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# Risk Assessment of Dietary Exposure to Aflatoxin Contamination in Spices

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## Abstract

This study highlights the daily consumption of spices in human diet and the potential of aflatoxin B1 (AFBI) contamination which attributable to the risk of developing liver cancer. All the AFBI contamination data in spices from various studies in Malaysia were considered for calculation of risk assessment by dietary exposure and margin of exposure (MOE). The mean dietary exposure to AFB1 ranges from 0.21-1.32 ng/kg-bw/day (overall mean, 0.59 ng/kg-bw/day), and 12.27 ng/kg-bw/day was the highest reported level of AFBI contamination. The MOEs derived from these dietary exposures at a benchmark dose lower confidence limit ($BMDL_{10}$) of 0.305 μg/kg-bw/day were 230-1,450 (overall mean, 520). The MOE of less than 10,000 indicates the risk of AFBI contamination in spices should be a high priority for risk management actions. Based on the tolerable daily intake (TDI) in Asia of 0.11-0.19 ng AFB1/kg-bw/day for liver cancer risk per 100,000 populations, the overall mean of 0.59 ng/kg-bw/day represents 3-536% of this TDI. Population risk for primary liver cancer attributable to AFB1 contamination in spices were 0.01-0.03 (0.1-0.7%) and 0-0.31 (0-6%) cancers/year/100,000 population, for mean and range of exposures. The risk, which was less than 1 cancer case/year/100,000 population, suggested that Malaysian population is not significantly at risk.

**Keywords:** Risk Assessment; Margin of Exposure; Aflatoxins; Spices; Malaysia

## Introduction

Aflatoxins (AF) are primarily produced by the food-borne fungi *Aspergillus flavus* and *Aspergillus parasiticus*, which colonize a variety of food commodities, including maize, oilseeds, spices, groundnuts, and tree nuts in tropical and subtropical regions of the world. They are found in foods as a result of fungal contamination both pre- and post-harvest, with the rate and degree of contamination dependent on temperature, humidity, soil and storage conditions. Additionally, when animals that are intended for dairy production consume AF-contaminated feed, a metabolite, aflatoxin M (AFM), is excreted in the milk [1, 2]. Aflatoxin B1 (AFB1), in particular, is well known as a potent liver carcinogen, (hepatocellular carcinoma, HCC) which risk is multiplicatively higher for individuals exposed to both AF and chronic infection with hepatitis B virus (HBV). AFBI is

considered as genotoxic and carcinogenic, classified as a Group 1 human carcinogen, and its presence in foods cannot be readily eliminated or avoided [3-5].

The most common causes of cancer death worldwide are cancers of lung, colorectal, stomach, liver and breast [6]. Methods for estimating cancer risk rely largely on epidemiological and toxicological data. Epidemiological studies provide statistical estimates of cancer risks in humans but are subject to certain limitations, including inadequate exposure data and confounding due to exposure to multiple agents. Toxicological studies can be conducted under controlled conditions but provide only indirect information on human cancer risks, since extrapolations need to be made from animal studies [7, 8]. In 2005, the European Food Safety Authority (EFSA's Scientific Committee) recommended use of the margin of exposure (MOE) approach for the safety assessment of carcinogens in foods. The MOE represents the ratio between the dose descriptor for tumor formation in animals or humans and the measured or estimated human exposure to that carcinogen. Application of the MOE-approach requires reliable animal carcinogenicity data or reliable epidemiological data including good quality exposure assessment. The MOEs for carcinogenic acrylamide (formed in variety of foods rich in carbohydrates), benzo[a]pyrene (representative of the polycyclic aromatic hydrocarbons, PAHs), furan and 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (formed during cooking), ethyl carbamate (formed in foods and beverages by fermentation), 1,3-dichloro-2-propanol (formed under a variety of conditions during the production of acid-hydrolysed vegetable proteins and of malt- and soy-based products), benzene (mainly an environmental contaminant) and aflatoxins have been calculated [9-12]. In general, MOE of 10,000 or higher, if it is based on the benchmark dose lower confidence limit ($BMDL_{10}$) from an animal carcinogenicity study, taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view and might be reasonably considered as a low priority for risk management actions [12].

In Malaysia, Leong, et al. reported an estimated dietary exposure of AFB1 in peanuts and peanut products as 0.36 and 8.8ng/kg-bw/day, representing the low and high-level of exposure, respectively. The derived MOE values ranged from 34-847, indicating that AFB1 would be of public health concern and might reasonably be considered as a high priority for risk management actions. In another study by Chin et al. exposure to AF among Malaysian population was estimated by analyzing AF in food composites prepared as ready for consumption [13, 14]. AF was detected in only 4.2% of the composite samples, which involved breakfast cereals (16.7%), peanut butter (33.3%) and peanuts (58.3%). Dietary exposure to AFB1 ranged from 24.3-34.00 ng/kg-bw/day, with peanuts being the main contributor. Estimated liver cancer risk from this exposure was 0.61-0.85 cancers/100,000 population/year, contributing 12.4%-17.3% of the liver cancer cases. Malaysia regulates the maximum limit (ML) of 15 µg/kg total AF for foods for further processing, and 5 µg/kg for all foods ready for consumption [15].

In the current study, the MOE for assessing risk of dietary exposure to AFB1 contamination in spices to consumers in Malaysia was calculated with reference to the approach by Benford, et al. [16]. The worldwide contamination of AF and ochratoxin A (OTA) in spices, including in Malaysia has been compiled and reported by Ali, et al. [17]. Contamination of AF in spices, along with rice as a staple food in Malaysia, even though contaminated at a very low level should be monitored closely as they are consumed daily.

## Spice Consumption

The popularity of highly spiced cuisine and consumer demand for more flavorful foods which are also low in sodium and fat have resulted in a continuing interest in the use of spices and herbs in food products [18]. Spices are used worldwide, particularly in the Asian and Middle Eastern countries, and considered protective against degenerative diseases, including cancer. The spices such as a jowan, caraway, fennel, cumin and anise are common, and are used to varying degrees in home recipes. Human consumption of these spices in some parts of the world is already established at relatively high levels. *In vivo* research has suggested efficacy of the aqueous and non-aqueous extracts of these spices that could provide a simple and effective strategy for preventing cancers, such as breast cancer [19].

