

chapter five

Ricin history, toxicity, adsorption, mobility, and palliative actions

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5.1 Introduction

Probably a few words in the United States are more associated with bioterrorism than the word “ricin.” Ricin is a naturally occurring, highly toxic protein produced in the seeds of the castor plant (*Ricinus communis*). The use of castor oil and castor toxin (ricin) goes back many thousands of years (Ogunniyi, 2006). The castor plant is thought to have originated in Africa, but there are wild forms of castor that grow in India. While there are reports of castor grown in the United States dating back to 1803 (Martin et al., 1976), Ogunniyi (2006) stated that “generally the toxicity of the castor seed is a reason why US farmers no longer grow the crop extensively.” India, China, and Brazil are the leaders in exported castor oil. The castor seed is not a bean as commonly reported because the castor plant is not a legume. It is thought that ricin is a plant-specific toxin that protects the castor plant and the seed of the castor plant from insect destruction. Ricin, however, is produced in the seed and nowhere else in the plant. Ricin notoriety (or infamy) is associated with the Markov case and from the numerous envelopes that contained a “white powder,” such as at the Dirksen Building in Washington, DC, and numerous post offices and business offices (CDC, 2003; Hale, 2014).

5.1.1 Ricin properties

Castor seeds contain 1%–5% ricin by weight (Franz and Jaax, 1997). Ricin is a dimeric cytotoxin that is produced and stored in castor seed. The toxin is comprised of two chains that are joined by a disulfide bond (Figure 5.1). The ricin A chain is the ribosome inactivator while the B chain is the galactose binder. The B chain attaches to the cell surface and the A chain enters and begins the enzymatic deactivation. A single molecule of ricin A chain can inactivate 1500 ribosomes/minute, causing rapid inhibition of protein synthesis leading to cell death (Audi et al., 2005). Wannemacher et al. (1990) reported that the median lethal dose to kill 50% of the population (LD_{50}) for intravenous ricin injection into mice was 3.7 $\mu\text{g}/\text{kg}$ of body weight and that it was 50–100 times more toxic when administered as an aerosol than when administered intravenously. Orally ingested ricin is much less toxic. The median lethal dose for orally ingested ricin in mice is 30 mg/kg or about 1000 times greater than for injected or inhaled ricin (Audi et al., 2005). The lethal oral ricin dose for humans was estimated to be 1–20 mg/kg of body weight (Audi et al., 2005). Vitetta et al. (2006) identified a site on ricin

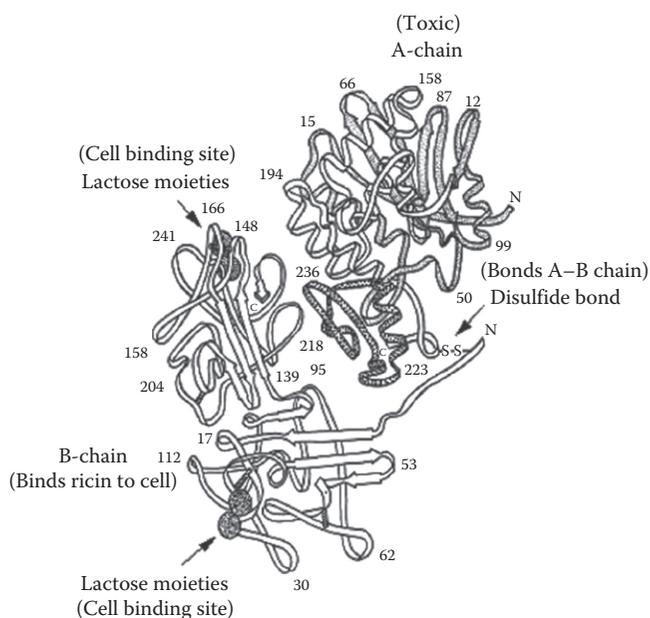


Figure 5.1 Ricin protein structure depicted as ribbons showing A-chain and B-chain subunits after Montfort et al. (1987). Lactose moieties on the B-chain allow ricin attachment to cell surfaces.

A-chain that induces vascular leak syndrome (VLS) in humans. There is no antidote to ricin poisoning, but Vitetta et al. (2006) developed a recombinant A-chain vaccine in which two amino acids in the ricin A-chain have been genetically engineered to inactivate the ribotoxic site and the VLS site. Buonocore et al. (2011) used liposome-encapsulated *N*-acetylcysteine to reverse ricin A-chain-induced hepatotoxicity in test animals. They concluded that liposome-encapsulated *N*-acetylcysteine might potentially serve as an effective treatment for ricin-induced liver injuries.

Ricin is a probable weapon of choice by terrorists because a large quantity of castor seed could easily be obtained by cultivation or other means. Separation of the toxin from castor seed can be accomplished using low-tech equipment and materials available in a kitchen or garage (see Hale, 2014 for a detailed list of events in which ricin was identified). Ricin is classified as a lectin, which is a protein that specifically binds to particular sugars (Zentz et al., 1978). Lectins are highly specific for particular sugar moieties. Ricin (RCA60) toxin and *Ricinus communis* Agglutinin (RCA120) are galactose-binding lectins. Other lectins specifically bind to mannose, fructose, or other sugars. The larger and much less toxic RCA120 lectin occurs with ricin in castor seed. Much of the research conducted on lectins concerns the specific binding to cell surfaces (d'Avila-Levy et al., 2004; Wigglesworth-Cooksey and Cooksey, 2005). Ricin has been attached to antibodies in research to kill cancer cells (de Virgilio and Degryse, 2014).

5.1.2 *Ricin used as a weapon*

As mentioned earlier, ricin is perceived to be a weapon of mass destruction. In actuality, ricin has limited mass destruction capabilities (Schep et al., 2009). Ricin is much more a weapon for individuals rather than for large masses of people. Targeted individuals and people in offices are much likelier to be potential victims of ricin intoxication than are the masses of people in an open stadium. This is based on the method of ricin uptake (inhaled, injected, or orally ingested) and amounts needed to cause harm. Ricin is a toxin and is not a disease-causing organism, such as anthrax. Anthrax or a neurotoxic gas such as sarin could more easily be deployed against crowds of people to produce a large number of fatalities.

5.1.2.1 *Antiquity*

The origin of the castor plant is thought to be Africa and castor seeds have been found in 6000-year-old Egyptian tombs (Hale, 2014). Castor oil has been used as a skin lotion, laxative, and to induce hair growth. In addition to the medicinal properties, castor oil was used as a lamp oil and a machine lubricant. Ricin has been found mixed with 35,000-year-old beeswax making it the earliest known use of a poison (d'Errico et al., 2012).

It has been speculated that the beeswax/ricin mixture was used to poison the tips of stones or spearheads to improve the lethality of those weapons (d'Errico et al., 2012).

5.1.2.2 Pre-9/11

The Markov case in which ricin was injected into the leg of a Bulgarian dissident is probably the most famous ricin poisoning event (Crompton and Gall, 1980). Georgi Markov was assassinated in London, England, when a ricin-filled platinum pellet was injected into his leg at Waterloo Bridge (Owen, 2000). Within the United States, one of the first incidents of ricin use as a terrorist weapon was the Minnesota Patriots Council in 1994 (Tucker and Pate, 2000). Castor is widely cultivated in Iraq and other semiarid countries. In 1989, approximately 10 L of concentrated ricin solution was manufactured at the Salman Pak biological weapons facility just south of Baghdad (Zilinskas, 1997). Even before the events of September 11, 2001 (9/11), the U.S. Centers for Disease Control and Prevention (CDC) identified ricin as a high-priority threat agent for biological and chemical terrorism. Ricin was placed on the CDC's "Category B" list because of its ease of dissemination and moderate morbidity.

5.1.2.3 Post-9/11

After the events of 9/11, biosecurity issues have been an increasing concern. These security issues led to the passage of the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (Bioterrorism Act). The Bioterrorism Act (2002) takes steps to protect the U.S. public from a threatened or actual terrorist attack. Biological and chemical threat agents are used in terrorism to create instability and paranoia within the population. A brief historic summary of biological and chemical weapon use is provided in Presley et al. (2008) with further details available in the U.S. Department of Defense "Bluebook" (USAMRID, 2011) and the *Medical Aspects of Chemical and Biological Warfare* (Sidell et al., 1997). Even before the events of September 11, 2001, the U.S. CDC had identified ricin as a high-priority threat material for biological terrorism. Ricin is considered a "Category B" biological threat agent because of its ease of dissemination, moderate morbidity rates, and low (2%–6% from ingestion) mortality rates (Arizona Department of Health Services, 2004). There were arrests in Great Britain in 2003 for the potential contamination of the military food supply with ricin (Risen and van Natta, 2003).

