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Mycotoxin Contamination of Maize in China

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Abstract: China is a major cereal-producing country and almost one third of the annual cereal yield is maize. The maize plant and kernel are prone to infection by fungal attack and are most likely to be contaminated with mycotoxins under suitable temperature and humidity conditions, during both the growing and storage period. A number of investigations conducted in China have demonstrated that maize had been infected by fungi and contaminated with mycotoxins to varying degrees. Although most of the maize produced in China is used as feed and raw materials for the chemistry industry, a small amount of maize is consumed directly by humans and the hazards of mycotoxin to humans cannot be ignored. The state of mycotoxin contamination of maize in China is analyzed in this review. Due to unfavorable weather and poor storage conditions, the high incidences of mycotoxin contamination of maize are of great concern to the Chinese. It is imperative for the national and local governments to increase investments on building large-scale modern warehouses and instructing farmers to grow, harvest, and store maize safely. Meanwhile, due to accumulative toxic effects of mycotoxins, quality control should be enforced to guarantee that animal products are safe for human consumption.

Keywords: contamination, fungi, maize, mycotoxins

Introduction

Maize is a grain widely consumed as feed, food, and industrial raw materials across the world. In 2014, China produced 215 million tons of maize, accounting for 35% of annual grains production (NBSPRC 2014). However, the maize plant is easily infected via fungal attack and likely to be contaminated with mycotoxins under suitable temperature and humidity conditions, during both the growing and storage period (Covarelli and others 2011). *Fusarium spp*. commonly colonize maize, and thus fumonisins, deoxynivalenol (DON), and zearalenone (ZEN) are frequently identified in maize grains and its derived products (Marín and others 2012).

One study has estimated that around 25% of crop and oil products worldwide were contaminated with various mycotoxins to different degrees (Fink-Gremmels 1999). The situation could be even worse in China due to poor storage conditions, although relevant data are not available at the present time.

The worldwide occurrence of aflatoxins (AFs), fumonisins, DON, and ZEN in maize and maize-based products, as well as the assessment of human exposure, has been well investigated and reviewed in the literature (Liu and others 2006; Lino and others 2007; Martins and others 2008; Scudamore and Patel 2009; Herrera and others 2010; Cano-Sancho and others 2012; Pleadin and others 2012; Escobar and others 2013). However, limited information is available on mycotoxin contamination of maize in China for 2 reasons. The 1st is the language barrier; almost all the papers on mycotoxin contamination of maize are written by Chinese researchers and published in Chinese journals using Chinese rather than English. Consequently, they are not easily understood by other researchers. The 2nd is the use of confidentiality agreements; the relevant grain safety and risk assessment projects, funded by some national government departments, are restricted by the involved sponsors from being published. Nevertheless, not all such departments carry out such confidential policies and some of the research studies can be found in Chinese journals. Table 1 summarizes different studies conducted on mycotoxin contamination of maize in China since 2000.

Due to diverse climate conditions in China, some maize-growing regions such as the Yangtze Delta Region are extremely humid during the rainy season. This promotes rapid growth and mycotoxin production by fungi during storage (Li and others 2014). The conditions of mycotoxin contamination of maize in the northern and southern regions of China are also different. Gong and others (2016) investigated, compared, and indicated that the incidence, maximum positive value, and average content of mycotoxin in maize in the northern regions were lower than those in the southern regions.

The Chinese government is concerned about mycotoxin contamination in cereals and has renewed the maximum residue limits (MRLs) and standard detection methods for major mycotoxins in foods in 2011 (CMH 2011). Table 2 compares the MRLs of mycotoxins in maize and its derived products with those of the European Food Safety Authority (EFSA) and other countries.

Some of the mycotoxins are formed by fungi encountered in the crop prior to harvesting. Fungal contamination by *Fusarium, Alternaria,* and *Aspergillus* species is often impossible to control. However, it is easier to prevent the formation of mycotoxins

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Table 1–Different studies conducted on investigation of mycotoxin contaminations of maize in China.

Province	Origin of maize samples	Analytical method	Mycotoxins	Total samples	Incidence (%)	Range of positive samples/maximum values (μg/kg)	Mean ±SEM of positive samples/mean (μg/kg)	Reference
Hubei	Barns	ELISA	FBs	60	32	–	17370	Xie and others 2001
			ST	60	85	–	21	
			FT	60	4.0	–	0.6	
			AFB_1	15	80	–	62	
Various regions	Feed plants Warehouses	ELISA	AFB_1	31	84	0.0 to 8.6	24.6 ± 0.3	Wang and others 2003
			FBs	22	77	0.0 to 5800	1150 ± 350	
			OTA	15	60	0.0 to 9.6	3.7 ± 1.0	
			T-2	16	88	0.0 to 50	29 ± 4.5	
			DON	13	100	200 to 2300	820 ± 170	
			ZEN	13	100	4.4 to 368	105 ± 36	
Various regions	Barns		ZEN	33	100	18 to 730	224	Li and others 2004
Northern regions	Feed companies	ELISA	FBs	68	100	80 to 30280	4810	Lv and others 2004
			DON	71	100	270 to 8600	3470	
			AFs	71	100	0.8 to 111	4.0	
			ZEN	69	89	0 to 6900	670	
			OTA	38	97	0.0 to 63	4.1	
			T-2	35	6.0	0.0 to 66	1.9	
Liaoning	Barns	HPLC	AFs	73	97	–	1.0	Liu and others 2006
Jiangsu	Supermarkets and farmers markets	ELISA	AFB_1	48	54	0.0 to 5.7	0.3 (Median)	Xu and others 2006
Henan	Barns	GC	DON	48	83	–	292 ± 4.4	Wang 2006
Hubei				46	78	–	33 ± 1.8	
Sichuan				48	83	–	52 ± 2.1	
Jilin				48	48	–	35 ± 2.4	
Guangxi				48	58	–	23 ± 1.7	
Guangdong				46	48	–	27 ± 1.8	
Sichuan	Feed companies	ELISA	FBs	27	100	960 to 21399	6882	Yang and others 2007
			DON	28	100	225 to 17163	3927	
			ZEN	27	74	0.0 to 1816	193	
			T-2	27	93	0.0 to 431	109	
			OTA	27	81	0.0 to 27	6.2	
			AFB_1	28	100	0.3 to 56	9.9	
Various provinces	Feed companies	ELISA	AFs	157	17.8	937	57	Zhang and liu 2007
		HPLC	AFB_1	157	15.9	714	48	
			AFB_2	157	2.5	14	7	
			AFG_1	157	6.4	223	36	
			AFG_2	157	1.3	3	2	
			ZEN	157	42	3514	376	
			DON	157	99	6088	1034	
			FBs	106	87	162000	6350	
			FB_1	106	87	117576	4879	
			FB_2	106	84	44415	1521	
			T-2	141	3.5	317	146	
			OTA	31	3.2	3	3	
Various provinces	Feed companies	ELISA HPLC	AFB_1	93	18	222	47	Zhang and liu 2008
			ZEN	97	42	955	211	
			DON	97	96	27852	991	
			FB_1	93	67	13025	2275	
			T-2	91	0	ND	ND	
			OTA	5	20	3	3	
Various provinces	Feed companies	ELISA	AFs	39	82	0.0 to 21	3.1	Ao and Chen 2008

(*Continued*)

Table 1–Continued.

