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TRICHOTHECENES AS ENVIRONMENTAL TOXICANTS

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=ABSTRACT=

The trichothecenes are a chemically related sesquiterpenoid fungal metabolites of Fusarium, Trichoderma, Stachybotrys and others, and at moment more than 70 kinds of derivatives are identified. Historically, they are identified as antifungal and phytotoxic compounds, but after the finding of T-2 toxin from Fusarium tricinctum, several trichothecenes are now considered to be natural toxicants in foodstuffs and feeds.

Among numerous trichothecene-producing fungi, F. graminearum (Gibberellazeae) and F. sporotrichioides are very important in the incidences of food-born diseases. The former fungus produces nivalenol and deoxynivalenol, and the latter produces T-2 toxin, HT-2 toxin, diacetoxyscirpenol and others.

Current developments of the methodology for trichotecene analysis revealed the natural occurrence of deoxynivalenol, nivalenol, T-2 toxin and some macrocyclic trichothecenes in foods, feeds, and their products. In Canada, the United States, England and South Africa, deoxynivalenol is the major toxicant in corn. barley, wheat and other cereals, and in Japan, Korea and Taiwan (ROC), both nivalenol and deoxynivalenol are detected in cereal grains and their products in high frequency. Several approaches on decontamination of these trichothecenes have revealed that milling and other food processesing are hard to remove these toxicants from cereals.

Toxicologically, the trichothecenes induce vomiting, diarrhea, dermal toxicity, hemorrhage in intestines, lung and other tissues, and impair the immunological function. Biochemically, they are a potent inhibitor of protein and DNA syntheses in animal cells.

The metabolic pathway of the trichothecenes is classified into 1) deacylation, 2) hydroxylation, 3) conjugation, and 4) depoxidation.

Long-term effects, combined effects with other chemicals, and transmission of the trichothecenes in edible tissues of farm animals to human, should be clarified to solve the

etiological evaluation of these naturaltoxins.

Key Words: *Trichothecenes, Fungaltoxin, Nivalenol, Deoxynivalenol, Food-born disease*

INTRODUCTION

The trichothecenes are a structurally closed group of sesquiterpenoid metabolites produced by several genera of imperfect fungi such as *Trichoderma, Trichothecium, Stachybotrys* and *Fusarium.* They have a spiro epoxy group on carbon 12 and 13, although one trichothecene, named verrucarin K, lacks this function. Firstly, Freeman and Morrison (1) isolated trichothecin from *T. roseum* as an antifungal agent. Harris et al. (2) isolated verrucarin A though J and roridin A though E from *Myrothecium roridum* and *M. verrucaria* as potent antifungal metabolites. Another trichothecene, named diacetoxyscirpenol, was isolated from *F. scirpi* as a phytotoxic compounds (3).

Toxicologically important finding was the discovery of T-2 toxin from *F. tricinctum* as the causative agent to the moldy corn toxicosis of farm animals in the United States (4). In Japan, nivalenol and deoxynivalenol were detected from the metabolite of *F. graminerum* as causative toxicants in red-mold toxicoses (5,6). Macrocyclic trichothecenes such as satratoxin H were identified as the causal agents in Stachybotryotoxicosis in the Central Europe (7).

These compounds, therefore, have been the subject of much research, the first major one focusing on their role in food and feed-borne diseases, that began appearing in 1971 (Bamburg and Strong, 1971) (8). Thereafter, several reviews and books are published as follows; Smalley and Strong (1974) (9), Bamburg (1976 and 1983) (10,11), Ong (1982) (12), and Ueno (1977a, 1977b, 1980a, 1980b, 1983, 1985a and 1985b) (13-19).

CHEMISTRY

The trichothecene mycotoxins are derivatives of a ring system named "trichothecane", as shown in Fig. 1. Naturally occurring trichothecenes contain an olefic bond at C-9, 10, and an epoxy ring at C-12, 13. Based on these chemical features, they are terminated as "12, 13-epoxytrichothecene". Fig. 2 shows the structure, stereochemistry and numbering of this family.

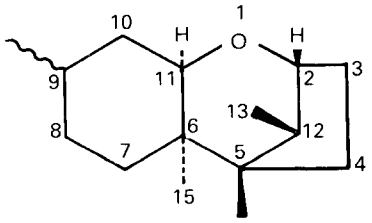

Fig. 1. Structure and numbering system of trichothecene

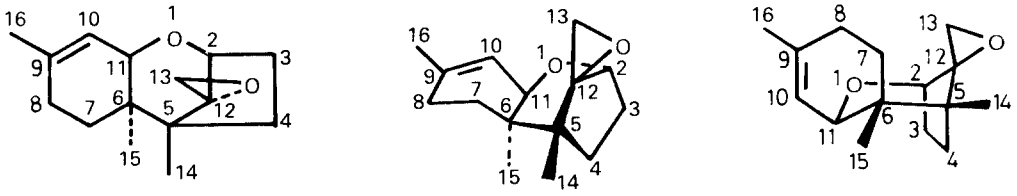

Fig. 2. Three representations of 12, 13-epoxytrichothec-9-ene

Generally, the carbon atoms at 3, 4, 7, 8, 14 and 15 are occupied by hydrogen, hydroxyl, acyl, additional epoxide, or macrocyclic ester linkages. At moment, more than 70 kinds of derivatives are identified from the fungal metabolites and their microbial biotransformed products. Fig. 3 shows the major trichothecene mycotoxins.