The primary concern about contamination of our food supply is that it is a very "low-technology" threat. This threat, however, continues to be largely ignored by the agricultural and environmental community. Brandenberger and McGlynn (2003) in the December 2003 Council for Agricultural Science and Technology Commentary stated, "Therefore, *any* disease-causing microbes present on fruits or vegetables are there because

of *inadvertent contamination...*” [Emphasis added]. The agricultural and environmental communities need to be aware of intentional contamination and available remediation methods. While there is abundant public geospatial information that could aid terrorists, it is not the greatest environmental vulnerability. “Human expertise” is a much more critical problem in the “more demanding attack planning part of the targeting problem” (Baker et al., 2004).

Toxins need not be “weaponized” to contaminate food. It was determined that the ricin in the October 15, 2003, letter was not in a weaponized form (CDC, 2003). The sophistication of these bioterrorists is in their subtle use of low-technology methods. Contamination of fresh fruits and vegetables with toxins would be an example of a sophisticated, low-technology terrorist attack. The food and feedstuffs supply chain is highly vulnerable to this type of attack from on-farm production through consumer sales. It is a standard practice to keep vegetables fresh in the supermarket with water rinses. If the water sources used to rinse fresh produce were contaminated by ricin, the consumer would be harmed without immediate knowledge. Our vulnerability to this form of covert biological attack against our food supply may be enhanced by the fact that the persons washing the fresh fruits and vegetables are generally low-paid employees, who might more easily be duped or persuaded to participate. The most common ricin toxicity events were mailed letters containing a “white powder.” About the same time as the September 11, 2001, attacks on the World Trade center in New York and the Pentagon in Washington, DC, there were ricin “attacks” in letters.

5.1.3 Modes of ricin dissemination

Biological and chemical threat agents associated with terrorism are directed not only toward vulnerable humans but also toward the environment that supports life. This chapter, however, focuses only on the threat to humans through our environment including food. More general and direct biological and chemical threats to humans are more thoroughly discussed in Kendall et al. (2008). The environment consists of three fundamental components—air, land, and water. As humans, we live in and interact with and depend on each of these environments for our health and well-being. Related to this chapter, biological and chemical threat agents in the environment were modeled and individual personal protection from threat agents is discussed in Kendall et al. (2008).

5.1.3.1 Soil

Ricin, as a natural protein, is a high-molecular-weight polymer like the synthetic polymers nylon 6–6 (poly adipic acid/hexamethylenediamine), polyacrylamide, and polyethylene glycol. Dissolved natural and synthetic

polymers are thought to nonspecifically sorb to surfaces via weak van der Waals bonds. The terms “sorb” or “sorption” are used here to indicate removal from solution without specifying a particular mechanism. Although each individual polymer bond is weak, the collective effect of multiple bond attachments can result in strong sorption. This is true especially for high-molecular-weight polymers. Polymer sorption is considered essentially irreversible in that most of the adsorbed polymer cannot be desorbed. Ricin has amino, carboxylic acid, and other functional side chain groups that cause the solubility to vary with pH. At a particular pH, a protein can be anionic, cationic, or have both anionic and cationic groups. Stryer (1975) noted that 20 different side chains commonly occur in proteins from the 20 essential amino acids. The isoelectric point of a protein is the pH where the molecule has a net charge of zero (i.e., equal number of anionic and cationic groups). The isoelectric point of ricin is 7.1 (Merck, 2001). At pH below the isoelectric point, ricin has a net positive charge while at pH above the isoelectric point, it has a net negative charge. A pH that maximizes the number of cationic groups is expected to maximize sorption to materials with a high cation exchange capacity (CEC). Similarly, a pH that maximizes the number of anionic groups would maximize sorption to a high anion exchange capacity (AEC) material.

5.1.3.2 Air

Some speculations were made that ricin could be released into the atmosphere causing injury to the general population. In that scenario, the key controlling factors are boundary layer depth and processes in the air. That scenario is improbable because vast quantities of ricin would be needed to likely produce a significant number of casualties. Of more concern is ricin-filled letters, in which the kinetic energy applied in opening mail can suspend microscopic ricin particles in the air. This can contaminate a small room or a person's face and inhaled air. There certainly have been many incidents in which ricin-laden letters have been sent through the mail.

Toxins, such as ricin, can move as very small particles in the air. Airborne ricin is a concern since inhaled ricin is ~1000 times more toxic than when orally ingested (Audi et al., 2005). Particle-tracking models provide the primary tools for predicting dispersion (Leggoe et al., 2008). To begin any useful effort in mitigating a terrorist incident, the modeling center must be promptly notified that an event has occurred and provided with an initial description of the source (Leggoe et al., 2008). Since ricin is often released in buildings, the volumes of contamination would be small. For ricin release, toxic exposure would mostly occur immediately after the initial release. Immediately after 9/11, aerial spray planes were grounded because there were concerns of aerial applications of biological terror agents.

5.1.3.3 *Water*

The introduction of ricin into surface waters and municipal water supplies poses several different problems. The nature of the problems posed will depend on the biological and physical properties of ricin and the nature of the water body. Water treatment processes would remove ricin, but small, treated water supplies and consumer products would remain vulnerable to ricin contamination. The large amount of ricin needed to effectively contaminate a large municipal water supply after treatment (e.g., water towers) would make that threat unlikely. The terrorism value of contaminating a municipal water supply might, however, remain attractive to terrorists even though the potential number of fatalities is minimal. The solubility of purified ricin in water is >3 mg/mL. The lethal oral ricin dose for a 70 kg person is 70–1400 mg (1–20 mg/kg body weight), which is 23–467 mL of a 3 mg ricin/mL solution. A 16 oz (473 mL) bottle of water could easily contain the maximum lethal oral dose of ricin for a 70 kg (154 lb) person. Only three 500 mg ricin pills made to look like vitamin tablets would be enough to exceed the maximum lethal oral dose. Because the ricin content is only 1%–5%, a greater weight of castor seeds is needed to get a lethal dose. Four castor seeds weigh about 1 g. Hence, 8 castor seeds with 5% ricin contain 100 mg of ricin, which is more than the minimum lethal dose for a 70 kg person. Only 112 castor seeds (28 g or 1 oz) with 5% ricin would be required for the 1400 mg maximum lethal dose for a 70 kg person. While the earth's surface is mostly water (78%), it is too saline to be potable. We depend on a small, vulnerable fraction of the earth's water (~3%) (Manahan, 2004) to sustain our lives. The environmental destruction caused by biological and chemical weapons would first endanger the surface water and might subsequently be transmitted to groundwater. The environmental effects of biological and chemical weapons would be catastrophic because we all depend on water. Not only would human life be compromised, but our plant and animal communities would also suffer.

5.1.3.4 *Clothing*

Ricin can contaminate clothing when it is applied as a liquid or when it is embedded within the fabric as fine particles of a solid powder. Greater amounts of ricin could be retained by clothing surfaces from ricin dissolved in water than from ricin powder. Ricin contact to the skin (except eyes and mucous membranes) is not toxic because ricin is too large to pass through the skin and oral ingestion of ricin from clothing is unlikely. Ricin toxicity from dried ricin on clothing, however, is a concern. Powdered ricin can be easily transferred to the eyes or airways after initial ricin contamination of clothing. Natural fibers, such as wool and cotton, are porous and are more amenable to ricin transfer than are synthetic fibers, such as nylon and polyester.

5.1.3.5 Food

There already have been limited biological attacks on fresh fruit and vegetables. The Rajneeshee cult contaminated salad bars in Oregon with *Salmonella typhimurium* to influence the outcome of an election (Torok et al., 1997). In 2003, there were arrests in England for the attempted contamination of military food supplies with ricin (BBC, 2003). These two examples demonstrate that intentional contamination of fresh fruit and vegetables can occur. The simplicity of contaminating food supplies is that it is a very “low-technology” threat.

Ricin need not be “weaponized” to contaminate food (Friedlander, 1997; U.S. Food and Drug Administration 2003). The letter containing ricin (dated approximately October 23, 2003) had not been weaponized (CDC, 2003). Terrorists can be clever by the subtle use of low-technology methods. Contamination of fresh fruits and vegetables by ricin (Franz and Jaax, 1997) would be an example of a sophisticated, low-technology terrorist attack. The food supply chain is vulnerable to this type of attack from farm production, transportation, and delivery all the way to consumer sales (BBC, 2003). It is a standard practice to keep vegetables fresh in the supermarket with water rinses. If the rinse water were contaminated with ricin, the consumer could be harmed without immediate knowledge. This type of biological attack on food security could easily occur because supermarket employees might contaminate the food or customers might surreptitiously contaminate the food as was done by the Rajneeshee cult.

