REVIEW ARTICLE

A Review on Hepatocellular Carcinoma Attributable to Chronic Aflatoxins' Exposure in Malaysia for the Last Two Decades

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ABSTRACT

High relative humidity and warm climate in Malaysia are essential for the thriving and flourishing of aflatoxin-producing fungi and subsequent contamination of various stored products with aflatoxins which are immunosuppressive, hepatotoxic, genotoxic, enterotoxic and highly carcinogenic proteins. The present review aimed to examine the associable incidence of hepatocellular carcinoma to prolong aflatoxins exposure from foods in Malaysia for the last two decades. The reports from the various literature showed that aflatoxins contamination of foods ranges from 0.004µg/kg to 547.5 µg/kg; attributable dietary exposure to aflatoxins ranges from 0.01ng/kgbw/day to 278.95ng/kgbw/day; estimated percentage incidence of hepatocellular carcinoma per 100,000 Malaysians associable to the reported dietary exposure levels is 0.0045 to 142.24% from 1998 to 2009 and 0.00039 to 51.84% from 2010 to 2020, indicating significant retardation in exposure levels. However, aflatoxin biomarkers in urine (AFM1) and serum (aflatoxin-albumin) range from 0.0024 to 5.34ng/ml and 0.20 to 23.16pg/mg, respectively; thus signifying an estimated 0.15 – 4.08% of liver cancer cases/100,000 population/year in Malaysia. It increases by 3.93% from 2012 to 2020, as the mean levels of biomarkers increased, indicating an increase in exposure to dietary AFB1 in Malaysia. However such increase in aflatoxin biomarkers contrary to the reported estimated dietary exposure, indicates there are other sources of dietary exposure to aflatoxins. Hence there is a need for more research works to cover the other possible sources of exposure such as meat and egg products that are highly consumed in Malaysia, but have not been previously investigated/screened for aflatoxins at consumer level.

Keywords: Aflatoxin exposure, Aflatoxicosis, Hepatocellular carcinoma, Immunosuppression, Malaysia

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INTRODUCTION

Aflatoxins are extremely toxic proteinous exometabolites, classified as group I hepatocarcinogens, secreted by filamentous fungi (aflatoxigenic fungi), particularly *Aspergillus* species section *Flavi* that commonly contaminate various stored food products. As a result, food contamination by these fungi is one of the most significant threats to public health, food safety, and the national economy in several countries. Although mycotoxigenic fungi may not survive food processing processes, research has shown that most mycotoxins, including aflatoxins, are heat-stable in nature (1) and are not denatured or inactivated during food preparation or cooking (2); hence they are generally passed to consumers intact. Thus, for humans' and animals' safety, the need for up-to-date information on the current level of contamination of foods/feeds by mycotoxins and strategies to combat them is of paramount importance.

Aflatoxins, being the most abundant (3) and most carcinogenic mycotoxins are of significant concern in global food safety. Research data showed that they

contaminate about 25% of the worlds food supply annually, leading to an estimated yearly human dietary exposure to about 4.5 billion of the world's population (4), thereby accounting for about 20% (one-sixth) of global deaths each year (4), with about 80% occurring in Africa and Southeast Asia (5). Hence, the need for continuous monitoring of aflatoxins in staples, especially in Africa and Southeast Asia, is very important.

Although many countries have set a minimum permissible limit of aflatoxins in various staples (foods and feeds), it is generally accepted that no dietary level of aflatoxin may be regarded safe dose in both humans and animals since it could lead to acute or chronic toxicities which could be fatal depending on the amount and length of exposure. One of the major adverse effects of long term aflatoxin exposure in humans is the development of hepatocellular carcinomas (HCC), the 3rd and 5th cause of cancer-related deaths in males and females respectively, worldwide (6). It has been shown that about 30% of the estimated annual global cases of HCC (25,200 to 155,000) associated with aflatoxin exposure occur in Southeast Asia (5). Therefore, reviews of relevant literature that will provide up-to-date information on HCC status in Southeast Asian countries are imperative.

