# Two Formats of Enzyme Immunoassay for 15-Acetyldeoxynivalenol Applied to Wheat

Ewald Usleber,*,† Elisabeth Schneider,† Erwin Märtlbauer,‡ and Gerhard Terplan†

Institute for Hygiene and Technology of Food of Animal Origin, Veterinary Faculty, University of Munich, 8000 Munich 22, Germany, and R-Biopharm GmbH, Rösslerstrasse 94, 6100 Darmstadt, Germany

Polyclonal antibodies against 15-acetyldeoxynivalenol (15-AcDON) were raised in rabbits after immunization with a 3-hemiglutaryl-15-AcDON (15-AcDON-HG) derivative coupled to human serum albumin. Using these antibodies and a 15-AcDON-HG–horseradish peroxidase conjugate in direct enzyme immunoassay (EIA), a microtiter plate EIA and a membrane-based dipstick EIA were established, with detection limits for 15-AcDON of 0.35 and 5 ng/mL, respectively. When specificity was checked on the basis of the 50% inhibition levels, relative cross-reactivities with 15-AcDON, diacetyl-DON, triacetyl-DON, 3-AcDON, and DON were found to be 100%, 602%, 0.85%, 0.18%, and 0.01%, respectively. Employing a simple extraction procedure, both test formats were applied to wheat. The detection limits for 15-AcDON in artificially contaminated wheat samples were at 50–100 ng/g; the recoveries for 15-AcDON in a concentration range from 50 to 600 ng/g were between 140.9% and 94.4%.

## INTRODUCTION

Among the trichothecene mycotoxins, the deoxynivalenol-type [DON (Yoshizawa and Morooka, 1973); vomitoxin (Vesonder et al., 1973)] toxins are most frequently found as contaminants on cereals (Tanaka et al., 1988). Typical adverse effects of DON (Figure 1) are feed refusal, lowered weight gain, and vomiting in animals, especially in swine (Ueno, 1983); immunodepressive effects have also been described (Tryphonas et al., 1986; Minervini et al., 1993). A monoacetyl analogue of DON, 15-acetyldeoxynivalenol [15-acetoxy-3,7-dihydroxy-12,13-epoxytrichothec-9-en-8-one (15-AcDON)], first detected in North American *Fusarium graminearum* isolates and characterized by Miller et al. (1983), has been found to occur naturally as a cocontaminant together with DON in "refusal factor corn" (Abbas et al., 1986). Although studies on 8-keto-trichothecenes hitherto focused mainly on DON and 3-AcDON (an isomer of 15-AcDON), the production of 15-AcDON by several *F. graminearum* and *Fusarium roseum* isolates (Pestka et al., 1985; Bennett et al., 1988; Sugiura et al., 1990) is well described. Incidence was found that DON-chemotype *Fusarium* strains from North America (Abbas et al., 1988; Mirocha et al., 1989; Miller et al., 1991) and from New Zealand (Lauren et al., 1992) produce 15-AcDON rather than 3-AcDON.

Observed adverse effects in mice after dietary exposure to 15-AcDON indicate that, compared to DON, 15-AcDON is only slightly less toxic *in vivo* (Pestka et al., 1986; Forsell et al., 1987; Pestka et al., 1987), although its *in vitro* toxicity is considerably lower (Forsell and Pestka, 1985). This is in agreement with the findings of Ohta et al. (1978), who reported rapid hydrolysis of acetylated DONs and other trichothecenes to their parent alcohols by microsomal nonspecific carboxyesterase from rabbit and rat livers. Thus, a contamination of cereals with 15-AcDON should be taken into consideration when the amount of DON in a diet does not account for the observed toxic effects, as described, e.g., by Foster et al. (1986).

In addition to physicochemical approaches [for reviews see, e.g., Scott (1982) and Cole (1986)], the use of immunochemical methods is of rapidly increasing importance for the analysis of trichothecenes and other mycotoxins [for reviews see, e.g., Morgan and Lee (1990), Chu (1992), and Scott (1993)]. A major advantage of the enzyme immunoassay (EIA) techniques is their potential as rapid screening methods. A range of enzyme immunoassays (EIA) for DON (Casale et al., 1988; Mills et al., 1990), 3-AcDON (Kemp et al., 1986), or multiacetyl derivatives (Xu et al., 1988; Ikebuchi et al., 1990) has been developed, but until now no EIA using antibodies against 15-AcDON has been described. Recently we produced antibodies against DON which showed high reactivity toward 15-AcDON (Usleber et al., 1991) when used in an EIA, but this test could not differentiate between the individual toxins. In this paper we describe the development of a sensitive EIA for 15-AcDON and its application to wheat. The assay was performed as a "conventional" microtiter plate system as well as a more rapid dipstick EIA, following procedures described by Schneider et al. (1991). The specificity of the assay was determined using a series of trichothecenes, including synthetic acetyl derivatives of DON.

