Trichothecenes: Natural Occurrence and Potential Hazard

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ABSTRACT

The trichothecene toxins are a chemical group of fungal metabolites characterized by a tetracyclic 12, 13-epoxy-trichothec-9-ene skeleton. There are over 40 naturally occurring derivatives produced predominantly by species of *Fusarium* but also produced by species of *Cephalosporium, Myrothecium, Trichoderma,* and *Stachybotrys.* The trichothecene derivatives most commonly encountered in feedstuff are: T-2 toxin, diacetoxyscirpenol (DAS), deoxynivalenol (vomitoxin) and nivalenol. Deoxynivalenol has been isolated from corn and mixed feed more frequently than the other derivatives reported, and moreover, it is frequently found together with the fungal estrogen zearalenone. Deoxynivalenol has frequently been associated with corn refused by swine, vomiting in dogs, and corn used by humans in South Africa. Deoxynivalenol is produced by *Fusarium roseum*, an organism that is commonly found infecting corn in the field and corn stored on-the-cob in cribs in the Midwest. The trichothecenes have been implicated in diseases of man called alimentary toxic aleukia in the USSR and have been found in corn in the Transkei where the incidence of esophageal cancer is high. Among domestic animals, trichothecenes such as T-2 toxin, DAS and deoxynivalenol have been implicated in diseases characterized by a hemorrhagic syndrome in cows and swine, vomiting in swine and dogs, decrease in avian egg production and body weight, refusal of feed by swine and infertility in swine. The data on the residue of the administered T-2 toxin in domestic animals suggests that trichothecenes can be transmitted into humans.

INTRODUCTION

Fungi imperfecti of the genera *Fusarium, Mycrothecium, Trichoderma, Cephalosporium, Trichothecium, Verticimonosporium* and *Stachybotrys* produce a group of chemically related toxic compounds called trichothecenes, characterized by a tetracyclic structure with a specific stereochemistry as shown in Figure 1. About 45 derivatives have been isolated from cultures of these fungi. Among those isolated, over 20 are known to be produced by species of *Fusarium* alone. Recently, baccharin, a very potent antileukemic trichothecene, has been isolated from a higher plant, *Baccharis megapotamica* (Asteraceae) (1).

The chemistry, biochemistry, and toxicology of trichothecenes have been extensively reviewed (2-6). The role of trichothecenes as an etiologic agent in mycotoxicoses was first elucidated by a group of investigators at the University of Wisconsin (7). They demonstrated the presence of T-2 toxin, a trichothecene produced by *F. tricinctum,* in moldy corn associated with illness and death of lactating cows in a herd in Wisconsin. However, the involvement of trichothecenes in several sporadic outbreaks of mycotoxicoses described as moldy corn toxicosis (7,8), alimentary toxic aleukia (ATA) (9), stachybotryotoxicosis (10,11) and fescue foot disease (12) was suspected for some time (2) because the signs of those diseases resembled toxicity as incited by trichothecenes. Wyatt et al. (13,14) reported signs in poultry which were identical to those produced by T-2 toxin in laboratory tests and strongly suggested the actual presence of at least some trichothecene derivative. In another report, Eppley et al. (15) tested 173 samples of corn collected from various grain elevators in the Midwest and found that 94 (54%) contained a "skin irritating factor", presumably T-2 toxin, ranging in concentration from 0.05 to 1.0 μg/g of corn (0.05-1.0 ppm). The presence of T-2 toxin was not demonstrated, however.

There are a number of cases reported describing the occurrence of trichothecenes in feedstuffs naturally contaminated. In this paper those cases have been reviewed in an attempt to elaborate the role of trichothecenes in mycotoxicoses and to evaluate the potential hazards of these natural toxic contaminants.

Natural Occurrence

Evidence for involvement of any trichothecene in moldy corn toxicosis was considered to be circumstantial until Hsu et al. (7) found 2 ppm of T-2 toxin in moldy corn associated with a lethal toxicosis in dairy cattle. In Japan several outbreaks of "red mold toxicoses" of humans and animals were described (16) and attributed to ingestion of grain infected by *Fusarium.* Although no trichothecene was isolated from the grain actually involved in the intoxication, a sample of barley naturally infected with *F. roseum* was shown to be contaminated with two trichothecenes, deoxynivalenol and nivalenol in the concentrations of 6.23 and 7.15 ppm, respectively (17). During recent years in the midwestern United States, corn infected with *F. roseum,* as well as other species of *Fusarium,* caused multiple problems when fed to swine: (a) hyperestrogenism, (b) emesis, and (c) refusal of feed. Such corn was associated with wet weather that preceded harvest. Vesdonder et al. (18,19) identified deoxynivalenol as one of the trichothecenes present in the corn causing emesis and refusal of feed. Hibbs et al. (20) reported the presence of T-2 toxin in a commercial pellet feed involved in a case of hemorrhagic syndrome in cattle. A moldy grain toxicosis involving ducks, geese, horses and swine has been noted by Greenway and Puls (21) in British Columbia, Canada. The clinical signs described by them included emesis and refusal of the feed in swine, fever and excessive salivation in horses, and emesis, thirst and death in ducks and geese; visible lesions of the esophagus, proventriculus, and gizzards of geese were

