permitted limit of 10 µg/kg. The minimum and maximum levels of total AFs detected in all samples were 2.51 and 98.87 µg/kg, respectively with a mean value of 16.43 µg/kg. Exposure assessment showed that the minimum and maximum daily AFs exposures were 0.044 and 2.805 µg/kg bw/d, respectively for Weanimix from rural households; these rates for Weanimix purchased from urban shops were 0.014 and 0.55 µg/kg bw/d, respectively. The chances of liver cancer development would increase to 0.6 per year if infants were fed on Weanimix prepared in rural households with minimum AF level of 7.9 µg/kg.
Conclusion: The infants fed on Weanimix prepared in rural households would be at a higher risk of AF exposure and liver cancer development than infants fed on Weanimix purchased from urban shops of Ghana.

Introduction

Complementary foods are given to infants when breast milk alone becomes insufficient in meeting their nutritional needs. The period of complementary feeding usually ranges from 6-24 months (Koo et al., 2018; WHO,

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2002). It is a critical period because malnutrition could set in if infants are not adequately fed on the appropriate complementary foods. Child malnutrition is prevalent in Ghana where 19% of children are stunted and 11% are underweight (Ghana Statistical Service et al., 2015). It is estimated that 80% of children less than five years suffer from some forms of under nutrition resulting in conditions such as protein-energy malnutrition, underweight, stunting, and wasting (Ghana Profiles, 2011).

The main causes of malnutrition in Ghanaian infants and children are known to be the lack of nutrient-dense complementary foods and frequent infections. Hence in 1987, one of the interventions for reducing protein-energy malnutrition, underweight, and stunting was the development of Weanimix formulation by Ghana's Food Research Institute and Nutrition Division of the Ministry of Health with support from The United Nations Children's Fund (UNICEF).

Weanimix is a flour product prepared from a mixture of four parts cereal (e.g. maize, rice, and millet) and one-part legume (e.g. soybeans, groundnuts, and cowpeas). The formulation is complementary because the amino acids that are limited in cereals are abundant in legumes and vice versa. In Ghana, the promotion of Weanimix as a better source of protein and energy than many traditional weaning foods resulted in its increased production and availability at maternal and child clinics, shops, supermarkets, open markets, and other outlets. Mothers have also been trained to prepare Weanimix at home using simple processing techniques.

Aflatoxins (AFs) are naturally-occurring metabolites mainly produced by the fungi *Aspergillus flavus* and *A. parasiticus*. Four main types of AFs are known, that is, B_1, B_2, G_1, G_2 (Iqbal et al., 2019). However, AFB_1 is the most common and the most toxic mycotoxin. Some studies have shown that maize and groundnuts, which are staple crops in Ghana and constitute the main ingredients in Weanimix are highly susceptible to AF contamination under favourable conditions (Hell et al., 2003).

The effects of AFs on growth retardation, immune suppression, and reduction in vitamin A and zinc bioavailability have been previously revealed (Pimpukdee et al., 2004). A significant dose-response relationship between exposure to AFs, stunting, and underweight has been found in epidemiological studies conducted in West Africa (Gong et al., 2004; Turner et al., 2007). Another important health effect of AFs is their link with liver cancer. In 2012, about 745 000 deaths were estimated to have been caused mostly by AF-induced hepatocellular carcinoma (HCC) in the world (Ferlay et al., 2015). In the same year, a total of 782 451 new liver cancer cases and 745 533 related deaths were estimated to occur per year (Wong et al., 2017). In Ghana, World Health Organization (WHO) estimates that 1 923 people had liver cancer in 2014 and this increased to 2 753 in 2018. Liver cancer was found to be the commonest cancer among Ghanaian males representing 21.1% of all cancers (Laryea et al., 2014).

Ghana has set standards for AFs where maximum regulatory level for total AFs in raw cereal, pulses, and legumes is 20 µg/kg and it is 4 µg/kg for groundnut paste. To address AFs problem in Ghana, some interventions have been initiated such as the National Aflatoxin Sensitisation and Management (NASAM) project and the Aflatoxin Policy and Technical Regulation Development Project. An important aspect of these projects is the need to generate evidence of AF prevalence and their health effects to influence policy decisions and consumer behaviour.

Thus, the major objective of this study was to assess the prevalence of AFs in Weanimix complementary foods purchased from shops in Accra, which is an urban centre; and to estimate the risk of liver cancer development in 6-12 months old infants.