Province	Origin of maize samples	Analytical method	Mycotoxins	Total samples	Incidence (%)	Range of positive samples/maximum values (μg/kg)	Mean ±SEM of positive samples/mean (μg/kg)	Reference
			OTA	32	100	0.7 to 16	6.6	
			T-2	32	94	0.0 to 57	20	
			ZEN	44	82	0.0 to 825	143	
			FBs	39	90	0.0 to 8020	1490	
			DON	44	100	60 to 4720	1280	
Various provinces	Feed companies	HPLC	AFB_1	97	20	289	39	Zhang and liu 2009
			ZEN	93	58	3112	388	
			DON	89	83	6120	1140	
			FB_1	78	72	9923	2302	
			OTA	73	15	19	6	
Anhui Henan	Barns	ELISA	DON	8	63	–	36 ± 54	Xiong 2010
				28	89		261 ± 352	
Various provinces	Feed companies	HPLC	AFB_1	83	9.6	16	7	Zhang and liu 2010
			ZEN	88	73	2662	390	
			DON	86	99	7871	1291	
			FB_1	84	91	8624	2426	
			OTA	83	7.2	18	6	
Various provinces	Feed companies	ELISA	ZEN DON	195	81	4904	198 ± 431	Zhen 2011
					98	21935	1447 ± 2171	
12 Provinces	Supermarkets/barns/farmers markets	UPLC-MS/MS	NIV	215	87	0.1 to 961	15	Ma and others 2011
			FX		79	0.2 to 134	5.0	
			ZEN		69	0.1 to 1151	49	
			DAS		60	0.3 to 86	1.7	
			HT-2		16	0.1 to 5	0.6	
			T-2		46	0.1 to 1	0.3	
			AFB_1		53	0.1 to 581	15	
			AFB_2		31	0.1 to 35	1.6	
			AFG_1		18	0.2 to 5.9	0.4	
			AFG_2		32	0.1 to 9.9	0.6	
6 Provinces	Barns	HPLC	AFB_1	279	75	0.2 to 864	40	Gao and others 2011
			AFB_2		59	0.0 to 45	3.8	
			AFG_1		41	0.3 to 211	3.5	
			AFG_2		20	0.0 to 12	0.5	
Beijing	Animal farms	HPLC	AFB_1	22	50	59	6.0 ± 4.0	Fan and others 2012
			AFB_2		9.1	12	0.6 ± 0.6	
			AFG_1		4.5	0.5	0.0 ± 0.0	
			AFG_2		9.1	0.5	0.0 ± 0.0	
Various provinces	Feed companies	HPLC	AFB_1	295	6	150	36	Wang and others 2013a
			ZEN	299	48	2858	658	
			DON	299	88	8702	1082	
			FBs	263	87	9554	1883	
			OTA	255	2	15	7	
Various provinces	Feed companies	ELISA	AFB_1	997	84	7.3	0.6	Du 2013
			ZEN		95	1672	198	
			DON		64	15063	1337	
Shanghai	Animal farms	ELISA	AFB_1	42	0	0	0	Wang and others 2013b
			DON	35	60	220 to 4330	1229	
			OTA	21	38	2.0 to 52	21	
			ZEN	42	52	25 to 4918	566	
			FBs	14	50	230 to 2780	910	
12 Provinces	Feed companies	ELISA	AFB_1	776	73	1.2	0.4	Du 2014

(*Continued*)

Table 1–Continued.

Province	Origin of maize samples	Analytical method	Mycotoxins	Total samples	Incidence (%)	Range of positive samples/maximum values (μg/kg)	Mean ±SEM of positive samples/mean (μg/kg)	Reference
			ZEN		97	1053	123	
			DON		89	8162	874	
			FB_1		80	63211	10807	
			T-2		66	124	24	
27 Provinces	Markets, feed companies, and animal farms	ELISA LC-MS	ZEN	622	29	12163	171 ± 587	Cheng and others 2014a
27 Provinces	Markets, feed companies, and animal farms	ELISA	T-2	125	25	217	7.9 ± 13	Cheng and others 2014b
27 Provinces	Markets, feed companies, and animal farms	ELISA LC-MS	AFB_1	622	29	232	3.1 ± 17	Cheng and others 2014c
27 Provinces	Markets, feed companies, and animal farms	ELISA LC-MS	OTA	125	24	56	7.1 ± 24	Cheng and others 2014d
Xizang	Farmers markets Supermarkets	ELISA LC-MS/MS	DON	80	43	253	24	Si 2014
			ZEN		96	1930	59	
Shandong	Feed companies	ELISA	AFB_1	87	99	0.0 to 183	8.8 ± 3.3	Fu 2014
			ZEN		100	1.2 to 8208	523 ± 172	
			DON		100	1.0 to 5051	294 ± 70	
27 Provinces	Markets, feed companies, and animal farms	ELISA	DON	287	82	4632	150 ± 32	Cheng and others 2015
Various provinces	Feed companies	ELISA	AFB_1	532	53	40	5.3	Du 2015
			ZEN	568	82	1050	160	
			DON	564	92	5064	934	
			FB_1	446	63	57564	4321	
			T-2	378	53	91	37	
12 Provinces	Feed companies	ELISA	AFB_1	461	77	110	4.1	Du 2016
			ZEN	467	84	2635	261	
S			DON	467	86	7000	1146	
			FB_1	434	64	37700	4285	
			T-2	446	79	53	31	
Various provinces	Barns and markets	ELISA	AFB_1	65	92	538	–	Gong and others 2016
			ZEN	65	86	1013	–	
			DON	64	94	8483	–	
Various provinces	Feed companies	ELISA	AFB_1	296	98	29	4.8	Ji and others 2016
			ZEN		93	1549	242	
			DON		99	4500	1035	

ND, not detected (below the quantification limit); LOQ, limit of quantification; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; GC, gas chromatography; UPLC-MS/MS, ultra-performance liquid chromatography-mass spectrometry/mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; LC-MS/MS, liquid chromatography-mass spectrometry/mass spectrometry; IAC-HPLC, immunoaffinity chromatography-high-performance liquid chromatography; UPLC-Q-TOF, ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry; FBs, fumonisin B-series; FB_1, fumonisin B_1; FB_2, fumonisin B_2; FB_3, fumonisin B_3; AFs, aflatoxins; AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; AFG_1, aflatoxin G_1; AFG_2, aflatoxin G_2; DON, deoxynivalenol; OTA, ochratoxin A; ZEN, zearalenone; T-2, T2 toxin; ST, sterigmatocystin; FX, fusarenon-X; DAS, diacetoxyscirpenol.

Table 2–Comparison of the MRLs of mycotoxins in maize and its products with EFSA and other countries.

Country or organization	Mycotoxins	MRLs μg/kg	Scope of application	Reference
China	AFB_1	20	Food	China's Ministry of Health 2011
	DON	1000		
	OTA	5		
	ZEN	60		
China	AFB_1	50	Feed	General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (AQSIQ) 2001
	DON	1000		
	OTA	100		General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (AQSIQ) 2006
	ZEN	500		
EFSA	AFB_1	2	Food	Food Standards Agency 2016
	DON	1750		
	OTA	5		
	ZEN	350		
	FB_1 and FB_2	4000		
EFSA	AFB_1	20	Feed	Food Standards Agency 2016
	DON	8000		
	OTA	250		
	ZEN	2000		
	FB_1 and FB_2	60000		
USDA	AFB_1	5	Food	United States Department of Agriculture (USDA) 2013
	OTA	5		
	DON	1750		
	FB_1 and FB_2	4000		
	ZEN	350		

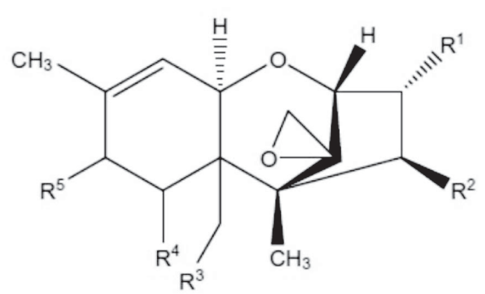
MRLs, maximum residue limits; AFB_1, aflatoxin B_1; DON, deoxynivalenol; OTA, ochratoxin A; ZEN, zearalenone; FB_1, fumonisin B_1; FB_2, fumonisin B_2.

produced during storage by species belonging to *Penicillium* and *Aspergillus* mainly by drying crops at harvest time (CAST 1989). Unfortunately, sometimes, this is not easily done, and even if performed correctly subsequent poor storage practices may result in additional fungi growth and mycotoxin formation (Stoev 2007; 2013a, 2013b). It has been reported that only 38.1% of farmers cared for maize storage conditions in China although more than 75.0% of farmers followed good agricultural practices (Liu and others 2016). An investigation on mycotoxin contamination of maize samples collected in Shandong Province at various stages in 2013 and 2014 showed that the incidences and average contents of FBs (FB_1, FB_2, and FB_3), AF (AFB_1, AFB_2, and AFG_1), DON, and ZEN increased from harvest stage to storage period, and the authors deduced that the increase in mycotoxin content was caused by fungal growth and reproduction (Wang and others 2016). Investigations also revealed that 100- to 500-fold or greater levels of fumonisin had been detected in insect-damaged maize kernels in comparison with those which were not insect-damaged, while closer to 10- to 30-fold higher levels of DON were determined in insect-damaged versus noninsect-damaged maize kernels (Dowd and Johnson 2010). The cocontamination of foods/feeds with known or unknown mycotoxins is being reported at an increasingly high rate (Stoev and others 2010a, b).

