Mycotoxin Contamination of Rice in China

Xiang Dong Sun, Ping Su, and Hong Shan

Abstract: Mycotoxin contamination in rice is generally lower than in other cereals such as corn or wheat. However, over 65% of the population in China consumes rice as a staple food. Due to the diversity of the climate across China, the southern region is characterized by high temperatures and humidities, especially in rainy season. Such conditions are optimal for the growth of fungi. The accumulative and transferrable characteristics of fungi mycotoxins pose a great potential threat as confirmed by high incidences of liver cancer in the Yangtze delta region. Major mycotoxins identified in China are aflatoxins and ochratoxin A, as well as fumonisins. The contents of aflatoxin B_1 (AFB_1) in rice are varied among different provinces and regions and generally less than 5 μg/kg. Although high incidences of positive aflatoxins samples have widely been detected, few samples were detected as exceeding the national's maximum residue limit (10 μg/kg). Limited information is available on risk assessment of human health hazards of mycotoxins in rice, children should be paid more attention to due to their having the highest mycotoxins exposure level, although the risks are generally at low levels from rice. Mycotoxins are mainly distributed in the outer layer of the paddy rice (also called rough rice, referring to whole rice grain with the hulls), and the AFB_1 content in bran is 8.4 times greater than that in brown rice (hulled rice). Further investigation should focus on isolation and identification of mycotoxins-producing fungal strains, especially unknown mycotoxigenic fungal strains determination. Infection resistant rice breeding of mycotoxigenic fungal species may be a fundamental approach to guaranteeing rice safety in China.

Keywords: China, contamination, mycotoxins, rice

Introduction

Mycotoxins are naturally occurring toxic contaminants in foods and feeds. They are secondary metabolites produced by filamentous fungi under appropriate temperature and humidity (Nielsen and others 2009). Mycotoxicosis can result through ingestion, inhalation, or skin contact with fungi. Apart from humans, other vertebrates can also be affected by mycotoxins.

Cereal grains can be infected by mycotoxigenic fungi preharvest or postharvest (Glenn 2007). Consequently, mycotoxins can be consumed by humans through contaminated plant origin food (cereal grains) or through the intake of animal origin food (meat, milk, and egg) (Fokunang and others 2006; Sirot and others 2013). Livestock fed with contaminated cereal grains or vegetables may accumulate mycotoxins and finally consumed by human; these animal products pose a great potential health risk to consumers.

In 2001, Reddy and Sathyanarayana listed 143 diverse fungal species that were reported in rice. Studies (Juan and others 2008b; Hoeltz and others 2009; Reddy and others 2009b; Sempere and Santamarina 2010) confirmed that the majority of mycotoxins were produced by molds of genera *Aspergillus, Penicillium,* and *Fusarium*. In 2008, Reddy and others published a review in which they summarized the major mycotoxins, including aflatoxins (AFs), fumonisins, ochratoxin A (OTA), deoxynivlenol, and zearalenone (ZEN), which had been detected in rice from different countries. In 2016, Ferre further summarized that AFs, citrinin, deoxynivalenol, sterigmatocystin, fumonisins, ZEN, cyclopiazonicacid, patulin, gliotoxin and some trichothecenes are the main mycotoxins that have been identified in rice with a high variable of contaminated varieties and at various infected levels. A number of studies have demonstrated that fungi are prone to grow in cereals such as rice and produce mycotoxins. Pitt and others (2013) summarized mycotoxin production in crops as affected by growing, harvesting, storage and processing. Pereira and others (2014) published a comprehensive review systematically describing mycotoxin occurrence in cereals and cereal foodstuffs, as well as analytical methods proposed for the detection of these mycotoxins. Marroquín-Cardona and others (2014) reviewed mycotoxins in a changing global environment and proposed strategies accordingly to reduce mycotoxin exposure, as well as global trends of mycotoxin regulation. These reviews provide a comprehensive description of worldwide mycotoxin contamination, regulation, analytical methods and control.

Many mycotoxins are proven to pose a potential threat to both human and animal health; some of them are very fatal and stable. These mycotoxins accumulate in animal bodies and remain highly active even through severe processing and treatment. They finally transfer to and accumulate inside human bodies in an active state through contaminated animal products. To ensure food safety, the European Union Commission has set strict maximum limits on mycotoxin levels. For instance, 2 μg/kg for aflatoxin B_1 (AFB_1) in rice, 5 μg/kg and 3 μg/kg for OTA in rice and their products, respectively, and 100 μg/kg for ZEN in cereals (European Commission Regulation 2006).

Rice (*Oryza sativa L.*) is the most important staple food in the Asian region. In China, more than 65% of the population consumes it (Zhang and others 2005). China ranks 1st in total annual rice production and accounts for 29.2% of the world's rice produced (milled) in 2015/16 (www.fao.org). Some studies have reported the contamination of rice with mycotoxins such as AFs and fumonisins, especially with AFs in certain areas of China (Tang 1999; Trucksee 2000; Liu and others 2006; Wang and Liu 2007; Sun and others 2011; Lai and others 2014a; Li and others 2014). As a result of inappropriate storage conditions, such as high temperature and high humidity, rice can be an ideal growth substrate for fungi. In recent years, there have been a number of reports

JFDS-2016-0950 Submitted 6/15/2016, Accepted 12/22/2016. *Authors are with Quality & Safety Inst. of Agricultural Products, Heilongjiang Academy of Agricultural Sciences, Harbin 150086, China. Authors are also with Laboratory of Quality & Safety Risk Assessment for Agro-products (Harbin), Ministry of Agriculture, Harbin 150086, China. Direct inquiries to author Sun (E-mail: xdsun65@yahoo.com).*

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Table 1–Different studies conducted on mycotoxins contamination in rice in China.

