Tissue Distribution of Deoxynivalenol in Swine Dosed Intravenously†

Dan B. Prelusky* and H. Locksley Trenholm

Animal Research Centre, Central Experimental Farm, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6

Following a single iv administration (1 mg/kg) of the mycotoxin deoxynivalenol (DON) to swine, tissue concentrations were monitored during the 24-h period postdosing. DON was detected in all tissues examined at the first sampling time (0.33 h); maximum concentrations were measured at 0.33–1.0 h, except with plasma, liver, and probably lung, which had evidently peaked earlier. The highest levels were detected in plasma, kidney, and liver (1–2 μg/g of wet tissue weight), followed by fat, lymph, lung, and adrenals (200–500 ng/g). Other tissues measured had substantially lower concentrations (20–165 ng/g; spleen, testes, brain, heart, muscle, skin, intestine, pancreas). The study showed a rapid distribution of DON to tissues and body fluids, with subsequent decline to trace or negligible levels by 24 h, except urine and bile, where elevated DON concentrations were observed throughout the study. The overall distribution profile indicates no extensive uptake or retention by any tissues occurred, suggesting that accumulation of residues in swine would not happen upon prolonged consumption of DON-contaminated feed at low levels.

INTRODUCTION

Infection of cereal crops, and consequently animal feedstuffs, by fungi capable of producing toxic secondary metabolites (mycotoxins) is a worldwide problem which is increasingly evident as monitoring/analytical procedures improve. Feeding contaminated grains can not only result in severe financial losses for producers, but there is increasing concern for human health over exposure to potentially toxic residues transmitted into animal-derived foods (Hayes, 1980; Wyatt, 1986; Wilson, 1978; Berry, 1988; Bullerman, 1986).

Grains contaminated with *Fusarium graminearum* may contain deoxynivalenol (DON; vomitoxin), one of the more routinely detected toxins, particularly in countries with a temperate climate (Trenholm et al., 1983; Coté et al., 1984; Ueno, 1986). DON produces a variety of well-documented symptoms, both subtle and overt, depending on the species exposed. Pigs, pigeons, and man seem to show the greatest sensitivity to this toxin (Forsyth et al., 1977; Trenholm et al., 1986; Ueno, 1977).

Most previous studies have shown no or only trace amounts (low parts per billion) of DON-derived residues being transmitted into animal products such as dairy milk (Prelusky et al., 1984) and poultry meats and eggs (Prelusky et al., 1987, 1989; El-Banna et al., 1983; Kubena et al., 1985). Coté et al. (1986), however, did detect significant levels (micrograms per milliliter) of the de-epoxide metabolite DOM-1 in milk of dairy cows following consumption of gram quantities of the toxin. With ruminants and poultry though, DON has been shown to be poorly absorbed, extensively metabolized, and rapidly cleared from biological tissues and fluids. In comparison, in pigs DON is efficiently absorbed, poorly metabolized, and eliminated at a comparatively slower rate than in these other farm species (Coppock et al., 1985; Prelusky et al., 1988). Studies also indicate that DON undergoes extensive tissue distribution in swine; consequently, prolonged ingestion of DON-contaminated feeds could result in the accumulation of residues in certain tissues.

The objective of the present study was to determine the pattern of DON distributions in individual tissues of swine during the 24-h period following a single iv dose of the toxin and examine the likelihood of residues accumulating in tissues possibly intended for human consumption.

EXPERIMENTAL PROCEDURES

Chemicals. Deoxynivalenol (>96% pure) was provided by Dr. J. D. Miller, Plant Research Centre, Agriculture Canada, Ottawa, Canada. The chemical purity was checked by reversed-phase high-pressure liquid chromatography. All solvents were distilled in glass and purchased from Anachemia. Aluminum oxide, neutral, was purchased from E. Merck, charcoal (Darco G 60) from J. T. Baker Chemical Co., and T-61 from Hoechst Canada Inc.

Animals. Twenty-five healthy Yorkshire barrows bred at the Animal Research Centre farm under pathogen-free conditions and of approximately 11–15 weeks of age (17–22 kg) were used. For the experiment, animals were moved into three group pens and allowed a short acclimatization period (24 h). Prior to dosing, pigs were fasted for 4 h; water was provided ad libitum throughout the study.

