Chapter Four

TOXINS

Toxins are natural poisons that include the most toxic substances known. Toxins are sometimes classified as chemical warfare agents and sometimes as biological warfare agents (SIPRI, 1972; Franke, 1976). Bacteria, fungi, dinoflagellates, algae, plants, and animals (e.g., corals, snails, frogs, arachnids, and snakes) produce toxins (U.S. Army, 1990; SIPRI, 1971; SIPRI, 1973; Raskova, 1971; Gill, 1982). Table 4.1 (SIPRI, 1973) compares the relative toxicity of chemicals and toxins, listed in order of toxic magnitude. As the table shows, sarin, a nerve agent, is set at 1,000 (an arbitrary scale), and many toxins are at the upper ranks of toxicity.

Human use of toxins has been both constructive and destructive. Toxins have been used to develop drugs, such as digitalis and physostigmine; as research probes to "dissect out" mechanisms of biological action (kainic acid, ryanodine); and to treat neurological disorders (botulinum toxin) and cancer (ricin). They also may be used for assassination and in warfare.

Toxins have been used periodically in warfare. The Moors may have used aconitine in warfare in the 14th century. It was perhaps used earlier in India and China. Aconitine was studied in World Wars I and II and tested in bullets (SIPRI, 1971; SIPRI, 1973). During World War II, toxins were produced and reached weapon status in several countries, perhaps with limited use in sabotage and special operations. Table 4.2 compares the lethality of some toxins and chemical agents in mice.

Although most countries signed treaties in the 1970s and 1980s prohibiting the use of biologic and toxin weapons, indications of toxin use were reported in Laos, Cambodia, and Afghanistan and were alleged during the Iran-Iraq War (Seagrave, 1981; Heyndrickx, 1984; House, 1982). Rapid development in the biological sciences has enabled the production of toxins outside their organisms of origin, e.g., placing the genetic information of the toxin in *E. coli* for expression (Gill, 1982).

Table 4.1

Relative Lethalities of Selected Natural and Synthetic Poisons

Relative Lethality[a] (Sarin = 1,000)	Synthetic Poisons[a]	Natural Poisons[b] Name	Source
10^{-4} to 10^{-3}		Botulinum toxin type A, α fraction	Botulinum toxin type A
10^{-3} to 10^{-2}		Botulinum toxin type A, crystalline	*Clostridium botulinum* bacteria
		Tetanus toxin, crystalline	*Clostridium tetani* bacteria
10^{-2} to 10^{-1}		Botulinum toxin type A, amorphous	*Clostridium botulinum* bacteria
10^{-1} to 10		Palytoxin	*Palythoa* zoanthid coelenterates
1 to 10			
10 to 10^2	Homocholine Tammelin-ester (3-trimethylammoniopropyl methylphosphonofluoridate iodide)	Ricin, crystalline	Castor beans, the seeds of *Ricinis communis*
	Dioxin (2,3,7,8-tetrachlorodibene-*p*-dioxin)	C-alkaloid E	Calabash-curare arrow poison
	35 SN^+ (O-ethyl S-2-trimethylammonioethyl methylphosphonothiolate iodide)	Saxitoxin	*Gonyaulax catanella* dinoflagellate marine algae
		Tetrodotoxin	Puffer fishes and certain salamanders
		Atelopidtoxin	*Atelopus zeteki*, a Panamanian arrow-poison frog
		Abrin, crystalline	Jequirity beans, the seeds of *Abrus precatorius*
		Indian cobra neurotoxin	Indian cobra venom
10^2 to 10^3	Ethylthioethyl-metasystox (OO-dimethyl S-2-(S'-ethylthioethylsulphonio)ethyl phosphorothiolate bromide)	Ricin, amorphous	Castor beans, the seeds of *Ricinis communis*
	Seleno VE (O-ethyl Se-2-diethylaminoethyl ethylphosphonoselenolate)	Kokór arrow poison	*Phyllobates aurotaenia*, a Colombian frog
	HC 3 (4,4'-*bis*(NN-dimethyl-N-2-hydroxyethylammonioacetyl)biphenyl dibromide)		

Table 4.1—Continued

Relative Lethality[a] (Sarin = 1,000)	Synthetic Poisons[a]	Natural Poisons[b] Name	Natural Poisons[b] Source
	VX (O-ethyl S-2 diisopropylaminoethyl methylphosphonothiolate)	Russell's viper venom	*Vipera russelli*
	Ro 3-0422 (3-(diethylphosphoryl)-1-methylquinolinium methosulphate)		
	TL 1236 (2-methyl-5-trimethylammoniophenyl N-methylcarbamate chloride)	Israeli scorpion venom	*Leiurus quinquestriatus*
	Gd-42 (O-ethyl S-2-(S'S'-methylethylsulphonio)ethyl methylphosphonothiolate methosulphate)		
	DCMQ (5-NN-dimethylcarbamoyl-1-methylquinolinium bromide)	α- Aminitin	*Amanita phalloides*, the death cap mushroom
	Phospholine (OO-diethyl S-2-trimethylammonioethyl phosphorothiolate iodide)		
	3152 CT (1-(3'-trimethylannoniophenoxy)-3-(-(3'-trimethylammoniophenoxy-5'-NN-dimethylcarbamoyl)propane diiodide)	Indian cobra venom	*Naja naja*
	Soman (1,2,2-trimethylpropyl methylphosphonofluoridate)		
		Brown widow spider venom	*Latrodectus geometricus*
		d-Tubocurarine	Tube-curare arrow poison
1,000	**Sarin (isopropyl methylphosphonofluoridate)**		
10^3 to 10^4	Tabun (ethyl NN-dimethylphosphoroamidocyanidate)	Aconitine	Roots of monkshood, *Aconitum napellus*
	Armin (O-ethyl O-4-nitrophenyl ethylphosphonate)	Physostigmine	Calabar bean, the seeds of *Physostigma venenosum*

Table 4.1—Continued

Relative Lethality[a] (Sarin = 1,000)	Synthetic Poisons[a]	Natural Poisons[b] Name	Natural Poisons[b] Source
	Gd-7 (O-ethyl S-2-ethylthioethyl methylphosphonothiolate)	North American scorpion venom	*Centruroides sculpturatus*
	Methyl fluoroacetate	Strychnine	*Stryhnos nuxvomica* bark or seeds
		Black widow spider venom	*Latrodectus mactans mactans*
		Ouabain	*Strophanthus gratus* seeds
10^4 to 10^5	Hydrogen cyanide	Nicotine	*Nicotiana* tobacco plants
	Cadmium oxide	Western diamondback rattlesnake venom	*Crotalus atrox*
	Mustard gas (*bis*(2-chloroethyl) sulphide		
	Parathion (OO-diethyl O-4 nitrophenyl phosphorothionate)		
	Lewisite (2-chlorovinyldichloroarsine)		
	Phosgene oxime		
	Arsine		
10^5 to 10^6	Cyanogen chloride	Bee venom	The honey bee *Apis mellifera*
	Chlorine		
	White arsenic		

SOURCE: SIPRI (1973).

[a]The "relative lethality" was determined as follows: Reported LD_{50} figures for the following combinations of route of administration and experimental animal were assembled from the cited literature: intravenous, mouse; subcutaneous, mouse; intravenous, rat; subcutaneous, rat; intravenous, guinea pig; intravenous, cat; intravenous, rabbit. Within each set, each agent's LD_{50} was converted into a lethality index relative to sarin, assigning a reference value of 1,000 to the sarin LD_{50} concerned. For example, the subcutaneous, mouse index for batrachotoxin is taken as 10 because its subcutaneous, mouse LD_{50} and that of sarin were around 0.002 and 0.2 mg/kg, respectively. In this table, the agents are ranked according to their lowest lethality index. When the animal parenteral LD_{50} was unavailable, the respiratory LCT_{50} was used instead, except for white arsenic, for which an oral LD_{50} was used. The respiratory LD_{50} of sarin in man is estimated to be about 1,000 µg.

[b]The venoms of *Vipera russelli*, *Leiurus quinquestriatus*, and *Latrodectus geometricus* appear to be, respectively, the most poisonous snake, scorpion, and spider venoms known.

Table 4.2

Comparative Lethality of Some Toxins and Chemical Agents in Mice

Agent	LD_{50} (µg/kg)[a]	Molecular Weight	Source
Botulinum toxin	0.001	150,000	Bacterium
Ricin	3	64,000	Plant
VX	15	267	Chemical agent
Soman	64	182	Chemical agent
Sarin	100	140	Chemical agent
Aconitine	100	647	Plant
T-2 toxin	1,210	466	Fungal toxin
Aflatoxin[b]			

SOURCE: Sidell, Takafuji, and Franz (1997), p. 609.

[a]Interperitoneal or intravenous.

[b]Franz did not report aflatoxin. The reviewer did not locate mouse toxicity data for this toxin.

Toxins have also been associated with public health and agricultural problems, primarily contamination of food (botulinum, mycotoxins), and, more recently, health problems arising from airborne contamination (Steyn, 1995; Coulombe, 1993; Hendry and Cole, 1993; DiPaolo et al., 1994; Ueno, 1983; Wang, Hatch, et al., 1996).

The diverse toxins available for military use span a wide range of effects, from immediate lethality to delayed illness and incapacity. Some toxins are highly stable, making them suitable for long-term storage in weapons and as persisting environmental hazards. They offer high potency, and many can be produced with modest technology investments. Toxins can be used as "strategic" weapons, as indicated in a 1970 World Health Organization (WHO) study, which indicated enormous casualties in urban settings from toxin use and chemicals (WHO, 1970).

Toxins are also quite suitable for tactical employment (U.S. Army, 1990). They may be distributed as aerosols, liquids, or powders, with attacks capable of covering tens to hundreds of square kilometers, and can be delivered by air- or ground-bursting munitions, aircraft spray tanks, or ground-based aerosol generators (U.S. Army, 1990). There are no volatile toxins (Sidell, Takafuji, and Franz, 1997, p. 609). According to Zilinskas (1997), an American arms control specialist who worked with UNSCOM inspecting Iraqi chemical and biological facilities, Iraq possessed the relevant systems and made a great effort to weaponize toxins.

Although the eye and respiratory systems are thought to be the primary routes of toxin exposure, skin and gastrointestinal exposures are also possible (Adams, 1989). Thus, sabotage might concentrate on food, water, and ventilation sys-

tems. Toxins have also been used in bullets and in other projectiles (SIPRI, 1973). Some toxins are capable of sustained surface contamination and may also represent a secondary aerosol hazard as soil is disturbed (Adams, 1989).

In general, toxin attacks are difficult to recognize. Most toxins are odorless, and their aerosols are not visible. Their potency and diversity have, to date, precluded the deployment of specific detector systems, although there are military systems that can detect aerosol clouds. Technology, such as microencapsulation, has the potential of altering delivery systems to permit skin intoxications, tailoring particle sizes, and making agents more resistant to environmental degradation (U.S. Army, 1990). Because the body has a limited number of ways to respond to chemicals and toxins, clinical recognition and diagnosis may not readily distinguish among different agents. It is not easy to demonstrate toxins in biological tissues or the environment, which may account for some of the controversy about their suspected use (Heyndrickx, 1984; Watson, Mirocha, and Hayes, 1984).

The defensive preparations coalition forces made (IOM, 1996; DSB, 1994) anticipated Iraq's postwar admission that it had developed and deployed biological weapons, including toxins, prior to the Gulf War (Marshall, 1997; PAC, 1996b; Zilinskas, 1997). The Iraqis had deployed botulinum toxin, the most toxic material known, in weapons, and Heyndrickx (1984) suspected that they also possessed and used trichothecene mycotoxins. Ricin had been the subject of advanced military research and development since World War I, and considerable unclassified public information about the development of weapons with ricin was available after World War II (OSRD, 1946; SIPRI, 1973, WHO, 1970). Thus, its properties were well known to the Iraqis. In addition, castor beans, ricin's basic source, are inexpensive and readily available.

Recent reports that aflatoxins (of which aflatoxin B_1 [AFB_1] is a hepatoxic material and suspected carcinogen) were produced and deployed in Scud warheads were surprising because this family of toxins had not generally been considered to be militarily useful (Marshall, 1997; Zilinskas, 1997; U.S. Army, 1990; SIPRI 1971).

Since the Gulf War, the occurrence of delayed and poorly understood illnesses in Gulf War veterans has raised the question of whether unrecognized toxin exposure may have played a role in such illnesses, either from clandestine use or from "fallout" from coalition attacks on Iraqi biological facilities (Riegle and D'Amato, 1994; GAO, 1997). Three toxins are reviewed in detail below: ricin (a plant toxin) and the trichothecenes and aflatoxins (two families of mycotoxins).[1]

---

[1]As noted earlier, anthrax and botulinum toxin are covered in Hilborne and Golomb (2000).

RICIN

Ricin, also known as Agent W, is the toxic protein derived from the castor bean plant. Ricin's properties have been known since ancient times, and it has a long history of accidental and intentional intoxication (Klain and Jaeger, 1990). Ricin was studied as a possible weapon in World War I, and the United States, Canada, and the United Kingdom developed it as a weapon during World War II (OSRD, 1946). Recent scientific interest in ricin stems from its possible use in cancer therapy, as a probe to study protein metabolism, and as a selective tool in neurophysiology research (Wellner, Hewetson, and Poli, 1995; de la Cruz et al., 1995). Ricin achieved some notoriety when it was used in a sophisticated pellet to kill the Bulgarian political dissident Georgi Markov in London and to injure another dissident in Paris (Klain and Jaeger, 1990). There is little information about the long-term consequences of ricin exposure, but its patterns of acute toxicity have been characterized (OSRD, 1946), as described below.

