SYMPOSIUM: BIOLOGICAL ACTION OF MYCOTOXINS

Biological Action of Mycotoxins[1]

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ABSTRACT

Mycotoxins are ubiquitous, mold-produced toxins that contaminate a wide variety of foods and feeds. Ingestion of mycotoxins cause a range of toxic responses, from acute toxicity to long-term or chronic health disorders. Some mycotoxins have caused outbreaks of human toxicoses, and at least one mycotoxin, aflatoxin B_1, is a presumed human hepatocarcinogen. As part of a comprehensive effort to curtail the adverse health effects posed by mycotoxins, substantial research has been conducted to determine the mechanism of action of mycotoxins in animals. This review presents some of the current knowledge on the biological action of four diverse classes of mycotoxins—aflatoxin B_1, tricothecenes, zearalenone, and fumonisin B_1—with particular emphasis on mechanisms of action.
(**Key words**: mycotoxin, mold, toxicity, biological action)

Abbreviation key: **AF** = aflatoxin (also used with B_1, M_1, Q_1, and P_1), **AFB_1–FAPyr** = AFB_1–formamidopyrimidine adduct, **AFB_1–N^7-Gua** = AFB_1–N^7-guanine adduct, **AFL** = aflatoxicol (used with H_1 and M_1), **ELEM** = equine leukoencephalomalacia, **FB_1** = fumonisin B_1, **GSH** = glutathione, **HCC** = hepatocellular carcinoma, **ZEN** = zearalenone, **γGT^+** = γ-glutamyl-transpeptidase-positive.

INTRODUCTION

Thousands of natural toxins exist that have known or potential adverse health effects in humans and animals. Natural toxins have been termed "nature's pesticides" because they often confer a protective or competitive advantage to the organism or plant that produces them (2). Because the diet contains at least 10,000 times more natural toxins exist compared with synthetic toxins (manmade toxins, such as pesticides or environmental chemicals), natural toxins probably pose the greater threat to human and animal health (1). One large group of natural toxins that are nearly universal contaminants of food and feed are the mycotoxins, the toxic secondary metabolites produced by fungi.

Mycotoxins are a diverse group of chemicals that elicit a wide range of toxic responses in animals and humans. Pre- or postharvest contamination of various food crops by mycotoxigenic fungi is a common problem; approximately 25% of the world's food supply is contaminated by mycotoxins annually (19). The severity of mycotoxin contamination of agricultural commodities varies yearly. Excessive moisture in the field and in storage, temperature extremes, humidity, drought, variations in harvesting practices, and insect infestation are major environmental factors that determine the severity of mycotoxin contamination. Although the actual resultant economic loss to agriculture is difficult to determine with accuracy, it is likely to be high.

In domestic animals, such as dairy cattle, swine, and poultry, mycotoxin contamination reduces growth efficiency, lowers feed conversion and reproductive rates, impairs resistance to infectious diseases, reduces vaccination efficacy, and induces pathologic damage to the liver and other organs. Many mycotoxins have been implicated in outbreaks of human diseases. Some are potent animal, and presumed human, carcinogens. For these reasons, mycotoxins pose a major threat to public and animal health, and determination of the biological mechanism of action of mycotoxins has

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understandably been the focus of much research. The biological action of four classes of mycotoxins that represent diverse toxicities to animals—aflatoxin (**AF**)B_1, tricothecenes, zearalenone (**ZEN**), and fumonisin B_1 (**FB_1**)—are considered in this overview.

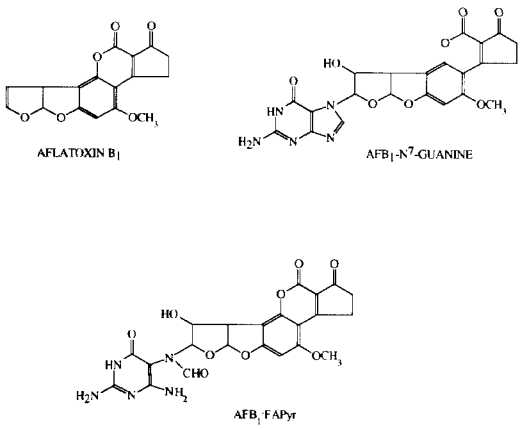


Figure 1. Chemical structure of aflatoxin (AF) AFB_1 and DNA adducts of AFB_1, AFB_1-N^7-guanine and formamidopyrimidine (AFB_1-FAPyr).