Animals (20) were given a single intravenous dose of 1.0 mg of DON/kg of body weight via the ear vein; five other pigs (controls) received the vehicle only (0.3 mL of 20% ethanol/water). For the collection of tissue samples for residue analysis, a group of five animals (four dosed; one control) was sacrificed by ear vein administration of T-61 (1.0 mL/10 kg) at each time of 0.33, 1, 3, 8, and 24 h postdosing. Tissues removed for analysis included blood, liver, kidney, heart, brain, spleen, muscle, fat (abdominal and back), lung, intestine, testes, adrenals, lymph, pancreas, and skin. Bile and urine were also collected at these times. All samples were frozen (−10 °C) until analyzed.

Analytical Methods. Preparation of samples for analysis of DON by HPLC/MS was carried out according to a method published previously, with some modification (Trenholm et al., 1985). Tissues (8 g) were thoroughly homogenized in 16 mL of water (1 part tissue to 2 parts water) by using a Teflon pestle. In instances when only smaller amounts of tissues were available, solvent volumes were used proportionately. To the homogenate was added 32 mL of acetonitrile (4 parts), and the mixture was blended in a high-speed blender for 5 min. Samples were then centrifuged (10 min at 2000*g*), and 5.6-mL aliquots of the supernatant, equivalent to 1.0 g of tissue, were used in the cleanup procedure.

Aliquot samples (5.6 mL), run in duplicate, were added to an alumina–charcoal column prepared and prewashed previously (Trenholm et al., 1985) and eluted with 10 mL of acetonitrile/

† Contribution No. 1692 ARC. Supported in part by the Ontario Ministry of Agriculture and Food (Project OP 1001) and the Ontario Pork Producers Marketing Board.

0021-8561/91/1439-0748$02.50/0 Published 1991 by the American Chemical Society

Table I. Recovery of Deoxynivalenol (DON) from Spiked Pig Tissues (100 ng/g) Prepared for HPLC/MS Analysis

	% DON recovery[a]		% DON recovery[a]
control (no tissue)	97.6 ± 1.9	liver	94.0 ± 3.9
plasma	92.8 ± 2.8	heart	89.7 ± 3.8
spleen	85.0 ± 4.2	brain	81.3 ± 2.1
pancreas	84.1 ± 5.5	testes	87.7 ± 4.1
muscle	86.7 ± 2.5	adrenals	82.5 ± 5.3
fat	79.8 ± 4.1	bile	81.3 ± 7.3
lung	89.6 ± 3.5	skin	81.8 ± 6.0
kidney	88.3 ± 4.7	urine	93.8 ± 2.1
intestine	84.4 ± 5.9		

[a] $\bar{x}$ ± SD, n = 5.

water (21:4), under vacuum. The eluent (~15 mL) was collected in a round-bottom test tube and evaporated to dryness (N_2, 50 °C). The resulting residue was redissolved in 200 μL of methanol/water (3:2) and transferred to 250-μL autosampler vials for analysis. One hundred microliters of sample, equivalent to 0.5 g of tissue, was injected into the LC/MS system.

Chromatographic equipment consisted of a Hewlett-Packard Model 1090 HPLC connected to a 25 cm × 4.6 mm i.d. column packed with reversed-phase C_{18} 5-μm particle size ODS-2 Spherisorb (Chromatography Sciences Co.). The mobile phase was methanol/water (40:60 v/v) containing 0.05 M ammonium acetate, at a flow rate of 1.0 mL/min. The LC system was connected to a HP Thermospray interface on a Model 5890 quadruple mass spectrometer. Operational parameters were as follows: vaporizer (tip) temperature was 195 ± 2 °C; ion source was maintained at 260 °C; analyzer manifold pressure remained at 2.3 × 10^{-6} Torr at a 1 mL/min solvent flow rate; electron energy was 155 + 800 eV; and the filament was "on" for electron-assisted ionization. For detection of DON, the MS was run in a selective ion monitoring (SIM) mode in which ions m/z 297 (M + 1) and 314 (M + NH_4) were observed.

Spiking of Samples. For recovery experiments, 8-g tissue samples obtained from control (nontreated) pigs not used in the current study were homogenized with 2 parts water and spiked with 0.8 μg of DON (in 100 μL of 20% methanol) to obtain a level of 100 ng/g of wet tissue. The spiked homogenates were then processed through the extraction/cleanup procedure detailed above. Analytical recoveries were calculated by comparing area under curve (AUC) values of extracted DON samples to those obtained following HPLC/MS detection of known standard amounts.

