Aflatoxins and Fumonisins in Corn from the High-Incidence Area for Human Hepatocellular Carcinoma in Guangxi, China

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A comparative study on the natural occurrence of aflatoxins and *Fusarium* toxins was conducted with corn samples from high- and low-incidence areas for human primary hepatocellular carcinoma (PHC) in Guangxi, China. In samples from the high-risk area, aflatoxin B_1 was the predominant toxin detected in terms of quantity and frequency, with its concentration ranging between 9 and 2496 μg/kg and an 85% incidence of contamination. Among the samples, 13 (76%) exceeded the Chinese regulation of 20 μg/kg for aflatoxin B_1 in corn and corn-based products intended for human consumption. Significant differences in aflatoxin B_1, B_2, and G_1 and total aflatoxin concentrations in corn between the areas were found ($P < 0.05$). The average daily intake of aflatoxin B_1 from corn in the high-risk area was 184.1 μg, and the probable daily intake is estimated to be 3.68 μg/kg of body weight/day, 3.20 times the TD_{50} in rats. Corn samples from both areas were simultaneously contaminated with fumonisins B_1, B_2, and B_3. Aflatoxin B_1 may play an important role in the development of PHC in Guangxi.

Keywords: *Aflatoxins; fumonisins; corn; primary hepatocellular carcinoma; risk assessment; China*

INTRODUCTION

Aflatoxins are a group of potently hepatotoxic, teratogenic, mutagenic, and carcinogenic mycotoxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* in a number of foods and feeds, particularly in corn, peanuts, and edible nuts. Aflatoxin B_1 is documented as a hepatic carcinogen in many animal species and is listed as a group I carcinogen by the International Agency for Research on Cancer (*1*), especially implicated in the cause of human primary hepatocellular carcinoma (PHC). The incidence of PHC varies widely throughout the world. It is one of the most common cancers in China, Saharan Africa, and Southeast Asia, such as the Philippines and Thailand, and causes at least 250000 deaths annually worldwide (*2*). According to the results of a five-year surveillance program initiated from the end of the 1980s to the beginning of the 1990s in China, the number of patients diagnosed annually with PHC was 110200, accounting for ~45% of the total PHC cases in the world (*3*). The incidence of PHC is geographically varied within China: high-risk regions are located in the provinces of Jiangsu, Zhejiang, Fujian, and Guangdong and in the Guangxi autonomous region (*4*). In some areas of Guangxi, the standardized rate of PHC is 120 per 100000 per year for men, which is >35 times the corresponding rate for men in the United States (*5*). Available studies indicate that the cause of PHC is multifactorial: genetic susceptibility and environmental exposures including chronic infection of hepatitis B virus (HBV), algal toxins in drinking water, and exposure to dietary aflatoxins, in particular aflatoxin B_1, are involved in the extraordinarily high rate of PHC (*5*, *6*). A multiplicative interaction between HBV and aflatoxins for the risk of PHC has been demonstrated in aflatoxins-positive subjects (*7*).

Although aflatoxin B_1 is a ubiquitous contaminant of several classes of commodities, contamination of corn likely poses the greatest health risk to humans worldwide. This is due primarily to the importance of this commodity as a food and feed source throughout the world, especially in PHC hyperendemic regions, where aflatoxins-contaminated corn is still a staple food for human consumption, as well as to the fact that corn is conducive to aflatoxigenic fungal infection and toxin production. On the other hand, corn invasion by *Fusarium* species and contamination with corresponding toxins such as fumonisins and trichothecenes have been reported universally (*8*). Fumonisin B_1 possesses a tumor-promoting activity in the rat liver medium-term hepatocarcinogenicity assay (*9*), and an increase in hepatocellular adenomas and carcinomas was detected in female B6C3F$_1$ mice fed a diet containing fumonisin B_1 (*10*). In addition, nivalenol is shown to be genotoxic in the gastrointestinal tract in mice (*11*). Studies on the relationship between mycotoxin exposure and PHC risk previously carried out in the Guangxi autonomous region focused only on aflatoxins (*12*, *13*). In this study we aim to describe the natural co-occurrence of aflatoxins and *Fusarium* mycotoxins in corn samples from Chongzuo County in the Guangxi autonomous region, China, an area with a high incidence of PHC, in comparison with that in samples from Guilin, an area with a low incidence of PHC. We also attempt to estimate the risk of people exposed to both aflatoxins