AFB_1

Aflatoxin B_1 (Figure 1) represents a group of mycotoxins produced by strains *Aspergillus flavus* and *Aspergillus parasiticus*. Of the known mycotoxins, AFB_1 had generated the greatest concern and has stimulated the most research effort because of its extreme toxicity and its widespread occurrence in staple foods and feeds (such as peanuts, corn, and cottonseed). For these reasons, AFB_1 currently is the only mycotoxin that is regulated by the FDA. In foods, the current "action level" (the concentration above which the commodity is condemned) is 20 ppb of total AF. The action level for the AFB_1 metabolite AFM_1 in milk is .5 ppb. Other regulatory guidelines for AFB_1 include 20 ppb in corn for dairy cows, 300 ppb in corn for finishing beef cattle and swine, and 100 ppb for breeding stock (24). The permissible amount of AFB_1 in cottonseed for beef cattle, swine, and poultry is 300 ppb (55). These regulatory levels generally preclude detectable AFB_1 in the various products from these animals. Worldwide, established tolerances for AFB_1 in animal feeds range from 10 to 600 ppb (65).

Prevention of *Aspergillus* infection in foods and feeds is the most desirable method of reducing AFB_1, although AFB_1 contamination often is unavoidable, even with the best agricultural practices. Therefore, several strategies have been developed to reduce postharvest product contamination of AFB_1. Some of these methods involve early identification and segregation of grossly contaminated kernels of corn or peanuts or use of electronic devices to identify and to reject grains that exhibit fluorescence that is due to AFB_1. Although these methods have been useful for peanuts, they have not been practical for decontaminating corn and cottonseed (54). Ammoniation has shown considerable promise as a means of treating contaminated cottonseed and corn because it can be used to treat large batches of product, and, importantly, ammoniation results in nearly complete elimination of AF and associated toxicity in commodities (54). Ammoniation has not yet achieved FDA approval for interstate shipments, but it is in use in some states. Another experimental strategy is the use of inorganic absorbent feed additives, which prevent absorption of mycotoxins in animals. For example, when added to feeds contaminated by AFB_1, hydrated sodium calcium aluminosilicate, an anticaking agent approved by the FDA, significantly reduced bioavailability of AFB_1 and many of the AFB_1-specific toxic effects in pigs (14).

A requisite step in the toxic and carcinogenic action of AFB_1 is its conversion to a variety of metabolites (Figure 2). Metabolism of AFB_1 is mediated principally by hepatic and extrahepatic microsomal cytochromes P-450, although other conversions have also been described (16). Most of the metabolic products are less toxic than parent AFB_1, the most prevalent of which is AFM_1, so named for its appearance in the milk of dairy cows that consume feed contaminated by AFB_1. Other detoxified metabolites produced from the P-450 oxidation of AFB_1 include AFQ_1 and AFP_1 (Figure 2).

Aflatoxin B_1 also is reduced by soluble NADPH-dependent cytosolic enzymes to produce aflatoxicol (**AFL**). Because this reaction is reversible, AFL may be a storage form of AFB_1 (56). The chronic and acute toxicity of