Although such condiments are generally used for the aesthetic properties they contribute to foods, and their potent chemopreventive/ antioxidant properties, spices and herbs can often be a major source of microbial contamination. The high levels of microbial and fungal contamination in spices and herbs reported by many studies suggest a need for better control in all aspects of the production, processing and usage of spices [18, 20-24].

The food balance sheets of the US Food and Agriculture Organization (FAO) estimated daily intake of spices ranged from 2 to 22 g/person/day worldwide. As

shown in Table 1, Malaysia is among the top 3 countries reported to have high spice consumption. However, the actual food consumption may be lower than the quantity shown, as food availability depends on the magnitude of wastage and losses of food in the household, e.g. during storage, in preparation and cooking, as plate-waste or quantities fed to domestic animals and pets, thrown or given away [25]. Spice consumption in Malaysia has increased from the year 1992 to 2007 compared to some other countries as shown in Figure 1. The consumption of spices increased from 5 and 4 g/person/day in 1992 and 1997, and to 9 and 13 g/person/day (125% and 44% increases) in 2002 and 2007 [25].

| Country | g/person/day |
|---|---|
| Bosnia and Herzegovina | 22 |
| Nepal | 16 |
| Malaysia, Grenada, United Arab Emirates | 13 |
| Peru | 12 |
| Brunei Darussalam | 11 |
| Sri Lanka, Jamaica | 10 |
| Hungary, Ghana | 9 |
| Seychelles, Kuwait, Dominica, Maldives | 8 |
| Saudi Arabia, Guyana, Cape Verde | 7 |
| Mauritius, Bangladesh, India | 6 |

Table 1: World Consumption of Spices-Ranks in 2007 (latest available data).
(Source: Food Security Data by Food Groups/Items, FAO, June 2012) [25].

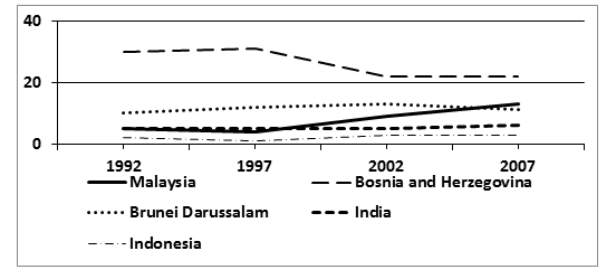


Figure 1: Spice Consumption from the Year 1992 to 2007 [25].

## Methodology

### Dietary Exposure to Aflatoxin B1 Contamination in Spices

Based on the statistical distribution of AFB1 contamination in foods for 2000 to 2006, Benford, et al. calculated the mean overall estimates of international dietary exposure to AFB1 for MOE studies [16, 26]. The dietary exposure to AFB1 contamination in spices (involving 4,704 number of samples with overall mean and maximum level of 1.5 and 96 µg/kg) for the 13 cluster diets was 0.07, 0.03, 0.06, 0.02, 0.04, 0.03, 0.10, 0.05, 0.03, 0.03, 0.01, 0.01 and 0.04 ng/kg-bw/day, respectively [27]. Cluster G (Malaysia, Indonesia, Thailand, Vietnam, Sri Lanka, Nepal, India and China) showed the highest exposure to AFB1 in spices, followed by cluster A (Mauritius), C (Algeria, Egypt, Jordan, Morocco, Syria and Tunisia) and H (Guatemala, Honduras, Mexico, Paraguay, Peru and El Salvador) which was 0.10, 0.07, 0.06 and 0.05 ng/kg bw/day, respectively. More recent data (1995-2013) on the contamination level of AF in spices reported by some individual countries worldwide has been compiled by Ali, et al. [17].

In the current study, only contamination of AFB1 in spices reported in Malaysia will be calculated for dietary exposure by multiplying the AFB1 contamination level with the intake of spices per day (13 g/person/day, FAO 2012) and dividing by body weight. The average body weight of 60 kg for adults was applied based on the average body weight of 62.65 kg for Malaysian adults reported by Ministry of Health Malaysia [28].

Dietary exposure (ng AFB1/kg-bw/day)

= Contamination level in food (ng/g) x Daily amount consumed (g/day)/Body weight (kg-bw)

Ali, et al. compiled all the reported research on AF in Malaysia (Tables 2 & 3) [29]. It was indicated at previous reports on high levels of AF in spices might be due to interferences if the analytical method used (mini column, TLC or HPLC) was not validated. Low contamination levels and difficulty in overcoming the interference problems in the analysis of spices have been reported [30-32]. High level of AFG2 contamination if compared to AFB1, AFB2 and AFGI levels was most probably due to interference with reference to the ability of AF-producing fungi [32]. Reddy, et al. reported that *A. flavus* strain isolated from pepper powder produced both AFB1 (2300 µg/kg) and AFB2 (780 µg/kg) but could not find any AFG1 and AFG2 producers among all the *A. flavus* strains screened [33]. However, overall high level of AF contamination in spices was possible if the safe level of moisture content was not observed. Further studies on the AF contamination in spices (Table 3), using reliable and validated analytical methods with efficient sample clean-up and low detection limit, showed relatively low levels when the interference problem was minimized. Contamination of AF in spices, as well as in traditional medicines with active ingredients consisting of herbs and spices, even though at low levels, should be examined

closely for the risk of prolonged exposure as these foods and supplements are taken frequently or daily [17, 32].