Materials and methods

Sampling

In February 2018, Weanimix complementary food samples were purchased from purposively selected popular supermarkets, shops, and open markets located in Adenta municipal, La Nkwantanan-Madina municipal and Ayawaso West municipal areas in the Greater Accra Region of Ghana. Packaged Weanimix, containing at least a combination of maize and groundnuts as ingredients, was identified in the shops by reading the list of ingredients information on the labels. The target products were purchased and prepared for AFs analysis. In total, 32 samples produced by 16 different processors were collected from 8 sales outlets.

AFs analysis

The method described by Stroka and Anklam (2002) was used in the determination of AFs in Weanimix samples. Test portions were extracted with a methanol:water (80:20 v/v) solution as solvent. The sample extract was filtered and 10 ml of filtrate diluted with Phosphate Buffered Saline (PBS) solution to a specified solvent concentration. This was applied to immunoaffinity column (R-Biopharm RHONE LTD EASI-EXTRACT®-AFLATOXIN) containing antibodies specific to AFs B_1, B_2, G_1, and G_2. AFs were removed from the immunoaffinity columns with neat methanol. AFs were quantified by Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) with post column derivatization involving bromination. The post column

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derivatization was achieved with pyrimidinum hydrobromide perbromide followed by fluorescence detection. The instrument system used for the HPLC analyses was from Agilent Technologies® (USA) infinity 1260 series which included an autosampler as well as a quaternary pump.

Approximately, 25 g of the test portion was weighed into a blender jar; then, 5 g of sodium chloride and 200 ml of methanol/water solvent were added. The mixture was then blended for 3 min with a high speed blender (Waring commercial blender, USA) and subsequently filtered through Whatman No.4 filter paper. Aliquots of 10 ml of the filtrate of each sample was pipetted into a beaker containing 60 ml of PBS, mixed with a plastic spatula/stirrer and applied onto an immunoaffinity column obtained from R-Biopharm. The filtrate was passed through the column at a flow rate of approximately 3 ml/min by gravity. Distilled water (15 ml) was applied in little portions of approximately 5 ml at a maximum flow rate of 5 ml/min and dried by passing air through the immunoaffinity column by means of a syringe for 10 seconds. AFs were eluted and quantified as described by Stroka and Anklam (2002). Limit of detection for AFB_1 and AFB_2 was 0.15 µg/kg and that for AFG_1 and AFG_2 was 0.13 µg/kg.

Risk assessment

Additional data for the assessment of risk associated with AFs in Weanimix was obtained from previously published studies. These were 1) total AFs levels in homemade Weanimix collected from rural households (Kumi et al., 2014); 2) breakfast cereal consumption data for infants in Ghana (USAID and GAIN, 2017); 3) mean standard weight for 6-12 months olds by sex (WHO, 2002); and 4) prevalence of hepatitis B ($HBsAg^+$) in Ghana (Ofori-Asenso and Agyeman, 2016).

The data was analyzed using (i) daily AF intake, which is the multiplication of AFs level in food and daily consumption of Weanimix, divided by the mean body weight. Thus, daily exposure of infants (6-12 months) to AFs was estimated based on the maximum, minimum, and mean levels of AFs detected in Weanimix, daily portion size of breakfast cereal for infants in Ghana, which was 0.046 kg/day (USAID and GAIN, 2017) and mean body weight for boys and girls (6-12 months), which were 8.8 and 8.2 kg, respectively; (ii) margin of exposure was calculated by dividing the Benchmark Dose Lower Confidence Limit, which is also known as $BMDL_{10}$ (µg/kg bw/day) by daily AF intake (EFSA, 2007); (iii) average Hepatocellular carcinoma (HCC) potency was estimated as [0.01 ($\%HBsAg^-$)+0.30 ($\%HBsAg^+$)], where $\%HBsAg^+$ represents proportion of populations with hepatitis B as well as $\%HBsAg^-$ represents proportion of population without hepatitis B (JECFA, 2001); (iv) population at risk involved estimating the cancer risk per 100 000 people and this is obtained by multiplying the daily AF exposure by the average HCC potency from individual potencies of $HBsAg^+$ and $HBsAg^-$ groups. In Ghana, the prevalence of $HBsAg^+$ was 12.3% (Ofori-Asenso and Agyeman, 2016) implying that the prevalence of $HBsAg^-$ was 100% minus 12.3% (Shephard, 2008); and (v) cancer risk was calculated for infants exposed to AFs in rural homemade Weanimix as well as Weanimix obtained from shops in an urban area. The data was also plotted using risk assessment in the 21^{st} Century (RISK21) visualization matrix tool (Embry et al., 2014). In the Risk 21 matrix tool, both exposure and toxicity (development of liver cancer) were evaluated.

