

# Chapter 34

## TRICHOTHECENE MYCOTOXINS

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## INTRODUCTION

Mycotoxins, by-products of fungal metabolism, have been implicated as causative agents of adverse health effects in humans and animals that have consumed fungus-infected agricultural products.<sup>1,2</sup> Consequently, fungi that produce mycotoxins, as well as the mycotoxins themselves, are potential problems from both public health and economic perspectives. The fungi are a vast assemblage of living organisms, but mycotoxin production is most commonly associated with the terrestrial filamentous fungi called the molds.<sup>3</sup> Various genera of toxigenic fungi are capable of producing such diverse mycotoxins as the aflatoxins, rubratoxins, ochratoxins, fumonisins, and trichothecenes.<sup>1,2</sup>

The trichothecenes are a very large family of chemically related toxins produced by various species of *Fusarium*, *Myrothecium*, *Trichoderma*, *Cephalosporium*, *Verticimonosporium*, and *Stachybotrys*.<sup>4</sup> They are markedly stable under different environ-

mental conditions. The distinguishing chemical feature of trichothecenes is the presence of a trichothecene ring, which contains an olefinic bond at C-9, 10; and an epoxide group at C-12, 12.<sup>5</sup> All trichothecenes are mycotoxins, but not all mycotoxins are trichothecenes. This family of mycotoxins causes multiorgan effects including emesis and diarrhea, weight loss, nervous disorders, cardiovascular alterations, immunodepression, hemostatic derangements, skin toxicity, decreased reproductive capacity, and bone marrow damage.<sup>4,6</sup>

In this chapter, we will concentrate on T-2 mycotoxin, a highly toxic trichothecene that, together with some closely related compounds, has been the causative agent of a number of illnesses in humans and domestic animals.<sup>1,2,4</sup> During the 1970s and 1980s, the trichothecene mycotoxins gained some notoriety as putative biological warfare agents when they were implicated in "yellow rain" attacks in Southeast Asia.<sup>7-11</sup>

## HISTORY AND MILITARY SIGNIFICANCE

Fungi that produce trichothecenes are plant pathogens and invade various agricultural products and plants. Since *Fusarium* and other related fungi infect important foodstuff, they have been associated worldwide with intoxication of humans and animals. Thus, these fungi have potential as biological weapons.

### Use in Biological Warfare

From 1974 to 1981, toxic agents were used by the Soviet Union and its client states in such Cold War sites as Afghanistan, Laos, and Kampuchea (Cambodia). Aerosol-and-droplet clouds were produced by delivery systems in the Soviet arsenal such as aircraft spray tanks, aircraft-launched rockets, bombs (exploding cylinders), canisters, a Soviet hand-held weapon (DH-10), and booby traps. Aircraft used for delivery included L-19s, AN-2s, T-28s, T-41s, MiG-21s (in Laos) and Soviet MI-24 helicopters (in Afghanistan and Laos).

Attacks in Laos (1975–1981) were directed against Hmong villagers and resistance forces who opposed the Lao People's Liberation Army and the North Vietnamese. In Kampuchea, North Vietnamese troops used 60-mm mortar shells; 120-mm shells; 107-mm rockets; M-79 grenade launchers containing chemicals; and chemical rockets, bombs, and

sprays delivered by T-28 aircraft (1979–1981) against Khmer Rouge troops. The chemical munitions were supplied by the Soviets and delivered by North Vietnamese or Laotian pilots. In Afghanistan, the chemical weapons were delivered by Soviet or Afghan pilots against Mujahidin guerrillas (1979–1981). Lethality of the attacks is documented by a minimum of 6,310 deaths in Laos (from 226 attacks); 981 deaths in Kampuchea (from 124 attacks); and 3,042 deaths in Afghanistan (from 47 attacks).<sup>7</sup> Trichothecenes appear to have been used in some of these attacks.

The air attacks in Laos have been described as "yellow rain" and consisted of a shower of sticky, yellow liquid that sounded like rain as it fell from the sky. Other accounts described a yellow cloud of dust or powder, a mist, smoke, or insect spray-like material. Liquid agent rapidly dried to a powder. In Laos, 50% to 81%<sup>7</sup> of attacks involved material associated with a yellow pigment. Other attacks were associated with red, green, white, or brown smoke or vapor. More than 80%<sup>7</sup> of attacks were delivered by air-to-surface rockets; the remainder, from aircraft spray tanks or bombs. Intelligence information and some of the victims' descriptions of symptoms raised the possibility that chemical warfare agents such as phosgene, sarin, soman, mustards, CS, phosgene oxime, or BZ may also

have been used. These agents may have been used in mixtures or alone, and with or without the trichothecenes.

Unconfirmed reports have implicated the use of trichothecenes in the 1964 Egyptian (or Russian) attacks on Yemeni Royalists in Yemen<sup>12</sup> and in combination with mustards during chemical warfare attacks in the Iran–Iraq War (1983–1984).<sup>13</sup> According to European sources, Soviet–Cuban forces in Cuba are said to have been equipped with mycotoxins, and a Cuban agent is said to have died of a hemorrhagic syndrome induced by a mycotoxin agent.<sup>14</sup>

### The Yellow Rain Controversy

Actual biological warfare use of trichothecenes in Southeast Asia and Afghanistan is strongly supported by the epidemiological and intelligence assessments and trichothecene assays, although reports in the open literature have discounted this contention. An article written by L. R. Ember,<sup>15</sup> published in 1984 in *Chemical Engineering News*, is the most exhaustive and authoritative account of the controversy surrounding the use of trichothecene mycotoxins in Southeast Asia during the 1970s.

The United States government, its allies, and journalists exhaustively studied the possibility that yellow rain attacks had occurred, based on evidence<sup>7,14,15</sup> such as the following:

- interviews of Hmong survivors of and eyewitnesses to lethal yellow rain attacks in Laos, who provided consistent descriptions of the episodes;
- interrogations of a defecting Laotian Air Force officer and North Vietnamese ground troops, who corroborated the descriptions of attacks and admitted using the chemicals;
- interrogations of prisoners of war, who admitted being involved in attacks where unconventional weapons were used (ie, in Afghanistan);
- laboratory confirmations of Soviet use of chemical agents, and
- the presence of Soviet-manufactured chemical agents and Soviet technicians in Laos.

The evidence supports the contention that trichothecene mycotoxins were used as biological warfare agents in Southeast Asia and Afghanistan by the former Soviet Union and its surrogates. The

Russians have not recently denied such use but have declined to discuss the subject.

In addition to the evidence stated above, elevated levels and naturally rare mixtures of trichothecene toxins were recovered from the surfaces of plants, fragments of plastic, and rocks in areas attacked<sup>9,11,15,16</sup>; and were detected in the blood of attack survivors and the tissues of a dead casualty.<sup>10,15</sup> Control samples that were taken (a) from an environment that had not been attacked, and during another season of the year,<sup>15</sup> and (b) from Hmong who had never been exposed to an attack were consistently negative.

The evidence that trichothecenes were used in Southeast Asia has been challenged: questions have been raised about the interview methodology used by U.S. Army physicians and U.S. State Department personnel in Hmong refugee camps in Thailand to obtain descriptions of the attacks. Some inconsistencies of specific individuals' stories were demonstrated, but the frequency of unreliable information has not been reported and is unlikely to be large enough to discredit all witnesses.<sup>15</sup> Symptom descriptions are generally consistent with known trichothecene effects.

The paucity of positive evidence of the presence of trichothecenes (5 positive environmental and 20 positive biomedical samples) has been used to challenge the belief that biological warfare attacks occurred, since only 10% of samples were positive. However, 32% of samples from victims were positive, a value too high for natural causes (eg, food contamination) to be used as an explanation, since 98% of controls in nonattack areas of Thailand were negative.<sup>17</sup> The 2% of samples that were positive could represent either a nonspecific result or low-prevalence food contamination. The paucity and type of control samples have also been questioned.

Some experts<sup>18–21</sup> have claimed that yellow rain was not a biological warfare attack at all, but that the yellow residue was caused by showers and deposits of bee feces—the result of massive bee swarming and cleansing–defecation flights over some areas of Southeast Asia. The presence of pollen in bee feces and some samples has not only added confusion<sup>18</sup> but is also the supporting evidence used by the skeptics. It is important to remember that persons caught in a shower of bee feces do not get sick and die. Although bee flights have occurred before and since 1982, reports of attacks of yellow rain and death in Asia have not.

Then what explains the symptoms consistent with trichothecene effects in the casualties, and the pollen and bee feces in some of the yellow spots on

vegetation in the area? Bee feces do not contain trichothecenes, yet pollen and trichothecenes without mold are found together in some samples from attack areas. The most likely explanation is that during biological warfare attacks, dispersed trichothecenes landed in pollen-containing areas.