Because of the multiple possible origins of fungal contamination, strategies for preventing fungal and mycotoxin contamination should be conducted at an integrative level along the food production chain (Robens and Cardwell 2003). In particular, 3 intervention steps may be performed. The 1st step is the prevention of contamination before any fungal infection. The 2nd step is at the stage of fungal invasion of maize and mycotoxin production, when the maize has been identified as heavily contaminated. The 3rd step is taken to reduce or deactivate the produced mycotoxins. There are 2 objectives to this report; the 1st is to systematically review research studies conducted on mycotoxin contamination of maize in China, relevant dietary exposure, and risk assessments of these mycotoxins regarding human health. The 2nd is to analyze situations and problems that have occurred in various regions under different climatic and storage conditions in China. Practical strategies to prevent mycotoxin contamination of maize are also proposed.

Deoxynivalenol

DON is a type B trichothecene mycotoxin which is mainly generated from the secondary metabolism of a variety of *Fusarium graminearum* and closely related species commonly found in plant crop products, especially feeds and foods. Figure 1 demonstrates the chemical structures of DON and its modified forms. Ingestion of DON may result in immuno-suppression due to inhibition of proteins and DNA synthesis (D'Mello and others 1999; Amuzie



Mycotoxin	R^1	R^2	R^3	R^4	R^5
DON	OH	H	OH	OH	O
DON3G	$C_6H_{11}O_6$	H	OH	OH	O
15ADON	OH	H	OAc	OH	O
3ADON	OAc	H	OH	OH	O
DON3GlcA	$C_6H_9O_7$	H	OH	OH	O
DON15GlcA	OH	H	$C_6H_9O_7$	OH	O

OAc = acetoxy group (Adapted from Broekaert and others 2015)

Figure 1–Structures of deoxynivalenol and its modified forms.

and others 2009; Wache and others 2009). DON exposure is associated with the occurrence of acute gastrointestinal diseases, which is why it is recognized as one of the key mycotoxins of great public health concern (IARC 2012; Ortiz and others 2013). Another concern is the cocontamination of foods with modified forms of DON (masked DON or covered DON) and their potentially additive or synergistic effects (Li and others 2011; Ma and others 2011; Han and others 2014). For example, A DONs (3-ADON and 15-ADON) may quickly deacetylate in mammals, producing almost the same toxicity as the parent DON (Han and others 2014).

Wang (2006) collected 284 maize samples from 6 provinces and detected DON contents using gas chromatography. His results showed an incidence of 67%, a range of positive samples at 10 to 3800 μg/kg, an average content of 26 μg/kg, and a mean of positive samples at 52 μg/kg for all 6 provinces. He indicated that the tendency of DON contamination was characterized by high positive rates and low DON content. He also found that the DON positive rate and mean content of disease-resistant cultivars (93% and 89 $\pm$ 5.8 μg/kg) were significantly higher than those of nondisease-resistant maize varieties (56% and 17 $\pm$ 3.1 μg/kg), the DON positive rate and mean content of genetically modified maize (97% and 244 $\pm$ 5.0 μg/kg) were dramatically greater than those which were not genetically modified (94% and 41 $\pm$ 3.4 μg/kg), and the DON positive rate and mean content of undamaged maize (66% and 26 $\pm$ 5.0 μg/kg) were significantly lower than those of damaged maize (75% and 40 $\pm$ 5.3 μg/kg). The author indicated that due to unclear mechanisms, further investigation was necessary. Zhang and Liu (2007) indicated that DON was the major mycotoxin which contaminated maize with 99% of incidence, 1034 μg/kg of average content, and 827 μg/kg of median value. This was further confirmed by results of the same authors (Zhang and Liu 2008) who detected an incidence rate of 96% for DON contamination in maize at an average content of 991 μg/kg, and a median value of 510 μg/kg. DON was also recorded with the greatest incidence rate compared with the other mycotoxins. However, the incidence and content levels fluctuated with climatic conditions; maize samples harvested in 2007 were a bit better than those in 2006 with lower incidence and average content. Zhang and Liu (2009) determined an incidence of 83% for DON in 2008, which was lower than that in 2007 and 2006 (96% and 99%, respectively). However, the average content of DON in 2008 (1140 μg/kg) was similar as both in 2007 and 2006 (991 and 1034 μg/kg, respectively). The incidence and average content of DON increased to 99% and 1291 μg/kg, respectively, in maize samples harvested in 2009 (Zhang and Liu 2010). Chen (2011) reported a 100% incidence for DON at an average content of 1320 μg/kg with a maize sample size of 47 collected from 18 provinces. This average content has already exceeded the national MRL (1000 μg/kg) (CMH 2011), which indicates a severe contamination of DON to the maize samples and the maximum content of DON in the maize samples even reached 7950 μg/kg. The author indicated that the contamination of DON in the maize and compound feed samples were more serious in 2009 in comparison with 2007 and 2008. Ma and others (2011) conducted a survey to investigate the natural occurrence of multimycotoxins in cereals and cereal-based products collected from 12 provinces. Their results showed that among 215 maize samples, 182 (85%) were detected as DON-positive with a mean value of 197 μg/kg (Table 3); 7 samples exceeded the national MRL of 1000 μg/kg (an average content of 1.8 times greater than 1000 μg/kg).

Table 3–Variation of field mycotoxins of feed maize during storage.

Detection items		DON	ZEN
Total sample		23	23
National MRLs (μg/kg)		1000	500
Rate of exceeding MRLs (%)	1st time	26.1	13.5
	2nd time	69.6	21.6
Mean (μg/kg)	1st time	740.2	310.2
	2nd time	2135.2	488.8
Mean of growth rates (%)	288.5	157.6	

Note: Maize samples were harvested in the fall, 2013, and 23 maize samples contaminated with DON and ZEN were selected and stored under cool and dry condition and tested in October, 2013 (1st time) and June, 2014 (2nd time), respectively.
Adapted from Cheng and others (2016).

Wang and others (2013a) conducted an investigation on mycotoxin contamination of feeds and feed materials from large-scale animal farms in Shanghai and found that the average content of DON (1229 μg/kg) in maize was greater than the national MRL. However, the sample size was only 35 for DON detection in this investigation. Fu (2014) documented a positive rate of 100% for DON contamination in maize samples collected from feed companies in Shandong Province at a sample size of 87, with 6.9% of the samples exceeding the national MRL. Wang and Du (2015) performed a nationwide survey to investigate mycotoxin contamination of feeds and feed raw materials from July to September of 2014 with a maize sample size of 336 and found that the incidence of DON (88%) and ZEN (88%) of maize samples were significantly greater than those of AFB_1 (70%), FB_1 (66%), and T-2 (40%). However, none of the mean values of these positive samples exceeded the national MRLs. Ji and others (2016) investigated mycotoxins contamination of feeds and feed raw materials from parts of China with a maize sample size of 296 harvested in 2015. Their results showed that 99% of maize samples had been contaminated by DON with an average content of 1035 μg/kg which was greater than the national MRL for feeds and feed raw materials. They also indicated that the positive mean value of DON in maize in 2015 was greater than that in 2014 (844 μg/kg).

Cheng and others (2016) investigated variations of field mycotoxins in feed maize at the storage stage using high performance liquid chromatography-mass spectrometry (HPLC-MS) and found that DON and ZEN contents of feed maize significantly increased under cool and dry storage conditions (Table 4). However, some data are confusing, such as the mean growth rate where the authors did not indicate whether it referred to the rate of exceeding MRLs or the mean values of the mycotoxin contents. After double-checking the data, it was observed that the mean growth rates (288% and 158% for DON and ZEN) did not fit any of the above items (rate of exceeding MRLs or mean values of the mycotoxins content).