Based on mean and maximum contamination levels of AFBI (Table 3), the dietary exposure and MOE for assessing the risk exposure to AFB1 contamination in spices to consumers' health in Malaysia was calculated.

| No. | Sample | Incidence (%) | Range/Level (µg/ kg) | | | | | Year reported, Institution and method[a] |
|---|---|---|---|---|---|---|---|---|
| | | | AFB1 | AFB2 | AFG1 | AFG2 | Total | |
| 1 | Chili sauce | 0/21 | nd[b] | | | | | 1976-1980 |
| 2 | Dried chili | 0/1 | nd | | | | | IMR[c] TLC[d] |
| 3 | Spices | 3/18 (17%) | 16-Feb | | | | | 1981-1984 MARDI[e] TLC/mini column |
| 4 | Spices | 155/155 (100%) | 4 - 400 | | | | | 1985-1995 MARDI TLC/mini column/ HPLC[f] |
| 5 | Black pepper | 51/51 -100% | 5 - >40 | | | | | |
| 6 | White pepper | 16/16 -100% | 5 - >40 | | | | | |
| 7 | Chili and products | 8/17 (47%) | 3 - 66 | | | | | 1995-1996 FQC[g] HPLC |
| 8 | Spices/chili & products | 0/6 | nd | | | | | 1996-1999 DOC[h] HPLC/TLC |
| 9 | Black and white pepper | -58.30% | | | | | 0.1- 25.8 | 2004-2012 MARDI IAC-HPLC[i] |
| 10 | Chili | 2/10 (20%) | 10.8 & 33.2 | 5 | 5.8 & 14.0 | 5.20 & 8.82 | | |

Table 2: Contamination levels of AF in Spices Reported in Earlier Studies in Malaysia.

- [a]Reference-Ali, et al. [29]
- [b]Not detected
- [c]Institute of Medical Research (IMR)
- [d]Thin Layer Chromatography (TLC)
- [e]Malaysian Agricultural Research and Development Institute (MARDI)
- [f]High Performance Liquid Chromatography (HPLC)
- [g]Food Quality Control Laboratory (FQC)
- [h]Department of Chemistry Laboratory (DOC)
- [i]Immunoaffinity column - High Performance Liquid Chromatography

| Samples (origin) | | Range AFB1 (Range Total AF) µg/kg | | Reference |
|---|---|---|---|---|
| **Commercial white and black pepper[a]:-** | 70/126 (55.5) | 0.1–4.9 (0.1–4.9) | - [b] | Jalili, et al. [34] |
| Malaysia | 47/90 (52.2) | | (2.67) [b] | |
| Singapore | 13/24 (54.2) | | -1.23 | |
| Australia | 3/12 (25.0) | | -0.96 | |
| White pepper seed | 10/24 (41.6) | 0.2-2.1 (0.2–4.5) | | |
| White pepper powder | 15/33 (45.5) | 0.1-3.4 (0.1–4.6) | | |
| Black pepper seed | 18/30 (60.0) | 0.1-3.5 (0.1–4.8) | | |
| Black pepper powder | 27/39 (69.2) | 0.4-4.9 (0.7–4.9) | | |
| **Spices [c]:-** | 14/15 (93.3) | | | Reddy, et al. [33] |
| Chili | 8/8 (100) | 0.58–4.64 | 2.62 | |
| pepper | 4/4 (100) | 0.65-2.1 | 1.2 | |
| Cumin | 2/3 (66.6) | 1.89–4.64 | 3.26 | |
| Chili[d] | 52/80 (65.0) | 0.2–56.61 (0.2–79.71) | 3.37 (4.56) | Jalili and Jinap, [35] |
| Chili[e] | 9/10 (90.0) 2/10 (AFB1) | 10.8–33.2 (5.85–44.2) | 4.40 (13.60) | Khayoon, et al.[36] |

| | | | | |
|---|---|---|---|---|
| **Spices[f] :** | 30/34 (85.0) | 0.01–7.68 | 1.38 (1.61)[g] | Ali, et al. [17] |
| | | (0.01–9.34) | 1.22 (1.42)[h] | |
| Mixed spices | 16/18 | 0.03–3.32 | | |
| | | (0.03–4.17) | | |
| Masala mixed spices | 4/4 | 0.05–3.88 | | |
| | | (0.51–4.41) | | |
| Dried chili | 1/2 | 3.18 (3.39) | | |
| Black pepper | 1/1 | 0.25 (0.28) | | |
| White pepper | 1/1 | nd (nd)[i] | | |
| Fennel | 2/2 | 0.01–4.37 | | |
| | | (0.01–5.29) | | |
| Cumin | 1/2 | 2.45 (3.20) | | |
| Turmeric | 2/2 | 0.05–0.18 | | |
| | | (0.05–0.21) | | |
| coriander | 1/1 | 7.68 (9.34) | | |
| Poppy seed | 1/1 | 0.05 (0.05) | | |
| **Spices[j] :** | 21/24 (88%) | 0.32–28.43 | 7.31(8.38)[g] | Ali, et al. [17] |
| | 20/24 (AFB1) | (0.32–31.17) | 6.09 (7.33)[h] | |
| Mixed spices | 11/11 | 2.44–11.03 | | |
| | | (3.72–15.31) | | |
| Dried chilli | 3/3 | 6.44–28.43 | | |
| | | (7.15–31.17) | | |
| Black pepper | 1/1 | 0.34 (0.34) | | |
| White pepper | 2/2 | 0.32–0.39 | | |
| | | (0.32–0.50) | | |
| Fennel | 2/2 | 0.38–21.74 | | |
| | | (1.23–24.05) | | |
| Cumin | 1/1 | nd (0.91) | | |
| Turmeric | 2/2 | 4.79–7.28 | | |
| | | (5.35–10.22) | | |
| Coriander | 1/1 | 10.97 (13.38) | | |
| Cinnamon | 1/1 | nd (nd) | | |

Table 3: Contamination levels of AF in Spices Marketed in Malaysia Analyzed by Validated Method.

- [a]Samples obtained from Kuala Lumpur, Putrajaya, and Selangor areas. A wide range of different brands. Products were from Malaysia, Australia and Singapore.
- [b]Data not available for AFB1. Mean level of total AF are taken as mean level of AFBI.
- [c]Samples obtained from Penang areas.
- [d]Samples obtained from Kuala Lumpur, Putrajaya, and Selangor areas.
- [e]Samples obtained from Penang areas.
- [f]Samples obtained from Penang areas.
- [g]Mean level of positive samples only.
- [h]Mean level of all the samples analyzed-taken for calculation of AFBI dietary exposure.
- [i]Not detected.
- [j]Samples obtained from Penang areas.

