Exposure assessment of aflatoxins in Thai peanut consumption

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Abstract: Aflatoxins (AFs) and aflatoxin B1 (AFB1) intake through consumption of peanuts and peanut products were estimated from 60 samples. The samples were collected from 2 retail and 2 wholesale markets in Bangkok during November 2013–January 2014. The results showed that 80% of raw peanut samples were contaminated with AFs while 100% of roasted and ground peanuts samples were contaminated. The highest concentration of AFs was found in ground peanuts at 362.48 ng g⁻¹ resulting in the highest mean concentration (68.22 ng g⁻¹). According to a food consumption database, the average intake of AFs was estimated at 0.49, 0.40 and 2.13 ng/kg bw/day for raw, roasted and ground peanuts, respectively. The potential risk for cancer was estimated at 0.01–0.12 cancer/year/100,000 persons. The results suggest that the current situation of aflatoxins contamination in peanuts and peanut products (especially in ground peanuts) has an adverse effect on the health of the Thai population.

Subjects: Agriculture and Food; Food Laws & Regulations; Food Safety Management

Keywords: risk assessment; aflatoxins; peanuts; aflatoxins intake; exposure assessment

1. Introduction
Various foodstuffs such as corn, wheat, rice, spices and ground nuts are often found to be contaminated with aflatoxins (AFs). The best-known common source of AFs contamination is in peanuts and peanut products. Unfortunately, peanuts are a popular ingredient in Thai cuisine including Pad Thai, green papaya salad (Som tum), spicy noodle (Tom Yum noodle soup), etc. AFs are secondary metabolites, difuranocoumarins, produced by Aspergillus flavus and A. parasiticus, commonly found in food and feeds and have been associated with various diseases such as aflatoxicosis, in livestock, domestic animals and humans throughout the world (Ashiq, 2015). The common forms of AFs (B1, B2, G1, G2) have been classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC). DNA-adducts are produced through the pathway of humans after AFs are ingested.

ABOUT THE AUTHORS
Our group conduct a research about mycotoxins including mycotoxin-producing fungi and mycotoxin determination such as aflatoxin, zearalenone and ochratoxin in food and feed.

PUBLIC INTEREST STATEMENT
Peanuts are often found to be contaminated with aflatoxins. Unfortunately, peanuts are a popular ingredient in Thai cuisine including Pad Thai, green papaya salad (Som tum), spicy noodle (Tom Yum noodle soup), etc. This article assesses the risk for cancer of peanut consumption by determines content of aflatoxins in peanuts sold in Bangkok markets. Thai peanuts are safe for general consumer. But high peanut consumption group should be aware. According to low amount of peanut in Thai dish, oversea travelers are safety to consume them.
Thus, gene mutation and malfunction of genes has occurred in humans and animals (International Agency for Research on Cancer [IARC], 1999).

The warm and humid weather in ASEAN countries including Thailand, means that AFs often contaminate various agricultural products including peanuts, corn, spices and other dry food (Leong, Rosma, Latiff, & Ahmad, 2011). Thus, this toxin is of great concern in Thailand. In the past, the Thai Food and Drug Administration has limited AFs content in food, but Thai peanuts have been reported to be contaminated at higher than regulation levels with AFs ranging from 4 to 576 ng g⁻¹ (average of 102 ng g⁻¹) (Lipigorngoson, Limtrakul, Suttajit, & Yoshizawa, 2003) and 82–92% of peanut samples were higher than the limit (Pichai, 2003). This hazard issue is a health concern for Thai consumers. Such levels of contamination result in a higher AFs content in common dishes such as Tom Yum noodle soup (0.01–17.3 ng g⁻¹), compared to the normal style (0.01–0.2 ng g⁻¹) (Promhirangul, 1999). Due to the heat resistance of AFs, normal cooking methods have a relatively low effect on the content of AFs, with most remaining in the cooked dishes. Thus, in order to control the AFs content in peanuts as the main ingredient in many Thai dishes and as a material for food processing, good agricultural practices must be applied to control the quality at harvest. The Thailand National Bureau of Agricultural Commodity and Food (ACFS) announced the maximum level of aflatoxins in peanuts kernel in 2014 (TAS 4702-2014), which followed a voluntary announcement since 2009, to reduce the content of AFs in peanuts. The aim of this study was to investigate the AFs content in peanuts sold in Bangkok markets and to determine the exposure to AFs from peanut consumption to support the implementation of prevention and control procedures for AFs in peanuts through voluntary action. In addition, the study compared the efficiency of the immunoassay column (IAC) IAC KU-AF02, which was developed for use as in-house method for the laboratory at Kasetsart University, with the standard IAC to clean-up AFs in peanuts samples.

