JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Review

pubs.acs.org/JAFC

Survey of Mycotoxins in U.S. Distiller's Dried Grains with Solubles from 2009 to 2011

Yanhong Zhang* and John Caupert

National Corn-to-Ethanol Research Center, Edwardsville, Illinois 62025, United States

ABSTRACT: Distiller's dried grains with solubles (DDGS) is a major coproduct of the fuel-ethanol industry and is becoming a popular low-cost ingredient for animal feed. Uncertainties regarding the risk factors in DDGS, such as level of mycotoxins, could limit its application in the animal feed industry. To provide a scientifically sound assessment of the prevalence and levels of mycotoxins in U.S. DDGS, we measured aflatoxins, deoxynivalenol, fumonisins, T-2 toxin, and zearalenone in 67 DDGS samples collected from 8 ethanol plants in the midwestern United States from 2009 to 2011. Among the five mycotoxins, deoxynivalenol was the main focus of the study because the crop year of 2009 was favorable for deoxynivalenol occurrence in corn. We learned that no more than 12% of the samples contained deoxynivalenol levels higher than the minimum advisory level for use in animal feed provided by the U.S. FDA, and the deoxynivalenol levels in all DDGS collected in 2011 were <2 mg/kg. Besides, intensive study showed that the enrichment of deoxynivalenol from contaminated corn to DDGS was about 3.5 times. With regard to the other mycotoxins in DDGS, the study suggested that (1) almost none of the DDGS samples produced in 2010 contained detectable aflatoxins and the highest level of aflatoxins in DDGS was 5.7 μg/kg; (2) no more than 6% of the samples contained fumonisin levels higher than the guidance level for feeding equids and rabbits provided by the U.S. FDA; (3) none of the samples contained T-2 higher than the detection limit; (4) most samples contained zearalenone levels between 100 and 300 μg/kg. This study was based on representative DDGS samples from the U.S. ethanol industry, and the data were collected using state-of-the-art analytical methodology. This study provided a comprehensive and scientifically sound assessment of the occurrence and levels of mycotoxins in DDGS produced from 2009 to early 2011 by the U.S. ethanol industry.

KEYWORDS: *DDGS, aflatoxins, deoxynivalenol, fumonisins, T-2 toxin and zearalenone*

INTRODUCTION

Mycotoxins are unavoidable contaminants in crops, and therefore they occur in commodities entering the marketing chain including those grains to be used in ethanol production.[1] Currently, corn (maize) is the primary commodity used for the production of ethanol in the United States. Several mycotoxins can potentially be found in corn including aflatoxins, deoxynivalenol, fumonisins, T-2 toxin, and zearalenone.[1] Most of these toxins can occur in corn, preharvest, and are present in the grain at harvest; however, such occurrence is dependent upon the unique environmental conditions that are conducive to the growth of specific molds that produce these mycotoxins during crop development. Therefore, mycotoxin contamination in corn is not an annual event because the appropriate environmental conditions are often lacking for the growth of the specific responsible fungi.[2−4] In 2009, the weather conditions for corn production in the United States were favorable for the growth of deoxynivalenol, and numerous papers showed data on detectable deoxynivalenol in corn, which eventually led to the concern of elevated deoxynivalenol level in distiller's dried grains with solubles (DDGS).[5]

During the corn-to-ethanol production process, approximately two-thirds of the grain, mainly starch, is fermented by yeast to produce ethanol and carbon dioxide, neither of which would contain mycotoxins if contaminated corn was used.[6] However, the remaining coproduct, DDGS, could potentially contain a higher concentration of any mycotoxin that was present in the grain prior to fermentation. The increased level of a given mycotoxin in DDGS was reported to be approximately 3 times as high as the level in the grain.[7−9] To safeguard the quality of DDGS, most ethanol plants perform mycotoxin screening on incoming corn as often as weekly, when it is known that the corn came out of a mycotoxin-prone crop year.