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Table 1. Concentration of Mycotoxins in Corn from the High PHC Prevalence Area, Chongzuo County, Guangxi Autonomous Region, China

sample code	farmers' code	location (town)	mycotoxin concn, μg/kg: aflatoxins B_1	aflatoxins B_2	aflatoxins G_1	aflatoxins total	fumonisins B_1	fumonisins B_2	fumonisins B_3	fumonisins total	nivalenol
CZ-98-C1	HZL	Tiaiping					116	72		238	
CZ-98-C2	NYN	Tiaiping	695	88	14	797	121	74		195	
CZ-98-C3	LSQ	Tiaiping	9		14	23					
CZ-98-C4	LZY	Tiaiping	15			15					
CZ-98-C5	HML	Tiaiping	9			9					
CZ-98-C6	CXC	Tiaiping	698	111		809					
CZ-98-C7	LGQ	Tiaiping	71	18	12	101	74			74	
CZ-98-C8	NXF	Tiaiping	103	11	13	127	718	390	196	1304	
CZ-98-C9	NHJ	Tiaiping	116	18	16	150					
CZ-98-C10	GYM	Tiaiping	2496	320		2816	780	654	252	1686	
CZ-98-C11	NWP	Tiaiping	185	29	18	232	76	56		132	
CZ-98-C12	NWS	Tiaiping	468	64	21	553	98			98	
CZ-98-C13	PHL	Madalu	2121	281		2402	198	88	58	344	
CZ-98-C14	HYJ	Nalong					1976	890	385	3251	216
CZ-98-C15	FAG	Nalong	171	24	12	207	116	75	53	244	246
CZ-98-C16	FQR	Nalong	150	21	12	183	440	114	82	636	172
CZ-98-C17	SRM	Zuozhou	215	28		243	71			71	
CZ-98-C18	SPZ	Zuozhou	11			11	104	104		208	
CZ-98-C19	ZB	Zuozhou					58			58	
CZ-98-C20	ZJQ	Zuozhou	290	55		345	709	305	279	1293	
mean			460	82	15	531	377	257	186	655	211
SD			732	102	3	840	515	281	128	890	37

and fumonisins, especially aflatoxin B_1 and fumonisin B_1, and to assess the possible risk to individuals consuming aflatoxin B_1- and fumonisin B_1-contaminated corn. To our knowledge, no attempts have been made to clarify the co-contamination of aflatoxins and *Fusarium* mycotoxins in cereals from the above-stated area.

MATERIALS AND METHODS

Corn Samples. A total of 40 corn kernel samples from the 1998 crop were collected in Chongzuo County and Guilin suburbs, Guangxi autonomous region, in October 1998. Among these, 20 samples were obtained by visiting 20 farmers' families with PHC patients selected randomly in four different towns (Tiaiping, Nalong, Madalu, and Zuozhou) in Chongzuo County. The remaining 20 samples were from 20 different farmers' families without PHC patients in the suburb of Guilin city, which lies ~400 km to the northeast of Chongzuo in the same autonomous region. There are no significant differences in dietary habits, lifestyle, or environmental conditions between the two areas. All corn samples were produced locally and intended for human consumption. The local farmers typically store the corn kernels in wood cribs outdoors or in stacked gunnysacks indoors or pile the corn kernels on the floor of a hut. Each sample consisted of multiple subsamples collected from different sampling sites of the container (3 layers and 5 points for each layer, totaling 15 sampling sites). The subsamples were pooled and mixed thoroughly by the collector. Approximately 500 g of each composite was collected, and ~200 g was used for analysis. Half of the entire composite was finely ground, and a 20 g of a test portion was taken for analysis. All ground and unground samples were kept in zip-lock plastic bags at −30 °C prior to analysis.

Chemicals and Reagents. Standards for aflatoxins B_1, B_2, G_1, and G_2 were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and those of fumonisins B_1, B_2 and B_3 were purchased from the Division of Food Science and Technology, Council for Scientific and Industrial Research (Pretoria, South Africa). Zearalenone and trichothecene mycotoxins including deoxynivalenol, nivalenol, and their acetylated derivatives such as 15-acetyl-, 3-acetyl- and 3,15-diacetyldeoxynivalenol, and 4-acetyl- and 4,15-diacetylnivalenol were prepared in the Laboratory of Mycotoxicology, Kagawa University, Japan. All organic solvents used for sample extraction were of reagent grade and distilled in a glass apparatus before use, and the solvents for high-performance liquid chromatography (HPLC) were of HPLC grade.