RESULTS

The recovery of DON from the various tissues ranged from 78 to 92%, at a spiked concentration of 100 ng/g (Table I). Recovery from control samples (no tissue) was near quantitative (97.6%). The extraction/cleanup method used in this study was efficient, and in combination with LC/SIM-MS, the minimal detection limit was calculated to be about 1 ng of DON/g of tissue (0.5 ng of DON injected on-column) at a signal-to-noise ratio of 3 (S/N = 3). Marginally higher limits (30–50 ng/g) obtained by this or similar column extraction procedures have been reported for grain samples, using UV detection for analysis (Lauren and Greenhalgh, 1978; Romer, 1986; Chang et al., 1984). The benefit of SIM-MS is that with samples which may contain more interferences from endogenous components being carried over (i.e., biological tissues) representative fragmentation ions can usually be successfully resolved amid all the background signals. This is particularly true for trichothecene structured toxins which tend to have a low UV absorbance. The advantage of better resolution is that smaller sample sizes (1–2 g of material) can be utilized and still achieve acceptable peak/noise (S/N) ratios for the compounds of interest.

The distribution of DON in the blood and tissues is summarized in Table II. Since little or no metabolism of DON in swine has been evident in previous studies, potential metabolites (i.e., DOM-1, conjugates) were not currently analyzed for (Prelusky et al., 1988; Friend et al., 1986). Measurable amounts of DON were detected in all tissues of the dosed group at the first sampling time (0.33 h). No residues were detected in tissues from control pigs at any time. The plasma profile displayed a rapid decrease in DON levels with time, as was to be expected following intravenous administration of the toxin and as reported previously (Coppock et al., 1985; Prelusky et al., 1988). Elimination half-life ($t_{1/2\beta}$), based on a limited number of points, was 3.90 h. Trace levels of the toxin (17.6 ± 6.6 ng/mL) were still detectable 24 h postdosing. In most tissues, residue concentrations peaked/plateaued between the 0.3- and 1.0-h sampling times. The exception appeared to be liver, and possibly lung, where DON levels had declined considerably by 1 h, suggesting peak levels had occurred at 0.33 h or earlier.

Kidney contained the highest level of DON, followed by liver, fat (abdominal and back), lymph, lung, adrenals, spleen, and testes (Table II). Lesser amounts were measured in brain, heart, muscle, and skin, and residues in the pancreas and intestine were the lowest detected. Subsequent decreases in DON levels were rapid in most tissues, measuring one-fourth to half peak levels by 3 h postdosing. Slightly different rates of clearance ($t_{1/2}$) from tissues resulted in a somewhat greater variation of tissue concentrations at later time points, but there was no evidence of significant accumulation or persistence observed for any tissues analyzed. Elimination rates of DON in individual tissues, presented as half-life of the toxin ($t_{1/2}$), are provided in Table II. Clearance appeared to occur fastest from the pancreas and slowest from back fat. DON peaked in the urine at approximately 1 h and in the bile between 3 and 8 h, and while declining rapidly thereafter, there were still significant levels measured in both biological fluids at 24 h postdosing.

DISCUSSION

Results indicate that DON is rapidly distributed to all tissues following intravenous administration to swine. The highest DON concentrations were measured in the kidney and liver (and correspondingly in urine and bile), which was probably associated with their role in the elimination of xenobiotics. Prelusky et al. (1988) observed that the greater proportion (>85%) of iv-dosed DON was excreted in the urine, with little evidence of metabolism, whereas Coppock et al. (1985) indicated that a high percentage of iv-administered DON that could not be accounted for in urine (43–72%) was possibly eliminated by extrarenal routes (hepatic, metabolic). The presence of DON in the bile confirms that some toxin does undergo hepatic excretion.

By 24 h it appeared most of the administered toxin had cleared from the body, although trace amounts of DON were measured in some tissues, typically those with the highest initial concentrations such as liver and kidney, and in back fat, where clearance of residues appeared marginally slower relative to that in the other tissues (Table II). DON was also detected in abdominal fat, lung, and lymph, but levels measured were near the detection limit (~1 ng/g of tissue). The presence of DON residues in animal tissues has been reported elsewhere. Twenty-four hours following an iv dose of 0.5 mg of DON/kg to swine, Coppock et al. (1985) could find low levels (24 ng/g) only in the kidney. At 96 h post oral administration of ^{14}C-labeled DON (10 mg/kg) to rats, Lake et al. (1987) detected radioactivity in all tissues tested, equivalent to approx-