Cooccurrence of mycotoxins of DON and its acetyl derivatives and their cumulative health risk were also investigated and assessed in China recently. Li and others (2011) performed a survey to investigate natural occurrence of masked DON and multimycotoxins in cereals harvested in 2007 and 2008. Their results showed that for 204 maize samples collected from 7 provinces, an incidence of 51% for DON was detected with a mean content at 319 μg/kg. The major acetyl derivative of DON appeared to be 15-acetyldeoxynivalenol (15-A DON) with an incidence of 47% and a mean content of 170 μg/kg, followed by DON 3-G with an incidence of 34% and an average content of 71 μg/kg. 3-Acetyldeoxynivalenol (3-A DON) appeared to be the minor acetyl derivative of DON with the lowest level of incidence (36%) and mean value (23 μg/kg) (Table 4). Similar results were also reported by Ma and others (2011); their data showed higher

Table 4–Occurrence of deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-A DON), 15-acetyldeoxynivalenol (15-A DON), and deoxynivalenol-3-glucoside (DON 3-G) in maize in various regions of China.

Province	Analytical methods	Mycotoxins	Total samples	Incidence (%)	Range (μg/kg)	Mean ±SD/Mean (μg/kg)	Conference
7 Provinces	UPLC-MS/MS	DON	204	50.5	1.6 to 4374	319	Li and others 2011
		3-A DON		35.5	0.8 to 368	23.1	
		15-A DON		47.3	1.5 to 1734	170	
		DON 3-G		34.0	2.0 to 499	70.7	
12 Provinces	UPLC-MS/MS	DON	215	84.7	0.1 to 2512	197	Ma and others 2011
		3-A DON		71.6	0.1 to 170	8.2	
		15-A DON		92.6	0.1 to 1519	68.7	
		DON 3-G		77.2	0.5 to 1979	46.7	
Shanghai	LC-MS/MS	DON[a]	132	58.3	0.5 to 584	78.9 ± 114	Han and others 2014
		DON[b]	50	100	0.5 to 584	116 ± 126	
		3-A DON[c]	50	100	0.7 to 8.4	2.0 ± 1.5	
		15-A DON[d]	50	100	0.5 to 242	23.9 ± 41.2	
		Total[e]	50	100	3.3 to 834	142 ± 152	

[a]The contamination levels of DON in wheat and maize samples collected from 2009 to 2012.
[b]The contamination levels of DON in wheat and maize samples collected from 2011 to 2012.
[c]The contamination levels of 3-DON in wheat and maize samples collected from 2011 to 2012.
[d]The contamination levels of 15-DON in wheat and maize samples collected from 2011 to 2012.
[e]Total was designed as the sum of the concentrations of DON, 3-ADON, and 15-ADON in the samples collected from 2011 to 2012.

incidence values and lower mean values in comparison with the relevant data of Li and others (2011).

In a project conducted by Han and others (2014), based on the concentration addition (CA) concept, they assessed the accumulative health risks of concomitant exposure via dietary intake (DI) to DON and its acetyl derivatives of 3-ADON and 15-ADON. The incidence of DON in maize samples was detected at 58% with concentrations ranging up to 584 μg/kg collected in the period of 2009 to 2012 (Table 4). The authors also compared the differences in health risks of DON, 3-A DON, and 15-A DON between maize and wheat and indicated that the average DI values for the 3 mycotoxins were 2.3 and 169 ng/kg body weight/d for maize and wheat, respectively. They hence concluded that less than 10% of the risks were due to the contamination of maize with mycotoxins.

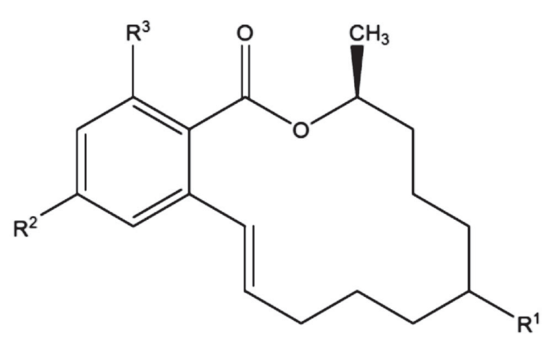
In general, from the literature reviewed, a DON incidence of 26% to 100% (Wang and others 2003; Lv and others 2004; Yang and others 2007; Ao and Chen 2008; Wang and others 2016) and an average content of positive maize samples of 23 to 3927 μg/kg (Wang 2006; Yang and others 2007) were documented. Maize intended for consumption is usually less contaminated by DON, with a lower incidence and average content than maize used as raw fed material. This is probably due to better hygienic conditions for maize intended for human consumption during packaging, transportation, and storage.

Mycotoxin-contaminated feeds have been confirmed to induce symptoms in animals, such as sickness, delay of growth, declining productivity, and even death. The economic loss resulting from the reason listed above forces owners of medium- and large-scale animal farms to seek safe feeds by requiring feed companies to provide inspection reports of their products. In addition, although maize as raw feed material is not directly consumed by humans, the potential risk of migration of mycotoxins from animal products to humans should not be ignored.

Zearalenone

ZEN is a mycotoxin produced by several *Fusarium* species, including *Fusarium graminearum (Fusarium roseum), Fusarium culmorum, Fusarium equiseti, Fusarium cerealis, Fusarium verticillioides, and Fusarium incarnatum* (Marin and others 2013), which grow on and attack crops under moist cool conditions during blooming in fields. Schneweis and others (2002) demonstrated the presence of the modified mycotoxin zearalenone-14-glucoside (ZEN14G) in naturally contaminated wheat for the 1st time. This result was a significant breakthrough because, although its presence had already been proved in *in vitro* studies with maize suspension cultures (Engelhardt and others 1988; Zill and others 1990) using indirect methods such as enzymatic hydrolysis (Gareis and others 1990), no study had confirmed the occurrence of modified ZEN14G in naturally contaminated grain before. Figure 2 demonstrates the chemical structures of ZEN and its modified forms.

All ZENs are estrogenic compounds and heat-stable up to 150 °C. The occurrence frequency of ZEN in maize (33%) and average content (15 μg/kg) are significantly higher among the grains for human consumption (EFSA 2011). ZEN is generally unaffected by cooking conditions. However, more than 40%



Mycotoxin	R^1	R^2	R^3
ZEN	O	OH	OH
ZEN14G	O	$C_6H_{11}O_6$	OH
ZEN14S	O	SO_4^-	OH
ZEN16G	O	OH	$C_6H_{11}O_6$
α-ZEL	OH	OH	OH
α-ZEL14G	OH	$C_6H_{11}O_6$	OH
β-ZEL	OH	OH	OH
β-ZEL14G	OH	$C_6H_{11}O_6$	OH

Figure 2–Structure of zearalenone and its modified forms. α-ZEL and β-ZEL (and their modified forms) differ in the position of the hydroxyl group at R^1, for α-ZEL the hydroxyl group is behind the page plane (hashed wedged bond) and for β-ZEL, it is in front of the plane (wedged bond). Adapted from Broekaert and others 2015.

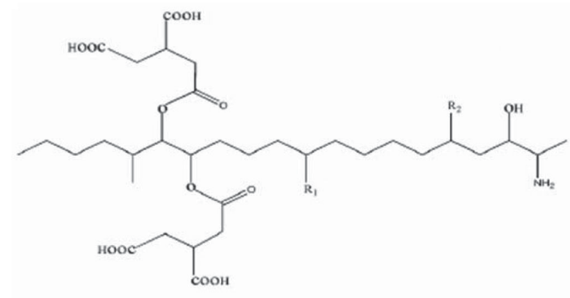
reduction of ZEN was reported under alkaline conditions or during extrusion-cooking (heating under high pressure) (EFSA 2011).