Mycotoxin Analysis. For the analysis of aflatoxins, all corn samples were qualitatively screened for aflatoxins according to the minicolumn method described previously (*14*). The positive minicolumn response for samples containing 5 μg/kg total aflatoxins and above was determined by visualizing the column under long-wave UV light. The aflatoxins-positive corn sample (20 g) was extracted with chloroform/water (10:1, v/v), cleaned-up on a Florisil column, and quantitatively determined for aflatoxins by HPLC (a Jasco PU-980 pump equipped with a Jasco AS-950 autosampler) with a Shimadzu RF-10 XL spectrofluorometer with excitation/emission wavelengths of 253 nm/415 nm after derivatization with trifluoroacetic acid (*14*, *15*). A short reversed phase column (TSK-gel ODS-80TM CTR, 5 μm particle size, 100 × 4.6 mm, Tosoh, Tokyo, Japan) thermostated at 40 °C was employed. For *Fusarium* toxin determination, a finely ground sample (20 g) was extracted with acetonitrile/water (3:1, v/v), defatted with *n*-hexane, and concentrated to dryness followed by chromatography on either a Florisil column for both trichothecenes and zearalenone or a Sep-Pak Accell plus QMA, strong anion-exchange cartridge for fumonisins (*16*−*19*). A Shimadzu GC/MS-QP 5000 gas chromatograph/mass spectrometer with selected ion monitoring mode was used for the quantitation and confirmation of trichothecenes as trimethylsilyl ethers, with scirpenetriol as an internal standard (*19*). Zearalenone was analyzed by HPLC with a fluorescence detector with excitation/emission wavelengths of 314 nm/450 nm. A Shimadzu LC-6A HPLC equipped with a fluorescence detector with excitation/emission wavelengths of 335 nm/440 nm were employed for fumonisins analysis after precolumn derivatization with *o*-phthaldialdehyde (*18*). Detection limits were 1 μg/kg for aflatoxins B_1 and B_2, 5 μg/kg for aflatoxins G_1 and G_2, 10 μg/kg for trichothecenes, 5 μg/kg for zearalenone, and 50 μg/kg for fumonisins.

RESULTS

Aflatoxins Contamination. The analytical results of aflatoxins and *Fusarium* toxins in corn from Chongzuo County are summarized in Table 1. Aflatoxin B_1 was the predominant toxin detected in terms of the concentration (9−2496 μg/kg, mean = 460 μg/kg) and the incidence of its contamination (85%, 17/20). In positive samples, 76% (13/17) contained aflatoxin B_1 at a level

Table 2. Comparison of Aflatoxins and Fumonisins in Corn from High- and Low-Incidence Areas for PHC in Guangxi

			toxin concn, μg/kg	
mycotoxin	area[a]	no. of positives (%)	range	av (SD)
aflatoxins				
B_1	H	17 (85%)	9−2496	460 (732)[b]
	L	1 (5%)		44 (−)
B_2	H	13 (65%)	11−320	82 (102)[b]
	L	1 (5%)		11 (−)
G_1	H	9 (45%)	12−21	15 (3)[b]
	L	0 (−)	0	0 (−)
total	H	17 (85%)	9−2816	531 (840)[b]
	L	1 (5%)		55 (−)
fumonisins				
B_1	H	15 (75%)	58−1976	377 (515)
	L	15 (75%)	72−294	129 (58)
B_2	H	11 (55%)	56−890	257 (281)
	L	7 (35%)	54−104	76 (23)
B_3	H	7 (35%)	53−385	186 (128)
	L	1 (5%)		70 (−)
total	H	15 (75%)	58−3251	655 (890)
	L	15 (75%)	72−468	170 (109)

[a] H, the area with high rate of primary hepatocellular carcinoma, Chongzuo County; L, the area with low rate of primary hepatocellular carcinoma, suburb of Guilin. [b] $P < 0.05$.