Table II. Distribution of Deoxynivalenol (DON) in Tissues following Intravenous Administration to Swine (1.0 mg of DON/kg)

	ng of DON/g of tissue[b] ± SD					$t_{1/2}$,[a] h
	0.33 h	1 h	3 h	8 h	24 h	
plasma	1337.3 ± 84.2	1044.3 ± 303.9	551.3 ± 161.6	155.5 ± 86.1	17.8 ± 6.6	3.90
kidney	1675.8 ± 391.9	1985.3 ± 725.2	930.5 ± 363.1	330.8 ± 160.7	10.0 ± 4.2	3.15
liver	1114.3 ± 153.9	735.3 ± 219.0	439.3 ± 99.2	207.3 ± 79.7	8.2 ± 5.5	3.48
abdominal fat	419.7 ± 127.1	491.6 ± 146.6	327.1 ± 228.7	78.0 ± 49.8	3.4 ± 3.4	3.30
back fat	224.7 ± 79.5	294.7 ± 95.9	133.7 ± 28.0	86.6 ± 24.2	12.4 ± 5.1	5.50
lymph	273.6 ± 142.8	292.3 ± 59.7	141.4 ± 80.9	48.7 ± 31.2	0.8 ± 1.0	2.77
lung	265.9 ± 58.7	203.2 ± 55.1	77.5 ± 14.5	35.3 ± 8.5	1.0 ± 1.2	3.04
adrenals	242.2 ± 142.0	237.0 ± 111.5	68.9 ± 67.2	22.1 ± 21.7	nd[c]	1.82
spleen	165.0 ± 32.6	148.9 ± 42.4	73.8 ± 26.2	9.6 ± 3.3	nd	1.82
testes	121.3 ± 33.8	123.8 ± 43.8	54.4 ± 16.2	15.2 ± 4.8	nd	2.47
brain	54.9 ± 17.3	51.5 ± 18.6	28.9 ± 7.3	15.6 ± 6.6	nd	4.70
heart	31.4 ± 10.6	48.0 ± 22.8	11.1 ± 4.9	2.0 ± 0.8	nd	1.75
muscle	32.1 ± 11.7	33.1 ± 8.5	19.1 ± 3.0	4.6 ± 2.4	nd	2.62
skin	31.0 ± 20.6	28.8 ± 13.5	16.1 ± 8.7	4.9 ± 3.4	nd	2.83
intestine	20.4 ± 5.8	17.5 ± 6.0	5.1 ± 3.8	1.6 ± 2.1	nd	2.07
pancreas	17.6 ± 8.0	18.7 ± 17.5	4.0 ± 2.6	nd	nd	1.16
urine	87892.5 ± 23311.4	139885.0 ± 136966.1	45750.0 ± 38993.5	15740.0 ± 12840.7	477.5 ± 510.6	
bile	1900.0 ± 589.7	5480.0 ± 2957.2	10935.0 ± 369.6	10962.5 ± 5527.8	1283.0 ± 678.5	

[a] Elimination half-lives of DON in tissues; estimations based on a limited number of data points. [b] $x \pm SD, n = 4$. [c] nd, not detected. Minimum detection limit 1 ng/g of tissue.

imately 18–69 ng/g of tissue (not including GIT), except for adrenals, which contained a much higher 481 ng equiv/g. While it is always precarious to compare species, it is of interest to note that following oral administration of [^{14}C]DON to laying hens (~1.4 mg/kg), tissues (GIT excluded) where residues persisted most were in the order of kidney, liver, and fat (Prelusky et al., 1986). This finding is similar to the current study.

Somewhat unexpected were the relatively high DON levels measured in the fat samples (back and abdominal). The physical characteristics of the compound would not indicate an affinity to fat. While DON is considered only marginally water soluble (Bamburg and Strong, 1971; Pohland et al., 1984) (~0.25 g/mL; Prelusky, unpublished results), it also has a very low water–lipid partition ratio (P, water/organic: ethyl acetate, 0.475; diethyl ether, 0.135; chloroform, 0.086; pentane, 0.049; hexane, 0.011; Prelusky, unpublished results). Of further interest was how quickly maximum DON concentrations were reached, especially considering the poor rate of blood perfusion of fat. With all other factors remaining equal, well-perfused tissues should take up residues much more rapidly than poorly perfused tissues, yet DON levels in fat peaked at approximately the same time as in the other tissues (except liver and lung). While there seems to be the potential for fat to behave as a storage depot for DON by virtue of its large volume (15.5% body weight), this does not occur to any considerable extent. Fat did account for a high proportion (about one-fifth) of the total residues estimated to be in the major organs (on the basis of organ weights as percent of body weight; data not shown), but even at peak levels (0.33–1.0 h) this amount was still only about 4% (35–45 μg of DON in fat/kg of body weight) of the administered dose.