5.1.4 Ricin sorption, movement, and degradation

Ricin is a water-soluble toxin that must be absorbed into the body to be toxic. Ricin is a large (>60 kDa), globular protein and is much too large for absorption through the skin. Dissolved ricin solutions can move through soils and ricin can adsorb to soil particles. Small ricin particles can move in air and be inhaled into human and animal airways and lungs. Heat, chemicals, ultraviolet radiation, and microbes can degrade ricin.

5.1.4.1 Ricin adsorption/retention by soil

Soil is a dynamic, natural three-phase system comprised of solids, liquids, and gas. The transport and fate of biological and chemical threat agents depend upon the state of the agent and how the agent reacts with the soil. Threat agents such as ricin can be applied as solids, can be mixed with the soil solids, or be suspended or dissolved in the aqueous phase surrounding the solids. Once ricin comes in contact with the soil, it may sorb to the soil, move with the water, or be degraded within the soil.

Several articles have reported that the clay component of soils and sediments effectively sorbs proteins. Sorption to natural soils and other

materials affects the migration and fate of toxins in the environment (Zartman et al., 2002). Soils contain inorganic minerals, organic matter, and microorganisms. These materials can sorb or degrade aqueous toxins. Soils contain a wide variety of materials with different sorptive properties and a wide range in particle size. Soil organic matter consists of a wide variety of materials that range from recalcitrant, highly altered materials, such as humus, to fresh plant material and animal remains. Humic acid, a component of humus, has been shown to sorb a variety of organic compounds, such as pesticides. Humic acid is the name (or misnomer) given to polydisperse biopolymers that occur in soil organic matter, sewage sludge, weathered lignite coal, and other organic materials.

The cation- and anion-exchange capacities of soils can strongly affect the sorption of many compounds. Temperate-region soils mostly contain minerals and materials with significant CECs. Most soils, however, contain minerals and materials with both a CEC and anion exchange capacity (AEC). Cationic compounds strongly adsorb to materials with a high CEC. Anionic compounds strongly adsorb to materials with a high AEC. Because like charges repel, small anionic compounds are not effectively sorbed by the negatively charged surfaces of high CEC materials and small cationic compounds are not effectively sorbed by high AEC materials. Large organic compounds, such as natural and synthetic polymers, sorb to a variety of materials. Proteins are natural polymers that can exist as cations, anions, or zwitterions depending on solution pH. The sorption of proteins and other charged polymers is affected both by charge and the polymeric character.

5.1.4.2 *Soil texture*

Soil texture is determined by the sand, silt, and clay content. Water movement into (infiltration) or through (permeability) a soil depends on textural class. Soil textural classes dominated by clay, such as sandy clay, clay, or silty clay, have low water infiltration rates and low permeabilities. Soil textural classes dominated by sand, such as sandy loam, loamy sand, or sand, have high water infiltration rates and high permeabilities. Silt textured soils have intermediate water infiltration rates and permeability. Sand, silt, and clay particles have different effects on the fate of biological and chemical threat agents in the environment. The sand (2–0.05 mm)—and silt (0.05–0.002 mm)—size fractions usually consist largely of quartz and feldspars. The clay (<0.002 mm) fraction usually consists mostly of silicate clay minerals and iron oxides. The clay-size minerals have a large specific surface area, which in part, explains the greater capacity of clays to adsorb a large variety of substances. The sand and silt fractions have low specific surface areas and are generally rather poor sorbents. Hence, soil texture has both physical and chemical effects on water and dissolved contaminants. A clayey soil both adsorbs contaminants and restricts surface water

movement into groundwater. Sandy soils neither readily adsorb contaminants nor restrict surface water movement into groundwater. Cationic chemicals readily sorb to negatively charged clay particles and inhibit cation movement within the soil. Sandy or silty soils, however, would allow biological and chemical threat agents to pass rapidly through the soil and contaminate shallow ground water (Zartman and Jaynes, 2014). Therefore, the soil texture may determine the environmental fate of biological and chemical threat agents. To sequester, sorb, and remove cationic materials, a clay-textured soil is advantageous. Sand- and silt-textured soils would facilitate the mobility of biological and chemical materials within the environment.

5.1.4.3 *Mineralogy*

The silicate clays are classified into 1:1 and 2:1 types based on crystal structure and chemistry. Silicate clays are phyllosilicate minerals with a sheet-like structure typical of the micas—muscovite and biotite. The silicate clays are classified according to the number of tetrahedral (SiO_4) silica units and octahedral (AlO_6 , MgO_6) alumina–magnesia units in the mineral structure. The tetrahedral and octahedral units are bonded together through shared oxygen or hydroxyl groups into a structural unit but are often described as if consisting of separate tetrahedral and octahedral layers. A 1:1 clay is a silicate clay with one silica tetrahedral layer and one alumina octahedral layer (Schulze, 1989). Kaolinite, also called “china clay,” is a 1:1 clay and an important industrial mineral used to produce porcelain and fine chinaware ceramics. Montmorillonite, vermiculite, and illite are examples of 2:1 silicate clays. Montmorillonite and vermiculite have high CECs and can expand the clay lattice by adding water or other substances between the individual clay layers. Montmorillonite is the principal mineral in most bentonites, which are mined worldwide and are extensively used commercial products. Montmorillonite is used as an excipient in pharmaceuticals, food products, animal feed, well-drilling fluids, metal casting, paints, pet litter, as well as many other uses. Illite (or clay mica) is a nonexpandable clay with an intermediate CEC. Kaolinite and illite are nonexpanding minerals. The “total” surface areas of smectites can be as high as $800 \text{ m}^2/\text{g}$. Minerals, such as quartz (SiO_2), have a fixed composition. In contrast, vermiculite and montmorillonite chemical compositions and CECs vary within limits for different samples. Most clays in temperate-region soils have significant CECs. Amorphous or noncrystalline clays, which are more common in tropical climates or in areas blanketed by volcanic deposits, have large surface areas and a pH-dependent charge (AEC at low pH, CEC at high pH). Iron oxide minerals, such as hematite, goethite, and ferrihydrite, also have a pH-dependent charge. Iron oxides occur in most soils but are more abundant in the soils of tropical regions.

The clay mineral, sepiolite, and sorbed collagen protein, and the extent of sorption were affected by solution pH and ionic strength (Perez-Castells et al., 1985). They reported that collagen is positively charged in acid solution, which increases electrostatic interactions between the negatively charged sepiolite clay surface and the positively charged protein. Ding and Henrichs (2002) concluded that electrostatic interactions dominated in protein adsorption to clay minerals such as goethite, illite, and montmorillonite. Jaynes et al. (2005) reported that several types of clay minerals common in soils were effective in binding ricin. They reported that ricin sorption was greatest at $\text{pH} \leq 7$ (i.e., below the ricin 7.1 isoelectric point), but much less ricin was sorbed at pH 10. At alkaline pH, ricin might sorb to positively charged minerals, such as hydrotalcites.

5.1.4.4 Organic matter

Organic matter in the soil also sorbs many ionic and nonionic chemicals. This is because the macromolecules that comprise organic matter contain both polar and nonpolar regions. These polar regions contain nitrogen, oxygen, and sulfur functional groups. On a weight-per-weight basis, organic matter has a greater CEC than inorganic clays. Organic matter, however, is less than 5% in most soils, and the more abundant inorganic clays can have a greater net sorptive capacity. The presence of organic matter within soils is specific to the soil depth and geographic region. Because organic matter is derived from plant material, it is generally greater near the soil surface than at greater depths. Soils in the hotter, drier areas (e.g., southwest United States) generally have very low soil organic matter contents. It is normal for soils in the southwestern United States to have less than 1% organic matter in the soil surface horizons. The soil organic matter percentages decrease significantly with depth. In contrast with these low organic matter levels, soils in the northeastern United States have higher soil organic matter levels. Soils in the northeast region have higher organic matter production since they have greater annual precipitation. Additionally, soils in the northeastern United States have lower mean annual temperatures, which results in less organic matter decomposition. The high organic matter production and the lower organic matter decay result in a greater net soil organic matter content.

