

Research Paper

Exposure Assessment of Multiple Mycotoxins in Black and White Sesame Seeds Consumed in Thailand

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ABSTRACT

This study was conducted to determine the occurrence of 16 well-recognized and emerging mycotoxins in black and white sesame seed samples sold in Thailand and to evaluate possible health risks to consumers. Samples were extracted and cleaned with a modified QuEChERS procedure. Multiple mycotoxins in sesame seed samples were analyzed with a validated liquid chromatography–tandem mass spectrometry method. The risk of mycotoxin exposure via dietary intake of sesame seeds was evaluated based on the hazard quotient, margin of exposure (MOE), and quantitative liver cancer risk established by European Food Safety Authority, the Food and Agriculture Organization of the United Nations, and the World Health Organization. Of the 200 samples, 21.5% were contaminated with mycotoxins, 19.5% were contaminated with a single mycotoxin, and 2% were contaminated with multiple mycotoxins. Although 9% of total samples were contaminated with aflatoxins (AFs), only one black sesame seed sample and one white sesame seed sample were above the regulatory limits for the European Union (2 µg/kg). The MOE values derived from consumption of black and white sesame seeds were generally <10,000, especially in the group consuming the most. The number of liver cancer cases over a lifetime associated with AFB₁ exposure based on the upper bound values for the group consuming high level of black and white sesame seeds (97.5 percentile) was estimated at more than 1 case per one million persons. Therefore, a potential risk to consumer health exists through the consumption of black and white sesame seeds and subsequent exposure to AFB₁. However, further evaluation with larger sample sizes is necessary for more accurate calculations. Continuous monitoring of mycotoxin contamination in sesame seeds with risk assessments is recommended.

HIGHLIGHTS

- Beauvericin, sterigmatocystin, and aflatoxins are frequently found in sesame seed samples.
- Mycotoxin contamination in most samples was below European Union stipulations.
- A potential risk to consumer health exists through consumption of sesame seeds.
- Evaluation of mycotoxin contamination is important to maintain consumer safety.

Key words: Black sesame seeds; Exposure assessment; Liquid chromatography–tandem mass spectrometry; Multiple mycotoxins; Thailand; White sesame seeds

Mycotoxins are natural secondary metabolites produced by several fungal species that are readily found in many kinds of agriculture commodities consumed at every stage of the food chain (35). Risk of contamination by mycotoxins increases under conditions that can enhance fungal growth and mycotoxin production, such as high humidity, suitable temperature, extreme weather conditions, large numbers of insects vectors, and plant damage under improper storage and processing conditions (35, 42). The Food and Agriculture Organization of the United Nations

(FAO) reported that 40% of the reduced life expectancy in developing countries is related to consumption of food contaminated with mycotoxins (26) and 25% of cereals were contaminated with mycotoxins (24). Mycotoxins contaminate many kinds of agriculture commodities such as fruit, cereal, spices, wheat, beans, and grains (32, 41). Mycotoxins also can be transferred from animals to humans through the consumption of contaminated animal products such as milk (23) and eggs (47). Mycotoxins are stable molecules that cannot be easily destroyed by heat treatment or during processing. Therefore, mycotoxin contamination is a major global issue and public health concern (7, 11, 35). The major groups of mycotoxins commonly found in food

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and feed are those produced by species of *Aspergillus*, *Penicillium*, *Fusarium*, and *Claviceps* (5). These mycotoxins are of great public health concern because of their high prevalence and toxicity (24).

Sesame (*Sesamum indicum* L.) is a plant whose seeds are well known as a healthy food with high nutritional value (13). These seeds are traditionally used in a wide variety of foods, especially vegetarian dishes, confectionery, and bakery products, as a source of nutrients, to enhance the taste, smell, and color of the food, and to make the food more attractive (2). Oil from sesame seeds is also used in salads, margarine, and animal feed (6). As urban lifestyles become increasingly hectic and stressful, this added stress can induce anxiety and exacerbate health problems. Thus, consumers are looking for effective ways to maintain health and wellness (3, 4, 27), often through nutritional values and functionality of their diets. The health benefits and disease prevention effects of sesame seeds are well established (13, 36). Minerals such as calcium, vitamins, and three bioactive components (sesamin, sesaminol, and sesamolin) found in sesame seeds are associated with lowering cholesterol, improvements in cardiovascular health, and prevention of high blood pressure, osteoporosis, and some cancers (9, 36, 37, 43, 46). Black sesame seeds and white sesame seeds are the most commonly available sesame seeds worldwide. Various toxigenic fungi, which can produce mycotoxins, are also found in sesame seeds (34), and mycotoxin contamination in sesame seeds from various countries has been reported (22, 28, 33). For consumer health protection, many organizations have established maximums for mycotoxin residues in various types of food commodities, including sesame seeds. The European Union (EU) (17) set the maximum concentration of aflatoxin B₁ (AFB₁) and total aflatoxins (AFs) in oilseeds intended for direct human consumption or use as an ingredient in foodstuffs at 2 and 4 µg/kg (ppb), respectively. The maximum concentration of total AFs allowed in Thailand for all food commodities is 20 ppb (25).