of >20 μg/kg (71−2496 μg/kg, mean = 598 μg/kg), the Chinese regulation for aflatoxin B_1 in corn and corn-based products for human consumption. It should be noted, moreover, that two samples contained a considerably high level of aflatoxin B_1 (2121 and 2496 μg/kg). In addition to aflatoxin B_1, aflatoxin B_2 at a level ranging from 11 to 320 μg/kg (mean = 82 μg/kg) was simultaneously detected in 13 (65%) samples. Nine samples were also positive for aflatoxin G_1, although at low levels. The average concentration of aflatoxin B_1 obtained in the present study was higher than those (90, 380, 205, and 181 μg/kg) reported previously (*12*, *13*, *20*). Only one sample from Guilin suburb was positive for aflatoxins B_1 and B_2 at levels of 44 and 11 μg/kg, respectively. Corn contamination by aflatoxins B_1, B_2, and G_1 and total aflatoxins from the high PHC prevalence area was significantly higher than that from low-risk area for PHC ($P < 0.05$, as shown in Table 2).

***Fusarium* Toxin Contamination.** The incidence of corn contamination with fumonisins was 75% for the high PHC prevalence area. In these fumonisins-positive samples, fumonisin B_1 was the major toxin detected at a concentration ranging from 58 to 1976 μg/kg (mean = 377 μg/kg) followed by B_2 (55% positive, mean = 257 μg/kg) and B_3 (35% positive, mean = 186 μg/kg) (Tables 1 and 2). Four corn samples were contaminated with total fumonisins at >1000 μg/kg. Fumonisin levels determined in corn samples from the suburb of Guilin were lower, ranging between 72 and 294 μg/kg (75% positive) for B_1 and between 54 and 104 μg/kg (35% positive) for B_2, with a small variation in toxin concentration among samples. No significant differences in fumonisin levels were found between the two areas ($P > 0.05$, Table 2). Fumonisin B_1 concentration in corn from Chongzuo County, either the maximum or the average, was insignificantly different from that reported in other regions of China (*17*).

Considering the co-occurrence of fumonisins with aflatoxins in corn from the high PHC prevalence area, 60% of the samples (12/20) were co-contaminated with both toxins, and the levels of aflatoxins were higher than those of fumonisins in more than half (7/12) of co-contaminated corns and vice versa in the remainder (Table 1). One sample (CZ-98-C10) with the highest level of aflatoxins (2816 μg/kg) was coincidentally contaminated with total fumonisins of >1000 μg/kg, but the most heavily fumonisin-contaminated sample (CZ-98-C14, 3251 μg/kg) was free from aflatoxins. Comparatively, samples from the towns of Tiaiping and Madalu were frequently contaminated with aflatoxins, whereas slightly more fumonisins were found in samples from the town of Nalong. Chemical analysis of corn samples from both areas showed the absence of detectable levels of zearalenone, deoxynivalenol, and its derivatives. Three samples from Nalong in Chongzuo County were positive for nivalenol at 216, 246, and 172 μg/kg, respectively.

Human Exposure to Aflatoxin B_1 and Fumonisin B_1. To evaluate the potential health risk of aflatoxin B_1 and fumonisin B_1 to populations in Chongzuo County, the probable daily intake (PDI) values of these toxins were estimated on the basis of their levels in corn, an average daily intake of corn product (400 g, the vast majority of corn consumption was 300−500 g per day per person), and a mean body weight of 50 kg. As indicated in Table 3, the daily aflatoxin B_1 intake is estimated to be between 3.60 and 998.40 μg (mean = 184.08 μg) for adults consuming foods made from 400 g of corn. The estimated PDI of aflatoxin B_1 is between 0.07 and 19.96 μg/kg of body weight (bw)/day (mean = 3.68 μg/kg of bw/day), 0.06−17.40 (average = 3.20) times the estimated TD_{50} of aflatoxin B_1 in the rat (1.15 μg/kg of bw/day), an indicator of carcinogenic potency of the dose at which 50% of the animals would have developed the tumors (*21*). It would certainly be considerably higher than the tolerable daily intake (TDI) for aflatoxin B_1. On the basis of the data stated above, it can be estimated that the annual human exposure to aflatoxin B_1 from corn is in the range of 1.31−364.40 mg (mean = 67.19 mg). Thus, human exposure to aflatoxin B_1 in Chongzuo County, either the highest or the average level, was much higher than described in other human aflatoxin investigations (*22*, *23*). With regard to fumonisin B_1, the daily exposure from consumption of corn-derived food is estimated at between 23.20 and 790.40 μg per person (mean = 150.80 μg per person), and the PDI value ranges from 0.46 to 15.81 μg/kg of bw/day (mean = 3.02 μg/kg of bw/day). It is impossible to make a further risk assessment for fumonisin B_1 as there are no TDIs for fumonisins available so far.