French scientists have reported the simultaneous synthesis of three trichothecene toxins by *Fusarium* growing on corn, but actual production of these toxins by *Fusarium* species in Southeast Asia has not been demonstrated, presumably because of high environmental temperature (ie, toxin production usually increases at low temperatures). Whether or not *Fusarium* toxin is produced in the high-mountain temperate regions of Laos inhabited by the Hmong remains unanswered. The presence of toxin on leaves without accompanying mold also is unexplained by critics of the trichothecene weapon hypothesis. In vivo studies have demonstrated that *F semitectum* var *semitectum* will grow on leaves in Southeast Asia, but have not shown that it will produce toxin in vivo.<sup>15</sup>

In support of the weapon hypothesis are the positive trichothecene analyses performed by two leading researchers<sup>9,10</sup> in the detection of trichothecenes; the Defense Research Establishment, Ottawa, Canada<sup>11,22</sup>; and the U.S. Army Chemical Research and Development Center, Edgewood, Maryland.<sup>23</sup> Negative results of analyses of biomedical and environmental samples from Southeast Asia have come from Porton Down Laboratory in England,<sup>17,24</sup> but according to the British, such results do not exclude sampling problems, including delay in sample collection after an attack, as a cause of the negative results.<sup>15</sup>

Proponents have been accused of analyzing samples that were purposely contaminated with toxin, either after collection or during the analysis. Other methodological criticisms include poor recovery (< 10% of one sample spiked with T-2 toxin); low precision of quantitative data when analyzing two portions of the same leaf; and lack of well-documented, confirming, replicate analyses in Mirocha's or a similarly equipped second laboratory.<sup>15</sup> The presence of polyethylene glycol in the sample analyzed by Rosen<sup>9</sup> also indicates that the trichothecene mixture detected was manufactured, not natural.

Many experts in the intelligence community,<sup>16</sup> academia,<sup>8,9</sup> the U.S. Department of State,<sup>7</sup> and the authors of this chapter believe that trichothecenes were used as biological weapons in Southeast Asia and Afghanistan. However, a weapon containing trichothecenes was not found in South-

east Asia, and the Soviets have not declared any stockpiles of trichothecenes among their chemical or biological weapons. Thus, it has not been possible for the United States to prove unequivocally that trichothecene mycotoxins were used as biological weapons.

## Weaponization

Trichothecene mycotoxins can be delivered as dusts, droplets, aerosols, or smoke from aircraft, rockets, missiles, artillery, mines, or portable sprayers. Because of their antipersonnel properties, ease of large-scale production, and apparent proven delivery by various aerial dispersal systems, the trichothecene mycotoxins (especially T-2 toxin) have an excellent potential for weaponization.

When delivered at low doses, trichothecene mycotoxins cause skin, eye, and gastrointestinal problems. In nanogram amounts,<sup>4,25</sup> they (T-2 toxin, in particular) cause severe skin irritation (erythema, edema, and necrosis).<sup>4,6</sup> Skin vesication has been observed in a number of humans exposed to yellow rain attacks.<sup>4,14,15</sup> T-2 toxin is about 400-fold more potent (50 ng vs 20 µg) than mustard in producing skin injury.<sup>26</sup> Lower-microgram quantities of trichothecene mycotoxins cause severe eye irritation, corneal damage, and impaired vision.<sup>4,16,26,27</sup> Emesis and diarrhea have been observed at amounts that are one fifth to one tenth the lethal doses of trichothecene mycotoxins.<sup>26</sup>

Depending on the species of experimental animal tested and the exposure procedure,<sup>28,29</sup> the lethality of T-2 toxin by aerosol exposure can be 10- to 50-fold greater than when injected parenterally.<sup>30</sup> With larger doses in humans, aerosolized trichothecenes may produce death within minutes to hours.<sup>7,14,15</sup> The term  $LCt_{50}$  (the concentration • time that is lethal to 50% of the exposed population) is used to describe exposure to vapors and aerosols; milligrams • minutes per cubic meter is the conventional unit of measurement.  $LCt_{50}$  and its relation to  $LD_{50}$  (the dose that is lethal to 50% of the exposed population) are discussed in detail in Chapter 5, Nerve Agents, and will not be further explicated here.

The toxicity of T-2 toxin by the inhalational route of exposure ( $LCt_{50}$  range: 200–5,800 mg•min/m<sup>3</sup>)<sup>28–30</sup> is similar to that observed for mustards or Lewisite ( $LCt_{50}$  range: 1,500–1,800 mg•min/m<sup>3</sup>).<sup>31</sup> However, the lethality of T-2 toxin by the dermal route ( $LD_{50}$  range: 2–12 mg/kg<sup>6</sup>) is higher than that for liquid Lewisite ( $LD_{50}$ : approximately 30 mg/

kg<sup>31(p39)</sup>) or liquid mustards (LD<sub>50</sub>: approximately 100 mg/kg<sup>31(p32)</sup>). Therefore, the trichothecene mycotoxins are considered to be primarily blister agents that, at lower exposure concentrations, can cause severe skin and eye irritation, and at larger doses can produce considerable incapacitation and death within minutes to hours.

By solid substrate fermentation, T-2 toxin can be produced at approximately 9 g/kg of substrate, with a yield of 2 to 3 g of crystalline product.<sup>32</sup> Several of the trichothecene mycotoxins have been produced in liquid culture at medium yields and large volumes of culture for extraction.<sup>33</sup> Thus, using existing state-of-the-art fermentation processes that

were developed for brewing and antibiotics, it would be fairly simple to produce ton quantities of a number of the trichothecene mycotoxins.

In Southeast Asia, most of the yellow rain attacks were delivered by aircraft or helicopter spray, bombs, and air-to-surface rockets. The attacks were described as a shower of sticky liquid, a yellow cloud of dust or powder, or a mist (like an insect spray).<sup>7,15</sup> The delivery of the trichothecene mycotoxins was similar in many aspects to the spraying of pesticides on agricultural crops. This would result in a very low-efficiency respiratory aerosol (1–5 μm particles)<sup>34</sup> but a highly effective droplet aerosol that could cause severe skin and eye irritation.

## DESCRIPTION OF THE AGENT

### Occurrence in Nature

Potentially hazardous concentrations of the trichothecene mycotoxins can occur naturally in moldy grains, cereals, and agricultural products.<sup>4,35</sup> Toxicogenic species of *Fusarium* occur worldwide in habitats as diverse as deserts, tidal salt flats, and alpine mountain regions.<sup>35</sup> For example, a food-related disease has been recorded in Russia from time to time, probably since the 19th century.<sup>36</sup> Over the period 1942 through 1947, more than 10% of the population in Orenburg, near Siberia, were fatally affected by overwintered millet, wheat, and barley.<sup>4,36</sup> The syndrome was officially named alimentary toxic aleukia (ATA). Extensive investigations in Russia indicated that a toxin from *Fusarium* species of fungi was the causative agent of ATA.<sup>36,37</sup> Subsequently, it was demonstrated that T-2 toxin, a potent trichothecene mycotoxin, was the likely agent.<sup>37</sup>

Stachybotryotoxicosis has been reported among farm workers in Russia, Yugoslavia, and Hungary.<sup>38,39</sup> This disease is caused by the presence of a mold, *Stachybotrys atra* (*S alternans*), on the hay fed to domestic animals. A macrocyclic trichothecene (satratoxin) produced by the *Stachybotrys* species of the mold may be in part responsible for this toxicosis.<sup>40</sup> The only literature citation on apparent human cases of stachybotryotoxicosis in the United States occurred in people living in a water-damaged house with a heavy infestation of *S atra*.<sup>41</sup>

Russian scientists have reported a case of “cotton lung disease,” which was brought about by the inhalation of cotton dust that was contaminated with *Dendrochium toxicum*. This fungus is consid-

ered to be synonymous with *Myrothecium verrucaria* (a natural producer of the verrucarins class of trichothecenes).<sup>42</sup>

The “red mold disease” of wheat and barley in Japan is prevalent in the region that faces the Pacific Ocean.<sup>4</sup> Toxic trichothecenes, including nivalenol, deoxynivalenol, and monoacetyl-nivalenol (fusarenon-X) from *Fusarium nivale*, can be isolated from moldy grains. In the suburbs of Tokyo, an illness similar to “red mold disease” was described in an outbreak of a food-borne disease, as a result of the consumption of *Fusarium*-infected rice.<sup>35</sup> Ingestion of moldy grains that are contaminated with trichothecenes has been associated with mycotoxicosis in domestic farm animals.<sup>4</sup>

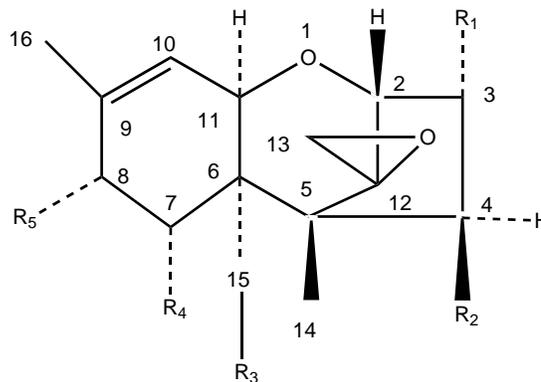


Fig. 34-1. The general structure, numbering system, and variable side groups of the tetracyclic trichothecene nucleus.