2. Material and methods

2.1. Samples

In total, 60 samples of raw, roasted and ground peanuts were purchased from Bangkok, Thailand and its vicinity (Khlong Toei, Bang Kapi, Song wat and Talaad Thai) during November 2013–January 2014. Song wat and Talaad Thai are large wholesale markets, particularly for agricultural products. Bang Kapi and Khlong Toei are also large fresh markets for general consumers. Five retail shops in each market were randomly chosen and peanuts (1–2 kg each sample) were purchased at each shop. Samples were sub-sampled to 25 g according to AOAC method 977.16.1977 (AOAC International, 2000). The test portions were stored at −20°C before aflatoxin analysis which was carried out within a week after sub-sampling.

2.2. Aflatoxins determination

The analytical method followed AOAC method 991.31–1994 (AOAC International, 2002). Briefly, 25 g of fine ground samples were extracted with 125 ml of 70% methanol and 5 g of NaCl. The extract was filtered, diluted with distilled water (15:30 ml), and filtered through a glass microfiber filter. Fifteen milliliters was passed through the IAC (AflaTest column, Vicam, MA, USA) at 1–2 drops per second. After the extract passed through the column, 10 ml of deionized water (DI water) was passed twice. Finally, aflatoxins in the IAC were eluted with 1 ml methanol and further analyzed using High Performance Liquid Chromatography (HPLC). For the in-house method, the AflaTest column was replaced by the KU-AF02 IAC, the same portion of extract was passed through following the same procedure.

2.3. HPLC analysis

Aflatoxins were analyzed using an HPLC-fluorescence detector (Water Corporation, USA). A symmetry C18 column (5 μm, 4.6 × 150 mm) with C18 guard column (Water Corporation, USA) was used to separate the solution. Photochemical derivatization (PHRED, AURA Industries, USA) was used to enhance the ability of the fluorescence detector to detect AFB₁ and AFG₁. The column was equilibrated with water:acetonitrile:methanol [60:20:20 (v/v)] which was the mobile phase. A flow rate of 1.0 ml/min
was used and the column oven temperature was 35°C. The fluorescence detector was set to excitation and emission wavelengths of 365 and 445 nm, respectively, adapted from AOAC International (2002).

2.4. Efficiency comparison between AflaTest and KU-AF02 IAC
Statistical analysis involved correlation analysis (SPSS statistics software, USA), a t-test (SPSS statistics software, USA) and Bland–Altman plots (Sigma plot, USA) to compare the results from the AflaTest and KU-AF02 IAC. A Bland–Altman plot (Bland & Altman, 1999) was used to compare the efficiency of, and agreement between, the in-house IAC and the AflaTest IAC to clean-up the sample. The x-axis shows the mean content of the two methods, whereas the y-axis is the mean difference of each paired sample, including the limits of agreement. The limit of agreement was defined as the mean difference plus and minus 1.96 times the standard deviation (SD) of the differences.

2.5. Aflatoxins exposure assessment
The intake of AFs was estimated using AFs contamination in peanuts from the previous results. Intakes of raw, roasted and ground peanuts were calculated separately. The peanut consumption data were obtained from the National Bureau of Agricultural Commodity and Food Standards (National Bureau of Agricultural Commodity & Food Standards [ACFS], 2006). The AFB$_1$ intake was calculated using the following equation:

$$\text{Exposure (ng/kg bw/day)} = \frac{\text{Concentration (ng g}^{-1}\text{) x consumption (g day}^{-1}\text{)}}{\text{Body weight (kg)}}$$