DISCUSSION

Considering the co-contamination of corn by aflatoxin B_1 and fumonisin B_1 in Chongzuo County, aflatoxin B_1 concentration was higher than that of fumonisin B_1 in 58% (7/12) of co-contaminated samples. Chongzuo County is located in the southern part of the Guangxi autonomous region of China. The average yearly temperature, relative humidity, precipitation, and rainy days are 21.9 °C, 79%, 1311 mm, and 148 days, respectively (*12*). On the basis of analysis over a 10-year period of climatic conditions in this area, >150 days per year have temperatures exceeding 30 °C and relative humidity >80%, optimal conditions that favor the growth of *Aspergillus* species and the production of associated mycotoxins. The heavy rain and high temperature normally coincide with the peak harvest months of corn.

Table 3. Risk Assessment of Aflatoxin B_1 and Fumonisin B_1 on Human Beings in the High-Risk Area for PHC, Chongzuo County

toxin	toxin concn in corn, μg/kg	toxin intake,[a] μg/person/day	PDI,[b] μg/kg of bw/day	ratio of PDI/TDI[c]	ratio of PDI/TD_{50} [d]
aflatoxin B_1	9−2496 (range)	3.60−998.40	0.07−19.96	181455−105053 (max toxin level)	0.06−17.40
	460 (av)	184.08	3.68	33455−19368	3.20
fumonisin B_1	58−1976 (range)	23.20−790.40	0.46−15.81		
	377 (av)	150.80	3.02		

[a] Calculated on the basis of daily average corn intake of 400 g. [b] Probable daily intake (PDI), calculated on the basis of the body weight (bw) of 50 kg. [c] Tolerable daily intake (TDI) of 0.11−0.19 ng/kg of bw/day in Asia, at a risk level of 10^{-5}. [d] TD_{50} of 1.15 μg/kg of bw/day in rat, the dose at which 50% of the animals would have developed tumors (*21*, *25*). No TDI and TD_{50} for fumonisin B_1 are available.

Hence, environmental conditions are responsible for the high levels of aflatoxins in corn from Chongzuo County.

Although a few studies have indicated that fumonisin B_1 was involved in hepatocellular and renal tubular carcinogeneses in rats and mice (*9*, *10*, *24*), it has not yet been established as to how it affects humans. The carcinogenicity of aflatoxin B_1 is estimated to be ∼4000 times higher than that of fumonisin B_1 (*25*). Therefore, aflatoxin B_1 seems to play a more important role than fumonisin B_1 in the development of PHC. A synergistic effect of fumonisin B_1 on aflatoxin B_1-initiated hepatocarcinogenicity was investigated in a medium-term rat hepatocarcinogenicity assay (*26*). In addition, some field studies have suggested an association of fumonisins exposure with human esophageal cancer (*9*, *27*). It is necessary to make a further study on bulk surveillance of fumonisins and aflatoxins contamination in corn in successive years with the aim of clarifying the relationship between the extraordinarily high incidence of PHC and coexposure to both carcinogenic toxins.

The present study indicates that the population in Chongzuo County definitely has a high exposure to aflatoxin B_1. Although a major effort was recently made to reduce exposure to aflatoxins by changing the staple food in the diet of the population from corn to rice, corn products still account for >80% (90% for the poorest members) of the total diet, and their consumption is also most likely a surrogate of aflatoxin B_1 exposure. It is also worth noting that high PHC prevalence villages were all in corn-consuming regions, whereas the incidence of PHC was very low in rice-eating regions. Rice contamination with aflatoxin B_1 was significantly lower than corn contamination (*13*). No epidemiological data on either the lowest concentration of aflatoxin B_1 in corn for the development of human PHC or the dose−response relationship between the aflatoxin B_1 intake and PHC in humans was available. Therefore, the risk assessment of aflatoxin B_1 on human beings was extrapolated from the data obtained from animal tests in a special reference to PHC. Yeh et al. (*5*) reported the role of HBV and aflatoxin B_1 in the development of liver cancer. A cohort study indicated that little association between the prevalence of HBsAg positivity and PHC mortality was found, but a positive and almost perfectly linear relationship between exposure to aflatoxin B_1 and PHC rate in Southern Guangxi, China, was found. Therefore, it is reasonable to conclude that dietary exposure to aflatoxin B_1 may be partially responsible for the unusually high rates of PHC in Chongzuo County, on the basis of the data demonstrated here in combination with those reported previously (*12*, *13*, *20*). Two-thirds of corn samples from this area contained aflatoxin B_1 at a level above the national regulation for aflatoxin B_1; therefore, it is urgent for the local government to take effective measures to lower the population's exposure to aflatoxin B_1.