## EXPERIMENTAL PROCEDURES

**Materials.** Nivalenol and fusarenon X were purchased from Waco Chemicals, Neuss, Germany. DON, 3-AcDON, 15-AcDON, and all other trichothecenes were obtained from Sigma Chemicals, Deisenhofen, Germany. Acetic anhydride, glutaric anhydride, human serum albumin (HSA), horseradish peroxidase (HRP), 3,3′,5,5′-tetramethylbenzidine (TMB), and all other chemicals (highest grade available) were also obtained from Sigma. Microtitration plates (Nunc immunoplate I) were purchased from Nunc, Wiesbaden, Germany. The dipstick membrane (Immunodyne immunoaffinity membrane) was obtained from Pall Filtrationstechnik, Dreieich, Germany.

**Preparation of Toxin Derivatives.** 15-AcDON (10 mg) was reacted with glutaric anhydride (100 mg) in pyridine (1 mL) at 100 °C for 2 h. The pyridine was removed under nitrogen flow and the residue dissolved in chloroform (15 mL) and extracted

* Address correspondence to this author at Lehrstuhl für Hygiene und Technologie der Milch, Universität München, Schellingstr. 10, 80799 München, Germany (telephone +49 89 21802256; fax +49 89 21802106).

† Institute for Hygiene and Technology of Food of Animal Origin.

‡ R-Biopharm GmbH.

0021-8561/93/1441-2019$04.00/0 

with 0.01 mol/l HCl (3 × 10 mL ). The chloroform was removed in a rotary evaporator and the remainder redissolved in methanol (2 mL). After characterization of the toxin derivative, distilled water (6 mL) was added and then the solution was lyophilized.

Diacetyldeoxynivalenol (DiacDON) and triacetyldeoxynivalenol (TriacDON) were prepared by reaction of DON (10 mg) with acetic anhydride (100 μL) in pyridine (1 mL) for 2 h at room temperature. The pyridine was evaporated under nitrogen and the remainder redissolved in 0.5 mL of methanol. DiacDON and TriacDON were isolated by isocratic reversed-phase high-performance liquid chromatography (HPLC; Table I). The UV absorbance was continuously monitored in a range from 190 to 370 nm by employing a Model 2140 photodiode array detector (LKB, Bromma, Sweden) and "wavescan EG" software (LKB). Fractions of 0.5 min were collected using a 201 fraction collector (Gilson, Villiers Le Bel, France).

The toxin derivatives were further checked by high-performance thin-layer chromatography (HPTLC) using Kieselgel 60 $F_{254}$ plates (Merck 5628) and solvent systems as listed in Table I. Toxin spots were detected under a UV lamp (254 nm), and then the plates were sprayed with a solution of 20% $AlCl_3$ in ethanol/water (50/50) and heated at 120 °C for 15 min. Toxins and derivatives (except for TriacDON) were visualized as blue fluorescent spots under UV light (366 nm). The concentration of the derivatives (in methanol) was estimated from the relative fluorescence of the spots compared to toxin standards and from the UV absorbance (in methanol) at 222 (15-AcDON-3-hemiglutarate), 223 (DiacDON), and 225 nm (TriacDON). The latter calculation was based on the assumption that the toxin derivatives had molar extinction coefficients of $\epsilon = 6000$, a value derived from literature data for other 8-ketotrichothecenes (Cole and Cox, 1981; Bennett and Shotwell, 1990).

**Conjugation of 15-AcDON-3-Hemiglutarate to Human Serum Albumin and Horseradish Peroxidase.** The immunogen and the enzyme conjugate were prepared following a scheme that employs a mixed anhydride method for the immunogen synthesis (Erlanger, 1980), and an activated ester method for the preparation of the enzyme conjugate (Kitagawa et al., 1981). For conjugation of the 15-AcDON-HG derivative to HSA, 3 mg of the derivative [in 1 mL of dimethylformamide (DMF)] were mixed with 5 μL isobutylchloroformate and 5 μL triethylamine at −5 °C for 15 min. Then the mixture was added to 6 mg of HSA (in 1.5 mL of 0.13 M $NaHCO_3$), and stirred at 4 °C. The pH of the reaction mixture was frequently controlled and adjusted to approximately 9 with 0.1 M NaOH (40 μL). After a reaction time of 4 h, the 15-AcDON–HSA conjugate was dialyzed against 3 × 5 L of phosphate-buffered saline (PBS, 0.15 M, pH 7.2) and stored lyophilized at −20 °C.

A 15-AcDON–HRP conjugate was prepared by reacting 2 mg of 15-AcDON-HG, 3.4 mg of *N*-hydroxysuccinimide, and 12 mg of dicyclohexylcarbodiimide in 300 μL of DMF at room temperature for 16 h. This intermediate was added to 24 mg of HRP (in 4 mL of 0.13 M $NaHCO_3$) and reacted for 2 h at room temperature, and then the conjugate was dialyzed against PBS (3 × 5 L). The peroxidase concentration of the conjugate, as determined photometrically at 403 nm, was 4.8 mg/mL. The 15-AcDON–HRP was stored lyophilized at −20 °C.