The present review aims to examine the HCC attributable to chronic aflatoxins exposure in foods in Malaysia, one of the Southeast Asian countries with suitable climatic conditions (warm and humid weather) for aflatoxigenic fungi to thrive and flourish. The specific objectives of the review were to identify and summarise the reported levels of aflatoxin exposure biomarkers in the serum and urine samples of Malaysians from 1998 to 2020, determine the HCC risks associable to the reported aflatoxin-biomarkers, and determine the associable dietary exposure risks and attributable HCC incidence. The chosen range (1998 – 2020) was selected for this study based on preliminary literature search which showed that most reports of aflatoxin in foods in Malaysia were published between 1998 to present; hence all the 21 years range was selected to ensure comprehensibility of the report in the present study. In addition, the data could help understand the effectiveness of the current aflatoxin intervention/control strategies in Malaysia and identify possible areas that need improvement.

MATERIALS AND METHODS

Search strategy

Published studies (available in Google scholar/Google/Scopus/PubMed search engines) reporting aflatoxins' contamination of foods or biomarkers of aflatoxin exposure in Malaysia from 1998 to 2020 were reviewed based on the guidelines of PRISMA (Preferred Reporting Items for Systematic-Review and Meta-Analyses) (7), using the following search keywords/phrases: (i) "aflatoxin" AND "Malaysia" AND "serum" AND "biomarker" OR "level" OR "exposure", (ii) "aflatoxin" AND "Malaysia" AND "urine" AND "biomarker" OR "level" OR "exposure", and (iii) "aflatoxin" AND "Malaysia" AND "food" AND "contamination" OR "level" OR "exposure".

Records obtained from various sources/search engines were first sorted according to their "titles" and "abstracts" to exclude possible duplicates. Next, screening the titles and abstracts of the various records were made based on this review's specific objectives/eligibility criteria to exclude records with the wrong population, subjects, or outcomes. All review papers were also excluded from this study. Finally, only full-text publications reporting incidence of aflatoxicosis, aflatoxin exposure in Malaysia, aflatoxin biomarkers in Malaysia, HCC attributed to aflatoxin exposure in Malaysia, or aflatoxin levels in Malaysian foods between 1998 to 2020 were included in the study.

Overall, a total of 6, 645 records were obtained from various sources: Google search (311 records), Google scholar (4542 records), Scopus (1325 records), PubMed (437 records) and reference list search (30 records). After applying the record selection criteria stated above, 2972 duplicates were obtained (based on title sorting of the harmonised records) and excluded from the study, and 3506 records were excluded as wrong population, subjects, or outcomes based review of titles and abstracts. Another 131 records (eight duplicates, 123 wrong population, subjects, or outcomes) were excluded after reviewing the full texts of the remaining 167 records. Thus, only 36 full texts records were included in the review. The summary of the record identification and selection criteria based on PRISMA guidelines are depicted in Fig. 1.

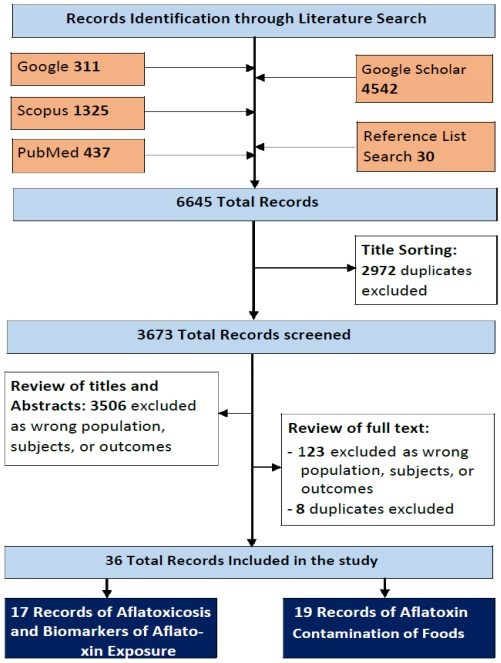

Fig. 1: Flow chart depicting the identification and selection of publications used in the study.

RESULTS AND DISCUSSION

Outbreaks of human aflatoxicosis: Global and Malaysian perspectives

Acute aflatoxicosis occur due to exposure to high doses of aflatoxins. In humans, it mainly affects the liver, gastrointestinal tract, kidney, heart, lungs, circulatory system and often the central nervous system. It is generally characterised by acute liver damage, jaundice, abdominal discomfort, vomiting, haemorrhage, oedema, liver failure and death (3).