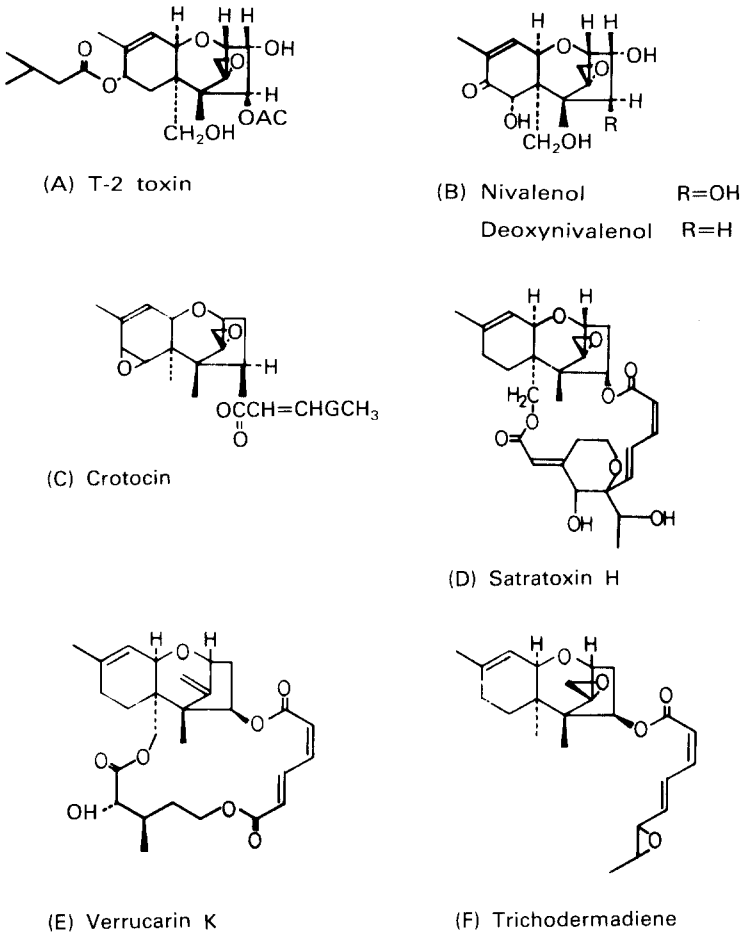

Fig. 3. Major trichothecene mycotoxins

The type A trichothecenes, represented by T-2 toxin, diacetoxyscrpenol and others, are the hydroxy and acyloxy substituted trichothecenes. The type B

trichothecenes are the C-8 keto compounds such as nivalenol and deoxynivalenol. The type C is the diepoxytrichothecene as crotocin. The type D are the macrocyclic trichothecenes (verrucarins etc.). The type E lacks the 12, 13-epoxy ring in their structures. The type F, represented by trichodermadiene and trichodermadienediol, is the macrocyclic-opened-ring trichothecenes. The last trichothecenes are the intermediates in the biosynthetic pathway of the macrocyclic trichothecenes.

Upon treatment of the acylated trichothecenes with bases, they are hydroxylated in their corresponding parent alcohols. For examples, T-2 toxin is converted into T-2 tetraol via HT-2 toxin, neosolaniol and T-2 triol. Fusarenon-X (4-acetyl nivalenol) and 3-acetyl deoxynivalenol give nivalenol and deoxynivalenol, respectively. The macrocyclic trichothecenes such as verrucarins turn to verrucarol.

The hydroxylated trichothecenes such as nivalenol and deoxynivalenol are soluble in polar solvents such as methanol and water. While, T-2 toxin and macrocyclic trichothecenes (verrucarins, roridins and satratoxins) are soluble in chloroform and ethyl acetate. Therefore, the trichothecene mycotoxins differ widely in their solubility by organic solvents.

TRICHOTHECENE-PRODUCING FUNGI

The majority of trichothecene-producing fungi found belong to the genus *Fusarium*. However, the taxonomy of *Fusarium* species is confusing due to many different classification systems. Recently, tha author (15) has reviewed the toxicity and taxomony of *Fusarium* species and attempted to identify synonymous species in the major classificaiton system, and the details of their toxigenicity were also reviewed by Marasas et al. (20).

Among numerous species of *Fusarium*, mycotoxicologically important ones are *F. graminearum* and *F. sporotrichioides*. *F. graminearum (Gibberella zeae* in sexual stage) is the potent plant pathogenic fungus which parasites barley, wheat, and corn, and the moldy grains, called scabby grains, are often toxic to animals and humans. The major mycotoxins are nivalenol, 4-acetyl nivalenol (fusarenon-X), deoxynivalenol, 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol, and zearalenone. As reviewed in the later section, nivalenol, deoxynivalenol and zearalenone are detected from the cereals and their products. Chemotaxonomically, this fungus is subdivided into two chemotypes; one is the producer of nivalenol and 4-acetyl nivalenol (fusarenon-X), and other is the producer of deoxynivalenol and 3-acetyl deoxynivalenol (21). In Canada, the United States, England, and the northern part of Japan (Hokkaido), deoxynivalenol is the major pollutant in cereal grains, while in the southern part (Shikoku) of Japan and Korea, both nivalenol and deoxynivalenol are detected as environmental toxicants from cereals and their products. It may suggest some geographical difference in their distribution in the world.

F. sporotrichioides is another important fungus in the trichothecene toxicosis. T-2 toxin was the first isolate from *F. tricinctum* in connection with moldy corn toxicosis in the United States (4), and the same toxin was isolated from *F. sporotrichioides* by the author, which was the major toxigenic *Fusarium* species in Alimentary Toxic Aleukia (ATA) in USSR (22). However, as suggested by Marasas et al. (20), *F. tricinctum* is very closed to *F. sporotrichioides*. Mycologically, the pathogenicity of *F. sporotrichioides* to plants are much weaker than that of *F. graminearum*. It is the reason why the trichothecenes (nivalenol and

deoxynivalenol) of *F. graminerau* are often. detected from cereals in compared to those of *F. sporotrichioides* (T-2 toxin et al.)

Stachybotrys atra (=S. alternanse) is the major toxic fungus which induces stachybotriotoxicosis in horses and other farm animals. This fungus produces satratoxins when parasited on hay and straw.

During the course of a search for tumor inhibitors, the macrocyclic trichothecenes, named baccharins, were isolated from the extract of a Brazillian shrub *Baccharis megapotamica* (23). This is the first report that the trichothecenes were found in the plant. Four trichothecenes, baccharin, baccharinol, isobaccharin and isobaccharinol, were fractionated from the plant extracts. However, the origin of these trichothecenes is presumed to be verrucarins and roridins, which are produced by soil fungi *M. verrucaria* and *M. roridum*. This suggests some ecological association between the plant and fungi.

ANALYTICAL METHODOLOGY OF TRICHOTHECENES

1. Thin-layer chromatography

Thin-layer chromatography (TLC) has always been a convenient method for mycotoxin analysis because of its low cost and simplicity, and several methods were applied for the screening of trichothecene-producing fungi and their metabolites. The trichothecenes on plates were detected by spraying H_2SO_4 solution, followed by heating over 100°C. They release purple spots on the plates. The type A toxins (T-2 toxin etc.) exhibit blue fluorescence under UV light (24). *p*-Anisaldehyde is often used for the detection on the plates. The type B toxins (nivalenol etc.) give blue fluorescence when heated with $AlCl_3$.

FDA procedure for the detection and quantitation of deoxynivalenol in cereals is as follows (25): A 50 g sample of wheat or corn was extracted with CH_3 CN/H_2O (86/15), followed by hexane participation and clean-up by charcoal-Al_2O_3-Celite column. On spraying 20% $AlCl_3$ solution and heating at 120° for 5 min, deoxynivalenol on the plate was quantitated by visual comparison of fluorodensitometer. This simple method is applicable for the natural occurrence of deoxynivalenol in cereals with the detection limit of 40 ppb (wheat) and 100 ppn (corn).

