Situation of mycotoxins Contamination in food and regulation on mycotoxins in Malaysia

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Abstract

The occurrence of mycotoxin in a large number of foods is a major health concern for Malaysia, as some mycotoxins such as aflatoxin are genotoxic carcinogenic substances where there is no threshold level below which no harmful effect is observed. For most mycotoxin, current scientific and technical knowledge and improvements in production and storage techniques do not prevent the development of moulds that produce these toxins. As such, setting limits that are practical and achievable and monitoring mycotoxins levels become necessary, which Malaysia has carried out. Monitoring of aflatoxin in raw peanuts imported into Malaysia in 2004 indicated a detection rate of 50 % and 6 % were detected with total aflatoxin exceeding 15 μg/kg [Maximum level in Malaysia's Food Regulation]. In 2004 and 2005, samples of raw peanut taken from retailed market showed 81 % were detected with aflatoxin and 38 % were detected with more than 15 μg/kg of total aflatoxin. The current practice of storage and the natural high temperatures and humidity levels contributed to the high occurrence at the retail market. In comparison roasted peanut in-shell was found to be much less contaminated. However, the rice was found to be relatively safe. A total of 96 rice sample taken in 2005 showed no trace of aflatoxin contamination. Only 18 out of 200 rice samples analyzed (9 %) contaminated with traces of zearalenone ranged between 6 to 11 μg/kg and none contaminated with ochratoxin. Determination of aflatoxin was performed by HPLC with photochemical reactor after cleaned up using immuno affinity column. Zearalenone and ochratoxin were determined by HPLC fluorescence detection after cleaned up using Immuno Affinity Column. In this paper, the mycotoxin monitoring results in 2004 and 2005 is reviewed and health hazard using risk assessment approached related to aflatoxin is assessed.

Key words: mycotoxin, aflatoxin, monitoring, risk assessment, Malaysia

Introduction

Mycotoxins are a group of compounds produced by some strains of certain fungi that can cause illness or death when ingested by man or animals. Aflatoxins are a type of mycotoxins that are classified as human genotoxic carcinogens substances where there is no threshold level below which no harmful effect is observed. Aflatoxins can be found contaminated in food and feed-stuffs, and are produced by the common fungi Aspergillus.
flavus and A. parasiticus. The aflatoxin problem is a worldwide phenomenon, but it is particularly severe in some developing countries, where food safety and security systems are not well developed to protect consumers against unsafe food products.

This fungal toxin has attracted worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and both international and domestic trade. Environmental factors such as high temperature and humidity, rainy seasons and improper storage practices, makes the tropical region prone to high levels of aflatoxin contamination. In Malaysia, mycotoxin research started in 1965 initiated by the Institute of Medical Research Malaysia, which dealt mainly with the aflatoxin contamination in food\textsuperscript{37}. The other agencies involved in aflatoxin analysis and research including Malaysia Agriculture Research and Developments Institute (MARDI), The Department of Chemistry of Malaysia, Ministry of Agriculture and Food Control Laboratory (Public Health Laboratory and Food Safety & Quality Laboratory), Ministry of Health Malaysia (MOH). The method of analysis used then was by Thin Layer Chromatography (TLC), silica mini-column cleaned up, ELIZA test kit, HPLC post column. The Malaysian Food Regulation (1985) Amendment 2003 specified that the maximum level of aflatoxins in food shall not be greater than 5 µg/kg in processed food and not greater than 15 µg/kg for groundnut for further processing\textsuperscript{31}.

**Samples collection, analysis and risk assessment**

**Sample collection** In 2004 and 2005, occurrence of aflatoxins in shell-roasted groundnuts, processed peanut, raw peanut and rice were investigated. The samples were obtained from the retailed markets, taken by the health districts office throughout Malaysia. A total of 116 raw peanut, 98 roasted peanut and 96 of rice samples were analyzed by National Public Health Laboratory. Determination of aflatoxins in groundnuts was performed according to ELISA screen kit and positive samples were further confirmed by HPLC with fluorescence detection after derivatisation with trifluoroacetic acid as well as by photochemical reactor enhancement (PHRED). While in 2003 and 2004, 200 rice samples were collected throughout the country for zearalenone and ochratoxin analysis.