Results

Totally, 18.8% of the samples had total AFs level above the Codex maximum limit of 10 µg/kg for processed foods. The minimum and maximum levels of total AFs detected in all samples were 2.51 and 98.87 µg/kg, respectively with a mean value of 16.43 µg/kg. All the samples that had total AF levels below 10 µg/kg had either Food and Drug Authority (FDA) registration number or GSA Certification Mark on the labels. Surprisingly, the sample with the highest level of AFs also had the GSA Certification Mark.

Table 1 showed the minimum and maximum daily AF intake in boys and girls if they were fed on Weanimix from rural households and urban shops. The minimum daily intake of AFs from Weanimix obtained from rural households was higher for girls than boys. Daily exposure to AFs from Weanimix obtained from urban shops was lower than exposure from Weanimix from rural households.

Two forms of risk characterization are presented in this study. The first is the margin of exposure (MOE) to AFs, which was estimated based on the Benchmark dose lower confidence limit ($BMDL_{10}$) for AFs, that is, 0.170 µg/kg bw/day. Table 2 shows the MOE based on sex of infants and source of Weanimix i.e. urban shops and rural households. The minimum and maximum MOEs for boys through consumption of Weanimix obtained from rural households were both lower than the values for Weanimix obtained from urban shops. At maximum AFs exposure, for example, MOE for girls who consumed Weanimix from rural households was 0.061 as compared to 0.31 for Weanimix from urban shops.

The results for the estimation of population of infants at risk of cancer development are shown in Table 3. The at risk population was expressed as cancers per year per 100 000 people per µg/kg bw/d of AFs intake.

Table 1: Infants' daily exposure to aflatoxins in Weanimix obtained from urban shops and rural households

Source of Weanimix	Daily exposure to aflatoxins (μg/kg bw/d)					
	Minimum		Maximum		Mean	
	Boys	*Girls*	*Boys*	*Girls*	*Boys*	*Girls*
Urban Shops	0.013	0.014	0.52	0.55	0.086	0.092
Rural Household (Kumi et al., 2014)	0.041	0.044	2.613	2.805	0.759	0.814

Table 2: Margin of Exposure estimation based on source of Weanimix, sex and level of aflatoxin exposure

Source of Weanimix	Margin of exposure to aflatoxins (μg/kg bw/d)					
	Minimum		Maximum		Mean	
	Boys	*Girls*	*Boys*	*Girls*	*Boys*	*Girls*
Urban Shops	13.08	12.14	0.33	0.31	1.98	1.85
Rural Household (Kumi et al., 2014)	4.146	3.864	0.065	0.061	0.224	0.209

Table 3: Proportion of 100 000 people in Ghana at risk of developing liver cancer due to the consumption of Weanimix contaminated with aflatoxins

Source of Weanimix	Cancers per year per 100 000 people per μg/kg bw/d of aflatoxin exposure					
	Minimum		Maximum		Mean	
	Boys	*Girls*	*Boys*	*Girls*	*Boys*	*Girls*
Urban Shops	0.00066	0.00064	0.02374	0.02512	0.0039	0.0042
Rural Household (Kumi et al., 2014)	0.00187	0.00201	0.11934	0.12810	0.0347	0.0372

Table 4 shows an extrapolation of the population at risk based on Ghana's total population of 29.46 million based on 2018 UN estimates. Risk of liver cancer development would be higher if infants were fed on Weanimix obtained from rural households compared to Weanimix from urban shops. For example, at the minimum level of AFs, infants would rarely develop liver cancer if they consumed Weanimix purchased from urban shops. However, the chances of liver cancer development increased to 0.6 if Weanimix from rural households was consumed.

Figure 1 shows a plot for infants' exposure to AFs and subsequent toxicity (liver cancer development) due to consumption of Weanimix obtained from urban shops and rural households. AF risk is the intersection of toxicity and exposure. The closer the point of intersection to the x-axis and the farther it is from the y-axis, the higher the toxicity and hence the higher the risk of cancer development.