With more health awareness, the consumption of superfoods such as sesame seeds in among the Thai population is gradually increasing (3, 4, 46). However, evaluations of mycotoxin contamination in sesame seed products consumed in Thailand are scarce. Thus, Thai consumers might be exposed to an underrecognized health risk from consumption of sesame seeds. This study was conducted to obtain information on the occurrence of 16 mycotoxins in black and white sesame seeds sold in Thailand: AFB₁, AFB₂, AFG₁, AFG₂, ochratoxin A (OTA), zearalenone (ZEA), beauvericin (BEA), fumonisin B₁ (FB₁), FB₂, T-2 toxin (T-2), diacetoxyscirpenol (DAS), sterigmatocystin (STC), deoxynivalenol (DON), nivalenol (NIV), alternariol (ALT), and citrinin (CTN). These data were used to compute the mycotoxin exposure of Thai consumers and were incorporated into a health risk assessment.

MATERIALS AND METHODS

Chemical and reagents. Standards for AFB₁, AFG₂, and STC were obtained from Makor Chemicals (Jerusalem, Israel), those for AFB₂, AFG₁, OTA, and BEA were obtained from

Fermentek (Jerusalem, Israel), and those for ZEA, FB₁, FB₂, T-2, DAS, DON, NIV, ALT, and CIT were obtained from Sigma-Aldrich (St. Louis, MO). Each working standard solution was prepared by mixing the individual standard dilution with 50% acetonitrile at a final concentration 1 µg/mL. The working standard solutions were stored at -20°C.

All solvents were analytical grade. Deionized distilled water was produced by the Milli-Q purification system (Millipore, Bedford, MA). Super high-performance liquid chromatography (HPLC) grade acetonitrile and formic acid were purchased from RCI Labscan (Bangkok, Thailand) and Fisher Scientific (Leicester, United Kingdom), respectively. C18 powder was purchased from Machery Nagel (Duren, Germany). Sodium chloride was purchased from Ajax Finechem (Seven Hills, New South Wales, Australia). Magnesium sulfate was purchased from Applichem GmbH (Darmstadt, Germany).

Sample collection. The 200 samples of sesame seeds (100 samples each of black and white sesame seeds, representing general Thai consumption habits) were randomly collected from supermarkets in Bangkok, Thailand. Only products with a label indicating packing in Thailand were included in the study. Information on the location of harvesting was not available. All samples were kept in plastic bags at 4°C until analysis.

Sample preparation. Samples extracts were obtained with a QuEChERS procedure modified from that Cunha et al. (12). A 2.5-g portion of the black or white sesame seed sample was added to a 50-mL plastic centrifuge tube, 5 mL of water containing 1% formic acid was added, and the tube was shaken by hand, vigorously vortexed, and then kept at room temperature to equilibrate the sample with the extraction solvent. After 1 h, 5 mL of acetonitrile was added, and the tube was then vortexed and placed on a mechanical shaker for 1 h. A QuEChERS salt mixture containing 4.0 ± 0.1 g of MgSO₄ and 1.0 ± 0.1 g of NaCl was added, and the tube was vigorously shaken by hand and then centrifuged at 1,968 × g for 10 min at 4°C. A 3-mL aliquot of supernatant was transferred into a screw-cap microtube, and 150 mg of C18 powder was added to clean the sample. The tube was then centrifuged at 15,811 × g for 10 min at 4°C, and the supernatant was collected and passed through 0.22-µm-pore-size nylon syringe filter and then injected onto a liquid chromatography–tandem mass spectrometry (LC-MS/MS) apparatus for analysis.

