Hybridoma Cell Line Production of a Specific Monoclonal Antibody to the Mycotoxins Zearalenone and α-Zearalenol

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A stabilized hybridoma cell line secreting anti-zearalenone monoclonal antibodies of subclass IgG_1 with κ light chains was produced following the fusion of hyperimmune spleen cells from a BALB/c mouse with NS-1 myeloma cells. A competitive indirect enzyme-linked immunosorbent assay (ELISA) employing this antibody detected zearalenone at 0.5 ng/mL. Cross-reactivity of the monoclonal antibody, as determined by relative concentrations (ng/mL) of zearalenone analogues required for 50% inhibition of antibody binding in the ELISA, was as follows: zearalenone, 4.0; α-zearalenol, 3.6; β-zearalenol, 14.4; α-zearalanol, 11.5; β-zearalanol, 16.4. When the monoclonal antibody was applied to the competitive direct ELISA of zearalenone in spiked (50–500 μg/kg) corn, the average recovery was 110%. Mean interwell and interassay coefficients of variation for the spiked samples were 3.3 and 4.0%, respectively. It is anticipated that this monoclonal antibody will find widespread use in the rapid screening of zearalenone and α-zearalenol in agricultural commodities.

Zearalenone [6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid lactone] is a secondary metabolite produced by members of the genus *Fusarium* (Mirocha et al., 1977). Its natural occurrence in corn, wheat, barley, and animal rations has been documented (Mirocha et al., 1971). While the low order of toxicity of zearalenone is not a major concern, its ability to exert estrogenic effects on the mammalian reproductive system (Hurd, 1977; Mirocha and Christensen, 1974) suggests that there is a need for routine screening in commodities slated for human and animal consumption. Manifestations of zearalenone-induced hyperestrogenism in female swine include enlargement of the uterus and nipples, vulvar swelling, vaginal prolapse, and infertility. Testicular atrophy and mammary gland enlargement have been observed in male swine following exposure to the compound. Two analogues of zearalenone, α- and β-zearalenol, are also estrogenic and have been detected naturally (Hagler et al., 1979; Richardson et al., 1985) and as in vivo metabolites in bovine milk (Mirocha et al., 1981).

Current methods for analysis of zearalenone in foods and feeds include thin-layer chromatography (TLC) (AOAC, 1984; Gimeno, 1983; Scott et al., 1978; Swanson et al., 1984), gas–liquid chromatography (GLC) (Scott et al., 1978; Thouvenot and Morfin, 1979), and liquid chromatography (Bennett et al, 1985, James et al., 1982; Scott et al., 1978; Turner et al., 1983). These methods require extensive extraction and sample cleanup and therefore are not readily applicable to routine screening of large numbers of samples for zearalenone. Immunoassays have recently been described as alternative methods for detection of zearalenone (Liu et al, 1985; Thouvenot and Morfin, 1983; Warner et al., 1986) and other mycotoxins (Pestka et al, 1980, 1981a, 1981b, 1982). A radioimmunoassay (RIA), developed for detection of zearalenone in clinical samples, employs swine anti-zearalenone antiserum and has a detection limit of 0.25 ng/assay (Thouvenot and Morfin, 1983). Recently, our laboratory reported on the application of competitive enzyme-linked immunosorbent assays to detection of zearalenone in corn, wheat, and pig rations using polyclonal rabbit antiserum (Liu et al., 1985; Warner et al., 1986).

The potential exists for improvement of immunochemical detection methods for zearalenone and other agricultural contaminants through the use of hybridoma technology. We describe here for the first time the production of a sensitive monoclonal antibody with specificity for zearalnone and α-zearalenol and its application to the analysis of corn.