Weaponization

Ricin is easily extracted from the cultivated plant *Ricinus communis*. The World War II effort led to refined ricin in a crystalline form, although it was the amorphous form that was used in high-explosive bombs and shells and in more specialized delivery systems, such as plastic containers and cluster bombs. Attaining effective particle sizes with ricin powder is difficult, and use of the material in water or suspended in glycerol or carbon tetrachloride is more effective (OSRD 1946; Franke, 1976). Because, as a protein, ricin degrades in the environment, it can also serve as a research surrogate for other agents of biological origin (OSRD, 1946; SIPRI, 1973).[2] World War II studies, conducted on unpurified material, found ricin to be 7 to 40 times as toxic as phosgene, and postwar comparisons found it to be comparable in toxicity to the nerve agents (Franke, 1976; OSRD, 1946; WHO, 1970).

The agent is difficult to detect. It is fairly stable in clear, dry weather, persisting in the soil or environment for up to three days. (U.S. Army, 1990; Sanches et al., 1993).[3]

Evidence from the Gulf War indicated that Iraq had previously developed and tested weapons containing ricin (Zilinskas, 1997). The reported delivery system—artillery shells with bursters—was not very sophisticated, compared to

---

[2]OSRD (1946) says that "Ricin was recognized as a prototype of toxic protein materials of bacterial origin which were known to have even greater toxicity but which were less conveniently prepared and handled." (The reviewer assumes that the microbial toxin was botulinum toxin.)

[3]This favorable weather may not have always occurred during the air and ground war period of the Gulf War.

World War II U.S. delivery systems (OSRD, 1946). There was no evidence that ricin was actually used in the Iran-Iraq conflict or in the Gulf War.

Detection

No military detectors have been deployed for ricin to date. During World War II, guinea pigs hypersensitized to ricin, so that they would develop anaphylaxis, were used to detect aerosols and assess persistence (OSRD, 1946). Efforts were made at that time to use hemaglutination to detect microgram amounts, but results were nonspecific. An anti-ricin precipitation reaction was also used.

More recently, competitive radioimmunoassays have been used in a chemotherapy project to detect ricin in the 50 to 100 pg/ml range. Several enzyme-linked immunoadsorbent assay (ELISA) systems are able to detect ricin in tissues, and immunocytochemical tests exist but are not highly sensitive (Wellner, Hewetson, and Poli, 1995). It appears possible, based on animal studies, to detect ricin aerosol exposures using ELISA techniques on material from oro-nasal swabs up to 24 hours after exposure (Franz and Jaax, 1997).

Regardless of the mechanism used, ricin cannot be detected long after it is used, because, as a protein, it degrades in the environment. Persistence for up to three days in dry weather was found in World War II studies (OSRD, 1946).

Physical and Chemical Characteristics

Ricin in dry form, depending on its purity, is either an amorphous solid or crystalline material. It is soluble in water and weak acid and forms stable suspensions in glycerol or carbon tetrachloride. It has no odor (OSRD, 1946; U.S. Army, 1990).

Ricin is a protein composed of two globular polypeptide chains linked by a disulfide bridge, with the enzymatically active A chain folding into a cleft in the globular B chain, which is responsible for adhesion to and transportation into the cell. The molecular weight is about 64,000. Chemicals that break the disulfide bond inactivate the toxin (Sanches et al., 1993).

Toxicology and Toxicokinetics

A great deal is known about the cellular and molecular mechanisms by which ricin interrupts protein synthesis within the cell. Basically, ricin inhibits protein synthesis by inactivating ribosomal RNA (Wellner, Hewetson, and Poli, 1995). Ricin's A subunit possesses the enzymatic biological activity, while the B unit is involved in binding the toxin to the cell surface receptors (galactose) and subsequent transport into the cell. After entry, the A unit in the cytosol inhibits protein synthesis by inactivating ribosomal RNA. The detailed mechanisms of

binding, transport, and RNA inhibition have been studied in great detail (Sandvig and Van Deurs, 1996; Simpson et al., 1996; Morino et al., 1995; Li, Frankel, and Ramakrishnan, 1992). Inhibition of protein synthesis in eukaryotes is caused by ricin's cleaving of an adenosine ribose bond in messenger RNA (Wellner, Hewetson, and Poli, 1995).

A single molecule in a cell can cause that cell to die. Ricin intoxication may induce "programmed cell death" (apoptosis), as noted in cultured pulmonary endothelial cells and in lymphatic tissues of poisoned rats (Leek et al., 1990; Hughes and Lindsay, 1996).

What remains unclear is how ricin causes injury and death in complex organisms (Wellner, Hewetson, and Poli, 1995; Klain and Jaeger, 1990). However, because mice can be protected from an intravenous ricin challenge by intracerebral anti-ricin antibodies, and because alterations of blood-brain barrier permeability increase ricin toxicity, the "lethal" target tissue is probably the central nervous system (Foxwell et al., 1985). This is not certain because studies of distribution in tissue seldom mention the brain, and it may be difficult to find ricin there anyway because a mouse can be killed by administration of only a picomole.

Exposure-Effect Relationships. The sensitivity of various animal species to ricin varies over a hundredfold range (Franz and Jaax, 1997). Toxicity and time of death vary considerably depending on the route of exposure, as exemplified by studies in mice: The inhalation LD_{50} is 3 to 5 µg/kg (absorbed dose), with death at 60 hours; the subcutaneous LD_{50} is 24 µg/kg, with death at 100 hours; and the oral LD_{50} is 20 mg/kg, with death at 85 hours (Franz and Jaax, 1997, p. 633).

Ricin can produce severe to fatal injury by contact with eyes or by ingestion, inhalation, or parenteral routes. Little has been reported, however, regarding dermal toxicity or chronic effects. According to the OSRD, dermal toxicity was not an issue for the U.S. researchers and production workers during World War II. Although ricin poisoning is noted for delayed onset of symptoms, larger doses produce a more rapid onset (OSRD, 1946; Balint, 1993).

Table 4.3 summarizes some selected exposure-effect data by route of exposure. No chronic exposure data are available, although ricin is immunogenic. Some World War II workers probably experienced brief allergic respiratory reactions (OSRD, 1946). The rapid development of immunity apparently protected World War II workers from major toxicity, in the same way that immunity thwarts chemotherapy with ricin. It has been shown in animals that when the immune system is impaired by T-cell depletion and ricin is repeatedly administered at sublethal levels (50 ng weekly for five weeks), deaths occur in 35 to 46 days. This indicates that an irreversible injury has occurred, perhaps to the heart or

Table 4.3

Exposure Effects of Ricin

	Dose	Species	Effect	Reference
Ocular	0.5 µg (particle)	Rabbit	Conjunctivitis for one week	OSRD (1946), p. 189
	1.5 mg (particle)	Rabbit	Serious eye injury	OSRD (1946), p. 189
Respiratory LCT_{50}	24 mg-min/m^3	Dog	Lethal pneumonia	OSRD (1946), p 188
	100 mg-min/m^3	Monkey	Lethal pneumonia	OSRD (1946) p. 188; Franz and Jaax (1997)
	30–70 mg-min/m^3 (est.)	Human	Lethal pneumonia	Franke (1976), OSRD (1946)
Pareneral	0.5–0.75 µg/kg	Human	Mild illness	Fodstad et al. (1984)
	0.1–0.3 µg/kg (est.)	Mouse, injected	LD_{50}	U.S. Army, (1990), pp. 73, 83, and 105; Gill (1982), p. 83
	30.27 µg/kg	Monkey	LD_{50}	Balint (1993)

brain, and demonstrates that cumulative toxicity can occur (Foxwell et al., 1985).[4]

The acute clinical picture varies by route of exposure. The most well-documented human experience with ricin concerns the ingestion of castor beans (Klain and Jaeger, 1990, reviewed 314 cases). There are fewer data on human exposures via other routes. Mild systemic illness (i.e., delayed onset of a "flulike" syndrome, with malaise, fatigue, muscle pain, and some nausea and vomiting) occurred in humans receiving 0.5 to 0.75 µg/kg of ricin intravenously as part of Phase I cancer chemotherapy trials. Onset of symptoms was in four to six hours and lasted two to four days. Muscle cramps, fatigue, and weakness were prominent problems (summarized by Wellner, Hewetson, and Poli, 1995; Fodstad et al., 1984). Similar systemic signs and symptoms occurred in World War II workers with mild exposures (OSRD, 1946; see "Respiratory System," below). Laboratory findings are nonspecific.

The fever ricin produces in mammals is consistent, predictable, and dose-related. Linear dose-response curves for several species have been developed (Balint, 1993). The suspected cause of the fever is release of endogenous (leukocyte) pyrogens.

[4]This raises the possibility of cumulative toxicity from sustained low-level exposures in immune-compromised humans. The U.S. personnel in the Gulf region are presumed to have been immuno-competent.

No data exist regarding mutagenic and teratogenic effects of ricin (Klain and Jaeger, 1990). However, in one case, a pregnant woman was poisoned with ricin, which suggested teratogenic effects.

Two cases of injection in humans are worth mentioning in some detail. In the first, severe headache and fever followed an intentional intramuscular injection of a castor bean extract (approximately 150 mg of ricin [2 mg/kg]) (Fine et al., 1992). After ten hours, the patient's pulse and blood pressure were elevated; he had a white count of 18,000/mm^3 (above normal) and a low sedimentation rate. Serum amylase and transaminases were slightly elevated, and bilirubin was also high, but creatine kinase was not, suggesting liver injury as the source of the enzyme elevation. The patient remained febrile for eight days without renal problems and was discharged home asymptomatic on day 10. The patient initially received supportive therapy with intravenous fluids and antibiotics.

The second case is the well-known 1978 murder of Georgi Markov by ricin (Klain and Jaeger, 1990). The toxin was contained in a pellet shot into the victim's thigh with an umbrella. At the time of the encounter, the patient noted only a stinging sensation. Later studies indicated the pellet contained 500 µg of ricin. After five hours, Markov complained of weakness. The next day, he developed fever and vomiting and had trouble speaking. When admitted to the hospital, he was hot and ill, with a fast, regular pulse. Lymph glands in his groin were swollen. The patient and staff had no idea he had been attacked. Markov's white blood cell count was 10,600/mm^3 (normal). On the third day, his blood pressure fell; his pulse rose to 160/min; and he was cold, sweaty, and dizzy. The white blood cell count rose to 26,300/mm^3 (quite elevated, with granulocytes predominating) and he was given plasma expanders. The following day, he became anuric, and acute tubular necrosis was suspected. Vomiting with hematemesis worsened. His white blood cell count rose to 32,200/mm^3, and he developed heart block, became confused, and died. Only at autopsy was the pellet discovered. Other autopsy findings included pulmonary edema, liver fatty change, and hemorrhage in the intestine (with necrosis) and in the lymph nodes, adrenals, pancreas, and heart. Another patient recovered from a similar attack after hospitalization for 12 days, the pellet in this case having released less toxin (Franz and Jaax, 1997; Klain and Jaeger, 1990).

Eye. No reports of human eye exposures or injuries were found (neither have eye signs been noted in systemic exposures) (Franz and Jaax, 1997; Klain and Jaeger, 1990). Animal studies report conjunctivitis lasting one week after ocular introduction of 0.5 µg of ricin and corneal lesions with keratitis lasting 11 days after exposure to 1.5 µg (OSRD, 1946). Ricin is potent enough to produce lethal illness via the eye (OSRD, 1946). Presumably, any systemic illness resulting from eye exposure would be associated with very intense conjunctivitis and corneal injury, including erythema, exudate, and corneal clouding.

Respiratory System. World War II animal studies indicated that the amounts of ricin for lethality were roughly the same for systemic or respiratory exposures (OSRD, 1946). Respiratory exposures produce effects confined to the respiratory tract, with modest systemic toxicity and lethality explicable by pulmonary failure (Franz and Jaax, 1997; Wilhelmson and Pitt, 1996). After exposing mice to aerosolized ricin, Doebler et al. (1995) found significant concentrations in the lungs and gastrointestinal tract, with only low tissue levels elsewhere; however, concentrations in the central nervous system were not assessed. No reports of serious human respiratory exposures were found. However, animals and humans exposed via other routes have experienced pulmonary congestion and edema. Only animal data exist concerning the pathology resulting from respiratory exposure. Studies in the UK and the United States have indicated that lesions were confined to the lungs, with intra-alveolar edema, acute alveolitis, and diffuse necrosis of epithelial linings (Griffiths et al., 1994; OSRD, 1946). Sublethal exposure in rats (a CT of 16.5 mg-min/m^3) sacrificed at 30 hours showed increased pulmonary water and albumin. Bronchoalveolar lavage showed leukocytosis, and there was a mild inflammatory response with minimal alveolar damage (Kokes et al., 1994). Rhesus monkeys exposed to lethal ricin aerosols in doses ranging from 21 to 42 µg/kg developed respiratory distress at 36 to 48 hours (Wilhelmsen and Pitt, 1996).[5] Pulmonary findings ranged from limited focal lesions to coalescing fibrinopurulent pneumonia, diffuse airway inflammation and necrosis, diffuse alveolar flooding, and peribronchial edema. All monkeys had purulent tracheitis, fibrinopurulent pleuritis, and purulent mediastinal lymphadenitis. There was no systemic lymphadenopathy. No bacterial role was identified. All monkeys died or were sacrificed. Activation and infiltration by leukocytes may play a role in the injury resulting from inhaled ricin (Assad et al., 1996).