Fluorodensitometric method proposed by Sano et al. (26) is briefly summarized as follows: the plate is treated with nicotinamide at elevated temperature, and treated with 2-acetylpyridine, followed by potassium hydroxide solution and formic acid. The trichothecenes appear a light blue fluorescent spots under UV light and the detection limits are 10-1500 ng/spot. This method is rather specific to the compounds containing epoxide function.

2. Gas-liquid chromatography

Several methods have been developed using gas-liquid chromatography (GLC) for the detection and quantification of trichothecenes. Formation of derivatives of those containing hydroxyl groups is important before GLC analysis. For derivatization of trichothecenes, the following reagents are commonly used: a combination of N-trimethylsilyl imidazole (TMSI) and trimethylchlorosilane (TMCS) in pyridine, chloroform, or ethyl acetate; a mixture of bis(trimethylsilyl) acetamide, IMCS and TMSI; heptafluorobutyrylimidazole (27, 28, 29). The sensitivity of TMS-derivatives of trichothecenes is 20-50 ng per injection by flame ionization detector. By electron capture detector, the sensitivity is as low as 2 pg per injection for silylated ethers of fusarenon-X, nivalenol, and deoxynivalenol.

3. Combination of GLC and mass spectrometry (GC-MS)

The combination of GLC and mass spectrometry (MS), using selected ion monitoring, is an useful tool for the quantitation and confirmation of trichothecenes in cereals and biological materials. The TMS-ether of T-2 toxin at m/z is 436, 350, 290, and 122 (m^+=538); TMS ether of diacetoxyscirpenol is m/z 378, 350, 290 and 106 (M^+=438); TMS ether of deoxynivalenol is m/z 422, 325, 295, 259, 235 and 197 (M^+=512).

Currently, Tanaka has proposed an improved method for the simultaneous detection of trichothecenes (nivalenol and deoxynivalenol) and zearalenone in cereals (30, 31), as summarized in Fig. 4. The *Fusarium* mycotoxins were extracted from cereals with CH_3CN-H_2O (3:1), defatted with *n*-hexane and purified by a two-step chromatography (Florisil/Sep-pak columns). The final methanol extract was divided into three parts A, B and C. The parts A and B were processed for GC/MS analysis of nivalenol and deoxynivalenol with electron capture detector, and the part C was used for high-performance liquid chromatographical (HPLC) analysis of zearalenone with fluorodetector. The detection limit of the trichothecenes was 2.0μg/Kg, and that of zearalenone is 1.0 μg/Kg with a recovery of over 90% (30). The introduction of a mixture of CH_3CN-H_2O (3:1) for the extraction of trichothecenes resulted in a high recovery rate with a low contamination of interfering materials in cereals, and the use of methanol as a dissolving solvent for column eluates increased the recovery of the trichothecenes. The chemical nature of the suspected toxins was confirmed by the selected ion monitoring of trichothecenes. Furtheremore, zearalenone in the methanol eluates was resolved on a porous silica gel column (Nucleosil 50-10) with 90% water-saturated chloroform-cyclohexane-acetonitril-ethanol (50:15:2:1) in HPLC, and quantitated by fluorodetector.

Employing this simultaneous detection method, we have carried out the survey of natural occurrences of nivalenol, deoxynivalenol, and zearalenone in cereal grains harvested in Japan, Korea, and other countries (30,31).

4. Quadrupole mass spectrometry/mass spectrometry

The rather new technique of tandem MS, or MS/MS, has been introduced for the identification of trichothecenes (32). MS/MS used one stage of mass separation to select the compound of interest from the matrix (usually as the molecular ion, protonated molecular ion, or molecular anion) and the second stage for anlysis after a collisionally activated dissociation (CAD) by collision with a target gas.

Ground and blended samples of grains were extracted with organic solvents, and a portion of the extract was directly analysed by MS/MS with the detection limit of 0.1 ppm.

Other physico-chemical methods with polarography and HPLC were reviewed by Pohland et al. (33).

5. Immunochemical assay

The trichothecene mycotoxins possess characteristic biological activities, and these features were applied for the biological assay of toxic fungal metabolites. Skin toxicity test, cytotoxicity tests with mammalian cells and protozoa, phytotoxicity test, and an inhibition of protein synthesis in rabbit reticulocytes were introduced, as reviewed by the author (34).

Recently, several immunoassay methods were introduced for T-2 toxin, diacetoxyscirpenol and deoxyverrucarol. Chu et al. (35) first reported the production of antibody directly in rabbits immunized with a bovine serum albumin-T-2 toxin hemisuccinate conjugate. This radioimmunoassay is sensitive to detect 3 ng/assay