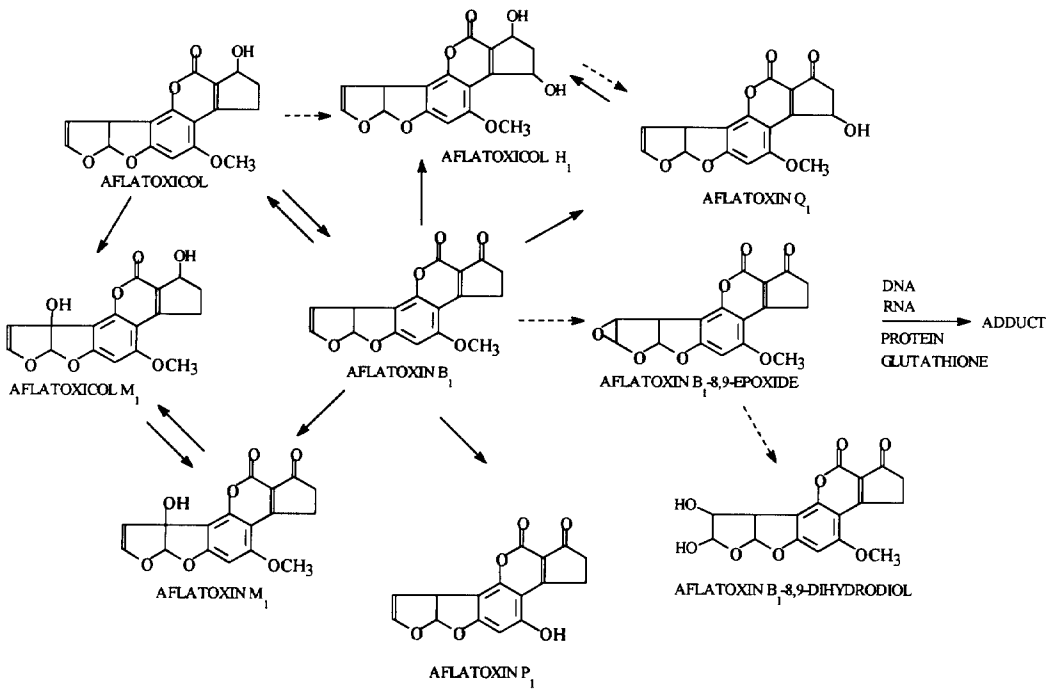


Figure 2. Metabolic scheme of aflatoxin B_1. The solid lines represent known pathways; the dashed lines signify presumed or proposed pathways.

AFL is equal to that of AFB_1; thus, this metabolite is not considered to be a detoxified metabolite. Furthermore, the mutagenic potency of AFL is nearly equal to that of AFB_1 (17). Two minor and relatively nontoxic reduced pentanone metabolites also exist, AFL-M_1 and AFL-H_1. The former metabolite also may be formed by the cytosolic reduction of AFM_1 (64).

Aflatoxin B_1 also is metabolized by cytochromes P-450 to the reputed proximate carcinogen, the AFB_1-8,9-epoxide. Presumably because of its extreme reactivity, the AFB_1-8,9-epoxide has been isolated only indirectly from biological systems as adducts of glutathione (**GSH**) or DNA bases. The AFB_1-8,9-epoxide can be inactivated by GSH, a reaction catalyzed by GSH S-transferase. Inactivation by GSH is an important AFB_1 detoxification pathway in a number of species, and the formation of AFB_1-GSH protects against the hepatocarcinogenic effects of AFB_1 (22, 43). Detoxification mediated by GST may be an important determinant in species resistance to AFB_1. Aflatoxin B_1 also may be detoxified via conjugation with sulfates and glucuronic acid (11). The AFB_1-8,9-epoxide also may be catalytically (by epoxide hydrolase) or spontaneously hydrolyzed to the AFB_1-8,9-dihydrodiol.

The carcinogenic and mutagenic action of AFB_1 probably is a result of the affinity of the electrophilic and highly reactive AFB_1-8,9-epoxide for cellular nucleophiles, such as DNA. Activated AFB_1 binds exclusively to guanyl residues, and the AFB_1-N^7-guanine adduct (**AFB_1-N^7-Gua**) is the most predominant (Figure 1). Other adducts have been isolated, of which the "ring-opened" derivative of AFB_1-N^7-Gua, AFB_1-formamidopyrimidine (**AFB_1-FAPyr**), is the most common (Figure 1). In hepatic DNA from livers of rats injected with AFB_1, approximately 80% of the adducts present are AFB_1-N^7-Gua, whereas the AFB_1-FAPyr constitutes approximately 7% (23). The formation of these adducts is the presumed first step in the development of heritable mutations from which tumors may arise. Repair of these genetic lesions occurs in living cells enzymatically or spontaneously, and the removed adducts is excreted in the urine.

The ring-opened adduct appears to be more resistant to DNA repair enzymes. In rats treated with a single dose of AFB_1, the AFB_1-N^7-Gua was rapidly removed with an apparent half-life of 7.5 h, whereas other adducts, such as AFB_1-FAPyr, were removed much more slowly (20).

Alkylation of DNA with AFB_1 also may result in the loss of a DNA base, resulting in an "apurinic site". In synthetic oligomers treated with AFB_1, at least twice as many apurinic sites were detected as AFB_1-N^7-Gua (13). Which of these genetic lesions ultimately is responsible for the carcinogenic action of AFB_1 is not known. Microsomally activated AFB_1 induced GC is converted to TA transversions in a strain of *Escherichia coli*, presumably as a result of AFB_1-N^7-Gua formation (26). In any event, AFB_1-specific mutations also exist in cellular protooncogenes and other loci in tumors from animals treated with AFB_1 (67).