World War II workers manifested two different clinical syndromes from presumptive low-level respiratory exposures (OSRD, 1946). First, among laboratory workers, the reaction resembled that of people hypersensitized to a foreign protein, with rapid onset of sneezing, coughing, and symptoms reminiscent of severe asthma. The reactions lasted less than one hour and were probably due to workers' becoming sensitized to ricin during work. One would not expect this syndrome in the Gulf War setting because prior ricin exposure was unlikely. The second syndrome arose in persons who inhaled low doses of ricin. Four to eight hours after exposure, there were fever, coughing, dyspnea, chest tightness, inflammation and burning of the trachea, aching joints, and nausea. Several hours later, profuse sweating occurred, usually coinciding with symptom

[5]The animals were exposed to 1.2 µm particles in an aerosol of 128–353 mg-min/m^3 (10 min) with absorbed dose calculated from respiratory parameters and impinger measurements.

abatement (OSRD, 1946). No follow-up or sustained observations were reported.

Gastrointestinal System. Ricin is less potent when delivered orally, although this route has produced the greatest human experience with the toxin (Franz and Jaax, 1997; Klain and Jaeger, 1990). Ishiguro et al. (1992) has shown that, in rats, active ricin is absorbed from the small bowel by blood and lymphatics, with the highest subsequent concentrations in the liver and spleen. Human oral exposures chiefly follow ingestion of castor beans (Klain and Jaeger, 1990). Symptom onset is often delayed but can range from hours to several days. The illness spectrum ranges from mild cases of weakness and prostration to, more commonly, nausea, vomiting, diarrhea, abdominal pain, and bleeding. Severe dehydration may occur, as can other constitutional symptoms, including fever, tachycardia, muscle cramps, dyspnea, lethargy, and confusion. In fatal cases, sudden collapse with hypotension and seizures can occur. Renal failure and evidence of hepatotoxicity are variable. The main findings are usually gastrointestinal (OSRD, 1946; Wellner, Hewetson, and Poli, 1995; Klain and Jaeger, 1990).

Autopsy studies of lethal castor bean poisonings show erosions and ulceration of stomach and small bowel with hemorrhagic inflammation of the stomach (Klain and Jaeger, 1990). Remote hemorrhage and necrosis of lymphatic tissues are common, but renal congestion and cerebral edema vary.

Exposure via other routes also produces gastrointestinal injury, especially in the liver, although histologic findings vary. Markov's autopsy findings include gastrointestinal necrosis and hemorrhage, with hepatic fatty change (Klain and Jaeger, 1990).

Dermal. Ricin does not have impressive dermal toxicity. It was not a clinical occupational-medicine problem with laboratory or production workers during U.S. World War II (OSRD, 1946), and animal research of that period did not report dermal toxicity. OSRD reports mentioned British observations that injection of ricin intradermally produced local inflammation and systemic effects similar to those of mild respiratory exposures (described above). It is unlikely that ricin in the Gulf operational environment could have produced dermal injury, and it would not explain later dermatological problems in Gulf veterans, based on World War II reports.

Nervous System. A prominent nonfocal neurological finding in serious ricin intoxications is the seizures noted in humans and animals (Klain and Jaeger, 1990; OSRD, 1946). Originally, hypoglycemia was suspected as the cause, but detailed studies indicate otherwise. The mechanism by which ricin induces seizures remains unknown, although experimental evidence exists for a central-nervous-system mechanism for ricin toxicity (Foxwell et al., 1985). Further,

personal communication from USAMRIID indicates that, in mice, rats and subhuman primates exposed to 5 to 10 LD_{50} of aerosolized ricin, no toxin-related lesions were seen in the brain or other nervous tissue. This conforms to some of the institute's published data, which show main pathologic findings from inhaled ricin are confined to the lungs.

Concerns about neuropathy following the Gulf War (Haley, Horn, et al., 1997—none of whose group had seizures) led to a special effort to find information about ricin's distribution to the brain and nervous system. Neurological disease, including neuropathy, has not been documented as a consequence of ricin exposure. However, it is known that, if introduced into the nervous system, ricin is extremely toxic and that axons can transport ricin into nerve cell bodies (De la Cruz et al., 1995). There is also some evidence from studies of rats that ricin can alter the "blood-nerve barrier" (Bouldin et al., 1990).

Intracerebral injection of ricin in young rats produces hydrocephalus. Periventricular cortical necrosis occurs, with typical features of neuronal degeneration, such as displaced nuclei and mitochondrial swelling and disintegration (Kaur and Ling, 1993). Brain hemorrhage and cerebral edema have been reported in human ricin poisoning cases (Klain and Jaeger, 1990).

Musculoskeletal. Clinically, muscle cramps and weakness are a common, early finding in ricin toxicity by all routes (OSRD, 1946; Klain and Jaeger, 1990; Fodstad et al., 1984). Some studies (e.g., Doebler et al., 1995) indicate substantial distribution of ricin to muscle tissue after parenteral administration, but the work of Fodstad et al. (1984) showed little distribution to muscle and suggested that some secondary mechanism was involved. There appear to be no reports demonstrating musculoskeletal pathology.

Other. Clinical evidence of cardiac injury with ricin includes reported cases of heart block, prolonged Q-T intervals on an electrocardiogram, and arrhythmias (Klain and Jaeger, 1990). Autopsy reports describe myocardial softening and necrosis and diffuse myocardial and systemic hemorrhage, raising the possibility of a selective effect on blood vessels (Klain and Jaeger, 1990).

Christiansen et al. (1994) conducted experiments with rabbits with intravenous doses of 44 µg/kg and a sublethal toxic dose one-half that (22µg/kg).[6] The higher dose produced significant systolic and diastolic blood pressure declines, while the lower dose did not produce a significant change in blood pressure. Heart rates (EKGs) were not significantly affected in either group. The study concluded that hypotension was peripheral in origin, not cardiac.

---

[6]The larger dose here is the minimum lethal dose, the lowest amount that killed rabbits in LD_{50} tests—48 hours of LD50 0.54 µm/kg.

There is no indication of bone marrow injury. Splenic hyperplasia occurs in animals given sublethal doses of ricin, while necrosis is seen in lethal exposures (OSRD, 1946; Klain and Jaeger, 1990). The specific mechanism causing hemorrhage is unknown, and no platelet abnormalities have been found following ricin exposure.

Swollen kidneys, renal failure, acute tubular necrosis, and acute renal failure have been reported (Klain and Jaeger, 1990; OSRD, 1946; Wellner, Hewetson, and Poli, 1995). Some of the renal findings may be due to hypovolemia and hypotension.

Combined Effects. Little information exists regarding the combined effects of ricin and medications or environmental factors. If stress or pretreatments are shown to alter the blood-brain barrier, they could increase sensitivity to ricin, based on Foxwell et al. (1985). For example, in mice, toxicity increased when ricin was administered with mannitol, which impairs the blood-brain barrier (Foxwell et al., 1985). Friedman et al. (1996) has demonstrated in animals that severe stress makes the blood-brain barrier more permeable.

Prevention and Treatment

No immunizations or treatments are available as yet for human use. Care is supportive. Active and passive immunizations show promise experimentally (Griffiths et al., 1995; Franz and Jaax, 1997). Drug therapy is in the very early stages of laboratory efforts (Franz and Jaax, 1997).

What to Look for in the Gulf Context

Ricin ingestion causes weakness, abdominal pain, and bloody diarrhea. However, there were few opportunities for food or water contamination in the Gulf. Thus, if any military exposures to ricin happened during the Gulf War, they would more likely have occurred via ocular, dermal, and respiratory routes. One would expect conjunctivitis (based on animal studies) and signs of persistent respiratory irritation from low-level exposures to ricin, generally followed by malaise, arthralgia, muscle aches, and a low fever, although these symptoms are not specific to ricin. One would not expect consequences from dermal exposure to ricin unless the agent gained entry through small cuts and abrasions. Muscle cramps and weakness are a distinctive finding common in low-level exposures (Fodstad et al., 1984).

There is no particular reason to think the 3rd Armored Cavalry regiment had any exposure to ricin or chemical agents, but its records give some idea of the background of illness in the region and the prevalence of illnesses that can also be produced by low levels of agents. Carefully collected medical data from this regiment during in the Gulf War showed increased respiratory illness rates

before the start of the air war. There was no increase in eye complaints, and there were few cases of fever of unknown origin (Wasserman et al., 1997). Examination of other unit records would be helpful.

Because ricin is immunogenic, individuals occupationally exposed to low levels during World War II may have developed ricin hypersensitivity, indicative of the presence of antibodies to ricin. Antibodies to ricin definitely developed in cancer patients given the agent intravenously (Fodstad et al., 1984). It might similarly be possible to document antibodies to ricin in persons who served in the Gulf. Although Fodstad's is not a routine study, his technique could be replicated.

At present, however, there is no evidence that ricin exposure occurred in the Gulf or that long-term illness is a consequence of low-level exposure. If such evidence is ever uncovered, it may be possible to test for exposure through antibody determinations in exposed persons. Although ricin should degrade in the environment over time, if canisters or filters from the Gulf War can be located, it might be valuable to examine them using antibody techniques to look for evidence of ricin.

Summary and Conclusions

Ricin is a potent plant toxin with delayed onset of effects and an aerosol toxicity equivalent to soman and sarin. It cannot be detected in the field and can persist in dry weather for up to three days but will environmentally degrade because of moisture, heat, light, and oxygen.

Ricin use had progressed to the point of unsophisticated weapon status in Iraq, but there is no evidence that it was used in the Gulf War against U.S. forces. Of the toxins of concern, ricin was probably the least stable and unlikely to withstand explosions and lengthy atmospheric transport to Saudi Arabia from releases in Iraq.

During the war, there were no mass outbreaks of the conjunctivitis and respiratory disease that ricin can cause, and such conditions are not specific to ricin in any case. Little is known about late long-term effects from clinically significant exposures; chronic low-level exposures have not been studied. An effort to follow up World War II workers and documented human poisonings would be helpful.

Some of the nonspecific general signs and symptoms of low-dose ricin exposure may resemble features of illnesses in Gulf War veterans, particularly muscle aches, sweating, and respiratory difficulties. Follow-up studies are lacking, so no evidence of ricin-induced recurrent or persistent illness after low-dose exposures was found.

Ricin is highly immunogenic. If reason to suspect ricin exposure of Gulf War patients increases, it may be useful to look for antibodies to ricin. Lacking information about the prevalence of such antibodies in the general population, suitable controls would be needed.

Neurological disease, including neuropathy, has not been documented as a consequence of ricin toxicity, although ricin is neurotoxic and can produce axonal degeneration. There are indications that neural factors are important in lethality and that ricin may alter the permeability of blood-neural barriers. Increased permeability of the blood-brain barrier enhances ricin toxicity. Further study of neural mechanisms in ricin toxicity should be pursued.

There is a remote possibility (based on an animal study by Foxwell et al., 1985) that cumulative toxicity from sustained subclinical ricin exposures could occur in immune-compromised subjects. The possibility that there were many such persons in the Gulf population seems very small.

Ricin should not be in the forefront of Gulf concerns, but other small countries and terrorists could turn to it as an inexpensive weapon, and thus further research on its mechanisms of action and treatment is warranted. The following areas in particular deserve more attention: the longer-term effects of ricin exposure, blood-brain barrier modulation of toxicity, neural mechanisms of ricin-related illness, and effects of ricin on disease resistance.

TRICHOTHECENE MYCOTOXINS

Trichothecene mycotoxins are produced by fungi (e.g., *Fusaria*, *Trichoderma*, *Myrothecium*, *Stachybotrys*); 60 are known. These were originally isolated as possible antifungal microbials or as antiplant agents. Analysis of trichothecene (and aflatoxin) exposures is complicated by their natural occurrence: Their presence alone does not prove a biological attack.

Iraq has admitted to possessing trichothecene mycotoxins and testing them in animals and has been accused of using them against Iran (UNSCOM, 1991, 1992, 1995; Zilinskas, 1997; Heyndrickx, 1984). The report of Iraqi possession of trichothecenes followed a considerable period of interest, attention, and controversy about their use in Southeast Asia (between 1974 and 1981, against Lao and Khmer populations by communist forces) and in Afghanistan (by Soviet forces) (Crocker, 1984; Haig, 1982; Schultz, 1982; Seagrave, 1981). Wannemacher and Wiener (1997), concluded that the Soviets and their clients have used trichothecenes, and the authors present a detailed review of the history of the subject and associated controversy. There may have been shortcomings in the epidemiological approaches (Hu et al., 1989). There were also many difficulties and inconsistencies in agent sampling, transport, and analysis.

These toxins, until discovered in Southeast Asian attack environments, had not been on the usual lists of potential toxin weapons (SIPRI, 1973). Analysts recognized that the toxins could produce the injuries encountered (Watson, Mirocha, and Hayes, 1984). Subsequent research identified properties of military significance, e.g., skin injury from nanogram amounts; eye injuries from micrograms; and serious central nervous system, respiratory, gastrointestinal, and hematological toxicity via multiple routes of exposure (Watson, Mirocha, and Hayes, 1984; Bunner et al., 1985; and Wannemacher and Wiener, 1997).

History

These mycotoxins have been poisoning people and animals for a long time. They grow well at low temperatures and frequently contaminate grain and other foodstuffs. They have been implicated in foodborne illnesses on several continents (Ueno et al., 1984). A large disease outbreak in the Soviet Union during World War II, which involved thousands and had high mortality, was eventually traced to the consumption of grain contaminated by *Fusaria* molds, which had been left in the fields over the winter. The disease, alimentary toxic aleukia, resembled a severe radiation injury with nausea, vomiting, diarrhea, leukopenia, hemorrhagic diathesis, and sepsis.