**Immunization.** For use as immunogen, 250 μg of 15-AcDON–HSA were emulsified in 2 mL of Freund's complete adjuvant/distilled water (3:1 by volume). Four rabbits (female chinchilla bastard) were immunized by using multisite intradermal injections. Booster injections, using the same composition and amount of immunogen, were given subcutaneously 10 and 24 weeks after the primary injection. Blood was collected from the A. auricularis magna. After centrifugation, the serum was precipitated with $(NH_4)_2SO_4$ (70% saturated), dialyzed against PBS (3 × 5 L), and stored frozen at −20 °C. The relative antibody titer was determined as described by Märtlbauer et al. (1988). Titer was defined as the antiserum dilution which gave 0.5 absorbance unit (concentration of 15-AcDON-HRP 500 ng/mL).

**Microtiter Plate EIA.** An appropriate dilution of the antiserum against 15-AcDON (1:2500) in 0.05 M carbonate–bicarbonate buffer (pH 9.6) was dispensed to the wells (100 μL) of a microtitration plate and incubated overnight at room temperature. Free protein-binding sites of the plate were blocked with 200 μL of sodium caseinate solution (2%, in PBS) for 30 min, and then the plate was washed and made semidry. To each well were added 50 μL of 15-AcDON toxin standard in PBS containing 10% methanol and 50 μL of 15-AcDON–HRP (48 ng/mL 1% sodium caseinate/PBS), and the plate was incubated for 2 h at room temperature. The plate was washed, and substrate solution (1 mM TMB and 3 mM $H_2O_2$ per liter of potassium citrate buffer, pH 3.9) was added (100 μL/well). After 15 min, the reaction was stopped with 1 M $H_2SO_4$ (100 μL/well), and the absorbance at 450 nm was measured (400 AT microplate reader, SLT, Crailsheim, Germany). To determine the test specificity, 21 structurally similiar trichothecenes (Table II) were tested for competition with the 15-AcDON–HRP under the conditions of the EIA. The relative cross-reactivity of each toxin was calculated on the basis of the concentration necessary to inhibit 50% binding of the 15-AcDON–HRP.

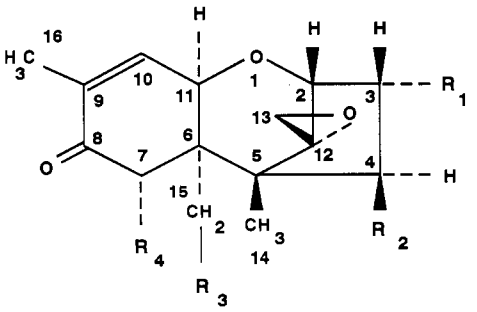
**Dipstick EIA.** The preparation of the dipsticks and the test protocol of the dipstick EIA for 15-AcDON (Figure 2) were essentially the same as described for other mycotoxins (Schneider et al., 1991). In brief, the protein-binding nylon membrane (1 × 1 cm) was mounted to a plastic support (1 × 5 cm). An area of about 4 mm in diameter of the membrane was coated with 2 μL of the antiserum against 15-AcDON (diluted 1:30 with PBS). Free protein-binding sites of the membrane were blocked by incubation in PBS containing 2% sodium caseinate for 15 min. Then the dipstick was dried (37 °C), ready for assay. For the detection of 15-AcDON by dipstick EIA, 1 mL of 15-AcDON standard or sample solution (in PBS containing 30% methanol) and 10 μL of 15-AcDON–HRP solution (1:100 in PBS) were mixed in a test tube. A dipstick was incubated in this mixture for 30 min at room temperature. Then the dipstick was washed with PBS and finally incubated in 1 mL of substrate solution for 3 min. The dot color intensity of the dipstick was compared to that of a negative control dipstick. The toxin concentrations that led to color intensity clearly distinguishable from the negative control by six test persons was set as the detection limit.

**Analysis of 15-AcDON in Wheat.** Portions of ground wheat (2 g) were artificially contaminated with a methanolic solution of 15-AcDON (20–30 μL). The toxin was extracted with 8 mL of methanol for 30 min, and then the solvent was filtered through a filter paper. For the dipstick test, 333 μL of the extract was mixed with 666 μL of PBS and assayed directly. For the microtiter plate assay, the extract was adjusted to a methanol content of 10% with PBS and then further diluted in PBS containing 10% methanol. The recovery of 15-AcDON was checked in a concentration range from 50 to 600 ng/g.

## RESULTS AND DISCUSSION

Through derivatization of 15-AcDON with glutaric anhydride, an additional side-chain ester was introduced into the toxin molecule. As a result of this modification, the haptenic properties of the toxin as present in the immunogen should be very similiar to that of diacetyl-DON. To further examine the specificity of the 15-AcDON assay, di- and triacetyl derivatives of DON (which were not commercially available) had to be prepared.