Province	Rice varieties or forms	Origin of rice samples	Analytical method	Mycotoxins	Total samples	Incidence (%)	Level range of positive samples (μg/kg)	Mean value of positive samples (μg/kg)	References
9 Cities	Rice	Markets	ELISA	AFB_1	161	5.6	1.45–17.71	5.13	Huang and others 2016
Guangdong	Rice	Markets	ELISA	AFB_1	106	24.53	0.15–5.64	2.18 ± 0.93	Zhang and others 2015
Heilongjiang					62	69	0.033–0.17	0.062	
Liaoning					30	96	<LOQ	<LOQ	
Jilin				AFs	59	39	0.030–0.98	0.12	
Guangdong					138	53	0.19–4.1	0.44	
Guangxi					67	81	0.032–21	1.3	
Hainan					14	93	0.032–0.71	0.23	
Heilongjiang					62	69	0.033–0.14	0.058	
Liaoning					30	96	<LOQ	<LOQ	
Jilin				AFB_1	59	39	0.030–0.90	0.11	
Guangdong					138	53	0.030–3.7	0.41	
Guangxi					67	81	0.032–20	1.2	
Hainan	Rice	Farmer stores, granaries, and markets	DLLME-HPLC		14	93	0.032–0.66	0.21	Lai and others 2015a
Heilongjiang					62	14	0.022	0.022	
Liaoning					30	6	<LOQ	<LOQ	
Jilin				AFB_2	59	15	0.086	0.086	
Guangdong					138	13	0.020–0.47	0.11	
Guangxi					67	37	0.029–1.6	0.19	
Hainan					14	14	0.051	0.051	
Heilongjiang					62	4.8	0.61	0.61	
Liaoning					30	0.0	ND	ND	
Jilin				OTA	59	8.5	0.30–0.30	0.30	
Guangdong					138	6.5	0.33–3.2	1.3	
Guangxi					67	1.5	<LOQ	<LOQ	
Hainan					14	0.0	ND	ND	
Hunan	Paddy rice	Fields	ELISA	AFB_1	80	100	0.82–6.98	2.37	Yuan and others 2015
Jiangxi	Rice	Supermarkets	ELISA	AFB_1	517	74.85	0.1–4.3	1.21	Tang and others 2015
Jiangxi	Rice	Supermarkets	LC-MS	FB_1	63	0	<LOQ	<LOQ	Qiu and others 2015
18 Cities	Rice and products	Supermarkets	ELISA	FB	95	3.16	0–740	20	Guo and others 2014
				AFs		35.3	0.10–18.00	0.55	
				AFB_1		33.3	0.02–1.80	0.13	
Guangdong	Rice	Supermarkets	UPLC	AFB_2	51	23.5	0.02–1.35	0.066	Li and others 2013
				AFG_1		15.7	0.04–13.45	0.31	
				AFG_2		2.4	0.02–1.40	0.047	

(*Continued*)

Table 1–Continued.

Province	Rice varieties or forms	Origin of rice samples	Analytical method	Mycotoxins	Total samples	Incidence (%)	Level range of positive samples (μg/kg)	Mean value of positive samples (μg/kg)	References
Henan					12	33.33	5.10-11.11	2.46 ± 3.88	
Hubei					43	18.60	1.24–77.05	6.40 ± 16.82	
Hunan					69	15.52	1.74–2095.08	52.93 ± 26.98	
Jiangxi	Paddy Rice	Barns	HPLC	ZEN	51	39.22	0.31–66.81	3.64 ± 10.99	Pan 2012
Fujian					14	50.00	1.09–14.45	2.74 ± 4.43	
Zhejiang					15	28.11	46.40–670.86	47.82 ± 17.27	
Jiangsu					48	25.00	1.29–203.00	5.15 ± 20.22	
Jilin	Paddy rice	Barns	ELISA	AFB_1	10	100	0–0.900	0.570 ± 0.195	Cai and others 2011
				AFB_1		3.9	0.5–2.9	1.7	
				ZEN		3.9	3.5–8.6	6.1	
Guangxi	Rice	Farmers markets	UPLC-MS/MS	FX	51	9.8	0.3–14.0	5.8	Li and others 2011
				NIV		9.8	1.9–56.8	25.2	
				DAS		9.8	3.3–55.5	33.6	
Guangxi	Rice	Markets	ELISA	AFB_1	34	8.8	–	1.0	Teng and others 2011
				AFB_1		50.0	0.10–6.9	0.89	
				AFB_2		27.78	0.1–0.8	0.24	
2 Provinces	Rice	Markets	HPLC	DON	18	44.44	0.1–2.1	0.74	Ma and others 2011
				T-2		61.11	0.1–1.3	0.47	
				ZEN		38.89	0.1–0.3	0.13	
Jiangxi	Rice	Supermarkets	HPLC	FB_1	40	100	0.11–1.64	0.87	Zhang and others 2010
8 Cities	Rice	Markets	ELISA	OTA	91	–	–	1.938 ± 1.328	Yang 2008
Hubei	Paddy rice	Barns	ELISA	AFB_1	30	100	0.6–8.1	3.3	Yu and others 2008
8 Provinces	Rice	Markets	HPLC	AFs	84	27.3	0.15–1.58	<0.6	Wang and Liu 2006
	Paddy rice	Barns	HPLC	AFs	37	97.3	–	0.88	
Liaoning	Paddy rice			AFG_1	15	93.7	–	–	Liu and others 2006
	Brown rice			AFG_2	33	91.7	–	–	
6 Provinces	Paddy rice	Barns	HPLC	OTA	284	2.11	0.34–1.47	0.9	Tan 2006
Hubei	Rice	Barns	ELISA	FB_1	49	32.65	10360–13640	12550	Lv and others 2005
				AFs	31	90.3	0–31	1.97	

(*Continued*)

Table 1–Continued.