LC-MS/MS analysis. LC analysis was performed with a binary pump system (1260, Agilent, Santa Clara, CA). Optimal HPLC conditions were achieved with the Zorbax Eclipse Plus Rapid Resolution High Definition C18 analytical column (4.6 mm by 50 mm by 1.8 µm; Agilent) and the Zorbax Eclipse Plus C18 guard column (4.6 mm by 5 mm by 1.8 µm; Agilent). The mobile phase consisted of water (mobile phase A) and acetonitrile (mobile phase B); both phases contained 5 mM ammonium formate with 0.2% formic acid. A time programmed gradient elution was set up as follow: 0 to 6.5 min with 5% A and 6.5 to 12 min with 95% A. Total run time was 12 min. The flow rate was 0.5 mL/min for all steps. The injection volume was 5 µL.

MS detection was conducted with a quadrupole analyzer (6460 triple, Agilent, Waldbronn, Germany) equipped with an electrospray ionization source running in positive ion mode under the multireaction monitoring mode alternating two transition reactions for each compound. The ionization source parameters were optimized as follows: capillary voltage, 3,500 V; dwell time, 100 ms; gas temperature, 320°C; gas flow rate, 10 L/min;

TABLE 1. MS/MS parameters for the determination of 16 mycotoxins

Analyte	Precursor ion (<i>m/z</i>)	Product ions (<i>m/z</i>)	Collision energy (eV)	Fragmentor (V)	Polarity
AFB ₁	313.07	285.1	21	150	Positive
	QPI ^a	241.0	35	150	
AFB ₂	315.09	287.1	25	160	Positive
	QPI	259.0	29	160	
AFG ₁	329.07	311	25	160	Positive
	QPI	243.0	43	160	
AFG ₂	331.08	313	25	180	Positive
	QPI	245.0	29	180	
OTA	404	192.9	48	130	Positive
	QPI	102.1	80	130	
ZEA	319.16	283	5	80	Positive
	QPI	187.0	17	80	
BEA	801.4	784.3	13	160	Positive
	QPI	244.1	35	160	
FB ₁	722.4	352.5	40	160	Positive
	QPI	334.4	45	160	
FB ₂	706.3	336.2	35	200	Positive
	QPI	318.3	40	200	
T-2	489.4	387.3	20	170	Positive
	QPI	245.2	26	170	
DAS	384.2	307.1	5	60	Positive
	QPI	199.0	13	60	
STC	325.1	309.9	25	160	Positive
	QPI	281.0	35	160	
DON	355.1	265.1	4	90	Negative
	QPI	59.1	10	90	
NIV	371.1	281	4	80	Negative
	QPI	59.1	10	80	
ALT	259.1	187.9	25	240	Positive
	QPI	160.1	33	240	
CTN	251.1	233.1	3	120	Positive
	QPI	205.1	20	120	

^a Qualitative product ion.

nebulizer pressure, 50 lb/in²; sheath gas temperature, 380°C; and sheath gas flow rate, 11 L/min. The MS parameters are listed in Table 1.

Analytical method validation. The method for determination of the 16 analytes was validated to assess the efficiency of the analytical method. The parameters assessed were linearity and working range, recovery, precision, limit of detection (LOD), limit of quantification (LOQ), and matrix effect (ME) according to the guidelines established by the EU (16, 18). The suitability of the analytical method for determination of the 16 analytes in black and white sesame seeds was quantitated by matrix match calibration from each category, and matrix match calibration curves were prepared in blank sesame seed matrix in three replicates with final concentrations of 1 to 50 µg/kg (1, 2.5, 5, 10, 25, and 50 µg/kg) for AFB₁, AFB₂, AFG₁, AFG₂, OTA, BEA, and STC, 1.5 to 100 µg/kg (1.5, 5, 10, 25, 50, and 100 µg/kg) for FB₁ and FB₂, 2 to 200 µg/kg (2, 5, 10, 50, 100, and 200 µg/kg) for ALT, CTN, DAS, and T2, 10 to 500 µg/kg (10, 20, 50, 75, 100, 250, and 500 µg/kg) for ZEA and DON, and 100 to 1,000 µg/kg (100, 200, 400, 500, 750, and 1,000 µg/kg) for NIV. Three concentrations and seven replicates of spiked samples for each of the 16 mycotoxins (and quality control sample) were used to determine intraday recovery. Samples were analyzed on five days (one set per day) to determine interday precision. The precision of the analytical method was recorded at the percent relative standard deviation (%RSD). Limits

of the analytical method were reported as the LOD and LOQ, which were determined by calculating three times the SD and 10 times the SD from 10 replicates of the quality control samples run within a single day. The MEs were evaluated by comparing the slope of the matrix matched calibration curve with the slope of the standard in the solvent calibration curve.