According to Greenhalgh et al. (1984), acetylation of DON with acetic anhydride and pyridine at room temperature yields mixtures of di- and triacetyl derivatives. This is in agreement with the observation that the hydroxyl group at R4 (Figure 1) in the DON molecule is less reactive than the hydroxyls at R1 and R3 (Blackwell et al., 1984; Usleber et al., 1991). In our experiments, two reaction products with retention times of 8.0 (DiacDON) and 11.1 min (TriacDON) were isolated by HPLC (Table I) in a ratio of about 70:30. The concentrations were estimated from TLC and from the UV absorbance (in MeOH) at 223 and 225 nm, assuming from the literature (Cole and Cox, 1981; Bennett and Shotwell, 1990) that molar extinction coefficients of ca. 6000 would be reasonable. By this calculation, a total amount of acetyl derivatives of approximately 8.5–9.0 mg was obtained from 10 mg of DON. The results obtained by HPLC and HPTLC for DiacDON



| Substance | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| DON | OH | H | OH | OH |
| 3-AcDON | Ac | H | OH | OH |
| 15-AcDON | OH | H | Ac | OH |
| DiacDON | Ac | H | Ac | OH |
| TriacDON | Ac | H | Ac | Ac |
| 15-AcDON-HG | HG | H | Ac | OH |
| Nivalenol | OH | OH | OH | OH |
| Fusarenon X | OH | Ac | OH | OH |

Ac = $O\text{-}\overset{O}{\overset{\|}{C}}\text{-}CH_3$ HG = $O\text{-}\overset{O}{\overset{\|}{C}}\text{-}(CH_2)_3\text{-}\overset{O}{\overset{\|}{C}}\text{-}OH$

**Figure 1.** Structure and side-chain residues of deoxynivalenol (DON) and several other 8-ketotrichothecenes.

**Table I. TLC, HPLC, and UV Characteristics of Deoxynivalenol (DON) and Several Derivatives (Figure 1)**

| compd | TLC $R_f$ value in mobile phase[a] A | B | C | D | reaction with $AlCl_3$ | HPLC retention time,[b] min | $A_{max}$ in MeOH, nm |
|---|---|---|---|---|---|---|---|
| DON | 0.11 | 0.05 | 0.36 | 0.32 | yes | 3.1 | 218 |
| 15-AcDON | 0.39 | nd[c] | 0.57 | 0.52 | yes | 3.9 | 220 |
| 3-AcDON | 0.46 | 0.31 | 0.72 | nd | yes | 4.2 | 220 |
| DiacDON | 0.86 | 0.81 | 0.83 | nd | yes | 8.0 | 223 |
| TriacDON | 0.91 | 0.86 | 0.87 | nd | no | 11.1 | 225 |
| 3-hemiglutaryl-15-AcDON | nd | nd | 0.77 | 0.16 | yes | nd | 222 |

[a] A, $CHCl_3$/MeOH 98/2; B, $CHCl_3$/acetone 95/5; C, ethyl acetate/*n*-hexane/acetic acid 75/25/5; D, ethyl acetate/*n*-hexane 75/25. [b] Column, Lichrospher RP 8 (125 × 4 nm, Merck); eluent, acetonitrile/water (40/60, 1 mL/min). [c] nd, not determined.

and TriacDON were in agreement with the expected polarities. Blue fluorescence under UV light (366 nm) after HPTLC plates were sprayed with $AlCl_3$ indicated that the hydroxyl at R4 and the keto group at C8 both were intact in the diacetyl derivative. Due to the esterification at R4, TriacDON gave a negative response. These results strongly suggest that the two compounds are 3,-15-diacetyldeoxynivalenol and 3,7,15-triacetyldeoxynivalenol.

As determined by HPTLC, derivatization of 15-AcDON with glutaric anhydride yielded a single reaction product; only trace amounts of unreacted 15-AcDON were detectable. The derivative showed blue fluorescence after treatment with $AlCl_3$. Marked changes of the $R_f$ values for the hemiglutaryl derivative relative to that of 15-AcDON, using neutral and acidic solvents (Table I), indicate the presence of a carboxyl group in the derivative. The absorbance maximum (in MeOH) of the derivative was at 222 nm. These results demonstrate that a 3-hemiglutaryl derivative of 15-AcDON has been formed.

Antibodies could be detected in the sera of all rabbits immunized with the 15-AcDON–HSA conjugate as early as 4 weeks after the initial exposure. High relative serum titers (> 1:1000000) were obtained from three rabbits after the first booster. The fourth animal had comparatively low titers but still in a range of about 1:100000. The serum that showed the highest affinity for 15-AcDON was used for the establishment of the immunoassay.