Province	Rice varieties or forms	Origin of rice samples	Analytical method	Mycotoxins	Total samples	Incidence (%)	Level range of positive samples (µg/kg)	Mean value of positive samples (µg/kg)	References
Various province	Paddy rice	Barns	ELISA	OTA		87.1	0–16	2.16	
				T-2		35.5	0–910	92.13	Fu and others 2004
				DON		3.2	0–770	24.84	
12 Provinces	Rice	Barns	ELISA	ST	542	72.0	0.01–260.17	13.9	Tian and Liu 2004
				FB	49	32.65	–	12000	
Hubei	Rice	Markets	ELISA	ST	60	50.00	–	105.00	
				FT	60	1.67	–	1.00	Xie and others 2001a
				AFB_1	22	77.27	–	80.64	
Jilin	Paddy rice	Barns	IC-ELISA	ST	59	74.6	1.02–214.38	21.04	Liu and others 2000
				ST	60	41.5	1.0–52.1	8.6	Li 2000
Shandong	Rice	Barns, markets	IC-ELISA	FT		3.7	0.4762–0.6345	–	
Shandong	Rice	Barns, markets	ELISA	FB	40	95.0	0–40000	27900	Yan and others 1999
				FB_1		37.93	3410–16790	11700	
Henan	Rice	Barns	ELISA	FT	68	–	–	–	Liao and others 1999
				ST		–	1.10–25.69	3.71	

ND, not detected (below the quantification limit); LOQ, limit of quantification.

from various countries on the occurrence of fungal contamination in rice with high levels of aflatoxin (Tanaka and others 2007; Reddy and others 2008; Reddy and others 2009a, 2009b; Reiter and others 2010; Aydin and others 2011; Bansal and others 2011; Hussaini and others 2011; Almeida and others 2012; Elena and others 2013; Ok and others 2014; Lai and others 2015a). Due to a vast area of land in China, rice is widely cultivated under different climate conditions and extensively contaminated by various fungi. However, little information is currently available on the aflatoxigenic fungal contamination of rice in China and on the ability of local fungal strains to produce mycotoxins (Liu and others 1981). Investigations conducted over the last 17 y revealed that the general condition of mycotoxin contamination of rice in China was safe, except in some special cases where the contamination levels were high and even exceeded the national maximum residue limits (MRLs). This may have been induced by unfavorable climatic and storage conditions. Table 1 summarizes different studies conducted on mycotoxins contamination in rice in China since 1999.

Postharvest rice storage is especially important for ensuring its safety. In advanced countries like Japan, rice is stored in warehouses where 13% to 14% moisture content which is equivalent to water activity levels of 0.65 to 0.70 is maintained (Tanaka and others 2007). The humidity and temperature of these warehouses are restrained between 70% and 75% and less than 15 °C, respectively (Tanaka and others 2007). Consequently, postharvest mycotoxin contamination seldom occurs in Japan (Tanaka and others 2007). In contrast, 70% to 80% of total rice yield in China is stored in farmer's simple barns which are unable to control temperature and humidity (Lai and others 2014b). Therefore, rice stored under these poor conditions is prone to be infected by molds and contaminated by their mycotoxins.

Due to diversified climatic conditions, some rice growing regions in China such as the Yangtze delta region are extremely humid during the rainy season, which enables large amounts of mycotoxigenic fungi to reproduce rapidly and produce mycotoxins in stored rice (Li and others 2014). The Chinese government pays great attention to mycotoxin contamination in cereals and has renewed the MRLs and the standard detection methods for major mycotoxins in foods in 2011 (China's Ministry of Health 2011). Table 2 compares the Chinese MRLs of mycotoxins in rice with EU and other countries. In recent years, the Ministry of Agriculture of the People's Republic of China has launched a series of risk assessment projects for agro-products, including mycotoxin contamination of rice assessments across the whole country. Dozens of research institutes and analytical organizations including my lab has been participating in these long-term consecutive dynamic assessment projects. Although the detailed results have not yet been made public, actions to promote food safety have already been taken by the government and the efforts are perceivable. Consequently, understanding the conditions of mycotoxin contamination in rice in China is fundamental for the local governments to take actions to reduce health risk and ensure food safety.

Table 2–Comparison of the MRLs of mycotoxins in rice with EU and other countries.

Countries or organization	Mycotoxins	MRLs μg/kg	Reference
China	AFB_1	10	China's Ministry of Health 2011
	OTA	5	
EU	AFB_1	2	European Commission Regulation 2006
	AFs	4	
	OTA	3	
	ZEN	100	
Brazil	OTA	50	Lai and others 2014a
Canada	AFs	15	Lai and others 2014a
India	AFB_1	30	Lai and others 2014a
Japan	AFB_1	10	Lai and others 2014a
Mexico	AFs	20	Lai and others 2014a
Russia	AFB_1	5	Lai and others 2014a
USA	AFs	15	Lai and others 2014a

Aflatoxins

Aflatoxins (AFs) are produced by fungi of genus *Aspergillus*, mainly *Aspergillus flavus, Aspergillus parasiticus* and rarely by *Aspergillus nomius* and *Aspergillus tamari* (Klich 2007; Iqbal and others 2012). These fungi are able to grow on different cereal grains and produce AFs before or during harvest, storage, handling, and shipment (Giray and others 2007; Reddy and others 2009a). The most important members are AFB_1, aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2). They have been classified as Group 1 carcinogens to humans by the International Agency for Research on Cancer (IARC 1993). Among them, AFB_1 has been confirmed the most toxic and carcinogenic (IARC 1993). Significant qualitative and quantitative differences exist in the aflatoxigenic capacity of isolates of *A. flavus* (Bezerra da Rocha and others 2014). Studies showed that only 50% of the strains of these species produce AFs; while up to 106 μg/kg of AFs can be produced by some of the aflatoxigenic isolates (Klich and Pitt 1988; Cotty and others 1994). AFs significantly increase the risk of liver cancer in chronic hepatitis B patients (Groopman and others 2008) and are considered a risk factor for hepatocellular cancer development in Asia (Scholl and Groopman 2008).

Xie and others (2001a) reported an average content of 80.64 μg/kg for AFB_1 in 17 rice samples out of 22 in Hubei province; this is the highest level of AFB_1 that has ever been reported in China. These data are not concrete because the authors applied the ELISA method to detect AFB_1 and judged the samples as positive when $AFB_1 \geq 10$ μg/kg. This high LOQ reveals that the above data is not very reliable. Another possible reason may be due to poor storage conditions where the collected rice samples were infected by mycotoxins-producing fungi thereby resulting in severe AFs contamination. However, high levels of AFB_1 contamination in rice have also been reported in Nigeria at 200.19 ± 320.98 μg/kg (Makun and others 2007) and 37.2 ± 14.0 μg/kg (Makun and others 2011), as well as in India at 60 to 600 μg/kg (Toteja and others 2006) in parboiled rice, due to inappropriate storage.

