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REVIEW ARTICLE

# The occurrence of aflatoxin in rice worldwide: a review

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**Abstract**

Rice is the second largest quantity staple international traded cereal and is produced in climatic areas favorable to aflatoxin production, which affects such trading. This review article is to show and explain the levels of aflatoxin contamination of rice worldwide; in general, the contamination is low and varies from country to country. However, the high daily intake of rice makes even these lower levels of concern, as aflatoxin $B_1$ is carcinogenic and has been correlated with hepatocellular carcinoma (HCC) incidence in some countries. In addition, the analytical procedures, the permissible limits used worldwide have been reviewed and discussed.

**Keywords**

Aflatoxin, economy, rice, health, worldwide contamination

**History**

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## Introduction

The aflatoxins are natural carcinogenic substances produced by several *Aspergillus* spp. (Ok et al., 2014; Tanaka et al., 2007) and contaminates different types of food commodities including animal feed and animal products (Elzupir et al., 2009a, b; Elzupir & Elhussein, 2010; Tchana et al., 2010) such as peanut (Elzupir et al., 2011), vegetable oils (Elzupir et al., 2010) and rice (Osman et al., 1999; Zhu et al., 2013) and can be secreted by women in their milk (Elzupir et al., 2012). These toxins and their metabolites are continuing matters of concern in terms of heath and economy for they present high health risks to both humans and animals (Andrade et al., 2012; Gong et al., 2012). In general *Aspergillus flavus* produce only aflatoxins $B_1$ ($AfB_1$) and $B_2$ ($AfB_2$) whereas *Aspergillus parasiticus* may produce the four main aflatoxins: $AfB_1$, aflatoxin $G_1$ ($AfG_1$), $AfB_2$ and aflatoxin $G_2$ ($AfG_2$). It is well established that the most commonly occurring one, $AfB_1$, is the most potent naturally occurring hepato-carcinogenic known to man and has been classified by the International Agency for Research on Cancer (IARC) as a group 1A, human carcinogen (IARC, 1993). It has both acute and chronic toxicity, followed by $AfG_1$ regarding to it is toxicity and abundance, while the toxicity of $AfB_2$ and $AfG_2$, which generally occur at lower levels, are also lower (Elzupir & Alamer, 2014; Hyun et al., 2007; Leong et al., 2012).

According to Global Environmental Monitoring System of the World Health Organization (Sales & Yoshizawa, 2005) rice (*Oryza sativa* or *Oryza glaberrima*) is the second level of cereal staples consumed food worldwide after wheat and consists of the major part of the diets for the half of the world. It is also reported that the rice is composed of 27% of the global diet and 20% of dietary protein intake in the developing countries (Ok et al., 2014). The food and agriculture organization of the United Nations in 2012 (Suarez-Bonnet et al., 2013) has reported that there are 156 million cultivated hectares of rice, producing 721 million metric tons globally in 2011. China was found to be the highest producer with 202.3 million metric tons and India with 154.5 whereas in Latin America and the Caribbean, Brazil produced the larger amount of rice of 12 million tons in 2009/2010 (Almeida et al., 2012); in Africa, rice is mainly produced in Egypt and Nigeria (Makun et al., 2011); and in Europe it is mainly produced in France and Spain (Suarez-Bonnet et al., 2013). In some countries like the Philippines rice formed 35% of total food intake with the provisional tolerable daily intake (PTDI) of up to 280 g per adult person (Sales & Yoshizawa, 2005), and in Vietnam of up to 500 g per adult (Nguyen et al., 2007).

Rice is usually produced in climatic environmental conditions favorable for fungal infection and growth and hence potential mycotoxin contamination before and after harvest (Sales & Yoshizawa, 2005). However, the contamination reported for rice with fungi is the lower than that for many other cereals (Tanaka et al., 2007). Even so that does not mean that fungal infected and mycotoxin contamination does not pose a real problem.