These toxins are also hazardous via other routes. Domestic animals and farmers manifested skin and respiratory irritation and systemic malaise from exposure to contaminated dusts and hay. Human illnesses have arisen from trichothecene mycotoxin contamination of houses and ventilation systems, resulting in so-called "sick building" syndrome (Croft et al., 1986; Jarvis, 1985; Smoragiewicz et al., 1993). One family so exposed was affected with nonspecific symptoms whose cause was not identified for months (*Myrothecium* and *Stachybotrys* were identified). For a time, several trichothecene mycotoxins were tested as anticancer agents in clinical trials (Thigpen et al., 1981; Bukowski et al., 1982; Yap et al., 1979; Diggs et al., 1978; Murphy et al., 1978; Goodwin et al., 1981). Some laboratory accidents have added to experience with human exposure (Wannemacher and Wiener, 1997). In addition, there is considerable information on the effects of trichothecene mycotoxins on economically important animals (Ueno et al., 1984).

Reports of communist attacks on Lao tribal people, and later on the Khmer, began in 1974 with aircraft and helicopter delivery of colored smokes, dusts, and droplets. People near these attacks had signs and symptoms that did not resemble known chemical warfare agents. Later similar attacks were reported in Cambodia and Afghanistan. Symptoms included vomiting, dizziness, seizures, hematemesis, respiratory distress, hypotension, and blisters. Survivors were ill for a long time with rashes, joint pains, fatigue, and memory problems (Haig, 1982; Schultz, 1982; Crossland and Townsend, 1984).

Investigative teams in refugee camps were puzzled, identifying a toxic epidermolysis without other expected findings from known chemical agents (House, 1979), but intelligence analysts recognized the similarities to trichothecene intoxication. Later, clinical examinations, autopsies, laboratory tests, and tissue samples showed trichothecene mycotoxins (and a propylene-glycol carrier) together with tissue damage compatible with trichothecene effects (Crocker, 1984; Watson, Mirocha, and Hayes, 1984; Rosen and Rosen, 1982; Stahl et al., 1985).

Chinese analysts attributed a higher toxicity to trichothecene mycotoxins than to nerve agents. They alleged that, between 1975 and 1982, 6,000 Laotians; 1,000 Cambodians; and 3,000 Afghans had died from attacks with what came to be known as "yellow rain" (Fang, 1983).

During the Iran-Iraq War, especially in the fighting around Majoon Island, colored smokes and powders were used against Iranian forces, perhaps reflecting combinations of agents. Although controversial in the scientific community, Heyndrickx (1984) found trichothecene mycotoxins in Iranian casualties who appeared to have sustained mustard injuries. Although other laboratories did not confirm these findings from the same material, Professor Heyndrickx argued that biological tissues had degraded the toxin over time.[7]

It is not known if, during the Gulf War, any of the Iraqi chemical and biological facilities hit by Allied fire contained trichothecenes. Trichothecenes are very resistant to environmental degradation and resist heat below 500°F; hence, the production of effects after long-distance transport following explosive release is possible but unlikely because the chemical would be very diffuse by that time (U.S. Army, 1990; Wannemacher and Wiener, 1997; Trusal, 1985). However, no events described during the war closely correspond to known acute effects of trichothecene syndromes. Lethal effects require substantial doses (milligrams), but eye and skin irritation can occur at much lower levels (U.S. Army, 1990; Wannemacher and Wiener, 1997; Coulombe, 1993), raising the remote possibility that low-level exposures might have been misinterpreted as being due to some other cause.

Weaponization

Production using contemporary fermentation methods similar to those of brewing and antibiotic production is easy and inexpensive, and conventional bioreactors can readily produce tons of these agents (Wannemacher and Wiener, 1997). AD Little (1986, Ch. 4) described the conditions defining pro-

[7]The professor also observed chemical casualties in Iran, and his treatment recommendations were a subject of controversy in 1997 and 1998 letters in *Lancet*.

duction. The large-scale production of *Fusaria* and trichothecenes for civil purposes in the former Soviet Union indicates the ease of large-scale production for other purposes (Buck et al., 1983). Formulations of T-2—one of the most potent trichothecenes—might also include polyethylene glycol, sodium lauryl sulfate, or dimethylsulfoxide (DMSO). These materials facilitate dispersal and handling of the toxin, possibly enhancing toxicity. Trichothecenes do not degrade to nontoxicity when exposed in the natural environment (for weeks at least) and are stable when stored. They can be delivered by mortars, artillery, free rockets, aerial bombs, and surface or aerial sprayers (Wannemacher and Wiener, 1997). Iraq possessed all the systems previously used to deliver trichothecenes.

T-2 is a skin-damaging agent of great potency (Bunner et al., 1985)—several hundred times more potent than mustards or lewisite (Wannemacher and Wiener, 1997). It is able to injure the eye in microgram amounts, which again indicates that it is more potent than mustards.

Toxicity by inhalation is comparable to mustards. NAS (1983) estimated that LC_{50} exposures of aerosols of 1 mg/m^3 or surface contamination or LD_{50} of 1 g/m^2 could readily be attained.

Trichothecenes readily result in vomiting, rather promptly at low concentrations, which might compromise the ability of exposed troops to use protective respirators. Other symptoms, including mild incapacitation, follow. Operationally, the persistence of trichothecenes makes them a threat even to military forces with protective equipment; Soviet troops in Afghanistan avoided operating in areas where these toxins were used (Fang, 1983). There are some indications that trichothecenes may have been used in combination with other agents in Southeast Asia and Afghanistan (Fang, 1983; Schultz, 1982).

Chemical and Physical Properties

The trichothecenes are classed as sesquiterpenes (Ueno, 1983). The members of this family of toxins vary depending on their side groups and include T-2, HT-2, nivalenol, deoxynivalenol, anguidine, diacetyoxyscirpenol (DAS), and crotocin.[8] When the toxins are extracted from fungal cultures, a yellow greasy residue remains. Had the various reported Asian attacks involved a crude extract containing some of that residue, the result might have been the yellow rain reported. The toxins are stable in air and light for weeks and can withstand

[8]Many other tricothecene toxins, such as verrucarin A, roridin A, satratoxin H, have greater intravenous and intraperitoneal toxicity in the mouse (Wannemacher and Wiener, 1997), but this review touches on them only occasionally (e.g., Croft, Jarvis, and Yatawara, 1986).

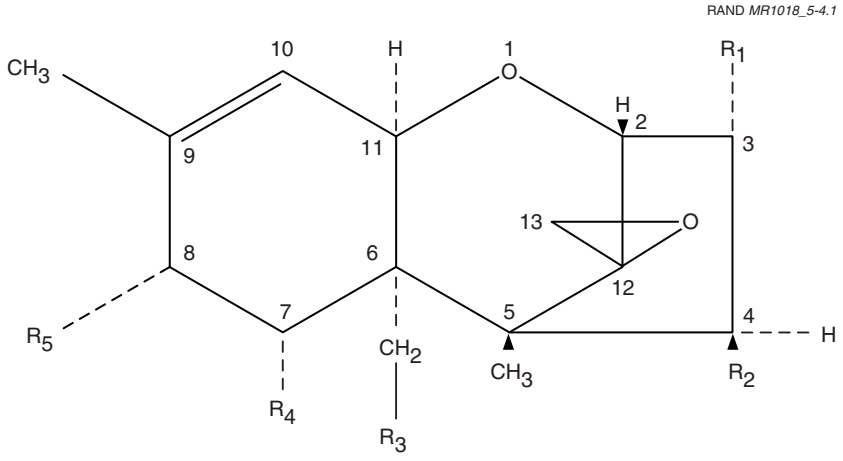
heat; a temperature of 500°F is required to destroy T-2 (Trusal, 1985; Wannemacher and Wiener, 1997).

These toxins can be inactivated with 3- to 5-percent hypochlorite solutions (Wannemacher and Wiener, 1997). The toxins are relatively insoluble in water but are soluble in acetone, chloroform, DMSO, glycols, ethanol, and other organic solvents. They have a peppery odor and negligible vapor pressure.

Figure 4.1 shows the general structure of trichothecene toxins. The olefinic bond at position 9-10 and the epoxide group at position 12-13 are important in the chemical and biological reactions of these agents.

Detection

No military field detection systems currently deployed can detect trichothecenes, although laboratory techniques (e.g., antibody-ELISA, gas chromatography or mass spectroscopy, and thin-layer chromatography coupled to fluorimetry) have been used. Biological detection systems using animals are neither specific nor easy (Fontelo et al., 1983; Mirocha et al., 1984; Thompson and Wannemacher, 1984; Rosen and Rosen, 1982; NAS, 1983). Wannemacher and Wiener (1997), reviewing confirmatory procedures, indicated that mass spectroscopy is the procedure of choice, requiring little specimen "cleanup" and enabling detection of one part per billion (ppb) of toxin. More-complex systems being evolved may detect 0.1 ppb.



SOURCE: AD Little (1986, Ch. 4).

Figure 4.1—General Structure of Trichothecene Toxins

Toxicology and Toxicokinetics

Mechanisms of Action. The many mechanisms by which trichothecenes produce toxicity are varied, and their relative importance in producing illness is not fully understood (Coulombe, 1993). They include the following:

- inhibition of protein synthesis, thought to be the most important effect (Ueno, 1983; Ueno et al., 1984; Tutelyan and Kravchenko, 1981)
- inhibition of DNA synthesis (Thompson and Wannemacher, 1984), which might contribute to their radiomimetic properties
- impairment of ribosome function (NAS, 1983; Coulombe, 1993; Tutelyan and Kravchenko, 1981)
- inhibition of mitochondrial protein synthesis (Pace et al., 1985)
- induction of reparable single strand breaks in DNA
- immunosuppression, allowing secondary and opportunistic bacterial infections and possibly delayed hypersensitivity (Ueno, 1983; Yarom et al., 1984; Jagadeesan et al., 1982).

Trichothecenes react readily with thiol groups and, at low concentrations, inhibit thiol enzymes (e.g., creatine kinase, lactate dehydrogenase) (Tutelyan and Kravchenko, 1981; Ueno et al., 1984). They can be incorporated into lipid or protein elements of cell membranes. Tissue culture studies show alteration of membrane function (Coulombe, 1993; Pfeifer and Irons, 1985). Sulfhydryl effects in cell membranes are important in cell-to-cell interactions in the immune system. T-2 toxin induces cell membrane injury with hemolysis, apparently via a free-radical mechanism (Segal et al., 1983; Coulombe, 1993).

Metabolism may be more important in detoxification than in producing toxicity. Unlike the aflatoxins that require metabolic activation, the trichothecenes are directly toxic without activation, as their prompt effects on the gastrointestinal mucosa with epithelial cell necrosis suggest (Busby and Wogan, 1979).

T-2 and other trichothecene toxins are deacetylated in the liver. Metabolites are also toxic but less so than T-2 (Ueno et al., 1984). Carboxyesterases (-SH serine esterases) in liver microsomes hydrolyze T-2 to the less potent HT-2. These enzymes may be clinically important. Inhibition of this enzyme by paraoxon (an organophosphate pesticide) in subclinical doses increases the toxicity of T-2 in mice (Johnsen et al., 1986). Other potent inhibitors of this enzyme are tri-o-cresyl phosphate (TOCP, an organophosphate), eserine (a carbamate), and diisopropyl fluorophosphate (DFP, a weak organophosphate nerve agent) (OSRD, 1946). These all inhibit hydrolysis of T-2 (Johnsen et al., 1986). This raises the strong possibility that similar compounds, such as PB; low levels of nerve agent; or other carbamate or organophosphate insecticides might enhance the toxicity of T-2 or other trichothecenes at low levels.

Exposure-Effect Relationships. T-2 toxin and other trichothecenes are absorbed slowly (12 to 24 hours) via the intact skin but rapidly through abraded skin. DMSO or similar penetrants can increase the rate of absorption, but even then the systemic toxicity appears slowly (Bunner et al., 1985; Schiefer, 1984; Kemppainen et al., 1986a, 1986b; Solberg et al., 1990).

The rapid appearance of symptoms after respiratory exposure in humans, along with the results of animal inhalation studies, indicates rapid absorption and high retention of aerosolized T-2 toxin, with the respiratory tree retaining small amounts (Creasia et al., 1987).[9] Tritium-labeled agent and immunoperoxidase studies have also been used to follow the distribution and disposition of T-2 toxin (Pace et al., 1985, Lee et al., 1984). Intramuscularly injected agent is distributed to liver, kidney, lung, and other tissues within 30 minutes. The plasma concentration has a biphasic course, with half-lives of 1.8 and 50 hours for the two phases. T-2 toxin and metabolites concentrate in bile with evidence of enterohepatic circulation. The liver and kidney are the main organs for detoxification. Oral intoxication showed T-2 toxin in the gastrointestinal tract and kidneys, but not in the liver, reflecting rapid hepatic metabolism. The brain showed a rapid uptake to levels higher than plasma but below many other tissues, with a rapid fall to levels similar to plasma in six hours. One would expect trichothecenes to enter the brain readily, since they are lipophilic (Wang, Wilson, and Fitzpatrick, 1992).