Fu and others (2004) performed a survey to investigate contamination situations of mycotoxins on grains. Thirty-one paddy rice samples were collected from different barns across China to determine mycotoxins using the ELISA method. The author's results showed a high incidence rate of AFs ($B_1 + B_2 + G_1 + G_2$) in rice at 90.3% with an average level of 1.97 μg/kg for the all 31 samples. They indicated that AFs and OTA were the dominant mycotoxins, and the infection of *Aspergillus* was during the rice growing season as well as transportation. The authors concluded that rice in China was generally safe, and that it is widely contaminated by AFs in the tropical, subtropical, and temperate regions. However, the contamination level in tropical regions is significantly greater than that in temperate ones.

Liu and others (2006) conducted a survey on aflatoxin contamination in stored maize and rice in Liaoning province. 37 whole grain rice samples stored from 1 to 14 y were collected, among them 16 samples were tested as whole grain rice (paddy) and all the 37 as dehusked rice (brown rice). Their results demonstrated that almost all rice samples collected contained AFs. The average contents in paddy rice and brown rice were 3.87 and 0.88 μg/kg, respectively. Through a dehusking treatment, 3-quarters of the total AFs in whole grain rice (3.87 μg/kg) can be reduced to as low as 0.88 μg/kg in brown rice. In a 10-y storage span, no significant increase of aflatoxin content was found in whole grain rice and brown rice. In over 92% of rice samples tested, AFG_1 was determined as the main type of aflatoxin. The aflatoxin content in rice is much lower than the regulated MRLs in foodstuffs in China as well as other countries. The authors indicated that the average aflatoxin content (0.88 μg/kg) of brown rice in Liaoning province was much lower than those of other regions in Asia (Tang and others 1998; Tang 1999) due to the cooler weather conditions in northern China which inhibited the growth of these aflatoxigenic fungi.

Wang and Liu (2007) randomly collected 283 samples of corn, peanut, rice, walnut and pine nut from local markets in 8 regions (Chongqing, Fujian, Guangdong, Guangxi, Hubei, Jiangsu, Shanghai and Zhejiang) of China and performed a survey to investigate mycotoxins contamination in foods. They reported that very few rice samples with AFs were detected, with an AFs contamination rate of 27.38% and, an average AFs content of 0.79 μg/kg in the rice samples. Their results showed that in 84 rice samples, 23 of them were detected as AFs positive with content range at 0.15 to 1.58 μg/kg. However, 16 out of 84 were detected as AFB_1 positive with content range at 0.15 to 3.22 μg/kg, 3 out of 84 were determined as AFB_2 positive with content range at 0.06 to 0.24 μg/kg, and 7 out of 84 were detected as AFG_1 positive with content range at 0.36 to 1.59 μg/kg. They consequently indicated that AFB_1 in foods might not be able to represent the contamination of AFs completely and suggested the establishment of total AFs besides AFB_1 in national and international standards. In another survey also conducted in Guangdong province, Huang and others (2007) reported that 8 and 3 out of 20 rice samples were detected as AFB_1 and AFB_2 positive, respectively. No AFG_1 and AFG_2 positive samples were determined in these samples. Although the authors applied Waters 600 high-performance liquid chromatography (HPLC) to detect the AFs, the content of AFB_1, AFB_2, AFG_1, and AFG_2 had not been reported. They further revealed that rates over the MRL for AFs were detected in more samples from farmer markets (21.6%) than that from supermarkets (5.4%).

Yu and others (2008) studied the distribution characteristic of AFB_1 in paddy, brown, and rice samples from 2 warehouses. Their results indicated that significant differences existed for AFB_1 content among rice samples collected from different layers and spots in the same warehouse. Greater AFB_1 contents were detected in the paddy samples collected from the middle layers (warehouse #1, an average content of 15.4 μg/kg; warehouse #2, an average content of 21.0 μg/kg) than that from the whole warehouse (warehouse #1, an average content of 12.4 μg/kg; warehouse #2, an average content of 17.3 μg/kg) and locations near the wall. Significant differences for AFB_1 content were also observed among rice, brown rice, and bran at 3.3, 32.2, and 270.9 μg/kg, respectively. They indicated that the AFB_1 content in bran is 8.4 times greater than that of the brown rice and accounts for 90% of the total AFB_1 content in the brown rice. This is an evidence that most of the AFB_1 exists in the outer layer (rice husk and bran) of the paddy rice and brown rice is not safe for human consumption unless it is well stored in modern warehouses.

Teng and others (2011) investigated AFB_1 contamination in rice in Guangxi province. They collected 34 rice samples from different supermarkets, stores, and roadside markets and determined the AFB_1 content by using the national standard method (ELISA). 3 samples were found to contain AFB_1 at 1 μg/kg and they were all from roadside markets. The authors hence inferred that the contamination of AFB_1 in their investigation was likely due to poor storage and transportation conditions.

Li and others (2014) investigated the occurrences of AFs, AFB_1, OTA, deoxynivalenol (DON), and ZEN for a total of 76 cereal and oil products (included 21 rice and derived products) collected from the Yangtze Delta region of China. Their results indicated that AFs and AFB_1 were determined in 14.5% of the samples and the contents ranging 1.1 to 35.0 μg/kg for AFs, and 1.0 to 32.2 μg/kg for AFB_1. Unfortunately, the authors did not provide specific data for rice and its derived products. Consequently, the above data can only be considered as a reference and cannot be applied in relative comparative studies.

Tang and others (2015) performed a dietary exposure assessment of AFB_1 in rice in Jiangxi province. Their results showed that the contents of AFB_1 in 517 rice samples were lower than the limits of "National Food Safety Standard -The Limits of Mycotoxins in Food" (National Food Safety Standards of China 2011). They assessed the dietary exposure to AFB_1 in rice of different high consumption groups, including 2- to 6-y-old children, standard adults, urban standard adults and rural standard adults at the P99 by Monte Carlo simulation (90% confidence interval). For the above groups, the contributions of AFB_1 at the P99 were 86.16 (74.19 to 102.91), 34.54 (29.74 to 41.25), 28.85 (24.84 to 34.46), 36.32 (31.27 to 43.38) ng/(kg bw•d), respectively, and the hepatocellular carainoma incidences were 2.671 (2.300 to 3.190), 1.071 (0.922 to 1.279), 0.894 (0.770 to 1.068), and 1.126 (0.969 to 1.345) cancers/(10^5 persons·year), respectively. They concluded that less dietary exposure risk to AFB_1 in rice existed in Jiangxi province; however, 2- to 6-y-old children have potential risk that deserves attention.

