APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1991, p. 672–677
0099-2240/91/030672-06$02.00/0

Vol. 57, No. 3

Immunochemical Assessment of Mycotoxins in 1989 Grain Foods: Evidence for Deoxynivalenol (Vomitoxin) Contamination

M. M. ABOUZIED,[1] J. I. AZCONA,[1] W. E. BRASELTON,[2] AND J. J. PESTKA[1,3]*

Department of Food Science and Human Nutrition,[1] Department of Pharmacology and Toxicology,[2] and Department of Microbiology and Public Health,[3] Michigan StateUniversity, East Lansing, Michigan 48824

Received 10 October 1990/Accepted 22 December 1990

To assess the potential for mycotoxin contamination of the human food supply following the 1988 U.S. drought, 92 grain food samples were purchased from retail outlets in the summer of 1989 and surveyed for aflatoxin B_1, zearalenone, and deoxynivalenol (DON [vomitoxin]) by monoclonal antibody-based competitive enzyme-linked immunosorbent assay (ELISA). Only one sample (buckwheat flour) was found to contain aflatoxin B_1 (12 ng/g), whereas zearalenone was found in 26% of the samples at a mean concentration of 19 ng/g. In contrast, the DON ELISA was positive in 50% of the samples at a detection level of 1.0 μg/g. Between 63 and 88% of corn cereals, wheat flour/muffin mixes, rice cereals, and corn meal/muffin mixes yielded positive results for DON, whereas 25 to 50% of oat cereals, wheat- and oat-based cookies/crackers, corn chips, popcorn, and mixed-grain cereals were positive for DON. The mean DON content of the positive samples was 4.0 μg/g, and the minimum and maximum levels were 1.2 and 19 μg/g, respectively. When positive ELISA samples were also analyzed by high-performance liquid chromatography, a strong correlation between the two methods was found. The presence of DON in the two highest samples, corn meal and mixed-grain cereal, which contained 19 and 16 μg/g, respectively, was quantitatively confirmed by gas chromatography-mass spectrometry. The results indicated that DON was present in 1989 retail food products at concentrations that exceeded those found in previous market surveys and that have been experimentally associated with impaired animal health.

Aflatoxin B_1 (AFB_1), zearalenone (ZEA), and deoxynivalenol (DON [vomitoxin]) are among the mycotoxins most commonly detected following fungal colonization of cereal grains in the field and during storage throughout the world. AFB_1 is an extremely potent liver carcinogen in experimental animals, and a correlation has been drawn between degree of AFB_1 exposure and human primary hepatocellular carcinoma in developing countries (2). ZEA, produced by members of the genus *Fusarium*, elicits estrogenic effects in mammalian reproductive systems and has been specifically associated with field cases of swine hyperestrogenism (18). DON is a trichothecene mycotoxin that is also produced by *Fusarium* spp. and that causes feed refusal, reduced weight gain, emesis, and altered immune function (20, 29).

Increased contamination of corn, wheat, and other cereal grains by fungi and their resultant mycotoxins have been associated with climatic factors such as drought or excessive rainfall (6). Of particular concern were high aflatoxin levels in commodities harvested in the United States during and after the severe 1988 drought. However, there is no published information on occurrence of aflatoxin or other mycotoxins in retail foods following this period. To assess the degree of mycotoxin carryover to the human food supply in the year following the drought, monoclonal antibody-based enzyme-linked immunosorbent assays (ELISAs) were used to survey for the presence of AFB_1, ZEA, and DON in grain-based food products purchased from retail outlets in 1989. The results suggested that AFB_1 and ZEA contamination were negligible, but there was widespread occurrence of DON in food products at levels that have been experimentally associated with impaired animal health.

* Corresponding author.

MATERIALS AND METHODS

Reagents. All inorganic chemicals and organic solvents were reagent grade or better. Ovalbumin (OA; crude and fraction VII), polyethylene sorbitan monolaurate (Tween 20), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydrogen peroxide, horseradish peroxidase (HRP), *N*-hydroxysuccinimide, dimethylformamide, and 1,3-dicyclohexylcarbodiimide were purchased from Sigma Chemical Co., St. Louis, Mo. Goat anti-mouse immunoglobulin G (IgG) conjugated to HRP was obtained from Cooper Biomedical, Malvern, Pa.

Monoclonal antibodies. Hybridoma cell lines 5C11, 2G3, and 6F5 were used to produce anti-AFB_1 (8), anti-ZEA (7), and anti-DON (3) monoclonal antibodies, respectively. These were scaled up as ascites and purified by ammonium sulfate precipitation (14, 15).

Preparation of mycotoxin conjugates. AFB_1 and ZEA were converted to carboxymethyl oximes by the procedures of Chu et al. (5) and Thouvenot and Morfin (27), respectively. The oximes were conjugated to HRP for use as marker ligands in the direct competitive ELISA by the method of Kitagawa et al. (16).