## Calculation of Margin of Exposure

The margin of exposure (MOE) approach compares the margin between a dose and an exposure causing cancer in animals or humans with the estimated human exposure to that substance. It uses a reference point, usually taken from an animal cancer bioassay in which the substance has been administered for most of the animal's life span. The reference point corresponds to a daily dose causing a low but measurable increase in the incidence of tumors. This reference point (also called a point of departure) is then divided by the estimate of human dietary exposure to the substance to give a dimensionless ratio that is the MOE [12]. Although AFB1 is reported to cause tumors at other sites in animal studies, such as lung, kidney and colon, dose response data were only available for the tumor incidence in the liver. EFSA concluded that the most adequate study for dose response modelling was

by Wogan, et al. where groups of male Fisher rats (the most sensitive animal strain and sex) were fed diets containing AFB1 (purity > 95%) until clinical deterioration of animals was observed [9, 37]. Tumor incidences (HCC) results of the study are given in Table 4. The daily intake was adjusted to 104 weeks of dosing and observation in order to compensate for the shorter study duration in some of the AFB1 fed groups. The method by European Chemicals Agency was used, by multiplying the dose applied with [(w1/104) (w2/104)] where w1 is the duration of dosing and w2 the duration of observation in weeks and 104 is the standard life span for rats and mice in weeks [38]. The bench mark dose (BMD) and the benchmark dose lower confidence limit (BMDL) values from the study of Wogan, et al. were considered to be the most suitable for providing a point of departure for calculating the MOE [9, 37].

This study adopted the dose response modelling reported by Benford, et al. for AFBI, where cancer dose response data were analyzed by dose response modelling in accordance with the International Program on Chemical Safety document "*Principles for modelling dose–response for the risk assessment of chemicals*" and the more recent guidance from EFSA [16, 39, 40]. Figure 2 shows key concepts for the BMD approach, where the $BMDL_{10}$ was used as a reference point on the dose response curve. It represents the lower bound of a 95% confidence interval on the benchmark dose that corresponds to a 10% increase in tumour incidence ($BMD_{10}$). Table 5 shows the seven conventional statistical dose response models (Logistic, Log-logistic, Gamma, Multistage, Probit, Log-Probit and Weibull) derived from the study of Wogan, et al., showing the $BMD_{10}$ and their lower 95% confidence bounds, $BMDL_{10}$ [37]. A $BMDL_{10}$ value of 0.305 µg/kg-bw/day was chosen as the best fit from the log-logistic model and had the highest log-likelihood value of-40.78 and the low AIC value of 87.55 compared to the other models. As quantitative measure of how close the model is to the data points; the higher the log-likelihood (less negative in this study), the better the fit of the model. Based on the best estimate of this BMDL value derived from the study of Wogan, et al. and the human exposure estimates, a range of MOE values can be derived [37].

MOE = $BMDL_{10}$ (µg/kg-bw/day)/
Exposure data (µg/kg-bw/day)

| Model | P value | II | AIC | $BMD_{10}$ | $BMDL_{10}$ |
|---|---|---|---|---|---|
| Logistic | 0.008 | -41.74 | 87.48 | 0.324 | 0.243 |
| Log-logistic | 0.008 | -40.78 | 87.55 | 0.434 | 0.305 |
| Log-probit | 0.008 | -41.08 | 88.16 | 0.436 | 0.306 |
| Probit | 0.007 | -42.29 | 88.58 | 0.291 | 0.221 |
| Gamma | 0.006 | -41.43 | 88.87 | 0.418 | 0.264 |
| Weibull | 0.004 | -42.07 | 90.15 | 0.358 | 0.21 |
| Multistage | 0.003 | -42.2 | 90.41 | 0.357 | 0.14 |

Table 4: Individual Model Results for AFB1-Induced Liver Tumors (in µg/kg-bw/day) for the complete data set of Wogan, et al. [a] [37].
[a]Adopted from Benford, et al. [16].

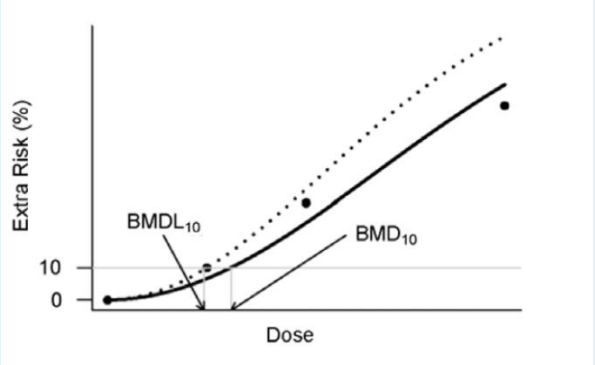


Figure 2: Key Concepts for the $BMD_{10}$ and $BMDL_{10}$ Approach.

| Concentration in diet (µg/kg) | Dose[a](µg/kg-bw/day) | Duration of dosing and observation (week) | Time adjusted dose[b] (µg/kg-bw/day) | Tumor incidence |
|---|---|---|---|---|
| 0 | 0 | 104 | 0 | 0/18 |
| 1 | 0.04 | 104 | 0.04 | 2/22 |
| 5 | 0.2 | 93 | 0.16 | 1/22 |
| 15 | 0.6 | 96 | 0.51 | 4/21 |
| 50 | 2 | 82 | 1.24 | 20/25 |
| 100 | 4 | 54 | 1.04 | 28/28 |

Table 5: Tumor incidences (hepatocellular carcinoma) in Male Fisher Rats after Dietary Administration of AFB1 [37].

- [a]Dose was calculated based on the assumption of 500 g male rats body weight and feed consumption of 20 g/day (concentration in the diet x 20/500).
- [b]Time adjusted dose was the dose which was adjusted to full duration of exposure (104 week), by multiplying the dose with $[week/104]^2$ [38].

### Population Risk for Primary Liver Cancer

Wu, et al. studied whether the current AF regulatory standards around the world adequately protect human health, since AFB1 is a potent liver carcinogen, and HCC risk is multiplicatively higher for individuals exposed to both AF and chronic infection with HBV [41]. Currently, most nations have a maximum limit (ML) of total AF in maize and peanuts ranging from 4 to 20μg/kg. The European Union sets a ML of 2 and 4 μg/kg for AFB1 and total AF in foods such as maize and peanuts, and in certain spices; dried, whole or ground *Capsicum spp*., including chilies, chili powder, cayenne pepper and paprika, white and black pepper, nutmeg, ginger and turmeric, the ML is 5 and 10 μg/kg for AFB1 and total AF [42, 43]. Other nations may not have a specific ML for AF in spices.

