Minireview

Trichothecenes in the environment: relevance to human health

Daniel L. Sudakin

Department of Environmental and Molecular Toxicology, Oregon State University, 333 Weniger, Corvallis, OR 97331-6502, USA

Received 16 December 2002; received in revised form 3 February 2003; accepted 4 February 2003

Abstract

Trichothecenes are agriculturally important mycotoxins of relevance to human health. Fungi capable of producing trichothecenes can be found throughout the world, and include certain species of Fusarium, Myrothecium, and Stachybotrys. The production of mycotoxins by these toxigenic species is determined by genetic factors and the environmental conditions of their growth. The environmental fate of trichothecenes may be affected by other microorganisms that can detoxify them. Deoxynivalenol and T-2 toxin are examples of trichothecenes that are detectable as natural and unavoidable contaminants of certain agricultural commodities as well as commercial foods. Current estimates of dietary exposure to deoxynivalenol and T-2 toxin are below thresholds for adverse effects that have been reported in experimental animal studies, although historical epidemics of human illness have rarely been described in association with consumption of food derived from heavily contaminated grains. The toxicodynamic properties of trichothecenes include inhibition of protein synthesis and immunomodulatory effects. Very little information is available relating to their toxicokinetics and toxicodynamics in humans. While there is general agreement that the diet represents an important source of human exposure to trichothecenes, risk assessment from non-dietary routes of exposure is complicated by the limited epidemiological data that are currently available.

Keywords: Mycotoxin; Trichothecene; Risk assessment; Toxicology; Epidemiology; Fusarium; Stachybotrys

1. Introduction

Mycotoxins represent a diverse group of secondary fungal metabolites, which vary widely in their chemistry and toxicology. In many cases, the biological effects of secondary fungal metabolites have been applied to benefit human health, as in the development of important pharmaceuticals including antibiotics (penicillin), immunosuppressants (cyclosporin A), and cholesterol-lowering medications (lovastatin). History also provides numerous examples of the potential for adverse effects associated with the ingestion of foods contaminated with mycotoxins, ranging from the vasoactive and neurotoxic effects of ergotamines to the carcinogenic effects of aflatoxins. Although most studies have investigated the toxicology of mycotoxins from dietary exposure, it has been speculated that other routes of exposure may also...
pose human health risks (Autrup et al., 1991). Trichothecenes are an example of mycotoxins that have been acknowledged as unavoidable contaminants of certain important agricultural commodities, but more recently have been suggested in association with adverse health effects from exposure in the indoor environment (American Academy of Pediatrics, 1998). The purpose of this article is to review the chemistry and toxicology of trichothecene mycotoxins, with an emphasis on human health effects from dietary as well as non-dietary routes of exposure.

2. Trichothecene chemistry

The trichothecenes are a group of structurally related mycotoxins with varying degrees of cytotoxic potency. They have a sesquiterpenoid ring structure, and can be classified according to the presence or absence of characteristic functional groups (World Health Organization, 1990). All trichothecenes contain an epoxide at the C_{12,13} position, which is responsible for their toxicological activity. Type A (T-2 toxin, HT-2 toxin diacetoxyscirpenol) and Type B (deoxynivalenol, nivalenol) trichothecenes are distinguished by the presence or absence of a carbonyl group at the C_9 position, respectively. Type C trichothecenes (erotoxin, baccharin) possess an additional epoxide group at the C_{7,8} or C_{9,10} position. Type D trichothecenes (satratoxin, roridin) contain a macrocyclic ring between the C_{4,15} positions. Chemical structures representative of the different classifications of trichothecenes appear in Fig. 1.

In general, trichothecenes are resistant to degradation by environmental factors including light and temperature. They are non-volatile compounds, and can be effectively deactivated under strong acid or alkaline conditions. The environmental fate of trichothecenes may be affected by the coexisting presence of bacteria and fungi that can alter their chemical structure and detoxify them (Jesenska and Sajbidorova, 1991; Beeton and Bull, 1989; Shima et al., 1997). Table 1 identifies genera and species of fungi and bacteria that have the capacity to rapidly and effectively degrade T-2 toxin.