In the latest survey conducted by Lai and others (2015a), 370 rice samples were collected from 6 provinces (South China: Guangdong, Guangxi and Hainan province; Northeast: Heilongjiang, Liaoning and Jilin province) in China and tested for AFs; 63.5% (235/370) of rice samples were reported to contain detectable amounts of AFs. In the positive samples, the average level of AFB_1 and total AFs were 0.60 and 0.65 μg/kg, respectively. AFB_1 and AFB_2 were present in samples from all 6 provinces and could be quantified in 87 and 32 samples, respectively. The authors indicated that differences exist in the contamination of AFs and AFB_1 in rice samples collected from various provinces. In rice samples collected from south China (Guangdong, Guangxi and Hainan province), the average content of AFs and AFB_1 was greater than those from the 3 northeast provinces (Heilongjiang, Liaoning and Jilin). In Liaoning province, 96.7% (29/30) of rice samples were contaminated with AFB_1 with no sample exceeding the LOQ (0.03 mg/kg). The authors attributed the high contamination levels of AFs in rice to climatic conditions in south China (very hot and wet all year round) which may favor fungal growth and AFs production.

In 2015, Lai and others (2015b) also conducted a study to investigate the potential of 127 strains of *Aspergillus flavus* isolated from Chinese rice grains to produce AFB_1 and AFB_2. They inoculated these strains onto rice grains and incubated at 28 °C for 21 days, then extracted and quantified the aflatoxin. Their results demonstrated that 37% of the strains produced AFB_1 and AFB_2 with AFB_1 levels at 175 to 124101 μg/kg and AFB_2 at 0 to 10329 μg/kg. The mean yields of AFB_1 and AFB_2 of these isolates were 5884 μg/kg and 1968 μg/kg, respectively. They pointed out that higher levels of AFB_1 were produced in rice than AFB_2 by most of the aflatoxigenic strains.

The conditions of aflatoxin contamination of rice in China are generally safe based on a number of investigations conducted by Chinese researchers. However, due to highly diversified climatic conditions in China, AFs contamination incidences and levels vary greatly from province to province, from year to year. Some areas with high humidity and temperature during the rice maturing period are especially favorable for the growth of aflatoxigenic strains. AFs are also produced extensively in rice under unfavorable weather conditions in field and during storage due to poor environmental situations. These all pose a great threat to the health of consumers hence requiring the research institutes and local governments to take appropriate actions to ensure commercial rice safety.

Ochratoxin A

Ochratoxin A (OTA) is mainly produced by *Aspergillus ochraceus*, *Aspergillus carbonarius,* and *Penicillium verrucosum* (EFSA 2006). It is a well-known nephrotoxic agent and has been associated with fatal human kidney disease, referred to as Balkan Endemic Nephropathy (BEN) and with an increased incidence of tumors of the upper urinary tract (JECFA 2001). The International Agency for Research on Cancer (IARC 1993) has classified OTA as possibly a carcinogen (group 2B), based on inadequate evidence for carcinogenicity in humans and sufficient evidence in animals.

In early 2005, Xie conducted a study and reported that a high OTA incidence rate of 87.1% (27/31) in rice samples from 5 provinces was detected, with an average OTA content of 2.48 μg/kg for all the 27 positive samples. He pointed out that AFs and OTA are the dominant mycotoxins in rice and the contamination of OTA should be paid more attention to.

In 2008, Yang investigated OTA contamination conditions of rice in China. The author collected 91 rice samples from 10 big cities across the country and determined the average content at 1.938 $\pm$ 1.33 μg/kg. The OTA contents of 2 samples reached 7.70 μg/kg and 6.03 μg/kg, respectively, which exceeded both the Chinese (5 μg/kg) and EU's MRLs (3 μg/kg). The average OTA contents of city 3 (4.07 μg/kg, n = 10) and city 5 (3.67 μg/kg, n = 10) were close to the Chinese MRL, but exceeded the EU's MRL. The author revealed that the MRL (5 μg/kg) exceeding samples were from cities Changsha, Harbin, Hefei, Nanning, Shenyang, and Zhengzhou.

Li and others (2014) sampled 76 cereal and oil products (including 21 rice and its derived products) in 5 cities from the Yangtze Delta region of China to investigate mycotoxin contamination. Their results showed that 14.5% of samples were contaminated with OTA at an average level of 3.5 μg/kg. However, they did not provide specific data for rice and its derived products.

As mentioned in the 2nd section, the survey of rice samples performed by Lai and others (2015a) not only included determination of AFs but also included OTA. Their results showed that 4.9% (18/370) of rice samples contained detectable amounts of OTA. The average content of OTA was 0.85 μg/kg in positive samples. 4.8% (3/62), 8.5% (5/59), 9.5% (9/138), and 1.5% (1/67) of samples from Heilongjiang, Jilin, Guangdong, and Guangxi province, respectively, can be detected as OTA positive. OTA was not determined in any of the rice samples from Liaoning and Hainan provinces. 6 samples were found and quantified for OTA from 4 provinces. Among the 18 OTA detectable rice samples, 6 of them had OTA contents exceeding the limits of quantification (LOQ, 0.3 μg/kg); one sample was from Heilongjiang, 2 from Jilin, and the rest 3 from Guangdong province. However, only one sample from Guangdong province had an OTA content greater than the maximum tolerable limit of 3.0 μg/kg set by the regulation of European Union Commission (European Commission Regulation 2006). The ratio of rice samples with OTA levels beyond the EU limit to those below was 0.3% (1/370). The authors concluded that the levels of the mycotoxin in rice are lower than regulatory limits and the rice commodities in China are generally safe.