DON-hemisuccinate was prepared by protection of the C-7 and C-15 hydroxyls with a cyclic boronate ester, esterification at the C-3 position, and then removal of boronate ester by a modification of the procedure of Casale et al. (3). Briefly, in a 2-ml reaction vial, 10 mg of DON was dissolved in 0.5 ml of pyridine, 7 mg of *n*-butyl boronate was added, and the mixture was stirred at room temperature for 30 min. Succinic anhydride (14 mg) was added and the vial was capped tightly and heated in a boiling water bath for 45 min. Pyridine was evaporated under N_2 and the residue was dissolved in 2 ml of water. The aqueous solution was washed

with 4 ml of chloroform four times, the H_2O fractions were extracted 10 times with 5 ml of ethyl ether, and then the ether fraction was evaporated under N_2. DON-hemisuccinate was conjugated to OA for use as a solid phase in the competitive indirect ELISA by the procedure of Kitagawa et al. (16).

Food samples. Ninety-two grain-based food products that included breakfast cereals, corn meal, wheat flour, and muffin mixes, popcorn, crackers, and mixed-grain cereals were purchased from mid-Michigan retail grocery outlets and natural food stores during August of 1989.

Sample preparation for ELISA. For initial ELISA screening, samples were ground and 25 g was extracted by shaking for 60 min with 100 ml of methanol-water (7:3). The extract was centrifuged and 4 ml of the supernatant was evaporated under N_2. The residue was dissolved in 5 ml of 0.01 M phosphate-buffered (pH 7.2) saline (PBS), filtered, and subjected to ELISA.

Sample preparation for DON confirmation. For subsequent confirmation, all samples testing positive for DON in the ELISA and selected negative samples were extracted with acetonitrile-water (9:1). Aliquots were evaporated and redissolved in PBS for quantitative DON ELISA. Additional aliquots were cleaned up by passage through activated carbon-alumina columns as described by Romer (23) for subsequent high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS) confirmation. Eluates were evaporated and dissolved in appropriate solvents for each method.

ELISA. Direct competitive ELISA was used to quantitate AFB_1 and ZEA (22, 30). Each well of microtiter plates (Immulon 2 Removawell strips; Dynatech Laboratories, Alexandria, Va.) was coated with 125 μl of specific mycotoxin monoclonal antibody (AFB_1 or ZEA monoclonal antibody) diluted 1:1,000 in 0.1 M carbonate buffer (pH 9.6). The antibody was dried onto the wells in a forced-air oven at 40°C. Wells were washed four times with PBS containing 0.05% (vol/vol) Tween 20 (PBS-Tween). Nonspecific binding was minimized by blocking the unbound sites of each well with 300 μl of PBS containing 1% (wt/vol) OA (PBS-OA) at 37°C for 30 min. After washing with PBS-Tween, mycotoxin standards or sample extracts (50 μl) were added to each well followed by 50 μl of mycotoxin-HRP conjugate (AFB_1-HRP or ZEA-HRP; 0.5 mg/ml) diluted 1:500 in PBS-OA, and plates were then incubated at 37°C for 60 min. The plates were washed eight times with PBS-Tween and 100 μl of ABTS substrate (400 μM ABTS, 0.009% [vol/vol] H_2O_2, 45 mM sodium citrate buffer, pH 4.0) was added. After 30 min, 100 μl of stopping reagent consisting of 0.1% (wt/vol) sodium azide in 0.3 M citric acid was added to each well. Absorbance of each well at 405 nm was measured with a Minireader II (Dynatech Laboratories). Mycotoxin content was calculated from standard competition curves comparing log mycotoxin concentration versus absorbance.

In the indirect ELISA for DON (4), microtiter plates were coated for 18 h at 4°C with 125 μl of DON-hemisuccinate-OA conjugate (5 μg/ml) in 0.01 M carbonate buffer (pH 9.6). The plates were washed four times with PBS-Tween. PBS-OA was added to each well in 200-μl aliquots and plates were incubated at 37°C for 30 min. After four washes with PBS-Tween, 50 μl of standard DON or sample extract was added to each well followed by 50 μl of a 1:200 dilution of DON monoclonal antibody in PBS-OA. Plates were then incubated for 1 h at 37°C. Anti-mouse IgG peroxidase diluted 1:500 in PBS-OA (100 μl) was added to each well, and plates were incubated for 30 min at 37°C. After the wells were washed eight times, bound peroxidase and mycotoxin content was determined with ABTS substrate as described above.

HPTLC. Following cleanup of acetonitrile-water extracts on charcoal-alumina columns (23), concentrated eluates were spotted on channeled HPTLC plates (10 by 10 cm; Whatman LHPK, Clifton, N.J.) and separated in chloroform-ethanol (95:5). DON was visualized by spraying with aluminum chloride as described by Trucksess et al. (28).

HPLC. HPLC was performed by a modification of the procedure of Chang et al. (4). The system consisted of a model 2300 HPLC pump, V4 variable-wavelength absorbance detector set at 224 nm (Isco Scientific, Lincoln, Neb.), and RP-18 Spheri-10 MPLC analytical column (22 cm by 4.6-cm inside diameter) and guard cartridge (Brownlee Labs, Santa Clara, Calif.). The mobile phase was 20% methanol in water and flow rate was 1.5 ml/min.