Table 4.4 gives effects for various acute exposure levels and pathways. Effects accumulate with repeated exposures. It has been shown, for example, that the effects of sustained low doses can accumulate to the clinical picture associated with alimentary toxic aleukia (Mayer 1953a, 1953 b; Lutsky et al., 1978). Or they can yield the more diffuse problems that Croft et al.(1986) and Jarvis (1985) reported: a case of ongoing illness for several years in a family of five, with recurring respiratory illness, flu syndromes, sore throats, diarrhea, cough, headaches, fatigue, and episodes of alopecia. One man had leg pains. Eventually, trichothecenes were identified in air ducts and ceiling material in the family's house. Material extracted from these areas was toxic to rats and mice. Croft cited other reports by Forgacs (1972) of toxin exposures producing similar symptoms with central nervous system and neuropsychiatric manifestations.[10]

Respiratory is high and comparable to parenteral injections. Oral and dermal lethal toxicities are lower but produce similar systemic effects (Creasia et al.,

---

[9]The LD_{50} was 0.24 mg/kg for young adult mice and 0.94 mg/kg for mature mice. For mice, inhalation was 10 times more toxic than systemic administration and 20 times more toxic than dermal administration.

[10]As noted earlier, the toxins were tricothecenes other than the main ones covered in this review, e.g., verrucarin A, B; satratoxin H, and trichoverrin A, B.

Table 4.4

Effects of Varied Trichothecene (T-2) Exposures

	Dose	Effect	Source
Skin Exposures	5–50 ng in liquid	Minimal erythema dose (guinea pig, rat)	Ueno (1983), Wannemacher et al. (1983)
	209 ng/cm^2 in liquid	Minimal erythema dose (monkey)	Wannemacher et al. (1983), Bunner et al. (1983)
	1 µg/cm^2	Irritation (guinea pig, rabbit)	Fairhurst et al. (1987)
	2 µg	Vesication, skin injury	Bunner (1983)
	0.25 mg/kg	Severe illness, diarrhea (monkey)	Bunner et al. (1983)
	1.5 mg/kg in DMSO	LD_{50} (rat; mean time to death, 19 hr.)	Wannemacher et al. (1983)
	4.2 mg/kg in methanol	LD_{50} (guinea pigs; mean time to death 190 hr.)	Wannemacher et al. (1983)
Eye	1 µg	Detectable corneal injury	USAMRIID (1983)
	>2 µg	Severe corneal injury, conjunctivitis	Bunner (1983)
Respiratory[a]	0.24 mg/kg (absorbed)	Mouse LD_{50}	Creasia et al. (1987)
	0.05 mg/kg (absorbed)	Rat LD_{50}	Bunner et al. (1985)
	0.6–2.0 mg/kg (absorbed)	Guinea pig LD_{50}	Wannemacher and Wieser (1997, p. 661)
	5,479 mg-min/m^3	Guinea pig LD_{50}	AD Little (1986)
	200–1,800 mg-min/m^3	Estimated LCT_{50}	Calculated from U.S. Army (1990)
Systemic Toxicity	500 µg/kg	Estimated human LD_{50}	U.S. Army (1990)
	470 µg/kg intramuscular	Rat LD_{50}	Bunner et al. (1985)
	1.17 mg/kg	Rat LD_{50}	Bunner et al. (1985)
	650 µg/kgintramuscular	Monkey LD_{20}	Cosgriff et al. (1986)
	790 µg/kg intravenous	Monkey LD_{50}	AD Little (1986)
	850 µg/kgintramuscular	Rat LD_{50}	Chan and Gentry (1984)
	111 mg/kgintramuscular	Rabbit LD_{50}	Chan and Gentry (1984)

Table 4.4—Continued

	Dose	Effect	Source
Oral Toxicity	0.1–0.2 mg/kg	Swine, emesis	Busby and Wogan (1979)
	0.1–1.0 mg/kg	Swine, diarrhea	Ueno (1983a)
	2.29 mg/kg	Rat LD_{50}	Bunner et al. (1985)
	3.06 mg/kg	Guinea pig LD_{50}	AD Little (1986)
	1.0 mg/kg[b]	Male monkey LD_{100}	Rukimi, Prasad, and Rao (1980)
	1 5 mg/kg	Estimated human LD_{50}	Calculated from U.S. Army (1990)

[a]No primate data were located.

[b]Per day for 15 days

1987; Bonomi et al., 1995; Wannemacher et al., 1993; Schiefer and Hancock, 1984).[11] For less-than-lethal dosages and for all routes of administration, sequelae of leukopenia, thrombocytopenia, bleeding tendency, weakness, diarrhea, dyspnea, recurrent infections, vomiting, anorexia, and weight loss are expected. Other sequelae of acute exposures include prolonged rashes, joint pains and fatigue (Schultz, 1982), fever, chills, hypotension, confusion, somnolence, seizures, memory loss, hallucinations, and burning erythema (Belt et al., 1979; Murphy et al., 1978; Yap, et al., 1979; Diggs et al., 1978; Bukowski et al., 1982; Thigpen et al., 1981; Crossland and Townsend, 1984).

Pathology and Pathophysiology

The clinical manifestations of trichothecene intoxication are derived from several sources. They are summarized here prior to more detailed treatment of individual organ systems.

Known effects from evidence other than the yellow rain attacks are nausea, vomiting, seizures, central nervous system dysfunction, chills, fever, hypothermia, hypotension, epithelial necrosis, myelosuppression, and gastroenteritis with hematemesis and melena (bloody vomiting and stools). In the yellow rain attacks, the Hmong victims were probably exposed by several routes, including dermal, respiratory, and oral (the last from swallowing larger particles trapped in upper airways and returned to the oropharynx by ciliary action (Wannemacher and Wiener, 1997). Vomiting was induced and lasted several days. There was a feeling of intense heat, itching and burning of the skin, dizziness, tachycardia, chest pain, headache, and decreased vision. Within hours, victims reported intense eye pain, red eyes, bleeding gums, and hematemesis. Trembling was common, and some patients had seizures. Severe itching ensued with the formation of small hard blisters, some of which were hemorrhagic, occasionally progressing to large bullae. Abdominal pain and bloody diarrhea continued (Watson, Mirocha, and Hayes, 1984).

Khmer yellow rain casualties had similar acute symptoms (Crossland and Townsend, 1984) with the following longer-term effects: intermittent weakness, anorexia, reduced memory and ability to concentrate, intermittent diarrhea, impotence, increased fatigue, cough and dyspnea, increased susceptibility to infection, and suspected increases in fetal abnormalities and spontaneous abortions (Haig, 1982; Schultz, 1982; Watson, Mirocha, and Hayes, 1984; Stahl et al., 1985; Crossland and Townsend, 1984). It must be noted that the Hmong cases with memory loss that Crossland described were not evaluated for the presence of toxins. These persons had undergone a harrowing experience,

[11] Note the NAS (1983) LD_{50} estimates under "Weaponization," above.

having been attacked, seen kinfolk die, fled, and become refugees. Severe apathy, confusion, and depression are common in survivors of natural or man-made disasters.

A limited autopsy was performed on a Kampuchean man injured in a toxic attack in February 1982, who died a month later having initially showed signs of recovery, then developing fever, jaundice, heoptysis, and anariax coma. Malaria was ruled out. The heart tissues showed interstitial myocardial hemorrhage and acute myocarditis, while the lungs showed only pulmonary edema. There was diffuse hepatitis with micronodular cirrhosis, as well as acute renal tubular necrosis. Tissues showed T-2 toxin in amounts ranging from 6.8 to 80 ppb, but there is little information with which to interpret the findings. The pathologist considered them to be compatible with mycotoxin poisoning (Stahl, Green, and Farnum, 1985).

Four stages were identified in the early Soviet *Fusaria* consumption incidents (a chronic oral exposure) (Mayer 1953a, 1953b):

- **Stage 1** begins within a few hours and lasts three to nine days. It consists of mild inflammation of the mouth and gastrointestinal tract, gastroenteritis, nausea, vomiting and diarrhea.
- **Stage 2** is a quiet period of two weeks or more with few symptoms even while contaminated grain was still being ingested. There were laboratory abnormalities in some patients, but most appeared well.
- **Stage 3** reveals the results of bone marrow aplasia with hemorrhagic diathesis, oral mucosal necrosis, and multiple infections.
- **Stage 4** is a period of convalescence requiring several months after ingestion stopped.

Grain elevator workers are in a complex environment with dusts, plant products, and trichothecenes. They frequently experience coughing, breathlessness, wheezing, fever, and dermatitis (Kemppainen et al., 1986b); similar problems occur in "sick" buildings (Hendy and Cole, 1993; Jarvis, 1985).

Respiratory. Rats, mice, and guinea pigs die rapidly from large respiratory exposures (1 to 12 hours) but show little sign of pulmonary injury, unlike direct effects on gastrointestinal mucosa (Creasia et al., 1987; Wannemacher and Wiener, 1997; Bunner et al., 1985). At low levels of respiratory exposure, coughing and upper respiratory irritation occur. Higher exposures produce pulmonary edema, collapse, hypoxia, and death within a few hours, or more indolent symptoms with later pulmonary hemorrhage, hypotension and shock, edema, or infections (Rukmini, Prasad, and Rao, 1980; Lutsky et al., 1978; Bunner, 1983; Bunner et al. 1985). Fifty Hmong survivors reported the following: smell of gunpowder or pepper (14 percent), rhinorrhea (28 percent), nasal

itching (14 percent), sore throat (40 percent), aphonia (26 percent), cough (60 percent), dyspnea (52 percent), severe chest pain (52 percent), and hemoptysis (18 percent). Systemic signs (vomiting, tachycardia, hypotension, etc.) follow. Oral or intravenous exposures result in pulmonary edema, hemorrhage, consolidation, and secondary pulmonary infection.

Toxicity by the respiratory route may be influenced by the material used to suspend the toxin (Creasia et al., 1987). Fibrinous exudate may be seen, and pulmonary fibrosis was a late complication in some of the trichothecene cancer trials (Goodwin et al., 1981). In contrast to inhaled ricin, where effects are confined to the lungs, respiratory exposure produces much less pulmonary change and pronounced systemic toxicity.

Eyes. Conjunctivitis begins several hours after exposure, although the mechanism of the immediate visual disturbances is unclear. Corneal changes begin at 12 hours, with the peak effect in 24 to 48 hours. Blurred vision continues, with recovery from mild injuries in three to seven days. Hmong yellow rain victims reported eye pain and burning (68 percent), blurred vision (58 percent), and tearing (47 percent). Eyelid edema and scleral inflammation are associated with more-intense exposures. Corneal thinning can follow toxin exposure, with irregularities lasting up to six months (Bunner, 1983).

Skin. The skin responds to nanogram amounts of toxin with edema and inflammation. T-2 administered with DMSO to animals produced almost no local reaction (Bunner et al., 1985), but the systemic effects were substantial, although delayed, and cutaneous LD_{50}s were elevated, compared to application without DMSO. Dermal application can produce the same effects as oral administration: bone marrow, thymus, and lymphatic changes and gastrointestinal effects (Schiefer, 1984; Wannemacher et al., 1983).

In T-2 laboratory accidents, vesication has not been a problem. Despite decontamination, a burning sensation developed from 4 to 24 hours in the contact area, followed by numbness. In cancer trials, erythema, burning stomatitis, and alopecia were common (Schiefer and Hancock, 1984; Murphy et al., 1978; Bukowski et al., 1982; Diggs et al., 1978; Belt et al., 1979; Yap et al., 1979; Thigpen et al., 1981; Goodwin et al., 1981). Hmong survivors reported persistent burning sensations, with tingling, itching, and pain lasting several hours. Some numbness lasted two days to several months in some victims. Scattered erythema was noted after a few hours, but only 23 percent reported blisters. In some cases, large hemorrhagic bullae occurred, with underlying necrosis. Necrotic areas sloughed easily when corpses were moved (Wannemacher and Wiener, 1997). Sequelae include secondary infections, hyperpigmentation, and recurrent rashes.

Gastrointestinal. T-2 and other trichothecenes readily injure the rapidly dividing cells of the gastrointestinal tract. Tissue responses include edema, cytolysis, and sloughing, with loss of gastric epithelium and villus tips (Lee et al., 1984; Rukmini, Prasad, and Rao, 1980). The trichothecene DAS given intravenously showed marked gastrointestinal tract necrosis (Coppock et al., 1985) and pancreatic damage resulting in hyperglycemia. Some jaundice was seen in yellow rain victims. The liver is involved in detoxification, but liver failure is rare (Lutsky et al., 1978). Liver enzymes and amylase rise initially but return to normal in three to seven days (Bunner et al., 1985). As a later consequence, the bowel may become less resistant to bacterial penetration, which can increase susceptibility to infection (Lutsky et al., 1978).

Nervous System. The central nervous system effects are striking. Animals and humans exposed via the respiratory route show early central nervous system signs and symptoms. Symptoms reported from cutaneous exposures—burning pain followed by numbness—suggest that these toxins may directly affect the peripheral nerves.

The early and sustained vomiting suggests direct central nervous system effects involving chemotactic and vomiting centers. Hallucinations are a distinctive feature of trichothecene intoxications. Headaches, drowsiness, anxiety, confusion, and seizures occur, but their mechanisms have not been studied (Yap et al., 1979; Thigpen et al., 1981; Bukowski et al., 1982).

There are few autopsy reports. DAS-poisoned swine showed cerebral hemorrhages (Coppock et al., 1985), while other animal studies showed meningeal bleeding and scattered petechial hemorrhages (Ueno et al., 1984). Experimental studies show alterations in levels of hydroxyindoleacetic acid and seratonin in the brain, with regional norepinephrine increases. Trichothecenes make the blood-brain barrier permeable to mannitol, although not dextran (Wang, Wilson, and Fitzpatrick, 1992). Intracerebral administration of T-2 decreased learning in mice, and intraperitoneal administration disturbed both learning ability and memory (Umeuchi et al., 1996).