To provide a scientifically sound assessment of the prevalence and levels of mycotoxins in DDGS produced from the midwestern United States from 2009 to 2011, we measured various mycotoxins, including aflatoxin, deoxynivalenol, fumonisin, T-2 toxin, and zearalenone, in DDGS produced from eight dry-grind ethanol plants in the midwestern United States between August 2009 and January 2011. Because the year 2009 was favorable for the occurrence of deoxynivalenol in corn, we specifically monitored the deoxynivalenol level in corn and DDGS from two ethanol plants for 14 consecutive days to better understand how to monitor and control deoxynivalenol accumulation in DDGS from contaminated corn.

MATERIALS AND METHODS

Sample Collection. DDGS samples were collected from eight ethanol plants in the midwestern United States every other month from August 2009 to January 2011. The sampling plan was designed to represent DDGS from corn produced in the crop years of 2008, 2009, and 2010. For example, the DDGS samples collected from August 2009 to January 2010 were likely produced from the mixture of corn

Received: August 25, 2011
Revised: December 6, 2011
Accepted: December 10, 2011

harvested in the crop years of 2008 and 2009, whereas the DDGS samples collected after January 2010 were likely produced from corn harvested in the crop year of 2009. Later in August of 2010, corn and DDGS samples were collected from the two ethanol plants with relatively high levels of deoxynivalenol in DDGS for 14 consecutive days to study how deoxynivalenol enriched from contaminated corn to DDGS.

About 2 kg of DDGS grab sample was collected at the ethanol plants immediately after they were produced, and about 2 kg of whole kernel corn was collected from the two ethanol plants before milling. After overnight shipment to the National Corn-to-Ethanol Research Center, the samples were immediately vacuum sealed and stored in a freezer at −20 °C.

Sample Testing. The mycotoxin tests were performed by Trilogy Analytical Laboratories (Washington, MO). Samples were analyzed for aflatoxins B_1, B_2, G_1, and G_2, deoxynivalenol, fumonisins B_1, B_2, and B_3, and zearalenone by high-performance liquid chromatography (HPLC) and for T-2 toxin by thin layer chromatography (TLC). Aflatoxins B_1, B_2, G_1, and G_2 were detected after extraction with acetonitrile/water (84:16), isolation using a solid phase cleanup column (Trilogy TC-M160) and detection with a fluorescence detector with a Kobra cell for postcolumn derivatization (AOAC 994.08).[10] Fumonisin B_1, B_2, and B_3 were detected after extraction with methanol/water (3:1), isolation using an immunoaffinity cleanup column and detection with a fluorescence detector with naphthalene dicarboxaldehyde (NDA) for precolumn derivatization (AOAC 2001.04).[11] Deoxynivalenol was detected after extraction with acetonitrile/water (84:16), isolation using a combination of solid phase (Trilogy TC-M160 and TC-C210) and immunoaffinity cleanup columns, and detection with an UV detector.[12] Detection of T-2 toxin was after extraction with acetonitrile/water (84:16), isolation using a combination of solid phase cleanup columns (Trilogy TC-M160 and TC-C210), and TLC detection.[13] Zearelenone was detected after extraction with acetonitrile/water (84:16), isolation using a combination of solid phase (Trilogy TC-M160) and immunoaffinity cleanup columns, and detection with a fluorescence detector.[14] The detection limits for the tests were 1 μg/kg for each aflatoxin, 0.1 mg/kg for deoxynivalenol, 0.1 mg/kg for each fumonisin, 0.1 mg/kg for T-2 toxin, and 0.05 mg/kg for zearalenone.

■ RESULTS AND DISCUSSION

The results for five mycotoxins in DDGS from eight ethanol plants are listed in Table 1A, and the results for deoxynivalenol in corn and DDGS from two ethanol plants are listed in Table 2.

Aflatoxins. The major fungus to produce aflatoxins, including aflatoxins B_1, B_2, G_1 and G_2, is *Aspergillus flavus*. Corn becomes susceptible to aflatoxin formation during growth under drought condition or in high moisture/humid storage.[15,16]

Aflatoxin B_1 was detected in DDGS collected in August and October 2009 with the highest level of 1.4 μg/kg. Aflatoxin B_1 was not detected in almost all DDGS samples collected since December 2009, and the highest level of aflatoxins in DDGS was 5.7 μg/kg in one DDGS collected in 2011. None of the other aflatoxin compounds, B_2, G_1, and G_2, were detected in any of the DDGS samples (Table 1A).