Risk assessment. The health risk to the Thai population from possible consumption of these mycotoxins was evaluated by conducting an exposure assessment and a risk characterization. The estimated daily intake (EDI) of mycotoxins for Thai people who consumed black and white sesame seeds was calculated:

$$EDI = CF \times IR / BW \quad (1)$$

where CF is the mean mycotoxin concentration in the sample (ng/g), IR is the sesame seed consumption rate by the Thai population (g/kg of body weight per day), and BW is the average body weight of an adult Thai person (65 kg). Based on actual sesame consumption, the population was separated into two groups, regular and heavy eaters, using mean consumption rate and consumption rate at the 97.5 percentile, respectively, for EDI calculations. The sesame seed consumption rates for regular and heavy eaters were obtained from the Thai National Bureau of Agricultural Commodity and Food Standards (38) and were 6.76 and 18 g/day, respectively. The exposure assessment assumed that when mycotoxin concentrations were below the LOD, the

TABLE 2. Performance characteristic of the analytical method

Analyte	Black sesame seeds				White sesame seeds			
	LOD (µg/kg)	LOQ (µg/kg)	R ²	Matrix effect (%)	LOD (µg/kg)	LOQ (µg/kg)	R ²	Matrix effect (%)
AFB ₁	0.40	1.33	0.9959	121.00	0.32	1.07	0.9992	122.70
AFB ₂	0.30	1.00	0.9972	107.67	0.25	0.83	0.9980	113.23
AFG ₁	0.50	1.67	0.9993	145.32	0.32	1.07	0.9991	145.19
AFG ₂	0.60	2.00	0.9902	138.11	0.50	1.67	0.9930	140.65
OTA	0.40	1.33	0.9916	127.34	0.22	0.73	0.9953	128.55
ZEA	10.50	35.0	0.9862	81.61	8.75	29.17	0.9924	85.32
BEA	0.25	0.83	0.9920	105.5	0.20	0.67	0.9988	101.8
FB ₁	0.50	1.67	0.9923	83.15	0.35	1.20	0.9945	89.47
FB ₂	0.75	2.50	0.9902	89.64	0.25	0.83	0.9926	93.52
T-2	2.50	8.35	0.9897	79.35	2.25	7.5	0.9969	87.64
DAS	2.32	7.73	0.9885	95.23	1.25	4.17	0.9951	107.36
STC	0.22	0.75	0.9917	79.88	0.75	2.50	0.9970	82.47
DON	15.45	51.50	0.9697	69.55	12.50	41.67	0.9885	77.32
NIV	32.00	106.67	0.9847	75.35	30.00	94.50	0.9887	80.65
ALT	0.68	2.30	0.9997	109.64	0.55	1.85	0.9993	103.48
CTN	0.70	2.33	0.9924	95.66	0.64	2.14	0.9941	92.68

concentration was half of the respective LOD, and when >60% of samples were below the LOD, both lower bounds (LBs) and upper bounds (UBs) were estimated by replacing those values in the nondetectable samples with 0 and the LOD, respectively, according to WHO guidelines (45).

Risk characterization for mycotoxins with the potential to have noncarcinogenic effects is typically expressed in terms of the hazard quotient (HQ), which is determined by dividing the EDI by the reference dose (RfD) for each contaminant:

$$HQ = EDI/RfD \quad (2)$$

where RfD is the health-based guidance value (ng/kg of body weight per day) for mycotoxins set by the Joint FAO/WHO Expert Committee on Food Additives (8). An HQ of <1 indicates that the exposure is less than the benchmark and is unlikely to result in an adverse effect.

For exposure to substances with genotoxic and carcinogenic properties, risk is estimated by two approaches using the margin of exposure (MOE) proposed by the European Food Safety Authority (EFSA) (19, 20) and the quantitative liver cancer risk approach proposed by the FAO and the World Health Organization (WHO) (44). The MOE is defined as the ratio between the benchmark dose lower confidence limit for 10% extra risk (BMDL₁₀) and the EDI of each mycotoxin:

$$MOE = BMDL_{10}/EDI \quad (3)$$

In this respect, high MOE values are desirable; those <10,000 indicate a threat to public health or an unacceptable risk of cancer.