Globally, several acute forms of human aflatoxicosis outbreaks have been reported from many countries such as India, Kenya, Uganda, and Tanzania. One of the first reports was that of Taiwan in 1967, which affected 26 people with 3 fatalities (8). In the early 1970s, aflatoxins were isolated in the autopsy of liver tissues of 23 children in Thailand and some children in Czechoslovakia and New Zealand who had died from Reye's syndrome (8). Almost within the same period, in 1972, a 45-year older man from Germany was reported to have died due to developed yellow atrophy of his liver after consuming mouldy nuts believed to contain aflatoxins (9). This was followed by the Indian outbreak of October 1974, in which 106 of 397 patients died following the consumption of maize contaminated by AFB1 and AFB2 (2000 – 6000 µg kg-1) (9). However, Kenyan episodes of acute human aflatoxicosis remain the most severe outbreaks worldwide that claimed the lives of over 600 people due to the consumption of aflatoxin-contaminated maize. These cases started from the early 1980s in which 12 out of 20 patients from Machakos died in 1981 (10,11), three peoples from Meru North died in 1987 (11), 16 peoples from Maua, Meru County died in 2001 (11), 125 out of 317 patients from Makueni, Kitui, Machakos and Thika died in 2004, and 53 peoples died in smaller outbreaks between 2005 and 2006 (11). Recent cases of human aflatoxicosis occurred in 2016 in Uganda and Tanzania, with case fatality rates of 30% out of 68 patients (12) and 75% out of four patients (13), respectively.

In Malaysia, a severe case attributed to aflatoxin occurred in Perak involving 13 children and 40 adult humans. All 13 children died from acute hepatic encephalopathy after consuming Chinese noodles. It was reported that *Aspergillus* fungi and aflatoxins contaminated the key ingredient of the noodles; wheat flour. Clinical manifestation started with Reye-like syndrome accompanied by other symptoms such as anorexia, diarrhoea, febrility, abdominal pain, dizziness, convulsions, and eventual stupor/coma (14). Pathological findings from post-mortem examinations of the liver, spleen, lungs, heart, kidneys, and brain showed damages characteristic of acute aflatoxicosis. There was encephalopathy, extensive coagulative liver and kidney necrosis, hepatocytic metaplasia, sclerosis of central vein, bile stagnation and giant cell formation (14). Death resulted due to acute renal and hepatic failure (14). In addition, a high level of aflatoxins was obtained in the sera, liver, and kidneys of the deceased.

Aflatoxin induced hepatocellular carcinoma (HCC)

Aflatoxin exposure generally occurs in two forms; occupational exposure through dermal and respiratory contacts and non-occupational exposure, which occurs through the consumption of aflatoxin-contaminated products (15). Both conditions of exposure can lead to acute or chronic diseases. The most common manifestation of chronic exposure to aflatoxins is HCC, which occurs mainly in synergy with the hepatitis B (HBV) or C (HCV) viruses and/or other factors (16). These viruses are highly prevalent in African and Asian countries (16). Many studies have reported a strong synergy between HBV and aflatoxins in developing liver cirrhosis and primary liver cancer in HCC patients (17). However, aflatoxins alone have been proven to cause HCC based on several shreds of evidence from experimental animal models treated with aflatoxins in which none of them failed to produce HCC (18). This implies that aflatoxins can induce HCC either in the presence or absence of hepatitis virus(es) (Fig. 2). However, in the hepatitis B positive population, the risk of HCC associated with aflatoxin exposure is 30 times higher compared to the hepatitis B negative population (5). Tumorigenesis of HCC attributable to aflatoxins usually occurs slowly and asymptomatically over the lifetime of the exposed subject as additional doses of aflatoxin are being ingested. In most cases, HCC becomes fully established when the subject is above 30 years of age (19). A study involving adults from five districts in Penang, Malaysia, showed that men above 30 years of age are 3.08 times more exposed to AFB1 ($p = 0.026$) than those between 18 to 30 years in the study (20).