GC-MS. For confirmation and quantification of DON by GC-MS, the compound was derivatized as the *tris*-trimethylsilyl ether (DON-*tris*-TMS). Samples were dissolved in 50 μl of CH_2Cl_2, mixed with 50 μl of Sylon BTZ (Supelco Inc., Bellefonte, Pa.), and allowed to react for 20 min at room temperature in a closed vial. The mixture was evaporated to dryness under a stream of N_2, and redissolved in 100 μl of cyclohexane-CH_2Cl_2 (1:1).

GC-MS was carried out on a Hewlett-Packard 5890-A GC and 5970 mass selective detector. The column was a 0.25- by 15-mm DB5 bonded phase capillary (J & W Scientific, Folsum, Calif.). The column temperature was 200°C for 1 min and then programmed to 250°C at 15°C/min. The electron impact ionization energy was 70 eV.

RESULTS

The presence of AFB_1, ZEA, and DON in foods was assayed by ELISA based on comparison of inhibition caused by methanolic extracts to standard curves for the three mycotoxins (Fig. 1). By using an ELISA with a detection level of 2.5 ng/g, AFB_1 was found in only a single sample. The sample, buckwheat flour, contained AFB_1 at a level of 12 ng/g and thus did not exceed the Food and Drug Administration action level of 20 ng/g. At a detection level of 5.0 ng/g, ZEA was detected in 26% of the samples at a mean level of 19 ng/g (Table 1). Corn cereals had the highest incidence of ZEA (88%) followed by mixed-grain cereals and oat products, 67 and 60%, respectively. There was no detectable ZEA in corn chips or popcorn (Table 1).

Fifty percent of the total samples were found to exceed 1 μg of DON per g during the initial ELISA screen of methanolic extracts. Additional samples were extracted with acetonitrile-water and the DON content was quantitated (Table 2). Corn cereals, corn meal/muffin mixes, wheat flour/muffin mixes, and rice cereals yielded positive results in 63 to 88% of the samples tested. Between 25 and 50% of oat cereals, wheat, and oat-based cookies/crackers, corn chips, popcorn, and mixed-grain cereals were positive for DON. The lowest number (9%) of positive samples was found in wheat cereals. The average concentration for ELISA-positive samples was 4.0 μg/g, with a maximum of 19 μg/g and a minimum of 1.2 μg/g. Four samples, corn meal, mixed-grain cereal, and two rice cereals, were the most heavily contaminated and had DON levels of 19, 16, 9.5, and 7.5 μg/g, respectively.

Because of the high incidence and levels of DON found in the ELISA survey, confirmatory analyses were performed

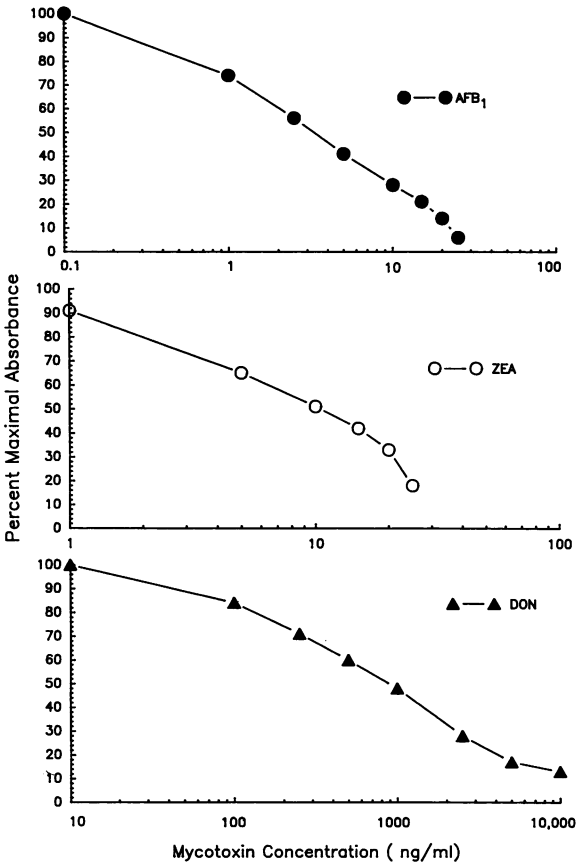

FIG. 1. ELISA standard curves for AFB_1, ZEA, and DON.

by several other methods to corroborate the results. Aliquots of acetonitrile-water extracts were cleaned up by passage through activated carbon-alumina columns and subjected to HPTLC, HPLC, and GC-MS. Figure 2 reveals that HPTLC also indicated the presence of DON in ELISA-positive samples selected from the major groups of grain

TABLE 1. ZEA content of grain-based food products determined by direct competitive ELISA

Item	No. of samples analyzed	No. (%) of positive samples	ZEA content in positive samples (ng/g)[a]		
			Maximum	Minimum	Avg
Corn cereals	8	7 (88)	20	5	12
Wheat cereals	12	2 (16)	30	27	28
Rice cereals	4	1 (25)	12	12	12
Oat cereals	5	3 (60)	22	9	16
Wheat & oat crackers/ cookies	18	3 (17)	16	10	12
Corn chips	6	0 (0)			
Popcorn	8	1 (12)	10	10	10
Wheat flour/muffin mix	17	2 (12)	14	12	13
Corn meal	11	3 (27)	100	8	38
Mixed-grain cereals	3	2 (67)	50	12	31
Total/mean	92	24 (26)	100	5	19[b]

[a] Data are means of six determinations. Detection limit of assay was 5 ng/g.
[b] Indicates mean for all food types. Mean for all positive samples was 19 ng/g.