Chen (2011) reported a 100% positive rate for ZEN at an average content of 291 μg/kg with a maize sample size of 38 samples collected from 18 provinces. This result indicated extensive contamination of ZEN in maize samples. However, the average content of ZEN has not exceeded the national MRL (500 μg/kg) (GAQSIQ 2006). In comparison, a light contamination condition of ZEN was reported by Ma and others (2011), with 69% of incidence, and 49 μg/kg of average content at a sample size of 215 collected from 12 provinces. A survey performed by Wang and others (2013a) on mycotoxins contamination of feeds and feed materials from large-scale animal farms in Shanghai showed that the mean value of ZEN (566 μg/kg) in positive maize samples was much greater than the national MRL for food (60 μg/kg) (CMH 2011). This average value was even higher than the national MRL for raw feed materials (500 μg/kg; GAQSIQ 2006). The authors hence concluded that ZEN was the major mycotoxin which contaminated maize and that the degree of contamination was very serious. Another investigation conducted by Du (2013) also showed that ZEN was the most serious mycotoxin detected in 997 maize samples collected from 12 provinces with an incidence of 95% and an average content of 198 μg/kg. However, the mean value of ZEN in her survey met the national MRL for raw feed materials. Furthermore, Du (2014) detected a ZEN incidence of 100% for maize samples collected from Shandong Province: 13% of the samples were determined to have exceeded the national MRL, and an overall average content of 523 μg/kg was calculated, also exceeding the national MRL. Then, a lower value was obtained by Rong and others (2015), who detected an average content of 478 μg/kg of ZEN in positive maize samples collected from Beijing, Shandong, Henan, Sichuan, and other areas, with an incidence at 94% for 112 samples in total. This average content value was close to the national MRL for raw feed materials (500 μg/kg; GAQSIQ 2006). In general, ZEN is the most frequent mycotoxin found in maize and often detected at an average level exceeding the national MRL. Consequently, actions need to be taken to prevent ZEN-contaminated maize from being used as raw feed material.

Fumonisins

Fumonisins are produced by *F. verticillioides* and *Fusarium proliferatum* (Gelderblom and others 1988), which are common fungal contaminants of maize and maize-derived products worldwide (Wang and others 2006). Other fungal species, including *F. napiforme, F. dlamini,* and *F. nygamai,* produce fumonisins as well (EFSA 2005). Fumonisins mainly occur in maize in comparison with the other grains. According to Gelderblom and others (1991), fumonisins have been classified into 4 groups, the A, B, C, and P-series; as they are a family of structurally related mycotoxins. The B-series has the most abundant analogs with fumonisin B_1 (FB_1) accounting for approximately 70% of the total fumonisins (Nelson and others 1993) followed by FB_2 (Shephard and others 1996; Lino and others 2006; Yazdanpanah and others 2006). From a toxicological point of view, FB_1 is the most important fumonisin. Since the 1st identification of FB_1, 28 diverse fumonisin analogs have been characterized (Rheeder and others 2002). Figure 3 demonstrates the structure of the fumonisin B-series (FBs).

Among those mycotoxins found in maize, fumonisins are also the most common ones, especially when grown in warmer regions. The species *F. verticillioides* and *F. proliferatum* can grow in a wide range of temperatures. These species require relatively high water activities (0.9 or higher) for growth (Pitt and Hocking 2009; Pitt and others 2012). Maize FBs are formed before harvest or at the early stage of storage in maize. The level of FBs will not rise during storage, except under extreme conditions (Marin and others 2013). FBs are heat-stable, and their contents cannot be greatly reduced during processes unless the temperature exceeds 150 °C. Little degradation of FBs has been reported during fermentation (EFSA 2005).



Mycotoxin	R_1	R_2
FB_1	OH	OH
FB_2	OH	H
FB_3	H	OH
FB_4	H	H

(Adapted from Bezuidenhout and others 1988)

Figure 3–Structure of fumonisin B-series.

A wide range of diseases, such as leukoencephalomalacia in horses, pulmonary edema and hydrothorax in pigs, and liver cancer in rats, have been associated with fumonisins (Marasas and others 1988; Harrison and others 1990; Kellerman and others 1990; Gelderblom and others 1991; Gelderblom and others 1996; Gelderblom and others 2001). Epidemiological studies indicate that fumonisins are associated with the high incidence of human esophageal cancer (OC) in some OC-endemic regions, although the effects of fumonisins on humans have not yet been established. Some areas of China have already been confirmed to be OC-endemic regions (Chu and Li 1994; Wang and others 2000).

FB_1 has been classified as potentially carcinogenic in humans (Group 2B) by the Intl. Agency for Research on Cancer (IARC) (IARC 2002). A maximum total fumonisin level of 4000 μg/kg in unprocessed maize has been set by the European Commission (EC) (EC 2005). A maximum fumonisin content of 2000 μg/kg for maize and maize-based products for human consumption has also been recommended by the Food and Drug Administration (FDA) of the United States (U.S. FDA 2001). It is believed that fumonisin formation mainly occurs in maize before harvest. However, evidence indicates that the toxins could be produced at the maize postharvest stage, especially when maize suffers from high temperatures and high relative humidity (Jackson and Jalonski 2004).

In early 1999, Yan and others investigated fumonisin contamination of major grains in Shandong Province as well as the capacity of fumonisin-producing mycotoxigenic fungi. Their results indicated that although the incidence of FBs in maize (26%) was lower than those of wheat (94%) and rice (95%), the content of FBs in maize (31 mg/kg) was greater than those of wheat (28 mg/kg) and rice (28 mg/kg). However, no significant

Table 5–Fumonisin contamination of maize samples collected from various regions in China.

Area	Samples		Level of FB_1 in positive samples[a]	
	Samples size	Positive/incidence (%)	Mean (μg/kg)	Range (μg/kg)
Northeastern Area	21	6/28.6	240 ± 40	210 to 290
Middle-eastern Area	24	16/66.7	740 ± 180	250 to 1800
Southeastern Area	20	6/30.0	470 ± 100	300 to 820

Note: Northeastern Area (Heilongjiang, Liaoning, and Jilin Province), Middle-eastern Area (Shandong and Henan Province), and Southeastern Area (Jiangsu and Zhejiang Province).
[a]Mean±standard derivation.
Adapted from Wang and others (2008b).

difference was observed between the content of FBs in maize and wheat, as well as rice. No significant difference was observed for average FB contents between freshly harvested maize and maize stored for 1 y. Zhang (2007) investigated fumonisins B_1 contamination of maize in 6 provinces and determined almost 100% incidence, except in Jilin Province (98%). The minimum average content of FB_1 in maize samples was 902 μg/kg from Jilin Province; while the maximum content was 19565 μg/kg in Sichuan Province. The author indicated that FB_1 content gradually increased from northern to southern provinces due to warm and humid climatic conditions. In a survey conducted by Zhang and Liu (2007), they reported FBs incidence level at 87% with maximum, mean, and median values at 161991, 6351, and 2864 μg/kg for the 106 maize samples collected from various regions of China.

Wang and others (2008a) performed a survey to detect fumonisin levels in a total of 104 maize kernel samples collected from Linxian County, a high-risk area for esophageal cancer in China. High-performance liquid chromatography coupled with an evaporative laser scattering detector (HPLC-ELSD) was applied to determine the content of mycotoxin fumonisins. These maize samples were collected from local households, granaries, wholesale markets (central markets), and retail markets (stores and supermarkets). FB_1 positive rates of 62%, 50%, 33%, 17%, and 0% were detected in the samples from households, granaries, central markets, stores, and supermarkets, respectively. The highest FB_1 contents (300 to 3200 μg/kg; 1420 μg/kg of mean value) were detected in samples from the granaries, followed by household (250 to 1800 μg/kg; 730 μg/kg of mean value), then central market (250 to 1100 μg/kg; 510 μg/kg of mean value), stores (220 to 340 μg/kg; 280 μg/kg of mean value), and the lowest from supermarkets. Seventy-five percent (18 out of 24 moldy samples) of contaminated samples were detected to possess high levels of FB_1 (280 to 3300 μg/kg; 1580 μg/kg of mean value) among the 80 maize kernel samples collected from local households, while 36% (20 out of 56) apparently healthy samples contained low levels of FB_1 (210 to 820 μg/kg; 460 μg/kg of mean value). The authors also collected a total of 115 maize-based foods and feed samples from local central markets. Their results showed that FB_1 was the only fumonisin detected by HPLC-ELSD in all feed and maize-based food samples collected in Linxian County, and the highest levels of FB_1 (300 to 3130 μg/kg; 1500 μg/kg of mean value) were determined in feed, followed by unprocessed food (310 to 630 μg/kg; 470 μg/kg of mean value) and processed food (210 to 280 μg/kg; 250 μg/kg of mean value). The positive incidences of FB_1 were 54%, 33%, and 18% in feed, unprocessed, and processed food, respectively. The authors hence concluded that low levels of FB_1 (< 2000 μg/kg) existed in maize-based food and feed from Linxian County. The average fumonisin contamination levels of the maize, feed, and maize-based food collected from various sources of Linxian County were found usually to be lower than those in other regions of China (Table 1). However, the authors had not discussed the correlation between esophageal cancer and fumonisin levels in maize and its derived products in this county. It seems that maize and its derived maize products are safe in terms of fumonisin contamination levels according to the guidance standards of U.S. Dept. of Agriculture (2013) and EFSA (2016) (Table 2). Consequently, there are probably major reasons other than contamination of fumonisins in maize and its derived products which causes the high risk of esophageal cancer in this area or county.