MATERIALS AND METHODS

Materials. All inorganic chemicals and organic solvents were of reagent grade or better. Sources: bovine serum albumin (BSA) (fatty acid free and fraction V), ovalbumin (OA) (crude and fraction VII), polyethylene sorbitan monolaurate (Tween 20), 2,2′-azinobis(3-ethylbenzthiazoline)sulfonic acid (ABTS), hydrogen peroxide, dicyclohexylcarbodiimide, *N*-hydroxysuccinimide, dimethylformamide (DMF), poly(ethylene glycol) (PEG) (MW 1450), insulin, oxaloacetate, hypoxanthine, aminopterin, thymidine, pristane, Sigma Chemical Co. (St. Louis, MO); tetrahydrofuran, Aldrich Chemical Co. (Milwaukee, WI); Freund's complete and incomplete adjuvants, Difco (Detroit, MI); goat anti-mouse IgG conjugated to horseradish peroxidase, Cooper Biomedical (Malvern, PA); Dulbecco's modified Eagle's medium (DMEM), penicillin/streptomycin solution (Pen/Strep) (100 000 U/mL), NCTC supplemental medium, fetal bovine serum (FBS), sodium pyruvate, Gibco Laboratories (Grand Island, NY); microculture plates (96- and 24-well plates), Costar (Cambridge, MA); microtiter plates, NUNC, Vangard International (Neptune, NJ). Zearalenone (Z), α-zearalenol, β-zearalenol, α-zearalanol, and β-zearalanol were generously supplied by International Minerals and Chemicals Corp. (Terre Haute, IN). Subclass identification kit was purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). The myeloma cell line P3/NS 1/1-Ag4-1 (NS-1) (ATCC TIB 18) was purchased from The American Type Culture Collection (Rockville, MD). Mice were purchased from Charles River Laboratories (Wilmington, MA).

Preparation of Conjugate Antigens. Zearalenone was conjugated to BSA (zearalenone–BSA) for use as immunogen and to OA (zearalenone–OA) for use as a solid-phase antigen in the competitive indirect ELISA (CI ELISA). Since zearalenone possesses no reactive groups for conju-

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gation, it was first converted to 6′-(carboxymethyl)zearalenone oxime (zearalenone oxime) by the method of Thouvenot and Morfin (1983) and then conjugated to BSA or OA (fraction VII) by the *N*-hydroxysuccinimide procedure of Kitagawa et al. (1981). The molar ratio of zearalenone conjugated to protein was determined spectrophotometrically, using an absorption maxima of 315 nm and an extinction coefficient of 4088. For each 1 mol of BSA and OA, 6 and 12.3 mol of zearalenone was conjugated to the two proteins, respectively. Zearalenone–BSA was lyophilized and stored at −80 °C, and zearalenone–OA was stored in small (0.5-mL) aliquots at −20 °C.

Immunization Protocol. Four BALB/c female mice, 8–10 weeks of age, weighing approximately 18 g, were given four intraperitoneal injections with zearalenone-BSA conjugate (250 μg/injection). The first injection consisted of 0.2 mL of conjugate in a 1:1 ratio of saline and Freund's complete adjuvant. The second injection (day 14) consisted of 0.2 mL of conjugate in saline and Freund's incomplete adjuvant (1:1). The third dose (day 28) was presented in saline (0.1 mL) alone. One week following the third injection, serum was collected from the retrobulbar plexus of each mouse and titers were determined by indirect ELISA. CI ELISA was also performed to determine which mouse produced the highest sensitivity antiserum. The spleen from the mouse with serum showing optimum relative inhibition was used for the subsequent fusion. Four weeks after the third injection and 4 days prior to fusion, this mouse was given a fourth injection of conjugate in saline (0.1 mL).