TABLE 2. DON content of grain-based food products determined by indirect competitive ELISA

Item	No. of samples analyzed	No. (%) of positive samples	DON content in positive samples (μg/g)[a]		
			Maximum	Minimum	Avg
Corn cereals	8	7 (88)	3.0	1.2	1.8
Wheat cereals	12	1 (8)	1.9	1.9	1.9
Rice cereals	4	3 (75)	9.5	3.9	6.8
Oat cereals	5	2 (40)	2.6	1.3	1.9
Wheat & oat crackers/ cookies	18	6 (33)	5.4	1.6	2.8
Corn chips	6	2 (33)	3.0	2.0	2.5
Popcorn	8	4 (50)	4.5	2.8	3.9
Wheat flour/muffin mix	17	13 (76)	5.8	1.5	4.0
Corn meal	11	7 (63)	19.0	2.0	5.8
Mixed-grain cereals	3	1 (3)	16.0	16.0	16.0
Total/mean	92	46 (50)	19	1.2	4.7[b]

[a] Data are means of six determinations. Detection limit was 1.0 μg/g.
[b] Indicates mean for all food types. The mean for all positive samples was 4.0 μg/kg.

foods. Similarly, ELISA-positive samples yielded absorbance peaks of retention time identical to that for DON standard in HPLC (Fig. 3). Based on HPLC quantitation, the average DON concentration for the positive samples was 4.1 μg/g, with a maximum of 17.5 μg/g and a minimum of 0.5 μg/g. A strong correlation (r^2 = 0.965) existed between ELISA and HPLC estimated for DON in the samples (Fig. 4). Of the 46 ELISA-positive samples, only 4 could not be verified by HPLC. Also, 1 of 20 negative samples was found to contain DON (1.1 μg/g).

For further confirmation, the two most highly contaminated samples of corn meal and mixed-grain cereal were subjected to quantitative GC-MS analysis. The GC retention

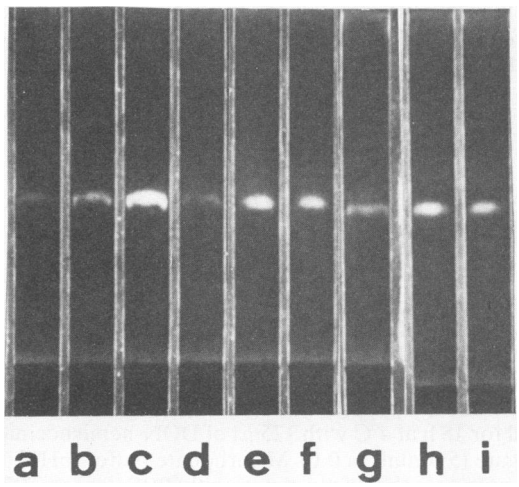

FIG. 2. HPTLC of DON in food samples. Extracts were cleaned up on a charcoal-alumina column, developed in chloroform-ethanol (95:5) on channeled HPTLC. DON was detected under UV light after spraying with aluminum chloride. Lanes: (a) DON, 100 ng; (b) DON, 250 ng; (c) DON, 1,000 ng; (d, e) corn meal samples; (f) seven-grain cereal; (g) whole wheat flour; (h, i) muffin mixes. Samples were 0.1-g (d, g) or 0.02-g (e, f, h, i) equivalent. Photo represents composite of two separate runs.