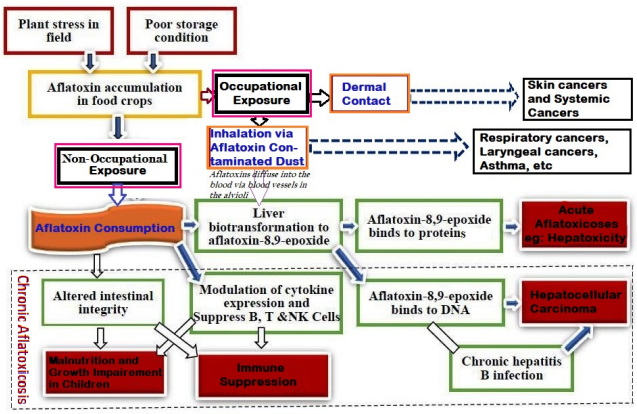

Fig. 2: Aflatoxin exposure and aflatoxicosis pathways in humans (adapted and modified from Negash (27)).

Globally, HCC is the 6th most common cancer (16), accounting for most cancer-related deaths, resulting in an estimated over 600,000 deaths and about 500, 000 new cases annually, with about 80% occurring in

Africa and South Asia (5). Research showed that HCC is respectively the 3rd and 5th cause of cancer-related deaths in males and females worldwide (6). However, from the Malaysian perspective, each year, about 13 HCC cases per 100,000 healthy people occur in Malaysia through Aflatoxin-contaminated maize only (21). According to Raihan and coworkers, yearly life lost due to HCC in Malaysia has increased by 31.5% from 1990 to 2018 (22).

Health effects of aflatoxin biomarkers exposure in Malaysia

Although a limited number of studies were conducted to assess biomarkers of aflatoxin exposure in Malaysia, it is noteworthy that all the studies point to a single fact that most Malaysian people are being exposed to dietary aflatoxins (Table I). All the biomarkers reported are the validated indicators of chronic aflatoxin exposure. Most of the studies have reported that more than 60% of tested samples were present with aflatoxin biomarkers. According to Leong and coworkers, many Malays are being exposed to dietary aflatoxins at lower doses, which is not enough to produce acute illness (20). As shown in Table I, the mean level of excretory aflatoxin M1 in the urine samples increases from 2012 to 2020, indicating an increase in exposure to dietary AFB1 in Malaysia. A recent study of the association between the excretory AFM1 in urine and types of diet consumption per day among 444 respondents in Selangor, Malaysia, further confirmed the reported excretory aflatoxin M1 level from the urine samples was due to dietary AFB1. Aflatoxin M1 was detected in 199 urine samples with 37 samples containing AFM1 above 0.64 ng/ml. It was found that cereals have the highest average dietary intake level (512.54 g per day) among all the respondents (23).

Based on the data presented in Table I, the estimated dietary exposure to AFB1 of the individuals in the reported population can be calculated using the regression equation (24), which describes the association/relation between urine AFM1 and dietary AFB1 as follows:

$$\text{Urine AFM1} = -0.042 + 0.0026\ \text{AFB1} \quad (1)$$

$$\therefore \text{Dietary AFB1} = \frac{((\text{Urine AFM1} + 0.042))}{0.0026} \quad (2)$$

The estimated population dietary exposure to AFB1, indicated by the reported urine AFM1, is between 0.29 to 7.81 ng/kgbw/day and the estimated populations' HCC cases per 100,000 Malaysian population per year range from 0.0073 cases to 0.2, which contribute 0.15 – 4.08% cancer cases per year. Overall, the reports showed that liver cancer cases attributable to aflatoxin exposure have increased by 3.93% from 2012 to 2020. This calls for more stringent preventive and/or control strategies to bring down Malaysia's liver cancer incidence rate. Similarly, a high prevalence (above 95% of tested samples) of serum aflatoxin-albumin was reported in two different studies with values ranging from 0.20 to 23.16pg/mg albumin (20,25). These values also suggest high exposure to dietary AFB1 in the study population because serum AFB1 represents only about 2-10% of ingested AFB1 (26). In fact, several studies showed that only about 1.4% to 2.3% of ingested aflatoxin (AFB1) becomes covalently bound to the serum protein albumin in both human and animal studies. This further indicates that the reported levels of serum aflatoxin (AFB1-albumin) in Malaysia may be of public health concern.

