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ABSTRACT:

Aflatoxins are secondary metabolites produced by the fungi Aspergillus flavus and A. parasiticus that grow on a variety of agricultural commodities and other foodstuffs. Aflatoxin B1 (AFB1), which is a highly toxic, hepatotoxic, mutagenic, teratogenic, carcinogenic and immunosuppressive compound, is the most toxic of all the aflatoxins produced by these fungi. Contamination of foodstuffs by AFB1 is a major food safety concern in developing countries in the tropics, because of its adverse effect on human and animal health; it can also negatively affect the exportation of foodstuffs, such as peanuts and grains to most developed countries. The levels of AFB1 in 204 food samples purchased from markets in Papua New Guinea (PNG) were determined using solid phase direct competitive enzyme immune-assay (EIA) kits. The results were analyzed according to the legal permissible limits for AFB1 in foods, recommended for PNG, Australia, Food & Drug Administration (FDA), European Union and the Codex Alimentarius. The mean AFB1 level in the 204 food samples was 2.80ppb and the Range was 0.0 – 29.30ppb. AFB1 was not detected in 21.6% of all the food samples. The AFB1 levels in 90.7% and 94.1% of the food samples were below the 10.0ppb and 15.0ppb legal permissible limits for PNG and Australia respectively. The dried roasted peanut group had the highest mean AFB1 level (6.1ppb), followed by the peanut butter (3.8ppb) and maize (3.3ppb) groups. There is need to advocate for continuous monitoring of AFB1 levels in foodstuffs in PNG.

Key words: Aflatoxin B1, food contaminants, Papua New Guinea

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INTRODUCTION:

Mycotoxins are secondary toxic metabolites produced by various fungi [1]. Aflatoxins are the most widely occurring mycotoxins produced by the fungi Aspergillus species [1 – 3]. Under favourable conditions of relative humidity, temperature and moisture content, Aspergillus flavus and A. Parasiticus can grow on a variety of agricultural crops, other foodstuffs and animal feeds [1 – 6]. This can occur before harvest, during post-harvest drying and storage or during food preparation [1, 3 – 8]. Aflatoxin B1 (AFB1), which is a highly toxic, hepatotoxic, mutagenic, teratogenic, carcinogenic and immunosuppressive compound, is the most toxic of all the aflatoxins produced by these fungi [1 – 8]. AFB1 contamination of peanuts, maize, cottonseeds, variety of grains, rice, cereals, dried fruits, wheat, chillies, cassava, and other foodstuffs have been reported in some tropical and subtropical regions of the world [6 – 12].

The Food and Agricultural Organization (FAO) and the Codex Alimentarius Commission proposed the legal permissible limit of 15.0ppb for AFB1 in foods for human consumption worldwide [13, 14]. However, a number of countries and organizations have set their own legal permissible limits [14]. Consumption of AFB1 contaminated foodstuffs is more common in developing countries with poorly implemented food safety regulations and limited consumer awareness of food safety [6, 9 – 12]. This is in contrast to the strict implementation of regulatory standards aimed at reducing the importation of AFB1 contaminated foods into the developed countries [6 – 8].

The limited published data on the prevalence of AFB1 contamination of food samples in Papua New Guinea (PNG) has been contradictory [9 – 12]. In 1972 AFB1 contamination was reported in 80% of food samples from East New Britain (ENB) and Markham Valley [9]. In 1975 AFB1 contamination was not detected in food samples purchased in Koki market in the National Capital District (NCD) of PNG [9, 10]. In 1991 four brands of peanut butter from the Markham Valley in PNG were highly contaminated with AFB1 ranging from 31.0 – 135.0ppb [11]. In surveys carried out in 1996, 2002 the PNG National Agricultural Research Institute (NARI) tested food samples, especially peanuts, purchased from markets in Sepik, Western Highlands, Morobe, ENB and NCD [9, 10]. According to these authors, AFB1 contamination ranging from 5.0 to 20.0ppb was reported in 35.0% of the food samples. The National Agricultural Research Institute (NARI) in PNG conducted a three years (2003 to 2005) bimonthly assessment of AFB1 levels in peanuts sold in markets and other outlets in NCD, Eastern Highlands, Morobe, and ENB provinces [15]. According to the authors the yearly mean results indicated that the AFB1

level in about 12.0% of peanut samples were greater than 10.0ppb, with a small percentage of highly contaminated samples [15].