After the intake of AFB$_1$ and AFs had been calculated, the estimated potency of liver cancer was determined using the model in Joint FAO/WHO Expert Committee on Food Additives [JECFA] (1998). According to Thai Liver Foundation data, 5% of the Thai population is infected with Hepatitis B (Ott, Stevens, Groeger, & Wiersma, 2012). Thus the potency of liver cancer in the Thai population can be estimated using the following equation:

$$\text{Average potency (cancers/year/100,000 people)} = (0.01 \times 95\%) + (0.30 \times 5\%) \times \text{AFB}_1 \text{ or AFs intake (ng/kg bw/day)}$$

3. Results and discussion

3.1. Aflatoxins contamination in peanuts
The results of AFB$_1$ and AFs contamination in peanuts are shown in Table 1. AFB$_1$ was detected in 55 samples while 56 samples were contaminated with AFs. Raw peanuts had the lowest frequency of AFB$_1$ and AFs contamination (75 and 80%, respectively) but the lowest mean concentrations of AFB$_1$ and AFs were in roasted peanuts (14.42 and 17.99, respectively), although a high frequency (100%) of AFB$_1$ and AFs in these peanuts was observed. AFG$_1$ and AFG$_2$ were not detected in roasted peanuts.

Fifteen out of 20 raw peanut samples were contaminated with AFB$_1$, ranging from not detected to 267.31 ng g$^{-1}$ with an average of 42.26 ng g$^{-1}$ (Table 2). Although the frequency of contamination in Thai raw peanuts (80% positive samples) in this survey was higher than in previous studies on raw peanuts in China, the concentration was lower than in China where contamination was from 0.01 to 720 ng g$^{-1}$ (25% positive samples) (Ding, Li, Bai, & Zhou, 2012) and 0.06 to 1390 ng g$^{-1}$ (15% of positive samples) (Wu et al., 2016). A higher quality of peanuts was found in developed countries such in Catalonia, Spain, where 8 samples out of 72 samples were contaminated with AFs with the highest concentration being 7.7 ng g$^{-1}$ (mean 2.7 ± 3.0 ng g$^{-1}$) (Cano-Sancho, Sanchis, Marin, & Ramos, 2013). Although the peanuts from Spain were high quality in terms of AFs contamination, pistachios had high levels of AFs contamination (mean $8.9 \pm 28.7$ ng g$^{-1}$ and maximum $108.3$ ng g$^{-1}$). These data indicated that limiting the AFs content is a promising way to achieve low levels of AFs contamination and to monitor the wholesomeness of food for consumer protection. Although the peanuts and peanuts products might not meet the different national regulations, the contamination level will be limited.
Thai roasted peanuts had a high occurrence of AFs contamination but a low concentration of AFs when compared with peanuts products from Brazil. The occurrence of AFs contamination in peanut products in Brazil was between 35 and 50% with the concentration being 0.05–113 ng g⁻¹ (Jager, Tedesco, Souto, & Oliveira, 2013; Magrine et al., 2011; Oliveira, Gonçalves, Rosim, & Fernandes, 2009). The highest contamination levels of AFB₁ and AFs were found in ground peanuts purchased from the Bangkok market which were in the ranges 0.81–323.41 and 0.93–362.48 ng g⁻¹, respectively, with averages of 56.10 and 68.22 ng g⁻¹, respectively. The high content of AFB₁ and AFs in ground peanuts resulted directly from the raw material. High quality raw peanuts are selected and packed for sale, while poor quality peanuts are roasted and further ground for sale. Thus, the content of AFB₁ and AFs in ground peanuts was higher than in raw peanuts.

The worldwide AFs limits in food and commodities can be classified into four categories (Table 3) depending on the contamination level of AFs. The first group (the European Union regulation for direct human consumption or use as an ingredient in foodstuffs) requires the AFs contamination in peanuts to be less than 4 ng g⁻¹. The second group (regulated limits set by Japan and China) allows AFs contamination of 4 ng g⁻¹ or higher but less than 10 ng g⁻¹. The AFs contamination levels for peanuts in Thailand are less than 20 ng g⁻¹. The last group consists of samples contaminated with AFs at levels of 20 ng g⁻¹ or more.