Table I: Biomarkers of aflatoxin exposure in Malaysia and their attributable cancer risks

Year	Location	Number and type of Subjects tested	Type of aflatoxin biomarker	Percentage of positive samples	Level of contamination (ng/ml)	Estimated Dietary AFB1 Exposure		[c] Estimated HCC (Cases/100,000 population/year)	[d] % Incidence of HCC per 100,000 population/year	Reference
						[a]ng/day	[b]ng/Kg bw/day			
2012	UPM, Malaysia	22 healthy non-academic staff	Urine AFM1	100%	R = 0.0289 – 0.1547 (M = 0.0421)	32.35	0.52	0.013	0.27	(28)
2012	Selangor/ Malaysia	160 subjects	Urine AFM1	61.3% (98/160)	R = 0 to 0.0747 (M ± SD = 0.0234 ± 0.0177) MR = 0.0057 to 0.0411	18.35 to 31.96	0.29 to 0.51	0.0073 to 0.013	0.15 to 0.27	(29)
2012	Malaysia	170 subjects	Serum AFB1-lysine adducts	97%	[a] R= 0.20 – 23.16 (M ± SD =7.67±4.54; Md=7.12)	-	-	-	-	(20)
2014	UPM/ Malaysia	71 subjects	Serum AFB1-lysine adduct	100%	[a] R = 1.13 to 18.85 (M ± SD = 6.85 ± 3.20)	-	-	-	-	(25)

CONTINUE

Table I: Biomarkers of aflatoxin exposure in Malaysia and their attributable cancer risks (CONT.)

Year	Location	Number and type of Subjects tested	Type of aflatoxin biomarker	Percentage of positive samples	Level of contamination (ng/ml)	Estimated Dietary AFB1 Exposure		[c] Estimated HCC (Cases/100, 000 population/year)	[d] % Incidence of HCC per 100, 000 population/year	Reference
						[a]ng/day	[b]ng/Kg bw/day			
2015	UPM/ Malaysia	71	Urine AFM1	18.57% (13/71)	R = 0.0024 to 0.1004 (M ± SD = 0.0188±0.0286)	34.23	0.55	0.014	0.29	(30)
2018	Selangor/ Malaysia	444 subjects	Urine AFM1	44.82% (199/444)	R= 0 to 5.34 M = 1.23	489.23	7.81	0.200	4.08	(23)
2020	Terengganu / Malaysia	206 adult subjects	Urine AFM1	40.8% (84/206)	R = 0.07 to 5.53 M = 0.589	242.69	3.87	0.097	1.97	(31)

- [a] Concentration is in pg/mg albumin
- M ± SD = Mean ± standard deviation
- R = Range
- M = Mean
- Md = Median
- MR = mean range
- [a]Values were calculated using the regression equation of (24)253 samples, AFM1 in urine = -0.042 + 0.0026AFB1, $R^2 = 0.65$
- [b] Estimated Dietary AFB1 Exposure (ng/kgbw/day) = Estimated Dietary AFB1 Exposure (ng/day) divided by the average body weight of adult Malaysian population (62.65kg) (33)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. Excluding AFB1 occurrence data higher than 15 mg/kg reduced exposure by 65%-91% to 2.27-11.99 ng/ kg b.w./day, reducing the cancer risk to 0.06-0.30 cancers/100,000 population/year (contributing 1.2%-6.1% liver cancer cases
- [c]Estimated HCC (Cases/100, 000 population/year) = population potency estimate of 0.025 cancers/100,000 population/year per ng/kg bw/day (33)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. Excluding AFB1 occurrence data higher than 15 mg/kg reduced exposure by 65%-91% to 2.27-11.99 ng/ kg b.w./day, reducing the cancer risk to 0.06-0.30 cancers/100,000 population/year (contributing 1.2%-6.1% liver cancer cases multiply by Estimated Dietary AFB1 Exposure (ng/kgbw/day).
- [d] % Incidence of HCC per 100, 000 population/year = [(Estimated HCC (Cases/100, 000 population/year) X 100%] divided by average liver cancer incidence in Malaysia = 4.9/100,000 population/year as reported in 2006 by the Ministry of Health, Malaysia.