Titration of Antiserum by Indirect ELISA. One hundred (100) microliters of zearalenone–OA (10 μg/mL dissolved in 0.15 M carbonate–bicarbonate buffer, pH 9.6) was added to each well of a 96-well microtiter plate and incubated overnight at 4 °C. Unbound conjugate was removed from the plate with four washes (250 μL) of 0.1 M phosphate-buffered saline (PBS, pH 7.2) containing 0.20% Tween 20 (v/v) (PBS–Tween). Unbound solid-phase sites were blocked by incubation with 300 μL of 1% (w/v) crude OA in PBS to each well for 30 min at 37 °C. The plate was washed four more times with PBS–Tween, and duplicate aliquots of antiserum, serially diluted in PBS (100 μL), were added to the wells and incubated for 1 h at 37 °C. Duplicate wells of serially diluted preimmune serum were used as a control. Unbound antibody was removed by washing four times with PBS–Tween, and 100 μL of goat anti-mouse IgG peroxidase conjugate (diluted 1:500 in 1% OA–PBS) was added to each well. Following incubation for 30 min at 37 °C, the plate was washed eight times with PBS–Tween. Bound peroxidase was determined with ABTS substrate as described previously (Pestka et al., 1982). Absorbance was read at 405 nm, and the end point titer for each serum was arbitrarily designated as the maximum dilution that gave twice or greater the absorbance of the same dilution nonimmune control serum.

Competitive Indirect ELISA (CI ELISA). CI ELISA was used to (1) determine the sensitivity of anti-zearalenone antibodies in mouse sera produced during the course of immunization, (2) identify culture wells containing hybridomas secreting the desired antibody following fusion and cloning, and (3) determine the sensitivity and specificity of the monoclonal antibody secreted by the stabilized cell line. Briefly, microtiter plates were coated with solid-phase zearalenone–OA and blocked with OA as described in the indirect titration procedure. Next, 50 μL of zearalenone (or zearalenone analogue) dissolved in 1% (v/v) methanol in PBS (1% MeOH–PBS) was simultaneously incubated with 50 μL of antiserum (diluted 1:100 in PBS) or 50 μL of crude hybridoma culture supernatant over the zearalenone-OA solid phase for 1 h at 37 °C. Bound antibody was then determined by the addition of anti-mouse IgG peroxidase conjugate as described above.

To identify cultures containing anti-zearalenone antibody in fusion wells and cloning wells, 50-μL aliquots of 1% MeOH–PBS were added as zearalenone-free blanks to each of two zearalenone–OA-coated wells and 50 μL aliquots of zearalenone (1 μg/mL in 1% MeOH–PBS) were added to two other zearalenone–OA-coated wells. To each of these four wells were added 50-μL aliquots of a single culture supernatant. Following a 2-h incubation period at 37 °C, wells were washed four times and then incubated for 1 h with goat anti-mouse IgG peroxidase conjugate. The assay was then completed as described above.

Monoclonal Antibody Production. Spleen cells and myeloma cells (NS-1) were fused with PEG by the method of Siraganian et al. (1983). The fused cells were suspended in Dulbecco's modified Eagle's medium containing 20% fetal bovine serum (20% FBS–DMEM) and supplemented with 1% NCTC, 5 mM oxaloacetate, 10 mM sodium pyruvate, and insulin (75.5 mg/L). Pen/Strep solution (100 U/mL) was added to minimize bacterial contamination. A feeder layer of spleen cells (final concentration 1×10^5 cells/mL) was mixed with the fused cells (Galfre and Milstein, 1981), and the 60 innermost wells of each of 15 microculture plates were seeded with 0.2 mL of this mixture; the outer wells were filled with sterile medium. Plates were incubated at 37 °C in an atmosphere of 8% CO_2.

Twenty-four hours following fusion, half of the supernatant fraction from each well was removed by aspiration, and 0.1 mL of hypoxanthine–aminopterin–thymidine (HAT) medium was added (Littlefield, 1964). The HAT medium was changed every 3 days for a 14-day period. The cultures were then fed fresh medium for 1 week with hypoxanthine–thymidine (HT) medium after which 20% (v/v) FBS–DMEM was subsequently used. CI ELISA screening of supernatants was performed over a 1-week period from day 12 to day 18 following fusion.