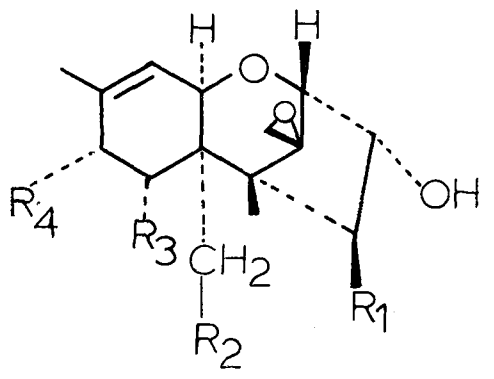

FIG. 1. Naturally occurring trichothecenes.

	R_1	R_2	R_3	R_4
Deoxynivalenol	H	OH	OH	=0
Nivalenol	OH	OH	OH	=0
T-2 toxin	$OCOCH_3$	$OCOCH_3$	H	-OCO CH_2-CH(CH_3)$_2$
Diacetoxyscirpenol	$OCOCH_3$	$OCOCH_3$	H	H

TABLE I

Natural Occurrence of Trichothecene Toxins in Feedstuff

Sample No.		Concentration μg/kg	Diagnosis	Feedstuff
FS-382	Diacetoxyscirpenol	500	Hemorrhagic bowel syndrome in swine	Mixed feed (Univ. Minn.)
FS-404	Diacetoxyscirpenol	380	Hemorrhagic bowel syndrome in swine	Mixed feed (Univ. Minn.)
FS-356	Deoxynivalenol[a]	1,800	Feed refused by swine	Maize kernels (Michigan)
FS-362	Deoxynivalenol	1,000	Feed refused by swine	Maize kernels (Indiana)
FS-398A	Deoxynivalenol	100	Feed refused by swine	Maize kernels (Ohio)
FS-463	Deoxynivalenol	40-60	Feed refused by swine and bloody stools	Commercial pelleted mixed feed
FS-417	T-2 toxin	76	Bloody stools, bovine	Mixed feed (Nebraska)
FS-483	Deoxynivalenol	1,000	Vomiting in dogs	Mixed feed (Iowa)
FS-489	Deoxynivalenol	1,000	Feed refused by swine	Mixed feed (Minnesota)
FS-416A	Deoxynivalenol	2,500	--	Maize kernels (So. Africa)
FS-516B	Deoxynivalenol	7,400	---	Maize kernels (Zambia)
FS-543B	Deoxynivalenol	75	Cows-CNS irritations irregular heat cycles	Maize (Nebraska)
FS-570A	Deoxynivalenol	25	Feed refused by swine	Ground corn (New York)
FS-570C	Deoxynivalenol	200	Dogs-refusal, emesis	Dry extruded dog food (NY)
FS-570D	Deoxynivalenol	120	Feed refused by swine	Ground corn (New York)
FS-570E	Deoxynivalenol	550	Feed refused by swine	Maize kernels (New York)

[a]Also referred to as vomitoxin (18,19).

also noted. This toxicosis was linked to T-2 toxin present at a level of ca. 25 ppm in barley used in the feedstuffs (22), although some question was raised as to the authenticity of the toxin identified.

A number of undiagnosed cases of animal toxicoses pass through veterinary diagnostic laboratories, which have no explanation as to causality except perhaps that the feed might be implicated; the signs of intoxication include abortion, diarrhea, emesis, loss of weight gain, hemorrhagia and death. Routinely, these feed samples are analyzed for toxic fungi and toxic components. During the past four years, we analyzed over 200 such samples of feeds and feedstuffs associated with such toxicoses in animals. Trichothecene mycotoxins were identified in some of these samples and are presented in Table I (23).