SAFETY

Aflatoxin B_1 is a known potent carcinogenic compound and fumonisin B_1 a major mycotoxin with cancer-promoting activity in animals; trichothecenes and zearalenone are known toxic substances. Consequently, sample collection, solvent extracts, and associated standards preparation should be handled with care. Sample extraction and analysis for aflatoxins should be kept from the light.

ABBREVIATIONS USED

HBV, hepatitis B virus; PDI, probable daily intake; PHC, primary hepatocellular carcinoma; TDI, tolerable daily intake.

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LITERATURE CITED

(1) IARC. *The Evaluation of the Carcinogenic Risk of Chemicals to Humans*; IARC Monograph Supplement 4; International Agency for Research on Cancer: Lyon, France, 1982.

(2) Waterhouse, J.; Shanmugaratnam, K.; Muir, C.; Powell, J. Cancer incidence in five continents. In *IARC Scientific Publications*; International Agency for Research on Cancer: Lyon, France, 1982; Vol. 4, No. 42.

(3) Center for Health Statistics Institute. *Selected Edition on the Health Statistics of China (1978−1990)*; Ministry of Health: Beijing, China, 1991; pp 78−79.

(4) Yu, S. Z. Primary prevention of hepatocellular carcinoma. *J. Gastroenterol. Hepatol.* **1995**, *10*, 674−682.

(5) Yeh, F. S.; Yu, M. C.; Mo, C. C.; Luo, S.; Tong, M. J.; Henderson, B. E. Hepatitis B virus, aflatoxin, and hepatocellular carcinoma in Southern Guangxi, China. *Cancer Res.* **1989**, *49*, 2506−2509.

(6) Ueno, Y.; Nagata, S.; Tsutsumi, T.; Hasegawa, A.; Watanabe, M. F.; Park, H. D.; Chen, G. C.; Chen, G.; Yu, S. Z. Detection of microcystins, blue-green algal toxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China. *Carcinogenesis* **1996**, *17*, 1317−1321.

(7) Ross, R. K.; Yuan, J. M.; Yu, M. C.; Wogan, G. N.; Qian, G. S.; Tu, J. T.; Groopman, J. D.; Gao, Y. T.; Henderson, B. E. Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet* **1992**, *339*, 943−946.

(8) Miller, J. D. Epidemiology of *Fusarium* ear diseases of cereals. In *Mycotoxins in Grains, Compounds Other Than Aflatoxins*; Miller, J. D., Trenholm, H. L., Eds.; Eagan Press: St. Paul, MN, 1994; pp 19−36.

(9) Gelderblom, W. C. A.; Jaskiewicz, K.; Marasas, W. F. O.; Thiel, P. G.; Horak, R. M.; Vleggaar, R.; Kriek, N. P. J. Fumonisins—novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* **1988**, *7*, 1806−1811.

(10) Howard, P. C.; Eppley, R. M.; Stack, M. E. Carcinogenicity of fumonisin B_1 in a two-year bioassay with Fischer 344 rats and B6C3F_1 mice. In *Mycotoxin Contamination: Health Risk and Prevention Project*; Akao, M., Kumagai, S., Goto, T., Kawai, K., Takahashi, H., Yabe, K., Yoshizawa, T., Koga, H., Eds.; Japanese Association of Mycotoxicology: Tokyo, Japan, 1999; pp 45−54.

(11) Tsuda, S.; Kosaka, Y.; Murakami, M.; Matsuo, H.; Matsusaka, N.; Taniguchi, K.; Sasaki, Y. F. Detection of nivalenol genotoxicity in culture cells and multiple mouse organs by the alkaline single-cell gel electrophoresis assay. *Mutat. Res.* **1998**, *415*, 191−200.

(12) Zhang, L. S.; Huang, Z. Y.; Liang, G. W.; Wei, J. Y.; Wu, J. L.; Meng, G. R.; Liu, Z. H.; Mo, Z. C.; Ye, F. S. Studies of the relationship between aflatoxin B_1 exposure and primary hepatocellular carcinoma. *Fusui Cancer Res.* **1985**, *12*, 53−60.