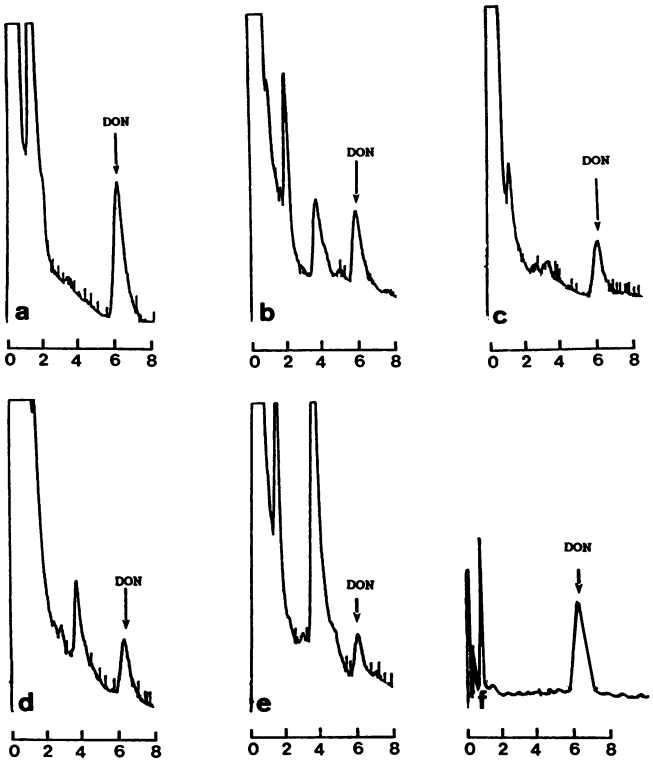

FIG. 3. HPLC chromatograms of DON in food samples. Chromatography was on an RP-18 Spheri-10 MPLC analytical column (22 by 4.6-cm inside diameter), using a mobile phase of 20% methanol, a flow rate of 1.5 ml/min, and absorbance detection at 224 nm. Samples were corn meal (a), rice cereal (b), muffin mix (c), popcorn (d), corn cereal (e), and DON standard (f).

times (Fig. 5) and mass spectra (Fig. 6) of the TMS derivatives were identical to those of reference standard DON-*tris*-TMS. DON concentrations in the corn meal and cereal sample were calculated to be 14.8 and 11.8 μg/g, respectively. These were consistent with ELISA estimates of 19 and 16 μg/g and HPLC estimates of 17.5 and 15.0 μg/g for the same samples, respectively. Characteristic ions are the molecular ion, M^+ = 512, *m/z* 422 (*M*-trimethylsilanol), and fragment ions at 325, 259, and 235. Ion *m/z* 73 and 147 are common in TMS derivatives, and *m/z* 103 indicates a TMS derivative of a primary alcohol formed by reaction of the C-15 position. HPLC peaks from corn meal and mixed-grain cereal were also collected and confirmed by GC-MS retention times and mass spectra.

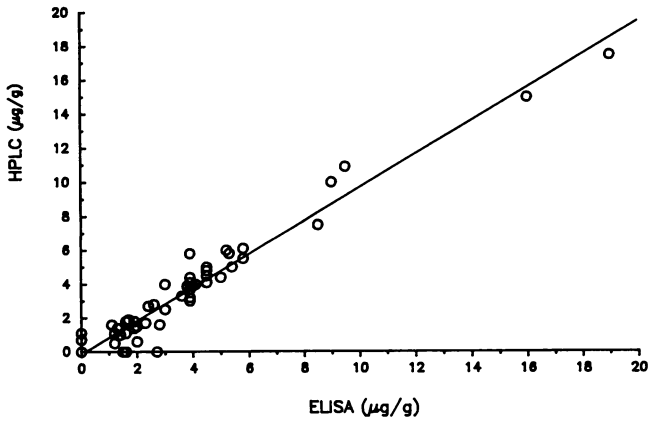

FIG. 4. Comparison of ELISA and HPLC quantitation of DON in food samples. Correlation coefficient (r^2) was 0.965 ($P < 0.01$).

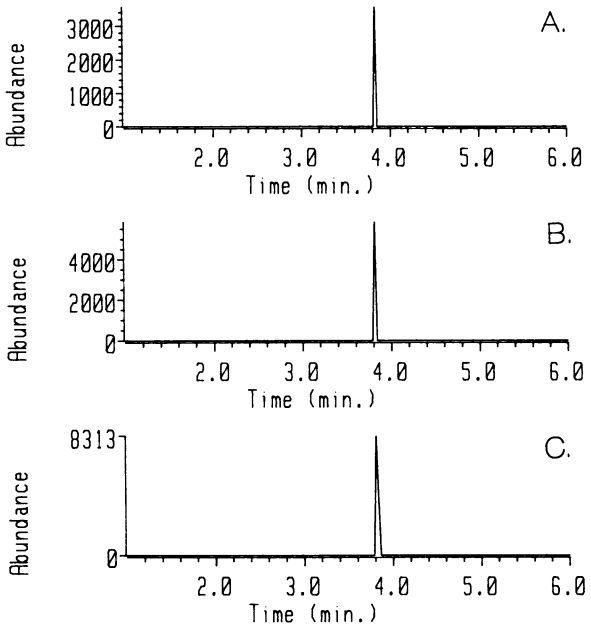

FIG. 5. Reconstructed ion chromatograms of *m/z* 512, the molecular ion of DON-*tris*-TMS: (A) 100-ng reference standard DON-*tris*-TMS; (B) 12.5-mg equivalents of corn meal extract; (C) 12.5-mg equivalents of seven-grain cereal. Chromatography was on a DB5 bonded phase capillary column (0.25 mm by 15 m) at 200°C for 1 min and then at 15°C/min to 250°C.