**TABLE 34-1**  
**SPECIFIC SIDE GROUPS OF THE MOST ABUNDANT TRICHOHECENE MYCOTOXINS**

Trichothecene	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
T-2 Toxin	-OH	-OCOCH <sub>3</sub>	-OCOCH <sub>3</sub>	-H	-OCOCH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>
HT-2 Toxin	-OH	-OH	-OCOCH <sub>3</sub>	-H	-OCOCH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>
4,15-Diacetoxyscripenol (DAS, also called anguidine)	-OH	-OCOCH <sub>3</sub>	-OCOCH <sub>3</sub>	-H	-H
Nivalenol	-OH	-OH	-OH	-OH	=O
Deoxynivalenol (DON)	-OH	-H	-OH	-OH	=O
Macrocyclics	-H	-O-R' -O-		-H	-H

R' : Macrocyclic ester or ester-ether bridge between carbons 4 and 15. The most abundant macrocyclic trichothecenes are verrucarins, roridins, and satratoxin H. Source for this statement: Jarvis BB. Macrocyclic trichothecenes. In: Sharma RP, Salunkhe DK, eds. *Mycotoxins and Phytoalexins*. Boca Raton, Fla: CRC Press; 1991: 361-421.

### Chemical and Physical Properties

The trichothecenes make up a family of closely related chemical compounds called *sesquiterpenoids* (Figure 34-1). The structures of close to 150 derivatives of the trichothecenes are described in the scientific literature.<sup>35,43</sup> The specific side structures of the most abundant of the naturally occurring trichothecenes are shown in Table 34-1. Because of its availability and relatively high toxicity, T-2 toxin has been the most extensively studied trichothecene mycotoxin.

The trichothecene mycotoxins are nonvolatile, low-molecular-weight (MW 250-550) compounds.<sup>43</sup> This group of mycotoxins is relatively insoluble in water but highly soluble in acetone, ethyl acetate, chloroform, dimethyl sulfoxide (DMSO), ethanol, methanol, and propylene glycol.<sup>43</sup> Purified trichothecenes generally have a low vapor pressure, but

they do vaporize when heated in organic solvents. Extraction of trichothecene mycotoxins from fungal cultures with organic solvents yields a yellow-brown liquid, which, if allowed to evaporate, forms a greasy, yellow crystalline product. Some experts<sup>10,16</sup> believe this extract to be the yellow contaminate of yellow rain. In contrast, highly purified toxins form white, crystalline products that have characteristic melting points.<sup>35</sup>

When maintained as either crystalline powders or liquid solutions, trichothecene mycotoxin compounds are stable when exposed to air, light, or both.<sup>35,44</sup> Moreover, these mycotoxins are not inactivated by autoclaving but require heating at 900°F for 10 minutes or 500°F for 30 minutes for complete inactivation. A 3% to 5% solution of sodium hypochlorite is an effective inactivation agent for them.<sup>44</sup> The efficacy of this solution can be increased by the addition of small amounts of alkali.

## TOXICOLOGY AND TOXICOKINETICS

The trichothecene mycotoxins are toxic to humans, other mammals, birds, fish, a variety of invertebrates, plants, and eukaryotic cells in general. The acute toxicity of the trichothecene mycotoxins varies somewhat with the particular toxin and animal species studied (Table 34-2). Differences are noted among the various species in their susceptibility to trichothecene mycotoxins, but they are small compared with the divergence obtained by diverse routes of administration of the toxins (Table 34-3). Once the trichothecene mycotoxins enter the systemic circulation, regardless of the route of exposure, they affect rapidly proliferating tissues.<sup>1,2,4,6,35,42,45</sup>

### Mechanism of Action

The trichothecene mycotoxins are cytotoxic to most eukaryotic cells.<sup>46</sup> A number of cytotoxicity assays have been developed and include survival and cloning assays, measuring protein and deoxyribonucleic acid (DNA) synthesis by radiolabeling procedures, and a neutral red cell-viability assay. A minimum of 24 to 48 hours is required to measure the effects of the trichothecene mycotoxins on cell viability.

These mycotoxins inhibit protein synthesis in a variety of eukaryotic cells.<sup>46-48</sup> Similar sensitivity to T-2 toxin was observed in established cell lines

TABLE 34-2

## RELATIVE ACUTE PARENTERAL TOXICITY OF THE MOST ABUNDANT TRICHOHECENE MYCOTOXINS

Trichothecenes Tested	Mammals Tested							
	Mouse	Rat	Guinea Pig	Rabbit	Cat	Dog	Pig	Monkey
	LD <sub>50</sub> (mg/kg)							
T-2 Toxin	5.2 (IV)	0.9 (IV)	1.0 (IV)	1.0 (IM)	< 0.5 (SC)	—	1.2 (IV)	0.8 (IM)
HT-2 Toxin	9.0 (IP)	—	—	—	—	—	—	—
4,15-Diacetoxy-scripenol (DAS)	12.0 (IV)	1.3 (IV)	—	1.0 (IV)	—	1.1 (IV)	0.38 (IV)	—
Nivalenol	6.3 (IV)	—	—	—	—	—	—	—
Deoxynivalenol (DON)	43 (SC)	—	—	—	—	—	—	—
Verrucaric acid	1.5 (IV)	0.8 (IV)	—	0.54 (IV)	—	—	—	—
Roridin A	1.0 (IV)	—	—	—	—	—	—	—
Satratoxin H	1.0 (IP)	—	—	—	—	—	—	—

Routes of administration of the mycotoxin: IV: intravenous; IM: intramuscular; SC: subcutaneous; IP: intraperitoneal  
—: Not determined

Data sources: (1) Ueno Y. Trichothecene mycotoxins: Mycology, chemistry, and toxicology. *Adv Nut Res.* 1989;3:301–353. (2) Wannemacher RW Jr, Bunner DL, Neufeld HA. Toxicity of trichothecenes and other related mycotoxins in laboratory animals. In: Smith JE, Henderson RS, eds. *Mycotoxins and Animal Foods*. Boca Raton, Fla: CRC Press; 1991: 499–552. (3) Sharma RP, Kim Y-W. Trichothecenes. In: Sharma RP, Salunkhe DK, eds. *Mycotoxins and Phytoalexins*. Boca Raton, Fla: CRC Press; 1991: 339–359. (4) Jarvis BB. Macrocyclic trichothecenes. In: Sharma RP, Salunkhe DK, eds. *Mycotoxins and Phytoalexins*. Boca Raton, Fla: CRC Press; 1991: 361–421.

TABLE 34-3

## COMPARATIVE TOXICITY OF T-2 TOXIN BY VARIOUS ROUTES OF ADMINISTRATION

Route of Administration	Mammals Tested						
	Mouse	Rat	Guinea Pig	Rabbit	Cat	Pig	Monkey
	T-2 Toxin LD <sub>50</sub> (mg/kg)						
Intravenous	4.2–7.3	0.7–1.2	1.0–2.0	—	—	1.2	—
Intraperitoneal	5.2–9.1	1.3–2.6	—	—	—	—	—
Subcutaneous	2.1–3.3	0.6–2.0	1.0–2.0	—	< 0.5	—	—
Intramuscular	—	0.5–0.9	1.0	1.1	—	—	0.8
Intragastric	9.6–10.5	2.3–5.2	3.1–5.3	—	—	—	—
Intranasal	—	0.6	—	—	—	—	—
Intratracheal	0.16	0.1	—	—	—	—	—
Inhalational	0.24	0.05	0.6–2.0	—	—	—	—
Intracerebral	—	0.01	—	—	—	—	—
Dermal in DMSO	6.6	4.3	2.2	10	—	—	> 8.0
Dermal in Methanol	—	> 380	> 80	—	—	—	—

DMSO: dimethyl sulfoxide  
—: Not determined

Data sources: (1) Ueno Y. Trichothecene mycotoxins: Mycology, chemistry, and toxicology. *Adv Nut Res.* 1989;3:301–353. (2) Wannemacher RW Jr, Bunner DL, Neufeld HA. Toxicity of trichothecenes and other related mycotoxins in laboratory animals. In: Smith JE, Henderson RS, eds. *Mycotoxins and Animal Foods*. Boca Raton, Fla: CRC Press; 1991: 499–552. (3) Sharma RP, Kim Y-W. Trichothecenes. In: Sharma RP, Salunkhe DK, eds. *Mycotoxins and Phytoalexins*. Boca Raton, Fla: CRC Press; 1991: 339–359.

and primary cell cultures.<sup>46,48</sup> Inhibition of protein synthesis is observed 5 minutes after exposure of Vero cells to T-2 toxin, with a maximal response noted by 60 minutes after the exposure.<sup>46</sup> Researchers<sup>47</sup> have concluded that the trichothecene mycotoxins act by inhibiting either the initiation or the elongation process of translation, by interfering with peptidyl transferase activity.