In comparison with the DDGS produced from 2006 to 2008 in the midwestern United States,[17] the results are very similar in that aflatoxins are not detected in most DDGS samples from the midwestern United States, and the highest level observed was <6 μg/kg. The U.S. FDA has set the lowest action level of 20 μg/kg aflatoxins in animal feeds and ingredients,[18] and the European Union Commission has recommended a guidance level of 5 μg/kg aflatoxins in complete feed.[19]

Deoxynivalenol. *Fusarium graminearum* is the principal deoxynivalenol-producing fungus in grains in the United States.[16] Deoxynivalenol may coexist with other toxins, such as zearalenone. The organism survives on old infested residue left on the field from the previous season, where a cold moist condition is favorable for the fungus to grow on corn. Generally, storage is not considered a potential source for contamination if the corn was mature and was stored at a moisture level of <14%.[15] Because the weather conditions in 2009 were favorable for the growth of deoxynivalenol in corn, elevated levels of deoxynivalenol in DDGS were expected.[5] However, the extent of deoxynivalenol contamination in DDGS was not systematically studied.

In this study, we learned that deoxynivalenol was detected in every DDGS sample collected (Table 1B). The detected level

Table 1. Aflatoxins (A), Deoxynivalenol (B), Fumonisins (C), and Zearalenone (D) in DDGS

	plant							
sampling time	1	2	3	4	5	6	7	8
				(A) Aflatoxins (μg/kg)				
2009-08	nd[a]	1.2[b]	1	1.3	2	nd	1.4	nd
2009-10	nd	1.2	1.1	1.4	1.3	nd	1	nd
2009-12	nd	nd	nd	nd	1.3	nd	nd	nd
2010-01	nd	nd	nd	SN[c]	1.5	nd	nd	nd
2010-03	nd	nd	nd	nd	SN	nd	nd	nd
2010-05	nd	1.1	nd	nd	SN	nd	nd	nd
2010-07	nd	nd	nd	nd	SN	nd	nd	nd
2010-09	nd	nd	nd	SN	nd	nd	nd	nd
				(B) Deoxynivalenol (mg/kg)				
2009-08	1.0	1.3	2.4	0.3	1.9	2.7	1.3	2.1
2009-10	1.7	2.0	2.3	1.6	1.1	2.3	0.7	1.9
2009-12	12.3	2.6	3.6	5.6	2.7	2.4	2.0	3.0
2010-01	10.4	3.9	1.9	SN	3.0	3.6	3.3	3.1
2010-03	9.4	3.1	2.4	6.3	SN	3.9	3.0	3.3
2010-05	5.9	3.1	2.4	3.9	SN	3.0	2.6	2.6
2010-07	9.1	3.0	2.7	5.0	SN	3.0	3.1	3.1
2010-09	4.5	2.3	2.4	SN	2.6	1.7	3.1	3.2
2011-01	2.1	0.8	0.5	1.7	0.4	1.0	0.6	0.3
				(C) Fumonisins (mg/kg)				
2011-01	nd	3.2	1.8	nd	1.9	nd	5.7	0.8
2009-08	0.8[d]	5.2	8.9	nd	1.8	1.8	5.4	3.3
2009-10	0.9	6.1	3.6	0.5	3.2	0.7	4.4	2.8
2009-12	0.8	0.6	3.7	0.2	0.5	0.2	0.3	0.5
2010-01	0.7	1.3	1.7	SN	1.5	0.2	0.2	nd
2010-03	0.7	0.7	2.2	0.2	SN	0.2	0.2	0.4
2010-05	0.3	0.5	1.7	0.2	SN	nd	0.3	0.2
2010-07	nd	0.4	0.9	nd	SN	nd	0.1	nd
2010-09	0.3	0.4	1.4	SN	1.1	0.1	nd	0.3
2011-01	0.2	1.6	0.9	nd	4.4	nd	nd	nd
				(D) Zearalenone (μg/kg)				
2009-08	102	225	234	118	136	270	161	256
2009-10	101	229	119	70	75	216	72	142
2009-12	469	311	334	123	560	116	133	202
2010-01	407	389	290	SN	245	209	114	261
2010-03	539	377	226	261	SN	212	154	228
2010-05	285	472	297	189	SN	161	117	309
2010-07	220	177	290	121	SN	108	130	161
2010-09	299	220	230	SN	244	113	61	263
2011-01	76.1	nd	nd	nd	nd	nd	nd	nd