The descriptions of chemotherapy patients (Thigpen et al., 1981; Yap et al., 1979), home exposures (Croft et al., 1986), and yellow rain cases (Watson, Mirocha, and Hayes, 1984; Crossland and Townsend, 1984) convey a picture of neurotoxicity, with somnolence, confusion, tremors, depression, weakness, malaise, and memory problems (some of which resemble findings in some Gulf veterans). In the cases just cited, however, symptoms appeared promptly, and there were other conspicuous indications of exposure.

Cardiovascular, Lymphatic, Hematologic. Hmong yellow rain victims reported chest pain, sometimes crushing, along with weakness. Animals poisoned with T-2 develop tachycardia and later bradycardia. Hypotension occurs early and

may persist for several days, sometimes proceeding to shock. Hypotension and orthostatic hypotension were common in chemotherapy patients (7 to 40 percent) (Yap et al., 1979; Thigpen et al., 1981; Murphy et al., 1978; Bukowski et al., 1982). Mucous membranes are bright red, reflecting vasodilation. Commonly, hemorrhagic foci are found throughout the myocardium (Ueno et al., 1984; Stahl et al., 1985), and the electrocardiogram may show a prolonged P-R interval and prolongation of the QRS and QT intervals, reflecting conduction system abnormalities and increased risk of arrhythmias.

Beginning with the alimentary toxic aleukia diagnoses, bone marrow and lymphatic system injury has been a consistent finding (Mayer 1953a, 1953b; Ueno et al., 1984). Cell culture studies show stem cells to be sensitive to T-2 toxin. Mycotoxins produce profound alterations in hemostasis, as noted in yellow-rain cases and documented by primate studies (Cosgriff et al., 1986). Prothrombin and activated partial thromboplastin times are increased early in intoxication from decreased coagulation factors. Lethal hemorrhage risk is greater because T-2 inhibits platelet aggregation (Yarom et al., 1984).

Other. There are clinical signs of muscle involvement. The Hmong complained of weakness, fatigability, tremors, and cramps. Animals show flaccid weakness after T-2 poisoning. The early elevation of serum creatine kinase could reflect muscle or cardiac injury, or both. Isoenzyme studies have not been reported (Bunner, 1983).

Impaired immunity and infection resistance is another effect of these toxins. The ability of leukocytes to kill bacteria is impaired (Yarom et al., 1984); immunoglobulin levels are depressed; and cell-mediated immunity is suppressed (Jagadeesan et al., 1982; Schiefer, 1984; Ueno et al., 1984).

Renal output decreases after T-2 intoxication, and the toxin is found in substantial amounts in the kidney. Observed tubular necrosis could be related to hypotension and liver disorders.

The endocrine effects of T-2 and other trichothecenes are not prominent. Adrenal cortical necrosis from T-2 exposure has been reported in rats (Thurman et al., 1986). Decreased spermatozoa production has been seen in several species.

Interactions. The literature on trichothecene interactions is limited. Combining aflatoxins and trichothecenes may increase toxicity (Schultz, 1982; U.S. Army, 1990). No reports emerged of studies examining combined inhalation exposures. There were indications of synergism in feeding studies of chickens (Huff et al., 1988). (However, a study of DAS and aflatoxin in lambs did not show any enhanced toxicity from combined oral exposures of these toxins (Harvey et al., 1995). There is a strong possibility that the severity of trichothecenes could be potentiated by exposure even to low levels of organophos-

phate pesticides, carbamate pesticides or pretreatments, or low levels of nerve agent, through inhibition of carboxyesterases involved in detoxification (Johnson and Read, 1987). Drugs inducing the increase of detoxifying enzymes, such as epoxide hydrolase or cytochrome P450, may favorably interact to decrease toxin severity. Such drugs as phenobarbital, metoclopramide, metochlopramide carbamazepine, metyrapone, and clofibrate have shown beneficial effects in animal models (Fricke, 1993; Wannemacher and Wiener, 1997).

What to Look for in the Gulf Context

Because of the high sensitivity of the skin and eyes to trichothecenes, injuries to these organs should be looked for in unit medical records. Conjunctivitis, erythema, burning skin, and blurred vision were followed by nausea, vomiting, and diarrhea might increase suspicion of trichothecene exposure.[12]

Summary and Recommendations

The trichothecenes are credible biological warfare toxins for some purposes. However, there is no proof or even a strong indication of their use against U.S. forces in the Gulf. With more concentrated exposure, hematological changes, seizures and other serious sequelae might have been expected.

Current information arises from clinically recognized exposures or laboratory research. Trichothecenes have multiple toxic effects with potential long-term consequences, such as central nervous system injury, immune suppression, and prolonged disability. The sequelae noted in the Hmong, e.g., long-term memory problems; the animal memory studies; and the story of the household exposure may resemble some features of the illnesses in Gulf War veterans, but the expected hematological alterations have not been reported among Gulf War patients. Furthermore, the Hmong effects resulted from substantial exposures with major short-term consequences. Little is known about the behavioral effects of sustained low-level exposures. The extreme sensitivity of the skin and eyes to T-2 and other trichothecenes makes it unlikely that delayed systemic illnesses in Gulf veterans represent a late effect of exposure to toxin "fallout." One would have expected an "epidemic" of painful dermatitis and conjunctivitis, as well as a number of other symptoms, which would have drawn attention to the exposure.

As in other cases, the AFIP should be consulted. The tests for trichothecenes are not routine, but have been used enough to be considered more than exper-

[12]However, troops dermally exposed to trichothecenes in DMSO might only have systemic symptoms, since little agent might remain in the skin after enhancement of transport by the solvent.

imental. The AFIP might be consulted about the possibility of detecting trichothecene metabolites in tissue specimens obtained from the Gulf and immediately after. If used protective mask filters from the war period become available, it might be possible to analyze them for the presence of trichothecenes, which are very stable molecules. Had trichothecenes been used, it is possible that their toxicity might have been increased by interactions with nerve agents or PB, although this has not been studied explicitly.

AFLATOXINS

This family of related toxins is produced by the molds *Aspergillus flavus* and *A. parasiticus*, which commonly contaminate food grains before and after harvest. Their toxicity was recognized in the 1960s, and it was later appreciated that they are a significant health problem for domestic animals and humans. The toxins are stable and survive cooking. Attention has focused on chronic exposure and illness from oral intake, although there have also been acute effects (Steyn, 1995; Coulombe, 1993; Bonomi et al., 1995). This review concentrates on AFB_1, the most toxic of the aflatoxin family, although the actual mixture Iraq weaponized is unknown. Aflatoxins show delayed acute toxicity (eight hours to several days) because most require metabolic activation (Daniels et al., 1990). However, most interest in aflatoxins arises from their carcinogenicity. They are implicated in the genesis of hepatocellular carcinoma, which is prevalent in tropical regions (Nigam et al., 1994, Groopman et al., 1996).

Aflatoxins do not appear to have attracted much of the military interest in toxins (SIPRI, 1973).[13] It was thus surprising when, after the War, Iraq informed the UN that it had produced aflatoxins and several trichothecene toxins (PAC, 1996a, 1996b; Zilinskas, 1997). Aflatoxins have, however, been mentioned as possibly enhancing the toxicity of trichothecene mycotoxins after the latter were recognized as military agents (U.S. Army, 1990; Schultz, 1982). Still, Iraq's placing aflatoxins in long-range missiles has surprised and puzzled analysts. Zilinskas (1997) offers three hypotheses:

1. The Iraqis discovered that aflatoxin possessed previously unknown properties useful in biological warfare.
2. The long-term potential for carcinogenesis was used to terrorize civilian populations.[14]

---

[13]Aflatoxins are not discussed in the extensive coverage of biological warfare and toxins in Sidell, Takafuji, and Franz (1997).

[14]During the Iran-Iraq War, fear of chemical warheads was a factor in the terror urban missile attacks inspired (Cordesman and Wagner, 1990).

3. Because aflatoxin is easy to produce, it was produced and deployed to meet toxin production quotas set by higher authorities.

This report examines the first and second hypotheses.

Although concerns about human exposure have focused on carcinogenic risks, acute aflatoxin toxicity has been recognized, primarily from oral exposure but also via the respiratory route (e.g., aflatoxin-contaminated grain dust) (Hendry and Cole, 1993; Zarba et al., 1992; Massey, 1996; Baxter et al., 1981, Autrup et al., 1993). Respiratory exposure would have been the most likely route in the Gulf. The available information indicates that the food and water supplies of U.S. forces in the Gulf theater were diverse but secure and did not present an opportunity for long-distance attack. Dermal exposure producing systemic toxicity from aflatoxins has not been described. Animal studies suggest that respiratory exposure is more toxic than oral exposure (Northup et al., 1995).

There is uncertainty about human clinical manifestations of low-dose respiratory exposure to aflatoxin, although both acute and chronic illnesses are expected. The symptom onset is delayed, and there is evidence of cumulative effects.

Although many humans are exposed to aflatoxin in food, gastrointestinal symptoms predominate; neuropathy, rashes, memory problems, and joint pain are not commonly reported. Aflatoxins do impair resistance to infection experimentally (Jakab et al., 1994; Raisuddin et al., 1993), although determining their role in increasing human infectious diseases has been difficult (Allen et al., 1992; Denning et al., 1995).

Weaponization

Information about military deployment of aflatoxin is apparently confined to the Iraqi experience, although the sources for this report were limited to the unclassified literature. Zilinskas (1997) was a member of the UNSCOM team that had access to Iraq and analyzed that country's biological warfare program, including toxin activity. It is evident that much of what the Iraqis chose to discuss could not be verified independently (Zilinskas, 1997).

Iraq began evaluating biological weapons in the late 1970s but began an earnest program in 1985. By April of 1991, Iraqi scientists had investigated the biological potential of five bacterial strains, one fungal strain, five viruses, and four toxins, while also developing two harmless bacteria for testing purposes. Major centers of development were Muthanna State Establishment (also the center of chemical weapon development) and Salman Pak, which became the biological warfare center, with production occurring at the Al Hakam Single Cell Protein

plant. Virus research was conducted at an animal disease research station at Al Manal (Zilinskas, 1997).

Substantial efforts went toward weaponizing aflatoxin, botulinum, ricin, and perhaps trichothecenes. Generally, the Iraqis manufactured crude solutions of toxins. Iraq developed a method of producing aflatoxin using cultured rice as a growth medium. UNSCOM was told that some 2,200 liters of aflatoxin were produced at Salman Pak. Some toxin was stored after weapons were filled.

The weapons filled with biologicals (at Al Muthanna) included 250- and 400-pound bombs (60 to 85 liters of toxin solution) (Zilinskas, 1997). An unknown number of 122 mm rockets were filled with aflatoxin as were some ten Scud warheads (Zilinskas, 1997). The UN inspectors were told that tests were made using toxin-filled and stimulant-filled 122-mm rockets, but as far as they knew such weapons were not deployed. Iraqi munitions used a simple burster charge to open the walls and disseminate the agent. The Iraqis also possessed several hundred Italian-made pesticide dispensers suitable for biological dissemination by aircraft or land vehicle. A MiG aircraft was modified for unmanned operation and fitted with a 2,200-liter tank to disseminate chemicals or toxins. It must be understood that UNSCOM has not independently verified most information Iraq provided pertaining to toxins.

The amount of toxin needed to produce severe illness or death (2 to 4 mg/kg) via oral routes is greater than for many military toxins. This level of toxicity places it in the second order of toxicity classification on a six-category scale, in which 1 indicates "extremely toxic" ($LD_{50} \leq 1$ mg/kg), 2 indicates "highly toxic" (1–50 mg/kg), and 6 indicates "harmless" (Proctor and Hughes, 1978). Such chemicals as lewisite, DFP, and the organophosphate pesticides parathion and isosustox fall in this category (SIPRI, 1973). The uncertain late cancer effects provide an implausible military motivation for use.

Although Zilinskas (1997) was not certain about what military effects would result from use of aflatoxins, they are capable of producing death, seizures, respiratory injury, nausea, vomiting, and liver failure, which would be militarily significant (Chao et al., 1991; Northup et al., 1995; Jakab et al., 1994; Bourgeois, 1971a, 1971b). Inhaled aflatoxins in microgram amounts are highly immunosuppressive (Jakab et al., 1994) (milligrams would be needed for humans), but this effect would not provide the predictable effects weapon developers favor (use in conjunction with an infectious agent might be an exception).

In sum, the Iraqis were well informed about the effects of biological weapons and admitted conducting extensive field trials. A variety of fairly unsophisticated delivery means existed, as well as toxin stocks not placed in weapons. No information is available about bombing results on known biological warfare facilities. There is no indication that Iraq employed biologicals during the war,

and there is no information about forward deployment of toxins in the theater. However, no detection system was deployed; no such system is available for aflatoxin, although there are mechanisms for detecting aflatoxin in the laboratory (Autrup et al., 1993; Harrison and Garner, 1991; Wang, 1996; Groopman et al., 1996; Ross et al., 1992). GAO (1997) suggested that aflatoxin exposure arising from U.S. attacks on chemical storage sites might have contributed to illnesses in Gulf War veterans but did not provide evidence to support the hypotheses.

The toxin is stable in the environment, is resistant to heat, and would be active after atmospheric transport from an attacked Iraqi depot. However, it is questionable how much significant toxicity would result after atmospheric dilution.