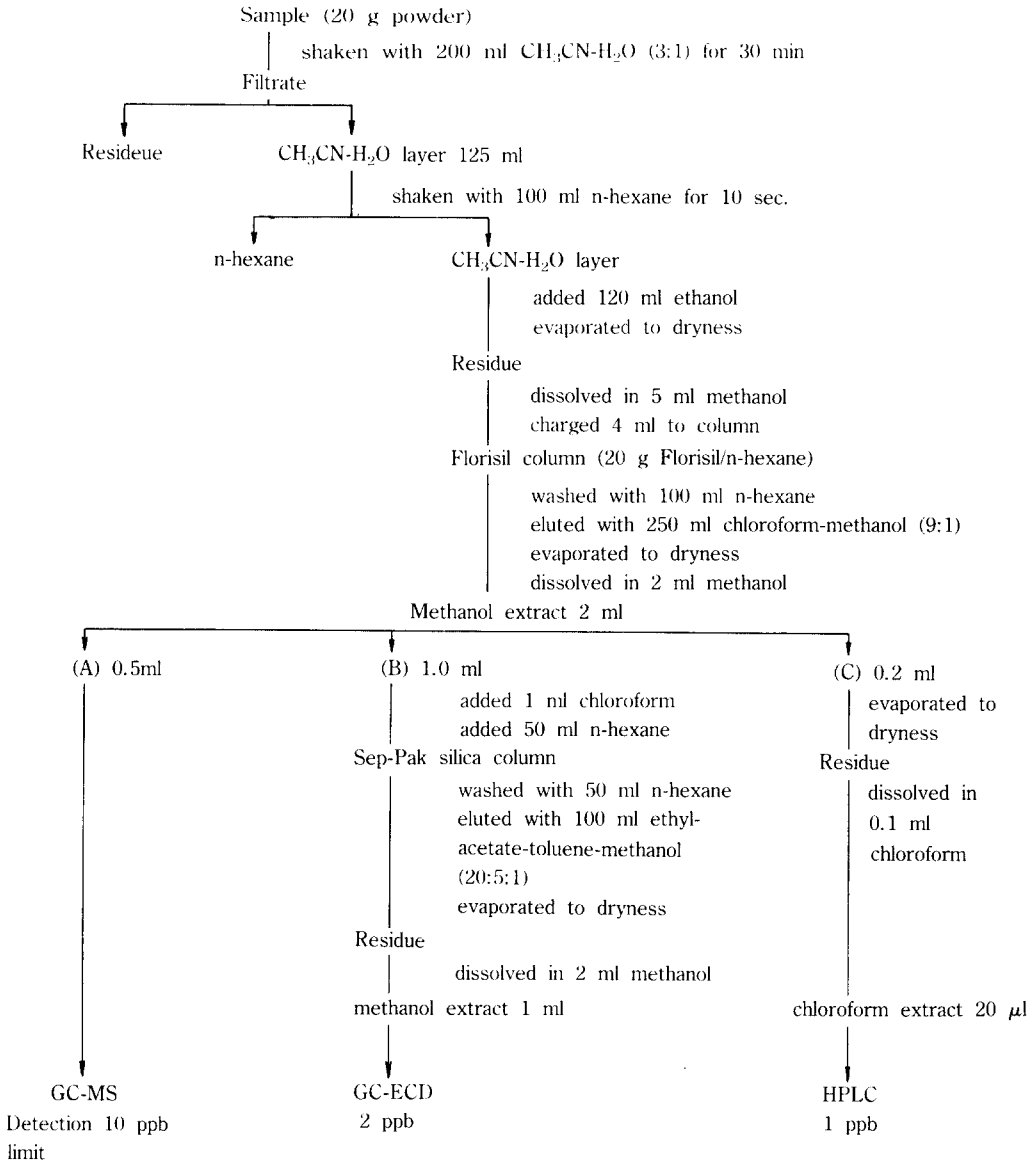

Fig. 4. Tanaka's method for the simultaneous determination of nivalenol, doexynivalenol and zearalenone in creals (Tanaka et al., 1984, with permission) [31, 32]

of T-2 toxin. Pestka et al. (36) developed a sensitive enzyme-linked immunoassay (ELISA) for T-2 toxin. The detection limit of this ELISA was about 2.5ng T-2 toxin/assay. The immunochemical methods were also developed for diacetoxyscirpenol (37) and deoxyverrucarol (38). Monoclonal antibody to T-2 toxin and its application for ELISA was currently reported (39).

This type of assay has the advantage of not requiring radioisotopes or expensive scintillation counters.

NATURAL OCCURRENCE

Of over 60 kinds of trichothecene compounds, T-2 toxin, nivalenol, deoxynivalenol, satratoxins G and H were detected in cereal grains and animal feed.

Interestingly, deoxynivalenol is the major toxicant in the United States, Canada, England and South Africa, as shown in Table 1.

Table 1. Natural occurrence of deoxynivalenol

Country	Substrates	samples	Positive	Contents (mg/kg)	References
Austria	corn	2	2	1.3-7.9	Vesonder et al. (40)
Canada	corn	2	1	7.9	ibid
	corn	105		0.20-0.62	Scott (41)
	cornmeal	35		0.11	ibid.
	corn flour	27		0.18	ibid.
	rye	8		0.10	ibid.
	rye flour	3		0.12	ibid.
	rye bread	4		0.058	ibid.
	wheat	939		0.03-0.74	ibid.
	wheat flour	43		0.40	ibid.
	wheat bran	14		0.17	ibid.
	cookies	35		0.08	ibid.
	crakers	20		0.27	ibid.
	baby cereals	30		0.043	ibid.
	wheat	77		0.32-8.53	Threnhold et al. (42)
England	feed barley	43	21	0.01-0.1	Gilbert et al. (43)
	malt barley	42	13	0.01-0.1	ibid.
	breweres corn	11	8	0.01-0.1	ibid.
Germany	cereals	42	16	0.03-2.0	Blaas et al. (44)
Hungry	corn	15	3	0.2-1.3	Bata et al. (45)
Roc (Taiwan)	wheat	12	9	0.53 (av)	Uneno et al. (46)
Japan	barley	6	6	0.02-0.11	Kuroda et al. (47)
	wheat	3	3	0.02-0.11	ibid.
	barley	25	19	tr-7.8	Kamimura et al. (27)
	wheat	18	15	tr-4.9	ibid.
	barley/wheat	205	153	0.06-49.6	Yoshizawa (49)
	parched barley flour	6	6	0.027-0.085	Yoshizawa wt al. (49)
	barley	50	42	0.05-1.54	Dohi et al. (50)
	barley	14	13	0.003-0.350	Tanaka et al. (30)
	wheat	9	6	0.005-0.740	ibid.
Korea	barley	28	26	0.004-0.508	Lee et aal. (51)
	malt	4	4	0.022-5.840	ibid.
South Africa	corn			1.3-7.4	Marasas et al. (52)
	corn kernals			0.42-2.50	Thiel et al. (53)
U.S.A.	corn	52	24	0.5-10	Vesonder et al. (54)
	mixed feed			10-50	Stahr et al. (55)
	wheat	33	31	1.782(av).	Hagler et al. (56)
	total feeds	342	274	0.1-41.6 (3.1 av.)	Côté et al. (57)

In the United States, Vesonder et al. (54), Hagler et al. (56), Côté et al. (57) and other groups surveyed the presence of trichothecenes in cereals and animal feeds.

As summarized in Table 2, Vesonder et al. (54) detected 0.5-10mg/Kg of deoxynivalenol in 24 out of 52 corn samples, and some corn samples were heavily

contaminated with 15-28mg/kg of this trichothecene.

Vomitoxin causes feed refusal and its name comes from its emetic effect on swine. Cool, wet climatic conditions in the fall of 1981 in the midwestern United States resulted in problems of moldy corn. Animal health problems and reduced growth performance were observed mainly in swine, fed with moldy rations. According to Côté et al. (57), the predominant clinical symptoms are, in decreasing frequency: reproductive problems (50%), feed refusal (43%), reduced weight gain (25%), diarrhea (17%), death (14%), and emesis (11%). They analysed the content of vomitoxin in various animal feed, and, as shown in Table 2, found that 274 (80%) of the 342 samples were positive in deoxynivalenol pollution. A total of 40 (12%) of the samples were found to contain zearalenone, but other mycotoxins were not detected. Most of the vomitoxin positive samples submitted were either corn (38%) or corn-based mixed feed (39%). Concentration of vomitoxin in these feeds ranged from 0.1 to 41.6 ppm, with a mean concentration of 2.5 to 4 ppm for all feeds and grains, except wheat.