At the protection level of 1 in 10,000 lifetime HCC cases in the population, it was reported that almost all AF regulations worldwide are adequately protective, with the exception of several nations in Africa and Latin America [41]. For the ML of AF contamination that would be allowable in maize and peanuts in order for increased lifetime liver cancer risk to be less than 1 in 10,000 and 100,000 in the population, Wu, et al. used the following formula [41]. The increased lifetime cancer risk from exposure to a carcinogen per unit time is calculated as

Risk = LADD x SF x (number of years life)

Where LADD is the adult individual's lifetime average daily dose of the carcinogen, and SF is the slope factor, or cancer potency factor of the carcinogen. Although (number of years life), or life expectancy differs from nation to nation, a value of 70 years was assumed in calculations.

For the slope factor (SF) for AF in a given nation, the weighted potency was based on summing the proportion of hepatitis B virus antigen positive (HBsAg+) and hepatitis B virus antigen negative (HBsAg-) individuals multiplied by their respective slope factors for aflatoxin-induced HCC. Wu, et al. used the values of 0.3 cancers/year/100,000 population per ng AFB1/kg-bw/day for HBsAg+ individuals and 0.01 cancers/year/100,000 population per ng AFB1/kg-bw/day for HBsAg- individuals [41].

The calculation used by Wu, et al. was in accordance with the report by the World Health Organization, which stated the fraction of the incidence of liver cancer in a population attributable to AF intake relates to AF potency estimates (risk per unit dose) and estimates of AF intake (dose per person) [41, 44]. The population risk for primary liver cancer can be estimated with an assumption that there is 25% carrier rate of hepatitis B in developing countries, and the value of 0.3 and 0.01 represent the potencies for people with HBV infection and without HBV, respectively, estimated from animal studies and epidemiological studies [44].

Population risk = Dietary exposure x Average potency
Average potency = (0.3 x 0.25) + (0.01 x 0.75)
= 0.0825 cancers/100,000 /year per ng AFB1 /kg-bw/day

In this study, we adopted the formula used by Chin, et al. to calculate the population risk for primary liver cancer of dietary exposure to AFBI contamination in spices marketed in Malaysia. Using the prevalence rate of HBsAg+ for adult Malaysian population of 5.24%, the average adult population potency will be [45]:-

Average potency = (0.3 x 0.0524) + (0.01 x 0.9476)
= 0.025 cancers/100,000 /year per ng AFB1 /kg-bw/day

The estimate of the burden of liver cancer attributable to dietary intake of AF can then be calculated using the formula:

% Liver cancer cases attributable to AF intake =

$$\frac{\text{Population risk per year /100,000 population}}{\text{Rate of liver cancer per year /100,000 population}} \times 100$$

Rate of liver cancer per year /100,000 population for Malaysian is based on age-standardized incidence rate for liver cancer of 4.9/100,000 population/year [46].

## Results and Discussion

### Dietary Exposure and MOE for AFB1 Contamination in Spices Marketed in Malaysia

Table 6 shows the calculated dietary exposure to AFB1 contamination in spices ranging from 0.21-1.32 ng/kg-bw/day, based on the mean level of contamination from different studies (overall mean, 0.59 ng/kg-bw/day). Taking the lowest and highest level of AFBI contamination (0.01 and 56.61 μg/kg) gave a dietary exposure range from 0.002-12.27 ng/kg-bw/day. Table 7 shows the MOEs derived from these dietary exposures to AFB1, ranging from 230-1,450 for the range of mean levels. For the lowest and highest level of AFBI contamination in spices, the derived MOEs were 25 and 152,500. Table 8 shows the data for exposure to AFB1 in foods (peanuts, maize, spices and all foods) so far reported in Malaysia including this study, compared to those reported from other

countries. From these data, we derived the MOEs for other studies, based on the calculation adopted in this study for the comparison of MOE values.

Kuiper-Goodman established a Tolerable Daily Intake (TDI) of 0.11-0.19 ng AFB1/kg-bw/day for liver cancer risk per 100,000 populations in Asia [47]. The risk of exposure to consumers for AFBI contamination in spice obtained in this study can be referred to this safety guideline. From Table 7, for the dietary exposure of 0.21-1.32 ng/kg-bw/day (overall mean, 0.59 ng/kg-bw/day), and 12.27 ng/kg-bw/day for the highest level of AFBI contamination, all the levels exceeded the TDI. The overall mean of 0.59 ng/kg-bw/day represents 310-536% of the TDI (3.1-5.4 x TDI) levels for AFB1, which should be considered as a matter of concern, if the risk of dietary exposure is based on the TDI for AFB1.

In our previous study, based on the mean level of 1.38 (range, 0.01-7.68) µg/kg AFB1 contamination in spices, the dietary exposure obtained was 0.09 (range, 0.001-0.45) ng/kg-bw/day, which represents 47–82% of the TDI levels for AFB1 [17]. The lower risk reported in previous study was due to the lower mean contamination level (1.38 µg/kg) and the assumption of a mean daily consumption of spices as 3g and a mean body weight (bw) as 50 kg/person [48]. The results reported in this study based on all the mean levels of AFB1 contamination data in spices from various studies so far available in Malaysia, a spice consumption of 13 g/person/day, and a mean bw of 60 kg/person would give a more representative risk assessment for dietary exposure to AFB1 contamination in spices for consumers in Malaysia [25].

The MOE is a mechanism for comparison of estimated risk between compounds and its magnitude. It gives an indication of the level of concern (the larger the MOE, the smaller the potential risk posed by exposure to the compound under consideration). Since it is not a precise quantification of risk, the level used for evaluation can be set at different levels. For MOE, as mentioned earlier, the value of 10,000 or higher, if it is based on the benchmark dose lower confidence limit ($BMDL_{10}$) from an animal carcinogenicity study, taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view and might be reasonably considered as a low priority for risk management actions [12]. From Table 7, the MOEs derived ranged from 1,450-230 for the range of mean levels and 25 for the highest level of AFBI contamination in spices. All the MOEs derived in this study were less than 10,000 indicating the risk as a high priority for risk management actions.