The number of raw and roasted peanut samples contaminated with AFs to a level higher than the Thai regulation was 10 samples whether clean-up occurred using either the AflaTest or KU-AF02. Interestingly, the number of ground peanut samples, with an AFs content over 20 ng g⁻¹, increased from 9 to 10 when the KU-AF02 was used. This circumstance occurred due to the IAC capacity—the KU-AF02 IAC had a higher capacity than the AflaTest IAC (500 ng g⁻¹ for KU-AF02). Moreover, AFG₂ was detected in some samples when the KU-AF02 was used but not when clean-up used the AflaTest IAC. At AFs contents less than 4 ng g⁻¹, both IACs could clean-up samples with similar criteria in all samples.

Table 1. Aflatoxin B₁ and total aflatoxins (AFs) contamination in peanuts from Bangkok and nearby markets with clean-up using AflaTest and KU-AF02

| Peanut type | Market | n | Positive | AflaTest IAC | KU-AF02 IAC |
|------------|--------|---|----------|--------------|-------------|
|            |        |   |          | AFB₁ (ng g⁻¹) | AFs (ng g⁻¹) | AFB₁ (ng g⁻¹) | AFs (ng g⁻¹) |
|            |        |   |          | Range      | Mean*       | Range      | Mean*       | Range      | Mean*       | Range      | Mean*       |
| Raw peanut | 1       | 5 | 5        | 0.32–89.87 | 35.78       | 0.34–103.59 | 42.61       | 0.31–93.79 | 37.48       | 0.32–106.48 | 43.76       |
|            | 2       | 5 | 4        | ND-17.9    | 5.80        | ND-18.2    | 7.04        | ND-14.98   | 5.89        | ND-17.85    | 7.14        |
|            | 3       | 5 | 2.3      | ND-1.95    | 1.24        | ND-2.46    | 1.11        | ND-1.91    | 0.81        | ND-2.38     | 1.07        |
|            | 4       | 5 | 4        | ND-267.3   | 85.87       | ND-303.5   | 101.84      | ND-312.04  | 121.71      | ND-359.29   | 144.22      |
| Total      |        | 20| 16       | ND-267.31  | 42.26       | ND-303.55  | 47.11       | ND-312.04  | 46.68       | ND-359.29   | 51.71       |
| Roasted peanut | 1 | 5 | 5        | 0.90–28.38 | 8.41        | 1.12–36.39 | 10.71       | 0.94–28.60 | 8.43        | 1.13–35.84  | 10.50       |
|            | 2       | 5 | 5        | 1.06–32.75 | 13.21       | 1.28–40.79 | 16.60       | 2.04–26.52 | 14.74       | 2.38–30.47  | 17.85       |
|            | 3       | 5 | 5        | 0.53–33.59 | 12.71       | 0.65–41.60 | 16.02       | 0.56–34.33 | 12.74       | 0.68–41.60  | 15.56       |
|            | 4       | 5 | 5        | 1.97–25.73 | 8.92        | 2.33–31.03 | 10.86       | 1.07–37.38 | 9.18        | 1.28–45.36  | 10.86       |
| Total      |        | 20| 20       | 0.53–33.59 | 10.81       | 0.65–41.60 | 13.50       | 0.56–37.38 | 11.27       | 0.68–45.36  | 13.68       |
| Ground peanut | 1 | 5 | 5        | 2.15–196.46 | 43.88      | 2.67–230.81 | 52.90      | 2.25–210.50 | 47.28       | 2.77–246.98 | 57.21       |
|            | 2       | 5 | 5        | 1.43–296.85 | 88.50      | 3.18–329.35 | 110.90     | 1.51–316.55 | 94.59       | 3.34–374.84 | 121.60      |
|            | 3       | 5 | 5        | 13.13–22.00 | 22.45      | 16.71–68.43 | 30.61     | 12.02–53.23 | 21.65       | 15.80–69.91 | 28.95       |
|            | 4       | 5 | 5        | 0.81–323.41 | 69.59      | 0.93–362.48 | 78.68      | 0.86–418.41 | 89.38       | 0.97–502.71 | 107.64      |
| Total      |        | 20| 20       | 0.81–323.41 | 56.10      | 0.93–362.48 | 68.22      | 0.86–418.41 | 63.22       | 0.97–502.71 | 78.80       |