In Canada, Scott and his group (41), and Trenhold et al.(42), demonstrated that Canadian cereals are contaminated with deoxynivalenol (Table 2). Average levels of deoxynivalenol that were determined by the Health Protection Branch Regional Laboratories in Canadian wheat for the years 1979-1982, showed geographic differences between Eastern and Western Canada: in Ontario soft winter wheat, the average of deoxynivalenol (ppm) was 0.06 (1979 crop year), 0.42 (1980), 0.22 (1981) and 0.74 (1982); in Quebec hard spring wheat, 0.93 (1980), 3.03 (1981) and 0.33 (1982); and in Western hard wheat 0.02-0.03 (1979-1982) (42). Corn, corn flour, rye, rye flour, and wheat flour were also contaminated (Table 2).

In England, Gilbert et al (43) surveyed extensively the content of deoxynivalenol in UK grown and imported cereals. In South Africa, Marasas et al (52). confirmed that deoxynivalenol, along with zearalenone, was often detected from corn. An interesting finding is the positive correlation between the incidence of esophageal cancer and the level of deoxynivalenol in corn samples of Transkei.

In China, scabby wheat toxicosis are often observed. During 1975-1980, 2450 samples of wheat head blight were collected from 21 provinces in China, and the presence of *Fusarium* species was investigated (68). The data revealed that the incidence of the *F. graminearum* was highest (94.5%) among 18 *Fusarium* spp identified. This fungus was widely distributed with high frequency, and is considered to be the dominant pathogenic species in China. Beside BD_1(=deoxynivalenol) Xu et al.(69) isolated a new trichothecene, named CBD_2, from *Fusarium*-contaminated field barley, grown in the suburbs of Shanghai. Chemical analysis of this toxin has revealed that it was 3-lactyl-deoxy-nivalenol.

In Japan, Yoshizawa (48), Kuroda et al. (47), Kamimura et al. (29) and Tanaka et al. (30) have extensively surveyed the contamination of cereals by trichothecenes. As summarized in Tables 1 and 2, both nivalenol and deoxynivalenol were detected from wheat, barley and other cereals. These two trichothecenes are, therefore, considered to be the major natural pollutants in grains. Recently, these two trichothecenes were detected in commercial purched barley flour (Mugi-kogashi or Hattaiko in Japanese) (49). This is one of the traditional foods substances that is commonly used in rural areas in Japan. A similar situation was exposed by Lee et al.(51), who analyezed Korean wheat and barley by using Tanaka's method, presented in Fig 4. A high level of both nivalenol and deoxynivalenol was detected from more than 90% of the total samples harvested in 1983 in Korea.

Table 2. Natural occurrence of nivalenol

Country	Substrates	Samples	Positives	Contents (mg/kg)	References
France	corn			4.28	Jammali et al. (58)
Germany	cereals	42	11	0.04-0.2	Blaas et al. (44)
Korea	barely	28	28	0.07-3.002	Lee et al. (51)
	malt	4	4	0.004-0.508	ibid.
Japan	barley	6	6	0.14-5.2	Kuroda et al. (27)
	wheat	3	3	0.01-0.14	ibid.
	barley	25	19	tr.-36.9	Kamimura et al. (27)
	wheat	18	15	tr.-7.8	ibid.
	barley/wheat	205	153	0.07-22.9	Yoshizawa (48)
	parched barley flour	6	6	0.037-0.190	Yoshizawa et al. (49)
	barley	50	50	0.05-11.44	Dohi et al. (50)
	barley	14	13	0.016-1.670	Tanaka et al. (30)
	wheat	9	3	0.005-1.630	ibid.
ROC (Taiwan)	wheat	12	6	0.074(av.)	Ueno et al. (46)

The natural occurrences of T-2 toxin were summarized in Table 3. T-2 toxin was detected from barley, corn, and feeds. But the incidence was much rare than those of deoxynivalenol and nivalenol.

Table 3. Natural occurrence of T-2 toxin

Country	Substrates	Samples	Positives	Contents(mg/kg)	References
Canada	barley	1	1	25	Puls et al. (59)
Hungary	corn	15	13	0.1-5.8	Bata et al. (45)
India	safflower seeds			3.5	Ghosal et al. (60)
USA	corn			2	Hsu et al. (61)
	mixed feed			0.076	Mirocha et al. (62)
	mixed feed			1	Stahr et al. (55)
	corn stalk			0.11	Mirocha et al. (63)
	farm-stored corn		8-16%	2	Stahr et al. (64)

In Table 4, the natural occurences of macrocyclic trichothecenes were summarized. Recently, the methodology for detection of these macrocycles were progressed, and as shown in Table 5, satratoxin G, H, and verrucarins were detected from animal feeds. However, exact contents of these macrocyclic trichothecenes in feeds were not estimated.

Table 4. Natural occurrence of macrocyclic trichothecenes

Country	Trichothecenes	Substrates	Contents	References
Hungary	satratoxin G	straw	unknown	Harrach et al. (65)
	satratoxin H	straw	ibid.	ibid.
	verrucarin J	straw	ibid.	Harrach et al. (66)
	satratoxins G, H	straw	ibid.	ibid.
USA	verrucarins B, J	house dust	ibid.	Jarvis et al. (67)
	trichoverrins A, B	ibid.	ibid.	ibid.
	satratoxin H	ibid.	ibid.	ibid.

COMPARATIVE TOXICITY OF NIVALENOL, DEOXYNIVALENOL AND T-2 TOXIN

Table 5 summarizes the toxicological characteristics of major trichothecenes, which are often detected from our food stuffs and animal feeds. The LD_{50} of nivalenol in mature mice is 5.0 mg/kg (p.o). While, this value decreases to 0.16 mg/kg in newborn mice. This indicates that immature animals are highly susceptible than the mature animals. The trichothecenes are highly cytotoxic to animal cells such as HeLa cells and protozoa. The mechanism of cytotoxicity is originated from their potent activity in inhibition of protein and DNA synthesis (34).

Table 5. Comparative toxicity of nivalenol, deoxynivalenol and T-2 toxin.