Cells in fusion wells with supernatants showing at least 40% inhibition in CI ELISA were transferred from 96-well to 24-well plates. Those cultures that still retained activity were expanded and frozen in liquid nitrogen. The culture exhibiting the highest percentage of inhibition was cloned by limiting dilution (Goding, 1983), at three cells/well, for both clonings. The cells were suspended in and fed with 20% macrophage-conditioned medium (Sugasawara et al, 1985). Stabilized cell lines were obtained following the two clonings. The cell line that secreted the most senstive antibody was identified by CI ELISA, and the antibody specificity was characterized in CI ELISA with respect to zearalenone analogues. The subclass of secreted antibody was identified with a kit according to the manufacturer's (Boehringer Mannheim) instructions. The 2G3-6E3-2E2 hybridoma was grown as ascites tumor in histocompatible mice injected 10 days previously with pristane (Galfre and Milstein, 1981).

Spiking and Extraction of Corn Samples for ELISA. Zearalenone-free homogeneous ground corn samples were provided by G. Bennett, Northern Regional Research Center (Peoria, IL). For spiking studies, zearalenone was dissolved in methanol (100 μg/mL) and was added to 10 g of ground corn to give final concentrations of 0, 50, 250, and 500 μg/kg. The corn was shaken in a stoppered bottle and the methanol allowed to evaporate overnight in a fume hood. The entire sample was shaken with methanol–water at a volume ratio of 70:30 for 5 min. Corn was filtered

Table I. Percent Inhibition of Mouse Antibody Binding to Zearalenone Solid Phase following Three Intraperitoneal Injections of Zearalenone-BSA as Determined by a Competitive Indirect ELISA

mouse no.	ng zearalenone/mL				
	500	50	5	0.5	0.05
1	76.1	62.3	25.2	7.8	5.5
2	86.5	73.9	24.9	7.0	5.6
3	71.4	67.7	57.1	19.0	9.9
4	78.5	55.0	32.5	19.1	4.3

through Whatman No. 4 filter paper and the filtrate used for ELISA analysis.

Competitive Direct ELISA (CD ELISA). The 2G3-6E3-2E2 ascites fluid was purified by 35% ammonium sulfate precipitation (Hebert et al., 1973) and used for CD ELISA of spiked corn extracts. CD ELISA was essentially the same as that described by Warner et al. (1986) except that (1) a 1:1600 dilution of monoclonal antibody (10 mg/mL) was used to coat the microtiter wells, (2) enzyme incubation times were reduced from 60 to 10 min, and (3) substrate incubation times were reduced from 30 to 10 min.

RESULTS AND DISCUSSION

Mouse Immunization. To determine whether the immunization protocol adequately stimulated plasma cell secretion of zearalenone-specific serum antibody, titers from each of four mice were performed 1 week following the third antigen injection; end point iters for all sera were 1:6400. CI ELISA was performed to verify whether the color development was due to a specific reaction involving anti-zearalenone antibodies. Zearalenone competed effectively with the solid phase for antibody binding in each of the sera (Table I), and the spleen from mouse 3 was subsequently used for fusion to the myeloma cells.

Hybridoma Preparation. Since the spleen from the mouse contained 3×10^8 cells, a "double fusion" was performed whereby 2×10^7 myeloma cells were fused with 2×10^8 spleen cells. The fusion was highly successful, and 100% fusion efficiency (number of wells with growing colonies/number of wells seeded) was obtained. All supernatant fractions from 878 wells were screened for desired antibody activity, and of these, 38 showed 40% or more inhibition when the antibody was incubated with 1 μg/mL zearalenone. When antibody activity was further tested in 24-well cultures following scale-up from the 96-well cultures, only four were found to have retained antibody activity. These cultures, identified as 1D6, 1F10, 2G3, and 7F11, were expanded and frozen in liquid nitrogen.

Cells from 2G3, which were cloned at 3 cells/well, yielded 44 positive subclones, which again showed at least 40% inhibition when culture supernatants were incubated with free zearalenone (1.0 μg/mL). Attempts to clone 2G3 at 1 or 2 cells/well were unsuccessful, so sequential clonings were carried out at 3 cells/well. Three subclones of 2G3 were recloned by limiting dilution. Only about 30% of the wells seeded with cells showed growth in the cloning plates. If cells are grown in small numbers, the fraction of wells with growth should follow the Poisson distribution (Lefkovits and Waldmann, 1979). To obtain a reasonable probability that wells with growth contain single clones, more than 37% of wells should have no growth. Thus, the percentage of wells that contained cells in both clonings strongly suggests that a true clone was isolated that secreted homogeneous antibodies.