5.1.4.5 Ion exchange capacity

As mentioned previously, there are many different kinds of clays; however, only phyllosilicate clays (1:1 and 2:1) and nonphyllosilicate materials will be discussed. Kaolinite is a low-CEC 1:1 clay that generally occurs in "old" highly weathered soils and is typical of the soil clay mineralogy that occurs in the southeastern United States. These soils typically also contain iron oxide minerals, a low CEC (2–10 centimoles per kilogram [cmol/kg] of material), and are not capable of sorbing large

quantities of ricin. While 1:1 silicate clays have low CECs at low soil pH (>5), these clays have an AEC. The AEC allows these clay minerals to sorb anions within the soil. The nonphyllosilicate iron oxide minerals, however, also have an AEC at acidic pH and are generally more effective anionic sorbents than 1:1 clays. The properties of 2:1 silicate clays vary depending on the CEC magnitude and location (tetrahedral or octahedral layer) of the charge. The total charge imparts a CEC (net negative charge) or AEC (net positive charge) to clays. Vermiculites are 2:1 silicate clays with a very large CEC and are not as expandable as smectites, such as montmorillonite and beidellite. The 2:1 clay minerals with most of the charge from tetrahedral layers (e.g., beidellite) are not as expandable as clays with most of the charge from the octahedral layer, such as montmorillonite. Soils that contain smectite clays have high CECs and have high shrink/swell capabilities. These soils expand when wet and shrink when dry. The high CECs and large surface areas of smectites contribute to the sorption of ricin as a cation and a polymer.

5.1.5 Adsorption/retention by building materials

Ricin sorption to some building materials can be similar to some soil minerals. Building materials, such as concrete, contain calcite (CaCO_3) that is typically present in soils in the southwestern United States. The sand and gravel aggregate used in concrete are like the sand and gravel fractions in soils. Fired brick is made using soil minerals, such as quartz, feldspars, and kaolinite, but the intense heat used in the firing process changes the mineralogy. Fired brick has lost most of the cation exchange and sorptive properties of the constituent soil materials used in manufacture. Bricks can be porous and might absorb dissolved ricin solutions. Wood is a common building material with sorptive properties that are controlled by the porosity and by the cellulose, hemicellulose, and lignin chemical constituents. Ricin sorption would be minimal to metal, glass, vinyl, ceramic tile, linoleum, and other nonporous materials used as building structural or exterior facing materials. Curtains and other fabric materials used in building interiors would have the greatest potential to sorb or retain ricin. In general, ricin sorption to building exterior surfaces should be minimal, but ricin sorption to interior surfaces and materials would be the greatest concern.

5.1.6 Adsorption/retention by clothing

For clothing, the type of fabric would control ricin sorption. The fibers of synthetic fabrics, such as nylon, consist of solid rods formed by extrusion of a polymer melt through a small orifice. Ricin sorption to synthetic fabrics is minimal (Zartman and Jaynes, 2014). In contrast, natural fibers

are hollow tubes with internal structures. Cotton fibers are cellulose seed hairs with primary and secondary cell walls and an interior lumen. Wool fibers are animal hairs with a complex interior structure formed of two helical proteins. Like other proteins, wool should have pH-dependent ion exchange properties.

5.1.7 Ricin adsorption/retention and food contamination

Food security issues have been an increasing homeland security and environmental concern. This concern is due to the simplicity of food supply contamination and that it is a very “low-technology” threat. Castor is easy to cultivate and is often used as an ornamental plant thereby making it accessible (Atlas, 1999). The grocery industry recognizes the food contamination problem can originate in the soil (Hennessy, 2000). In 2003, the U.S. Food and Drug Administration stated “the agency concludes that there is a high likelihood, over the course of a year, which a significant number of people will be affected by an act of food terrorism...” (U.S. Food and Drug Administration, 2003). The FDA Food Safety Modernization Act (FSMA) was signed into law on January 4, 2011, to further limit potential food contamination (U.S. Food and Drug Administration, 2011). Food safety was further emphasized by the Council for Agricultural Science and Technology, which stated that “recent outbreaks of food-related illnesses have increased many people’s concern about the safety of fresh fruits and vegetables...” (Brandenberger and McGlynn, 2003).

5.1.7.1 Surfaces

From studies on ricin sorption to fruits and vegetables, it is clear that fruit/vegetable surfaces influence ricin sorption. Vegetables with porous, non-waxy surfaces retain significantly more ricin than vegetables that have smooth, waxy surfaces (Zartman et al., 2003). Once sorbed, most of the ricin sorbed to the fruits and vegetables might persist through multiple aqueous rinses. After fruits and vegetables have been contaminated with ricin, it is not certain that aqueous washing will render it safe or acceptable for human consumption (Zartman et al., 2003). Although the residual ricin might not pose a significant health risk to the general population, babies and people with compromised health may be at a risk for ricin intoxication.

5.1.7.2 Temperature

Ricin is a protein that can be denatured with heat. Therefore, foods contaminated with ricin heated above 80°C should not be a problem. Fresh fruits or vegetables that are consumed cold or at room temperature would be problematic. While certainly not a food product, the meal remaining after castor oil is extracted from castor seeds can be used as an insecticide. From an industrial perspective, this meal is heated and

or chemically treated to render it nontoxic (Barnes et al., 2009). Typically castor meal is heated to 100°C to insure the ricin protein is denatured.

5.1.8 Ricin movement through soils

As mentioned in [Section 5.1.4.1](#), soil texture, mineralogy, and organic matter all influence ricin movement within soils. Ricin attacks most likely will be small in extent due to the relatively large amounts of ricin needed to cause illness or death. Ricin introduced in sandy soils, such as that which occur in Florida, would move rapidly with the soil water due to lack of ricin sorption sites. As the soil clay and organic matter increase, ricin is less readily transported. Even for soils with similar clay contents, the mineralogy would influence ricin movement. The kaolinitic clays of the southeast United States would allow greater movement and transmission of ricin than would the illitic clays of the Midwest or the montmorillonitic clays of the west (Zartman et al., 2008).

5.1.9 Ricin movement in dust

Ricin is more readily sorbed to the clay fraction of the soil than either the sand or silt fractions. Ricin powder can easily be transported long distances with the prevailing wind (Zartman et al., 2005). This was a concern with the US troop movement into Iraq. If the Iraqis had sufficient ricin to spread along the incursion path to Bagdad, the disabilities and death of coalition warfighters might have been horrific.

5.1.10 Degradation of ricin

Ricin is a dimeric cytotoxin that can be chemically or microbiologically degraded into nontoxic substances. For typical laboratory cleanup, sodium hypochlorite (Bleach) can be used to decompose or denature ricin (Mackinnon and Alderton, 2000). Alternatively, other oxidation agents, such as hydrogen peroxide, will decompose or denature ricin. Strong acids, such as nitric or sulfuric, are also capable of destroying ricin. de Oliviera et al. (2010) reported that microbiota in ruminates are able to degrade ricin, but ricin inhibits ruminant microbial growth. Ricin is deactivated irreversibly by heating. Heating above the critical temperature can denature ricin and eliminate the capacity of ricin to inhibit protein synthesis.

5.1.11 Modeling ricin transport

Ricin can exist as solid particles, sorbed to a solid, or dissolved in a liquid media. Ricin often occurs in a powdered form that could be disseminated into the air as an aerosol. While ricin is soluble in water and does

not exert a significant vapor pressure, in nonaqueous media, ricin would exist as a solid. Relative amounts of dissolved versus adsorbed ricin can vary dynamically with quantity added and sorbent material. Solid ricin particles can move independently of soil particles. Ricin adsorbed to soil particles can only move with the soil particle. Dissolved ricin can move by diffusion, convection, or by both modes.

The partitioning of ricin between the solid and liquid phases in soil can be written as

$$C = \rho_b C_a + \theta_v C_l \quad (5.1)$$

where

C is the total ricin content (M/L^3)

ρ_b is the soil bulk density (M/L^3)

C_a is the adsorbed ricin concentration expressed as mass of ricin per mass of dry soil (M/M)

θ_v is the volumetric water content (L^3/L^3)

C_l is the dissolved ricin concentration expressed as mass of ricin per volume of soil solution (M/L^3)

Note that fundamental units M (mass), L (length), and T (time) are used.

5.1.11.1 Diffusion

Diffusion is defined as movement by a gradient from an area of high concentration to an area of low concentration. The gradient is the difference in ricin concentration divided by the distance between the two concentration areas. Molecular diffusion for steady-state transport is generally written as

$$J = -D \frac{\partial C}{\partial z} \quad (5.2)$$

where

J is the solute flux density ($M/L^2/T$)

D is the molecular diffusion coefficient (L^2/T)

z is the distance (L)

For transient-state conditions, the conservation of mass equation in one-dimension without generation or consumption is

$$\frac{\partial C}{\partial t} = -\frac{\partial J}{\partial z} \quad (5.3)$$

where t is the time. Combining Equation 5.2 with Equation 5.3 gives the following:

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial z} \left(D \frac{\partial C}{\partial z} \right) \quad (5.4)$$

This equation presents change of ricin concentration with time and the molecular diffusion coefficient in the z direction. Ricin would not be expected to diffuse to any great extent, due to the small diffusion coefficient.