Toxicity	Nivalenol	Deoxynivalenol	T-2 toxin
LD_{50} (mg/Kg)			
Mouse (adult) male po	5.0	46	10.5
(new born) sc	0.16		0.15
Pecking duckling sc	2.0	27	
Cytotoxicity (μg/ml)			
Protozoa *(Tetrahymena)*	0.5	4.6	0.01
Mammalian cells (HeLa)	0.3	1.5	0.01
Emetics (MED, mg/Kg)			
Peking duckling sc	1.0	10	0.1
Cat sc			0.1
Dog sc		0.1	
Feed refusal (mg/kg feed)			
Swine		ca 10	
Dermal irritation (μg/spot)			
Rat	1.0	75	0.01

Another toxicological properties are the induction of emetics, vomiting, and feed refusals. These toxic effects were often observed in scabby grain intoxications in men and farm animals.

The relationship betweeen the chemical structure and toxicological activities is complicated. In general, the macrocyclic trichothecenes such as verrucarins, and satratoxins are highest in the toxicity, and followed by T-2 toxin, and the Type B toxins (nivalenol et al.) It means that the macrocyclic ring may regulate their affinity to animal cells, The hydroxy and acyl residues of the trichothecenes give a great influence on their activities. For example, only one difference of deoxynivalenol in compared to nivalenol is a lack of a hydroxyl residue at C-4 (Fig 3). This chemical character leads to a marked reduction in the toxicity of deoxynivalenol, as shown in Table 5. As mentioned in the section "NATURAL OCCURRENCES", deoxynivalenol is the major pollutant in the U.S.A., Canada, England, and South Africa. While, both nivalenol and deoxynivalenol are often detected in the grains harvested in Japan and Korea (Table 2). Since the toxicity of nivalenol is about 10-fold higher than deoxynivalenol, we have to pay much attentions to the natural occurrences of nivalenol in our foodstuffs. Another important function of the trichothecenes is the epoxide ring at C-12,13. Upon reduction of the epoxide ring of deoxynivalenol by anaerobic dermal flora, deoxynivalenol turns to C-12, 13 de-epoxy deoxynivalenol. This metabolite loses its biological and toxicological activities. Therefore, this epoxide ring is essential for their toxicological activities. For the evaluation of trichothecenes toxicity, metabolic pathways should be clarified. At moment, their metabolic pathways are classified; 1) deacylation, 2)

hydroxylation, 3) conjugation, and 4) depoxydation.

For example, T-2 toxin and 4-acetyl-nivalenol are deacylated by hepatic microsmal esterases into HT-2 toxin and nivalenol are deacylated by hepatic microsomal esterases into HT-2 toxin and nivalenol, respectively (70). The cytochrome P-450 catalyses the hydroxylation of T-2 toxin and HT-2 toxin into 3-hydroxy-T-2 and 3-hydroxy HT-2 toxins (71). In some fungal cultures, T-2 toxin is conjugated with acetyl residue. A part of deoxynivalenol administered in swine was recovered as glucuronide conjugate. Toxicologically important pathway is the depoxydation reaction, which was recently observed in dermal flora.

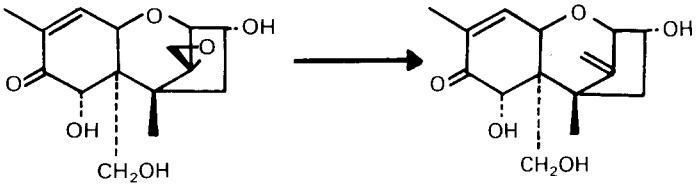

Fig. 5. Depoxidation reaction of deoxynivalenol

REFERENCES

1. G.G. Freeman and J.E. Morrison, Trichothecin: An antifungal product of *T. roseum* *Nature*, 162:30, (1948)
2. E. Harris, W. Loeffer, H.P. Stahelin, Ch. Stoll, Ch. Tamm and D. Wiesinger: Über die Verrucarine und Roridine, eine Gruppe von cytostatische hochwirksamen Antibiotica aus *Myrothecium*-Arten. *Helv. Chim. Acta*,45:839, (1962)
3. H.P. Sigg, R. Mault, E. Flury and D. Hauser: Die Konstitution von Diacetoxyscirpenol. *Helv. Chim. Acta*, 48:962, (1965)
4. J.R. Bamburg, N.V. Riggs and F. M. Strong: The structures of toxins from two strains of *Fusarium tricinctum*. *Tetrahedron*, 24:3329, (1968)
5. T. Tatsuno, Y. Fujimoto and Y. Morita: Toxicological research on substances from *Fusarium nivale*. III. The structure of nivalenol and its monoacetate. *Tetrahedron Lett., 33:2823, (1962)*
6. T. Yoshizawa and N. Morooka: Deoxynivalenol and its monoacetate, new mycotoxins from *Fusarium roseum* and moldy barley. *Agric. Biol. Chem.*, 33:2933, (1973)
7. R.M. Eppley and W.J. Bailey: 12, 13-Epoxy-delta-trichothecenes as the probable mycotoxins responsible for stachybotryotoxicosis. *Science*, 181:758, (1973)
8. J.R. Bamburg and F.M. Strong: 13, 13-Epoxytrichothecenes. In S. Kadis, A. Ciegler, S.J. Ajl (Eds.), Microbial Toxins, Vol. VII, Academic Press, New York, 1971, p.207.
9. E.B. Smalley and F.M. Strong: Toxic trichothecenes, In I.F.H. Purchase (Ed.), Mycotoxins, Elsevier, Amsterdam, 1974, p.199.
10. J.R. Bamburg: Chemical and biological studies of the trichothecene mycotoxins.*Adv. Chem.*,149:144, (1976)
11. J.R. Bamburg: Biological and biochemical action of trichothecene mycotoxins, In F.F.Hahn (Ed.), Progress in Molecular and Subcellular Biology, Vol. 8. Springer-Verlag, Berlin and Heiderburg, 1983, p.41.
12. C.W. Ong: Trichothecenes-A review, *Heterocycles*, 19:1685, (1982)
13. Y. Ueno: Mode of action of trichothecenes, *Pure Appl. Chem.*, 49:1737, (1977a)
14. Y. Ueno: Trichothecenes Overview, In J.R. Rodricks, C. W. Hesseltine and .A. mehlman (Eds.), Mycotoxins in Human and Animal Health, Pathotox, Ill., 1977b, p.189.
15. Y. Ueno: Trichothecene mycotoxins: Mycology, Chemistry and Toxicology. *Adv. Nutr. Res.*, 3:301, (1980a)