3. Toxigenic fungi and trichothecenes

There are several species of toxigenic fungi that are capable of producing trichothecenes. *Fusarium graminearum* is an important species found throughout the world. The pathogenic growth of this species on agricultural commodities, including wheat and corn, can result in contamination by the trichothecene deoxynivalenol as well as other classes of mycotoxins (Miller, 2002). *Fusarium sporotrichioides* and *Fusarium poae*, both of which are post-harvest contaminants of certain agricultural commodities, are also species of economic importance capable of producing the potent trichothecene T-2 toxin. The production of trichothecenes by strains of these toxigenic species is not uniform, and the role of genetic factors is beginning to be elucidated (Ward et al., 2002). In addition to fungal genetics, trichothecene production is significantly influenced by environmental conditions including temperature, humidity, and growth substrate (Mateo et al., 2002; Nikulin et al., 1994).

Several common soil fungi, including *Myrothecium roridum* and *Stachybotrys chartarum*, are capable of producing macrocyclic trichothecenes. Although much concern has recently been generated in association with the detection of *S. chartarum* in indoor environments (Etzel, 2000), the assessment of hazard and exposure in these reports has consistently focused on toxigenic fungi (Dearborn et al., 2002), in contrast to the trichothecenes that certain species can produce. This distinction complicates their interpretation, because it is well established that the environmental detection of a toxigenic fungal species does not necessarily confirm the presence of mycotoxins (Tuomi et al., 2000). In one laboratory investigation of *S. chartarum* strains collected from indoor environments, the fungi producing the highest concentrations of macrocyclic trichothecenes were derived from the residences of healthy individuals (Jarvis et al., 1998). Approximately, one-third of *S. chartarum* species that have been analyzed are capable of producing macrocyclic trichothecenes (Jarvis, 2002).

Some publications have reported that *Trichoderma* species are trichothecene producers (Etzel,
2000; Novotny and Dixit, 2000), although a recent investigation of over 150 fungal strains of *Trichoderma* found only one isolate that was capable of producing detectable levels (Nielsen and Thran, 2001). In US, certain non-toxigenic species of *Trichoderma* (*Trichoderma harzianum* ATCC, *T. harzianum* Rifai T-22, and *Trichoderma polysporum* ATCC) have been registered for use as biopesticides by the Environmental Protection Agency, because of their ability to function as competitive inhibitors of both toxigenic and pathogenic fungal growth on food crops.

4. Trichotheccenes in the diet and environment

Dietary ingestion represents the most common route of human exposure to trichotheccenes. Deoxynivalenol is frequently detected in agricultural commodities such as wheat, rye, barley, oats, and other cereals (Trucksess et al., 1995; Eskola et al., 2001). Deoxynivalenol is also frequently detectable at low levels in certain commercial foods including beer and other fermented beverages, breakfast cereals, bread, and related products (Wolf-Hall and Schwarz, 2002; Schollenberger et al., 1999). Estimated daily intake of deoxynivalenol ranges from 0.77 to 2.4 mcg/kg body weight per day based upon dietary habits in Africa and the Middle East (FAO/WHO Expert Committee on Food Additives, 2001). In US, the mean dietary intake of deoxynivalenol has been estimated to be 0.49 mcg/kg body weight per day (United States Depart-

Table 1

<table>
<thead>
<tr>
<th>Environmental fungi and bacteria capable of detoxifying T-2 toxin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fungi</strong></td>
<td><strong>Bacteria</strong></td>
</tr>
<tr>
<td>Alternaria sp.</td>
<td>Pseudomonas sp.</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>Arthrobacter sp.</td>
</tr>
<tr>
<td>Aspergillus candidus</td>
<td>Blastobacter natatorius</td>
</tr>
<tr>
<td>Cladosporium cladosporioides</td>
<td>Agrobacterium sp.</td>
</tr>
<tr>
<td>Cladosporium macrocarpum</td>
<td></td>
</tr>
<tr>
<td>Rhodotorula sp.</td>
<td></td>
</tr>
<tr>
<td>Ulocladium sp.</td>
<td></td>
</tr>
</tbody>
</table>
ment of Agriculture, 1996). These regional estimates are in proximity to the provisional maximum tolerable daily intake (PMTDI) value of 1 mcg/kg per day, which was established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), although the committee acknowledged that considerable uncertainty exists in the estimates of dietary intake.