AF can show effects as an acute toxicant, which would require the use of a high-percentile concentration for the dietary exposure and MOE calculation (as a single exposure may lead to a serious sickness or fatality). However, AF is always show effects as a chronic toxicant over a longer period of time, where consumers would experience many different concentrations over a lifetime of consumption. Thus, in this study (Table 6) we consider all the AFBI contamination data in spices from various studies so far available in Malaysia, which include the overall mean, range of mean levels, and the maximum level of contamination for the calculation of dietary exposure and MOE to give the most representative risk assessment. As shown in Table 6, almost all types of spices including peppers and other spices from a wide range (brand, origin and location) were included in this study in order to evaluate the dietary exposure of AFB1 to Malaysian population. However, areas of sampling may be restricted to Penang, Kuala Lumpur, Putra Jaya and Selangor. In future studies, sample from other areas should be included, and should also include spice samples in fresh or wet form, as well as spices in cooked food ready for consumption. A proper survey of spice intake should be carried out to get the actual amount of daily intake among consumers in Malaysia [49, 50].

In this study and other related studies (Table 6), the spice samples analyzed were those marketed or sold in retail shops or markets, thus the reduction of AF that occurs during the milling and industrial processing was covered. On the other hand, the reduction during the cooking process was not covered, thus need to be studied in future to more accurately estimate the dietary exposure to AFB1 in spices consumed by consumers. However, it had been reported that AF content in spices did not decrease during cooking [51]. Sakuma, et al. studied the reduction of AF in rice after cooking [52]. Assuming that the AFB1 concentration of rice was at LOD (0.1 ng/g) in Japan, they demonstrated that the cooking processing factors for rice was 93.8% and that value was used in the exposure assessment. It was estimated that the dietary exposure to AFB1 in rice at the 95th percentile would be 1.20-2.34 ng/kg-bw/day. The derived MOE was 209-107 based on a benchmarked dose of the $BMDL_{10}$. This result suggested the risk for AFB1 would be significant for high consumers (high intake of staple foods), even though the overall AF contamination was at LOD, thus the regulation of AFB1 for staple foods should be more restricted than for other foods.

Table 8 shows the dietary exposure to AFBI and the derived MOEs in other foods so far available for risk assessment studies in Malaysia and some other countries,

as compared to the results obtained for spices in this study. Dietary exposure to AFBI in peanuts and its products from studies in Malaysia (0.36-8.89, 9.00, 26.20 and 24.37-34.00 ng/kg-bw/day) and the MOEs (9-13, 12, 34 and 34-847), represented a higher calculated risk than in spices (0.59, 0.21-1.32 and 0.002-12.27 ng/kg-bw/day and MOEs of 520, 1,450-230 and 152,500-25). Dietary exposure and MOE of AFBI in spices for cluster G (which include Malaysia) based on international contamination data sources were 0.10 ng/kg-bw/day and MOE of 3,050. On the other hand, the dietary exposure and MOE of AFBI in peanut and maize reported by Liu and Wu were high [2]. Both data sets may not be representative for the risk assessment of AFBI in food to consumers in Malaysia due to insufficient AFBI contamination data and other factors involved in the calculation of dietary exposure specifically for the Malaysian population. Compared to other countries, dietary exposure to AFB1 in Europe and USA was lower than in Malaysia, and was higher in Africa. The dietary exposure to AFB1 in Malaysia as shown in Table 9, was not as high as reported for Asian countries (0.3-53 ng/kg-bw/day) [16].

Overall, the values of dietary exposure and the derived MOEs for AFB1 contamination in spices, as well as in peanuts and products, suggest that risk management or some preventive measures should be considered to control the AF contamination in foods in Malaysia.

| | Range AFB1 µg/kg (ng/g) | Mean AFB1 µg/kg (ng/g) | Dietary exposure to AFB1[a] (ng/kg-bw/day) |
|---|---|---|---|
| White and black pepper[b] | 0.58–4.64 | 2.67 | 0.58 |
| | | 1.23 | 0.27 |
| | | 0.96 | 0.21 |
| Chili | | 2.62 | 0.57 |
| Pepper | 0.65-2.1 | 1.2 | 0.26 |
| Cumin | 1.89–4.64 | 3.26 | 0.71 |
| Chili | 0.20–56.61 | 3.37 | 0.73 |
| Chili | 10.8–33.2 | 4.4 | 0.95 |
| Spices | 0.01–7.68 | 1.22 | 0.26 |
| Spices | 0.32–28.43 | 6.09 | 1.32 |
| Mean of exposure | | | 0.59 |
| Range of mean exposure | | | 0.21–1.32 |
| Range of exposure | 0.01–56.61 | | 0.002–12.27 |

Table 6: Dietary Exposure to AFB1 Contamination in Spices Marketed in Malaysia.

- [a]Dietary exposure to AFBI for the consumption of 13 g spices/day and average body weight of 60 kg.
- [b]See Table 4 for description of source data.

| Exposure Data (ng/kg-bw/day) | MOE[a] |
|---|---|
| Mean of exposure: 0.59 | 520 |
| Range of mean exposure: 0.21 – 1.32 | 1,450-230 |
| Lowest and highest level: 0.002 – 12.27 | 152,500-25 |

Table 7: MOE Derived from Estimates of Dietary Exposure to AFB1 Contamination in Spices.

[a]MOE = $BMDL_{10}$ / Exposure data; MOE value was rounded-up.

$BMDL_{10}$ = 0.305 µg/kg-bw/day (305 ng/kg-bw/day)

## Population risk for primary liver cancer

The Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) estimated potency values for AFB1 from the epidemiological data that showed a positive association between AF and liver cancer. However, studies in which no association was detected, or in which the association was negative, were not used, thus may lead to an overestimate of AF potency. The estimated potency values corresponded to 0.3 cancers/year per 100,000 population per ng AFB1/kg-bw/day (uncertainty range: 0.05-0.5) in HBsAg+ individuals and 0.01 cancers/year per 100,000 population per ng AFB1/kg-bw/day (uncertainty range: 0.002-0.03) in HBsAg- individuals [41, 44, 53].