The FAO and WHO have recommended the quantitative liver cancer risk assessment for an estimation of the likelihood of cancer associated with a given level of exposure averaged over a lifetime (44). The population risk associated with exposure to a single carcinogen (cancer cases per year per 100,000 people) is estimated by multiplying an average exposure concentration by the average potency. The average potency depends on the hepatitis B antigen (HBsAg⁺) prevalence rate in the population, and the assumption used was that approximately 5% of Thai people are affected by hepatitis B (40). For example, the average potency of AFB₁ for the Thai population can be calculated as follows:

$$\text{Average potency} = (0.3 \times \text{HBsAg}^+ \text{ prevalence rate}) + (0.01 \times \text{HBsAg}^- \text{ prevalence rate}) \quad (4)$$

The quantitative liver cancer risk approach was calculated and expressed in terms of the extra cases per 100,000 individuals per year. Thereafter, the lifetime cancer risk impact from exposure to AFB₁ in this study was quantified by multiplying the population risk by the average life expectancy. The average life expectancy of Thai people was reported as 75.5 years (29). The quantitative liver cancer risk estimates are expressed in extra hepatocarcinoma cases both per 100,000 individuals per year and per million individuals for a lifetime. Criteria for exposure to risk of liver cancer is 1 case of liver cancer per 1,000,000 per life period (14).

RESULTS AND DISCUSSION

Method validation. The aim of this study was to use an easy, quick, precise, and reliable method to determine the extent of recognized and emerging mycotoxin contamination in black and white sesame seeds. The QuEChERS extraction procedure with LC-MS/MS was chosen for the simultaneous determination of 16 mycotoxins in black and white sesame seed samples. Performance of the analytical method was validated in the black and white sesame seeds according to the guidelines established by the EU (16, 18). Matrix match calibration curves were constructed for quantification of all mycotoxins. All of the method validation parameters of this study were sound. Correlation coefficients (R^2) ≥ 0.9902 . LODs and LOQs were 0.20 to 32 and 0.67 to 106.67 µg/kg, respectively (Table 2). Recovery and intraday and interday precision are shown in Table 3. A matrix effect was found for both signal suppression and enhancement for the 16 mycotoxins in black and white sesame seed samples (Table 2). Based on the validation results, this method had acceptable recovery and interday and intraday precision in accordance with standard criteria for ensuring reliable data on contaminants.

TABLE 3. Accuracy and precision study for black and white sesame seeds

Mycotoxin ($\mu\text{g/kg}$)	Black sesame seeds			White sesame seeds		
	Recovery (%) ($n = 7$)	Intraday precision (%RSD) ($n = 7$)	Interday precision (%RSD) ($n = 18$)	Recovery (%) ($n = 7$)	Intraday precision (%RSD) ($n = 7$)	Interday precision (%RSD) ($n = 18$)
AFB ₁						
1	81.5	6.4	7.8	83.7	6.5	7.9
10	85.6	5.3	8.5	88.9	4.1	5.3
40	87.2	7.5	6.6	89.6	5.7	4.7
AFB ₂						
1	84.9	2.5	6.4	88.5	4.6	6.9
10	87.2	3.1	3.9	90.3	3.8	4.8
40	88.6	1.9	4.7	89.7	7.6	3.1
AFG ₁						
1	83.6	6.8	7.5	82.4	5.2	6.3
10	87.6	5.3	6.5	85.8	4.7	5.5
40	90.5	7.4	8.1	91.6	3.4	4.6
AFG ₂						
1	80.7	3.4	8.3	85.9	5.5	7.4
10	83.5	2.7	6.5	91.7	7.3	4.7
40	88.6	1.7	5.7	92.4	6.8	3.5
OTA						
1	84.5	2.6	8.5	90.6	3.2	5.8
10	87.7	2.1	7.6	87.5	4.1	6.1
40	88.3	3.2	6.1	93.2	5.7	7.7
ZEA						
50	78.4	3.3	5.9	81.3	8.8	8.7
250	80.2	4.6	3.7	83.8	5.5	8.3
500	83.4	3.4	4.5	85.5	4.6	6.2
BEA						
1	91.1	3.5	4.7	92.6	2.8	4.6
10	95.2	2.3	4.5	95.5	3.4	3.9
40	93.7	1.8	3.6	106.1	2.5	4.2
FB ₁						
2	82.3	2.1	5.7	85.7	5.7	5.5
20	83.1	1.6	8.7	88.4	4.8	5.8
100	87.6	1.9	3.9	91.5	5.4	4.7
FB ₂						
2	81.6	2.3	7.4	83.3	3.1	6.2
20	80.8	1.8	4.7	85.1	6.3	5.4
100	85.2	2.1	3.5	92.4	4.9	4.8
T-2						
5	85.3	4.2	5.8	88.6	5.2	5.7
50	84.4	3.3	6.1	92.7	4.9	8.7
200	87.9	4.2	7.7	97.3	7.7	3.9
DAS						
10	87.7	2.7	8.7	82.1	2.2	4.5
100	89.6	2.7	8.3	89.6	3.5	4.7
250	86.8	2	6.2	88.7	2.9	4.2
STC						
1	82.6	3.4	8.8	89.5	7.1	5.1
10	83.3	2.9	5.5	94.2	5.9	3.9
40	88.1	2.2	4.6	103.6	4.6	3.6