Substantial inhibition of ribonucleic acid (RNA) synthesis (86% inhibition) by trichothecene mycotoxin was observed in human (HeLa) cells,<sup>47</sup> but T-2 toxin had minor effects (15% inhibition) on RNA synthesis in Vero cells.<sup>46</sup> The trichothecene mycotoxin-related inhibition of RNA synthesis is probably a secondary effect of the inhibition of protein synthesis. Scheduled DNA synthesis is strongly inhibited in various types of cells that are exposed to trichothecene mycotoxins. In mice or rats treated with trichothecene mycotoxins, DNA synthesis in all tissues studied was suppressed, although to a lesser degree than protein synthesis.<sup>49</sup> The pattern by which DNA synthesis is inhibited by the trichothecene mycotoxins is consistent with the primary effect of these toxins on protein synthesis. In appropriate cell models, for the most part, trichothecene mycotoxins demonstrate neither mutagenic activity nor the capacity to damage DNA.<sup>50</sup>

Studies with radiolabeled trichothecene mycotoxins suggest that the toxin interaction with cells is best viewed as (1) a free, bidirectional movement of these low-molecular-weight chemicals across the plasma membrane; and (2) specific, high-affinity binding to ribosomes.<sup>51</sup> Thus, further evidence indicates that the primary toxic effects of the trichothecene mycotoxins is caused by their properties as potent inhibitors of protein synthesis.

Since the trichothecene mycotoxins are amphiphilic molecules, an investigation<sup>52</sup> that focused on various kinds of interaction with cellular membranes concluded that T-2 exerts multiple effects on the cell membrane. Lipid peroxidation is increased in liver, spleen, kidney, and thymus; and bone marrow when rats are treated with a single, oral dose of T-2 toxin.<sup>53</sup> These observations led to the suggestion that the trichothecene mycotoxins might induce some alterations in membrane structure, which consequently stimulates lipid peroxidation. Once trichothecene mycotoxins cross the plasma membrane barrier, they enter the cell, where they can interact with a number of targets, including ribosomes<sup>47</sup> and mitochondria.<sup>54</sup>

These toxins inhibit electron transport activity, as the inhibition of succinic dehydrogenase activity and mitochondrial protein synthesis implies.

Toxin-stimulated alteration in mitochondrial membranes contributes to the effects on cellular energetics and cellular cytotoxicity. Although initial investigations on the mechanism of action of the trichothecene mycotoxins suggested that the inhibition of protein synthesis as the principal mechanism of action, the above observations indicate that the effects of these toxins are much more diverse.

## Metabolism

Compared with some of the other mycotoxins such as aflatoxin, the trichothecenes do not appear to require metabolic activation to exert their biological activity.<sup>50</sup> After direct dermal application or oral ingestion, the trichothecene mycotoxins can cause rapid irritation to the skin or intestinal mucosa. In cell-free systems or single cells in culture, these mycotoxins cause a rapid inhibition of protein synthesis and polyribosomal disaggregation.<sup>35,47,50</sup> Thus, we can postulate that the trichothecene mycotoxins have molecular capability of direct reaction with cellular components. Despite this direct effect, it is possible to measure the toxicokinetics and the metabolism of the trichothecene mycotoxins.

The lipophilic nature of these toxins suggests that they are easily absorbed through skin, gut, and pulmonary mucosa. Absorption of a single, oral dose of T-2 toxin is rapid, with concentration of labeled toxin peaking in the blood within 1 hour.<sup>55</sup> This indicates that the trichothecene mycotoxins rapidly pass through the intestinal mucosa. The inhaled median lethal dose of T-2 toxin is equal to<sup>29</sup> or less than<sup>28,30</sup> the systemic dose. Mice, rats, and guinea pigs die rapidly (within 1–12 h) after exposure to high concentrations of aerosolized mycotoxin, with no apparent lung lesions or pulmonary edema.<sup>28–30</sup> This finding is in contrast to the effect of an oral dose of T-2 toxin, which causes direct damage to the intestinal mucosa.<sup>55</sup>

From these data, we can conclude that the trichothecene mycotoxins very rapidly cross the pulmonary and intestinal mucosa and enter the systemic circulation to induce the toxin-related toxicoses. In contrast, trichothecene mycotoxins are only slowly absorbed through skin, especially when applied as a dust or powder.<sup>56</sup> Systemic toxicity and lethality can be produced by dermal exposure to higher concentrations of T-2 toxin, however, especially if the mycotoxin is dissolved in a penetrant such as DMSO.<sup>6</sup>

Various cell culture lines and ruminal bacteria metabolize T-2 toxin by deacylation of specific

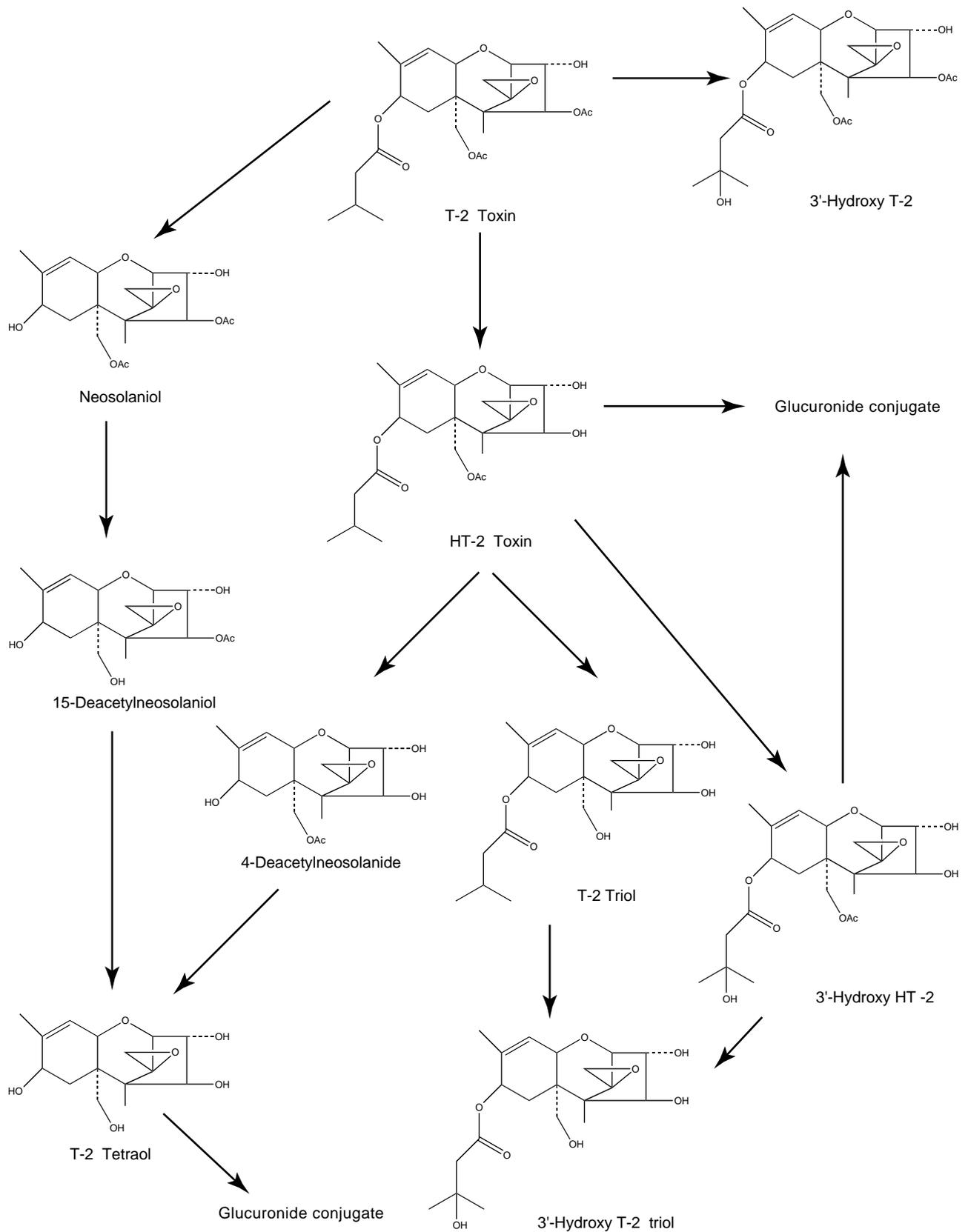


Fig. 34-2. Metabolic pathway of T-2 toxin both in vitro and in vivo.