Forty-five stabilized cell lines were obtained, of which four were subclones of 2G3-5D4, two were subclones of 2G3-5D6, and the rest were subclones of 2G3-6E3. Supernatant collected from 2G3-6E3-2E2 exhibited the highest percentage of inhibition (89%) in CI ELISA.

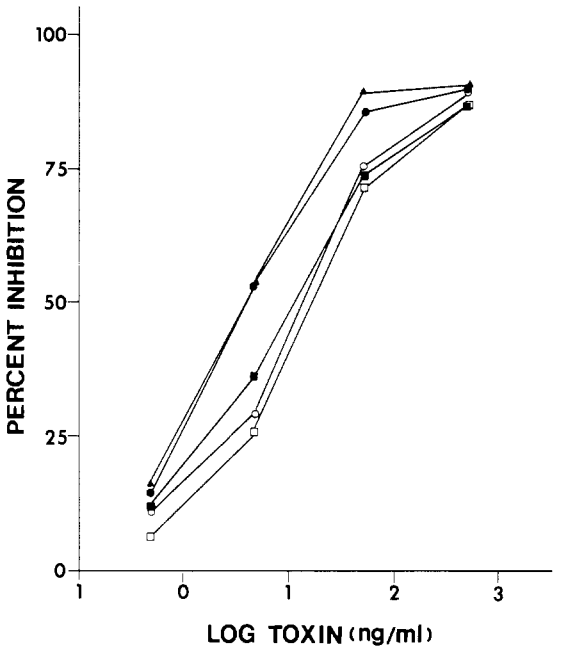

Figure 1. Specificity of 2G3-6E3-2EZ monoclonal antibody in CI ELISA. Concentration of zearalenone and analogues required to inhibit 50% of antibody binding: (●) zearalenone, 4.0 ng/mL; (▲) α-zearalenol, 3.6 ng/mL; (○) β-zearalenol, 14.4 ng/mL; (■) α-zearalanol, 11.5 ng/mL; (□) β-zearalanol, 16.4 ng/mL.

Table II. Comparison of Cross-Reactivities among Various Anti-Zearalenone Antibodies toward Zearalenone Analogues

analogue	% cross-reactivity[a]			
	monoclonal[b]	rabbit[c]	pig[d]	pig[e]
zearalenone	100	100	100	100
α-zearalenol	107	50	33	100
β-zearalenol	29	12	25	44
α-zearalanol	35	6	6	53
β-zearalanol	25	3	10	44

[a] Cross-reactivity defined as (zearalenone concn for 50% inhibn)/(zearalenone analogue concn for 50% inhibn) × 100. [b] Described in this paper (indirect ELISA). [c] Liu et al. (1985) (indirect ELISA). [d] Pestka et al. (1985) (indirect ELISA). [e] Thouvenot and Morfin (1983) (RIA).

Monoclonal Antibody Characterization. The subclass of 2G3-6E3-2E2 was identified as IgG_1, and the light chains were identified as κ chains. Sensitivity and specificity of the antibody were determined at the same time in a CI ELISA. The limit of detection was determined to be 0.5 ng/mL or 25 pg/assay (Figure 1), lower than previously reported immunoassays for zearalenone. Liu et al. (1985) determined that the limit of detection for the CI ELISA with rabbit antiserum was 5 ng/mL or 0.25 ng/assay whereas the limit of detection for the same assay employing porcine antiserum was 10 ng/mL or 0.5 ng/assay (Pestka et al., 1985). A 0.25 ng/assay detection limit for zearalenone was previously found for RIA employing porcine anti-zearalenone antiserum (Thouvenot and Morfin, 1983).