From the point of view of carcinogenicity and toxicity, it is becoming apparent that the producing spp. of $AfB_1$, i.e. *A. flavus and parasiticus* are some of the most important contaminants of rice, worldwide. Hence, this review attempts

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to describe the occurrence and levels of aflatoxin in the global production of rice, also the methods and techniques used for the analysis, and to estimate the aflatoxin-PTDI by populations in selected countries and it is involvement in hepatocellular carcinoma (HCC) as reported by the IARC (2014).

## Methodology

### Criteria used to selected articles

We have used PubMed and the Web of Science to perform a literature search using ''aflatoxin'' and ''rice'' as keywords. The selected articles were published during 2004–2014 period. The publications selected from the search were aimed to cover the presence and levels of aflatoxin in rice as based on title and/or abstract of the paper selected by the search. Twenty-five articles of 259 have been chosen based on these criteria.

### Analytical procedure

In general, high performance liquid chromatography (HPLC) with fluorescence detection is used for separation, detection and quantitation of aflatoxins in agricultural commodities in 15 studies. In order to increase the sensitivity of detection of $AfB_1$ and $AfG_1$ the used of derivatization, with Kobra cell which uses post column bromination, has developed (Almeida et al., 2012; Fredlunda et al., 2009; Ghali et al., 2010; Makun et al., 2011; Nguyen et al., 2007; Reiter et al., 2010). Other methods used are enzyme-linked immunosorbent assays (ELISAs) in five studies and thin-layer chromatography (TLC) in one (Table 1).

For extraction of aflatoxins from solid matrices, e.g. cereals, polar aqueous media containing large amounts of methanol or acetonitrile has been widely used (Almeida et al., 2012; Bansal et al., 2011; Fredlunda et al., 2009; Ghali et al., 2010; Iqbal et al., 2012; Makun et al., 2011; Nguyen et al., 2007; Park et al., 2004; Reddy et al., 2011; Reiter et al., 2010; Ruadrew et al., 2013; Villa & Markaki, 2009; Wang & Liu, 2007), whereas the use of the moderate polar solvents of ethanol and chloroform are seldom (Sales & Yoshizawa, 2005; Toteja et al., 2006). For clean-up purposes, this may be followed by solid phase extraction (SPE) chromatography (Ok et al., 2014) or immune-affinity column chromatography (Sales & Yoshizawa, 2005) in order to remove interfering substances and enhance HPLC performance (Table 1).

## Results

### Levels and incidence

Table 2 has shown the presence of aflatoxin in rice documented in different region in the world.

### Asia

Aflatoxin $B_1$ was found in all 29 rice samples collected from three different areas of Huantai, Huaian, and Fusui in China and found to have an average contamination of around 0.5–0.6 µg/kg (Sun et al., 2011). These results are similar to studies conducted previously by Wang & Liu 2007 in China which shown contamination with an average of 0.79 µg/kg. In South Korea, the level of $AfB_1$ is much lower, of 88 samples, 5 was found to be contaminated with lower mean values of 4.8 ng/kg (Park et al., 2004), these results are consistent with recent investigations in South Korea (Ok et al., 2014).

In rice from India higher levels of aflatoxins have been reported. A survey covering 12 states have shown that of 1511 samples, 256 exceed the Indian permissible limit of 30 µg/kg for total aflatoxin and 930 were above 5 µg/kg with a median concentration of 45 µg/kg (Toteja et al., 2006). Recently another survey covered 20 states. In this investigation, 1200 rice samples were analyzed, the level of contamination with $AfB_1$ was found to be between 0.1 and 308 µg/kg with the frequency of contamination being up to 67.8% (Reddy et al., 2009). More recently, acceptable levels of aflatoxin have reported in Punjab in India (Siruguri et al., 2012). The distribution of aflatoxins in Malaysia are comparable with that of China and Philippines, 9 of the 13 samples have shown $AfB_1$ contamination with an average level of 1.75 µg/kg (Reddy et al., 2011) but in Japan the rice (83 samples) is free from all types of aflatoxin (Kumagai et al., 2008); the same results were found earlier by Sugita-Konishi et al., 2006.