[a]nd, not detected. [b]The detected mycotoxin was aflatoxin B_1. [c]SN, sample not available. [d]The detected fumonisins include fumonisins B_1, B_2, and B_3.

Table 2. Deoxynivalenol (Milligrams per Kilogram) in Corn and DDGS from Plants 1 and 4

	plant 1		plant 4	
sampling day	corn	DDGS	corn	DDGS
1	1.7	9.3	1.3	4.6
2	2.4	9.0	1.0	5.2
3	1.8	8.2	1.8	5.6
4	1.4	7.8	1.1	4.7
5	2.1	7.3	1.0	4.5
6	2.8	7.7	1.0	4.5
7	2.5	8.7	1.1	3.2
8	2.9	7.8	1.4	4.3
9	2.5	8.4	1.6	4.3
10	2.2	8.3	1.4	4.8
11	2.6	6.2	1.9	4.0
12	2.3	7.3	0.9	4.4
13	1.8	6.3	1.2	3.5
14	2.2	8.0	1.1	4.3
mean	**2.2**	**7.9**	**1.3**	**4.4**
RSD (%)	19	11	25	14

of deoxynivalenol in DDGS ranged from 0.3 to 12.3 mg/kg. Five DDGS samples from plant 1 and three DDGS samples from plant 4 contained deoxynivalenol at >5 mg/kg, which is the FDA advisory level for deoxynivalenol in animal feeds.[18] Overall, about 12% of the 67 samples studied contained deoxynivalenol levels higher than the minimum advisory level by FDA, and those samples were from two ethanol plants (plants 1 and 4). However, the European Union Commission set a guidance level of 0.9 mg/kg deoxynivalenol in complete feed, which is close to the U.S. FDA advisory level if 20% DDGS inclusion is used in the animal ration.[19]

With respect to the temporal trend, the deoxynivalenol level in DDGS from the eight ethanol plants increased from August 2009 to January 2010, then stayed unchanged or slightly decreased from March 2010 to September 2010, then drastically decreased in January 2011 (Figure 1A). The deoxynivalenol in DDGS increasing trend from August 2009 to January 2010 can be explained by the utilization of more and more deoxynivalenol contaminated corn produced from 2009. Whereas the temporal trend of deoxynivalenol in DDGS was similar for the eight plants, only two ethanol plants (plants 1 and 4) showed certain level of deoxynivalenol contamination in DDGS.

In comparison with the DDGS produced from 2006 to 2008 in the midwestern United States,[17] the deoxynivalenol in DDGS from 2006 to 2008 was around 1 mg/kg, and the deoxynivalenol in DDGS from 2009 to 2010 was >2 mg/kg.

Fumonisins. The major producer, *Fusarium verticillioides*, is capable of producing the fumonisins, mainly B_1, B_2, and B_3.[20] Corn is the major commodity affected by the fungi that produce the toxins. The exact conditions for this disease are unknown, but it is suggested that drought stress followed by warm, wet weather during flowering seems to be important. It is reported that the organism is present virtually in every seed and is present in the corn plant throughout its growth and that, sometimes, there is a considerable amount of fumonisins present in symptomless kernels of corn.