T-2 and HT-2 toxins are also occasionally detected as unavoidable contaminants of certain agricultural commodities (including wheat, corn, oats, rice, and rye) as well as some commercial foods (Schollenberger et al., 1999). Based upon dietary habits in Europe, JECFA has estimated that the daily intake of T-2 and HT-2 toxins is 7.6 and 8.7 ng/kg body weight per day, respectively. A wider margin is apparent between estimated dietary intake and PMTDI for T-2 toxin (60 ng/kg per day), although similar uncertainty in the assessment of dietary exposure and residue concentrations in commodities has been acknowledged by JECFA Expert Committee.

In addition to their presence in the outdoor environment, certain trichothecenes have been detected indoors in association with water-damaged building materials. Various trichothecenes were detected in 19% of visibly moldy bulk samples in one investigation, which used a semi-quantitative analytical methodology (Tuomi et al., 2000). Serratotoxins have been detected in quantities ranging from 2 to 15 ng/cm² in building materials heavily contaminated by S. chartarum (Nielsen et al., 1998). Trichothecenes have not been quantified in indoor air of water-damaged building environments, and this leads to difficulties in the assessment of risk from inhalation routes of exposure. In epidemiological studies that have measured fungal spores in indoor air, species capable of producing trichothecenes (including S. chartarum) have usually comprised a small proportion of the total fungal load (Sorensen et al., 1996). This observation is probably related to the bioaerosol properties of S. chartarum spores, which are large, sticky, and require mechanical disturbance for dispersion (Burge, 2001). Stachybotrys spores have been detected as a minor component of indoor and outdoor air throughout the world, ranging from temperate to arid and desert climates (Shelton et al., 2002; Al Suwaine et al., 1999; Khan et al., 1999).

5. Trichothecene toxicodynamics

Toxicodynamic studies have demonstrated that the trichothecenes function as inhibitors of eukaryotic protein synthesis. Trichothecenes bind to the 60S ribosomal subunit and interact with the enzyme peptidyltransferase. This interaction leads to varying degrees of inhibition of peptide bond formation depending upon the chemical structure of the specific trichothecene (Cundliffe and Davies, 1977). The C₁₂,₁₃ epoxide group common to trichothecenes is necessary for protein synthesis inhibition, while certain substitutions of the ring structure at the C₃ and C₄ positions lead to additional effects (Feinberg and McLaughlin, 1989). The more potent trichothecenes (including T-2 toxin) may inhibit the initiation of protein synthesis as well as the elongation and termination phases (Murthy et al., 1985).

More recent mechanistic investigations have provided further insight to the toxicodynamics of trichothecenes. Animal studies have consistently reported immunomodulatory effects, ranging from suppression to stimulation of the immune system (Bondy and Pestka, 2000). Oral doses of 10 mg/kg of T-2 toxin in a murine model resulted in lymphocyte apoptosis in thymus tissue, mesenteric lymph nodes, and Peyers's patches (Nagata et al., 2001). Dose-dependent induction of mRNA expression for certain proinflammatory cytokines (TNF-alpha, IL-6, and IL-1 beta) was reported in a murine model after oral doses of deoxynivalenol ranging from 1 to 25 mg/kg (Zhou et al., 1999). The lowest-observable effect level for changes in immune parameters is 0.029 mg/kg body weight per day for T-2 toxin, based upon a 3-week dietary study in pigs (Rafai et al., 1995). A no-observable effect level of 100 mcg/kg body weight per day has been reported for deoxynivalenol, based upon a 2-year feeding study in mice (Iverson et al., 1995).

In human tissues, cytotoxic effects of trichothecenes on megakaryocyte progenitor cells, as well as on red and white blood cell progenitors, have been
reported in vitro (Froquet et al., 2001; Lautraite et al., 1997; Rio et al., 1997). Similar to the findings in certain animal models, both stimulatory and inhibitory effects on immunological parameters have been described in vitro, in response to varying levels of exposure to trichothecenes. In a study of cultured human lymphocytes, enhanced immunoglobulin production was observed in cells exposed to lower doses of trichothecenes, whereas decreased immunoglobulin production was noted at higher doses (Thuvander et al., 1999). Additive as well as antagonistic effects on lymphocyte proliferation and immunoglobulin production were observed upon exposure to combinations of Types A and B trichothecenes in this investigation, although synergistic effects were not apparent.