Contamination of rice with OTA has also been widely reported in other countries including Nigeria, Morocco, Portugal, Spain, Pakistan, Jordan, Korea, Chile, Egypt, Turkey, Vietnam, and the United Kingdom (Abdelhamid 1990; Scudamore and others1997; Park and others 2005; González and others 2006; Makun and others 2007; Nguyen and others 2007; Juan and others 2008a, 2008b; Vega and others 2009; Salem and Ahmad 2010; Aydin and others 2011; Majeed and others 2013; Iqbal and others 2016). Among these countries, mean values of 141.7 $\pm$ 25.4, 12.6 $\pm$ 3.4, 8.50 $\pm$ 0.60, and 3.9 μg/kg for OTA were reported from Nigeria, Morocco, Pakistan, and Korea, respectively. These contamination levels are higher than those reported in China. Even though the OTA contamination levels of rice are normally lower in China than the other countries and it is generally considered safe upon data available in recent years, close attention is still needed on the occurrence and change tendency of OTA.

Zearalenone

Zearalenone (ZEN) is a secondary metabolite mainly produced by *Fusarium graminearum*. Under moist cool field conditions, *Fusarium* species grow and invade crops during blooming, but under poor storage conditions, they also grow and produce toxins at the postharvest stage (EFSA 2011a). Although ZEN is biologically potent, since it is rarely toxic, classifying it as a toxin is considered inappropriate (Bezerra da Rocha and others 2014). It has been concluded that ZEN is not classifiable regarding its carcinogenicity to humans (Group 3) by IARC (IARC 1993) and was proposed to be better fit the classification of a nonsteroidal estrogen or a mycoestrogen (Bennet and Klich 2003).

The natural occurrence of ZEN in rice has only been identified in a few of studies. Ma and others (2011) conducted a survey to investigate multimycotoxins contamination of cereals collected from various provinces in China. Rice samples were collected from Hunan and Jiangxi provinces and the ZEN contents were reported at average levels of 0.17 μg/kg and 0.10 μg/kg, respectively. Pan (2012) conducted a survey in 7 provinces in the central and south of China to investigate the contamination of ZEN in rice. The results revealed that the average content, incidence rate, and over MRL rate of ZEN for the 252 samples in total was 17.31 μg/kg, 25.4%, and 9.52%, respectively. His results showed that the ZEN contamination incidence in Fujian province reached a high level

Table 3–Contamination situation of ZEN in rice grown in different seasons from central and south of China. Adapted from Pan (2012).

	Total samples	Positive rate (%)	Exceed MRLs rate (%)	Range of positive samples (μg/kg)	Mean (μg/kg)
Early indica rice	16	12.50	6.25	46.40–670.80	44.83 ± 44.16^a
Medium indica rice	43	18.60	2.33	1.24–77.05	6.40 ± 4.44^b
Late indica rice	13	53.85	0.00	0.50–14.45	2.95 ± 4.81^b

Table 4–Major mycotoxigenic fungal species found in rice in China.

Mycotoxins	Classification	Fungal species	References
Aflatoxins	Aflatoxin B_1	*Aspergillus flavus*	Xie and others 2001a, 2001b; Lai and others 2015b
	Aflatoxin B_2	*Aspergillus parasiticus*	
	Aflatoxin G_1		
	Aflatoxin G_2		
Fumonisins	Fumonisin B_1	*Fusarium fujikuroi*	Yan and others 1999
	Fumonisin B_2	*Fusarium graminearum*	Li and others 2000
		Fusarium proliferatum	Sun and others 2005
		Fusarium oxysporum	Hou 2013
		Fusarium verticillioides	
Sterigmatocystin		*Aspergillus nidulans*	Li and others 2000
		Aspergillus versicolor	Xie and others 2001b; Sun and others 2005
Fumitremorgin		*Fusarium moniliforme*	Zhang and others 2001; Sun and others 2005

of 50% and the average contents in Hunan and Zhejiang provinces reached 52.93 and 47.82 μg/kg, respectively. Although these data points are lower than the national MRL for cereals (60 μg/kg), the maximum contents of 2095.08 and 670.86 μg/kg in the above 2 provinces far exceed the National MRL. ZEN contamination of rice grown in different seasons was also studied in this work and the author demonstrated that the average ZEN content in early Indica rice was significantly greater than that of the medium and late ones, whereas no significant difference was observed between the medium and late ones (Table 3). In a study of determination method performed by Wang and others (2016), they collected naturally contaminated samples (corn, wheat, and rice) on local markets (Nanchang, Jiangxi province) and detected contamination of mycotoxin ZEN. Their results showed that ZEN was not detectable in only 5 rice samples whereas it was mostly detectable in both the 15 corn and 15 wheat samples. Their results indicated that the contamination of ZEN in rice is possibly lower than in corn and wheat.

Based on limited information available from literature, it seems like ZEN is not a serious concern among mycotoxin contamination of rice in China. However, conditions of ZEN contamination in rice have attracted our attention in 2 provinces, namely Hunan and Zhejiang, their relatively high average contents and ranges of positive samples need continuous monitoring.

Fusarium Mycotoxins

Fumonisins (FB) are mainly produced by *Fusarium* species from the *Liseola* section. The major producing species are *Fusarium verticillioides* (*syn. Fusarium moniliforme*) and *Fusarium proliferatum*. Fumonisins are suspected to be a possible contributory risk factor for primary liver cancer (Ueno and others 1997) since they promote hepatocarcinogenesis in rats (Rilay and others 1994). From a toxicological point of view, fumonisin B_1 (FB_1) is the most important fumonisin. The IARC has classified FB_1 as "possibly carcinogenic to humans" (class 2B) (IARC 2002).

A survey conducted by Yu and others (1999) showed that FB was undetectable in the 40 rice samples collected in Sichuan province using both ELISA and HPLC methods. However, 27 out of 70 corn samples were detected FB positive with an average content of 0.68 mg/kg. Lv and others (2005) investigated contamination situations of fumonisins and the mycotoxins producing *Fusarium* in main grains in Hubei province. Their results showed that an average FB_1 content of 12.55 mg/kg was detected, whereas *Fusarium* species had not been determined whatsoever in fresh rice (current year) or stale rice (last year) samples. They concluded that this phenomenon was consistent with reports from other provinces and speculated that during harvest, processing, and storage, the *Fusarium* species were substituted by other species due to the change of environmental conditions, hence the mycoflora on rice was changed however the FB left in the rice.