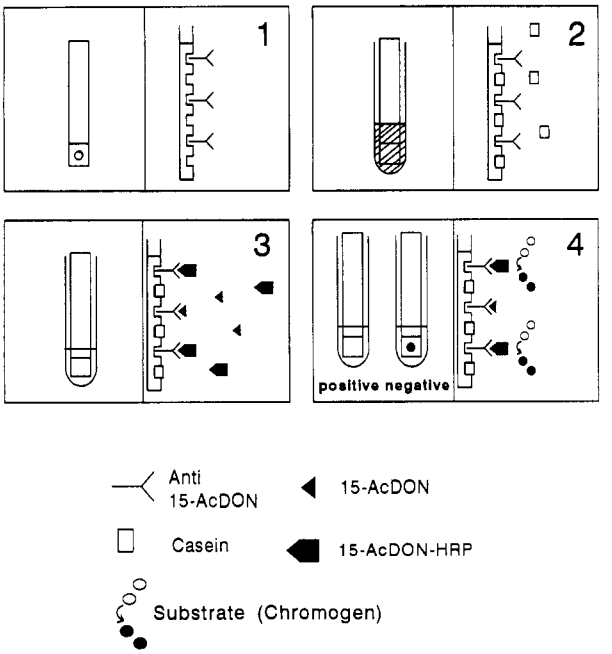



**Figure 2.** Schematic representation of the dipstick EIA preparation (1, 2) and the assay performance (3, 4). (1) Coating of the membrane with antibodies against 15-AcDON; (2) blocking of free protein binding sites with casein/PBS solution; (3) competitive binding of free 15-AcDON in sample extract and added 15-AcDON–HRP; (4) color reaction in substrate solution ($H_2O_2$ + TMB).

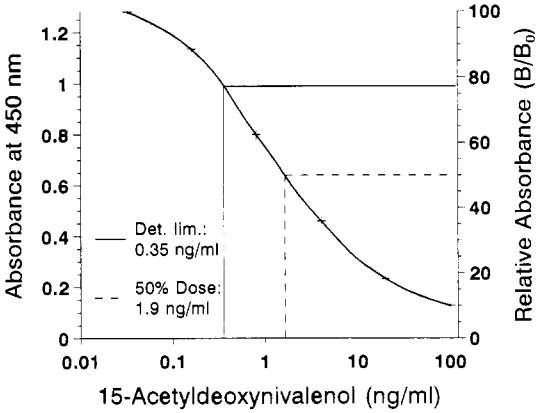



**Figure 3.** Standard curve of the microtiter plate EIA for the detection of 15-acetyldeoxynivalenol. The *x*-axis (log scale) indicates the toxin concentration. The corresponding absorbance values at 450 nm are plotted on the left *y*-axis. The right *y*-axis indicates the relative binding ($B/B_0$), expressed as (absorbance units corresponding to a standard toxin concentration/absorbance of toxin negative buffer solution) × 100. Each point represents the mean of four replicates (coefficients of variation were between 2.5% and 4.6%). The detection limit and the 50% dose are given as described in the text.

Using the microtiter plate format, the intraassay (fourfold determinations) and interassay coefficients of variation were usually below 6.5% and 12%, respectively. The detection limit for 15-AcDON in buffer solution was at 350 pg/mL, as determined by Student's *t*-test (onesided, $n = 4$, 95% confidence limit). A typical standard curve for the detection of 15-AcDON by microtiter plate EIA is given in Figure 3.

**Table II. Cross-Reactivity of the Antiserum against 15-Acetyldeoxynivalenol (15-AcDON) to Various Trichothecenes**

| toxin | MW | 50% inhibition dose ng/mL | 50% inhibition dose nmol/mL | cross-reactivity,[a] % |
|---|---|---|---|---|
| 15-AcDON | 338 | 1.89 | 0.0056 | 100 |
| DON | 296 | 14130 | 47.74 | 0.01 |
| 3-AcDON | 338 | 1020 | 3.02 | 0.18 |
| 3,15-DiacDON[b] | 380 | 0.35 | 0.00093 | 602 |
| 3,7,15-TriacDON[b] | 422 | 279.7 | 0.66 | 0.85 |
| nivalenol (NIV) | 312 | –[c] | – | – |
| 4-AcNIV (fusarenon X) | 354 | – | – | – |

[a] [(nmol/mL 15-AcDON)/(nmol/mL toxin)] × 100. [b] Prepared as described in the text. [c] –, no reactivity at a concentration of 100 μg/mL; other toxins tested but showing no reactivity at this level were T-2 toxin, HT-2, Iso T-2, acetyl T-2, T-2 triol, T-2 tetraol tetracetate, verrucarol, 4,15-diacetylverrucarol, scirpentriol, 15-acetoxyscirpenol, diacetoxyscirpenol (DAS), 3-acetyl-DAS, neosolaniol, trichothecin, and trichothecolone.

The lower sensitivity of a competitive immunoassay using visual evaluation is mainly a result of the high antibody density at the membrane dot, required for sufficient color development of the negative control. The detection limit for 15-AcDON using the dipstick EIA was more than 1 order of magnitude higher, at a toxin concentration of ca. 5 ng/mL. At this toxin level, the dot color development on the dipstick membrane was sufficiently reduced to be scored positive by all test persons when compared to the negative control. Toxin levels of about 20–25 ng/mL resulted in a complete suppression of the dot color development.