Direct calculation using the mean dietary exposure to AFB1 in spices of 0.59 ng/kg-bw/day reported in this study will give an estimated potency of 0.18 cancers/year per 100,000 populations in HBsAg+ individuals and 0.01 cancers/year per 100,000 populations in HBsAg- individuals. However as mentioned earlier, few factors were considered in the formula to calculate the overall estimate of liver cancer risk (cases/100,000 population/year) and percent of cancer incidence

attributable to dietary AF, which involved both HBsAg+ and HBsAg- individuals in the population. As shown in Table 8, the calculated population risk for primary liver cancer attributable to AFB1 contamination in spices were 0.0-0.03 (0.1-0.7%) and 0-0.31 (0-6%) cancers/year/100,000 population, respectively for mean and range of exposures from various studies in Malaysia. The risk was low (less than 1 cancer case/year per 100,000 population), as compared to the overall incidence rate registered for liver cancer in Malaysia which was 4.9 per 100,000 for both males and females [46]. It indicates that Malaysian populations are not significantly at risk of developing primary liver cancer attributable to the AF exposure in spices alone.

For contamination of AFB1 in peanuts and its products so far reported in Malaysia, the calculated population risk for primary liver cancer (Table 8) from the few studies conducted in Malaysia were 0.01-0.22 (1.8-4.5%), 0.22 (4.5%), 0.66 (13.4%) and 0.61-0.85 (12.4-17.3%) cancers/year/100,000 population, which were all less than 1 cancer case/year/100,000 population. Malaysian population consumes large amounts of peanuts directly or as ingredients included in special sweets and cookies prepared daily and especially during the "Ramadan" fasting month and festival days. Nuts such as pistachio, hazelnut, walnut, cashew, and almonds are widely consumed in Malaysia [33, 50]. Chin, et al. concluded that current regulatory control for AF in Malaysia (5 µg/kg for all foods ready for consumption) is adequate in protecting Malaysians' health, referring to the data obtained from his study where the calculated population risk for primary liver cancer was reduced from 0.61-0.85 (12.4-17.3%) to 0.06- 0.30 (1.2-6.1%) when excluding AFB1 occurrence data higher than 15 µg/kg, and to 0.01-0.26 (0.2-5.2%) when excluding AFB1 occurrence data higher than 5 µg/kg [14]. The same conclusion should be applicable to the regulatory control of AF in spices as the level of contamination was relatively low (Tables 3 & 4).

Study from Japan estimated that the dietary exposure to AFB1 in rice at the 95th percentile would be 1.20-2.34 ng/kg/day, hence, the cancer risk was estimated to be 0.021-0.040 cancer/100,000 population [52]. It was suggested that the risk for AFB1 would be significant for high consumers (high intake of staple foods), even though the overall AF contamination was at limit of detection (LOD), thus the regulation of AFB1 for staple foods should be more restricted than for other foods. While further studies on the risk assessment of AFB1 contamination in staple foods such as rice in Malaysia should be carried out, it may also be important to monitor AF contamination of spices, since they are important ingredients in almost all dishes and consumed frequently, 2 to 4 times a day. Health impacts by AF, while not negligible, do not justify AF being a top public health priority. Better understanding and quantification of the health impacts of AF exposure can create a more convincing case for prioritizing the reduction of AF exposure, particularly in developing countries where uncontrolled exposure is highest [41].

| Dietary AFB1 exposure, ng/kg-bw/day (MOE)[a] | Estimated liver cancer risk (cases/100,000 population/year)[b] | Cancer incidence attributable to dietary AF (%)[c] | Reference (Type of diet/food) Remarks |
|---|---|---|---|
| 24.37 – 34.00 (13-9) | 0.61 – 0.85 | 12.4 – 17.3% | Chin, et al.[d] (Peanut) [14] |
| 28.81 – 58.02[e] (11-5) | 0.72 – 1.45 | 14.7 – 29.6% | Data for total AF[e] |
| 2.27 – 11.99 (134-25) | 0.06 – 0.30 | 1.2 – 6.1% | Excluding AFB1 occurrence data higher than 15 µg/kg |
| 2.65 – 32.02[e] (115-10) | 0.07 – 0.80 | 1.4 – 16.3% | |
| 0.47 – 10.26 (649-30) | 0.01 – 0.26 | 0.2 – 5.2% | Excluding AFB1 occurrence data higher than 5 µg/kg |
| 0.61 – 30.09[e] (500-10) | 0.02 – 0.75 | 0.3 – 15.4% | |
| 0.36 – 8.89 (847-34) | 0.01 – 0.22 | 1.8 – 4.5% | Leong, et al.[f] (Peanut) [13] |
| | 0.03 – 0.73[g] | | Data by author[g] |
| 9.00 (34) | 0.22 | 4.50% | Arzandeh, et al.,[h] (Peanut) [54] |
| 10.69[e] (28) | 0.27 | 5.50% | Data for total AF[e] |
| 0.59 (520) | 0.01 | 0.30% | This study[i] (Spices) |

| | | | |
|---|---|---|---|
| 0.21 – 1.32 (1,450-230) | 0.01 – 0.03 | 0.1 – 0.7% | |
| 0.002 – 12.27 (152,500-25) | 0 – 0.31 | 0 – 6.3% | |
| 26.20 (12) | 0.66 | 13.40% | Mohd Redzwan, et al.[j] (Foods) [55] |
| 15 – 140 (20-2) | 0.38 – 3.50 | 7.7 – 71.4% | Liu and Wu[k] [2] |
| | 4.5 – 42[g] | | (Peanut and maize) |
| | 0.15 – 1.4[g] | | Data by author[g] |
| 0.10 (Cluster G)i (3,050) | 0.002 | 0.05% | Benford, et al.[l] (Spices) [16] |
| 0.93 – 2.45 (Europe) (328-124) | | | Benford, et al.[am] (Foods) [16] |
| 2.7 (United States) (113) | | | |
| 0.3 – 53 (Asia) (1,017-6) | | | |
| 3.5 – 180 (Africa) (87-2) | | | |

Table 8: MOE and Estimated Liver Cancer Risk and Incidence Derived from Estimates of Dietary Exposure Data to AF Contamination in Foods for Malaysians' Health.