5.1.11.2 Convection

Convection is movement of a substance with a carrier, such as air or water. Ricin moves as a particle in the air or as a solute dissolved in soil water. The kinetic energy produced by opening a ricin-laden envelop would cause ricin particles to be ejected into the air. One-dimensional flow rate, J_z , in the z direction can be represented by

$$J_z = -q_z C_a \quad (5.5)$$

where

q_z is the air flow rate (L/T)

C_a is the ricin concentration expressed as the mass per volume of air (M/L³)

For ricin moved as a convective liquid, Equation 5.5 can be rewritten as

$$J_z = -v\theta_v C_l \quad (5.6)$$

where

v is the average water velocity (L/T)

θ_v is the volumetric water content (L³/L³)

C_l is the dissolved ricin concentration expressed as the mass of ricin per volume of soil solution (M/L³) in the z direction

If applied to the soil surface, the mathematical solution for the ricin solution would be as follows:

$$C(z, t) = \left[\frac{C_0 Z}{2\sqrt{\pi D t^3}} \right] \left[\exp \left\{ -\frac{(z - vt)^2}{4Dt} \right\} \right] \quad (5.7)$$

given the appropriate boundary conditions of Jury et al. (1991).

Application of the Equation 5.7 to real-world problems is very limited. This type of flow assumes sharp wetting fronts of solution passing through the soil as “piston flow,” which seldom, if ever, occurs in the environment. The diffusion portion of the diffusion–convection advances or retards the flow and precludes the sharp line of demarcation. Due to dispersion, preferential flow and fingering, solute flow is unstable and has diffuse flow boundaries (Hillel, 1998). Examples of ricin breakthrough curve data will be shown in the results [Section 5.2.2](#) later in this document.

5.2 Results and discussion

5.2.1 Ricin adsorption to soil clays and other natural materials

Montmorillonite, illite, and kaolinite are common clay minerals in temperate-region soils and ricin adsorption to montmorillonite was much greater than to illite or kaolinite ([Figure 5.2](#)). Montmorillonite is an expandable clay mineral with a much larger specific surface area (630 m²/g) and CEC than illite (20 m²/g) or kaolinite (11 m²/g) and these properties probably account for the greater ricin adsorption. Soils typically contain other minerals and materials, such as iron oxides, carbonates, and organic matter. The other soil materials adsorbed ricin ([Figure 5.3](#)), but not nearly as effectively as montmorillonite ([Figure 5.2](#)). Of the iron oxide minerals, the high surface area ferrihydrite (227 m²/g) more effectively adsorbed

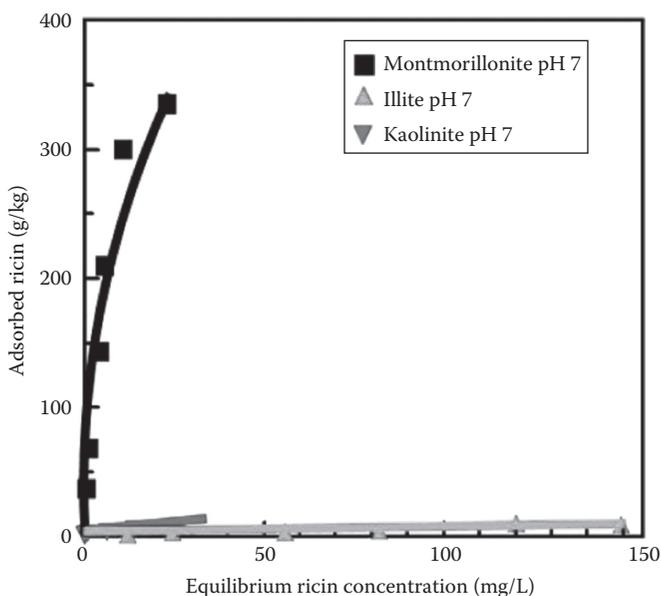


Figure 5.2 Ricin adsorption to common soil clay minerals at pH 7.

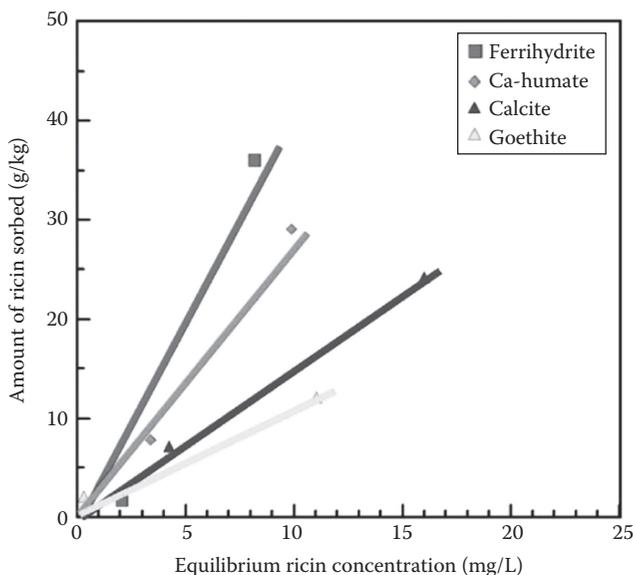


Figure 5.3 Ricin adsorption at neutral pH to nonsilicate soil materials: calcite (CaCO_3), ferrihydrite and goethite are iron oxides, and Ca-humate is soil organic matter or humus.

ricin than goethite ($31 \text{ m}^2/\text{g}$). Calcite, a carbonate mineral common in arid and semiarid region soils, also effectively adsorbed ricin. Calcium humate, a common type of soil organic matter, also effectively adsorbed ricin.

5.2.2 Ricin and peanut lectin adsorption and movement through soil

Ricin (mw = 65 kDa) and peanut lectin (PNA, mw = 110 kDa) are both globular plant proteins and galactose-binding lectins. Peanut lectin is similar to ricin and was used as a nontoxic simulant in experiments before ricin use. Ricin adsorption to montmorillonite, however, was much greater than peanut lectin (Figure 5.4). Peanut lectin is a larger molecule than ricin and greater adsorption might be expected. Differences in the amino acid composition between ricin and peanut lectin might explain the difference in adsorption. The ratio of basic amino acids (histidine, lysine, and arginine) to acidic amino acids (glutamate, aspartate) in ricin (1.02) is much greater than in peanut lectin (0.39). Basic amino acids are positively charged at neutral to acidic pH, which would favor ricin adsorption to a negatively charged high-CEC mineral like montmorillonite. Ricin and peanut lectin solutions at neutral pH were eluted through loamy fine sand and sandy

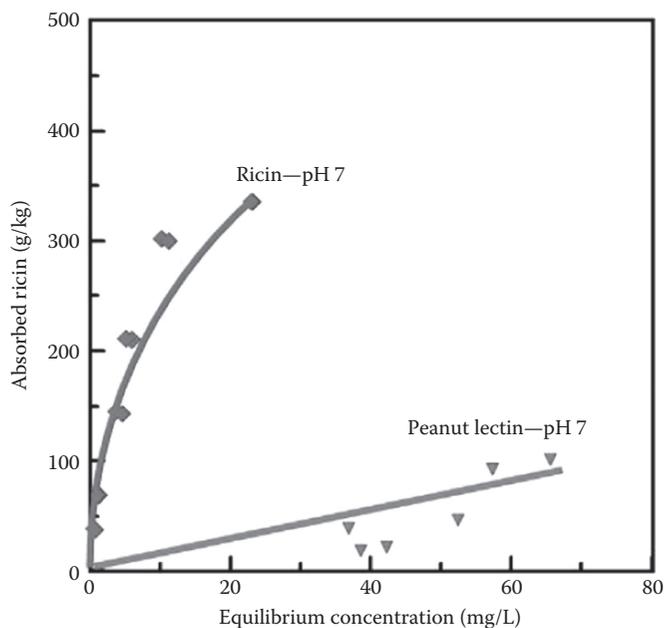


Figure 5.4 Relative adsorption of castor seed lectin (ricin) and peanut lectin (PNA) to montmorillonite at pH 7. Ricin (mw = 60 kDa) and peanut lectin (mw = 100 kDa) are both galactose-binding globular proteins.

clay loam soil columns (Figure 5.5). Soil column pore volume is the volume of all of the water-filled voids in a water-saturated soil column (Skaggs and Leij, 2002). Breakthrough is achieved when the initial (C_0) and eluted concentrations (C_e) of ricin and peanut lectin are equal (e.g., $C_e/C_0 = 1$). For the loamy fine sand columns, peanut lectin reached breakthrough in only 10 pore volumes, but 50 pore volumes were not quite enough ($C_e/C_0 = 0.9$) to achieve ricin breakthrough (Figure 5.5). The loamy fine sand (13% clay) soil column adsorbed more ricin than peanut lectin, which is consistent with the montmorillonite adsorption isotherms (Figure 5.4). For the sandy clay loam (28% clay) soil columns, peanut lectin achieved breakthrough in 55 pore volumes, but ricin breakthrough was not achieved ($C_e/C_0 = 0.1$) after 130 pore volumes. Even a relatively sandy soil, like loamy fine sand, can adsorb and limit the mobility of ricin.