deepoxidylation (ie, removal of the oxygen from the epoxide ring at the C-12, 13 position to yield a carbon-carbon double bond) and oxidization of the C-3' and C-4' positions on the isovaleryl side chains of T-2 toxin and HT-2 toxin, a metabolite (Figure 34-2).<sup>57-59</sup> A number of different cell types contain the metabolic processes necessary to metabolize trichothecene mycotoxins.

Pharmacokinetic studies<sup>60,61</sup> have demonstrated T-2 toxin in the plasma of animals that were administered this mycotoxin both intravascularly and by aerosol. As plasma concentrations of the parent trichothecene mycotoxin decrease, the deacylated and hydroxylated metabolites and their glucuronide conjugates rapidly appear and disappear from circulation. From these various observations, we can conclude that the pharmacokinetics of the trichothecene mycotoxins are functions of the rate of absorption into the general circulation, metabolism, tissue distribution, and excretion.

Tissue-distribution studies<sup>55</sup> suggest that the liver is the major organ for metabolism of the trichothecene mycotoxins. The bile and the gastrointestinal tract contained large amounts of radioactivity after intravascular, intramuscular, oral, or dermal administration of radiolabeled T-2 toxin. Although the liver is the major organ for the metabolism of the trichothecene mycotoxins, other tissues such as the intestine are capable of metabolic alteration of these toxins. After an intravenous dose of T-2 toxin, 95% of the total radioactivity was excreted in the urine and feces, in a ratio of 3 to 1.<sup>61</sup> The majority of the excreted products were either metabolites or glucuronide conjugates of the metabolites.

Regardless of the route of administration or the species of animal tested, the trichothecene mycotoxins were rapidly metabolized and excreted in urine

and feces. The route of exposure to the toxins and the species can, however, influence the pattern of metabolites that are excreted in the urine. The deacetylated and hydroxylated metabolites appear to be present in most of the species that have been evaluated to date.

A microsomal, nonspecific carboxylesterase [EC 3.1.1.1] from liver selectively hydrolyses the C-4 acetyl group of T-2 toxin to yield HT-2 toxin.<sup>62</sup> In addition to hepatic microsomes, the trichothecene-specific carboxylesterase activity has been detected in brain, kidney, spleen, intestine, white blood cells, and erythrocytes. These findings emphasize the importance of carboxylesterase in detoxifying the trichothecene mycotoxins. A hepatic cytochrome, P-450, is responsible for catalyzing the hydroxylation of the C-3' and C-4' positions on the isovaleryl side chain of the T-2 and HT-2 toxins.<sup>59</sup> When oxygen is removed from the epoxide group of a trichothecene mycotoxin to yield the carbon-carbon bond, deepoxy metabolites are formed. The deepoxy metabolites are essentially nontoxic.<sup>58</sup> This latter observation indicates that epoxide reduction is a single-step detoxification reaction for trichothecene mycotoxins.

Four hours after swine received intravenous tritium-labeled T-2 toxin, glucuronide conjugates represented 63% of the metabolic residues in urine, and 77% in bile.<sup>63</sup> The formation of glucuronide conjugates generally results in the elimination of toxicological activity of xenobiotics, which in certain species could represent a major route of detoxification of trichothecene mycotoxins.

In summary, then, very little of the parent trichothecene mycotoxin is excreted intact. Rather, elimination by detoxification of the toxin is the result of extensive and rapid biotransformation.

## CLINICAL DISEASE

The degree of illness in an individual exposed to trichothecene mycotoxins could be affected by a number of factors, including the nutritional status of the host, liver damage, intestinal infections, route of toxin administration, and stress.

The pathological effects and clinical signs for many toxic materials can vary with the route and type (acute, single dose vs chronic, subacute doses) of exposure. For the trichothecene mycotoxins, however, a number of the toxic responses are similar, regardless of the route of exposure. As we discussed earlier in this chapter, once they enter the systemic circulation, trichothecene mycotoxins affect rapidly proliferating tissue regardless of the

route of exposure.

In contrast, the symptoms and clinical signs of trichothecene intoxication can vary depending on whether the exposure is acute or chronic. Acute exposure to trichothecene mycotoxins used as biological warfare agents is the major concern for military medicine, but for continuity and historical implications, chronic intoxication will also be addressed in this chapter.

### Acute Effects

Acute oral, parenteral, dermal, or aerosol exposures to trichothecene mycotoxins produce gastric

and intestinal lesions. Hematopoietic and immunosuppressive effects are radiomimetic. Central nervous system toxicity causes anorexia, lassitude, and nausea; suppression of reproductive organ function; and acute vascular effects leading to hypotension and shock. While a number of toxic effects are common to different routes of exposure, route-specific effects have been observed in animal models. Examples of local, route-specific effects include the following:

- dermal exposure: local cutaneous necrosis and inflammation<sup>6</sup>;
- oral exposure: lesions to the upper gastrointestinal tract<sup>64</sup>; and
- ocular exposure: corneal injury.<sup>6</sup>

In Southeast Asia during the 1970s, symptoms began within minutes after an exploding munition (air-to-surface rocket, aerial bomb, cylinder) caused a yellow, oily, droplet mist to fall on individuals within 100 m of the explosion site. The falling droplet rain was inhaled, swallowed, and collected on skin and clothing; contaminated the terrain and food and water supply; and caused humans and animals to become acutely ill and to die after a variable period.<sup>7</sup> Massive cutaneous contact was prevalent when the sources of exposure were sprays or coarse mists that were used deliberately to contaminate humans and the environment. Although the suspected trichothecene mycotoxin attacks in Southeast Asia would have involved multiple routes of exposure, we can postulate that the skin would have been the major site for deposition of an aerosol spray or coarse mist.

Early symptoms and signs included severe nausea, vomiting, burning superficial skin discomfort, lethargy, weakness, dizziness, and loss of coordination. Within minutes to hours, diarrhea—at first watery brown and later grossly bloody—began. During the first 3 to 12 hours, dyspnea, coughing, sore mouth, bleeding gums, epistaxis, hematemesis, abdominal pain, and central chest pain could occur. The exposed cutaneous areas could become red, tender, swollen, painful, or pruritic, in any combination. Small or large vesicles and bullae might form; and petechiae, ecchymoses, and black, leathery areas of necrosis might appear. After death, the necrotic areas might slough easily when the corpse was moved.

Marked anorexia and dehydration were frequent. Dying patients became hypothermic and hypotensive, and developed tachycardia. A bloody ooze from the nares and mouth and an associated hematochezia

occurred in severely poisoned individuals. Death could occur within minutes, hours, or days, and was often preceded by tremors, seizures, and coma, in any combination.

The most common symptoms in both Southeast Asia and Afghanistan included vomiting (71%); diarrhea (53%); skin irritation, burning, and itching (44%); rash or blisters (33%); bleeding (52%); and dyspnea (48%).<sup>7,15,27</sup> All of the symptoms listed could be attributed to trichothecene mycotoxin toxicity.

### *Dermal Exposure*

Similar cutaneous irritations have been observed in numerous accidental and experimental settings:

- Individuals who were exposed to hay or hay dust contaminated with trichothecene-producing molds developed severe cutaneous irritations.<sup>38</sup>
- In working up large batches of fungal cultures from trichothecene-producing organisms, laboratory personnel suffered facial inflammation followed by desquamation of the skin and considerable local irritation.<sup>65</sup>
- When trichothecene mycotoxins of relatively low toxicity (crotoxin and trichotecin) were applied to the volar surface of human forearm or to the human head, reddening and irritation occurred within a few hours of exposure, and was followed by inflammation or scrubbing that healed in 1 to 2 weeks.<sup>66</sup>
- The hands of two laboratory workers were exposed to crude ethyl acetate extracts containing T-2 toxin (approximately 200 µg/mL) when the extract accidentally got inside their plastic gloves.<sup>66</sup> Even though the workers thoroughly washed their hands with a mild detergent within 2 minutes after contact, they experienced severe cutaneous irritations.