The metabolism of AFB_1, specifically the balance between activation and detoxification pathways in an animal, and the formation and repair of genetic lesions induced by this mycotoxin appear to be critical determinants of species sensitivity. Specific relationships have been derived from a number of comparative studies in which sensitive species (rat and rainbow trout) have been compared with resistant species (mouse and salmon) with respect to endpoints that define these processes. For example, the in vivo binding of AFB_1 to hepatic DNA was 40 times greater in the rat than in the more resistant mouse (45). A likely mechanism underlying the relative resistance of the mouse is that it efficiently detoxifies the AFB_1-8,9-epoxide via GSH S-transferase (50).

Toxic effects of AFB_1 are either acute or chronic, depending in large part on the dose and duration of exposure. Aflatoxin B_1 is acutely toxic to a number of cell types, plants, invertebrates, and various vertebrate species. Cattle, swine, and poultry are the farm animals primarily affected by AFB_1. Common observations 1 to 2 d following acute exposure to AFB_1 include malaise, loss of appetite, and eventually lower growth rates. Because many of these sequelae are not specific for AF, field diagnoses often are difficult. Approximately .2 ppm of AFB_1 causes reduced weight gains in cattle, swine, and poultry; between 2 and 10 ppm of AFB_1 resulted in decreased egg production, hepatic necrosis, hemorrhage, and death in poultry (57).

In the bovine, acute AFB_1 decreased rumen motility (31). In a review on that subject, studies were described showing that AFB_1 reduced cellulose breakdown and production of VFA and ammonia either in vivo or in artificial rumen environments (47). Decreases in feed conversions, breeding efficiency, and milk production and health problems resulted when a dairy herd was exposed chronically to corn contaminated by AF at 120 ppb (31).

The liver is the organ most severely affected by AFB_1, and the primary lesions include hemorrhagic necrosis, fatty infiltration, and bile duct proliferation. In pigs, guinea pigs, and dogs, these effects most commonly occur in the centrilobular region, whereas in ducklings and rats, the periportal region is the site of action. Acute toxicities for AFB_1 have been tabulated extensively (11). In vertebrate species, at least a 10-fold variation occurs in susceptibility to the acute effects of AFB_1, and no species appear to be totally resistant. Poultry, rainbow trout, and monkeys are particularly sensitive to the acute effects of AFB_1; oral median lethal doses are .34 to .56, 3.0, and .81 mg/kg, respectively. The mouse, hamster, and rat are much less sensitive; these species have oral median lethal doses of 9.0, 10.2, and 1.3 mg/kg, respectively (56). Significant differences occurred in potency of AFB_1 in age, strain, gender, and route of administration.

Aflatoxin B_1 is carcinogenic in a wide variety of animals. As is the case following acute exposures, the major target organ is the liver, although tumors in other organs result from long-term dietary exposure to AFB_1. Aflatoxin B_1 at .4 ppb fed over 14 mo resulted in a 14% incidence of hepatocellular carcinomas (**HCC**) in rainbow trout, the animal species known to be most sensitive to the carcinogenic effects of this mycotoxin (42). By contrast, tumor incidence was only 5% during a similar time in Fischer rats exposed to 5 ppm of AFB_1 (83).

Considerable epidemiological data also support the hypothesis that dietary AFB_1 is an important risk factor for human HCC. In many geographical areas where incidence of HCC is high, such as sub-Saharan Africa and Southeast Asia, a linear relationship exists between AFB_1 contamination of food and the incidence

of HCC (78). However, a firm determination of the role of AFB_1 in human cancer is confounded by the coincidence of hepatitis B virus infection, which is another reputed factor for HCC in humans in these regions. Further confirming evidence on the role of AFB_1 in HCC has been obtained through the use of new, sensitive methods to detect specific "biomarkers" of human exposure to AFB_1, such as adducts of DNA (3, 30), or serum albumin (63). Measurement of these biomarkers in samples of blood or urine has allowed direct determination of actual AFB_1 exposure in populations, which is an improvement over random dietary analysis for AFB_1 and imprecise dietary recall surveys. Thus, the use of biomarkers should significantly improve AFB_1 risk assessment.