Since 2005 no published data is available on the level of AFB1 in foodstuffs sold in PNG. The major aim of the present study was to assess the prevalence of AFB1 contamination in some foodstuffs sold in Papua New Guinea.

METHODOLOGY:

A total of 204 food samples were purchased from markets, trade-stores, supermarkets, street vendors and other food outlets in Port Moresby & NCD (Port Moresby-NCD), Lae in Morobe Province and Wewak in East Sepik Province. All the food samples were collected between June 2011 and March 2012. A high speed electronic food processor was used to ground each food sample into fine powder, which was then passed through a sieve to obtain the finely grounded particles. The fine powder was then collected and stored in a plastic zip-lock bag in the refrigerator at 4°C until required for extraction.

The extraction was carried out in a fume cupboard in dim light. Two grams of the fine powder was extracted with 10ml of 70.0% methanol; the extract was filtered through Whatman # 1 filter paper. The filtrate obtained was used for analysis of AFB1 [16]. The commercial Helica Aflatoxin B1 solid phase direct competitive enzyme immune-assay (EIA 96 Microwell plates) kit was used to determine the AFB1 level in each filtrate [16]. All reagents used were of analytical grade. Assay procedures for AFB1 in filtrate, standards and quality control samples were carried out as indicated in the instructional protocol of the manufacturer [16]. Analysis was carried out in the Micronutrient Laboratory, Division of Basic Medical Sciences, School of Medicine and Health Sciences, University of Papua New Guinea.

The results obtained in this study were analyzed using the various legal lower permissible limits of AFB1 in foods recommended in various countries as follows: Papua New Guinea AFB1 below 10.0ppb [15]; European Union (EU) AFB1 below 2.0ppb; Australia AFB1 below 15.0ppb; Food and Drug Administration (FDA) Unites States of America AFB1 below 20.0ppb; foodstuffs with AFB1 greater than or equals to 20.0ppb are considered as highly contaminated [14].

RESULTS:

The 204 food samples were assayed in duplicate for AFB1 levels and the mean value for each sample was calculated. Of the 204 food samples 184 (90.2%) samples were from Port Moresby-NCD, 14 (6.9%) were from Lae in Morobe province and 6 (2.9%) were from Wewak in East Sepik province. All the samples from Lae and Wewak were peanuts. The mean and range AFB1 levels for the 184 samples from Port Moresby-NCD were 2.7ppb and 0.0

to 29.3ppb respectively. The mean AFB1 for the 14 peanut samples from Lae city was 3.2ppb and range was 0.3 – 22.6ppb. For the 6 samples from Wewak the mean AFB1 was 0.5ppb and range was 0.2 to 0.6ppb. There was no statistically significant difference between the AFB1 levels in the Port Moresby-NCD samples and the Lae samples. The levels of AFB1 in 166 (90.2%), 13 (92.9%) and 6 (100%) of the food samples from Port Moresby-NCD, Lae and Wewak were below 10.0ppb. The three sets of data from Port Moresby-NCD, Lae and Wewak were pooled together for further analysis.

The mean and median AFB1 levels for the 204 food samples were 2.80ppb and 0.84ppb respectively. The Range was 0.0 – 29.30ppb and the Interquartile Range was 0.18 – 2.06ppb. Table 1 shows the percent distribution of all the food samples according to the legal lower permissible limits for AFB1 levels in foods by country.

Table 1: Percent distribution of all the food samples (n = 204) according to the legal lower permissible limits for AFB1 levels in foods by country

Legal lower permissible limits for AFB1 in foods		**Percent (n) of foodstuffs**
Not detected (ND)	0.0ppb	21.6 (44)
European Union (EU)	< 2.0ppb	74.0 (151)
Papua New Guinea (PNG)	< 10.0ppb	90.7 (185)
Australia (AU)	< 15.0ppb	94.1 (192)
Food & Drug Administration (FDA) USA	< 20.0ppb	96.6 (197)
Highly Contaminated (HC)	≥20.0ppb	3.4 (7)

(NB: ppb = part per billion; 1.0ppb = 1.0pg/mg, or 1.0ug/kg)

AFB1 was not detected in 21.6% of all the food samples. The AFB1 levels in 90.7% (185) of all the food samples were below the 10.0ppb legal lower permissible limits for PNG. A total of 94.1% (192) of all the food samples were below the 15.0ppb legal lower permissible limit for Australia. According to the FDA legal lower permissible limits 3.4% (7) of all the food samples were highly contaminate with AFB1.