16. Y. Ueno: Toxicological evaluation of trichothecene mycotoxins, In D. Baker and T. Wadstrom (Eds.), Natural Toxins, Pergamon, New York, 1980b, p.663.
17. Y. Ueno (Ed.): Trichothecenes: Chemical, Biological and Toxicological Aspects, Elsevier, Amsterdam-Oxford-New York, 1983.
18. Y. Ueno: The Toxicology of Mycotoxins, CRC Press, Boca Raton, 1985a (inpress)
19. Y. Ueno: Trichothecenes in Food, In P. krogh (Ed.), Mycotoxins in Food: Occurrence an Implications, Academic Press, New York, 1985b, (in press)
20. W. F.O. Marasas, P.E. Nelson and T.A. Toussoun (Eds.): Toxigenic *Fusarium* species. The Pennsylvania State University Press, 1984.
21. M. Ichinoe, H. Kurata, Y. Sugiura and Y. Ueno: Chemotaxonomy of *Gibberella zeae* with special reference to production of trichothecenes and zearalenone. *Appl. Environ. Microbiol.*, 46:1364, (1983)
22. Y. Ueno, N. Sato, K. Ishii, K. Sakai and M. Enomoto: Toxicological approaches to the metabolites of Fusaria. V. Neosolaniol, T-2 toxin and butenolide, toxic metabolites of *F. sporotrichioides* NRRL 3510 and *F. poae* 3287. *Jpn. J. Exp. Med.*, 42:461, (1972)
23. B.B. Jarvis, R.M. Eppley and E.P. Mazzola: Chemistry and bioproduction of macrocyclic trichothecenes. In Y. Ueno (Ed.), Trichothecenes: Chemical, Biological and Toxicological Aspects, Elsevier, AmsterdamOxford-New York, 1983, p.20.
24. Y. Ueno, N. Sato, K. Ishii, K. Sakai, H. Tsunoda and M. Enomoto: Biological and chemical detection of trichothecene mycotoxins of *Fusarium* species. *Appl. Microbiol.* 25:699, (1973)
25. M. Trucksess, S. Nesheim and R. Eppley: Thin-layer chromatographic deter-ination of deoxynivalenol in wheat and corn. *J. Assoc. Off. Anal. Chem.*, 67:40, (1984)
26. A. Sano, Y. Asabe, S. Takitani and Y. Ueno: Fluorodensitometric determination of trichothecene mycotoxins with nicotiamide and 2-acetyl-Pyridine on a sillica gel layer. *J. Chromatogr.*, 235:257, (1982)
27. H. Kamimura, M. Nishizima, K. Yasuda, K. Saito, A. Ibe, T. Nagayama, H. Ushiyama and Y. Naoi: Simultaneous detection of several *Fusarium* mycotoxins in cereals, grains and foodstuffs. *J. Assoc. Off. Anal. Chem.*, 64:1973, (1981)
28. T.R. Romer, T.M. Boling and J.L. MacDonald: Gas-liquid chromatographic determination of T-2 toxin and diacetoxyscirpenol in corn and mixed feeds. *J. Assoc. Off. Anal. Chem.*, 61:801, (1978)
29. P.M. Scott: Assessemnt of quantitative methods for determination of trichothecenes in grains and grain products. *J. Assoc. Anal. Chem.*, 65:876, (1982)
30. T. Tanaka, A. Hasegawa, Y. Matsui, K. Ishii and Y. Ueno: Improved methodology for the simultaneous detection of the trichothecenes deoxynivalenol and nivalenol in cereals. *Food Addt. Contami.*, in press (1985).
31. T. Tanaka, A. Hasegawa, Y. Matui, U.S. Lee and Y. Ueno: Rapid and sensitive determination of zearalenone in cereals by high-performance liquid chromatography with fluoresence detection. J. Chromatogr., in press (1985).
32. R.D. Plattner and G.A. Bennett: Rapid detection of *Fusarium* mycotoxins in grains by quadrupole mass spectrometry/mass spctrometry. *J. Assoc. Off. Anal. Chem.*, 66:1470, (1983)
33. A. Pohland, C. Thorpe and J. Sphon: The analytical chemistry of deoxynivalenol. In H. Kurata and Y. Ueno (Eds.), Toxigenic Fungi: their toxins and healthe hazard. Elsevier, Amsterdam-Oxford-New York-Tokyo, 1984, p. 227.
34. Y. Ueno: Biological detection of trichothecenes, In Y. Ueno (Ed.), Trichothecenes-Chemical, Biological and Toxicological Aspects, Elsevier-Amsterdam-Oxford-New York, 1983, p. 125.
35. M.S. Chu, S. Grossman, R.D. Wei and C.J. Mirocha: Production of antibody against T-2 toxin. *Appl. Environ. Microbiol., 37:104, (1979)*
36. J.J. Pestka, S.C. Lee, H.P. Lan and F.S. Chu: Enzyme-linked immunosorbent assay for T-2 toxin. *J. Am. Oil Chem. Soc.*, 58:1981, (940A)