**HPLC analysis in National Public Health Laboratory, MOH Malaysia** Analyses of aflatoxin were performed with Shimadzu HPLC system using Inertisil ODS 3V (250 x 4.6 mm) column, mobile phase consisted of water: methanol: acetonitrile (70:20:10, v/v/v) with flow rate of 1 mL/min and chromatograph at 365 nm excitation and 450nm emission. Extraction of the samples prior to HPLC analysis were using immuno affinity column (IAC, VICAM). Limit of quantification (LOQ) for total aflatoxins was 1.2 µg/kg (0.4 µg/kg for AFB\textsubscript{1} and AFG\textsubscript{1} and 0.2 µg/kg for AFB\textsubscript{2} and AFG\textsubscript{2}). Linear range of aflatoxins calibration curve was between 0.2 to 100 ng/mL of aflatoxins. The chromatograms for the standard solution of aflatoxin mixture of B\textsubscript{1} and G\textsubscript{1} at 0.4 µg/kg; B\textsubscript{2} and G\textsubscript{2} at 0.2 µg/kg analyzed by HPLC with photochemical reactor enhancement and pre derivatization are shown in Fig. 1 and 2, respectively.

For zearalenone analyses, rice samples were extracted with acetonitrile – water (9:1, v/v) and sodium chloride. The extract was centrifuge and diluted with phosphate buffered saline containing tween 20 to reduce interferences materials. Then, the solution was applied to immunoaffinity column, washed with phosphate buffered saline including tween 20 before eluted with methanol and concentrated to dryness. The dried elutant was dissolved in methanol –water (3:7, v/v) before introduce to HPLC. As for Ochratoxin determination, the samples were extracted with methanol – 1 % NaHCO\textsubscript{3} (7:3, v/v), filtered and diluted with 0.01 % tween 20 in phosphate buffered saline and then filter again with glass fiber filter under
Fig. 1. Chromatogram of mixed aflatoxin standard (0.4 µg/kg for AFB1 and AFG1 and 0.2 µg/kg for AFB2 and AFG2). Column Inertsil ODS 3V (250 x 4.6 mm), mobile phase consisted of water: methanol: acetonitrile (70:20:10, v/v/v) with flow rate of 1 mL/min and chromatograph at 365 nm excitation and 450 nm emission (photochemical reactor enhancement).

Fig. 2. Chromatogram of mixed aflatoxin standard (0.4 µg/kg for AFB1 and AFG1 and 0.2 µg/kg for AFB2 and AFG2). Column Inertsil ODS 3V (250 x 4.6 mm), mobile phase consisted of water: methanol: acetonitrile (70:20:10, v/v/v) with flow rate of 1 mL/min and chromatograph at 365 nm excitation and 450 nm emission (trifluoroacetic acid pre derivatization).

vacuum condition. Then the solution was applied to immunoaffinity column, washed using Phosphate buffered saline- 0.01 % tween 20 and Phosphate buffered saline (PBS) before eluted with methanol and evaporate to dryness. The elutant was dissolved with acetonitrile-water-acetic acid (30:70:1, v/v/v) before HPLC.
**Hazard assessment of aflatoxin**  
Aflatoxins are both acutely and chronically toxic to animals, including human. Liver is the primary target organ in most species. Human exposure to aflatoxin is principally through ingestion of contaminated foods. Epidemiological studies in Africa and Asia supported a positive correlation between aflatoxin ingestion and occurrence of human primary liver cancer. Studies revealed that the coexistence of hepatitis B virus infections contribute to higher incidence of liver cancer in aflatoxin exposed population. According to Groopman and Kensler (2005) they were two major cohort case-studies incidence report (Shanghai and Taiwan studies) using aflatoxin-acid nucleic adduct as biomarker to address the relation of aflatoxin exposure to human hepatocellular carcinoma. Since aflatoxins are carcinogenic, there is no NOEL and no ADI allocated. Therefore, potency estimates which was derived from animal toxicity and epidemiological studies for human liver cancer resulting from exposure to aflatoxin B\(_1\) have been proposed by JECFA (1997)\(^5\)-\(^6\). Potency value of 0.3 cancers/year per 100,000 population per ng aflatoxin/kg body weight per day for positives hepatitis B individuals while potency value of 0.01 cancer for non hepatitis individual has been estimated by JECFA. Studies by Medical Science Study Center, University Science of Malaysia shows in Malaysia, the hepatitis B virus carriers vary from 5% in West Malaysia to 13% in Sabah and Sarawak\(^8\). Margin of Exposure (MOE) which is the ratio between a dose leading to tumors in experimental animals and the human intake for aflatoxin based on Benchmark Dose Level 10 values (BMDL\(_{10}\)) was 0.16 µg/kg bw/day\(^1\). This value is based on BMD calculation as modeled on hepatocellular carcinoma in male rats\(^9\).