DISCUSSION

The ELISA methods used in this survey are rapid, simple, and inexpensive in terms of reagents and equipment. The approach allows the facile screening of food products for mycotoxins and other contaminants (19). ELISA has been previously validated for the screening of AFB_1 (22) and ZEA (30) in naturally contaminated samples. The results presented here demonstrate for the first time that ELISA recovery of DON in naturally contaminated foods correlated well with HPLC analysis, and this was further verified by HPTLC and GC-MS. It should be mentioned that there was a 12% false-positive rate for the DON ELISA when compared with HPLC. False-positives included cookies, corn cereal, and muffin mix and therefore were not relegated to a single group. We have recently shown that the DON monoclonal antibody cross-reacted with several putative trichothecene precursors and shunt metabolites produced by *Fusarium* spp. (1). Thus, false-positives might be explained by the possible presence of other *Fusarium* metabolites in these samples. Notably, of 20 negative samples analyzed by HPLC, a single sample was found to exceed 1 μg/g when reanalyzed by HPLC. This particular product, popcorn, contained cheese flavoring that may have interfered with the ELISA.

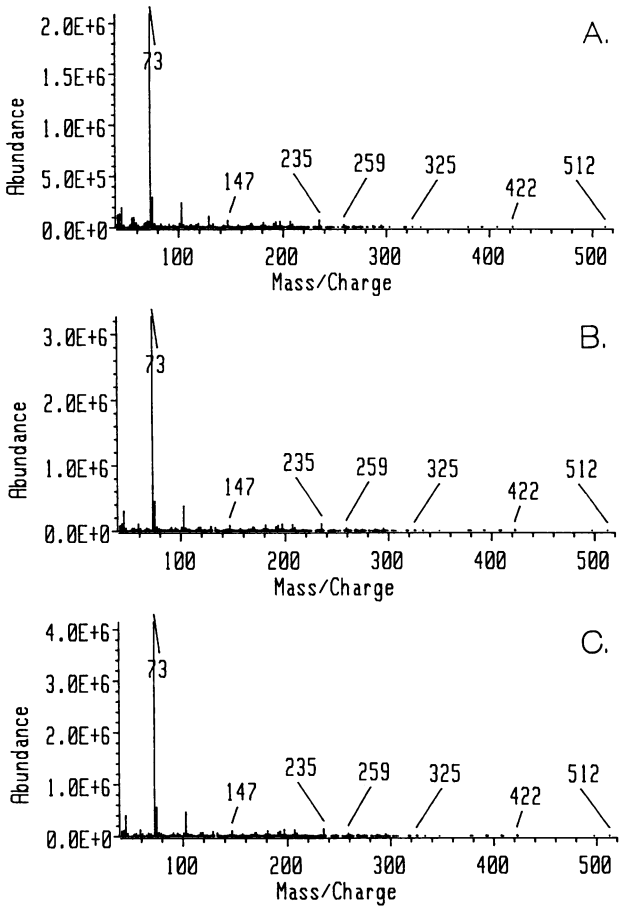

FIG. 6. Electron impact (70 eV) mass spectra of compounds eluting at 3.81 min under conditions given in the legend to Fig. 5: (A) 100-ng reference standard DON-*tris*-TMS; (B) 12.5-mg equivalents of corn meal extract; (C) 12.5-mg equivalents of seven-grain cereal.

The virtual absence of AFB_1 in food products was unanticipated in light of the dire predictions by the lay press following the 1988 U.S. drought. This was likely due to effective surveillance and diversion of AFB_1-contaminated raw materials by food processors. Interestingly, the single AFB_1-positive sample, buckwheat, was obtained from the "chemical-free" section of a natural foods store.

The levels of ZEA found in samples were consistent with those found previously by our group and others (9, 24, 31). The mean level for positive samples, 19 ng/g, would not be expected to present a hazard to the consumer based on a recent risk assessment for ZEA (17).

The data suggest that there was a high incidence of DON in cereal grain products, consistent with reports of worldwide occurrence of DON and other trichothecene mycotoxins (25). For example, Brumley et al. (1a) determined that >80% of samples of wheat, corn flour, corn meal, and snack foods contained DON in the range of 0.3 to 9.0 μg/g. In a retail product survey, Trucksess et al. (28) found that DON was detected in 60% of 60 breakfast cereals analyzed. In a recent survey of cereals from The Netherlands, Tanaka et al. (26) found that 90, 79, and 62% of samples were contaminated with DON, nivalenol, and ZEA, respectively, with a maximum DON concentration of 3.2 μg/g.

One major difference between this report and the above-described studies is that, in addition to contamination of corn and wheat products, DON was found in oat- and rice-based products. A second major difference between this and previous studies was the high mean level of DON found in positive samples (>4 μg/g) and the observation that two foods from the market shelf exceeded 10 μg/g. One (corn meal) was a major brand purchased from a grocery store, while another (seven-grain cereal) was obtained from a natural foods store. Conceivably, the severe drought conditions that occurred in the United States in 1988 contributed to these apparently unique findings. We are unaware of any surveys that were conducted for DON in raw agricultural commodities during the 1988 harvest that might support this possibility.