TABLE 3. *Continued*

Mycotoxin ($\mu\text{g/kg}$)	Black sesame seeds			White sesame seeds		
	Recovery (%) ($n = 7$)	Intraday precision (%RSD) ($n = 7$)	Interday precision (%RSD) ($n = 18$)	Recovery (%) ($n = 7$)	Intraday precision (%RSD) ($n = 7$)	Interday precision (%RSD) ($n = 18$)
DON						
50	73.6	6.3	2.8	79.2	6.5	8.3
250	72.2	5.7	3.4	85.4	3.8	6.5
500	78.6	4.9	2.5	88.7	4.1	5.7
NIV						
100	75.2	7.7	5.7	78.5	5.2	8.5
500	78.3	5.4	4.8	86.8	6.1	7.6
1,000	79.8	4.9	5.4	89.3	7.6	6.1
ALT						
2	83.2	3	3.1	90.1	4.2	5.9
20	85.9	2.2	6.3	93.9	3.8	3.7
100	86.2	2.7	4.9	89.7	3.5	4.5
CTN						
5	82.5	3.5	5.2	88.6	4.4	4.7
50	85.7	1.7	4.9	91.5	5.3	4.5
200	80.4	2.3	7.7	94.7	3.9	3.6

Occurrence of selected mycotoxins in black and white sesame seeds. One hundred samples each of black and white sesame seeds were collected from supermarkets in Bangkok. All samples were domestically packed, but the planting area was not specified. Of the 200 total samples, 37 samples (37%) of black and 18 samples (18%) of white sesame seeds were contaminated with mycotoxins. Among the positive samples, seven black sesame seed samples and one white sesame seed sample were contaminated with more than one mycotoxin. BEA was the most frequently detected toxin in both black and white sesame seed samples (25 and 10%, respectively). AFB₁ and STC were also detected in white sesame seeds (3 and 6%, respectively), whereas AFB₁, AFB₂, AFG₁, and STC were found in black sesame seeds (7, 2, 6, and 7%, respectively) (Table 4). Four of the toxin-positive black sesame seed samples were cocontaminated with AFB₁ and AFG₁, with a mean total AF concentration of 1.98 $\mu\text{g/kg}$. The concentrations of AFB₁ in the contaminated black and white sesame samples were

0.84 to 2.08 and 1.02 to 2.17 $\mu\text{g/kg}$, respectively. Among the AFB₁-positive samples, only one was had concentrations above the EU regulatory limit (2 $\mu\text{g/kg}$). However, none of the AF concentrations in any sesame seed samples exceeded the legal limit prescribed by the EU (17) and the Thai Ministry of Public Health (25). The mean concentration of AFs in our study was lower than those previously reported in black sesame seed samples by Chinnaphuti et al. (10), who found that 92.28% of tested samples were contaminated with AFs at 0.40 to 179.40 $\mu\text{g/kg}$. Kollia et al. (33) reported mean AF concentrations of 8.6 $\mu\text{g/kg}$ in white sesame seeds and 0.1 $\mu\text{g/kg}$ in black sesame seeds collected from a Greek market. Hosseininia et al. (28) reported that >50% of tested sesame seed samples imported into Khorasan, Iran were contaminated with AFs at a >1 $\mu\text{g/kg}$. Fapohunda et al. (22) reported contamination with major mycotoxins such as AFs, FBs, DON, ZEA, and several emerging mycotoxins, including BEA, in sesame seed samples collected from Nigeria; BEA had the highest

TABLE 4. *Occurrence of mycotoxins in black and white sesame seeds*

Mycotoxin	Black sesame seeds ($n = 100$)				White sesame seeds ($n = 100$)		
	EU maximum concn ($\mu\text{g/kg}$)	No. of positive samples	Concn ($\mu\text{g/kg}$)		No. of positive samples	Concn ($\mu\text{g/kg}$)	
			Range	Mean ^a		Range	Mean ^a
AFB ₁	2	7	0.84–2.08	1.4	3	1.02–2.17	1.47
AFB ₂	4 ^b	2	1.07–1.57	1.32	0	<LOD ^c	<LOD
AFG ₁	4 ^b	6	0.74–2.52	1.22	0	<LOD	<LOD
BEA	NA ^d	25	1.78–23.71	8.45	10	1.39–37.82	9.32
STC	NA	7	9.52–14.78	11.23	6	1.33–7.87	5.19

^a Mean concentrations were calculated from positive samples.