With concentrations of zearalenone and zearalenone analogues required to inhibit 50% of the binding of the monoclonal antibody in the CI ELISA (Figure 1) as a basis for comparison, the relative cross-reactivities for zearalenone, α-zearalenol, β-zearalenol, α-zearalanol, and β-zearalanol were determined (Table II). The antibody apparently reacted as well with α-zearalenol as zearalenone

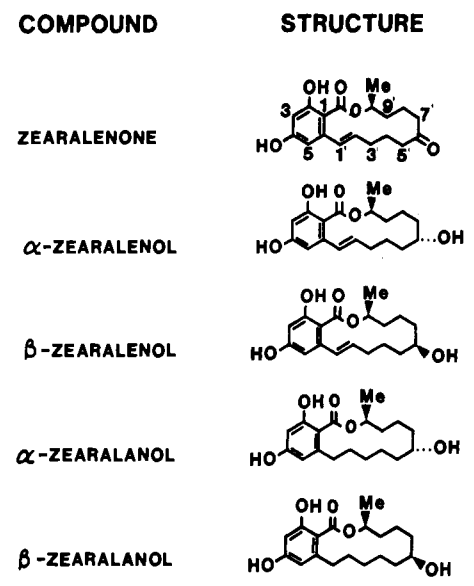

Figure 2. Structures of zearalenone and analogues tested for cross-reactivity.

but reacted to a lesser extent with β-zearalenol and the zearalanols, suggesting that the C6′ position and double bond at the C1′–C2′ position were predominant in determining the specificity of the monoclonal antibody. It is interesting to note that the pig antiserum employed in the RIA (Thouvenot and Morfin, 1983) shows a nearly identical spectrum of cross-reactivity as the monoclonal antibody (Table II). In contrast, rabbit and pig antiserum (Table II) in the CI ELISA show a similar ability to discriminate the double bond at the C1′–C2′ position but lesser cross-reactivity with the β configuration (Figure 2). Since α- and β-zearalenol are major animal metabolites of zearalenone (Mirocha et al., 1981; Ueno and Tashiro, 1981) and they can occur naturally (Hagler et al, 1979; Richardson et al., 1985), the ability of the monoclonal antibody to react strongly with these metabolites is particularly advantageous when detection of these analogues is desirable.

CD ELISA of Spiked Corn. CD ELISA results obtained on zearalenone-spiked corn are summarized in Table III. Competition by methanol–water extracts of spiked corn was compared directly to standard competition curves in extractant for zearalenone quantitation. Recoveries for samples containing 0, 50, 250, and 500 μg/kg of zearalenone were 106, 112, and 113, respectively. Mean recovery was 110%, and mean interwell coefficient of variation was 3.3%. The mean interassay coefficient of variation was 4.0%.

CD ELISAs of zearalenone in corn conducted with monoclonal antibody revealed a much lower degree of variability than was found previously with polyclonal antibodies (Warner et al., 1986). Both the monoclonal assay described here and the polyclonal CD ELISA conducted by Warner et al. (1986) obtained recoveries in excess of 100%. Overestimation of zearalenone may be the result of partial interference by the sample corn extract. Nevertheless, the CD ELISA provides a simple quantitative method for the rapid (20-min) screening of zearalenone in corn and potentially other cereal grains at levels that cause hyperestrogenism. Samples containing as little as 50 μg/kg zearalenone caused a visually distinct inhibition of absorbance in the final enzyme assay.

Conclusion. Hybridoma-based immunoassays offer simple alternatives for the routine detection of low molecular weight chemical contaminants in agricultural materials. Monoclonal antibodies have been produced against benzo[a]pyrene (Wallin et al., 1984) and various steroids (Eshhar et al., 1979; Fantl et al., 1981; Fantl et al., 1982; Kohen et al., 1982). Monoclonal antibodies have recently been produced to several mycotoxins, only some of which actually exhibited improved sensitivity and specificity in immunoassays when compared to polyclonal sera (Gendloff et al., 1986; Hunter et al., 1985; Woychik et al., 1984). The present paper is the first published report of a monoclonal antibody to zearalenone, a potential hazard to animal and human health, and further demonstrates that antibodies with enhanced specificity and sensitivity for haptens can be produced using hybridoma technology. The 2G3-6E3-2E2 hybridoma line offers a continuing supply of antibody with high sensitivity and specificity for zearalenone and its analogues that should be readily applicable to the screening of cereal grains by competitive direct ELISA as demonstrated here.