The cross-reactivities of the assay with other trichothecenes, as determined by microtiter plate EIA, are summarized in Table II. It is interesting to note that DiacDON inhibited antibody binding to 15-AcDON-HRP at a 6-fold lower concentration than 15-AcDON, whereas the TriacDON derivative had a relative cross-reactivity of less than 1%. High concentrations of 3-AcDON and DON in the microgram per milliliter range were required to reach 50% inhibition. These findings indicate the following degree of importance for recognition by the antibodies (in decreasing order): acetyl side chain at R1 > acetyl side chain at R3 > intact hydroxyl function at R4. However, trichothecenes lacking the 8-keto function (e.g., T-2 toxins, scirpenols) or having a bulkier substituent at R2 (e.g., nivalenol, fusarenon X), showed no reactivity at levels as high as 100 μg/mL. In the dipstick assay, the same order of cross-reactivity was observed.

Due to the high sensitivity of both test formats, low levels of 15-AcDON could be detected in wheat using dilutions of the raw extracts, without any cleanup steps necessary. To check the influence of sample matrices, serial dilutions of toxin-negative wheat extracts had been assayed. For the microtiter plate test, extracts equivalent to 10 mg/mL of wheat gave absorbance values of about 90–100% of the negative control buffer solution. When dilutions corresponding to 3 mg/mL or less of wheat were assayed, practically no matrix effects on the absorbance were measured. The recoveries obtained from samples spiked with 15-AcDON are listed in Table III. The detection limit for 15-AcDON in wheat was at 50–100 ng/g; toxin concentrations higher than 200 ng/g could be determined quantitatively. This is in about the same range as found earlier for a 3-AcDON assay (Usleber et al., 1992).

Compared to the microtiter plate EIA, the dipstick EIA was considerably less subject to matrix interferences and methanol content of the extract. For blank wheat samples, an extract dilution of 1:3 (with PBS) was sufficient to give dot color intensities virtually identical with that obtained for the buffer control solution. Therefore, despite the lower test sensitivity of this membrane-based assay, the detection limit for 15-AcDON by dipstick EIA (50–100 ng/g) was in the same range as that of the microtiter plate system. Toxin concentrations higher than 500 ng/g resulted in a complete suppression of the dot color development. Including the time necessary for the extraction procedure, the total assay time was still less than 2 h.

**Table III. Recovery of 15-Acetyldeoxynivalenol from Artificially Contaminated Wheat Samples (Microtiter Plate EIA)**

| toxin added, ng/g | toxin found av, ng/g | SD, ng/g | coeff of variation, % | recovery, % | n |
|---|---|---|---|---|---|
| 50 | 70.4 | 10.75 | 15.27 | 140.9 | 4 |
| 100 | 119.9 | 15.83 | 13.21 | 119.8 | 6 |
| 200 | 199.4 | 11.91 | 5.97 | 99.7 | 6 |
| 300 | 287.9 | 25.69 | 8.92 | 96.0 | 6 |
| 400 | 371.7 | 26.16 | 7.04 | 92.9 | 4 |
| 600 | 566.4 | 64.35 | 11.36 | 94.4 | 4 |

In conclusion, we have developed two immunochemical test formats for 15-AcDON that are both sensitive and easy to perform. The dipstick assay could be used as a rapid qualitative screening test for this toxin in wheat, especially if the number of samples is small (<10). If large numbers of wheat samples have to be analyzed, the microtiter plate assay offers several advantages, including quantitative determination of the toxin content and automatization.

ACKNOWLEDGMENT

We thank M. Djeffal, M. Lorber, and M. Straka for excellent technical assistance.

LITERATURE CITED

Abbas, H. K.; Mirocha, C. J.; Tuite, J. Natural Occurrence of Deoxynivalenol, 15-Acetyldeoxynivalenol, and Zearalenone in Refusal Factor Corn Stored Since 1972. *J. Appl. Environ. Microbiol.* **1986**, *51*, 841–843.

Abbas, H. K.; Mirocha, C. J.; Meronuck, R. A.; Pokorny, J. D.; Gould, S. L.; Kommedahl, T. Mycotoxins and *Fusarium* spp. Associated with Infected Ears in Corn in Minnesota. *J. Appl. Environ. Microbiol.* **1988**, *54*, 1930–1933.

Bennett, G. A.; Shotwell, O. L. Criteria for Determining Purity of *Fusarium* Mycotoxins. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 270–275.

Bennett, G. A.; Wicklow, D. T.; Caldwell, R. W.; Smalley, E. B. Distribution of Trichothecenes and Zearalenone in *Fusarium graminearum*: Rotted Corn Ears Grown in a Controlled Environment. *J. Agric. Food Chem.* **1988**, *36*, 639–642.