**Calculation aflatoxin intake for risk assessment**  
In addition to information about aflatoxin toxicity, exposure assessment is another main component of risk assessment. Aflatoxin concentration data from the survey conducted in 2004 and 2005 for raw peanut and roasted groundnut, which represent random samples taken from each state in Malaysia was used for dietary exposure. The mean value of total aflatoxin for raw peanuts and roasted groundnut was 119 and 2.28 µg/kg respectively. Not detected results were assumed as equal to 0.6 µg/kg, which was half of the limit of quantification and this value is used in deriving the mean concentration. From the survey, the mean value was used in calculation of exposure assessment for peanut to represent situation of aflatoxin contamination. The limited number concentration data from peanut butter (10 samples) and satay gravy (5 samples) which were analyzed in 2006 were also included in the intake assessment. Food Consumption Statistic of Malaysia 2002/2003 of adult population (18-59 years old), which is based on Food Frequency Questionnaire (FFQ) was used as food consumption data\(^2\). In FFQ method, a semi-quantifiable serving size as reference (food album) was used during this survey and presented as the estimated consumption of each food (g/day).

**Discussion**

**Mycotoxin incidences in Malaysian food**  
Of 116 raw peanut samples analyzed, 22 samples (19%) were free of aflatoxin contamination. 58 raw peanut samples (50%) were contaminated with single or in combination of aflatoxin B\(_1\), aflatoxin B\(_2\), aflatoxin G\(_1\) and aflatoxin G\(_2\), with a concentration range of between limit of quantification and below 15 µg/kg. 44 raw peanut samples (38%) were found to have exceeded maximum level of 15 µg/kg prescribed in the Malaysian Food Regulation 1985. Aflatoxins B\(_1\) was predominantly present. Fig. 3 shows occurrence of aflatoxin in raw peanut samples taken from the national monitoring in 2004 and 2005 in comparison with the aflatoxin occurrence data taken from imported raw peanuts samples in 2004. Monitoring of aflatoxin in raw peanuts imported into Malaysia in 2004 indicated a detection rate of 50% and 6% were detected with total aflatoxin exceeding 15 µg/kg. For processed peanut including roasted groundnut, 17 out of 98 samples
(17%) were detected with aflatoxins where 3 samples (3%), contravened the Malaysian Food Regulation 1985. The aflatoxins level found were above 5 μg/kg (Fig. 4). Rice samples shown none of 96 samples were contaminated with aflatoxins (< 1.2 μg/kg).

For other mycotoxin, only 18 out of 200 rice samples analyzed (9%) were detected with traces of zearalenone ranging between 6 to 11 μg/kg and none were contaminated with ochratoxin A (< 0.5 μg/kg).

**Intake of Aflatoxin from Consumption of Peanuts in the Malaysian Diet**

Due to limitations in the data, various assumptions were made in calculating the dietary exposure of the Malaysian population to aflatoxin from consuming peanuts. Firstly, it was assumed that aflatoxin in raw peanut was not reduced from food preparations or due to various cooking style i.e. reduction from processing is not taken into consideration. Secondly, peanuts sampled are considered representatives except for satay gravy and peanut butter which was based on limited number of samples. And thirdly, the consumption of nuts of 57 g/day is attributed solely to peanuts, even though a portion maybe attributed to the consumption of other types of nuts. As such the food consumption data representing current eating pattern showed that the consumption of peanuts among Malaysian population is on the high side. The food consumption also meant for ready peanut to be consumed so the consumption of raw peanut in the cooking estimated to be much lower and consumption of 4.12 g/day nuts was chosen.