In South Asia in Philippines, the total aflatoxin were found in 74 of 78 samples with a mean level of 1.53µg/kg (Sales & Yoshizawa, 2005), and it was found to be higher in Vietnam (51/100) with a mean of 3.31 µg/kg (Nguyen et al., 2007). In Pakistan, the paddy, parboiled, brown, white, and broken rice showed higher contamination with an average range between 7.10 and 16.35 µg/kg and the frequency ranged from 33 to 64% (Iqbal et al., 2012).

Contamination levels and frequency of $AfB_1$ in West Asia are similar to that of South Asia. Of the 100 Turkish rice samples, 32 were found contaminated higher than the EC Regulations and Turkish Food Codex limit for total aflatoxin of 4 µg/kg (Aydin et al., 2011). And in Iran, 59 of the 71 samples have been found contaminated with $AfB_1$ at an average level 2.1 µg/kg (Mazaheri, 2009). These results are consistent with others reported by Mardani et al. (2011).

### Europe

Only four papers represented the aflatoxin in rice. In Europe, four countries were included: Scotland, Austria, Spain and Sweden. Aflatoxin was found to be reported in all samples from these countries. In Scotland, the brown rice (Asian origin sourced) has shown contamination with an average 14.7 µg/kg of total aflatoxin (Ruadrew et al., 2013). The results in Austria in central Europe have shown that 15 of 81 samples (imported mainly from Asian countries, only one sample from Spain and one from Egypt) have been contaminated with an average level 1.97 µg/kg of total aflatoxin (Reiter et al., 2010). The same results were found in Sweden, (mainly ethnical origin) the contamination ranged between 0.1 and 50.7 µg/kg of total aflatoxin (Fredlunda et al., 2009). However, the level of contamination was higher in both of imported and locally sampled in Spain and Mexico; the aflatoxin was detected in 66 of 67 with a mean concentration 37.3 µg/kg (Suarez-Bonnet et al., 2013).

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Table 1. Techniques and analytical procedures used for determination of aflatoxin in rice samples.

| Region | Country | Tech | Derivatization | Extraction | Clean up | Limit of detection PPB | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | B1 | B2 | G1 | G2 | |
| North Africa | Tunisia | HPLC | Kobracell | MeOH: $H_2O$ | Immunoaffinity column | 0.05 | 0.025 | 0.025 | 0.025 | Ghali et al., 2010 |
| West Africa | Ivory Coast | ELISA | – | Rhone Diagnostics | Immunoaffinity column | – | – | – | – | Sangare-Tigori et al., 2006 |
| | Nigeria | HPLC | Kobracell | ACN: $H_2O$ | dichloromethane | 0.02 | 0.01 | 0.01 | 0.06 | Makun et al., 2011 |
| North America and Latin America | Canada | HPLC | PBPB | MeOH: $H_2O$ | immunoaffinity column | 0.002 | 0.05 | – | – | Bansal et al., 2011 |
| | Brazil | HPLC | Kobracell | MeOH: $H_2O$ | Immunoaffinity column | 0.03 | 0.01 | 0.01 | 0.01 | Almeida et al., 2012 |
| South Asia | Philippines | HPLC | TFA | EtOH: $H_2O$ | Immunoaffinity column | 0.025 | 0.025 | 0.25 | 0.025 | Sales & Yoshizawa, 2005 |
| | Vietnam | HPLC | Kobracell | ACN: $H_2O$ | Chloroform | 0.07 | – | – | – | Nguyen et al., 2007 |
| | India | ELISA | – | MeOH: $H_2O$ | PBST–BSA | 0.02 | – | – | – | Reddy et al., 2009 |
| | India | HPLC | TFA | Official Method 990.33 | Immunoaffinity column | 0.2 | 0.2 | 0.2 | 0.2 | Siruguri et al., 2012 |
| | India | TLC | – | $CHCl_3$: $H_2O$ | SPE | 5 | – | – | – | Toteja et al., 2006 |
| | Pakistan | HPLC | TFA | ACN: $H_2O$ | SPE | 0.04 | 0.04 | 0.04 | 0.04 | Iqbal et al., 2012 |
| Western Pacific | China | HPLC | TFA | ACN: $H_2O$ | Multifunctional purifying column | 0.012 | 0.008 | 0.036 | 0.024 | Wang & Liu, 2007 |
| | China | ELISA | – | | Jiangsu Microbe Institute KITS | 0.1 | – | – | – | Sun et al., 2011 |
| | Malaysia | ELISA | – | MeOH: $H_2O$ | – | – | – | – | – | Reddy et al., 2011 |
| | Korea | HPLC | TFA | MeOH: $H_2O$ | SPE | 0.03 | 0.03 | 0.05 | 0.05 | Ok et al., 2014 |
| | Korea | ELISA | – | MeOH: $H_2O$ | AFB1-horseradish peroxidase conjugate | – | – | – | – | Park et al., 2004 |
| Central Europe | Austria | HPLC | Kobracell | MeOH: $H_2O$ | Immunoaffinity column | 0.1 | 0.1 | 0.15 | 0.16 | Reiter et al., 2010 |
| South Europe | Spain | HPLC | TFA | MeOH: $H_2O$ | Immunoaffinity column | 0.5 | 0.6 | 0.4 | 0.4 | Suarez-Bonnet et al., 2013 |
| | Greece | HPLC | TFA | MeOH: $H_2O$ | SPE | 0.4 | – | – | – | Villa & Markaki, 2009 |
| North Europe | Sweden | HPLC | Kobracell | ACN: $H_2O$ | SPE | <0.03 | <0.03 | <0.03 | <0.03 | Fredlunda et al., 2009 |
| | Scotland | HPLC | Bromination | MeOH: $H_2O$ | SPE | – | – | – | – | Ruadrew et al., 2013 |