Blackwell, B. A.; Greenhalgh, R.; Bain, A. D. Carbon-13 and Proton Nuclear Magnetic Assignments of Deoxynivalenol and Other Mycotoxins from *Fusarium graminearum*. *J. Agric. Food Chem.* **1984**, *32*, 1078–1083.

Casale, W. L.; Pestka, J. J.; Hart, L. P. Enzyme-Linked Immunosorbent Assay Employing Monoclonal Antibody Specific for Deoxynivalenol (Vomitoxin) and Several Analogues. *J. Agric. Food Chem.* **1988**, *36*, 663–668.

Chu, F. S. Recent Progress on Analytical Techniques for Mycotoxins in Feedstuffs. *J. Anim. Sci.* **1992**, *70*, 3950–3963.

Cole, R. J., Ed. *Modern Methods in the Analysis and Structural Elucidation of Mycotoxins*; Academic Press: Orlando, FL, 1986.

Cole, R. J., Cox, R. J., Eds. *Handbook of Toxic Fungal Metabolites*; Academic Press: New York, 1981.

Erlanger, B. F. The preparation of antigenic hapten-carrier conjugates: a survey. *Methods Enzymol.* **1980**, *70*, 85–103.

Forsell, J. H.; Pestka, J. J. Relation of 8-Ketotrichothecene and Zearalenone Analog Structure to Inhibition of Mitogen-Induced Human Lymphocyte Blastogenesis. *J. Appl. Environ. Microbiol.* **1985**, *50*, 1304–1307.

Forsell, J. H.; Jensen, R.; Tai, J. H.; Witt, M.; Lin, W. S.; Pestka, J. J. Comparison of Acute Toxicities of Deoxynivalenol (Vomitoxin) and 15-Acetyl-Deoxynivalenol in the B6C3F1 Mouse. *Food Chem. Toxicol.* **1987**, *25*, 155–162.

Foster, B. C.; Trenholm, H. L.; Elliot, J. I.; Thompson, B. K.; Hartin, K. E. Evaluation of Different Sources of Deoxynivalenol (Vomitoxin) Fed to Swine. *Can. J. Anim. Sci.* **1986**, *66*, 1149–1154.

Greenhalgh, R.; Gilbert, J.; King, R. R.; Blackwell, B. A.; Startin, J. R.; Sheperd, M. J. Synthesis, Characterization, and Occurrence in Bread and Cereal Products of an Isomer of 4-Deoxynivalenol (Vomitoxin). *J. Agric. Food Chem.* **1984**, *32*, 1416–1420.

Ikebuchi, H.; Teshima, R.; Hirai, K.; Sato, M.; Ichinoe, M.; Terao, T. Production and Characterization of Monoclonal Antibodies to Nivalenol Tetraacetate and their Application to Enzyme-Linked Immunoassay of Nivalenol. *Biol. Chem. Hoppe-Seyler* **1990**, *371*, 31–36.

Kemp, H. A.; Mills, E. N. C.; Morgan, M. R. A. Enzyme-Linked Immunosorbent Assay Applied to Rice. *J. Sci. Food Agric.* **1986**, *37*, 888–894.

Kitagawa, T.; Shimozono, T.; Aikawa, T.; Yoshida, T.; Nishimura, H. Preparation and Characterization of Hetero-Bifunctional Crosslinking Reagents for Protein Modifications. *Chem. Pharm. Bull.* **1981**, *29*, 1130–1135.

Lauren, D. R.; Sayer, S. T.; diMenna, M. E. Trichothecene Production by *Fusarium* Species Isolated from Grain and Pasture Troughout New Zealand. *Mycopathologia* **1992**, *120*, 167–176.

Märtlbauer, E.; Gareis, M.; Terplan, G. Enzyme Immunoassay for the Macrocyclic Trichothecene Roridin A: Production, Properties, and Use of Rabbit Antibodies. *J. Appl. Environ. Microbiol.* **1988**, *54*, 225–230.

Miller, J. D.; Taylor, A.; Greenhalgh, R. Production of Deoxynivalenol and Related Compounds in Liquid Culture by *Fusarium graminearum*. *Can. J. Microbiol.* **1983**, *29*, 1171–1178.

Miller, J. D.; Greenhalgh, R.; Wang, Y.; Lu, M. Trichothecene Chemotypes of Three *Fusarium* Species. *Mycologia* **1991**, *83*, 121–130.

Mills, E. N. C.; Alcock, S. M.; Lee, H. A.; Morgan, M. R. A. An Enzyme-Linked Immunosorbent Assay for Deoxynivalenol in Wheat, Utilizing Novel Hapten Derivatization Procedures. *Food Agric. Immunol.* **1990**, *2*, 109–118.

Minervini, F.; Dong, W.; Pestka, J. J. *In vitro* Vomitoxin Exposure Alters IgA and IgM secretion by CH12LX B Cells. Relationship to Prolifertion and Macromolecular Synthesis. *Mycopathologia* **1993**, *121*, 33–40.