Based on the above limitations, total dietary exposure of aflatoxin from consumption of peanuts in Malaysian is estimated about 10 ng/day/ kg body weight, as showed Table 1. This gives an overestimate of the actual exposure.

![Aflatoxin incidences in raw peanut 2004-2005](image)

**Fig. 3.** Occurrence of aflatoxin in raw peanut from monitoring data in 2004 and 2005 and import data 2004
Fig. 4: Occurrence of Aflatoxin in Roasted Peanut in 2004 and 2005.

**Risk Estimate**  The risk for Malaysian population was calculated based on JECFA’s cancer potency estimate for hepatitis B positives individual of 0.3 cancers per year per 100,000 populations per ng aflatoxin per kg body weight and hepatitis B negative individuals of 0.01 cancers. In Malaysia, it is estimated potency cancer risk of consumed food contaminated with aflatoxin in positive hepatitis B individuals were 3 cancers per 100,000 population while 0.1 cancer per 100,000 population of negatives hepatitis B individual. Expressing the cancer risk in Malaysian population based on the assumption that 13% of the Malaysian population carries the hepatitis B virus; the risk estimated was 0.47 cancers per 100,000 populations due to intake of aflatoxin. The MOE calculated based on Benchmark Dose Level 10 values (BMDL10) of 0.16 μg/kg bw/day is far below 10,000 indicating exposure to aflatoxin is of public health concern.

Table 1. Dietary exposure to aflatoxin in Malaysia diet for 18-59 year olds with the average body weight 63 kg.

<table>
<thead>
<tr>
<th>Food type</th>
<th>Mean analytical level (μg/kg)</th>
<th>Food consumption (kg/day)</th>
<th>Estimated mean dietary exposure of aflatoxin (μg/day)</th>
<th>% of total exposure from all foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut, roasted</td>
<td>2.28</td>
<td>0.057</td>
<td>0.13</td>
<td>19</td>
</tr>
<tr>
<td>Peanut, raw</td>
<td>119</td>
<td>0.004</td>
<td>0.48</td>
<td>72</td>
</tr>
<tr>
<td>Peanut butter</td>
<td>5.0</td>
<td>0.003</td>
<td>0.02</td>
<td>3</td>
</tr>
<tr>
<td>Satay sauce</td>
<td>9.1</td>
<td>0.004</td>
<td>0.04</td>
<td>6</td>
</tr>
<tr>
<td>Total dietary exposure (μg/day)</td>
<td></td>
<td></td>
<td>0.66</td>
<td>100</td>
</tr>
<tr>
<td>Total dietary exposure (μg/day/ kg bw)</td>
<td></td>
<td></td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

The outcome of the preliminary risk assessment of aflatoxin in Malaysian food showed that there was a perceived appreciable health risk among the population, even though the aflatoxin assessment was performed based on assumptions, which gives an overestimate of the risk. As such, the risk assessment needs to be refined by collecting more accurate concentration and intake data to obtain a more realistic estimation of the risk.

While the complete elimination of aflatoxin in food is extremely unlikely, efforts to reduce aflatoxin in foods the lowest levels that are technologically feasible should be made. Some control measures have already been undertaken by the Malaysian authority. This includes imposing health certificate requirements declaring the level of aflatoxin on every consignment of imported peanuts, as Malaysia is a net importer of peanuts. Studies are also being undertaken to better understand local practices that may contribute to the occurrence moulds that formed aflatoxin. In addition, guidelines and education initiatives are being undertaken to facilitate traders’ and retailers’ understanding on GMP and improve practices of storage, temperatures and humidity to reduce aflatoxin occurrence in foods. To minimize risk of aflatoxin exposure, close tripartite cooperation among the traders, the public and the government is essential.

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References