5.2.3 Ricin adsorption to activated carbon and building materials

Ricin adsorption to decolorizing activated carbon (Norit-A) was 50 times less than to montmorillonite (Figure 5.2) and comparable to kaolinite (Figure 5.6). Ricin adsorption to powdered quartz, glass, and concrete

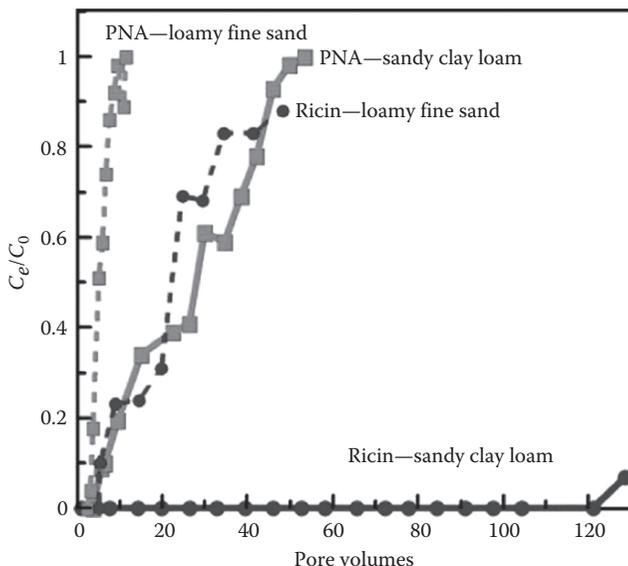


Figure 5.5 Breakthrough curves for elution of ricin and peanut lectin (PNA) through loamy fine sand (13% clay) and sandy clay loam (28% clay) soil columns. Ricin was strongly retained in the sandy clay loam soil column and breakthrough ($C_e/C_0 = 1$) was not achieved after 130 pore volumes.

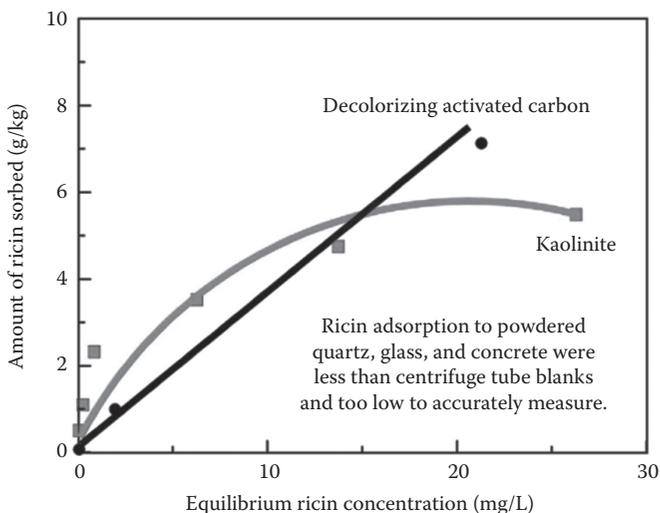


Figure 5.6 Adsorption of ricin to decolorizing activated carbon and kaolinite from neutral (pH ~7) solution. Ricin adsorption to decolorizing activated carbon and kaolinite were low and about 100 times less than montmorillonite. Ricin adsorption to quartz, glass, and concrete were even less than kaolinite.

was also measured, but the values were too low for accurate measurement. A blank correction was used because small amounts of ricin adsorbed to the centrifuge tubes used in the adsorption experiments. Equilibrium ricin concentrations in centrifuge tubes with added quartz, glass, and concrete powders were equal to or greater than that in centrifuge tube blanks. Building materials used in exterior surfaces should not retain ricin, but fabrics and other materials in building interiors might act differently.

5.2.4 Ricin adsorption kinetics to montmorillonite and effect of ionic strength

Timed ricin concentration measurements for ricin/montmorillonite suspensions were made (pH 7) in an attempt to measure the rate of ricin adsorption. After only 1 minute (Figure 5.7a), ricin adsorption was complete. Ricin adsorption was clearly too rapid for the experimental method and equipment used. The amount of adsorbed ricin slightly decreased between 1 and 240 minutes. This suggests that ricin is very rapidly adsorbed to montmorillonite and is later reorganized during equilibration with some ricin desorption. Cation exchange is a rapid process that might explain the rapid adsorption of ricin. Increasing the ionic strength from 0.02 to 0.5 (seawater ionic strength = 0.7) greatly reduced ricin adsorption to montmorillonite (Figure 5.7b). The decreased ricin adsorption with increased ionic strength also suggests that ion exchange is an important process in ricin adsorption. The greater Na^+ concentrations with increased ionic strength compete against ricin for cation exchange sites. It also suggests that high ionic strength solutions might be used to desorb adsorbed ricin.

5.2.5 Ricin adsorption to clays, pH, CEC, and Langmuir model fit

Ricin adsorption to montmorillonite was measured at different pH using buffer solutions to control pH. Ricin adsorption at pH 5 was greater than at pH 7, which in turn was greater than at pH 10 (Figure 5.8). The acidic pH 5 buffer solution should impart a net positive charge to ricin (isoelectric point pH 7.1), which could enhance adsorption to negatively charged montmorillonite particles. The ricin adsorption data were fitted to the Langmuir adsorption model, and monolayer ricin adsorption capacities (X_m) were calculated from the Langmuir fit parameters. The good fit of the adsorption data to the Langmuir adsorption model suggests that ricin adsorbs to a surface. Ricin adsorption to montmorillonite was five times greater at pH 5 than at pH 10 and three times greater at pH 7 than at pH 10. The pH 10 buffer solution should impart a net negative charge

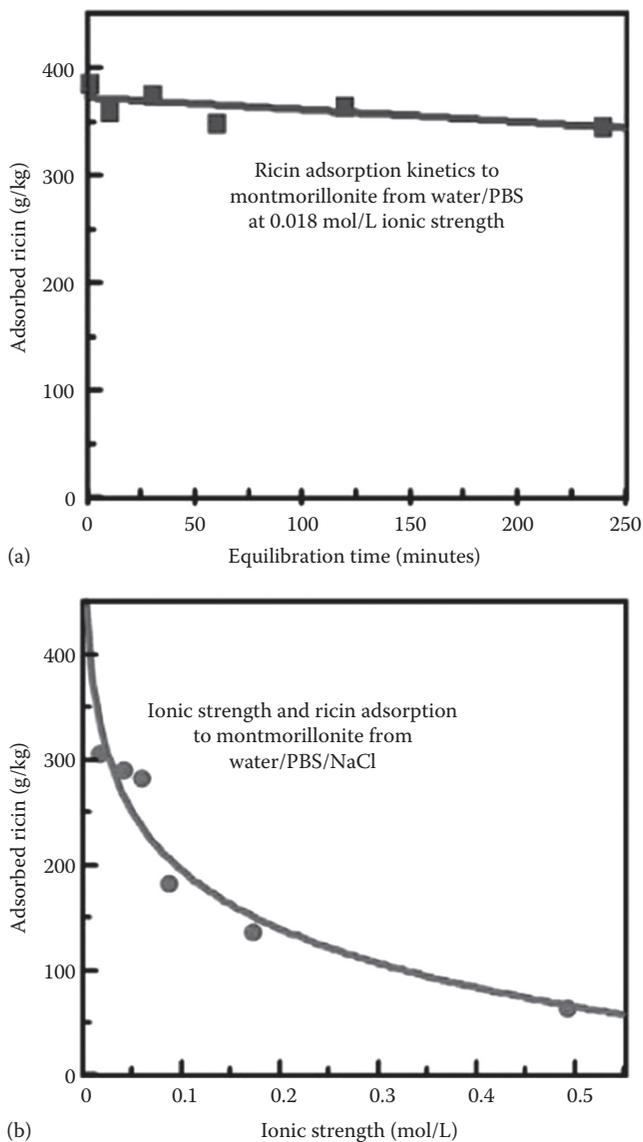


Figure 5.7 (a) Rate of ricin adsorption (kinetics) to montmorillonite and (b) effect of solution ionic strength on ricin adsorption.