It appears accumulation in tissues may be precluded by an initial rapid elimination phase. Prelusky et al. (1988) reported that up to 40% of an iv dose could be excreted within 1 h, and 75% within 3 h, depending on the individual animal. Furthermore, limited tissue uptake of DON, followed by its slow release from the more poorly perfused tissues, would explain earlier observations in which a very high proportion of DON was excreted within the initial 6 h (80–95%) but that trace DON levels were still detected in blood, urine, and bile for at least 24 h postdosing (Prelusky et al., 1988).

In studies by both Coppock et al. (1985) and Prelusky et al. (1988), it was shown that DON administered to swine has an apparent volume of distribution (V_d) of 1.02–1.62 L/kg, indicating that the toxin could be sequestered to specific tissues to account for its rapid disappearance from plasma. Consequently, there were concerns that swine consuming DON-contaminated feed over a prolonged period could accumulate residues in tissues, which could then be introduced into the human food chain. The current study, however, does not support tissue accumulation of DON. While several tissues do show comparatively higher DON concentrations, these elevated levels are transient and cannot account for the dose administered. It would appear that the high V_d is a combination of extensive distribution within the body, a significant first-pass elimination effect (urine, bile) as suggested by data in earlier studies (Coppock et al., 1985; Prelusky et al., 1988), and the rapid although limited and temporary sequestration of residues into certain tissues.

ACKNOWLEDGMENT

We express our sincere appreciation to Nicholas Zabolotny for preparation of the samples and to K. E. Hartin and staff, L. Robinson, J. Shackelton, and F. Fisher, for the animal work involved.

LITERATURE CITED

Bamburg, J. R.; Strong, F. M. 12,13-Epoxytrichothecenes. In *Microbial Toxins: Algal and Fungal Toxins*; Kadis, S., Ciegler, A., Ajl, S. J., Eds.; Academic Press: New York, 1971; Vol. VII, pp 207–292.

Berry, C. L. The pathology of mycotoxins. *J. Pathol.* **1988**, *154*, 301.

Bullerman, L. B. Mycotoxins and food safety. *Food Technol.* **1986**, *40*, 59.

Chang, H. L.; DeVries, J. W.; Larson, P. A.; Patel, H. H. Rapid determination of deoxynivalenol (vomitoxin) by liquid chromatography using Romer column cleanup. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 52.

Coppock, R. W.; Swanson, S. P.; Gelberg, H. B.; Koritz, G. D.; Hoffman, W. E.; Buck, W. B.; Vesonder, B. S. Preliminary study of the pharmacokinetic and toxicopathy of deoxynivalenol (vomitoxin) in swine. *Am. J. Vet. Res.* **1985**, *46*, 169.

Coté, L.-M.; Reynolds, J. D.; Vesonder, R. F.; Buck, W. B.; Swanson, S. P.; Coffey, R. T.; Brown, D. C. Survey of vomitoxin-contaminated feed grains in midwestern United States and associated health problems in swine. *J. Am. Vet. Med. Assoc.* **1984**, *184*, 189.

Coté, L.-M.; Dahlem, A. M.; Yoshizawa, T.; Swanson, S. P.; Buck, W. B. Excretion of deoxynivalenol and its metabolite, DOM-1, in milk, urine and feces of lactating dairy cattle. *J. Dairy Sci.* **1986**, *69*, 2416.

El-Banna, A. A.; Hamilton, R. M. G.; Scott, P. M.; Trenholm, H. L. Nontransmission of deoxynivalenol (vomitoxin) to eggs and meat in chickens fed deoxynivalenol contaminated diets. *J. Agric. Food Chem.* **1983**, *31*, 1381.

Forsyth, D. M.; Yoshizawa, T.; Morooka, N.; Truite, J. Emetic and refusal activity of deoxynivalenol to swine. *Appl. Environ. Microbiol.* **1977**, *34*, 547.

Friend, D. W.; Trenholm, H. L.; Thompson, B. K.; Prelusky, D. B.; Hartin, K. E. Effect of Deoxynivalenol (DON)-contaminated diet fed to growing-finishing pigs on their performance at market weight, nitrogen retention and DON excretion. *Can. J. Anim. Sci.* **1986**, *66*, 1075.