Table 4: Proportion of Ghana's total population at risk of developing liver cancer due to the consumption of Weanimix contaminated with aflatoxins

Source of Weanimix	Cancers per year per Ghana's population per μg/kg bw/d of aflatoxin exposure					
	Minimum		Maximum		Mean	
	Boys	*Girls*	*Boys*	*Girls*	*Boys*	*Girls*
Urban Shops	0.19	0.19	6.99	7.4	1.14	1.2
Rural Household (Kumi et al., 2014)	0.55	0.59	35	37.7	10.2	10.96

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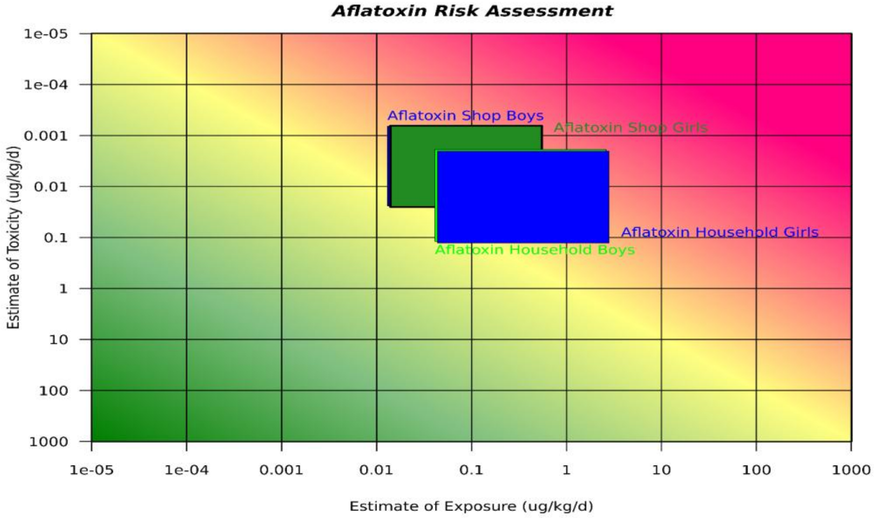

Figure 1: Risk 21 matrix showing relationship between aflatoxin exposure and risk of liver cancer development if infant boys and girls consumed Weanimix from obtained from rural households and urban shops.

Discussion

This study showed that Weanimix infant complementary foods prepared from maize and groundnuts are potential sources of AFs. About 63% of the tested samples had total AFs levels below 10 µg/kg, which is an indication that most of the commercial processors whose products we sampled are making efforts to comply with the Codex maximum limits for AFs in processed foods. The result obtained from the current investigation is similar to findings of Blankson et al. (2019) who showed that 4% of the breakfast cereals analyzed in Ghana had no detectable levels of AFs. Differently, Kumi et al. (2014) revealed that all the 36 (i.e. 100%) homemade Weanimix samples in rural Ghana had AFs ranging from 7.9 to 500 µg/kg. The findings imply that it is possible to substantially reduce AFs to safer levels if appropriate control measures are put in place. Further enquiries are needed to determine the best practices that commercial processors have in place for the prevention and control of AFs in Weanimix.

According to this study, 81.2% of Weanimix samples had level of AFB_1 ranging from 2.51 to 80.09 µg/kg. A similar research indicated that 71% of infant cereal products purchased from shops in Accra, Ghana had AFB_1 ranging from 0.18-36.1 µg/kg (Blankson and Mills-Robertson, 2016). Also, AFs levels ranging from 19 to 70 µg/kg were detected in cereal-based foods in Libya (Aidoo et al., 2011). Comparing the findings of the current study and some previous ones, it can be stated that varying proportions of the cereal-based products analyzed had AFs above the Codex maximum limit of 10 µg/kg. This is a major cause for concern considering the fact that the products tested were obtained from shops that are expected to be selling food products certified as safe. It calls for increased enforcement of AFs standards through monitoring and post market surveillance by the Food and Drugs Authority and Ghana Standards Authority to ensure that certified products on markets are safe.

The differences in the maximum levels of AFs detected in some previously mentioned studies could be due to the AF management practices applied as well as the combination of ingredients used in the preparation of the products. For example, it is possible that a product that contained maize and groundnuts (as in Weanimix), which are both highly susceptible to AFs contamination could have higher levels of the toxins than a product made from either maize or groundnuts alone (Adetunji et al., 2014; Bediako et al., 2019; Gruber-Dorninger et al., 2018; Hell et al., 2003). The extremely high level of AFs detected by Kumi et al. (2014) in the homemade Weanimix in Ghana could be as a result of limited public knowledge of AF control strategies as compared to commercial producers who are required to comply with AFs standards before their products could be certified by regulatory agencies or even be marketed by certain supermarkets. The higher levels of AFs in homemade Weanimix may be also related the relatively low public concern about AFs compared to other food safety issues such as unhygienic food environments, bacterial contamination, excessive use of food additives, high pesticide residues, and harmful adulterants (Omari et al., 2018).