Although the majority of interest in the possible health effects has correctly focused on dietary exposure to AFB_1, workers in food and grain production and in harvest, transport, and processing industries also are exposed to considerable amounts of airborne, respirable grain dusts contaminated by AFB_1. For example, airborne dust sampled in a corn processing plant contained 107 ng/m^3 AFB_1, and the daily occupational exposure to this toxin was estimated to be between 40 and 856 ng (10). In another survey, concentrations of AFB_1 in smaller, more easily retained airborne grain particles, contained more AFB_1 than that in larger grain particles; AFB_1 in particles under 7 μm were as high as 1814 ppb, whereas AFB_1, ranging from 7 to 11 μm had an average of 695 ppb (69).

A small number of studies indicate that exposure to airborne AFB_1 may adversely affect health of those exposed. For example, airborne peanut dust contaminated with AFB_1 was linked to increases in liver and lung cancer in Dutch peanut processing workers relative to unexposed cohorts (35). An earlier survey established that these workers were exposed continually to between .04 and 2.5 μg of AFB_1/wk (77). In laboratory studies, AFB_1 was converted to stable metabolites and activated to mutagenic and DNA binding intermediates by cells of the mammalian respiratory epithelium (5, 6, 18). In cultured airway epithelia from a variety of mammalian species, AFB_1 forms AFB_1-N^7-Gua and AFB_1-FAPyr in patterns similar to those in hepatic systems (6).

TRICOTHECENES

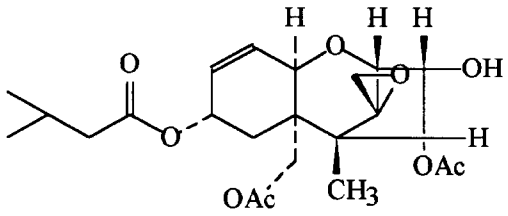
Tricothecenes are a group of over 150 structurally related compounds produced by several genera of fungi, the most important being *Fusarium*. *Fusarium sporotrichioides* and *Fusarium graminearum* are the most common tricothecene producers. In this country, tricothecenes commonly infect corn grown in the upper Midwest. Low temperatures, high moisture, and humidity appear to increase toxin production. Tricothecenes possess the tetracyclic 12,13-epoxy-tricothecane skeleton of the sesquiterpenoids (75). Examples of important tricothecenes include T-2 toxin, nivalenol, and deoxynivalenol (Figure 3). Of these compounds, T-2 toxin has been the most widely studied, because it was one of the first tricothecenes discovered in grains, although deoxynivalenol (vomitoxin) is the most common contaminant of cereal grains such as wheat and barley in many countries worldwide (74).

The toxic potency of several tricothecenes in laboratory animals has been tabulated (74, 75). The most acutely toxic tricothecene, verrucarin J, had a median lethal dose of .5 mg/kg i.v. in mice. In mice, toxin T-2 and nivalenol are nearly equitoxic; median lethal doses are 5.2 and 4.1 mg/kg (i.p.), respectively. The in vivo toxicity of T-2 toxin and nivalenol are approximately 10 times that of deoxynivalenol. In a variety of in vitro systems, T-2 consistently has been the most toxic, followed by nivalenol and then deoxynivalenol (75).

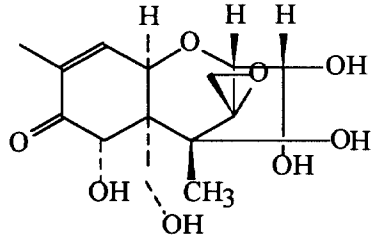
The tricothecenes produce a wide variety of toxic effects: acute tricothecene toxicity is characterized by gastrointestinal disturbances, such as vomiting, diarrhea, and inflammation. Dermal irritation, feed refusal, abortion, and hematological sequelae, such as anemia and leukopenia, also are common. In cattle, dietary T-2 toxin at .64 ppm for 20 d resulted in death and bloody feces, enteritis, and abomasal and ruminal ulcers (57). In poultry, as low as 5 ppm of tricothecenes resulted in oral necrosis and reduced BW gains (12). Egg production and shell quality were decreased when poultry were fed a diet of 20 ppm of T-2 toxin (57). Tricothecenes also are toxic when administered dermally. Toxic reactions, which were similar to those observed when the tricothecenes were administered systematically, were observed

when applied doses ranged from 10^{-11} mol (for T-2 toxin, verrucarin A) to 10^{-7} mol (deoxynivalenol) (75).