In a study on a new analytical method, Wang and others (2008b) applied the HPLC-ELSD method to detect fumonisins in maize from different areas of China. Their results showed that FB_1 was the main contaminant in the samples and the overall level of fumonisin contamination was relatively low (Table 5).

Wei (2013) conducted a project to investigate the effect of various factors on fumonisin contamination in 369 maize samples collected from 6 provinces including Inner Mongolia, Gansu, Ningxia, Henan, Hebei, and Shandong. The author also designed a test to explore the effects of pests, pathogenic bacteria, and climatic conditions on maize ear rot and fumonisin contamination at the silking stage. Cotton bollworms and maize borers were employed as test insects and 2 strains of *F. verticillioides* with different toxin-producing abilities were inoculated or coinoculated in the maize samples. The results showed that pests and pathogens increased the occurrence of maize ear rot and the level of fumonisins. The degree of maize ear rot was greater with inoculation of high-toxin-producing *F. verticillioides* in comparison with that of the low-toxin-producing one. No absolute relationship existed between the varieties of insect and the levels of fumonisin accumulation; the combination of insect varieties with climatic conditions determined the degree of fumonisin contamination. Varieties of *F. verticillioides* with different toxin-producing abilities directly determined fumonisin contents in maize. Different varieties of maize exhibited different insect- and pathogen-resistant capacities. The author consequently indicated that the incidence and degree of maize ear rot and fumonisin contamination could be greatly reduced through the selection of known insect- and pathogen-resistant maize varieties.

The author also investigated the natural occurrence of FB_1 and B_2 in maize sampled from Inner Mongolia, Gansu, Ningxia, Henan, Hebei, and Shandong Province. Significant differences were observed for fumonisin levels in the 6 provinces in terms of incidences and mean values. The incidences of fumonisin were the highest in Liaocheng, Shandong province (81% for FB_1 and 68% for FB_2) when compared to those of Inner Mongolia (FB_1: 54%, FB_2: 43%), Henan (FB_1: 50%, FB_2: 20%), Hebei (FB_1: 39%, FB_2: 22%), Gansu (25% for FB_1 and 16% for FB_2), and Ningxia (19% for FB_1 and 42% for FB_2). The author indicated that the mean values of fumonisins in Inner Mongolia, Gansu, Ningxia, Henan, Hebei, and Shandong Provinces were 1399, 175, 373, 354, 251, and 2496 μg/kg, respectively. Although the samples from Shandong were detected with the highest total fumonisins ($FB_1 + FB_2$)

content, they did not exceed the MRL of the EFSA and USDA of 4000 μg/kg for food, not to mention the MRL of the EFSA of 60000 μg/kg for feed. Since no MRLs have been set up for fumonisins in both food and feed by Chinese authorities, per the EFSA limit of 4000 μg/kg, the total fumonisin contents of all 6 provinces were considered safe for human consumption.

Li and others (2015) investigated natural occurrences of fumonisins B_1 and B_2 in a total of 146 maize samples collected from 3 major maize-producing provinces, including Gansu, Sichuan, and Guizhou after a maize harvest in 2012 using high-performance liquid chromatography coupled with fluorescence detection following *o*-phthalaldehyde derivatization. They obtained an incidence and mean value of fumonisins ($FB_1 + FB_2$) contamination for all the 146 collected maize samples at 40% and 497 μg/kg, respectively. The mean contents of total fumonisin contamination of maize samples from Guizhou, Sichuan, and Gansu were detected at 754, 527, and 202 μg/kg, respectively; and 3.4% of the maize samples were determined to possess total fumonisins levels greater than the limit of 2000 μg/kg set by the U.S. FDA, and 1.4% was detected exceeding the 4000 μg/kg of the MRL recommended by the EC. The authors hence suggested that further studies should focus on the accumulative mechanism of fumonisin contamination and the climatic conditions and other factors influencing fumonisins production.

Wang and others (2016) investigated various mycotoxin contaminations of 520 maize samples in Shandong Province harvested during 2013 to 2014 using liquid chromatography time-of-flight mass spectrometry. Their results showed that 481 out of 520 maize samples were detected for FB, with an incidence of 93% and an average content of FBs ($FB_1 + FB_2 + FB_3$) of 2528 μg /kg, far exceeding the maximum limit of the FDA (2000 μg/kg) with 33% over the standard rate. They concluded that FBs are the main risk factor of mycotoxin contamination. They also investigated multimycotoxin contamination of maize and indicated that 90% of the samples were detected for multimycotoxin contamination; 1.0%, 2.7%, 14%, 22%, 44%, and 6.0% of the samples were detected with 7, 6, 5, 4, 3, and 2 kinds of mycotoxins contamination, respectively. Xu and others (2006) determined that 48 commercial maize samples in Nanjing City possessed a 54% and 60% incidence of AFB_1 and FBs contamination, respectively, and 33% cocontamination of both AFB_1 and FBs. In general, fumonisin contamination in maize can be detected throughout the country and is often detected alongside other mycotoxins. Due to the potential risk of OC associated with fumonisins, it is necessary to further study the occurrence conditions and develop specific prevention and control methods.

Aflatoxins

AFs are toxic, carcinogenic, and immunosuppressive secondary metabolites primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus*. *A. flavus* is of universal occurrence in tropical and subtropical climates, and occurs in all major crops, but especially maize, peanuts, and cottonseed. *A. parasiticus* is uncommon or even unknown in Southeast Asia, and is commonly found only in peanuts (Pitt and Hocking 2009). They were 1st noted in the early 1960s, and extensive experimental and epidemiological evidence confirmed that they result in liver cancer. Both *A. flavus* and *A. parasiticus* produce aflatoxin B_1 (AFB_1) and B_2; *A. parasiticus* produces B and G AFs (EFSA 2007). Among these naturally occurring mycotoxins, AFB_1 is believed to be the most toxic AF and the most potent carcinogen. AFs frequently contaminate foods as a result of fungal growth and reproduction during pre- and postharvest periods. Environmental factors such as temperature, humidity, and other storage conditions, as well as water activity, concurrent mycobiota, and physical damage of the grains, affect the rate and degree of contamination (EFSA 2007; Pitt and others 2012).

Liu and others (2006) investigated AF contamination in stored maize in Liaoning Province and found 71 out of 73 maize samples to be positive, with an incidence of 97%. The content of AFs was significantly higher in 2-y maize samples (1.2 μg/kg) than that of 1-y ones (0.8 μg/kg). The authors indicated that AFG_1 was detected as the main type of AF in over 40% of all the stored maize samples. They concluded that the average content of AF (1.0 μg/kg) was much lower than other regions in Asia and the MRLs established by most countries.