6. Trichothecene toxicokinetics

Very little data are available on the toxicokinetics of trichothecenes in humans. Most studies have been conducted in laboratory animals and domestic livestock, where interspecies variation in pharmacokinetic parameters and susceptibility to trichothecenes has been consistently reported (Trenholm et al., 1989). It is unknown whether the metabolic and elimination pathways that have been described in these studies are predictive of trichothecene toxicokinetics in humans.

The oral bioavailability of trichothecenes is generally low as a result of physiological instability and first-pass metabolism (Yagen and Bialer, 1993). Intestinal microorganisms present an additional pathway for the detoxification of ingested trichothecenes in some animal species. Reduction of the trichothecene C\textsubscript{12,13} epoxide has been demonstrated to occur within the gastrointestinal tract of rats, cattle, and swine (Swanson et al., 1988).

Studies of dermal exposure in the rat have reported T-2 toxin to be among the more potent trichothecenes, with a threshold for irritant effects at contamination densities of 0.5 mcg/cm\textsuperscript{2} (Fairhurst et al., 1987). A slow rate of penetration of T-2 toxin across human skin has been described in vitro, with a low risk of systemic toxicity from doses up to 2.6 mcg/cm\textsuperscript{2} (Maxwell et al., 1986).

Many studies of dermal exposure to trichothecenes have utilized dimethyl sulfoxide or other solvent vehicles as an absorption enhancer (Pang et al., 1987b), which limits their external validity to human environmental exposures (Schiefer et al., 1986).

The inhalation toxicology of aerosolized T-2 toxin has been investigated in experimental studies of swine and guinea pigs. Systemic absorption and toxicity have been reported in these investigations, with LD\textsubscript{50} < 5 mg/kg body weight based upon estimated absorbed doses (Marrs et al., 1986; Pang et al., 1987a). Interestingly, pathologic findings in the lungs have been relatively unremarkable in these inhalation studies, in comparison to the extent of necrosis that has been described in the gastrointestinal tract and lymphatic organs. It has been suggested that inhalation represents a more potent route than oral or even parenteral exposure to trichothecenes (Creasia et al., 1987); however, this has not been consistently demonstrated (Marrs et al., 1986; Pang et al., 1987a). It is important to note that these investigations have been conducted using different animal models and different methods of toxin administration.

Some investigators have reported dose-dependent injury and pathology in the rat and mouse lungs from direct airway instillation of aqueous suspensions containing toxigenic S. chartarum spores (Nikulin et al., 1997; Yike et al., 2002). The doses administered in these experimental studies greatly exceed spore levels that have been measured in ambient air. Histopathological changes have been confined to the lung in these investigations, with no evidence of extensive necrosis in other target tissues that have previously been demonstrated to be affected by inhalation exposure to trichothecenes (including thymus, spleen, and intestines). In contrast to the direct pulmonary effects of high-dose inhalation exposure in experimental studies, the systemic bioavailability of trichothecenes from inhaled toxigenic fungal spores has not been measured from environmentally relevant levels of exposure, or under physiologically based exposure conditions.

In vivo experiments with different animal species indicate that orally or parenterally administered T-2 toxin does not bioaccumulate. Short
elimination half-lives (less than 30 min) have been reported for T-2 toxin in swine, cattle, and dogs (Yagen and Bialer, 1993). Elimination half-lives ranging from 3 to 5 h have been measured for deoxynivalenol in swine and cattle (Rotter et al., 1996). Major metabolic pathways for trichothecenes include oxidation, de-epoxidation, hydrolysis, and glucuronide conjugation (Yagen and Bialer, 1993). Enterohepatic re-circulation of T-2 toxin and its metabolites has been reported in the rat (Coddington et al., 1989). In vitro studies of human blood suggest that carboxylesterases may be important in the hydrolysis of T-2 toxin to more polar and less toxic metabolites (Johnsen et al., 1988).