HPLC, high performance liquid chromatography; ELISA, the enzyme-linked immunosorbent assay; SPE, solid phase extraction; TFA, trifluoroacetic acid.

Table 2. The occurrence of aflatoxin in rice worldwide for the period 2004–14.

| Region | Country | Aflatoxin | No. of sample/ contaminated sample | Average (μg/kg) | Range of contamination (μg/kg) | Reference |
|---|---|---|---|---|---|---|
| North Africa | Tunisia | Tot. | 11/0 | – | – | Ghali et al., 2010 |
| West Africa | Ivory Coast | AfB1 | 10/10 | 4.5 | >1.5–10 | Sangare-Tigori et al., 2006 |
| | Nigeria | Tot. | 21/21 | 82.5 ± 16.9 | 27.7–371.9 | Makun et al., 2011 |
| North America and Latin America | Canada | AfB1 | 99/56 | 0.34 | – | Bansal et al., 2011 |
| | | AfB1 | 100/43 | 0.39 | – | Bansal et al., 2011 |
| | | AfB2 | 100/23 | 0.08 | 0.002–0.63 | Bansal et al., 2011 |
| | Brazil | Tot. | 230/135 | 13.13 | – | Almeida et al., 2012 |
| South Asia | Philippines | Tot. | 78/74 | 1.53 | NR–8.66 | Sales & Yoshizawa, 2005 |
| | Vietnam | AfB1 | 100/35 | 3.31 | NR–29.8 | Nguyen et al., 2007 |
| | India | AfB1 | 1200/814 | – | 0.1–308.0 | Reddy et al., 2009 |
| | Pakistan | Tot. | 413/185 | 11.2 ± 3.91 | NR – 68.3 | Iqbal et al., 2012 |
| Western Pacific | China | Tot. | 84/23 | 0.79 | 0.15–3.88 | Wang & Liu, 2007 |
| | Malaysia | AfB1 | 13/9 | 1.75 | 0.68–3.79 | Reddy et al., 2011 |
| | Korea | AfB1 | 88/5 | 4.8 | 2.1–7.7 | Park et al., 2004 |
| Central Europe | Austria | Tot. | 81/15 | 1.97 | 0.45–9.86 | Reiter et al., 2010 |
| North Europe | Scotland | Tot. | 3/1 | 14.7 | 14.7 | Ruadrew et al., 2013 |

NR, not reported for the lowest contaminated sample.
Tot., total aflatoxin (B1 + B2 + G1 + G2). –, results not available.