In a survey performed by Chen (2011), the author detected a 100% positive rate for AFB_1 at an average content of 6.0 μg/kg with a maize sample size of 26 collected from 18 provinces. This result reveals extensive contamination of AFB_1 in maize samples. However, the average content of AFB_1 did not exceed the national MRL (50 μg/kg) (AQSIQ 2006). In another survey conducted by Gao and others (2011), the authors investigated AFs (AFB_1, AFB_2, AFG_1, and AFG_2) contamination of maize collected from 6 provinces, with a sample size of 279 and 76% of the samples were detected as positive for AFs, with an average content of 44 μg/kg. They divided the 6 provinces into 3 regions: north, central, and south, and indicated that the incidences were different among various provinces. High incidences were very common. Among these 6 provinces, Hubei, Guangdong, and Sichuan recorded the highest incidences of 94%, 92%, and 90%, respectively, followed by Guangxi and Jilin, with 88% and 52%, respectively, while Henan recorded the lowest value at 37% (Table 6). For the average content, the central provinces Sichuan and Hubei also recorded the highest content at 108 and 71 μg/kg, followed by Guangxi and Henan with 40 and 8.1 μg/kg, respectively, while Guangdong and Jilin recorded the lowest levels at 3.7 and 1.2 μg/kg, respectively. The authors concluded that, in general, the incidence and average content increased from the north to the south due to warm and humid weather conditions in the southern provinces. It was observed that the AF contamination was more serious in the central provinces, Hubei and Sichuan, with the highest average contents (71 and 108 μg/kg) and the range of positive samples at 0.3 to 888 and 0.3 to 769 μg/kg; the average contents of these 2 provinces as well as that of Guangxi Province (40 μg/kg) far exceeded the national MRL (20 μg/kg for food maize and 50 μg/kg for feed maize). They pointed out that AFB_1 contributed the most to the total AF (AFB_1, AFB_2, AFG_1, and AFG_2) contamination.

Fan and others (2012) conducted a survey to investigate AF contamination of feeds and feedstuffs in the Beijing area with incidences of 50%, 9.1%, 4.5%, and 9.1% for AFB_1, AFB_2, AFG_1, and AFG_2 at average contents of 6.0, 0.6, 0.0, and 0.0 μg/kg, respectively, in positive maize samples. The authors hence concluded that AFB_1 was the most serious mycotoxin that contaminated maize. Cheng and others (2014c) investigated AF contamination of raw feed materials using ELISA kit screening and LC-MS verification. Their results showed that 29% of maize samples were positive for AFB_1 with an average content of 3.1 μg/kg. A significant correlation (correlation coefficient 0.6) was found between AFB_1 and AFB_2. In addition, AFG_1 and AFG_2 were not detected in any of the maize samples. The authors pointed out that *A. parasiticus*, responsible for AFG_1 and AFG_2, is rarely found in China.

Gao and others (2013) investigated the correlations of incidence and contamination content between any 2 of AFs AFB_1, AFB_2, AFG_1, and AFG_2 and found that AFB_1 has the highest incidence.

Table 6–Comparison of aflatoxin contamination of maize in different regions of China.

Regions	Province	Total sample	Incidence (%)	Range of content (μg/kg)	Mean of positive samples (μg/kg)
North	Jilin	46	52.17	0.20 to 8.19	1.15
	Henan	46	36.96	0.34 to 69.66	8.06
Central	Hubei	48	93.75	0.31 to 888.30	70.98
	Sichuan	42	90.48	0.31 to 769.19	107.93
South	Guangdong	49	91.84	0.35 to 31.42	3.70
	Guangxi	48	87.50	0.47 to 330.44	39.65

Note: Adapted from Gao and others 2011.

Furthermore, no AFB_2 has been detected alone as it is always detected along with AFB_1. Correlations were found for both incidence and content between any of the 2 AFs, and the AFB_2 contamination was closely related to AFB_1. In addition, AFG_1 and AFG_2 were inhibited when AFB_1 was determined at higher concentration.

Fungal Diversity and Relevant Mycotoxins

Maize has been confirmed to be a good substrate for fungal growth, such as *A. flavus* (Shephard 2008; Reddy and others 2009), *F. verticillioides* (Nikiema and others 2004; Domijan and others 2005), and mycotoxin production (Reddy 2009; Reddy and others 2009). Early in 1967, Lopez and Christensen found that at 19% moisture content and temperatures of 25 or 35 °C, *A. flavus* became the predominant species of the mycoflora, while Trenk and Hartman (1970) indicated that at 26% moisture and temperatures of 30 and 35 °C, optimal capacity of AFs production was obtained. *F. verticillioides* produces great levels of fumonisins at 20 to 26 °C (JECFA 2002) preharvest.

Incidence and levels of mycotoxin contamination in maize is the most common investigation project funded by various government departments in China; little work is done on the identification of fungal species and the relationships or interactions among them as well as their impacts on mycotoxin production. This is partly so because identification of fungal species is often complicated due to processes such as gene mapping requiring expensive equipment and partly because the government urgently and rapidly demands the data of mycotoxin contamination in cereals in order to take quick action.

Still, a few investigations have been performed to identify fungal species which grow and reproduce in maize, and to explore the potential of toxin production capacity. Yan and others (1999) isolated 987 strains of *Fusarium moniliforme* (= *F. verticillioides*) from maize samples collected in Shandong Province, and selected 20 strains to detect their toxin-producing capacity by weighing 10 g maize in a 100 mL Erlenmeyer flask, adding 10 mL distilled water, sterilizing at 121 °C for 1 h, inoculating 1 mL fungus suspension in the flask, culturing for 2 wk at 25 °C, and then culturing at 15 °C for another 2 wk. Their result showed that 18 out of 20 of the strains were toxigenic strains, and 13 strains produced greater than 50 mg/kg of FBs, while the maximum capacity of toxigenic strain produced FBs at 59 mg/kg. Xu and others (2009) conducted a project to investigate fungal invasion of wheat and maize harvested from 4 provinces of China in order to provide the data of fungal strains for further toxigenicity tests in predictive microbiological studies. Their results showed that all 84 maize samples were detected as fungi-positive and *Fusarium sp.* was the dominant species. The 3 fungi which infected maize the most were *Aspergillus niger* (40%), *Fusarium spp.* (24%), and *A. flavus* (16%); *Fusarium sp.* (62%), *A. niger* (19%), and *Rhizopus sp.* (12%); *Fusarium sp.* (32%), *Trichoderma sp.* (17%), and *A. niger* (15%); and *Rhizopus sp.* (37%), *Trichoderma sp.* (29%), and *Fusarium sp.* (12%) in Anhui, Jiangsu, Hebei, and Henan provinces, respectively. The authors indicated that maize samples collected from the 4 provinces above had been seriously infected by fungi; hence, actions must to be taken to protect cereals from fungal invasion during storage. Feng (2011) isolated 23 strains of *F. proliferatum* from maize samples in Liaoning Province and inoculated maize kernels to determine their capability of producing FB_1 and FB_2. The results showed that 57% of these strains were high-yield strains (≥500 mg/kg) and all the strains produced relatively high levels of FB_1 and FB_2. The capability of *F. verticillioides* and *F. proliferatum* to produce FB_1 and FB_2 was significantly affected by temperature, and 20 °C is optimal to produce the above toxins. At 20 °C, FB_1 and FB_2 producing abilities of *F. verticillioides* and *F. proliferatum* were observed to be inhibited by *F. graminearum*. FB_1 and FB_2 produced by *F. proliferatum* increased in the presence of *F. graminearum* at 25 and 30 °C. However, the effect of *F. graminearum* on FB_1 and FB_2 production of *F. verticillioides* was not significant. Cheng (2012) conducted research on variations of microbial diversity and control of microorganisms in wheat and maize during storage. The results showed that the total number of fungi was less than 10^3 cfu/g and the main fungi detected in freshly harvested maize with less than 10% moisture content were *Aspergillus candidus, Fusarium sp.,* and *Rhizopus nigricans*. Moisture contents of the maize samples were adjusted to 10%, 15%, and 19%, and they were then stored at 20, 25, 30, and 35 °C and relative humidity of 70% to 80%, 80% to 90%, and greater than 90% for 21 d. *Penicillium*, *Aspergillus*, *Fusarium sp.*, and *Rhizopus* were the dominant fungal species when the moisture content of maize samples was 10%, the above fungal species, excluding *Rhizopus*, were the dominant species when the moisture content was 15%, and *Aspergillus glaucus* and *A. flavus* were dominant at a moisture content of greater than 19%. Greater diversity and lower dominance index were present in the maize at lower temperatures and moisture contents, while lower diversity and greater dominance index were detected in the maize samples with high moisture content. A negative correlation was found between fungal diversity index and moisture content in the maize samples, while a positive correlation was observed between fungal dominance index and moisture content. Author Cheng (2012) hence indicated that the major fungi which resulted in moldy maize were *Penicillium* and *Aspergillus*, also including *A. glaucus* and *A. flavus*. Li (2015) studied fungal diversity and mycotoxin contamination in maize and wheat under various storage conditions using storage environment simulation and analyzed fungal cultures using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR–DGGE) and HPLC techniques to investigate the changing pattern of total number of microbial colonies and the quality changing trend of maize during storage. The maize samples with initial moisture contents of 12%, 14%, 16%, 18%, and 20% were stored at 30 °C and 75%, 84%, and 92% relative humidity for 35 d. The results showed that the growth of *A. flavus* and toxin-producing capacity was inhibited by other microorganisms under the conditions of medium-to-high relative humidity. A very significant positive correlation was found between the total number of *A. flavus*, *A. niger*, and AFB_1 ($P<0.001$) when maize was stored at 30 °C and 75% relative humidity for 35 d. A significantly positive correlation was observed between the total number of *A. niger* and AFB_1 in the maize samples stored at 30 °C and 84% of relative humidity. Under the condition of low humidity, the ability of ZEN production inhibited by *Trichoderma viride* was significantly increased with the rising initial moisture content; while under the conditions of medium-to-high relative humidity, the inhibition capacity of *T. viride* to ZEN increased rapidly with the increasing initial moisture content of the maize samples. ZEN was strongly inhibited by *T. viride* at the medium-to-high initial moisture content. When maize was stored at 75% relative humidity for 28 d, the content of ZEN was negatively correlated with the number of *F. moniliforme*, *T. viride*, and fungi, respectively. At 92% relative humidity, the content of ZEN was positively correlated with the numbers of fungi, *T. viride,* and *F. moniliforme*. A significant positive correlation was found between *T. viride*, moisture content, and ZEN at a relative humidity of 84%. A negative correlation was also observed between ZEN and the number of fungi and *F. moniliforme*, respectively. The results showed that ZEN was strongly influenced by biological and environmental factors under medium and low humidity conditions. In addition, ZEN was significantly affected by *F. moniliforme* under high humidity condition.