These observations provide evidence that when human skin is exposed *in vivo* to small amounts of trichothecene mycotoxins, severe cutaneous irritations develop and can last 1 to 2 weeks after acute exposure.

A number of animal models have been used to assess local and systemic toxicity and lethality from skin exposure to trichothecenes.<sup>6</sup> In a dermal study that used a mouse model, necrosis in the skin was present by 6 hours after dermal application

of T-2 toxin, with inflammation observed by 12 hours. The hairless guinea pig is an excellent model to illustrate the local skin lesions produced by a dermal application of T-2 toxin (Figure 34-3). The lesions are easily identified by 24 hours after the exposure, with maximal response at 48 hours. Some small lesions are still present 14 days after exposure to the toxin. From this experimental evidence, we can postulate that dermal exposure to trichothecene mycotoxins played a major role in the clinical illnesses that were seen following the yellow rain attacks.

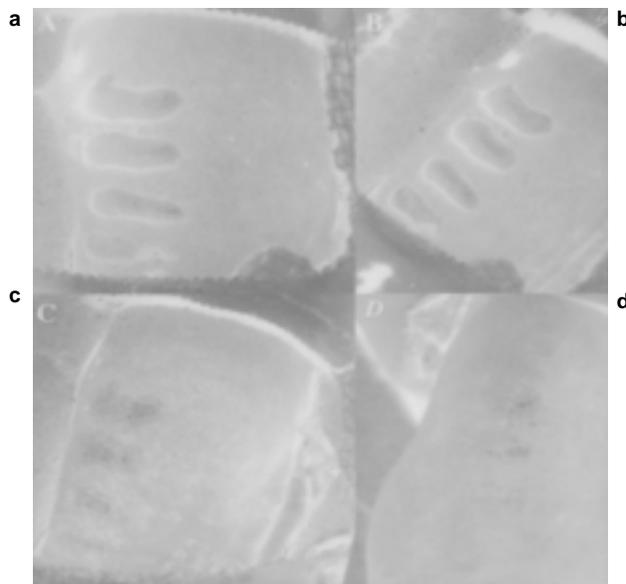
### Ocular Exposure

Victims of yellow rain attacks frequently reported tearing, eye pain, conjunctivitis, burning sensations about the eyes, and blurred vision for up to 1 week.<sup>7,16</sup> A Canadian Forces medical team interviewed Khmer Rouge casualties after a chemical/toxin attack at Tuol Chrey, Kampuchea.<sup>27</sup> Soldiers located 100 to 300 m from the artillery impact had onset of symptoms 2 to 5 minutes after exposure; these, likewise, included tearing, burning sensations, and blurred vision that lasted from 8 to 14 days. Analysis of autopsy samples from one of the casualties identified T-2, HT-2, and diacetoxyscripenol (DAS, also called anguidine) in his tissues. When the culture filtrates containing trichothecenes were instilled into the conjunctival sacs of rabbits, reddening and edema of the conjunctive membrane were observed within 1 or 2 days. Later, the cornea became opaque and developed scars that persisted for as long as 5 months.<sup>67</sup>

From these reports, we conclude that trichothecene mycotoxins can cause severe eye injury that can lead to a marked impairment of vision. This could be a severe incapacitating problem for unprotected military personnel. No systemic toxicity has been documented from the instillation of trichothecene mycotoxins into the eye of experimental animals, however.

### Respiratory Exposure

Victims of yellow rain reported a variety of upper respiratory signs and symptoms.<sup>7,27</sup> The major subdivisions of the respiratory tract that were affected include the nose (itching, pain, rhinorrhea, and epistaxis); the throat (sore/pain, aphonia, and voice change); and the tracheobronchial tree (cough, hemoptysis, dyspnea, and deep chest pain or pressure or both). Agricultural workers who were exposed to hay or hay dust contaminated with tricho-



**Fig. 34-3.** Skin lesions on the back of a hairless guinea pig at (a) 1, (b) 2, (c) 7, and (d) 14 days after application of (bottom to top) 25, 50, 100, or 200 ng of T-2 toxin in 2  $\mu$ L of methanol.

thecene mycotoxins developed similar signs and symptoms of upper respiratory injury. The descriptions of the yellow rain attacks in Southeast Asia (ie, the droplets, heavy mist, vapor), suggest that the aerosols were larger than 1 to 4  $\mu$ m—the particle size required for deposition in the alveoli. Thus, respiratory tract exposure from the larger-particle aerosols would involve mycotoxin deposition in the upper respiratory and tracheobronchial region, followed by secondary gastrointestinal tract exposure after clearance from the lungs.

We can postulate that multiple routes of exposure (topical, upper respiratory, and secondary enteral) to trichothecene mycotoxins occurred in victims of the yellow rain attacks. The symptoms of vomiting, diarrhea, melena, abdominal pain, and acute gastroenteritis with hematemesis<sup>7</sup> could be related to ingestion of toxin that was deposited in the upper respiratory tract and tracheobronchial region. Autopsies in the field of victims who died 24 to 48 hours after a yellow rain attack disclosed severe gastroenteritis with bleeding in the lower esophagus, stomach, and duodenum.<sup>27</sup> In humans, many of the acute enteral effects (from either yellow rain or contaminated hay and dust particles) of the trichothecene mycotoxins are probably the result of secondary ingestion of toxins that originally were deposited in the respiratory tract by large-particle aerosol.

## Chronic Toxicity

Chronic exposure to subacute doses of trichothecene mycotoxins is not thought to be an effect of biological warfare. This type of exposure, however, was responsible for ATA toxicosis in humans and mycotoxicosis in domestic animals. In addition, chronic toxicity has been iatrogenically induced when repeated subacute doses of a trichothecene mycotoxin were administered intravenously to cancer patients as a chemotherapy for colon adenocarcinoma.

### *Alimentary Toxic Aleukia Toxicosis*

The clinical course of ATA is divided into four stages. The *first stage* develops immediately or several days after consumption of grain products that are contaminated with trichothecene mycotoxins. Inflammation of the gastric and intestinal mucosa causes vomiting, diarrhea, and abdominal pain. In most cases, excessive salivation, headache, dizziness, weakness, fatigue, and tachycardia accompany this stage, and fever and sweating may also be present.<sup>36</sup>

The disease progresses to the *second stage*—the leukopenic or latent stage—which is characterized by leukopenia, granulopenia, and progressive lymphocytosis. When the ingestion of the toxin-contaminated food is not interrupted or if large doses are consumed, the next stage develops.<sup>36</sup>

The *third stage* is characterized by the appearance of a bright red, or dark cherry-red, petechial rash on the skin of the chest and other areas of the body. At first, the petechiae are localized in small areas, but they then spread and become more numerous.

In the most severe cases, intensive ulceration and gangrenous processes develop in the larynx, leading to aphonia and death by strangulation. At the same time, affected individuals have severe hemorrhagic diathesis of the nasal, oral, gastric, and intestinal mucosa.<sup>36</sup>

As the necrotic lesions heal and the body temperature falls, the *fourth stage*—the recovery stage—begins. During this period, exposed patients are susceptible to various secondary infections, including pneumonia. Convalescence is prolonged and can last for several weeks. Usually, 2 months or more are required for the blood-forming capacity of the bone marrow to return to normal.<sup>36</sup>

### *Cancer Chemotherapy*

The inhibitory effect of trichothecene mycotoxins on rapidly dividing cells was the basis for their evaluation as antitumor chemotherapy drugs during the late 1970s and early 1980s.<sup>68</sup> Phase I and phase II clinical evaluations of DAS (anguidine) in patients with cancer disclosed significant toxicity with intravenous doses 3.0 mg/m<sup>2</sup> (0.077 mg/kg) daily for 5 days, particularly in patients with hepatic metastases. The signs and symptoms included nausea, vomiting, diarrhea, burning erythema, confusion, ataxia, chills, fever, hypotension, and hair loss.<sup>69,70</sup> Antitumor activity of the trichothecenes was minimal or absent in the patients treated with DAS. Because of the marked toxicity of the drug, the life-threatening hypotensive effects, and the poor tolerance by patients, the evaluation of trichothecenes as chemotherapeutic drugs was discontinued.