Table 3. The provisional tolerable daily intake (PTDI) of aflatoxin in rice/ng/60 kg bodyweight/person in various countries.

| Region | Country | Af in rice (μg/kg) | Rice daily intake/g | PTDI | HCC cases[a] | Reference |
|---|---|---|---|---|---|---|
| West Africa | Ivory Coast | 4.5 | 429 | 32.18 | 2237 | Sangare-Tigori et al., 2006 |
| | Brazil | 13.13 | 100 | 21.88 | 9678 | Almeida et al., 2012 |
| Southeast Asia | Philippines | 0.37 | 280 | 1.73 | 7734 | Sales & Yoshizawa, 2005 |
| | Vietnam | 3.31 | 500 | 27.58 | 21 997 | Nguyen et al., 2007 |
| Western Pacific (Asia) | Korea | 3.25 | 181 | 9.8 | 16 900 | Ok et al., 2014 |
| | China | 0.6 | 210 | 2.1 | 394 770 | Sun et al., 2011 |
| Central Europe | Austria | 1.97 | 10 | 0.33 | 955 | Reiter et al., 2010 |
| South-Western Europe | Spain | 37.3 | 16 | 9.95 | 5522 | Suarez-Bonnet et al., 2013 |
| Northern Europe | Sweden | 2.2 | 9 | 0.33 | 490 | Fredlunda et al., 2009 |

Af, total aflatoxin; PTDI, provisional tolerable daily intake of Af in rice.
HCC, hepatocellular carcinoma.
[a]HCC cases reported in 2012 by the international agency for research on cancer (IARC).



## Africa

In Africa, there were no aflatoxin ($AfB_1$, $AfB_2$, $AfG_1$ and $AfG_2$) detected in rice (10 samples) in Tunisia (Ghali et al., 2010) but in west Africa in the Ivory Coast (10 samples) and Nigeria (21 samples) the aflatoxin have been detected in all analysed samples with an average levels of 4.5 μg/kg of $AfB_1$ (Sangare-Tigoribi et al., 2006) and 82.5 μg/kg of total aflatoxin (Makun et al., 2011), respectively.

## America and Latin America

Aflatoxin ($AfB_1$ and or $AfB_1 + AfB_2$) in rice in Canada (imported from the United States and Asian countries) was found in 99 of 200 samples with an average level of 0.34–0.39 μg/kg for $AfB_1$ and 0.08 μg/kg for $AfB_2$ (Bansal et al., 2011). In Brazil, the contamination was much higher, the total aflatoxin was found in 135 of 230 samples with a mean contamination of 13.13 μg/kg (Almeida et al., 2012).

## The provisional tolerable daily intake

The data about rice consumption by humans were mentioned in eight articles listed in Table 3. Rice is the principle staple cereal in Asia, part of Africa and South America. The provisional tolerable daily intake has been calculated by assuming that 60 kg is the average of bodyweight of persons in these areas. To determine the hepatocarcinogenic effect of aflatoxin in rice, we have considered the provisional tolerable daily intake (PTDI) and the HCC cases in selected countries. The data of HCC in these countries were taken from the Globocan database (IARC, 2014). The coefficient of correlation (linear regression) between the HCC and the PTDI is weak ($R^2 = 0.073$), but become more significant when data from Asian countries was plotted alone ($R^2 = 0.6189$), but when Spain, Brazil and Ivory Coast are included, as they are thought to have high PTDI values, the curve is diffused again, suggesting that there are special nutritional habits that skew the results, and other factors such as Hepatitis B virus and aflatoxin in other commodities such as peanut are involved.

## Legal limit

The survey of selected articles has indicated that there is no particular regulation related to aflatoxin in rice. These data were compared with local permissible levels and/or European Commission of 2 μg/kg for $AfB_1$ or 4 μg/kg for total aflatoxins in food (Fredlunda et al., 2009; Ghali et al., 2010; Ruadrew et al., 2013; Sales & Yoshizawa, 2005; Suarez-Bonnet et al., 2013) or current regulation of European Commission of 10 μg/kg for total aflatoxins in food (Makun et al., 2011).