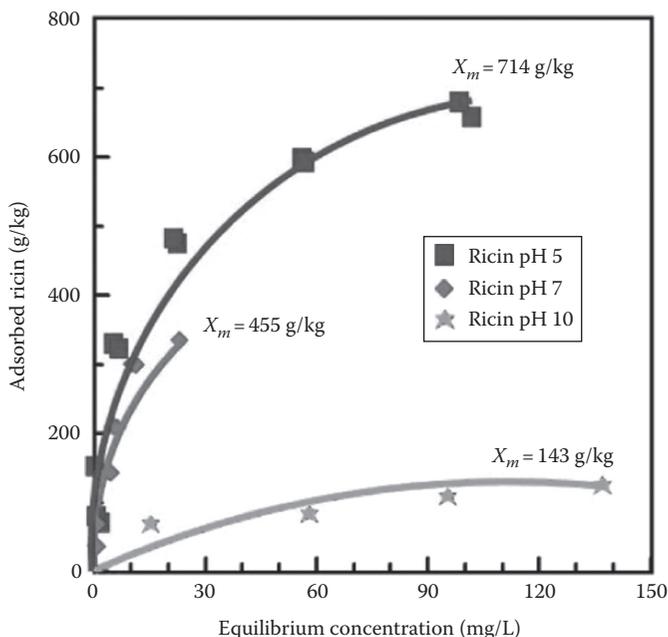


Figure 5.8 Ricin adsorption to montmorillonite at pH 5, 7, and 10. The adsorption data were fitted to the Langmuir adsorption model and monolayer (X_m) adsorption capacities were calculated.

to ricin, which could prevent or limit adsorption to negatively charged montmorillonite particles. Most (>80%) of the charge site on montmorillonite particles is on permanent negatively charged sites on interlayer surfaces, but montmorillonite particles also have pH-dependent exchange sites at the edges of particles. The relative amounts of ricin adsorbed at pH 5 and pH 10 suggest that ricin might adsorb to montmorillonite edge sites only at pH 10. Montmorillonite samples vary in CEC and the number of negatively charged sites. Clay Minerals Society sample SWy-2 (CEC = 87 cmol/kg) is a low-charge montmorillonite from Wyoming and sample SAz-1 (CEC = 130 cmol/kg) is a high-charge montmorillonite from Arizona. Ricin adsorption isotherms with pH 5 buffer were prepared for SWy-2 and SAz-1 (Figure 5.9). Despite the greater SAz-1 CEC, comparable amounts of ricin adsorbed to SWy-2 and SAz-1 and calculated Langmuir monolayer adsorption capacities were approximately the same. Based on montmorillonite, illite, and kaolinite CECs and the relative amounts of ricin adsorbed (Figure 5.2), CEC is an important factor in ricin adsorption. The 50% greater CEC of SAz-1 relative to SWy-2, however, did not increase ricin adsorption. Ricin molecules are large polymers and numerous relatively weak van der Waals bonds might contribute to ricin adsorption.

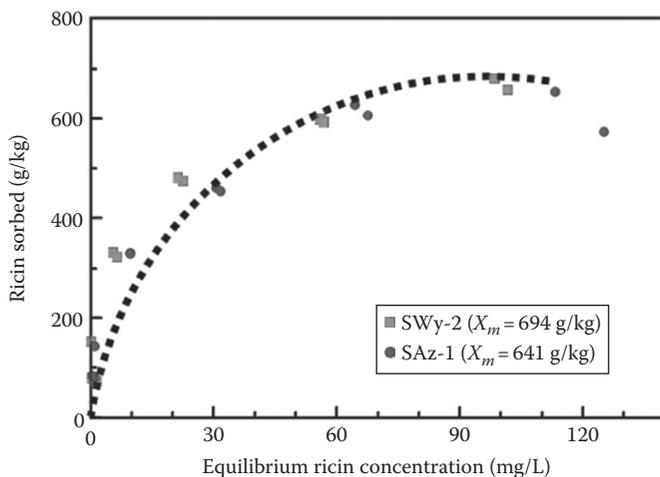


Figure 5.9 Ricin adsorption to low- (SWy-2) and high-charge (SAz-1) reference montmorillonite samples at pH 5. Monolayer ricin adsorption capacities (X_m) were calculated from a fit of adsorption data to the Langmuir adsorption model.

5.2.6 X-ray diffraction, ricin adsorption, and interlayer montmorillonite expansion

Montmorillonite samples were suspended in pH 4 buffer solutions with or without ricin and were dried as oriented films on glass slides. Drying clays on glass slides causes the clay platelets to lie flat on the glass surface. An oriented clay film makes it possible to measure interlayer expansion using x-ray diffraction. The montmorillonite with only pH 4 buffer yielded a 12 Å interlayer spacing characteristic of a K⁺-saturated montmorillonite (Figure 5.10). The K⁺ was derived from the potassium hydrogen phthalate in the pH 4 buffer. With both pH 4 buffer and ricin added, 34.6 and 23.1 Å spacings were produced that indicate interlayer expansion of montmorillonite. In contrast, montmorillonite treated with pH 10 buffer and ricin (not shown, Figure 6a, Jaynes et al., 2005) showed no expansion. No expansion at pH 10 indicates that ricin did not adsorb to montmorillonite interlayers, but some ricin (143 g/kg) did adsorb to montmorillonite at pH 10 (Figure 5.8). Significant ricin adsorption without interlayer expansion suggests that ricin must adsorb to montmorillonite edge sites at pH 10. The interlayer expansion of montmorillonite by water absorption is illustrated in Figure 5.11. An air-dried, K⁺-saturated montmorillonite sample yields a 12 Å interlayer spacing. When water is added to the K⁺-montmorillonite, the interlayers expand and physically separate. In a dilute montmorillonite suspension, the distance between interlayers

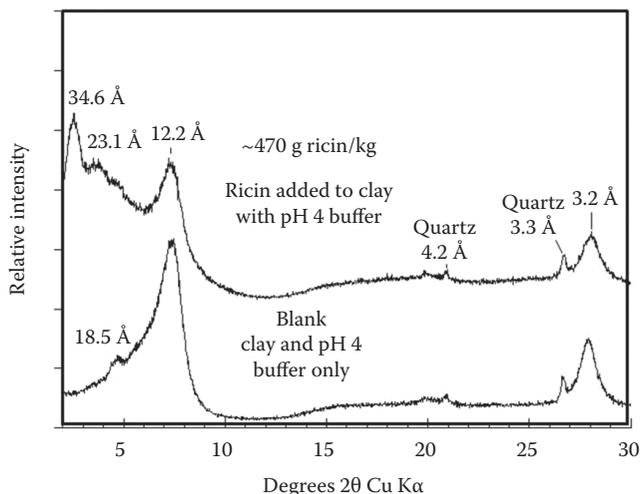


Figure 5.10 Ricin adsorption to montmorillonite from pH 4 buffer solution (potassium hydrogen phthalate) and interlayer expansion of montmorillonite.

can be indefinitely large. The large separation between montmorillonite layers allows large molecules, such as ricin, to move between layers and adsorb to interlayer surfaces.

5.2.7 Ricin adsorption and desorption from soil clay minerals

Adsorbed ricin might later be partially desorbed using wash solutions. Ricin was initially adsorbed to montmorillonite, illite, and kaolinite samples from pH 7 PBS solutions (Figure 5.12). The clay samples were then sequentially washed with PBS, pH 10 buffer, and once more with pH 10 buffer. Ricin concentrations in the wash solutions were measured to calculate the amounts of residual ricin still adsorbed to the clays. The PBS wash was not very effective in desorbing ricin, but the pH 10 washes desorbed greater amounts of ricin. Alkaline solutions, such as the pH 10 buffer, can effectively remove adsorbed ricin.

5.2.8 Ricin sorption/desorption from textiles, fruits, and vegetables

Ricin adsorption and desorption was examined for textiles (Figure 5.13). Ricin from pH 7 PBS solution was adsorbed to samples of wool, nylon, and cotton fabric. Initial ricin sorption by wool, cotton, and nylon were 4, 2.5, and 2.3 g/kg, which were about 100-fold less than montmorillonite.

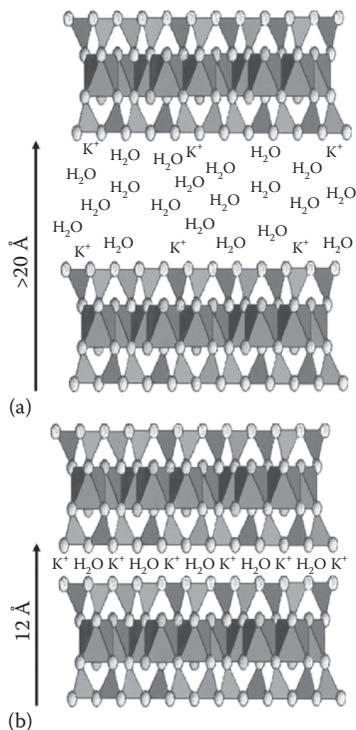


Figure 5.11 Illustration of K-montmorillonite interlayer expansion (a) suspended in water and (b) under air-dry conditions. The pH 4 buffer used in Figure 5.10 contained potassium hydrogen phthalate, which saturated the exchange sites with K^+ cations.