Note: ND = not detected.  
*Mean of positive samples.
3.2. Efficiency comparison between AflaTest and KU-AF02 IAC

The efficiency of the AflaTest and KU-AF02 IACs was compared using a Bland–Altman plot shown in Figure 1. Two samples of AFB1 (Figure 1(a)) and three samples of AFs (Figure 1(b)) were beyond the limit lines (±1.96 SD). These samples contained amounts of AFB1 and AFs higher than 300 ng g\(^{-1}\), as at this concentration the AflaTest IAC had poor accuracy according to its specifications (capacity 0–300 ng g\(^{-1}\)). The result in Figure 1(b) indicates that the capacity of the KU-AF02 was not different to the commercial IAC particularly in the range below 300 ng g\(^{-1}\). Mean difference values of data increased when approaching the positive area if the AFB1 concentration was higher than 250 ng g\(^{-1}\). At lower AFB1 concentrations (than 40 ng g\(^{-1}\)), there was no difference between the AflaTest and the KU-AF02 IAC for the clean-up step. Overall, the Bland–Altman plot indicated that the KU-AF02 IAC was in agreement with the AflaTest IAC when the concentration of AFB1 was lower than 250 ng g\(^{-1}\). At higher concentrations, the KU-AF02 IAC exhibited better performance to clean-up and recover AFB1 from the sample compared to the AflaTest IAC. Although the Bland–Altman plot showed differences from using the two IACs at high concentrations of AFB1 and AFs in samples, the values in the paired-sample t-test did not indicate a significant difference (p > 0.05) with a high correlation (>0.99). Since the AflaTest is the standard method of aflatoxin determination (AOAC International, 2002), the data generated from this method was used for exposure assessment.

3.3. Exposure assessment of aflatoxins in peanut consumption

According to the peanut consumption data from the ACFS collected during 2003–2004, Thai consumption of raw, roasted and ground peanuts averaged 0.74, 1.31 and 1.28 g per day, respectively,
with the 97.5 percentile of consumption being 6.93, 12.84 and 8.06 g. These values were used to estimate the exposure of AFB$_1$ and AFs in peanut consumption based on an equal peanut consumption between age groups. The exposures to AFB$_1$ and AFs were estimated and are shown in Table 4.

The highest exposures of AFB$_1$ and AFs were obtained with children (aged between 6 and 9 years) who consumed ground peanuts at the 97.5 percentile level of AFB$_1$ and AFs contamination. The children group that has been exposed to AFB$_1$ (11.24 ng/kg bw/day) and AFs (13.68 ng/kg bw/day) was higher than for teenagers and adults by about 2.5 times (4.88 and 4.31 ng/kg bw/day for AFB$_1$ and 5.94 and 5.24 ng/kg bw/day for AFs). A recent survey of AFB$_1$ in peanuts from Malaysia showed double the AFB$_1$ exposure as compared with the current study (24.3–34.0 ng/kg bw/day) (Chin, Abdullah, & Sugita-Konishi, 2012) which was also about double that in previous studies in Penang in 2010 and 2011 (Arzandeh, Selamat, & Lioe, 2010; Leong et al., 2011). Chinese children and adults were exposed to AFB$_1$ 0.21 and 0.10 ng/kg bw/day) in their consumption of local peanuts (Ding et al., 2012) while 0.10–13.7 ng/kg bw/day were ingested by Brazilians (Jager et al., 2013). Although the level of AFs contamination in Brazil peanuts was lower than in Thai peanuts, the exposure to AFs was higher. Brazilians consumed higher amounts of peanuts than Thais (0.73–112.73 g/day). The peanut consumption of Thais resulted in a higher exposure to AFB$_1$ than in Thai consumption of spices that had been imported from China (0.06–3.879 ng/kg bw/day) (Zhao, Schaffner, & Yue, 2013).