## DIAGNOSIS

### **Battlefield Diagnosis**

In the absence of a biological detector or a particular characteristic of the aerosol (such as color or odor), diagnosis of an attack with trichothecene would depend on clinical observations of casualties and identification of the toxins in biological or environmental samples. This would involve a combined effort between the medical and chemical units in the field. The early signs and symptoms of an aerosol exposure to trichothecene mycotoxins would depend on particle size and toxin concentration. For a large-particle aerosol (particles > 10 μm, found in mist, fog, and dust; similar to that used in Southeast Asia), the signs and symptoms would include rhinorrhea, sore throat, blurred vi-

sion, vomiting, diarrhea, skin irritation (burning and itching), and dyspnea. Early (0–8 h) signs and symptoms from a deep-respiratory aerosol exposure (from aerosol particles in the 1- to 4-μm range) have not been fully evaluated but could include vomiting, diarrhea, skin irritation, and blurred vision.

Later signs and symptoms (8–24 h) would probably be similar (except for the degree of skin rash and blisters) for both large-particle and deep-respiratory aerosol exposure to trichothecene mycotoxins. They could include continued nausea and vomiting, diarrhea, burning erythema, skin rash and blisters, confusion, ataxia, chills, fever, hypotension, and bleeding.

Nonspecific changes in serum chemistry and hematology occurred in monkeys exposed to an

acute dose of T-2 toxin. Alterations in serum chemistry included elevations in serum creatinine, serum enzymes (especially creatine kinase), potassium, phosphorous, and serum amino acids; and, due to decreased coagulation factors, elevations in prothrombin time and partial thromboplastin time. An initial rise in the absolute number of neutrophils and lymphocytes may occur within hours, followed by a decrease in lymphocyte counts by 48 hours. Survival beyond several days may be associated with a fall in all blood cellular elements.<sup>6</sup> Although it is likely that these acute changes will also be seen in humans, careful clinical observations of human victims of acute trichothecene mycotoxicosis have not been reported to date. In patients with chronic toxicity (ie, ALA) resulting from repeated ingestion of contaminated bread, pancytopenia is an important part of the clinical picture.<sup>36</sup>

In the yellow rain attacks in Southeast Asia, diagnosis of the causative agent was difficult and involved ruling out the presence of conventional chemical warfare agents. Contamination of the environment and clothing by nerve and blistering agents would be absent, and these were, in fact, not detectable in such samples from Southeast Asia. Sarin, soman, or other nerve agents could be missed unless thickened soman or VX was used.

The following events should suggest to medical officers that a biological warfare attack with trichothecene mycotoxins has occurred:

- clinical findings that match the symptoms listed above;
- high attack and fatality rates;
- all types of dead animals; and
- onset of symptoms after a yellow rain or red, green, or white smoke or vapor attack.

At present, we do not have a fieldable identification kit for any of the trichothecene mycotoxins. Several commercial immunoassay kits are marketed for the detection of trichothecene mycotoxins (T-2 toxin, deoxynivalenol, and their metabolites) in grain extracts or culture filtrates of *Fusarium* species.<sup>71,72</sup> These kits have not been evaluated against biomedical samples that contain typical concentrations of the mycotoxins, however. Screening tests for presumptive identification of trichothecene mycotoxins in the biomedical samples would probably involve bioassays, thin-layer chromatography, or immunological assays, in any combination. At a national laboratory, confirmatory methodology would involve the use of various combinations of gas chromatography, high-performance liquid chro-

matography, mass spectrometry, and nuclear magnetic resonance spectrometry.

In areas that have experienced a yellow rain attack, environmental assays have been in the range of 1 to 150 parts per million (ppm) and blood samples in the range of 1 to 296 parts per billion (ppb).<sup>8-10,16,22</sup> In the laboratory, at 10 and 50 minutes after an intramuscular exposure to 0.4 mg/kg of T-2 toxin in the dog, plasma concentrations of T-2 toxin were 150 and 25 ppb, and for HT-2 toxin were 50 and 75 ppb, respectively.<sup>60</sup> Thus, any screening procedure for trichothecene mycotoxins in biomedical samples must have detection limits of 1 to 100 ppb. Most of the analytical procedures require extraction and cleanup treatment to remove interfering substances.<sup>73</sup>

Screening tests for the trichothecene mycotoxins are generally simple and rapid but, with the exception of the immunochemical methods, are nonspecific. A number of bioassay systems have been used for the identification of trichothecene mycotoxins.<sup>73</sup> Although most of these systems are very simple, they are not specific, their sensitivity is generally relatively low compared to other methods, and they require that the laboratory maintain vertebrates, invertebrates, plants, or cell cultures. Thin-layer chromatography (TLC) is one of the simplest and earliest analytical methods developed for mycotoxin analysis. Detection limits for trichothecene mycotoxins by TLC is 0.2 to 5 ppm (0.2 to 5 µg/mL). Therefore, extracts from biomedical samples would have to be concentrated 10- to 1,000-fold to screen for trichothecene mycotoxins.

To overcome the difficulties encountered with the bioassays and TLC methods, immunoassays using specific polyclonal and monoclonal antibodies have been developed for most of the major trichothecene mycotoxins and their metabolites.<sup>73</sup> These antibodies have been used to produce simple, sensitive, and specific radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs) for the mycotoxins. In the presence of the sample matrix, the lower detection limits for identification of trichothecene mycotoxins by RIA is about 2 to 5 ppb<sup>73</sup> and by ELISA, 1 ppb.<sup>74</sup> We conclude that immunoassays are useful tools for screening biomedical samples for evidence of a biological warfare attack with trichothecene mycotoxins.

### Confirmatory Procedures

Gas-liquid chromatography (GLC) is one of the most commonly used methods for the identification of the trichothecene mycotoxins in both agricultural

products and biomedical samples.<sup>75</sup> Before GLC analysis, the polar groups in mycotoxin molecules must first be converted to their esters or ethers. Extensive treatment to clean up the sample is required before derivatization and subsequent analysis can be performed. By the most sensitive procedures, the detection limit for trichothecene mycotoxins is 10 ppb. If the analysis is on a sample that contains an unknown toxic material, such as those from the yellow rain attacks, then the GLC method can only provide presumptive evidence of a trichothecene mycotoxin exposure. Confirmation will require the identification with more definitive physicochemical procedures.

Mass spectrometry (MS) is the physicochemical method of choice for characterizing, identifying, and confirming the presence of trichothecene mycotoxins.<sup>76,77</sup> Picogram quantities of trichothecene mycotoxins are readily detectable by MS methods. In some cases, extensive cleanup steps are unnecessary.

The combination of GLC and MS techniques (GLC-MS) has proven to be a more-specific method for identifying mycotoxins than is GLC alone.<sup>76,77</sup> As a result, the GLC-MS method has become the standard for identifying trichothecene mycotoxins in agricultural products as well as in biomedical samples. As little as 1 ppb of T-2 toxin can be identified without extensive cleanup.<sup>76</sup> One major drawback of this methodology is the time-consuming derivatization step that trichothecene mycotoxin identification by GLC-MS requires. A high-performance liquid chromatography-mass spectrometry (HPLC-MS) procedure was described in 1991 and provides a specific and reliable method for the identification of trichothecene mycotoxins without derivatization.<sup>78</sup> The HPLC-MS procedure achieves sensitivity at the 0.1-ppb level. This technology will require further evaluation and development, but it appears to be a promising approach for the rapid confirmation of trichothecene mycotoxins in a biomedical sample.

## MEDICAL MANAGEMENT

### Individual and Unit

The immediate use of protective clothing and mask at the first sign of a yellow rain-like attack should protect an individual from the lethal effects of this mycotoxin. The mask can be applied in less than 9 seconds and can be worn at first sighting of an incoming rocket or enemy aircraft. Contaminated battle dress uniforms (BDUs) should be removed before protective clothing is donned. Since the area covered with agent is likely to be small, another helpful tactic is to leave the area after taking samples to document the attack. Vulnerability is increased by lack of protective clothing, mask, or training (as was demonstrated in Laos) or by a surprise biological warfare attack (such as a night or an undetected attack). A lightweight face mask, outfitted with filters that block the penetration of aerosol particles 3 to 4  $\mu\text{m}$  or larger, should provide respiratory protection against yellow rain. Only 1% or 2% of aerosolized T-2 toxin penetrated nuclear, biological, chemical protective covers (NBC-PC).<sup>79</sup> Regular BDUs would offer some protection, but the degree would be functions of the age and condition of the fabric, and the type of environmental conditions.