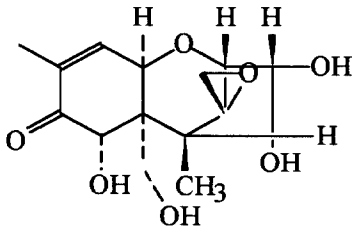
Alimentary toxic aleukia, which is the most common human tricothecene mycotoxicosis, follows a multistage pathogenesis. Shortly after ingestion of contaminated cereal grains, a burning sensation in the mouth, tongue, throat, esophagus, and stomach and gastrointestinal disturbances are the initial observations (66). A



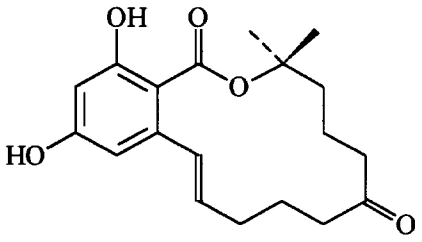
T-2 TOXIN



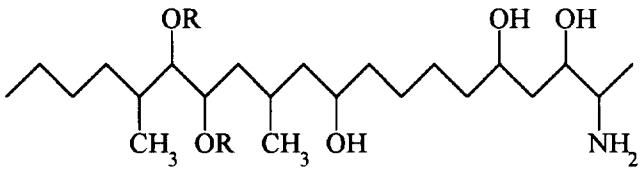
NIVALENOL



DEOXYNIVALENOL



ZEARALENONE



R= $COCH_2CH(CO_2H)CH_2CO_2H$

FUMONISIN B_1

Figure 3. Chemical structures of T-2 toxin, nivalenol, deoxynivalenol, zearalenone, and fumonisin B_1. AC = Acetate.

severe leukopenia and granulopenia may then follow, which may progress to white cell counts as low as 100 cells/μl of blood (66). Continuous exposure to trichothecenes then results in rashes on the skin, which may progress to severe necrotic lesions.

The actual mechanisms of these toxic actions of the tricothecenes are not clear. Investigators studying the mechanisms of toxicity thus far have focused on the effects of these mycotoxins on protein and macromolecular synthesis, membrane function, and immune parameters. Ueno et al. (76) initially observed that a number of tricothecene mycotoxins inhibited protein synthesis in eukaryotic cells. The toxin inhibited all steps in protein synthesis (initiation, elongation, and termination) in intact ribosomes (21). Structure-activity studies indicated that the C-4 or C-5 positions or the isovaleryl group at the C-8 position of T-2 were important for inhibition of protein synthesis in vitro because this mycotoxin was more potent than HT-2, neosolaniol, or T-2 tetrol (72). A substituent at the C-3 position to T-2, such as an acetyl group, making acetyl T-2 for example, resulted in a diminution of inhibition of in vitro protein synthesis. Protein synthesis inhibition likely is a result of binding of the toxin to ribosomes. The binding of T-2 to ribosomes is specific and saturable (.3 n*M*), and the stoichiometry is one toxin molecule bound per ribosome (48).

Because T-2 toxin is an amphipathic molecule, it could be incorporated into the lipid or protein moieties of cellular plasma membranes, thereby interfering with membrane function (59). The in vitro treatment of L-6 myoblasts with 8.5×10^{-12} *M* T-2 toxin reduced the uptake of Ca, glucose, Leu, and Tyr within 10 min of exposure (9). These effects were independent of protein synthesis inhibition. Effects were similar for erythrocytes treated with T-2 but at a much higher concentration (32). Recently, human fibroblasts exposed to T-2 (1 μ*M*) accumulated lucifer yellow dye to the same extent as control cells, indicating that T-2 had no effect on membrane function, at least with respect to this indicator (40).

The toxin significantly alters several immune parameters, and the major effects appear to be associated with the cellular immune response, specifically including inhibition of the mitogen response, reductions of protein, DNA, and interleukin-2 synthesis in normal spleen cells treated with concanavalin A (73), thymic atrophy (60, 74), and reductions in plaque-forming spleen cells (73). In animals, resistance to many challenges that are dependent on cellular immune-response was decreased, such as resistance to mycobacterial infections and skin grafts in mice (37, 61), *Salmonella* in mice (71), and aspergillosis in rabbits (52). The effects of tricothecenes on the immune system have been reviewed recently (66).