Mirocha, C. J.; Abbas, H. K.; Windels, C. E.; Xie, W. Variation in Deoxynivalenol, 15-Acetyldeoxynivalenol, 3-Acetyldeoxynivalenol, and Zearalenone Production by *Fusarium graminearum* Isolates. *J. Appl. Environ. Microbiol.* **1989**, *55*, 1315–1316.

Morgan, M. R. A.; Lee, H. A. Mycotoxins and Natural Food Toxicants. In *Development and Application of Immunoassays for Food Analysis*; Rittenburg, J. H., Ed.; Elsevier Applied Science: London, 1990; Chapter 6.

Ohta, M.; Matsumoto, H.; Ishii, K.; Ueno, Y. Metabolism of Trichothecene Mycotoxins. II. Substrate Specifity of Microsomal Deacetylation of Trichothecenes. *J. Biochem.* **1978**, *84*, 697–706.

Pestka, J. J.; El-Bahrawy, A.; Hart, P. L. Deoxynivalenol and 15-Monoacetyl Deoxynivalenol Production by *Fusarium graminearum* R6576 in Liquid Media. *Mycopathologia* **1985**, *91*, 23–28.

Pestka, J. J.; Lin, W. S.; Forsell, J. H. Decreased Feed Consumption and Body-Weight Gains in the B6C3F$_1$ Mouse after Dietary Exposure to 15-Acetyldeoxynivalenol. *Food Chem. Toxicol.* **1986**, *24*, 1309–1313.

Pestka, J. J.; Lin, W.-S.; Miller, E. R. Emetic Activity of the Trichothecene 15-Acetyldeoxynivalenol in Swine. *Food Chem Toxicol.* **1987**, *25*, 855–858.

Schneider, E.; Dietrich, R.; Märtlbauer, E., Usleber, E.; Terplan, G. Detection of Aflatoxins, Trichothecenes, Ochratoxin A, and Zearalenone by Test Strip Enzyme Immunoassay: a Rapid Method for Screening Cereals for Mycotoxins. *Food Agric. Immunol.* **1991**, *3*, 185–193.

Scott, P. M. Assessment of Quantitative Methods for Determination of Trichothecenes in Grains and Grain Products. *J. Assoc. Off. Anal. Chem.* **1982**, *65*, 867–883.

Scott, P. M. General Referee Report on Mycotoxins. *J. AOAC Int.* **1993**, *76*, 112–119.

Sugiura, Y.; Watanabe, Y.; Tanaka, T.; Yamamoto, S.; Ueno, Y. Occurrence of *Gibberella zeae* Strains that Produce Both Nivalenol and Deoxynivalenol. *J. Appl. Environ. Microbiol.* **1990**, *56*, 3047–3051.

Tanaka, T., Hasegawa, A.; Yamamoto, S.; Lee, U.-S.; Sugiura, Y.; Ueno, Y. Worldwide Contamination of Cereals by the *Fusarium* Mycotoxins Nivalenol, Deoxynivalenol, and Zearalenone. 1: Survey of 19 Countries. *J. Agric. Food Chem.* **1988**, *36*, 979–983.

Tryphonas, H.; Iverson, F.; So, Y.; Nera, E. A.; McGuire, P. F.; O'Grady, L.; Clayson, D. B.; Scott, P. M. Effects of Deoxynivalenol (Vomitoxin) on the Humoral and Cellular Immunity of Mice. *Toxicol. Lett.* **1986**, *30*, 137–150.

Ueno, Y., Ed. *Trichothecenes—Chemical, Biological and Toxicological Aspects*; Elsevier Science Publishing: New York, 1983.

Usleber, E.; Märtlbauer, E.; Dietrich, R.; Terplan, G. Direct Enzyme-Linked Immunosorbent Assay for the Detection of the 8-Ketotrichothecene Mycotoxins Deoxynivalenol, 3-Acetyldeoxynivalenol, and 15-Acetyldeoxynivalenol in Buffer Solutions. *J. Agric. Food Chem.* **1991**, *39*, 2091–2095.

Usleber, E.; Renz, V.; Märtlbauer, E.; Terplan, G. Studies on the Application of Enzyme Immunoassays for the *Fusarium* Mycotoxins Deoxynivalenol, 3-Acetyldeoxynivalenol, and Zearalenone. *J. Vet. Med. B* **1992**, *39*, 617–627.

Vesonder, R. F.; Ciegler, A.; Jensen, A. H. Isolation of the Emetic Principle from *Fusarium*-Infected Corn. *J. Appl. Microbiol.* **1973**, *26*, 1008–1010.

Xu, Y.-C.; Zhang, G. S.; Chu, F. S. Enzyme-Linked Immunosorbent Assay for Deoxynivalenol in Corn and Wheat. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 945–949.

Yoshizawa, T.; Morooka, N. Deoxynivalenol and its Monoacetate: New Mycotoxins from *Fusarium roseum* and Mouldy Barley. *Agric. Biol. Chem.* **1973**, *37*, 2933–2934.

Received for review April 20, 1993. Accepted July 26, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, October 1, 1993.