One question arises after carefully consideration of the above data, the average content of 12.55 mg/kg of FB_1 seems too high in comparison with the results of other countries such as Korea (mean value of FB_1 at 54.4 μg/kg) (Park and others 2005), Nigeria (mean value of FB_1 at 0.2 μg/kg) (Makun and others 2011), Iran (FB_1 at 98 to 711 μg/kg) (Dowling 1997), and Japan (FB_1 at 0.061 to 0.101 μg/kg) (Kushiro and others 2008). A difference in FB_1 contents by a factor of 100 between China and other countries (except Iran) results in the suspicion that the data from China may be incorrect due to an inadequate determination method or even incorrect calculations. However, these high fumonisin levels were also reported by Li and others (2000) (27.9 mg/kg) and Xie and others (2001a) (12 mg/kg). In addition, another study performed in Iran demonstrated even greater values, the average FB_1 contamination level of fresh and stored rice collected from 15 different zones ranged from 0 to 56.2 and 4.3 to 56.2 mg/kg, respectively, using HPLC method (Khosravi and others 2013). In comparison, Zhang and others (2010) reported an average contamination level of 0.87 μg/kg for FB_1 in Jiangxi province. One difference between the above normal values and the extreme values from China is the determination methods, the extremely great values are all obtained using ELISA method, whereas the normal data is mostly determined using HPLC detection. It is noticeable that similar data obtained in Iran was also employed HPLC method. Consequently, it is probably true that the high contamination levels of FB reported in rice from both China and Iran are incorrect. Further investigation is needed to confirm the extremely high contamination levels of fumonisins in these areas of China through comparative methods by using HPLC and ELISA.

Qiu and others (2015) conducted a survey to investigate FB_1 contamination in marketed rice (63 samples in total from 5 cities) in Jiangxi province and no FB_1 positive samples was detected. They consequently concluded that no FB_1 was found in commercial rice from Jiangxi province.

Besides the above discussed mycotoxin contamination in rice, some rarely covered ones in rice have also been reported in China. In a survey conducted in Henan province, Liao and others (1999) determined sterigmatocystin (ST) and fumitremorgin (FT) in rice samples. However, the authors did not provide the data of incidence rates of ST and FT as well as mean value of FT; they just mentioned that the positive rate of FT contaminated rice was between that of wheat (7.21%) and corn (2.41%). Li and others (2000) reported an average ST content of 8.6 μg/kg in rice. Liu and others (2000) found that the positive rate of ST in stale rice samples was significantly greater than that of fresh rice. They also reported a 100% positive rate of ST in the strains which was much higher in comparison with those in rice samples and an average ST content of 1559.04 μg/kg in these strains. Xie and others (2001a) also reported the detection of ST at 105 μg/kg in Hubei province, as well as FT at 1.00 μg/kg. Tian and Liu (2004) conducted a survey on ST contamination of rice in 5 regions (included 12 provinces) and reported the positive rates at 62.2, 84.0, 81.2, 86.3, and 55.8%, and average contamination levels of 10.4, 38.4, 24.5, 5.1, and 4.3 μg/kg, respectively, for the Northeast, Northwest, East, Central, and Southwest regions. Xie (2005) reported an average of T-2 toxin and deoxynivalenol (DON) contents of 92.13 and 24.84 μg/kg, respectively, from various provinces. He indicated that the contaminations of T-2 and DON mainly occurred in temperate and warm regions. However, T-2 was also reported in the northernmost province of Heilongjiang at an average content of 0.17 μg/kg (Feng and others 2004). Other mycotoxins contamination of rice confirmed in China including nivalenol (NIV), diacetoxyscirpenol (DAS), HT-2 toxin (HT-2), 3-acetyldeoxynivalenol (3A DON), 15-acetyldeoxynivalenol (15A DON), deoxynivalenol-3-glucoside (DON 3G), and fusarenon-X (FX) have also been reported (Ma and others 2011). Li and others (2011) further reported detection of FX, NIV, and DAS in rice from Guangxi province at median values of 5.5, 23.5, and 35.2 μg/kg, respectively.

Major Mycotoxigenic Fungal Species

In 1986, Yin and others separated and identified 30 genera and 84 species of fungi from the ripening paddy rice in the fields and the paddy rice stored for 2 y from 25 provinces of China. Among them 26 species were identified as dominant, including *Aspergillus glaucus*, *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus nidulans, and Aspergillus versicolor.* Fortunately, most of these species are not mycotoxigenic fungi. This is an early well established comprehensive work on fungal identification and classification in rice from different regions of China. Zhou and others (2008) and Tang and others (2009) identified nonmycotoxigenic fungi *Aspergillus glaucus* and *Aspergillus candidus* as the main spoilage fungi in stored paddy rice that affect rice storage safety. Liao and others (1999) and Xie and others (2001b) reported that the major mycotoxigenic fungi identified in Hubei and Henan provinces are *Aspergillus nidulans* and *Aspergillus versicolor*. Hou (2013) isolated 216 strains of *Fusarium spp.* from infected rice panicle samples in Guangxi province and then identified and divided these strains into 5 species: *F. fujikuroi, F. graminearum, F. proliferatum, F. oxysporum,* and *F. verticillioides.* The fumonisin content in the crude toxin of *F. fujikuroi*, *F. verticillioides*, *F. graminearum*, *F. proliferatum*, and *F. oxysporum,* were 5379.03, 638.62, 139.39, 13.87, and 2.16 ppm, respectively. A total of 127 strains of *A. flavus*, which were isolated from rice samples collected from 12 provinces in China were selected by Lai and others (2015b) to test for aflatoxin production in cultures of rice grain. Their results showed that 47 out of 127 (37%) tested isolates produced AFs at levels ranging from 175 to 124101 μg/kg for AFB_1 and 0 to 10329 μg/kg for AFB_2. They indicated that the percentage of aflatoxigenic strains over total *A. flavus* strains differed significantly in different provinces and the mean yields of the aflatoxin-producing isolates were 5884, 1968, and 7852 μg/kg for AFB_1, AFB_2, and AFs (AFB_1+ AFB_2), respectively. The authors pointed out that the high yields of AFs in rice grain cultures by *A. flavus* strains might be due to incubation under optimal conditions whereas the actual levels of AFs in rice samples in China were very low. Table 4 lists major mycotoxigenic fungal species found in rice in China. In general, literature on mycotoxigenic fungal species isolation and identification from rice is still very limited in China and more work need to be done to clarify the specific species occur in various regions.