^b EU maximum for total aflatoxins.

^c LOD, limit of detection.

^d NA, not available.

TABLE 5. Estimate daily intake (EDI) and hazard quotient (HQ) of mycotoxins in black and white sesame seed samples

Mycotoxin	Index	Value (ng/kg of body wt/day) ^a							
		Black sesame seeds				White sesame seeds			
		Mean		97.5 percentile		Mean		97.5 percentile	
		UB	LB	UB	LB	UB	LB	UB	LB
AFB ₁	EDI	0.05	0.01	0.13	0.03	0.04	0.00	0.10	0.01
AFB ₂	EDI	0.03	0.00	0.09	0.01	0.03	0.00	0.07	0.00
AFG ₁	EDI	0.06	0.01	0.15	0.02	0.03	0.00	0.09	0.00
AFG ₂	EDI	0.06	0.00	0.13	0.00	0.05	0.00	0.14	0.00
AFs	EDI	0.05	0.01	0.17	0.01	0.04	0.00	0.10	0.00
STC	EDI	0.10	0.08	0.27	0.22	0.11	0.03	0.28	0.09
BEA	EDI	0.24	0.22	0.64	0.59	0.12	0.10	0.31	0.26
	HQ	0.40	0.37	1.06	0.98	0.19	0.16	0.51	0.43

^a Mean concentrations were calculated from all samples. The undetected values and unquantified values were replaced by zero and the LOD, respectively, for the lower bound estimate (LB) and were replaced by the LOD and LOQ, respectively, for the upper bound estimate (UB).

prevalence (66.67%), with concentrations of 0.08 to 4.2 µg/kg. Our findings are consistent with previous reports, indicating that BEA can occur in sesame seeds might be increasing in other agricultural products (8, 42). In contrast, Abbas et al. (1) reported that none of the tested sesame seeds grown in Mississippi were contaminated with AFs or fumonisins.

Health risk assessment. Risk assessment is an important tool for health safety and management. Assessment of the health risks associated with mycotoxins in contaminated samples is needed to understand the probability of adverse health effects. The exposure assessment in this study was reported as the EDI. The mean and 97.5 percentile EDI and HQ values are shown in Table 5. The EDI values were also used to compare with either the tolerable daily intake (TDI) or the provisional maximum (PM) TDI as guidance values to evaluate the health risk from chronic exposure to noncarcinogenic mycotoxins. Because the FAO and WHO have not yet set a PMTDI for BEA, we cannot directly evaluate the concentration of BEA that represents a risk for Thai consumers. Therefore, we

used the health-based guidance value of BEA with reference to the PMTDI of T-2 and its metabolite HT-2 at 60 ng/kg of body weight per day for indirect risk evaluation (15). The HQ of BEA calculated from concentrations as the LB and UB estimates for both regular and heavy eaters in adult populations were <1, which indicated no apparent risk to consumers. The mean and 97.5 percentile EDI values obtained in our study for the intake of AFB₁ from black and white sesame seeds were 0.01 to 0.05 and 0.03 to 0.13, and 0.00 to 0.04 and 0.01 to 0.10 ng/kg of body weight per day, respectively.

AFB₁, an IARC (International Agency for Research on Cancer) group I carcinogen, poses a risk for adverse health outcomes at all exposure concentrations (30). The EFSA (19, 20) assumed that the carcinogenic potency of total AFs would be similar to that of AFB₁. STC is a biogenic precursor of AFB₁ that has the same toxicological properties and is considered a carcinogenic compound that can induce tumors in several animal species (21, 39). Taking into account the available information on genotoxicity, carcinogenicity, and DNA adduct formation, the WHO concluded that STC is genotoxic and carcinogenic (45).

TABLE 6. Margin of exposure (MOE) of mycotoxins in black and white sesame seed samples

Mycotoxin	MOE ^a							
	Black sesame seeds				White sesame seeds			
	Mean		97.5 percentile		Mean		97.5 percentile	
	UB	LB	UB	LB	UB	LB	UB	LB
AFB ₁	3,477.91	16,679.75	1,306.15	6,264.17	4,604.55	37,150.35	1,729.26	13,952.02
AFB ₂	5,108.17	62,869.82	1,918.40	23,611.11	6,538.46		2,455.56	
AFG ₁	3,010.34	22,391.99	1,130.55	8,409.44	5,108.17		1,918.40	
AFG ₂	2,724.36		1,270.99		3,269.23		1,227.78	
AFs	3,384.30	33,359.50	1,023.15	12,528.34	4,591.62	148,601.40	1,724.41	55,808.08
STC	1,557,147.31	1,957,330.20	584,795.32	735,086.23	1,512,744.88	4,930,966.47	568,119.74	1,851,851.85

^a LB, lower bound; <LOD and <LOQ = 0. UB, upper bound; <LOD and <LOQ = LOD and LOQ, respectively.