Sample No. FS-382 and FS-404 were mixed feed obtained from the College of Veterinary Medicine of the University of Minnesota and were associated with an idiopathic condition in swine called hemorrhagic bowel syndrome (24). Each of the samples represented a separated incidence of this disease which occurred among experimental animals at the University. Diacetoxyscirpenol, another trichothecene produced by species of *Fusarium*, was isolated from these samples and was the only toxic component found. Weaver et al. (25) reproduced hemorrhagic bowel lesions in swine by the administration of pure diacetoxyscirpenol intravenously.

Sample No. 417 was associated with bloody stools in cattle; analyses revealed 78 ppb of T-2 toxin.

Four whole kernel corn samples (FS-356, 363, 398) and one commercial mixed feed sample (FS-463), which were refused by swine, were referred to our laboratory for analysis. The corn samples originated in Michigan, Indiana, and Ohio and the mixed feed in Minnesota. Analysis showed the presence of deoxynivalenol (vomitoxin) at levels from 50 to 1800 ppb (the percentage recovery was not estimated). The identity of deoxynivalenol was authenticated by mass spectroscopy. Although Vesonder et al. (18,19) reported deoxynivalenol in corn, this was the first report of its occurrence in a commercial feed sample. All samples analyzed also contained zearalenone; isolates of *F. roseum* which produce deoxynivalenol usually synthesize zearalenone.

Samples consisting of hand-selected 100% moldy maize kernels (FS-516B) from 1973/74 Zambian crop and from maize harvested in South Africa (FS-516A) were found to be heavily contaminated with deoxynivalenol and zearalenone (26).

A corn sample (FS-543B) associated with intoxiciation in cows was submitted by the Veterinary Clinic in Arizona. The clinical signs described were central nervous system irritations, muscle tremors, irregular heat cycles and even death. We found the sample to be contaminated with deoxynivalenol; zearalenone was also detected in this sample. Samples FS-570A-D associated with feed refusal and emesis were contaminated with deoxynivalenol and zearalenone; the sample FS-570A which had been totally refused by swine was found to contain rather low amounts of deoxynivalenol.

Deoxynivalenol and zearalenone were also found in maize samples in some areas in the Transkei. These samples were collected in the areas of incidences of esophageal cancer in human, and were naturally contaminated with *Fusarium* (W.F.O. Marasas, private communication).

Recently, Siegfried (27) reported that he isolated 31.5 ppm of diacetoxyscirpenol from maize (used as feed); however, a detailed account of this occurrence is not given.

Potential Hazards

Toxicological problems in humans and animals associated with consumption of moldy grains have long been recognized; and those particularly suspected of being trichothecene-toxicoses are listed in Table II. Moldy corn toxicosis as described by Hsu et al. (7) and Smalley et al. (8) in northern climates is associated with many different fungi, predominant among which is *F. tricinctum*. The signs of this disease are similar to those described by Forgac (10), namely general digestive disorders, bloody diarrhea, and hemorrhagic lesions in the stomach, heart, intestine, lungs, bladder and kidneys.

ATA in man was described by Joffe in the Soviet Union (9). This disease was associated with moldy millet primarily, but also with wheat, rye, oats, and buckwheat. The signs described were "typical spots on the skin, leukopenia, agranulocytosis, necrotic angina, hemorrhagic diathesis, sepsis and exhaustion of the bone marrow." Of the fungi involved, *F. sporotrichioides* (sny. *F. tricinctum*) and *F. poae* were thought to be involved, and the toxins were described as poaefusarin and sporofusarin, steroidal compounds. More than 10% of the population in the Orenburg district of the Soviet Union was affected at one time.

The symptoms and signs of this disease resembled toxicity of the trichothecenes as reported in the United States. An authentic sample of poaefusarin was obtained and analyzed for the steroids. Modern analytical methods were used to identify the constituents, but no trace of a steroid could be found. However, 2.5% of the sample was

TABLE II

Role of Trichothecenes in Mycotoxises

Toxicosis	Location	Animals affected	Fungus	Mycotoxins
Taumelgetreide (45)	Siberia	Horse	*G. saubinetti*	Trichothecenes
Alimentary toxic aleukia (9)	USSR	Man	*F. sporotrichioides*	T-2 toxin
Stachybotryotoxicosis (10)	Europe	Horse	*Stachybotrys atra*	Satratoxins
Bean-hull toxicosis (46)	Japan	Horse	*F. solani*	T-2 toxin, Neosolaniol
Red-mold toxicosis (16)	Japan	Horse, Swine, Cow	*F. graminearum*	Deoxynivalenol, Nivalenol
Moldy corn toxicosis (2,3)	USA	Swine, Cow	*F. roseum* *F. tricinctum*	Deoxynivalenol, T-2 toxin, Diacetoxyscirpenol
Oesophageal cancer (27)	Transkei	Man	*F. graminearum* *F. moniliforme*	Deoxynivalenol
Degnala disease	India	Cow	*F. equiseti*	Trichothecenes
Dendrodochiotoxicosis[a] (47)	Europe	Horse	*D. toxicum*	Trichothecenes
Fusariotoxicosis (21)	Canada	Ducks, Geese, Horse, Swine	*Fusarium* spp.	T-2 toxin