Risk Assessment of Mycotoxins in Human Health in China

Risk assessment of mycotoxins contaminated rice in human health in China is still limited due to long term grain shortage. However, in the past 10 y, with increased grain output, the Chinese government is aware of the significance of food safety and has been launching projects on risk assessment of mycotoxins, pesticides, and heavy metals in grains as well as in animal products and vegetables in human health hazards through the Ministry of Agriculture. Due to political reasons, these results are not open to public. Consequently, limited information is available in this field.

Yang (2008) conducted an investigation and risk assessment of OTA in main foods in China and obtained average OTA exposure levels of 0.4771, 0.3282, 0.0428, and 0.0067 μg/(kg·d) for adults from rice, wheat products, beans, and nuts, respectively. The total average OTA intake was estimated at 0.8548 μg/(kg·d) with the margin of safety of OTA at 94%. They concluded that rice accounted for 55.8% of the total OTA intake and in general the OTA exposure in main food in China was safe. In another risk assessment investigation, Guo and others (2014) determined fumonisins contamination in major food products in China and assessed the dietary exposure risk. Their results indicated that the overall average fumonisins intakes in various sex-age groups ranged from 0.162 to 0.369μg/(kg·d), far lower than the provisional maximum tolerable daily intake of 2 μg/(kg·d), which was established by the Joint Food and Agriculture Organization and World Health Organization Expert Committee on Food Additives (JECFA) (Bolger and others 2001). In addition, the average intake of fumonisins in rice products for the group of 2- to 4-y-old and 4- to 7-y-old children were estimated to be the highest levels among the all age groups at 0.133 and 0.129 μg/(kg·d), respectively, which accounted for 37.3% and 36.2% of the total fumonisins intake including rice products, wheat products, other cereal products, dry beans, and nuts. In comparison, the average intake of fumonisins in wheat products for the group of 2- to 4-y-old and 4- to 7-y-old children were also estimated to be the highest levels among the all age groups at 0.1595 and 0.171 μg/(kg·d), respectively, which accounted for 44.7% and 48.0% of the total fumonisins intake. They hence concluded that the risk of public health hazards induced by fumonisins in food was low in China and the health risk of rice products was even lower than wheat products.

In a study performed by Tang and others (2015), the authors assessed dietary exposure of AFB_1 in rice in Jiangxi province. Dietary exposure of AFB_1 intake was assessed at general consumption and high consumption levels and the data was evaluated using @ Risk software based on Monte Carlo simulation. Their results indicated that the dietary exposure of AFB_1 intake for 2- to 6-y-old children, standard adults, urban standard adults, and rural standard adults at the P99 were 86.16 (74.19 to 102.91), 34.54(29.74 to 41.25), 28.85 (24.84 to 34.46), and 36.32 (31.27 to 43.38) ng/(kg bw/d), respectively. The hepatocellular carcinoma incidences were 2.671 (2.300 to 3.190), 1.071 (0.922 to 1.279), 0.894 (0.770 to 1.068), and 1.126 (0.969 to 1.345) cancers/(105 persons/year). They consequently concluded that the dietary exposure risk of AFB_1 in rice was low in Jiangxi province; however, 2- to 6-y-old children had an inherent risk that should be paid attention to. Due to the highly variable mycotoxin content of rice and other grains in different seasons and regions all over China, and the advance in toxicological data sets for mycotoxins, more sophisticated and practical human health risk assessment models will be developed. However, interactions of mycotoxins and emerging human epidemics of various chronic and infectious diseases are still to pose great challenges to researchers and governments.

Conclusions

Due to inappropriate storage conditions, rice can be an ideal substrate for mycotoxin producing fungi and most of the aflatoxigenic *A. flavus* strains isolated from rice collected in China showed a high capacity for aflatoxin production. Due to greatly diversified climatic conditions in different regions of China, such as Sichuan province which is located in a basin and has climate features with high temperature and humidity in summer and the Yangtze Delta region which is extremely humid in the rainy season and is appropriate for mold growing, as well as poor rice storage conditions of farmers, these unfavorable situations enable fungi to grow rapidly and produce mycotoxins. Consequently, high risk of mycotoxin contamination is unavoidable. Although the Chinese government has updated the national standard of maximum residue limits for major mycotoxins in rice, more mycotoxins should be included to ensure rice safety. From the above data reviewed, it can be concluded that rice in China is generally safe. However, the data obtained from open source is very limited possibly due to the government's confidential policy. Another phenomenon is that researchers put more emphasizes on mycotoxins detection rather than the mycotoxin producing strains identification. Some research data showed that AFB_1 in rice might not be able to completely represent the contamination of AFs; hence, it is necessary to establish the MRL for total AFs besides AFB_1 in national and international standards. In general, more efforts need to be put into risk assessment on mycotoxin contamination of rice especially in those hot and humid regions. The local governments also need to increase investment to build more modern rice storage warehouses with air conditioning to keep rice under optimal temperature and humidity, along with a pest controlling system to prevent postharvest mycotoxin contamination. Limited studies on risk assessment of human health hazards revealed that children are at greater risk than the other age groups, and consequently need to be paid more attention to. Further investigation should focus on isolation and identification of mycotoxin producing fungi strains, especially determination and masked mycotoxin isolation of unknown mycotoxin producing fungal strains. Rice breeding of infection resistance to mycotoxigenic fungal species is probably a fundamental approach to guarantee Chinese rice safety. A better approach is the combination of survey for mycotoxins with intake estimations and risk assessment of exposures of human health hazards to populations by monitoring the appropriate biomarkers. This parallel approach would provide a better foundation for developing effective preventative strategies of mycotoxin contamination.

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