7. Epidemiological reports in humans

Reports of human illness associated with ingestion pathways of exposure to trichothecenes date back to 1930s in the former Soviet Union, where an epidemic that was termed alimentary toxic aleukia (ATA) was described (Joffe, 1986a). The epidemic occurred under conditions of near famine, where the population was forced to consume over-wintered grain that was contaminated with species of F. poae and F. sporotrichioides. Subsequent analyses of these strains found them to be high producers of T-2 toxin. One strain of F. sporotrichioides that was isolated from the epidemic produced 4.1 g of T-2 toxin/kg of infected millet (Joffe, 1986a).

Four stages of ATA were described (Joffe, 1986a). The first stage was characterized by severe gastrointestinal symptoms lasting for 3–9 days. The second stage was characterized by improvement of symptoms, and the concurrent development of anemia, thrombocytopenia, and leukopenia over a period of several weeks. With persistent dietary exposure, a third stage was described, characterized by necrotic lesions in the airways and gastrointestinal tract, as well as infectious and hemorrhagic complications. A fourth stage of convalescence was reported, where removal from exposure eventually led to resolution of necrotic lesions and hematologic abnormalities.

There have not been any foodborne outbreaks of human illness consistent with ATA since that time, although epidemics of gastrointestinal illness have occasionally been reported in association with the ingestion of foods contaminated by toxigenic fungi and trichothecenes (Wang et al., 1993). In one such study, T-2 toxin was measured in contaminated wheat samples at concentrations ranging from 2 to 4 mg/kg (Bhat et al., 1989). This greatly exceeds levels that have been measured in representative wheat samples from various countries, which have ranged from 0.1 to 60 μg/kg (Food and Agriculture Organization and World Health Organization, 2001).

The cytotoxic effects of trichothecenes that were observed in association with ATA led some investigators to evaluate whether they may have a therapeutic role in the treatment of cancer. Diacetoxyscirpenol, a potent Type A trichothecene, was investigated as a chemotherapy drug in individuals with advanced malignancies. A Phase I clinical trial found no evidence of physiological activity or drug-related toxicity at daily intravenous doses of < 2.4 mg/m² for 5 consecutive days (Murphy et al., 1978). At higher doses, adverse effects were reported including gastrointestinal symptoms, myelosuppression, and in a minority of subjects, hypotension and transient neurological symptoms. Pharmacokinetic parameters were not published from these clinical trials. The drug was not found to have therapeutic efficacy, and was not investigated beyond the Phase II stage.

Trichothecenes have also been investigated because of concerns about their potential misuse as agents of biological or chemical warfare. In late 1970s and early 1980s, it was alleged that weaponized, aerosolized trichothecenes had been used on civilian and refugee populations in Laos, Kampuchea, and Afghanistan. A variety of symptoms were reported in association with these allegations including bleeding, nausea, fever, dyspnea, dizziness, and vertigo (Joffe, 1986b). These incidents became known as "yellow rain", because of descriptive reports of witnesses, although the allegation that trichothecenes were responsible for the reported symptoms is controversial (Joffe, 1986b; Ember, 1984). Inconsistencies were identified with respect to the manner in which information was collected from witnesses as well as the
results of laboratory analyses for trichothecenes in environmental and biological samples (Hu et al., 1989; Marshall, 1986).

More recently, reports of associations between trichothecenes and building-related illness have been published. A cluster of cases of pulmonary hemorrhage in infants was reported in association with residential exposure to S. chartarum and other fungi in a case-control study (Etzel et al., 1998). An extensive review of this investigation was conducted by the Centers for Disease Control and Prevention (CDC) which identified shortcomings in the analysis and reporting of data, leading to the conclusion that the association was not confirmed (Centers for Disease Control and Prevention, 2000). Additional case reports of pulmonary hemosiderosis and hemorrhage in infants and children in association with S. chartarum and other toxigenic fungi have been described since that time (Elidemir et al., 1999). The role of toxigenic fungi and other identified environmental risk factors (including environmental tobacco smoke) in infant pulmonary hemorrhage continues to be investigated (Dearborn et al., 2002).