Two topical skin protectants (TPS1 and TSP2) are in advanced development for protection against chemical warfare agents. When applied to the skin of rabbits 60 minutes before exposure to 50  $\mu\text{g}$  of T-

2 toxin, both topical skin protectants completely protected the rabbits from the dermal irritating effects of this mycotoxin for at least 6 hours.<sup>80</sup>

As soon as individuals or units suspect that they have been exposed to a mycotoxin attack, they should remove their BDUs, wash their contaminated skin with soap and water, and then rinse with water. Washing the contaminated area of the skin within 4 to 6 hours after exposure to T-2 toxin removed 80% to 98% of the toxin and prevented dermal lesions and death in experimental animals.<sup>25</sup> Contaminated BDUs as well as wash waste from personnel decontamination should be exposed to household bleach (5% sodium hypochlorite) for 6 hours or more to inactivate any residue mycotoxin.

Two skin decontamination kits, the M258A1 and the M238A1, have been designed for the removal and detoxification of chemical warfare agents. The M258A1 kit is the currently fielded standard. When evaluated against trichothecene mycotoxins, however, the M238A1 kit effectively removed T-2 toxin from the skin of rats but did not detoxify this biological warfare agent.<sup>81</sup> Several of the components of the M258A1 kit are themselves highly toxic, caustic compounds that caused dermal irritation and lethality in rats and rabbits.<sup>82</sup>

A second-generation skin decontamination kit, the XM291, has been developed, and contains an XE-555 resin material as the active component. This skin decontamination kit is efficacious against most

chemical warfare agents and presents no serious human factor or human safety problems. The XE-556 resin, a similar but different formulation, was effective in the physical removal of T-2 toxin from the skin of rabbits and guinea pigs.<sup>83</sup> The foregoing observations suggest that the skin decontamination kits that were designed specifically for removal and detoxification of chemical warfare agents could also afford a significant degree of protection through the physical removal of mycotoxins from the skin of exposed individuals.

### Specific or Supportive Therapy

No specific therapy for trichothecene-induced mycotoxicosis is known or is presently under experimental evaluation. Several therapeutic approaches have been evaluated in animal models. It is perhaps significant, however, that although experimental procedures for treatment of systemic exposure have been successful in reducing mortality in animal models, they have not been tested in primates. Thus, these treatments are not available for field use for humans exposed to trichothecene mycotoxins.

Individuals exposed to a yellow rain-like attack should be treated with standardized clinical toxicology and emergency medicine practices for ingestion of toxic compounds. After an aerosol exposure to a yellow rain-like attack, mycotoxins will be trapped in the nose, throat, and upper respiratory tract. The particles will be returned by ciliary action to be swallowed, resulting in a significant oral exposure. Superactive charcoal has a very high maximal binding capacity (0.48 mg of T-2 toxin per 1 mg of charcoal), and treatment either immediately or 1 hour after oral or parenteral exposure to T-2 toxin significantly improves the survival of mice.<sup>84</sup> Superactivated charcoal with magnesium sulfate is stocked in the chemical and biological warfare kits of U.S. Army field hospitals.

Symptomatic measures for the treatment of exposure to trichothecene mycotoxins are modeled after the care of casualties of mustard poisoning.<sup>85</sup> Irrigation of the eyes with large volumes of isotonic saline may assist in the mechanical removal of trichothecene mycotoxins, but would have limited useful therapeutic effects. After the skin has been decontaminated, some erythema may appear, accompanied by burning and itching. Most casualties whose skin has been treated with soap and water within 12 hours of exposure will have mild dermal effects; these should be relieved by calamine and other lotion or cream, such as 0.25% camphor and methanol.

Limited data are available on the respiratory effects of inhaled trichothecene mycotoxins, although acute pulmonary edema is one of the serious, often lethal consequences of a yellow rain attack.<sup>16,27</sup> One of the major symptoms following the yellow rain attacks was an upper respiratory irritation (sore throat, hoarseness, nonproductive cough),<sup>7,16,27</sup> which can be relieved by steam inhalation, codeine, or another substance to suppress the cough, and other simple measures.<sup>85</sup> A casualty who develops severe respiratory symptoms should be under the care of a physician skilled in respiratory care.

The early use of high doses of systemic glucocorticosteroids increases survival time by decreasing the primary injury and the shocklike state that follows exposure to trichothecene mycotoxins.<sup>86</sup> A selective platelet activating factor antagonist, BN 52021, can prolong the survival of rats exposed to a lethal intravenous dose of T-2 toxin.<sup>87</sup> This finding suggests that platelet activating factor is an important mediator of T-2 toxicosis. Dosing before and after the exposure with diphenhydramine (an antihistaminic agent) or naloxone (an opioid antagonist) prolonged the survival times of mice exposed subcutaneously or topically with lethal doses of T-2 toxin.<sup>88</sup>

We can postulate that a number of bioregulators are the mediators of the shocklike state of trichothecene mycotoxicosis. Methylthiazolidine-4-carboxylate increased hepatic glutathione content and enhanced the survival of mice after an acute intraperitoneal exposure to T-2 toxin.<sup>89</sup> The protective effects of this drug may be the result of increased detoxification and excretion of the glucuronide conjugate of T-2 toxin. A general therapeutic protocol that included combinations of metoclopramide, activated charcoal, magnesium sulfate, dexamethasone, sodium phosphate (which had very little effect), sodium bicarbonate, and normal saline as the therapeutic agents was evaluated in swine given an intravenous LD<sub>50</sub> dose of T-2 toxin.<sup>90</sup> All treatment groups showed improved survival times when compared with the nontreated T-2 controls.

### Prophylaxis

The mycotoxins are low-molecular-weight compounds that must be conjugated to a carrier protein to produce an effective antigen.<sup>73</sup> When T-2 toxin is conjugated to a protein, it develops relatively low antibody titers and is still a marked skin irritant.<sup>91</sup> This would preclude mycotoxins' use as immunogens in the production of protective immunity. To circumvent such problems, a deoxy-

verrucarol (DOVE)-protein conjugate was used to immunize rabbits.<sup>92</sup> Antibody titers to DOVE developed rapidly after immunization, but they were highly specific for DOVE rather than a common trichothecene backbone.<sup>92</sup>

Another approach was to develop antibody-based vaccines (anti-idiotypic) against T-2 toxin. Protective monoclonal antitoxin antibodies were first generated and then used to induce specific monoclonal anti-idiotypic antibodies. When mice were immunized with specific monoclonal anti-idiotypic antibodies, they developed neutralizing antibodies and were protected against challenge with a lethal dose of T-2 toxin.<sup>93</sup> Thus, it would be feasible to develop a despeciated monoclonal anti-idiotypic antibody that could be a vaccine candidate against T-2 toxin.

Several monoclonal antibodies against T-2 toxin will protect against the T-2-induced cytotoxicity in various cell lines.<sup>94,95</sup> When a monoclonal anti-

body against T-2 toxin (15H6) was given to rats (250 mg/kg) 30 minutes before or 15 minutes after a lethal dose of mycotoxin, it conferred 100% survival.<sup>94</sup> Thus, monoclonal antibodies do have some prophylactic and therapeutic value against T-2 toxicosis, but very large quantities are required for protection.

Prophylactic induction of enzymes involved in the conjugation of xenobiotics reduced or prevented the acute toxic effects of T-2 toxin in the rat, while inhibition of these enzymes resulted in a higher toxicity for this trichothecene.<sup>96</sup> Pretreatment with flavonoids,<sup>97</sup> ascorbic acid,<sup>98</sup> vitamin E,<sup>99</sup> selenium,<sup>100</sup> or chemoprotective compounds such as emetine<sup>101</sup> that block trichothecene-cell association all reduce acute toxicity of these mycotoxins. However, none of these chemoprotective treatments have undergone extensive efficacy studies to evaluate their ability to protect against an aerosol or dermal exposure to trichothecene mycotoxins.

## SUMMARY

Trichothecene mycotoxins are noted for their marked stability under different environmental conditions. On a weight-for-weight basis, they are less toxic than other toxins such as ricin, botulinum, and staphylococcal enterotoxin B, but trichothecene mycotoxins are proven lethal agents in warfare. Symptoms include vomiting, pain, weakness, dizziness, ataxia, anorexia, diarrhea, bleeding, skin redness, blistering, and gangrene, as well as shock and rapid death. Sensitive immunoassays and chemical procedures are available for the identifi-

cation of trichothecene mycotoxins in biological samples, but no detection kits have been fielded.

Prevention of exposure is the only current defense, with a protective mask and clothing worn when under attack. Previous successful lethal attacks have always occurred against unprotected civilians and soldiers. Skin decontamination with water and soap can be used effectively up to 6 hours after exposure. Experimental treatments for systemic toxicity are being investigated, but no therapy is available for humans.

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