Although the DON levels found here would not present an acute toxicity risk (10, 29), there is potential for chronic effects upon extended exposure. The presence of DON in feed in the low microgram-per-gram range is sufficient to cause reduced weight gain and feed refusal in several animal species (12, 13, 19). The U.S. Food and Drug Administration has designated 2 μg of DON per g as a "level of concern" for wheat products intended for human consumption and, relatedly, the Canadian government has set guidelines of 1 and 2 μg/g for softwheat intended for infant foods and for humans, respectively (29). Of additional concern is the capacity of DON to alter immunity, particularly mucosal immune function (20). Levels as low as 2 μg/g in diet cause elevation of serum IgA in mice (12) and exposure to 25 μg/g can induce symptoms of an IgA nephropathy-like glomerulonephritis in mice with clinical signs that include glomerular IgA accumulation (21), hematuria, and elevated circulating IgA immune complexes (8a). Little is known of the potential immunotoxicity of DON to humans, although in vitro studies indicate that human and animal lymphocytes are similarly susceptible to the toxic effects of trichothecene mycotoxins (10). Estimation of any potential risks to humans ingesting DON-contaminated grains must take into account the fact that these represent only one portion of a balanced diet.

In summary, the data presented here demonstrate that 1989 retail grain-based foods exhibited negligible AFB_1 and ZEA levels, but had high levels of DON contamination. Thus, from a microbiological standpoint it appears that *Fusarium* contamination with concurrent trichothecene elaboration may have occurred in cereal grains as a result of the 1988 drought. The data suggest the need for regular screening of grains and grain-based food products for DON by producers and processors, particularly during years when crops are under stress by extreme weather conditions. Further elucidation of possible chronic and immunological effects in humans and animals exposed to DON at the levels found here is also necessary.

ACKNOWLEDGMENTS

This work was supported by USDA grant 59-32U4-7-103, U.S. Public Health Service grant ES-03358, and the Michigan State University Agricultural Experiment Station. J.I.A. is recipient of a postdoctoral fellowship from the Ministerio de Educacion y Ciencia, Spain.

We thank L. Trenholm and M. Trucksess for valuable advice concerning this work. We thank D. Klein for assistance with manuscript preparation.

REFERENCES

1. **Abouzied, M., M. Beremand, S. McCormick, and J. Pestka.** J. Food Protect., in press.

1a. **Brumley, W. C., M. W. Trucksess, S. H. Adler, C. K. Cohen,**

K. D. White, and J. A. Sphon. 1985. Negative ion chemical ionization mass spectrometry of deoxynivalenol (DON): application to identification of DON in grains and snack foods after quantitation/isolation by thin layer chromatography. J. Agric. Food Chem. **33**:326–330.

2. **Busby, W. F., Jr., and G. F. Wogan.** 1979. Mycotoxins and mycotoxicoses, p. 519–610. *In* H. Riemann and F. L. Bryan (ed.), Food-borne infections and intoxications, 2nd ed. Academic Press, Inc., New York.
3. **Casale, W. L., J. J. Pestka, and L. P. Hart.** 1988. Enzyme-linked immunosorbent assay employing monoclonal antibody specific for deoxynivalenol (vomitoxin) and several analogues. J. Agric. Food Chem. **36**:663–668.
4. **Chang, H. L., J. W. DeVries, P. A. Larson, and H. H. Patel.** 1984. Rapid determination of deoxynivalenol (vomitoxin) by liquid chromatography using modified Romer column cleanup. J. Assoc. Off. Anal. Chem. **67**:52–54.
5. **Chu, F. S., M.-T. S. Hsia, and P. Sun.** 1977. Preparation and characterization of aflatoxin B_1-1(-CO-carboxymethyl)-oxime. J. Assoc. Off. Anal. Chem. **60**:791–794.
6. **Council for Agricultural Science and Technology.** 1989. Mycotoxins—economic and health risks, p. 53–69. Council for Agricultural Science and Technology, Ames, Iowa.
7. **Dixon, D. E., R. L. Warner, B. P. Ram, L. P. Hart, and J. J. Pestka.** 1987. Hybridoma cell line produces a monoclonal antibody to the mycotoxins zearalenone and α-zearalenol. J. Agric. Food Chem. **35**:122–126.
8. **Dixon-Holland, D. W., J. J. Pestka, B. A. Bidigare, W. L. Casale, R. L. Warner, and L. P. Hart.** 1988. Production of sensitive monoclonal antibodies to aflatoxin B_1 and aflatoxin M_1 and their application to ELISA of naturally contaminated foods. J. Food Protect. **51**:201–204.

8a. **Dong, W., and J. Pestka.** 1990. Induction of experimental IgA nephropathy by dietary exposure to vomitoxin. FASEB J. **4**:A1885.