37. F.S. Chu, M.Y. Chen Liang and G.S. Zang: Production and characterization of antibody against diacetoxyscripenol. *Appl. Environ. Microbiol.*, 48:777, (1984)
38. F.S. Chu, G.S. Zang, M.D. Williams and B.B. Jarvis: Production and characterization of antibody against deoxyverrucarol. *Appl. Environ. Microbiol.*, 38:781, (1984),
39. K.W. Hunter,Jr., A.A. Brinfield, M. Miller, F.D. Finkelman and S.F. Chu: Preparation and characterization of monoclonal antibodies to the trichothecene mycotoxin T-2. *Appl.Environ.Mivrobiol*49:168, (1985)
40. R.F. Vesonder and A. Ciegler: Natural occurrence of vomitoxin in Austrarian and Canadian corn.*Europian J. Appl. Environ. Microbiol.*, 48:781 (1984)
41. P.M. Scott: The occurrence of vomitoxin (deoxynivalenol, DON) in Canadian grains. In H. Kurata and Y. Ueno (Eds.), Toxigenic Fungi:their toxins and health hazazd, Elsevier, Amsterdam-Oxford-New York, Tokyo, 1984, p.182.
42. H.L. Trenhold, W.P. Cochrane, H. Cohen, J.I. Elliot, E.R. Farnworth, D.W. Friend, R.K.G Hamiltion, J. E., Standish and B.K. Thompton: Survey of vomitoxin contamination of 1980 Ontario Winter Crops. Results of survey and feeding trails. *J. Assoc. Off. Anal. Chem.*, 66:92, (1983)
43. J.Gilbert, M.J. Shepherd and J.R. Startin: A survey of the occurrence of the trichothecene mycotoxins deoxynivalenol (vomitoxin) in UK grown and in imported maize by combined gas chromatography-mass spectrometry. *J. Sci. Food Agric.*, 34:86, (1983)
44. W. Blass, M. Kellert, S. Steinmeyer, R. Tienbach and R. Weber: Untersuchung von Cerealien auf Deoxynivalenol und Nivalenol im unteren ug/kg Bereich. *Lebnsm. Unters. Forsch.*, 179:104 (1984)
45. A. Beta, A. Vanyi and R. Lasztity: Simultaneous detection of some fusariotoxins by mass-liquid chromatography. *J. Assoc. Off. Anal. Chem.*,
46. Y. Ueno: unpublished.
47. H. Kuroda, T. Mori, C. Nishioka. H. Okasak and M. Takagi: Studies on gas chromatographic detection of trichothecene mycotoxins in food. *J. Food Hyg. Soc. Jpn.*, 20 (1979) 137. (in Japanese)
48. T. Yoshizawa, Natural occurrence of *Fusarium* toxins in japan. In H. Kurata and Y. Ueno (Eds.): Toxigenic Fungi: their Toxins and Health Hazard, Elsevier, Amsterdam-Oxford-New York-Tokyo, 1984, p292.
49. T. Yoshizawa and H. Hosokawa: Natural occurrence of deoxynivalenol and nivalenol, trichothecene mycotoxins, in commercial foods. *J. Food Hyg. Soc. Jpn.*, 24 (1983) 413.
50. Y. Dohi, F. Watanugi, H. Kita, K. Kosaka, M. Ichinoe and K. Ohba: Determination of trichothecene mycotoxins in barley by FID-GC after clean-up on anion-exchange Sephadex. *J. Food Hyg. Soc. Jpn.*, 25:1, (1984) (in Japanese)
51. U.S. Lee, H.S. Jang, T. Tanaka, A. Hasegawa, Y.J. Oh and Y. Ueno: Co-existance of the *Fusarium* mycotoxins, nivalenol, deoxynivalenol and zearalenone, in korean cereals harvested in 1983. *Food Add. Contami.*, in press (1985)
52. W.F.O. Marasas, N.P.J. Kriek, S.J. van Rensburg, M. Steyn and G.C. van Schalkwyk: Occurrence of zearalenone and deoxynivalenol, mycotoxins produced by *Fusarium graminearum* Schwabe, in maize in Southern Africa. *South Afr. Sci.* 73:346, (1977)
53. P.G. Thiel, C.J. Meyer and W.F.O. Marasas: Natural occurrence of moniliformin together with deoxynivalenol and zearalenone in Transkei corn. *J. Agric. Food Chem.*, 30:308, (1982)
54. R.F. Vesonder, A. Ciegler, R.F. Rogers, K. A. Burmeister, K.J. Bothast and A.H. Jensen: Survey of 1977 crop year preharvest corn for vomitoxin. *Appl. Environ. Microbiol.*, 36:885, (1978)
55. H.M. Stahr, A.A. Kraft and M. Schuh: The determination of T-2 toxin, diacetoxyscirpenol, and deoxynivalenol in foods and feeds. *Appl. Spectr.*, 33:294, (1979)
56. W.H. Hagler, Jr., K. Tyckowska and P.B. Hamilton: Simultaneous occurrence of deoxynivalenol, zearalenone, and aflatoxin in 1982 scabby wheat from the midwestern United States. *Appl. Environ. Microbiol.*, 47:151, (1984)

57. L.M. Côté, J.D. Reynolds, R.F. Vesonder, W.B. Buck, S.P. Swanson, R.T. Coffey, and D.C. Brown: Survey of vomitoxin-contaminated feed grains in midwestern United States, and associated health problems in swine. *J. Amer. Vet. Med. Assoc.*, 184:189 (1984)
58. M. Jemmali, Y. Ueno, K. Ishii, C. Frayssinet and M. Etienne: Natural occurrence of trichothecenes (nivalenol, deoxynivalenol, T-2, and zearalenone) in corn. *Experientia,* 34:1333, (1978)
59. R. Puls and J.A. Greenway: Fusariotoxicosis from barley in British Colombia. II. Analysis and toxicity of suspected barley. *Can. J. comp.Med,*. 40:16, (1976)
60. S. Ghosal, D.K. Chakbabarti and K.C.B. Chaudhary: The occurrence of 12, 13-epoxytrichothecenes in seeds of safflower infected with *Fusarium oxysporum F.* sp. *carthami. Experientia,* 33:574, (1977)
61. I.C. Hsu, E.B. Smalley, F.M. Strong and W.E Ribelin: Identification of T-2 toxin in moldy corn associated with a lethal toxicoses in dairy cattle. *Appl. Microbiol.*, 24:684, (1972)
62. C.J. Mirocha, S.V. Pathre, B. Schauerhamer and C.M. Christensen: Natural occurrence of *Fusarium* toxins in feedstuff. *Appl. Microbiol., 32:553, (1976)*
63. C.J. Mirocha, B. Schauerhamer, C.M. Christensen and T. Kommedhal: Zearalenone deoxynivalenol and T-2 toxin associated with stalk rot in corn. *Appl. Environ. Microbiol.*, 38:557, :1979)
64. H.M. Stahr, P.F. Ross and W. Oboiha: Some mycotoxins levels in farmstored corn. *J. Agric. Food Chem.*, 29:207, (1981)
65. B. Harrach, A. Bata, E. Bajmocy and M. Benko: Isolation of satratoxins from the bedding straw of a sheep flock with fatal stachybotryotoxicosis. *Appl. Environ. Microbiol.*, 45:1419, (1983)
66. B. Harrach, A. Bata, F. Vezer and B. Burcucs: Isolation of verrucarin J, satratoxins G and H from *Stachybotrys atra* infected bedding straw of sports horses with mass disease. Proc. 9th Congr. Hung. Soc. Microbiol., Budapest, 23-25 August, 1983, p. 1401
67. B, Jarvis: personal communication.
68. All China Corporation of Research Wheat scab. Studies on *Fusarium* speciesrefecting wheat species in China. *Bull. Shanghai Teachers College,* 3 (1984) 69 (in Chinese).
69. Y. Xu, X. Huang and Y. Cai, Isolation and structure CBD_2-a new trichothecene toxin. *Acta. Microbiol. Sinica.*, 22:35, (1982) (in Chinese)
70. M. Ohta, K. Ishii, and Y. Ueno: Metabolism of trichothecene mycotoxins. I. Micosomal deacetylation of T-2 toxin in animal tissues. *J. Biochem.* (Tokyo), 82:1519, (1977)
71. J. Kobayashi, K. Ishii, N. Horikoshi, Y. Ueno, T. Tanaka, and A. Hasegawa: The hydroxyl reactions in the metabolism of T-2 toxin by hepatic cytochrome P-450, Proceeding of 105th annual meeting of Pharmaceutical Society of Japan. (1985).