Chemical Characteristics

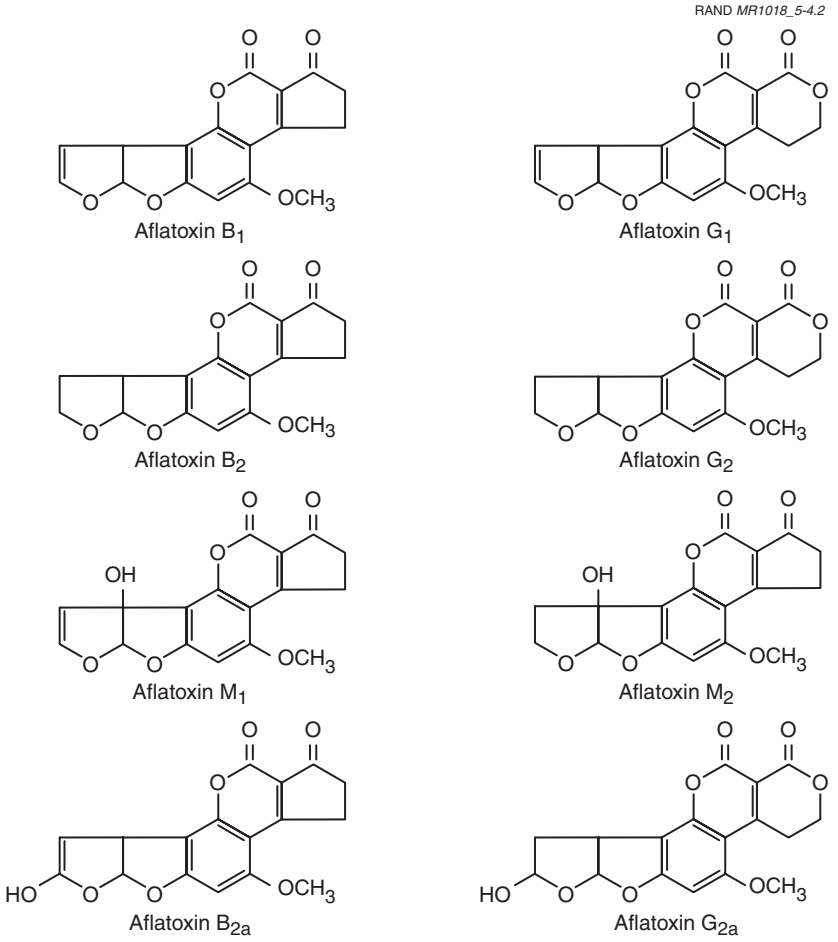
Figure 4.2 shows the chemical structures of eight of the 17 aflatoxins. The top four—B_1, B_2, G, and G_2,—are the four most commonly mentioned. All occur naturally. The "B" and "G" designations of these toxins relate to the fluorescence color in ultraviolet light (blue or green) while the subscripts refer to chromatographic mobility.

Mechanisms of Action

The concern about aflatoxin producing cancer in humans and animals has produced an extensive literature on the metabolism of aflatoxin and the biochemical reactions of the metabolites (Steyn, 1995; Coulombe et al., 1991, McLean and Dutton, 1995; Tutelyan and Kravchenko, 1981). Active metabolites act on several cell structures (e.g., mitochondria, lysosomes, endoplasmic reticulum), but the cancer concern has focused attention on the effects on the nucleus and DNA.

Activated AFB_1 attacks nucleic acids with the formation of adducts that can act like point mutations, damaging DNA and impairing RNA and protein synthesis (McLean and Dutton, 1995). Proteins, including receptors and those with important intracellular functions, may also be nonspecifically but irreversibly bound by toxins, producing diverse loss of function (e.g., enzyme inactivation) (Tutelyan and Kravchenko, 1981).

Acute mycotoxin injury inhibits cellular energy production. The aflatoxins act on the electron transport system, interfering with the cytochrome system (Tutelyan and Kravchenko, 1981), depleting ATP, inhibiting ATPase, and causing mitochondrial swelling (Sajan et al., 1995). The effect of aflatoxin on the electron transport system may not require activation of the toxin. Recent studies draw attention to mitochondrial disease and injury, producing liver failure



SOURCE: Reprinted with permission from McLean and Dutton (1995).

Figure 4.2—Chemical Structures of Several Aflatoxins

and associated disorders (e.g., Reyes syndrome), with brain and liver injury (Schafer and Sorrel, 1997). Carbohydrate and lipid metabolism are impaired, and hepatic glycogen stores are depleted, with a secondary rise in blood sugar. Lipids accumulate in the liver and fatty oxidation decreases, perhaps secondary to mitochondrial injury. These effects occur at levels lower than those producing RNA and growth effects (McLean and Dutton, 1995; Tutelyan and Kravchenko, 1981; Verma and Choudhary, 1995).

Activation. Metabolic activation is required to produce toxicity from AFB_1. After crossing the cell membrane, the molecule is activated by microsomal

mixed-function oxidases involving the cytochrome P450 enzymes and nicotinamide adenine dinucleotide phosphate reductase (NADPH) and oxygen. The active and toxic AFB_1 8,9-epoxide (Figure 4.3) is the result. This active molecule has a short half-life and binds to DNA and other structures in the endoplasmic reticulum. Other NADPH reactions can reversibly produce aflatoxicol, which can serve as a sink or source of AFB_1 in the cell. The microsomal monoxygenase system may transform AFB_1 into more polar molecules, such as AFM_1, Q_1, or P_1, which can be eliminated by liver cells (McLean and Dutton, 1995). The situation is complex, and there are other concepts of toxicity involving more indirect mechanisms associated with membrane actions involving lipid peroxidases and aldehydes (Shen et al., 1994; Tutelyan and Kravchenko, 1981); see Figure 4.4.

Detoxification. Aflatoxins are also detoxified by mechanisms that deal with xenobiotics—leading to conjugation with glucuronic acid, sulfates, or glutathione. The major route for AFB_1 detoxification is conjugation of the epoxide with glutathione (through glutathione S transferase) and subsequent excretion in bile (McLean and Dutton, 1995). This means that toxicity may vary depending on intracellular glutathione stores in various tissues, which can vary considerably with circadian effects or depletion by other factors—diet, smoking, alcohol, and medications (Tsutsumi and Miyazaki, 1994). Other aflatoxins appear to be primarily eliminated via glucuronide or sulfate conjugation. Various species differences in sensitivity to aflatoxins may reflect differences in detoxification mechanisms (McLean and Dutton, 1995).

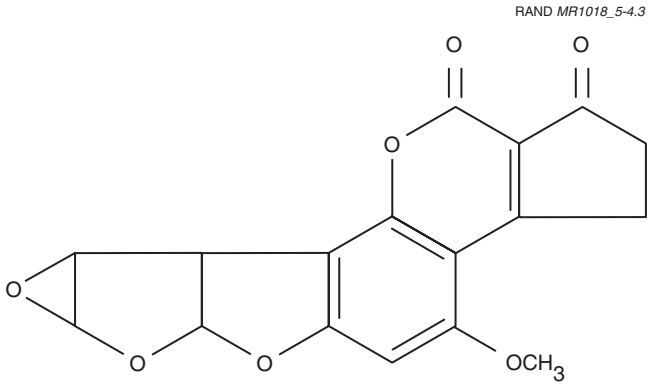
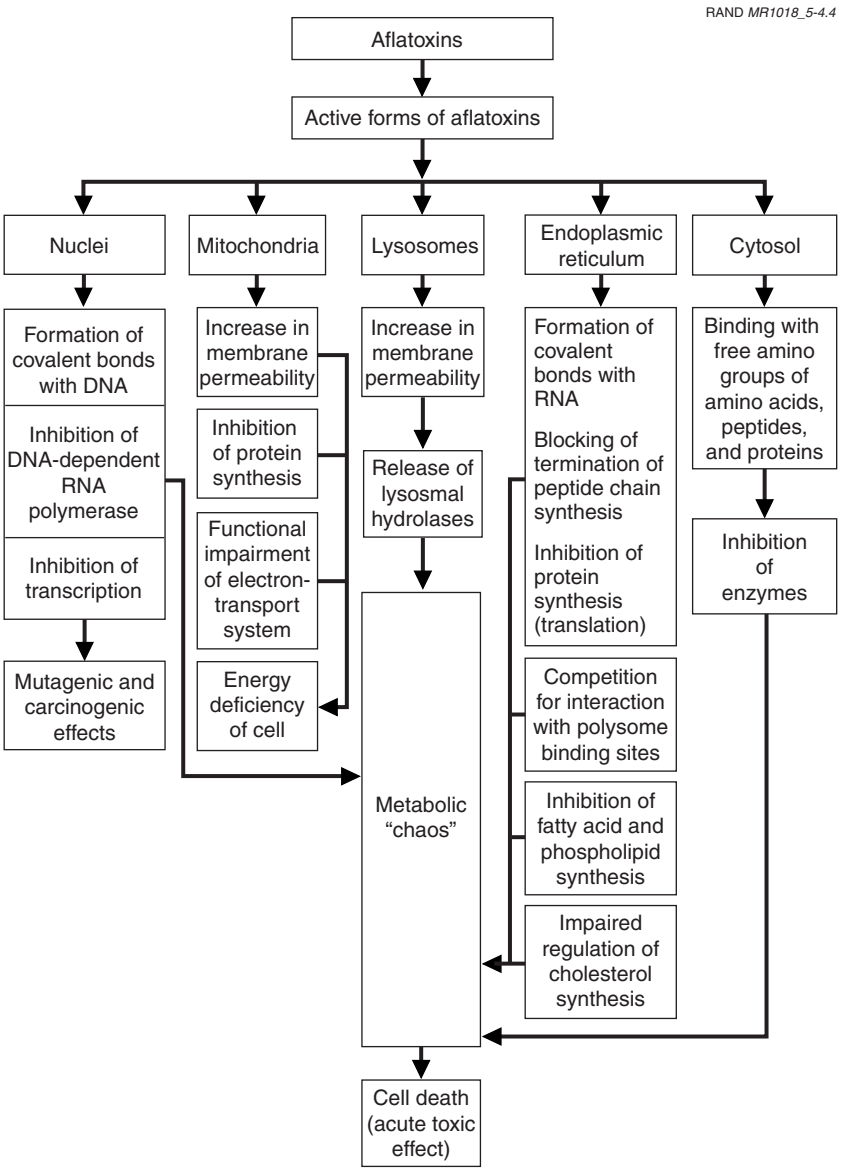


Figure 4.3—Activated AFB_1 Molecule



SOURCE: Tutelyan and Kravchenko (1981).

Figure 4.4—Mechanism of Effects of Aflatoxins on Cell

Exposure-Effect Relationships and Clinical Manifestations

Gastrointestinal. In Southeast Asia, intoxication occurs from ingestion of food, chiefly rice and noodles, that has been contaminated by the fungus. Outbreaks of illness attributed to oral intoxication by aflatoxin (Bourgeois et al., 1971b)

have been reported in humans. Some 40 cases in Thai children were characterized by abrupt onset of coma or convulsions, fever, respiratory distress, vomiting, and death within 72 hours. Serum transaminases were elevated; prothrombin times were prolonged; and blood sugars were lowered. Pathology findings showed neuronolysis; cerebral edema; fatty infiltrations of liver, kidney, and heart; and lysis of lymphatic tissues (Chao et al., 1991; Chao, 1992).

In monkeys, a Reyes-like syndrome develops, with fatty degeneration of the liver (and encephalopathy) (Bourgeois et al., 1971a). Young monkeys were given 0, 1.5, 4.5, 13.5, or 40.5 mg/kg of AFB_1 orally. Doses of 1.5 mg/kg were not fatal, and no unusual clinical signs were noted. Deaths began at 4.5 mg/kg, with others sick. All animals at higher levels died. Cough, vomiting, diarrhea, and coma were the key clinical findings. Laboratory findings were similar to those in the Thai children. Pathology was similar but also showed bile duct hyperplasia.

A well-studied outbreak occurred in Malaysia in 1988, with severe illness occurring in several towns that was eventually traced to noodles prepared from aflatoxin-contaminated grain. The noodles were also discovered to be contaminated with boric acid used as bleach. It took an average of eight hours (a range of 3 to 16 hours) from eating the noodles to the onset of symptoms. The illness began with vomiting (in 100 percent of cases), followed by seizures (82.4 percent), hematemesis (82.4 percent), fever (17 percent), diarrhea (23 percent), and abdominal pain. Liver and renal failure ensued, as did coma and respiratory failure. The outbreak killed 13 children, and another 45 persons had milder symptoms. The mortality rate was high, despite supportive modern medical care (Chao et al., 1991; Lye et al., 1995; Chao, 1992; Harrison and Garner, 1991). The estimated lethal amount was 2 mg/kg (Harrison and Garner, 1991).[15]

High doses result in multiple injuries. Those the Malay children sustained included gastric erosions, although these may have been related to the boric acid simultaneously ingested (Chao et al., 1991). Chao et al. made some effort to distinguish between the effects of aflatoxin and those of boric acid. They noted the patients lacked the "boiled lobster" skin changes of heavy boric acid poisoning but believed that the diarrhea, renal problems, and metabolic acidosis probably reflected boric acid effects. Overall, the researchers considered the toxicity to be primarily an effect of the aflatoxin. The most common findings in human and animal poisoning is liver injury (Lye 1991; Fernandez, Ramos, et al., 1995; Fernandez, Verde, et al., 1995), including macrovesicular steatosis (also a finding in Reyes syndrome), bile duct metaplasia, and centrilobular coagulative necrosis (Chao et al., 1991). AFB_1 metabolizing enzymes are present in intesti-

[15]As noted previously, this falls in the "highly toxic" category described by Proctor and Hughes (1978)—less toxic than most nerve agents but in the same category as lewisite and parathion.

nal epithelial cells, which can sustain mild injury from low-dose AFB_1 ingestion, impairing nutritional intake in animals (Guengerich et al., 1996). No follow-up reports are available to reveal what sequelae, if any, occurred in the survivors of acute exposures.