Table III. Recovery of Zearalenone from Spiked Corn by Competitive Direct Monoclonal ELISA

zearalenone added, μg/kg	sample[a]	recovery[b] μg/kg	recovery[b] %	interwell CV,[c,d] %
0	1	ND[e]		
0	2	ND		
0	3	ND		
50	1	54.5 ± 9	109	3.8
50	2	49.4 ± 5	99	2.4
50	3	54.5 ± 11	109	5.2
250	1	292 ± 13	117	1.7
250	2	267 ± 20	107	2.9
250	3	279 ± 29	112	4.2
500	1	579 ± 43	116	4.2
500	2	553 ± 34	111	3.6
500	3	606 ± 22	112	2

[a] Each sample was spiked separately and then extracted and assayed in replicates of four. [b] Interassay coefficient of variation (n = 3) for 50, 250, and 500 μg/kg were 4.5, 3.7, and 3.7%, respectively. Mean interassay coefficient of variation was 4%. [c] Coefficient of variation. [d] Mean interwell CV was 3.33%. [e] None detected.

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Registry No. Zearalenone, 17924-92-4; α-zearalenol, 36455-72-8; β-zearalenol, 71030-11-0; α-zearalanol, 26538-44-3; β-zearalanol, 42422-68-4.

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Effect of Milling on Decontamination of *Fusarium* Mycotoxins Nivalenol, Deoxynivalenol, and Zearalenone in Korean Wheat

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Samples of wheat naturally contaminated with *Fusarium* mycotoxins were obtained from fields and mills in Korea and were milled by the Bühler test mill and an industrial-scale mill, respectively. Each of the milling fractions was analyzed for nivalenol (NIV) and deoxynivalenol (DON) by gas chromatography with an electron capture detector and for ZEN by high-performance liquid chromatography with a fluorescence detector. NIV, DON, and ZEN were found throughout all fractions, but ZEN was not detected in break and reduction flour fractions in the industrial mill. The highest concentration of NIV was found in the bran, and DON and ZEN were in the shorts. The lowest concentration of NIV was found in the reduction flour, and DON and ZEN were in the break flour. Milling was not effective in removing NIV, DON, and ZEN from the naturally contaminated wheat, but the effect on its concentration in the samples varied.

The determination of concentrations of *Fusarium* mycotoxins such as nivalenol (NIV), deoxynivalenol (vomitoxin, DON), and zearalenone (F-2 toxin, ZEN) in cereals has been carried out recently in several countries with varied results. Accumulated data have revealed DON to be the major toxicant in scabby grains in the United States (Hagler et al., 1984), Canada (Scott, 1984), England (Osborne and Willis, 1984), Austria (Vesonder and Ciegler, 1979), and South Africa (Marasas et al., 1979), while both NIV and DON were detected from cereal products in Japan (Tanaka et al., 1985c), China (Ueno et al., 1986), USSR (Ueno et al., 1986), and West Germany (Blaas et al., 1984). Regarding the occurrence of ZEN, this mycotoxin has been widely detected throughout the world in cereals, mixed feeds, and other products (Mirocha et al., 1977). *Fusarium graminearum* is the major causative fungus of NIV, DON, and ZEN contamination of grains (Ichinoe et al., 1983; Neish and Cohen, 1981). Young et al. (1984) reported that the concentration of DON was reduced during the milling for industrially milled and pilot-milled wheat. Others have also reported various concentration of DON in different milling fractions (Seitz et al., 1985; Abbas et al., 1985; Scott

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