Aflatoxin contamination of staple foods in Malaysia and the attributable cancer risks (1998 – 2020)

Various literature reported aflatoxin contamination of staple foods in Malaysia were reviewed for two decades from 1998 to 2020. Food items reported by the various literature within these 20 years include barley, rice, maize, wheat, peanuts and nut products, spices, milk and other dairy products (Table II). The rate of detection of aflatoxins in the various food categories was higher in peanuts and spices, in which the percentage of positive samples is above 50% in most of the studies. Also, peanuts have the highest contamination level ranging from 0.30 to 547.50µg/kg, followed by the cereals with a contamination range of 0.01 to 436.25µg/kg, spices with a range of 0.10 to 101.20µg/kg, and dairy products with the least contamination ranging from 0.004ppb to 100.50ppb. However, in terms of levels of dietary exposure to aflatoxins (ng/kgbw/day) which is determined by the consumption of the foods per capita, rice has the highest attributable dietary exposure to aflatoxins ranging from 0.8ng/kgbw/day to 278.95ng/kgbw/day followed by corn with a range of dietary exposure of 0.38ng/kgbw/day to 47.73ng/kgbw/day, spices with a dietary exposure range of 0.021ng/kgbw/day to 21.0ng/kgbw/day, milk with a range of 0.00077ng/kgbw/day to 19.34ng/kgbw/day, peanuts with a range of 0.0086ng/kgbw/day to 8.89ng/kgbw/day, and other cereals having the least attributable dietary exposure range of 0.01ng/kgbw/day to 2.99ng/kg bw/day. These aflatoxin exposure levels could account for an estimated % incidence of HCC per 100,000 population in Malaysia, ranging from 0.41 to 142.24%, 0.19 to 24.29%, 0.011 to 10.82%, 0.00039 to 9.8%, 0.0045 to 4.49%, and 0.0019 to 1.53% due to consumption of rice, corn, spices, milk, peanuts, and other cereals respectively (Table II). Overall, the estimated dietary exposure to aflatoxins in Malaysia could account for an estimated incidence of HCC per 100,000 Malaysian population ranging from 0.0045 to 142.24% (mean range = 0.22 to 24.93%) between 1998 to 2009 and 0.00039 to 51.84% (mean range = 1.43 to 5.23%) between 2010 to 2020; which indicates a significant reduction in the dietary exposure levels.

Compared to the biomarkers in urine and serum, the reported level of aflatoxin in Malaysian foods signified low exposure to aflatoxins. But it should be noted that aflatoxin research for many products at consumer levels in Malaysia is still inadequate. As noted in the present review, there are many foods that are highly consumed in Malaysia (e.g., chicken meat and eggs) that could serve as potential sources of exposure to aflatoxins, yet there were no reports of aflatoxin screening from these products within the selected range (1998 to 2020) for

this review. Hence, aflatoxin studies that could cover the unreported food products will be helpful in estimating the extent of aflatoxin exposure in Malaysia.

Based on the various literature analysed in this review, some of the factors that were identified as being responsible for aflatoxin contamination of food products in Malaysia include a high level of fungal spores in some imported food products, which are not being given much emphasis while checking/screening of imported foods by the relevant authorities (50), poor storage facilities (e.g. leaky roofs and insufficient ventilation outlets) used by some of the staple's sellers trading in open markets (non-supermarket shops), and inter transportation of commodities under warm temperatures and high relative humidity among the stakeholders. Other factors include storage of the food commodities such as peanuts in polythene bags (which are known to enhance deterioration by accumulating heat within them and preventing good airflow) instead of jute bags by most retailers, fluctuations in the relative humidity and temperatures in the open markets where the commodities are being sold, and the general lack of knowledge of fungi and aflatoxins by most retailers which lead to poor handling/hygienic practices of the commodities (50). This, therefore, calls for proper intervention/preventive and control strategies to address these issues to reduce/prevent the exposure risks to these toxic carcinogens.