Northup et al. (1995) indicate that the oral LD_{50} for guinea pigs is 1 mg/kg for AFB_1.

Respiratory. No estimates for an acute human lethal or incapacitating respiratory dose are available. There are also no descriptions of acute human or other primate respiratory exposures. The human lung does possess the enzymes necessary to activate AFB_1 (Massey, 1996). At high levels of exposure, enough aflatoxin might be absorbed to produce the serious systemic illness seen in Malay children, with seizures, vomiting, coma, hepatorenal failure, and death. At low levels of exposure, there might be few obvious acute effects or only mild respiratory symptoms. However, there is some indication in animal studies that acute respiratory effects are possible at doses much lower than those required for dangerous oral intoxication (Cresia et al., 1987; Bunner et al., 1985). It may be that the Iraqis discovered some effects of that kind, and that is why they weaponized the toxin.

There has been concern about an increased risk of lung and liver cancer among humans chronically exposed to low-level AFB_1 in grain dust at mixed oral and respiratory intake levels of 0.04 to 2.5 µg per week (Massey, 1996; Autrup et al., 1993; Coulombe, 1993). Mycotoxins have been shown to have mitogenic effects at levels well below clinical toxicity (Griffiths, Rea, et al., 1996). Other than the cancer risks, however, respiratory disease or systemic illness from chronic respiratory exposure has not been found (Coulombe, 1993); Autrup et al., 1993).

There have been some significant studies on animals, although caution must be exercised in generalizing these findings to humans. Sensitivity to aflatoxins varies tenfold among species because of metabolic differences and the balance between activation and detoxification mechanisms (Coulombe et al., 1991), and species vary as to the sites where aflatoxin is activated.

Experimental animals have shown a range of aflatoxin responses from mild inflammation to more striking illness with tracheal epithelial damage, alveolar injury, and pulmonary hemorrhage (Coulombe et al., 1991; Coulombe, 1993; Jakab et al., 1994). Guinea pigs, after a large aerosol exposure, developed hemorrhage and exfoliation of epithelial cells at six hours (Northup et al., 1995). Of particular interest, since the doses are in a range of potential military interest because of high potency, is a report that guinea pigs exposed for four hours to nanogram amounts by aerosol produced hemorrhage and exfoliation of respiratory cells (Northup et al., 1995). Intratracheal aflatoxin in rats appears in the blood in 3 to 12 hours (Coulombe et al., 1991). Minor bronchial mucosal

damage occurred in rats that were intratracheally exposed to 300 µg/kg microcrystalline AFB_1, but bronchiolitis occurred with intratracheal dust aflatoxin delivery (Coulombe et al., 1991).

Northup and Kilburn (1978; as cited by Hendry and Cole, 1993) reported tracheal-bronchial cell destruction in hamsters and guinea pigs from acute inhalation of aflatoxin.

Aflatoxin in rats was also retained longer when adsorbed on dust particles than when delivered in its microcrystalline form (Coulombe et al., 1991). Aflatoxins are known for their impairment of resistance to infections, so secondary respiratory and other infections might be expected. For example, macrophage function was impaired for two weeks following exposure to AFB_1 aerosol in rats (16 µg/kg) (Jakab et al., 1994). Pulmonary pathology also occurs experimentally with oral aflatoxicosis, and there was some bronchopneumonia in the Malay outbreak (Fernandez, Ramos, et al., 1995; Fernandez, Verde, et al., 1995; Chao et al., 1991).

Rats given intratracheal aflatoxin B and G for 30 weeks developed carcinomas of the liver, intestine, and kidney (as cited in Hendry and Cole, 1993: Northup and Kilburn, 1978).

Nervous and Musculoskeletal Systems. Aflatoxin is rapidly distributed to gray matter (Larsson and Tjalve, 1996), although no histopathologic descriptions of changes arising from aflatoxin exposure were found in the literature, other than the previously noted cerebral edema and neuronolysis noted in Thai and Malay children and in monkey experiments (Chao et al., 1991; Bourgeois et al., 1971a, 1971b). Animals and humans with high exposures to aflatoxin have seizures. The Malay cases showed widespread edema, with petechial hemorrhages in the white matter; however, these patients were on respirators for prolonged periods, and the brains were necrotic with impression of hypoxic encephalopathy (Chao et al., 1991). The mechanism for neurotoxicity is not clear, although brain cells have high metabolic rates, so disturbances in mitochondria and energy metabolism would be significant. No data have emerged to suggest that peripheral neuropathy is a problem arising from aflatoxin exposure, and there are no reports of musculoskeletal problems.

Studies in mice show that "nontoxic" low-level exposure to AFB_1 reduced brain levels of serotonin and catecholamines (Kimbrough, Llewellyn, and Weekley, 1992). Although the clinical significance of this observation is unknown, it is noteworthy that this type of exposure to AFB_1 affects these important neurotransmitters.

Cardiovascular and Hematologic. No reports of characteristic cardiovascular findings were found. Cardiac hemorrhages have been described in cattle acutely poisoned by aflatoxin (Rajendran et al., 1992). Fatty degeneration of

heart muscle (especially atrial and conduction systems) was seen in Thai children efficiently poisoned by AFB (Bourgeois, Olson, et al., 1971).

Hematologic problems, although noted, do not seem prominent. *In vitro* studies have shown dose-related inhibition of myelopoiesis in several marrow culture models (Cukrova et al., 1991; Dugyala et al., 1994). Aflatoxins impair phagocytosis by alveolar macrophages (Richard and Thurston, 1975). The impaired production of prothrombin in the liver in serious intoxications may contribute to the observed bleeding in other tissues.

Other Sites and Systems. Because aflatoxins are soluble in DMSO (McLean and Dutton, 1995), it might be possible to deliver the toxin dissolved in DMSO through the skin. There is no information about acute or chronic cutaneous effects or hazards arising from cutaneous exposure.

Although the conjunctiva binds toxins, there is no information about inflammation or other acute or chronic toxic effects on the eye (Larsson and Tjalve, 1996).

Renal pathology is seen in acute toxicity, but does not appear to be a feature in chronic exposures. Autopsy data show swollen pale cortices with congested medullary regions. Aflatoxins M_1 and M_2 were found more often than B_1 in renal tissue (Chao et al., 1991). Bourgeois, Olson, et al. (1971) noted fatty degeneration of kidneys with proximale tubule damage.

At levels below clinical illness, aflatoxins are immunosuppressive and impair humoral and cell-mediated immunity (Griffiths et al., 1996; Raisuddin et al., 1993; Cysewski et al., 1978; Dimitri and Gabal, 1996). Although acute effects of immunosuppression in animals reversed after two weeks (Jakab et al., 1994), longer exposure has resulted in loss of suppression of toxoplasma cysts (Venturini et al., 1996). It would not be surprising to see reactivation of quiescent infections, such as herpes.

Cross-Systemic and Chronic Effects. Because of human health concerns, efforts are made to keep aflatoxins at low levels in food and milk. Interestingly, mice fed low levels of AFB_1 and AFG_1, within human exposure limits, showed signs of liver and kidney cytotoxicity, although species differences may play a role in this observation (Ankrah et al., 1993). Studies addressing chronic human exposure in occupationally exposed workers to unmeasured AFB in food show increased rates of liver cancer, impaired child health and development, and increased infections from long-term exposure (Groopman et al., 1996). No reports of neuropathy, chronic brain syndromes, skin problems, or arthropathy from chronic oral intake were available. Animal studies have found weight loss, illness, and reproductive problems from respiratory intake (Coulombe, 1993; Aulerich et al., 1993; Bonomi et al., 1995). Teratogenic effects have been observed (Raisuddin et al., 1993).

Combined Interactions

As noted before, there has been discussion of enhanced toxicity from combined exposure to trichothecenes and aflatoxins (U.S. Army, 1990). No reports of combined respiratory exposures were found. Some animal-feeding studies (chickens) found synergism (Huff et al., 1988), and others did not (Harvey et al., 1995), although the latter study showed some synergism in weight loss of a liver enzyme. We have not found a study that looked at synergism in acute respiratory exposures, which would be helpful in understanding the significance of this possible synergism.

What to Look for in the Gulf Context

The intended use of Iraqi aflatoxin weapons is unclear (Zilinskas, 1997). The toxin is stable enough to survive transport through the atmosphere and to persist trapped in dust, creating a secondary inhalation hazard. It is not clear that such transport and contamination occurred, but at low levels it would have been difficult to recognize or detect. The carcinogenic effects of aflatoxins take many years, and the risk from an acute exposure via missile attack does not seem enough to make it a credible objective.

There is insufficient information about respiratory effects in primates. Northup's finding of respiratory injury in animals with nanogram amounts of aflatoxin aerosol raises the possibility that respiratory toxicity is much greater than the better known oral toxicity, which might reflect the intended Iraqi use. Because the entire cardiac output passes the lungs, it might be possible to produce seizures, coma, and liver failure via the respiratory route.

Dramatic illness would have been noticed in the Gulf War if exposure to aflatoxin took place. There are no documented clinical reports of acute symptomatic lower-level human exposures, but extrapolation from animal studies suggests that low doses might produce respiratory irritation, nausea, malaise, and anorexia—symptoms not specifically associated with toxins or other chemical agents, where eye or skin problems would typically be expected. At levels comparable to those grain workers are exposed to, there might be no symptoms, although tissue and immune effects may occur.

Compared to most of the agents under review, it is hard to describe a "typical" aflatoxin case. As a result, it would be difficult to tie individual Gulf War illness cases to aflatoxin poisoning, even if it had occurred.

It is unknown what symptoms a combined low-level trichothecene and aflatoxin exposure would display. Although U.S. Army (1990) mentions synergistic effects, there are some data that prove a synergistic effect (Huff et al., 1988) and some that suggest that such a synergy does not always occur (Harvey et al., 1995). Studies of combined respiratory exposures would be helpful.

Summary, Conclusions, and Recommendations

Why Iraq developed weapon systems for aflatoxin remains speculative; respiratory toxicity at low levels and immunosuppression at low doses may provide hints, although there is little information about primate respiratory toxicity. Oral exposure in Malaysia led to deaths; if exposure at similar levels could be achieved through inhalation, pulmonary and systemic effects would be delayed, and there would have been no detection means (and no effective therapy). The agent, if spread in the vicinity of U.S. troops, would be stable enough to provide recurrent exposures. Although the estimated lethal dose of 2 to 4 mg/kg is not as toxic as some substances, it would be possible to create an aerosol that would deliver the 140 mg of toxin sufficient to kill a 150-pound person (if the Malay experience with children can be generalized). Of course, a full-blown outbreak of this nature would have been noted during the Gulf War. Lower levels of exposure might resemble acute oral exposure in animals, with vomiting, malaise, and nonspecific signs and symptoms.

There is no clear clinical picture that would make recognition of low-level aflatoxin exposure easy. However, there is also no information that aflatoxin was present in the Gulf War, and no descriptions of Gulf War illnesses resemble what might be expected from aflatoxin exposure.

Several steps might still be taken to assess the possibility of aflatoxin exposure in the Gulf. These include following up on a report that aflatoxin antibodies could be detected in exposed persons by measuring antibody levels in Gulf personnel and controls (Autrup et al., 1993). The antibody level in the Danish controls was low compared to that in Kenyans with high dietary intake, so finding antibodies in Gulf war veterans would not be conclusive proof of exposure to military toxin, since food exposure alone could promote antibody formation. Blood and tissue samples proximate to the Gulf deployment would be most useful, but care in study design and use of controls would be necessary. The antibody measurements are not routine but have apparently had substantial use.

Harrison and Garner (1991) detected aflatoxin and adducts in formalin-fixed pathology specimens a long time after the event in Malaysia. AFIP could consider analyzing some of its preserved tissues from Gulf cases for aflatoxins and adducts. Also, an analysis could be made of whether material from other Gulf War veterans shows adducts from aflatoxin. Adducts in Gulf War tissue material higher than those in controls would not prove a particular source of aflatoxin exposure but, if found, would require more research on this toxin.

Aflatoxin is sufficiently stable that it might still be detectable in clothing, equipment, filters, and mask canisters from the Gulf War, if they can be located. It would be useful to ask Malaysian health officials about any long-term effects

in the less severe cases from the 1988 outbreak. Such a follow-up period would be about two years longer than the current Gulf War period of observation.

The aflatoxins are potent and poorly understood. They do not seem a likely explanation for the pattern of illnesses in Gulf War veterans, but it does at least appear possible to detect exposure of U.S. personnel to low levels of aflatoxins. Aflatoxins are a poorly understood agent, so further research on their possible military threats should be considered.

The respiratory toxicity of aflatoxins in primates and other species should be evaluated seriously, with some selective evaluation of combined toxicities (e.g., with trichothecenes or infectious agents). A better understanding of the mechanisms of central nervous system toxicity and immune suppression would be helpful (e.g., do aflatoxins alter responses to leishmaniasis, malaria, sand fly fever?).

Aflatoxins are known carcinogens. The induction of cancer has generally been seen in populations with sustained exposures to fairly high dietary levels of the toxin after many years (and in some situations in populations with a high prevalence of chronic hepatitis B infections). A short (few weeks), low-level exposure to aflatoxins should have little risk of increased cancer because the incremental additional amount compared to the background level in Western diets would be small.