The WHO has set 30 μg/kg for aflatoxin in food, and this high limit has been used in several countries for aflatoxin in rice without any regards to the daily intake of rice (Nguyen et al., 2007). A comparable regulations have been reported in Malaysia of 35 μg/kg (Reddy et al., 2011), and in India of 30 μg/kg (Reddy et al., 2009). These limits have been set for food in general, but it may not be suitable for a global staple food like rice.

The lowest legal limit of 2 μg/kg for $AfB_1$ has set in Tunisia and the EU (Ghali et al., 2010), then Iran (5 μg/kg) followed by South Korea (10 μg/kg) (Mazaheri, 2009; Park et al., 2004).

## Discussion

Aflatoxin has high impact on human and animal health. Therefore, the large economy lost in international trade of cereal and crops could be attributed to legislation controlling the permissible exposure levels of this toxin to humans and animals. Rice is one of the most consumed cereals worldwide. Herein, all the presented studies have shown the widespread occurrence of aflatoxin ($AfB_1$ or Total Af) in rice for everywhere in the world, except in Tunisia and Japan.

The estimated levels of aflatoxin in rice are not high as in peanut or other cereal commodities, for example the average of total aflatoxin in peanut butter in Sudan is up to 200 μg/kg (Elzupir et al., 2011), and the average of $AfB_1$ in groundnut in DR Congo is 229.07 (Makun et al., 2012). However, that does not mean the aflatoxin in rice has no contribution in general health because of high daily intake of rice in certain regions of the world (Table 2). The values of PTDI have shown significant correlation with HCC incidence in Asian countries which includes Philippines, Vietnam, Korea and China. The most important point herein is the average of contamination in these countries does not exceed the European Commission limit of 10 μg/kg for aflatoxin in food; in fact the highest average reported in Korea is 3.25 μg/kg. Therefore, indicating the PTDI of Af in rice as shown in Table 2 could be a major factor for chronic disease causative role of aflatoxin. This observation suggested the importance for the particular regulation of aflatoxin in rice with respect to the PTDI.

Generally, the levels of aflatoxin in rice are varied through the world even within some countries in the same region. For example, Japan did not suffer from any high values of contamination but in nearby India and Philippines the cases are completely different. This is due to good strategies followed by Japanese to avoid fungal growth and aflatoxin production (Tanaka et al., 2007). On the other hand, the rice in such countries like Philippines, India, Nigeria and Spain contains high levels of contamination, which may lead to aflatoxicosis and/or contributed to HCC in these countries. The effect of these levels may be extended to other countries importing this rice.

Specifically, the higher reported contamination was found in India (Toteja et al., 2006) followed by Spain in Europe (Suarez-Bonnet et al., 2013) and Nigeria in West Africa (Makun et al., 2011). The reported levels have exceeds the US-FDA legal limit of aflatoxin in food of 20 μg/kg. Significant levels higher than the EC regulation of 10 μg/kg for aflatoxin in food, were found in Scotland (imported from Asia) (Ruadrew et al., 2013), Brazil (Almeida et al., 2012) and Pakistan (Iqbal et al., 2012) (Table 3). The remaining countries have reported lowest value of contamination.

In term of consumption, rice is consumed widely in Vietnam as shown in Table 2 (Nguyen et al., 2007), then Ivory Coast (Sangare-Tigoribi et al., 2006), Philippines (Sales & Yoshizawa, 2005), China (Sun et al., 2011), Korea (Ok et al., 2014) and Brazil (Almeida et al., 2012). And rare in Europe, may be this explain the less attention of Europe countries to manage and control the aflatoxin in rice as the higher contamination have been reported (Suarez-Bonnet et al., 2013).

To conclude, the rice worldwide has low levels of aflatoxin contamination which are mostly lower than locally produced rice and the international permissible levels. But the aflatoxin in some countries, e.g. Nigeria is high, and may pose serious health problem. Further, the effect attributed to aflatoxin in rice in other regions in the world is still unclear as the low level does not absolutely mean safer rice food regarding to its high consumption.

Finally, in spite of lower levels of aflatoxins permitted by regulations in particular countries, more investigations are required to find out the effect of long-time exposure, and estimates of daily intake of contaminated rice are recommended.

## Declaration of interest

The authors report no declaration of interest.

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