Fumonisins were detected in almost all DDGS samples. The fumonisin level in DDGS ranged from not detected to 8.9 mg/kg. Two DDGS samples from plant 2, one DDGS sample from

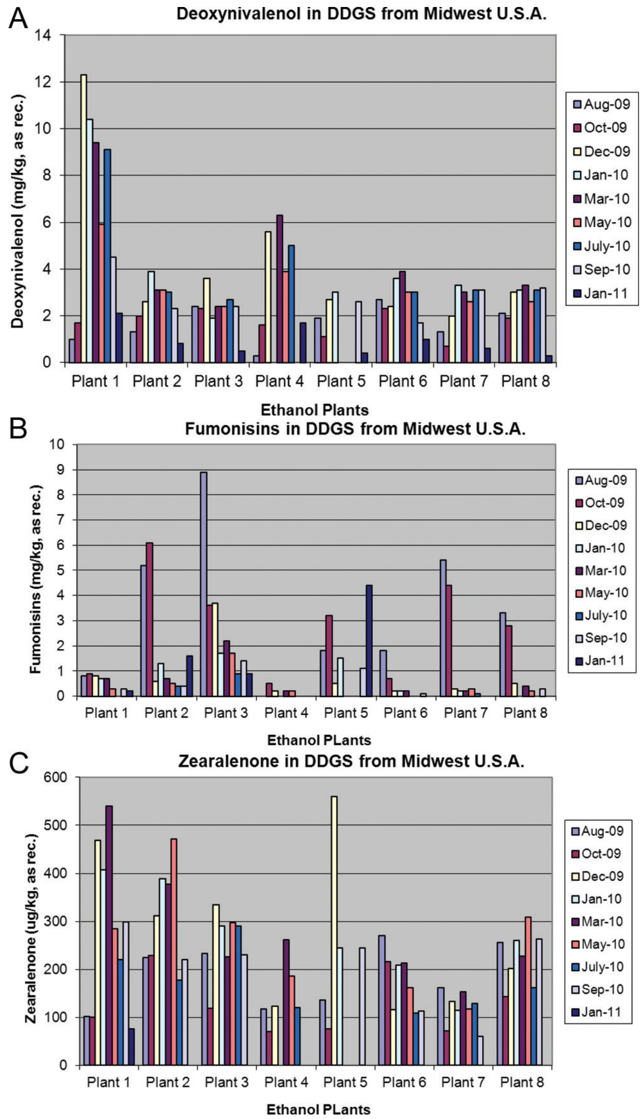

Figure 1. Temporal changes of deoxynivalenol (A), fumonisins (B), and zearalenone (C) in DDGS.

plant 3, and one DDGS sample from plant 7 contained fumonisins at >5 mg/kg, which is the FDA lowest guidance level for fumonisins in animal feeds.[18] In total, no more than 6% of the 67 samples studied contained fumonisin levels higher than the guidance level for feeding equids and rabbits by the U.S. FDA and European Union Commission, and the 6% of DDGS samples with elevated fumonisins were from three ethanol plants (plants 2, 3, and 7).

Different from the temporal trend of deoxynivalenol in DDGS, the fumonisin level in DDGS showed relatively high values in August 2009 and October 2009 and stayed fairly low afterward (Figure 1B). The plants producing DDGS with relatively high levels of deoxynivalenol had DDGS with relatively low levels of fumonisins.

In comparison with the DDGS produced from 2006 to 2008 in the midwestern United States,[17] only 6% of the DDGS from 2009 to 2010 contained fumonisin levels higher than the guidance level for feeding equids and rabbits by the U.S. FDA, whereas about 12% of the DDGS from 2006 to 2008 contained fumonisin levels higher than the recommendation level by FDA.

dx.doi.org/10.1021/jf203429f | J. Agric.Food Chem. XXXX, XXX, XXX–XXX

T-2. This mycotoxin is a member of fungal metabolites known as the trichothecenes. *Fusarium sporotrichioides* is the principal fungus responsible for the production of T-2. The production of T-2 is greatest with increased humidity and temperatures of 6−24 °C.[16]

None of the DDGS samples tested in this study were found to contain levels above the detection limit of 0.1 mg/kg, which is similar to the observation with the DDGS produced from 2006 to 2008.[17]

Zearalenone. This is an estrogenic fungal metabolite. The major fungus responsible for producing this toxin is *Fusarium graminearum*.[16] A moist and cool growing condition is favorable for this fungus to grow, the same conditions favorable for deoxynivalenol. For storage, controlling moisture at <14% is important to avoid contamination.