- [a]Using BMDL10 = 0.305 µg/kg-bw/day (305 ng/kg-bw/day) as adopted in this study.
- [b]Calculated based on general adult population potency estimate of 0.025 cancers/100,000 population/year per ng/kg bw/day [46].
- [c]Based on age-standardised incidence rate for liver cancer of 4.9/100,000 population/year [46].
- [d]Based on consumption of peanut from 56.9 g/day for mean population to 324g/day for the 97.5th percentile, and for 62.65 kg body weight. Study by Chin, et al. indicated that current ML for AF (5 µg/kg for all foods ready for consumption) is adequate in protecting Malaysians' health [14].
- [e]Data for total AF.
- [f]Additional calculation added here based on individual type of low and high level of peanut intake (mean intake of 0.77 g/d) among Penang adults, and 60 kg body weight.
- [g]Data (figure) as reported by author in the reference.
- [h]Based on the average Malaysian consumes 56.90 g/day of peanut and the mean concentrations of total AF and AFBI (11.28 and 9.00 ng/g), for 60 kg body weight.
- [i]Based on mean of exposure, range of mean exposure, and lowest and highest level of AFB1 contamination in spices (Table 8).
- [j]Based on extrapolating the mean level of AFM in urine to get the estimated dietary AFB1 exposure (0.0262 µg/kg-bw/day) and for average body weight of 62.65 kg.
- [k]Based on AF exposure data in different countries from multiple sources. Contamination data for the consumption of 35 and 18 g/day of peanut and maize, for 60 kg-bw [4,27,31]. Data from author based on chronic HBV prevalence of 5%, and for HBV-postive (4.5–42) and HB-negative (0.15-1.4) individuals [45] .
- [l]Based on the statistical distribution of AFB1 contamination in foods for 2000 to 2006 worldwide, for overall estimates of international dietary. Exposure to AFB1 (upper-bound scenarios) for MOE studies. Data for spices only [26].
- [m]Based on the statistical distribution of AFB1 contamination in foods for 2000 to 2006 worldwide [26]. Data for all food sources.

## Preventive Measures

The contribution of AF exposure to the liver cancer has been documented [3-5]. Other risk factors include obesity, type 2 diabetes, cirrhosis related to heavy alcohol consumption, nonalcoholic fatty liver disease (associated with obesity), smoking, genetic characteristics of the virus, as well as age and sex of the infected person. Not all risk factors for HCC, including synergistic roles between AF and other carcinogens, are clearly understood. For example, AF also appears to have a synergistic effect on hepatitis C virus (HCV)-induced liver cancer, although the

quantitative relationship is not as well established as that for AF and HBV in inducing HCC [56]. The primary causes of liver cancer can be prevented through public health measures including vaccination, sanitary medical practices, healthy lifestyle choices, and environmental management strategies. The WHO recommends that all countries include the HBV vaccine in routine infant immunization programs. Effective preventive strategies also include limiting alcohol consumption and avoiding smoking. Other approaches to reduce liver cancer in less economically developed countries include reducing AF contamination of foods and preventing and treating parasitic liver fluke infections [57]. According to the WHO data published in May 2014, liver cancer death in Malaysia reached 1,733 or 1.36% of total death. The average annual age-standardized incidence rates (ASR) is 7.78 per 100,000 of population, ranks Malaysia at number 52 in the world [58].

Adopting measures to reduce dietary exposure to AF is crucial for public health. Although it is impossible to completely eliminate AF in food worldwide, it is possible to significantly reduce levels and dramatically reduce liver cancer incidence worldwide. The challenge remains to deliver these interventions to places of the world where they are most needed. AF is a controllable risk factor in foods but some of the exporting countries for these foods may have limited resources to implement most of AF control strategies. Globally, most of the countries have nominally established maximum allowable AF standards in foods but there is little if any enforcement of these standards in many rural areas. In reality, the food in subsistence farming and local food markets is rarely formally inspected. Strict AF standards can even lead to large economic losses for poor food-exporting nations when trading with other nations [59]. Malaysia imports most of its foods including peanuts, some spices and rice, thus will depend in part on the strategies adopted by exporting countries to control the contamination of AF, even though imported food samples at the point of entry are taken at random and analyzed by government laboratories in Malaysia [29]. The presence of AF in the food chain is a serious matter but not knowing its impact to the health should be a matter of concern to the government. A recent survey in Malaysia reported low awareness and knowledge among the public on the problems associated with fungal and AF contamination in the diets [60]. Similar observations were also reported by several studies in African countries [61-64]. This situation requires local authorities in Malaysia to have a more stringent food safety system, enforcement of the existing regulatory control and dissemination of knowledge of AF contamination in foods and its health impacts on the population through awareness programs. Wu and Khlangwiset described multiple public health interventions to control the burden of AF in the body and to prevent HCC [65]. These interventions can be grouped into three categories: agricultural, dietary, and clinical. Agricultural interventions can be applied either in the field (pre-harvest) or in storage and transportation (post-harvest) to reduce AF levels in key crops. They can thus be considered as primary interventions. Dietary (contamination control of AF in foods) and clinical interventions (vaccination against HBV) can be considered as secondary interventions.

## Conclusion

This study highlights the daily consumption of spices in human diet and the potential of AFBI contamination which attributable to the risk of developing liver cancer to consumers in Malaysia. To the best of our knowledge, this is the first report on risk assessment of AFB1 in spices. The result of the study for dietary exposure to AFB1 and the derived MOEs indicated that AFB1 contamination in spices would be of concern from the public health point of view and might reasonably be considered as a high priority for risk management actions. Based on the dietary exposure, the calculated population risk for primary liver cancer attributable to AFB1 contamination were 0.01-0.03 (0.1-0.7%) and 0-0.31 (0-6%) cancers/year/100,000 population, for mean and range of exposures. The risk was low (less than 1 cancer case/year/ 100,000 population) which indicates that Malaysian population are not significantly at risk of developing primary liver cancer attributable to the AF exposure in spices alone. However, considering the liver cancer risk attributable to AFB1 contamination in spices and other foods, there is a need for local authority in Malaysia to have a more stringent food safety system, enforcement of the existing regulatory control and dissemination of knowledge, and awareness program on AF contamination in foods and its health impacts on the population. Further studies are needed to clarify other factors involved in risk assessment of AF contamination in foods in Malaysia, such as contamination of AFB1 in rice.

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## Conflict of Interest

The authors declare that this study has no conflict of interest.

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