Mycotoxins in Human Health in China

Risk assessment data of mycotoxin contamination of maize in human health in China are still limited due to the government's confidentiality policy. Over the last 5 y, the Chinese Ministry of Agriculture has launched a series of projects on risk assessment of mycotoxin contamination of agro-products, such as maize, in human health. Dozens of research institutes, comprised mainly of laboratories of quality and safety risk assessment for agro-products established by the Ministry of Agriculture, including our laboratory, have participated in these projects.

Wang and others (2007) performed a survey on DON contamination in resident meals and an exposure assessment in 6 provinces of China. They determined the average contents of DON to be 26 μg/kg in maize and 50 μg/kg in wheat. In the range of 95th percentile of DON contamination, the revealed amounts in meals are lower than tolerable daily intake in the urban and rural populations and various populations of age groups. They concluded that the contamination situation of DON in cereals showed a trend of high incidences and low concentrations of contamination. Ninety-five percent of the entire population in the 6 provinces was safe in terms of DON intake. Children were at potential risk due to their low body weight and singular dietary structure.

Wang and Liu (2007) conducted an assessment on the dietary exposure of AFs in Chinese residents and analyzed the main contributing foods and the risk of liver cancer. Their results indicated that the mean dietary exposure levels of AFs in an average Chinese resident, 2- to 6-y-old children, urban standard population, and rural standard population were 665, 415, 488, and 749 ng/person·d, respectively, and the dietary AF exposure levels of high intake consumers in those resident groups (97.5 percentile) were 24787, 16544, 17358, and 29370 ng/person·d, respectively. The dietary exposure of AFs in rural residents was greater than that of the urban residents. They concluded that the status of dietary exposure of AFs in 2- to 6-y-old children was a major concern. Sources of dietary exposure of AFs were mainly from maize and rice for Chinese residents. The risk of liver cancer due to dietary exposure of AFs in rural residents was greater than that in urban residents.

A case study on risk assessment of the cooccurrence of DON and its acetyl derivatives, including 3-ADON and 15-ADON, based on the CA concept was performed by Han and others (2014) in Shanghai City, with 1269 participants and 330 wheat and maize samples. Probabilistic analysis using a Monte Carlo simulation was applied in this assessment and no health risks were shown by the results to the population in Shanghai considering individual mycotoxins. However, the authors pointed out that a potential cumulative health risk for the local population existed, based on the combined consideration of DON with either 3-ADON or 15-ADON or both. The DI values were up to 1087 ng/kg body weight/d in the 95th percentile, exceeding the Provisional Maximum Tolerable Daily Intake (PMTDI) of 1000 ng/kg body weight/d and hence posing potential health risk for the population in Shanghai. Consequently, the authors indicated the necessity of cooccurring risk assessments on wheat and maize. In the investigation of Li and others (2015), they collected 146 maize samples from 3 provinces and assessed the possible level of exposure to fumonisins for the consumers (0.1 mg/kg of body weight per day), and concluded that this level was within the PMTDI of 2.0 mg/kg of body weight per day set up by the Joint FAO/WHO Expert Committee on Food Additives (Bolger and others 2001). The authors indicated that the average probable daily intake exposure to total fumonisins for the 3 provinces was significantly lower than those reported in Brazil (1.6 mg/kg of body weight) (van der Westhuizen and others 2003) and some provinces of China (0.1, 0.3, and 1.1 mg/kg of body weight) (Gong and others 2009; Feng and others 2011; Wei and others 2013). Wang and others (2015) conducted a larger scale study on coexposure of DON and ZEN in domestic wheat flour and maize-based products, and their result showed that children were at a high risk to both of the evaluated mycotoxins above. They also suggested an evaluation of existing Chinese regulatory limits based on their risk assessment results, as well as the establishment of specific tolerance limits in foods intended for children. Sun and Wu (2016) performed a risk assessment project to evaluate dietary exposure to DON and its derivatives from cereals including raw maize and maize-based foods in China. They investigated the dietary exposure to DON, including its acetylated derivatives, and other type B trichothecenes from cereals and cereal-based products in Chinese populations using probabilistic estimation. Their result revealed that 75% of children and 90% of the general population and adults were under the PMTDI value of 1 mg/kg bw/d, while 99% of the 3 populations were under the group acute reference dose of 8 mg/kg bw/d for DON and its acetylated derivatives. They indicated that concern should be paid to high-end exposure to DON and its derivatives, especially for children. The authors also suggested a combination of rigorous formulation of maximum limits for DON and its derivatives in the relevant foodstuffs with enhanced monitoring as an effective way to reduce potential risk.

Conclusion

Maize is prone to be invaded by fungi both during growth in the field and during the postharvest storage stage, and this situation significantly increases the potential for multitoxin contamination. The production of mycotoxins is usually unavoidable and depends upon the environmental conditions during maize growth and its subsequent storage conditions. Due to poor economic conditions of many farmers at present, maize is extensively stored in open areas or simple barns which suffer from rain or snow; only around 35% of maize harvested in China in 2015 was stored in national warehouses. As indicated by numerous literature published in China, maize is extensively contaminated by various fungi as well as their mycotoxins. The contamination of maize/maize-based feeds with various mycotoxins is a serious threat to animals or humans living in China, which can result in many health problems and foodborne diseases.

Although information on the effects of specific mycotoxins or mycotoxin combinations in the course of various human/animal diseases is limited, a considerable amount of evidence supporting the association between mycotoxins and certain animal syndromes or livestock diseases is available. In addition, the hazards to humans from these mycotoxins produced in maize have continuously proven that precautions must be taken. Since most of the maize in China is consumed as raw feed material, attention should be paid to the transfer of mycotoxins from animal products such as meat and milk to the human body, and also to the effect of cumulative toxicity. Consequently, growing disease- and insect-resistant varieties of maize, as well as adequate application of pesticide and fungicide, are effective approaches to reducing preharvest mycotoxin contamination. Furthermore, performing good postharvest practices of storage will enable maize to maintain a safe status for both human consumption and application as raw feed material.

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