Zearalenone was detected in all DDGS samples. The zearalenone level in DDGS ranged from not detected to 560 μg/kg. No action levels, advisory levels, or guidance levels for zearalenone are available from the U.S. FDA; however, the European Commission Recommendation gave the lowest guidance level for zearalenone in complete feedstuffs of 0.25 mg/kg.[19] For most ethanol plants, it seemed that the temporal trend of zearalenone in DDGS was similar to that of deoxynivalenol level in DDGS (Figure 1C). When the level of zearalenone was plotted against deoxynivalenol in DDGS for each plant, the correlation was not strong (Figure 2).

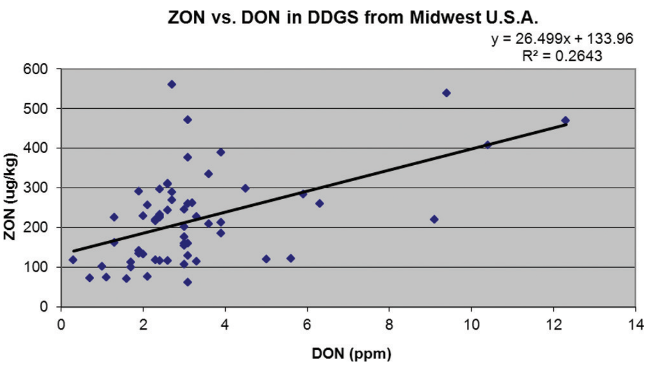

Figure 2. Zearalenone in DDGS versus deoxynivalenol in DDGS.

In comparison with the DDGS produced from 2006 to 2008 in the midwestern United States,[17] most DDGS from 2006 to 2008 contained zearalenone levels of <100 μg/kg; the DDGS from 2009 to 2011 contained zearalenone from not detected to 300 μg/kg.

Deoxynivalenol Enriched from Corn to DDGS. For plant 1, the mean of deoxynivalenol in corn was 2.2 mg/kg with a coefficient of variation (CV) of 19%, and the mean of deoxynivalenol in DDGS was 7.9 mg/kg with a CV of 19%. For plant 4, the mean of deoxynivalenol in corn was 1.3 mg/kg with a CV of 25%, and the mean of deoxynivalenol in DDGS was 4.4 mg/kg with a CV of 14%. On the basis of the mean values, the enrichment of deoxynivalenol from corn to DDGS was calculated as 3.5 times for samples from both plants.

About 2 kg of each sample was collected from a medium-sized ethanol plant (about 36 t) daily for 14 days, and after grinding, about 5 g of ground material was used for deoxynivalenol testing. Our data showed that the variation of deoxynivalenol in DDGS within 14 days was about 10% for both plants, and the variation of deoxynivalenol in corn within 14 days was about 20%. Considering that mycotoxins tend not to homogeneously distribute among grains,[16] the sampling and testing procedure used here was effective and representative to monitor the mycotoxin quality of DDGS from an ethanol plant. It is as expected that the deoxynivalenol in DDGS was more homogeneous than that in corn, because the corn to DDGS production involves a great amount of milling and mixing to homogenize the mycotoxins in DDGS.

The data from the two ethanol plants, which do not have identical processing parameters, confirmed that the enrichment of deoxynivalenol from corn to DDGS was about 3.5 times. This suggests that it is effective for an ethanol plant to monitor incoming corn frequently to safeguard the quality of the DDGS they produce.

AUTHOR INFORMATION

Corresponding Author

*Postal address: National Corn-to-Ethanol Research Center, 400 University Park Drive, Edwardsville, IL 62025. Phone: (618) 659-6737, ext. 224. E-mail: yzhang@ethanolresearch.com.

ACKNOWLEDGMENTS

We thank Dave Summers, a faculty assistant from the Illinois Institute of Rural Affairs, for help with the sample preparation for this project.

REFERENCES

(1) Whitlow, L. W.; Hagler, W. H. Jr. Mycotoxins in feeds. *Feedstuffs Reference Issue* **2006**, *77*, 69−79.

(2) Payne, G. A. Process of contamination by aflatoxin-producing fungi and their impact on crops. In *Mycotoxins in Agriculture and Food Safety*; Sinha, K. K. S., Bhatnagar, D., Eds.; Dekker: New York, 1998; pp 279−306.