For further interpretation of the results the food samples were separated into ten food groups

shown in Table 2. The mean, range and median values of AFB1 in the various food groups are presented in Table 2. The dried roasted peanut group has the highest mean AFB1 level (6.1ppb), followed by the mean AFB1 levels in the peanut butter (3.8ppb) and maize (3.3ppb) groups.

The range for AFB1 was highest in the dried roasted peanut group (0.0 – 29.3ppb), followed by the maize group (0.0 – 19.7ppb) and the peanut butter group (0.0 – 16.7ppb).

The percent distribution of the AFB1 levels, in food samples in the ten food groups, according to the legal lower permissible limits for AFB1 in foods is presented in Table 3. In the dried roasted peanut group the AFB1 levels in 67.4% of the peanuts were below 2.0ppb, 76.1% were below 10.0ppb and 80.4% were below 15.0ppb. A total of 15.2% of the peanut samples were highly contaminated. For the peanut butter group, the AFB1 levels in 53.8% of the samples were below 2.0ppb and 92.3% were below 10.0ppb and 15.0ppb. None of the peanut butter samples were highly contaminated with AFB1. In the maize group the AFB1 levels in 45.8% of the samples were below 2.0ppb and 91.7% were below 10.0ppb and 15.0ppb.

Table. 2: Mean, range and median values of AFB1 levels in the ten food groups

Food Groups	Mean (ppb)	Range (ppb)	Median (ppb)
Peanut butter (n = 13)	3.8	0.0 – 16.7	1.9
Dried roasted Peanuts (n = 46)	6.1	0.0 – 29.3	1.5
Seeds & Beans (n = 18)	2.0	0.0 – 10.6	0.7
Maize (n = 24)	3.3	0.0 – 19.7	2.0
Flours (n = 10)	0.4	0.0 – 0.9	0.3
Rice (n = 12)	0.5	0.0 – 1.7	0.5
Bread & Biscuits (n = 24)	0.8	0.0 – 8.6	0.1
Cereals & Oats (n = 16)	1.1	0.1 – 4.1	1.0
Sago & Starches (n = 13)	0.5	0.0 – 1.6	0.3
Other foodstuffs (n = 28)	3.0	0.0 – 12.8	1.2

Table 3: Percent distribution of the AFB1 levels in the ten food groups according to the legal lower permissible limits for AFB1 in foods by country

		Food Groups**									
Legal lower permissible limits for AFB1 in foods		Peanut butters (n =13)	Dried roasted peanuts (n = 46)	Seeds & Beans (n=18)	Maize (n=24)	Flours (n=10)	Rice (n=12)	Bread & Biscuits (n=24)	Cereals & Oats (n=16)	Sago & Starches (n=13)	Other foodstuffs (n=28)
Not detected	0.0ppb	38.5% (5)	6.5% (3)	16.7% (3)	12.5% (3)	50.0% (5)	41.7% (5)	50.0% (12)	0.0	30.8% (4)	13.3% (4)
European Union	<2.0ppb	53.8% (7)	67.4% (31)	72.2% (13)	45.8% (11)	100% (10)	100% (12)	95.8% (23)	93.8% (15)	100% (13)	57.1% (16)
PNG	<10.0ppb	92.3% (12)	76.1% (35)	88.9% (16)	91.7% (22)	100% (10)	100% (12)	100% (24)	100% (16)	100% (13)	89.3% (25)
Australia	<15.0ppb	92.3% (12)	80.4% (37)	100% (18)	91.7% (22)	100% (10)	100% (12)	100% (24)	100% (16)	100% (13)	100% (28)
FDA USA	<20.0ppb	100% (13)	84.8% (39)	100% (18)	100% (24)	100% (10)	100% (12)	100% (24)	100% (16)	100% (13)	100% (28)
HC*	≥20.0ppb	0	15.2% (7)	0	0	0	0	0	0	0	0