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This study also assessed the level of infants' exposure to AFs if they consumed Weanimix purchased from shops in an urban centre and Weanimix prepared in rural households. The exposure of infants to AFs through the consumption of Weanimix purchased from shops ranged from 0.014 to 0.55 µg/kg bw/d. This is similar to infants' daily exposure of 0.011 to 0.852 µg/kg bw/d found in processed cereal-based complementary foods in Ghana (Blankson and Mills-Robertson, 2016). Kumi et al. (2014) found that the daily exposure to AFs from consumption of rural homemade Weanimix, ranged from 0.044 to 2.805 µg/kg bw/d, which was higher than the ranges found in the present work and also study of Blankson and Mills-Robertson (2016).

We found that the mean daily exposure to AFs was 0.086 and 0.092 µg/kg bw/d in boys and girls, respectively. These findings are in line with the mean daily exposure of 0.146 µg/kg bw/d reported by Blankson and Mills-Robertson (2016). However, our result was lower than the mean daily exposure of 0.899 for infants who consumed cereal-based products in Accra, Ghana (Kortei et al., 2019). It was reported that mean daily exposure to AFs was 0.786 µg/kg bw/d for children who consumed maize-based complementary foods in Tanzania (Kimanya et al., 2014). Also, a mean exposure to AFs of 1.909 µg/kg bw/d was obtained for infants in Nigeria (Adetunji et al., 2017).

According to EFSA (2007), MOE of 10 000 or more indicates situations of low public health concern. Hence, in this study, all the MOEs were far below 10 000 suggesting that AF exposure in infants through the consumption of Weanimix obtained from both urban shops and rural households pose a high public health risk. However, the risk is higher if infants were fed on Weanimix obtained from rural households than that from urban shops. As an inference, the smaller the MOE, the larger the potential risk posed by dietary AF exposure. The risk 21 matrix also depicts that infants fed on Weanimix prepared in rural households were at a higher risk of daily exposure to AFs and higher toxicity as depicted by the blue rectangle compared to infants who ate Weanimix from urban shops as seen in the deep green rectangle. This demonstrates an increased risk of AF exposure from homemade Weanimix. Even at the minimum total AFs level of 7.9 µg/kg as found in Weanimix from rural household, at least one person is at risk of developing liver cancer in Ghana.

Our findings call for the need to review downward the current AF standards for processed foods, which is largely based on Codex standard of 10 µg/kg, because exposure is directly proportional to the frequency and amount of contaminated products consumed. It is even more important because cereal and legume (especially maize and groundnuts) products are staple foods that are readily available and consumed frequently by all age groups in Ghana. It will benefit infants and children below 12 years who are more vulnerable to the effects of AFs because of their immature immune systems and high intake of foods and water per kg body weight (Lombard, 2014). Furthermore, risk factors for liver cancer such as hepatitis B, HIV, and tuberculosis are prevalent in Ghana hence, it is important to keep AFs in Weanimix and other staple foods below regulatory limits so as to protect public health. Ghana is not the first country to do this downward review of AF standards because already in Europe, the maximum AF limit for cereals designated for infant foods has been set below 0.025 µg/kg (International Food Policy Research Institute, 2018).

Whereas some commercial processors are producing Weanimix that meet Ghana and Codex standards, others are producing and marketing Weanimix contaminated with unacceptable levels of AFs. Food processors have a great responsibility in ensuring the safety of their products; however they require training on best practices for AF management as well as regular monitoring and inspection by regulatory agencies. Prevention or reduction of AF contamination can be achieved by the application of measures such as good agricultural practices like planting healthy seeds, pest management, soil management, timely harvesting, irrigation, etc. (Cleveland et al., 2003; Munkvold, 2003).

Conclusion

AFs were found at varying concentrations in Weanimix purchased from urban centre shops as well as that produced by individuals in rural households in Ghana. The contamination rate of AFs was higher in Weanimix obtained from rural households. Exposure assessment and risk characterization suggest that infants fed on Weanimix prepared in rural households would be at a higher risk of AF exposure and liver cancer development than infants fed on Weanimix purchased from urban shops. Increased effort by official regulators and all food producers is necessary to reduce AFs to levels below the limits of detection. Further studies are however, required to examine the best practices that the complying processors are currently using for the prevention and control of AFs contamination in Ghana.

Author contributions

R.O. and G.A. designed the study and collected food samples for analysis; G.A. conducted laboratory analysis; R.O. analyzed the data and wrote the manuscript. Both authors read, revised, and approved the final manuscript.

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Conflicts of interest

The authors declared that there is no conflict of interest in the study.

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