[a]P.G. Tulpule, private communication.

made up of the T-2 toxin, an amount sufficient to explain the toxicity found in the rat and rabbit skin tests. In addition, neosolaniol (0.14%), T-2 tetraol (0.6%), and zearalenone (0.43%) were present as constituents of the sample (28).

The role of deoxynivalenol has been in part elucidated as one of the causative agents of emesis and feed refusal in swine. Fusariotoxicosis in Canada has been attributed to T-2 toxin since the latter was present in large amounts in the barley consumed by the affected population. Toxicosis such as Taumelgetreide, Stachybotryotoxicosis, Dendrodochiotoxicosis, Bean-hull toxicosis are often linked with trichothecene-toxicoses since the fungus responsible for contamination elaborates trichothecenes in pure cultures; however, no evidence demonstrating the presence of trichothecene in the field samples suspected of causing the toxicosis has yet been reported. All these cases of intoxication have a common denominator; i.e., the signs and symptoms described are similar to those induced in animals by administering known pure trichothecene or cultures of known trichothecene-producing fungus, mainly *Fusarium*.

Definitive studies on the effects of specific trichothecene in domestic farm animals are rare for most trichothecenes, although some studies have been conducted by feeding whole cultures of known trichothecene-producing *Fusarium* species (7,29,30), pure T-2 toxin (31) and deoxynivalenol (32) to animals. On the basis of information on the cause-and-effect relationship available today, the toxin concentration of the naturally occurring trichothecenes in contaminated feedstuffs so far reported does not appear to be high enough to explain the natural symptomatology or that induced in experimental animals given pure toxins. This discrepancy can be attributed to the problems associated with analyses and probably synergistic effects of more than one toxin. Apart from this quantitative aspect of the occurrence, the qualitative relationship between symptomatology and the occurrence is more than coincidental, and is sufficient to cause concern about the potential hazard of these highly toxic natural contaminants.

The toxicity studies have indicated that most trichothecenes are acutely toxic to animals (2,6). Recent studies on the distribution of radiolabeled T-2 toxin in swine (33) and chickens (34) have indicated that a measurable portion of the radioactivity was retained in the edible tissues. Moreover, ca. 18 ppb of the radioactive T-2 toxin administered was transmitted into the edible portion of the egg in laying hens (35); 15 to 80 ppb of T-2 toxin administered was shown to be transmitted into the cow's milk when a pregnant holstein cow was intubated with T-2 toxin (36).

The radioactivity distribution experiments do not lend themselves to establish the identity of the residue as T-2 toxin. However, these experiments suggest that there exists a potential risk of transmitting trichothecenes or metabolic derivatives into humans since meat, eggs and milk constitute main items of diet. The in vivo experiments have demonstrated the presence of T-2 toxin and its derivatives such as neosolaniol, HT-2 toxin, and T-2 tetraol in the excreta of broiler chicks dosed with T-2 toxin (37). The dried poultry excreta is often used as an animal feed ingredient in some poultry operations. Since the toxins in the ingested feed are concentrated in the excreta (34,38), the excreta becomes a potential reservoir for trichothecenes. There is little data available on the chronic effects of trichothecenes at subacute levels except in swine. Swine fed T-2 toxin at 12 ppm for three months developed extreme congestion in the intestines and bile duct and signs of infertility (39). It has also been found that animals dosed with trichothecenes become vulnerable to infectious diseases (40). Such predisposition of animals to secondary diseases further complicates the problem.

Schoental (41) reported that pure crystalline T-2 toxin is carcinogenic to rats, in which it induced benign and malignant tumors of the gastrointestinal tract, pancreas, and brain. She also suggested that the irritant metabolites of *Fusaria* may play a role in the development of tumors of the digestive tract (42,43,44). Deoxynivalenol has been found in corn used by humans in the Transkei where the incidence of esophageal cancer is high. Additional experiments might elucidate more clearly what role deoxynivalenol and perhaps other trichothecenes have in this disease.

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