Table II: Cancer risk attributable to dietary aflatoxin exposure in Malaysia

Year	Location	Number and Type of Food Samples	Percentage of Positive Samples	Level of Aflatoxins (ng/g)	[1] Estimated Level of dietary Exposure to AFB1 (ng/kgbw/day)	[2] Attributable Primary Liver Cancer (HCC) per 100,000/ year	[3] % Incidence of the HCC per 100,000	Reference
1998	Malaysian Markets	84 samples of rice grains	- AFG1 = 2.4% - AFG2 = 3.6%	R= 0.369 – 77.5	[c] 1.33 – 278.95	0.033 – 6.97	0.67 – 142.24	(34)
1998	Malaysian Markets	83 wheat flour	- AFB1 = 1.2% - AFB2 = 3.8% - AFG1 = 3.6% - AFG2 = 13.25%	-R= 2.5 – 6.2 - R= 112.5 – 252.5 - R= 25 – 289.38 - R= 162.50 – 436.25	[d] 1.21 – 2.99	0.030 – 0.075	0.62 – 1.53	(34)
1999	Malaysia and Philippines	- 22 peanuts - 8 corns	- 63.64 % - 100%	- R= 1 – 378 - R= 1 – 130	- [e] 0.012 – 4.65 - [d] 0.38 – 47.73	- 0.0003 – 0.12 - 0.0095 – 1.19	- 0.0061 – 2.45 - 0.19 – 24.29	(35)
2003	Perak	210 groundnut samples	- AFB1 = 61% - AFG1 = 56% - TAF = 92%	- [a] R= 0.7 – 547.5 - [a] R= 0.3 – 376 - [a] R= 0.3 – 762.1	- [e] 0.0086 – 6.73	0.00022 – 0.17	0.0045 – 3.47	(36)
2009	Malaysia	126 commercial white and black pepper	55.5% (70/126)	R= 0.1 – 4.9	[f] 0.021 – 1.02	0.00053 – 0.026	0.011 – 0.53	(37)
2010	Malaysia	60 cereal samples	NS	R= 0.01 – 5.9	[d] 0.0038 – 2.24	0.000095 – 0.056	0.0019– 1.14	(38)

CONTINUE

Table II: Cancer risk attributable to dietary aflatoxin exposure in Malaysia (CONT.)

Year	Location	Number and Type of Food Samples	Percentage of Positive Samples	Level of Aflatoxins (ng/g)	[1] Estimated Level of dietary Exposure to AFB1 (ng/kgbw/day)	[2] Attributable Primary Liver Cancer (HCC) per 100,000/year	[3] % Incidence of the HCC per 100,000	Reference
2010	Penang	196 nuts and their products	TAF = 16.3% (32/196)	R= 16. 6 – 711 M =17.2 – 350	[e] 0.21	0.0053	0.11	(41)
2011	Malaysian Markets	- 30 rice samples - 10 wheat - 5 maize samples	- 33.33% (10/30) - 30% (3/10) - 20% (1/5)	-M± SD = 2.120 ± 0.260 -M ± SD = 3.160 ± 0.230 -M ± SD = 0.250	- [c] 2.44 – 3.13 - [d] 1.11 – 1.29 - [d] 0.095	- 0.061 – 0.078 - 0.028 – 0.032 - 0.0048	- 1.24 – 1.59 - 0.57 – 0.65 - 0.098	(42)
2011	Penang	128 nuts and their products	57%	R = 4 – 222	*0.36 to 8.89	0.009 – 0.22	0.18 – 4.49	(43)
2012	Malaysian Open and S/markets	80 chilli samples	TAF = 65% (52 samples)	R = 0.2 – 101.2	[f] 0.042 - 21	0.0011 – 0.53	0.022 – 10.82	(44)
2012	Malaysia	10 chilli	- TAF = 90% - AFB1 = 20%	M = 13.6 M = 4.40	[f] 0.91	0.023	0.47	(45)
2012	Malaysia	236 ready to eat foods composites	NS	R = 0.430 – 32.420	* 24.3 to 34.00	0.61-0.85	12.45 – 17.35	(33)
2013	Selangor	50 red rice	92%	R = 0.610 –77.330	[c] 0.8 – 101.58	0.02 – 2.54	0.41– 51.84	(46)
2015	Penang	34 commercially processed spices	- TAF = 88% - AFB1 = 83%	- TAF: M = 8, 380 - AFB1: M= 7, 310	*M = 0.09	0.0023	0.047	(47)
2017	Terengganu	53 milk and dairy products	AFM1 = 35.8%	R = 3.5 – 100.5	[g] 0.67- 19.34	0.17 – 0.48	3.47 – 9.8	(48)
2017	Penang	33 dairy products	AFM1 = 10%	[b] 0.004 – 0.01	[g] 0.00077 – 0.019	0.000019 – 0.00048	0.00039 – 0.0098	(49)
2018	Malaysia	- 13 peanuts from importers - 81 raw and peanuts - based samples from retailers - 84 raw and peanuts - based samples from the manufacturer	- 0% - 38% - 22%	- M = 0.00 - M = 120.7 (MD=1. 4) - M= 20.5	0.00 [h] 8.61 [h] 1.46	0.00 0.22 0.037	0.00 4.49 0.76	(50)