9. **Eppley, R. M., L. Stoloff, M. W. Trucksess, and C. W. Chung.** 1974. Survey of corn for Fusarium toxins. J. Assoc. Off. Anal. Chem. **57**:632–635.
10. **Forsell, J. H., R. Jensen, J.-H. Tai, M. Witt, W. S. Lin, and J. J. Pestka.** 1987. Comparison of acute toxicities of deoxynivalenol (vomitoxin) and 15-acetyldeoxynivalenol in the B6C3F1 mouse. Food Chem. Toxicol. **25**:155–162.
11. **Forsell, J. H., and J. J. Pestka.** 1985. Relation of 8-ketotrichothecene and zearalenone analog structure to inhibition of mitogen-induced human lymphocyte blastogenesis. Appl. Environ. Microbiol. **50**:1304–1307.
12. **Forsell, J. H., M. F. Witt, J.-H. Tai, R. Jensen, and J. J. Pestka.** 1986. Effects of 8-wk exposure of the B6C3F1 mouse to dietary deoxynivalenol (vomitoxin) and zearalenone. Food Chem. Toxicol. **24**:213–219.
13. **Forsyth, D. M., T. Yoshizawa, N. Morooka, and J. Tuite.** 1977. Emetic and refusal activity of deoxynivalenol to swine. Appl. Environ. Microbiol. **34**:547–552.
14. **Galfre, G., and C. Milstein.** 1983. Preparation of monoclonal antibodies; strategies and procedures. Methods Enzymol. **73**:1–40.
15. **Goding, J. W.** 1980. Antibody production by hybridomas. J. Immunol. Methods **39**:285–308.
16. **Kitagawa, T., T. Shimozona, T. Aikawa, T. Yoshida, and H. Nichimura.** 1981. Preparation and characterization of heterobifunctional cross linking reagents for protein modifications. Chem. Pharm. Bull. **29**:1130–1135.
17. **Kuiper-Goodman, T., P. M. Scott, and H. Watanabe.** 1987. Risk assessment of the mycotoxin zearalenone. Regul. Toxicol. Pharmacol. **7**:253–306.
18. **Mirocha, C. J., S. V. Pathre, and C. M. Christensen.** 1977. Zearalenone, p. 345–364. *In* J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman (ed.) Mycotoxins in human and animal Health. Pathotox Publishers, Park Forest South, Ill.
19. **Pestka, J. J.** 1988. Enhanced surveillance of foodborne mycotoxins by immunochemical assay. J. Assoc. Off. Anal. Chem. **71**:1075–1081.
20. **Pestka, J. J., and G. S. Bondy.** 1990. Alteration of immune function following dietary vomitoxin exposure. Can. J. Physiol. Pharmacol. **68**:1009–1016.
21. **Pestka, J. J., M. A. Moorman, and R. L. Warner.** 1989. Dysregulation of IgA production and IgA nephropathy induced by the trichothecene vomitoxin. Food Chem. Toxicol. **6**:361–368.
22. **Ram, B. P., L. P. Hart, O. L. Shotwell, and J. J. Pestka.** 1986. Enzyme-linked immunosorbent assay of aflatoxin B_1 in naturally contaminated corn and cottonseed. J. Assoc. Off. Anal. Chem. **69**:904–907.
23. **Romer, T. R.** 1986. Use of small charcoal/alumina cleanup columns in determination of trichothecene mycotoxins in foods and feeds. J. Assoc. Off. Anal. Chem. **69**:699–703.
24. **Scott, P. M., T. Panalaks, S. Kanhere, and W. F. Miles.** 1978. Determination of zearalenone in cornflakes and other corn-based foods by thin layer chromatography, high pressure liquid chromatography and gas-liquid chromatography/high resolution mass spectroscopy. J. Assoc. Off. Anal. Chem. **61**:593–600.
25. **Tanaka, T., A. Hasegawa, S. Yamamato, U. S. Lee, Y. Sugiura, and Y. Ueno.** 1988. Worldwide contamination of cereals by the Fusarium mycotoxins nivalenol, deoxynivalenol, and zearalenone. 1. Survey of 19 countries. J. Agric. Food Chem. **36**:979.
26. **Tanaka, T., S. Yamamato, A. N. Hasegawa, J. R. Breslin, Y. Sugiura, and Y. Ueno.** 1990. A survey of the natural occurrence of Fusarium mycotoxins, deoxynivalenol, nivalenol, and zearalenone in cereals harvested in the Netherlands. Mycopathologia **110**:19–22.
27. **Thouvenot, D. R., and R. F. Morfin.** 1983. Radioimmunoassay for zearalenone and zearalenol in human serum: production, properties, and use of porcine antibodies. Appl. Environ. Microbiol. **45**:16–23.
28. **Trucksess, M. W., M. T. Flood, and S. N. Page.** 1986. Thin layer chromatographic determinations of deoxynivalenol in processed grain products. J. Assoc. Off. Anal. Chem. **69**:35–36.
29. **Ueno, Y.** 1987. Trichothecenes in food, p. 123–147. *In* P. Krogh (ed.), Mycotoxins in food. Academic Press, Inc., New York.
30. **Warner, R., B. P. Ram, L. P. Hart, and J. J. Pestka.** 1986. Screening for zearalenone in corn by competitive direct enzyme-linked immunosorbent assay. J. Agric. Food Chem. **34**: 714–717.
31. **Warner, R. L., and J. J. Pestka.** 1987. ELISA survey of retail grain-based food products for zearalenone and aflatoxin B_1. J. Food Protect. **50**:502–503.