ZEN

Zearalenone is a phenolic resorcyclic acid lactone (Figure 3) produced by strains of *Fusarium*, primarily by *F. graminearum*, *F. sporotrichiodes*, and others. Zearalenone is a natural contaminant of corn, wheat, barley, oats, sorghum, and hay. Toxin production is promoted by high humidity and low temperatures, as is common in the upper Midwest during autumn harvest. Zearalenone is a nearly universal corn contaminant and often occurs in the same samples with tricothecenes (44). Despite its dissimilarity to steroid compounds, ZEN produces potent hyperestrogenic responses in susceptible animals. Thus, the toxicity of this compound is unique among known mycotoxins.

Swine are most affected by ZEN, but other animals, such as cattle, poultry, and laboratory animals, are also affected, but to a lesser degree. Symptoms of ZEN poisoning include uterine enlargement and swollen vulva and mammae (51). In pigs, symptoms of hyperestrogenism generally appear when contamination of ZEN in corn exceeds 1 ppm, but it can occur at concentrations as low as .1 ppm (49). In ewes, ZEN exposure resulted in declines in ovulation rate and cycle length and an increase in duration of estrus but did not affect pregnancy rate or embryonic loss (68). Young male pigs exposed to ZEN undergo symptoms of "feminization", such as enlarged nipples and testicular atrophy (51).

Dairy heifers exposed to ZEN have reduced conception rates (19). However, transmission of ZEN or its metabolites into milk appears to be minimal (58). In rats, decreased growth rate, food intake, fertility, resorptions, stillbirths, abortion, and bone malformations in fetuses also are sequelae of ZEN ingestion at

10 mg/kg (4). Egg production in hens is reduced by ZEN (8).

Zearalenone elicits permanent reproductive tract alterations. In newborn female mice, ZEN treatment (1 μg/d for 5 d) resulted in significant ovary-dependent reproductive tract alterations at 8 mo posttreatment: the majority of treated mice lacked corpus lutea and uterine glands and exhibited squamous metaplasia of the uterine luminal epithelium (81). Ovariectomized mice treated with ZEN showed none of these ovary-dependent alterations.

The mechanisms of the estrogenic effect of ZEN appears to be mediated via binding of this mycotoxin or its metabolites to the cytoplasmic estrogen receptor. *Trans-* and *cis-*ZEN and two ZEN derivatives competed with 17β-estradiol for binding with the cytosolic receptor in rat uterine tissue (41). In that study, the binding affinity of ZEN to the receptors was only .1 that of estradiol. Translocation of the ZEN receptor complex into immature rat uterine nuclei induced synthesis of a protein with identical properties to that following translocation of natural estrogen receptor (38). Interestingly, ZEN appears to have a greater affinity for estrogen receptors from animals that are more susceptible to the estrogenic effects of the mycotoxin. The affinity of ZEN to uterine and oviduct estrogen receptors followed this order: pig, rat, and chicken (25).

FUMONISINS

A group of mycotoxins that were discovered recently have been associated with toxicity and mortality in horses and pigs following ingestion of corn-based feeds that were contaminated by *Fusaria*. One such animal disease is the neurotoxic syndrome equine leukoencephalomalacia (**ELEM**). The ELEM syndrome often is epizootic and almost always is associated with ingestion of moldy corn. Research on the possible effects of *Fusarium* contamination of foods and feeds began in earnest following initial laboratory studies showing that cultures of *Fusarium moniliforme*, the most common fungal contaminant of corn, caused cancer in rats (36). Shortly thereafter, a group of compounds named fumonisins were isolated and characterized (7, 27). Fumonisin B_1 is the most toxic representative of the fumonisins (Figure 3).