Some studies have reported pulmonary and non-specific building-related symptoms in association with airborne exposure to toxigenic fungi (particularly S. chartarum) in buildings with a history of moisture problems (Hodgson et al., 1998; Johanning et al., 1996). The assessment of exposure in these studies has been based primarily upon air and surface sampling for toxigenic fungi, in contrast to the detection or quantification of trichothecenes in indoor air. The levels of airborne fungi measured in these investigations are much lower than the concentrations that have been reported in agricultural environments in cases of organic dust toxic syndrome (pulmonary mycotoxicosis; Malmberg et al., 1993). Although some investigators have concluded that building-related symptoms have been caused by inhalation of mycotoxins, the limitations to these studies have been the subject of further review and discussion (Fung et al., 1998; Page and Trout, 1998). While there is general agreement that fungi pose immunological and infectious risks to sensitive individuals, the weight and quality of the current scientific evidence does not support a causal relationship between inhalation exposure to trichothecenes in indoor environments and specific health effects (American College of Occupational and Environmental Medicine, 2002; Page and Trout, 2001; Kuhn and Ghannoum, 2003).

There is good agreement that further research is needed to understand the human health impact of trichothecenes from dietary as well as non-dietary routes of exposure (Council for Agricultural Science and Technology, 2003). Progress has been made in the development of analytical methods for the detection of trichothecenes in agricultural commodities, but further investigation of their precision and accuracy is needed (Krska et al., 2001). The ability to reliably detect and quantify different mycotoxins in building materials remains a challenge (Tuomi et al., 2001). Laboratory investigations have reported membrane filter sampling methods for the detection of airborne trichothecenes (Pasanen et al., 1992), but such methods have not yet been applied to air samples from water-damaged buildings with fungal contamination. While sensitive and specific analytical techniques have been utilized to detect and quantify airborne mycotoxins in agricultural environments (Selim et al., 1998), the extent to which mycotoxins are present in indoor air has not been measured in epidemiological studies.

In the future, risk assessment may be significantly enhanced through the utilization of biomarkers of exposure to trichothecenes, which have been applied in animal studies (Yagen and Bialer, 1993), and have been developed for the analysis of human matrices including blood and urine (Begley et al., 1986; Black et al., 1986). Although such biomarkers have not been utilized in epidemiological studies of human illness, an immunoaffinity column-high-performance liquid chromatography (IAC-HPLC) technique was recently explored in the assessment of human urine samples from individuals with low- and high levels of estimated dietary exposure to deoxynivalenol (Meky et al., 2003). Preliminary results were confirmed with mass spectrometry, revealing that deoxynivalenol was detectable in urine samples obtained from every study subject. The mean level of urinary deoxynivalenol was found to be higher in the
group of subjects with high levels of estimated dietary exposure. The development of a biomarker of exposure to deoxynivalenol represents a significant advance, as such markers have been proven to be important for understanding the human health implications of other agriculturally important mycotoxins such as aflatoxins (Groopman and Kensler, 1999). As indicators of internal dose, biomarkers could provide much needed insight to the absorption, metabolism, and elimination of trichothecenes in humans. In addition to providing a more direct assessment of exposure than is currently possible through environmental measurements, the application of biomarkers may help identify underlying susceptibility factors, define the implications of combined exposure to different trichothecenes, and clarify the mechanisms linking exposure and disease in humans.

8. Conclusions

Toxigenic fungi capable of producing trichothecenes can be found in indoor and outdoor environments throughout the world. The genetic and environmental factors that can affect the production of trichothecenes by certain fungal species are becoming clearer. While the effects of trichothecenes have been extensively studied in animals, the toxicology of these important mycotoxins remains largely unexplored in humans. As natural and unavoidable contaminants of important agricultural commodities, trichothecenes represent an important area of focus for food safety. Reports of foodborne illness from trichothecenes are rare, and have been associated with levels of exposure that greatly exceed current estimates of dietary intake. More recent reports suggesting human health risks from non-dietary routes of exposure are difficult to objectively interpret, as the assessment of hazard and exposure in these epidemiological studies has primarily focused on toxigenic fungi, not trichothecenes. Progress has recently been made in the development of biomarkers of exposure to certain trichothecenes. Through the refinement, validation, and integration of analytical methods to detect trichothecenes in environmental, food, and biological samples, future studies should provide much needed insight in understanding dose and response in humans.

References


Truckess, M.W., Thomas, F., Young, K., Stack, M.E., Fulgueras, W.J., Page, S.W., 1995. Survey of deoxynivalenol...
nol in U.S. 1993 wheat and barley crops by enzyme-linked immunosorbent assay. J. AOAC Int. 78, 631–635.