The FAO Regional Office for Asia and the Pacific (RAP) reported in 2013 that 92.6% of AFs intake by Thai consumers from their average consumption of various foods was due to peanut consumption. Moreover, 99% of the 97.5 percentile of various food consumers intake of AFs was from the consumption of peanuts. Therefore, almost all AFs exposure in Thailand was from peanut consumption. A similar contribution by peanuts as the major source of AFB$_1$ exposure was reported in Malaysia, where 83.8–99.7% of AFB$_1$ exposure came from peanut consumption (Chin et al., 2012). The adult urban Lebanese population was exposed to AFB$_1$ at levels of 0.63–1.46 ng/kg bw/day after consumed various food (cereal-based products, nuts, seeds, olives and dried dates) (Raad, Nasreddine, Hilan, Bartosik, & Parent-Massin, 2014). Thai adults were exposed to levels of AFB$_1$ about nine times.
Higher than the Lebanese, even though only peanut consumption was assessed. Higher levels of AFB1 exposure were observed with the consumption of breakfast cereal by child, teenager and adult Greeks (6.45–10.75, 4.3–6.6 and 3.07–6.14 ng/kg w/day, respectively) (Villa & Markaki, 2009). Although the Greek intake of AFB1 was mostly from contaminated breakfast cereal, the AFB1 exposure was lower than from Thai peanut consumption.

3.4. Estimated risk of liver cancer from AFB1 in peanuts

After the AFB1 exposure through peanut consumption in Thai was assessed, the exposure value was then used to estimate the risk of peanut consumption. The risk estimation of AFB1 intake cannot be determined because the no observed adverse effect level (NOAEL) and low observed adverse effect level (LOAEL) cannot be observed. Thus the model of potency liver cancer, developed by JECFA, was used to express the risk of AFB1 consumption. Thus, the population risk was derived by combining estimates of aflatoxin exposure (dose per person) and estimates of aflatoxin potency (risk per unit dose) from individual potencies of hepatitis B surface antigen positive and negative groups. The Thai population of liver cancer potency was 0.0245 cancers/100,000 population/year on the basis of a hepatitis B prevalence rate of 5% for the Thai population. Thai potency of liver cancer ranged from 0.01 to 0.03 cancers/year/100,000 people for average consumption, while at 97.5 percentile consumption, the potency of liver cancer was four times higher. Although at 97.5 percentile, ground peanut consumption showed the highest potency, this estimated value was lower than the dietary intake of Malaysians at lower and upper bounds by five and seven times, respectively (Chin et al., 2012).

Beside the estimated risk cancer, the percentage of cancer incidents was also estimated. The cancer case percentage attributable to aflatoxin intake was derived from the target population risk per year/100,000 population divided by the rate of liver cancer per year/100,000 population, multiplied by 100. The rate of liver cancer of Thais was 36.9 and 15.2 cancers/year/100,000 population for males and females, respectively. This cancer rate varied depending on the region; with the highest cancer rate estimated in northeast Thailand, with rates of 37.6 and 16 cancers/year/100,000 for males and females, respectively (Srivatanakul, 2001). Although the estimated potency of liver cancer values from peanut consumption seem high, it is only 0.01–0.43% of the causes of liver cancer incidence in Thailand (Table 5). Liu and Wu (2010) reported that out of the 550,000–600,000 new liver cancer cases worldwide each year, 4.6–28.2% may be attributed to aflatoxin exposure. This percentage is higher than for Thais due to the high exposure of Africans to aflatoxins.

### Table 5. Risk estimation of liver cancer and percentage cancer incidence attributed to AFB1 for peanut consumption at the mean and 97.5 percentile in Thailand

| Product         | Potency of liver cancer (cancers/year/100,000 people) | Cancer incidence attributable to aflatoxin (%) |
|-----------------|-------------------------------------------------------|---------------------------------------------|
|                 | Mean | 97.5th | Mean | 97.5th | Mean | 97.5th |
| Raw peanuts     | 0.01 | 0.04   | 0.01 | 0.05   | 0.03 | 0.13   |
| Roasted peanuts | 0.01 | 0.02   | 0.01 | 0.03   | 0.03 | 0.07   |
| Ground peanuts  | 0.03 | 0.12   | 0.04 | 0.16   | 0.10 | 0.39   |
| Peanuts         | 0.02 | 0.06   | 0.03 | 0.08   | 0.07 | 0.20   |

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Competing Interests
The authors declare no competing interest.
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