Recently, fumonisins have been linked positively to ELEM (39, 46), which is characterized by facial paralysis, nervousness, lameness, ataxia, and inability to eat or to drink. The principal pathologic lesions include severe cerebral edema, focal malacia, and liquefaction of cerebral white matter. The onset of such severe symptoms can be as short as a few hours. Fourteen of 18 horses fed a corn-based feed contaminated with 37 to 122 ppm of FB_1 developed fatal ELEM (82). Hepatic involvement often is coincident with central nervous system involvement in horses and swine. Two reports also have described the development of pulmonary edema, hydrothorax, or both in swine receiving either intravenous injections of FB_1 or by ingesting feed contaminated by FB_1 (33, 62). In swine, lower doses of FB_1 resulted in a slowly progressive hepatic necrosis; higher doses resulted in acute pulmonary edema coincident with hepatic toxicity (34). That fumonisins are potent inhibitors of sphingosine biosynthesis in cultured hepatocytes (80) has been postulated to account for the hepatotoxic (34) and central nervous system effects of this toxin (53). Compared with AFB_1, however, FB_1 is much less toxic to cultured hepatocytes (29).

Aside from its acute effects, FB_1 also appears to possess tumor-initiating and tumor-promoting activity. Initial studies showed that a diet containing culture material inoculated with *F. moniliforme* was carcinogenic in rats (36). In later studies with purified FB_1, a promotional effect on the incidence of diethylnitrosamine-induced, γ-glutamyltranspeptidase-positive (**γ-GT$^+$**) hepatic foci in rats was noted (27). Short-term dietary FB_1 induced a high incidence (66%) of HCC (28). Metastases to the heart, lungs, or kidneys also were observed. Symptoms of hepatic involvement, such as macronodular cirrhosis and cholangiofibrosis, also were observed.

In a recent study, Gelderblom et al. (29) noted that FB_1 is probably only a modest initiator of liver tumors, because γ-GT$^+$ foci and hepatocellular nodules were observed only after prolonged feeding of .1% FB_1 in rats. Those authors (29) postulated that the carcinogenic effect of FB_1 likely involves promotion and the selection of initiated hepatocytes, events that occur during the postinitiation phase of hepatocarcinogenesis.

The mechanism of fumonisin carcinogenicity does not appear to involve interaction with DNA. Neither FB_1 nor FB_2 elicited unscheduled DNA repair in primary rat hepatocyte cultures treated either in vitro or in vivo (29). Therefore, the carcinogenic activity of fumonisins may be mediated via epigenetic mechanisms, as is the case of peroxisome proliferators.

Research on the health effects of fumonisins has essentially only just begun, and information on the mechanism of fumonisin toxicity or on the mechanisms underlying species sensitivity is only beginning to be compiled. More information is needed to obtain a fuller understanding of the extent of the adverse effects of fumonisins in human and animal health. Because corn is a staple in many parts of the world and because *Fusarium* contamination of corn is nearly universal, fumonisins likely are involved in human toxicoses and other health effects. Corn contaminated by *Fusarium* has been epidemiologically associated with human esophageal cancer in some regions of South Africa (70).

CONCLUSIONS

Although much information has been obtained regarding the action of several classes of mycotoxins, future research topics should continue to address several areas of critical concern. For example, the continued development of simple, rapid, and more sensitive detection methods will aid in efforts to prevent contaminated commodities from reaching the market. New kits that allow simple and rapid mycotoxin detection without the need for expensive equipment or extensive training recently have been developed.

More information is needed on decontamination strategies. Although ammoniation is effective for detoxification of AFB_1, fumonisins apparently are not affected by this treatment (80). In addition, little information exists on the stability of tricothecenes and ZEN to food processing.

Determination of the actual risk posed to humans who consume foods contaminated by mycotoxin is of critical concern. Laboratory and epidemiological approaches are important in addressing this question. In the case of AFB_1, comparative species studies have been essential to obtain some understanding of the mechanisms underlying sensitivity and resistance to the carcinogenic effects of this mycotoxin. Molecular biological approaches have begun to provide some information on the role of AFB_1 in mutations in specific cancer-causing genes in humans and other animals. Mechanism studies on the fumonisins will be advanced with the identification of appropriate animal models of the toxic effects observed in domestic animals.

New methods to detect specific biomarkers in noninvasive samples, such as urine, are beginning to be an important tool in measurement of actual human exposure in populations that are particularly at risk. Biomarkers for other potentially carcinogenic mycotoxins to which humans are exposed, such as fumonisins, will likewise allow greater determination of risk. Identification of potentially synergistic risk factors coincident with mycotoxin exposure, such as other foodborne toxins, infectious agents, and synthetic chemicals, will also be important in these efforts.

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