(13) Zhang, L. S.; Huang, Z. Y.; Liang, G. W. Studies of the relationship between aflatoxin B_1 exposure and primary hepatocellular carcinoma in Fusui, Guangxi autonomous. *Learned J. Guangxi Medical Coll.* **1990**, *7*, 12−17.

(14) Arim, R. H.; Aguinaldo, A. R.; Yoshizawa, T. Application of a modified minicolumn to detection of aflatoxins in corn. *Mycotoxins* **1999**, *48*, 53−57.

(15) Ali, N.; Sardjono; Yamashita, A.; Yoshizawa, T. Natural co-occurrence of aflatoxins and *Fusarium* mycotoxins (fumonisins, deoxynivalenol, nivalenol and zearalenone) in corn from Indonesia. *Food Addit. Contam.* **1998**, *15*, 377−384.

(16) Luo, Y.; Yoshizawa, T.; Katayama, T. Comparative study on the natural occurrence of *Fusarium* mycotoxins (trichothecenes and zearalenone) in corn and wheat from high- and low-risk areas for human esophageal cancer in China. *Appl. Environ. Microbiol.* **1990**, *56*, 3723−3726.

(17) Yoshizawa, T.; Yamashita, A.; Luo, Y. Fumonisin occurrence in corn from high- and low-risk areas for human esophageal cancer in China. *Appl. Environ. Microbiol.* **1994**, *60*, 1626−1629.

(18) Gao, H. P.; Yoshizawa, T. Further study on *Fusarium* mycotoxins in corn and wheat from a high-risk area for human esophageal cancer in China. *Mycotoxins* **1997**, *45*, 51−55.

(19) Li, F. Q.; Luo, X. Y.; Yoshizawa, T. Mycotoxins (trichothecenes, zearalenone and fumonisins) in cereals associated with human red-mold intoxications stored since 1989 and 1991 in China. *Nat. Toxins* **1999**, *7*, 93−97.

(20) Zhang, L. S.; Liang, G. W. Status of aflatoxin B_1 in the development of hepatocellular carcinoma in Fusui. *Fusui Cancer Res.* **1980**, *1*, 26−38.

(21) Kuiper-Goodman, T. Uncertainties in the risk assessment of three mycotoxins: aflatoxin, ochratoxin, and zearalenone. *Can. J. Physiol. Pharmacol.* **1990**, *68*, 1017−1024.

(22) Groopman, J. D.; Zhu, J. Q.; Donanue, P. R.; Pikul, A.; Zhang, L. S.; Chen, J. S. Molecular dosimetry of urinary aflatoxin-DNA adducts in people living in Guangxi autonomous region, People's Republic of China. *Cancer Res.* **1992**, *52*, 45−52.

(23) Yeh, F. S.; Shen, K. N. Epidemiology and early diagnosis of primary liver cancer in China. *Adv. Cancer Res.* **1986**, *47*, 297−329.

(24) Collines, T. F. X.; Sprando, R. L.; Black, T. N.; Shackelford, M. E.; Laborde, J. B.; Hansen, D. K.; Eppley, R. M.; Trucksess, M. W.; Howard, P. C.; Bryant, M. A.; Ruggles, D. I.; Olejnik, N.; Rorie, J. I. Effects of fumonisin B_1 in pregnant rats. Part 2. *Food Chem. Toxicol.* **1998**, *36*, 673−685.

(25) Kuiper-Goodman, T. Prevention of human mycotoxicoses through risk assessment and risk management. In *Mycotoxins in Grains, Compounds Other Than Aflatoxins*; Miller, J. D., Trenholm, H. L., Eds.; Eagan Press: St. Paul, MN, 1994; pp 439−469.

(26) Wogan, G. E. Aflatoxins as risk factors for hepatocarcinogenesis in humans. *Cancer Res.* **1992**, *52*, 2114−2118.

(27) Yoshizawa, T.; Gao, H. P. Risk assessment of mycotoxins in staple foods from the high-risk area for human esophageal cancer in China. In *Mycotoxin Contamination: Health Risk and Prevention Project*; Akao, M., Kumagai, S., Goto, T., Kawai, K., Takahashi, H., Yabe, K., Yoshizawa, T., Koga, H., Eds.; Japanese Association of Mycotoxicology: Tokyo, Japan, 1999; pp 55−62.

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