Note:

- Reported levels of AFB1 in foods (mean or range) were used in computing the estimated primary cancer attributable to aflatoxins exposure/100,000 population. Where the level of AFB1 was not given, the levels of total aflatoxins or AFM1 were used. However, palaces where AFM1 was used, the values were divided by 10 since AFM1 is 10 times less carcinogenic than AFB1.
- Abbreviations used include: AFB1 = Aflatoxin B1; AFB2 = Aflatoxin B2; AFG1 = Aflatoxin G1; AFG2 = Aflatoxin G2; AFM1 = Aflatoxin M1; H= Highest contamination value; M = Mean; MD = Median; MR = Mean Range; NS = Not stated; R = Range; SD = Standard deviation; TAF = Total aflatoxins;
- [1] Estimated Level of dietary Exposure to AFB1 (ng/kgbw/day) = [level of contamination in food (ng/g) x Daily amount of food consumed per person (g/day)] ч (Average Body weight (kg-bw), 62.65kg (13)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. Excluding AFB1 occurrence data higher than 15 mg/kg reduced exposure by 65%-91% to 2.27-11.99 ng/ kg b.w./day, reducing the cancer risk to 0.06-0.30 cancers/100,000 population/year (contributing 1.2%-6.1% liver cancer cases)
- [2] Attributable Primary Liver Cancer (HCC) per 100,000/year = Estimated Level of dietary Exposure to AFB1 (ng/kgbw/day) x the average Malaysian population potency estimate of 0.025 cancers/100,000 population/year per ng/kg b.w./day (13)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. Excluding AFB1 occurrence data higher than 15 mg/kg reduced exposure by 65%-91% to 2.27-11.99 ng/ kg b.w./day, reducing the cancer risk to 0.06-0.30 cancers/100,000 population/year (contributing 1.2%-6.1% liver cancer cases.
- [3] % Incidence of the HCC per 100,000 = [Attributable Primary Liver Cancer (HCC) per 100,000/year X 100%] divided by average liver cancer incidence in Malaysia= 4.9/100,000 population/year (as reported by Ministry of Health, Malaysia)
- * Exposure data was provided by the author
- [a] Concentration expressed in ng/L
- [b] Concentration expressed in ng/mg albumin
- [c] **to** [h] represent information of the reported average population's daily consumption of the stated food item per person (g/person/day) as follows: [c] Rice = 225.5g ; [d] Cereals = 23.8g; [e] Peanut = 0.77g (10); [f] Spices = 13g; [g] Milk = 120.55; and [h] Peanut = 4.47g

CONCLUSION

The reported aflatoxin levels in Malaysian foods signify a decrease in dietary exposure and liver cancer cases from 24.93% to 5.23% from 1998 to 2020. On the contrary, the reported levels of aflatoxin biomarkers from human body fluids signified a 3.93% increase in exposure levels to aflatoxins in Malaysia from 2012 to 2020; thus suggesting other potential possible sources of dietary exposure to aflatoxins such as in meats, fish, potatoes and many other food items yet to be reported. Based on the identified factors that promote aflatoxin contamination in Malaysia and consequent dietary exposure, it is recommended to enhance monitoring of marketed products, especially those produced locally and sold to consumers directly in local markets, without undergoing the necessary fungal/aflatoxin screening being imposed on imported food products. Fungal bioburden should be given much emphasis while screening imported food products from importers. In addition, there is a need for educational interventions on the knowledge of fungi and aflatoxins and factors that promote their presence in foods at various stakeholders level to improve their level of awareness, attitudes and hygienic practices toward handling and processing food commodities to avoid or reduce fungal/aflatoxin contaminations of the commercial foods.

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