*HC = highly contaminated; **All values are cumulative, thus do not add up to 100%

DISCUSSION:

In the present study the solid phase direct competitive enzyme immune-assay (EIA 96 Microwell plates) technique was used to determine the AFB1 level in the filtrates. This technique is more specific for AFB1 and has a higher sensitivity and precision compared to the Thin Layer Chromatography (TLC) and the High Performance Liquid Chromatography (HPLC) [14, 16].

The mean AFB1 levels in food samples from Port Moresby-NCD, Lae and Wewak were below the 10.0ppb legal lower permissible limits for PNG. These values were lower than the mean AFB1 levels reported for foodstuffs in Nigeria [8], Ghana [8, 17] and Turkey [18]. The 9.8% and 7.1% of foodstuffs from Port Moresby-NCD and Lae respectively with AFB1 levels greater than 10.0ppb were lower than the 12.0% AFB1 contaminated foodstuffs reported for PNG [15].

Although AFB1 was not detected in 21.6% of the 204 food samples, a total of 26.0% had AFB1 levels greater than the 2.0ppb legal lower permissible limit for the European Union. This should be of concern to program planners wising to establish trade relationship with countries in the European Union.

The 6.1ppb mean AFB1 level obtained for the dried roasted peanut group in the present study was higher than the mean AFB1 values reported for peanuts in NCD [11], but lower than the values reported for peanuts sold in most cities in Nigeria, Ghana and Cameroon [8, 12, 17]. The AFB1 range (0.0 – 29.3ppb) obtained for the dried peanut group was also lower than the range reported for AFB1 in peanuts sold in Nigeria [8, 17], Botswana, India and Argentina [8]. For the peanut butter group the mean AFB1 level (3.8ppb) was similar to values reported peanut butter samples in NCD [15]. The range (0.0 – 16.7ppb) for the peanut butter group was lower than the values reported for Bangladesh [8], Nigeria [8, 17] and India [8]. In the present study AFB1 was detected in all the food groups in PNG, which contradicts sections of the reports by others [10, 11]. One of the reasons for this discrepancy might be due to the difference in methodology. The EIA method for assay of AFB1 is a faster and more advanced technique than the TLC and HPLC methods.

The 23.9% of peanut samples with AFB1 levels greater than 10.0ppb in the dried roasted peanut group obtained in this study was higher than the 11.0% and slightly higher than the 22.0% peanut samples reported in the NARI survey in 2005 [15]. This high level of AFB1 contamination of peanuts should be of concern

to program planners in the health and agricultural sectors in PNG. Peanut is one of five major cash crops cultivated by small and medium scale farmers in PNG. It is also a major component of the diet consumed in both rural and urban households. Regular consumption of small amounts of AFB1 contaminated foodstuffs can lead to liver disease and other Aflatoxin related disorders [4, 5]. Thus, the urgent need for intensive nutrition education, food safety information and awareness campaigns to advocate for proper implementation of recommended guidelines to reduce the infestation of peanuts by fungi.

In order to effectively address these issues, between 2003 and 2005, the NARI in collaboration with stakeholders implemented the "Aflatoxin Contamination and Public Awareness Program on Better Handling Practices", a project that was funded by PNG Agricultural Innovations Grant Facility (AIGF) [15]. The outcome of this project (AIGF 1035) apart from the bimonthly collection of peanut samples for analysis of AFB1, included production of advocacy materials and newsletters in the local PNG language [19, 20]. The long term impact of this project on the small scale peanut farmers has not been fully assessed because the project was not accompanied by effective monitoring [15].

CONCLUSION:

In summary, our data indicates that the AFB1 level was greater than 10.0ppb in 23.9% of the peanuts in the dried roasted peanut group. All the highly contaminated food samples were in the dried roasted peanut group. These findings strongly suggest the urgent need for implementation of well structured projects similar to AIGF-1035 across PNG. The projects should also include nutrition education for farmers and consumers, and efficient, sustainable, monitoring systems.

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