(3) Munkvold, G. P.; Desjardins, A. E. Fumonisins in maize: can we reduce their occurrence? *Plant Dis.* **1997**, *81*, 556−565.

(4) Sutton, J. C. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. *Can. J. Plant Pathol* **1982**, 3 (Suppl.), 39−44.

(5) Hurburgh, C. Impact of the cold 2009 growing season, hailstorms, and storage problems on corn DDGS quality. *Fuel Ethanol Workshop*, 2010 (presentation).

(6) Ingledew, M. W. Improvements in alcohol technology through advancements in fermentation technology. In *The Alcohol Textbook*; Lyons, T. P., Kelsall, D. R., Murtagh, J. E., Eds.; Nottingham University Press: Notingham, U.K., 2006.

(7) Bennett, G. A.; Richard, J. L. Influence of processing on *Fusarium* mycotoxins in contaminated grains. *Food Technol.* **1996**, *50*, 235−238.8.

(8) Bothast, R. J.; Bennett, G. A.; Vancauwenberge, J. E.; Richard, J. L. Fate of fumonisin B_1 in naturally contaminated corn during ethanol fermentation. *Appl. Environ. Microbiol.* **1992**, *58*, 233−236.

(9) Schafsma, A. W.; Limay-Rios, V.; Paul, D. E.; Miller, J. D. Mycotoxins in fuel ethanol co-products derived from maize: a mass balance for deoxynivalenol. *J. Sci. Food Agric.* **2009**, *89*, 1574−1580.

(10) AOAC 994.08. *Official Methods of Analysis of AOAC International*, 17th ed.; AOAC: Washington, DC, 2000; Chapter 49, pp 26−27.

(11) AOAC Official Method 2001.04. *Official Methods of Analysis of AOAC International*, 18th ed.; AOAC: Washington, DC, 2001.

(12) MacDonald, S. J.; Chan, D.; Brereton, P.; Damant, A.; Wood, R. Determination of deoxynivalenol in cereals and cereal products by immunoaffinity column cleanup with liquid chromatography: interlaboratory study. *J. AOAC Int.* **2005**, *88* (4), 1197−1204.

(13) Romer, T. R. Use of small charcoal/alumina cleanup columns in determination of trichothecene mycotoxins in foods and feeds. *J. Assoc. Off. Anal. Chem.* **1986**, *69* (4), 699−703.

(14) MacDonald, S. J.; Anderson, S.; Brereton, P.; Wood, R.; Damant, A. Determination of zearalenone in barley, maize and wheat flour, polenta, and maize-based baby food by immunoaffinity column cleanup with liquid chromatography: interlaboratory study. *J. AOAC Int.* **2005**, *88* (6), 1733−1740.

(15) Richard, J. L. Mycotoxins − an overview. *Romer Labs' Guide to Mycotoxins*; 2000; Vol.*1*

(16) Council for Agricultural Science and Technology (CAST). Mycotoxins: risks in plant, animal, and human systems; Task Force Report 1392003.

(17) Zhang, Y.; Caupert, V. J.; Imerman, P. M.; Richard, J. L.; Shurson, G. C. The occurrence and concentration of mycotoxins in U.S. distiller's dried grains with solubles. *J. Agric. Food Chem.* **2009**, *57*, 9828−9837.

(18) FDA websites: Aflatoxins in feeds and feed ingredients: URL http://www.cfsan.fda.gov/~lrd/fdaact.html#afl. Fumonisins in feeds and feed ingredients: URL http://www.cfsan.fda.gov/~dms/fumongu2.html. Deoxynivalenol (DON) in feeds and feed ingredients: URL http://www.cfsan.fda.gov/~dms/graingui.html.

(19) Europe Union Commission Recommendation. *Off. J. Eur. Union* **2006**, *L 229/7*.

(20) Voss, K. A.; Smith, G. W.; Haschek, W.M.. Fumonisins: toxicokinetics, mechanism of action and toxicity. *Anim. Feed Sci. Technol.* **2007**, *137*, 299−325.