Hayes, A. W. Mycotoxins: A review of biological effects and their role in human diseases. *Clin. Toxicol.* **1980**, *17*, 45.

Kubena, L. F.; Swanson, S. P.; Harvey, R. B.; Fletcher, O. J.; Rowe, L. D.; Phillips, T. D. Effects of feeding deoxynivalenol (DON, vomitoxin) contaminated wheat to growing chicks. *Poult. Sci.* **1985**, *64*, 1649.

Lake, B. G.; Phillips, J. C.; Walters, D. G.; Bayley, D. L.; Cook, M. W.; Thomas, L. V.; Gilbert, J.; Startin, J. R.; Baldwin, N. C. P.; Bycroft, B. W.; Dewick, P. M. Studies on the metabolism of deoxynivalenol in the rat. *Food Chem. Toxicol.* **1987**, *25*, 589.

Lauren, D. R.; Greenhalgh, R. Simultaneous analysis of nivalenol and deoxynivalenol in cereals by liquid chromatography. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 479.

Pohland, A.; Thorpe, C.; Sphon, J. The analytical chemistry of deoxynivalenol. In *Toxigenic Fungi—Their Toxins and Health Hazards*; Kurata, H., Ueno, Y., Eds.; Elsevier: New York, 1984; Vol. 7, pp 217–230.

Prelusky, D. B.; Trenholm, H. L.; Lawrence, G. A.; Scott, P. M. Nontransmission of deoxynivalenol (vomitoxin) to milk following oral administration to dairy cows. *J. Environ. Sci. Health* **1984**, *B19*, 593.

Prelusky, D. B.; Hamilton, R. M. G.; Trenholm, H. L.; Miller, J. D. Tissue distribution and excretion of radioactivity following administration of ^{14}C-labelled deoxynivalenol to white Leghorn hens. *Fundam. Appl. Toxicol.* **1986**, *7*, 635.

Prelusky, D. B.; Trenholm, H. L.; Hamilton, R. M. G.; Miller, J. D. Transmission of ^{14}C-deoxynivalenol to eggs following oral administration to laying hens. *J. Agric. Food Chem.* **1987**, *35*, 182.

Prelusky, D. B.; Hartin, K. E.; Trenholm, H. L.; Miller, J. D. Pharmacokinetic fate of ^{14}C-labelled deoxynivalenol in swine. *Fundam. Appl. Toxicol.* **1988**, *10*, 276.

Prelusky, D. B.; Hamilton, R. M. G.; Trenholm, H. L. Transmission of residues to eggs following long-term administration of ^{14}C-labelled deoxynivalenol to laying hens. *Poult. Sci.* **1989**, *68*, 744.

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*, 699.

Trenholm, H. L.; Cochrane, W. B.; Cohen, H.; Elliot, J. I.; Farnworth, E. R.; Friend, D. W.; Hamilton, R. M. G.; Standish, J. F.; Thompson, B. K. Survey of vomitoxin contamination of 1980 Ontario white winter wheat crop. Results of survey and feeding trials. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 92.

Trenholm, H. L.; Warner, R. M.; Prelusky, D. B. Assessment of extraction procedures in the analysis of naturally contaminated grain products for deoxynivalenol (vomitoxin). *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 645.

Trenholm, H. L.; Friend, D. W.; Hamilton, R. M. G.; Thompson, B. K.; Hartin, K. E. Incidence and toxicology of deoxynivalenol as an emerging mycotoxin problem. *Proceedings*, VI International Conference on Mycoses; Scientific Publication 479; Pan American Health Organization, World Health Organization: Washington, DC, 1986; pp 76–82.

Ueno, Y. Mode of action of trichothecenes. *Pure Appl. Chem.* **1977**, *49*, 1737.

Ueno, Y. Trichothecenes as environmental toxicants. In *Reviews in Environmental Toxicology 2*; Hodgson, E., Ed.; Elsevier: Amsterdam, 1986; pp 303–341.

Wilson, B. J. Hazards of mycotoxins to public health. *J. Food Prot.* **1978**, *41*, 375.

Wyatt, R. D. Moulds and mycotoxins—Concepts of importance to the feed industry. *Poult. Adviser* **1986**, Feb, 53.

Received for review June 29, 1990. Accepted November 5, 1990.

Registry No. DON, 51481-10-8.