TABLE 7. Quantitative liver cancer risk from exposure to AFB₁ in black and white sesame seeds

Mycotoxin	Quantitative liver cancer risk (cases/yr/100,000 persons) ^a							
	Black sesame seeds				White sesame seeds			
	Mean		97.5 percentile		Mean		97.5 percentile	
	UB	LB	UB	LB	UB	LB	UB	LB
AFB ₁	0.0012	0.0002	0.0032	0.0007	0.0009	0.0001	0.0024	0.0003
AFB ₂	0.0008	0.0001	0.0022	0.0002	0.0006	0.0000	0.0017	0.0000
AFG ₁	0.0014	0.0002	0.0037	0.0005	0.0008	0.0000	0.0022	0.0000
AFG ₂	0.0015	0.0000	0.0033	0.0000	0.0013	0.0000	0.0034	0.0000
AFs	0.0012	0.0001	0.0041	0.0003	0.0009	0.0000	0.0024	0.0001
STC	0.0025	0.0020	0.0067	0.0053	0.0026	0.0008	0.0069	0.0021

^a LB, lower bound; <LOD and <LOQ = 0. UB, upper bound; <LOD and <LOQ = LOD and LOQ, respectively.

Therefore, we evaluated the health risk associated with total AF, AFB₁, and STC exposure using the MOE and the quantitative liver cancer risk approach. The rat BMDL₁₀ for AFB₁ and STC is 170 and 160 ng/kg of body weight per day, respectively (45). Currently, an MOE of $\geq 10,000$ based on the animal BMDL₁₀ is considered of low public health concern, whereas an MOE of $<10,000$ indicates high concern for cancer risk in humans exposed to a genotoxic carcinogen (20). In this study, the MOE of total AFs and AFB₁ based on UBs for both mean and 97.5 percentile black and white sesame seed exposures are $<10,000$ (Table 6), which indicates a risk to public health and thus should be a priority for risk management. In contrast, the MOE of STC based on LB and UB values were $>10,000$ for all groups of consumers of black and white sesame seeds. Therefore, exposure of Thai consumers to STC is of minimal concern.

Liver cancer is ranked second in terms of incidence, prevalence, and mortality in the Thai population, with incidence rates in men and women of 32.2 and 11.5 cases per 100,000 people, respectively (31). Based on the LB and UB estimations of AFB₁ concentrations in this study, the theoretical incidence rates of liver cancer through black sesame seed consumption for Thailand's regular and heavy eater groups were 0.0002 to 0.0012 and 0.0007 to 0.0032 cases per 100,000 people per year, respectively (Table 7). The estimated cancer risk from consumption of white sesame seeds evaluated in the same manner was 0.0001 to 0.0009 and 0.0003 to 0.0024 cases per 100,000 people per year, respectively. Thus, estimates of cancer risk to Thai people from consumption of black and white sesame seeds over lifetime exposure were 0.151 to 2.416 and 0.075 to 1.812 cases per 1 million people. Our results indicate that the lifetime risk of liver cancer from AFB₁ exposure based on UBs at high consumption of black and white sesame seeds (97.5 percentile) is estimated to be >1 liver cancer case per 1 million people. Although the risk of mycotoxin exposure via the consumption of black and white sesame seeds is low for Thai consumers, both the MOE approach and lifetime cancer risk values revealed that long-term consumption of sesame seeds, especially at the UB and high

consumption rate, might pose a significant cancer risk to human health.

This study was conducted to evaluate the health risks of exposure to mycotoxins from consumption of sesame seeds in Thailand. BEA, STC, and AFs (especially AFB₁) were the most frequently found contaminants. Although most of the toxin concentrations found in sesame seed samples were lower than the EU standard values, long-term consumption of sesame seeds might have some adverse effects on the health of consumers, especially those who consumer high amounts of these seeds. With regards to consumer health, continued monitoring of mycotoxin contamination in sesame seeds concomitant with risk assessment is recommended. This approach can be used to plan, design, and manage systems for mycotoxin control strategies, minimizing the negative health impacts of mycotoxins.

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