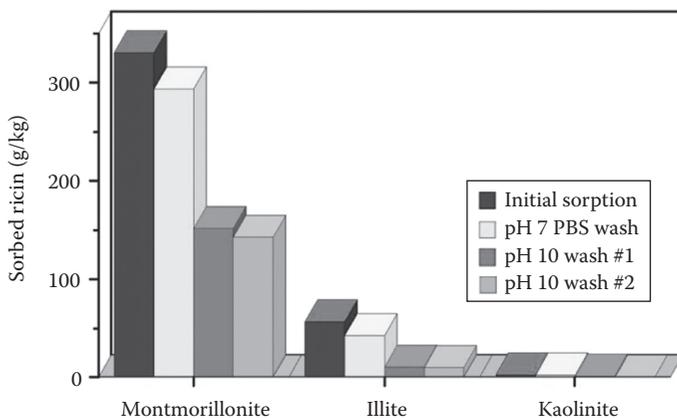


Figure 5.12 Ricin adsorption and desorption from common silicate clay minerals in soils.

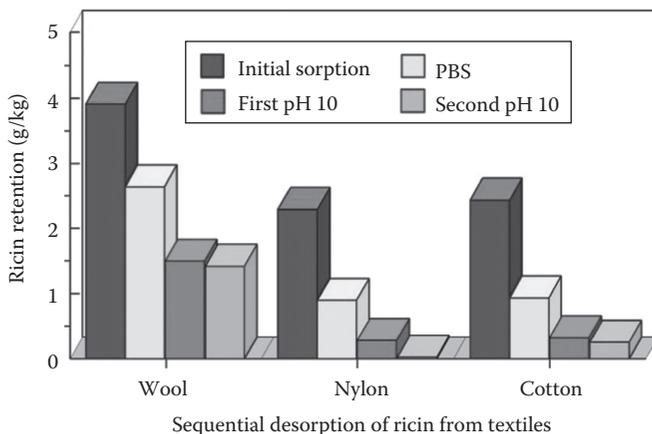


Figure 5.13 Ricin sorption to wool, nylon, and cotton textiles and sequential desorption with pH 7 phosphate buffered saline (PBS) and pH 10 buffer.

Ricin desorption by PBS was much less than desorption by pH 10 buffer. The use of an alkaline detergent in a washing machine might be as effective as pH 10 buffer in desorbing ricin. Wool is a protein and only about one-half of sorbed ricin was desorbed after one PBS and two pH 10 buffer washes. The wash treatments desorbed most of the ricin from cotton and nylon. Nearly all sorbed ricin was removed from the synthetic fabric, nylon, by the wash treatments. Ricin sorbed to the natural fabrics, wool and cotton, was more resistant to desorption. An Army Combat Uniform (ACU) was obtained and tested for the contact transfer of ricin from contaminated soil. The ACU fabric is a 50% cotton/50% nylon blend. Clothing is more prone to contamination at knee and elbow joints because significant pressure is usually applied to clothing in those areas and contact with contaminants is more likely. The greater applied pressure at knees and elbows can facilitate greater contaminant transfer from soils or other contacted surfaces. Checkai et al. (2011) developed a method to test the contact transfer of liquid chemical agents to clothing from contaminated soil. A similar procedure was used to measure contact transfer of aqueous ricin from contaminated soil to ACU fabric. A 1 kg weight was used to apply pressure to 93 mm diameter disks of ACU. The initial amount of ricin transferred to 93 mm ACU disks (Figure 5.14) was approximately 2 g/kg, which is comparable to cotton and nylon in Figure 5.13. A second pH 10 buffer wash likely would have reduced ACU-sorbed ricin in Figure 5.14 to levels intermediate between cotton and nylon (Figure 5.13). The alkaline detergent, greater water volume, and multiple water rinses used in a laundry washing machine would probably more effectively remove sorbed ricin than the pH 10 buffer washes.

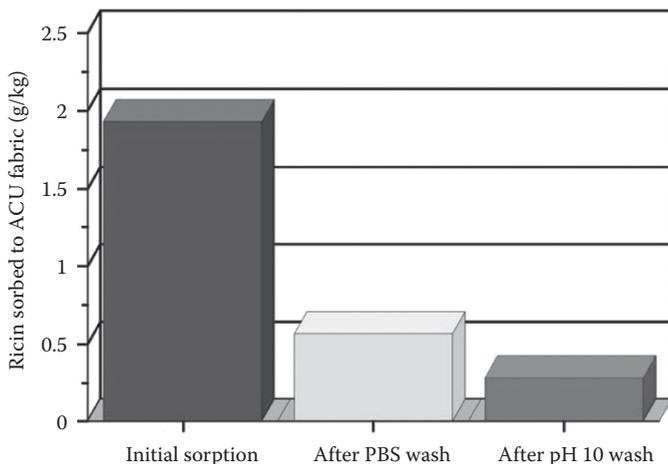


Figure 5.14 Contact transfer of ricin to Army Combat Uniform (ACU) textile from sand moistened with ricin solution. Desorption of ricin from ACU with pH 7 phosphate buffered saline solution (PBS) and pH 10 buffer.

Fruits and vegetables on grocery store shelves and salad bars might be intentionally contaminated with ricin. Grocery stores commonly use an aqueous spray to keep displayed fruits and vegetables fresh and prevent dehydration. The wash could be contaminated with ricin and adsorb to fruits and vegetables. Fruits and vegetables were dipped into a solution of ricin dissolved in pH 7 PBS and ricin amounts initially sorbed were measured (Figure 5.15). Broccoli, lettuce, carrots, and celery retained the most ricin. The more spherical fruits, blueberries, tomatoes, and grapes retained the least ricin. The amounts of ricin sorbed by fruits and vegetables (mg/kg units) were much less than fabrics and about 1000 times less than soil minerals (g/kg units). The fruits and vegetables were sequentially washed to desorb ricin with two PBS and one pH 10 buffer wash. The pH 7 PBS washes were not as effective in removing ricin as the pH 10 buffer. The effect of the different wash treatments suggest that washing with water (like PBS washes) would not be very effective and that washing with an alkaline detergent (like pH 10 buffer washes) would be needed to effectively remove ricin. The Figure 5.15 data indicate that fruits and vegetables contaminated by a grocery produce spray would not likely cause harm. Such an event could produce terror, but few, if any, casualties. A very large quantity of fruits and vegetables would have to be consumed to ingest a lethal oral dose of ricin. Washing fruits and vegetables before consumption would greatly reduce any risk.

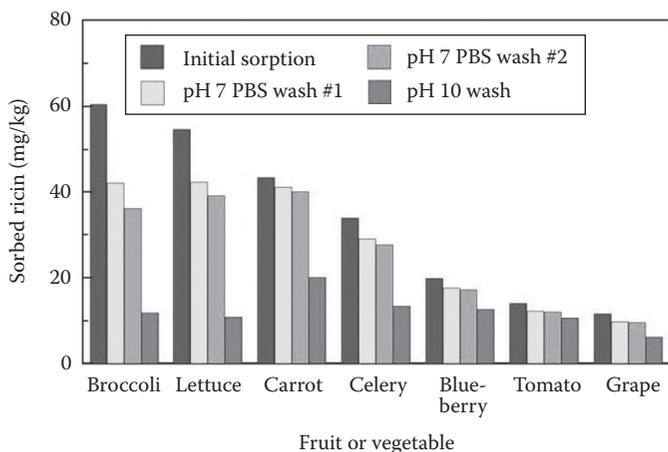


Figure 5.15 Ricin sorption and sequential desorption from fruits and vegetables. Note that the sorbed ricin units, mg/kg, are 1000 times less than the g/kg units used for clay minerals.

5.2.9 Ricin movement and concentration in soil dust and effect of surfactants

Ricin inhaled into the lungs is about 1000 times more toxic than orally ingested ricin. A dust generator/dust fraction collector assembly was built to examine the transport of ricin particles in soil dust (Figure 5.16a). The dust generator design was modified from the much larger USDA-ARS dust generator (Zobeck et al., 1997). Soil dust generated by wind or by passing vehicles at a ricin-contaminated site might carry respirable ricin particles to passing military personnel or civilians. The rotating dust

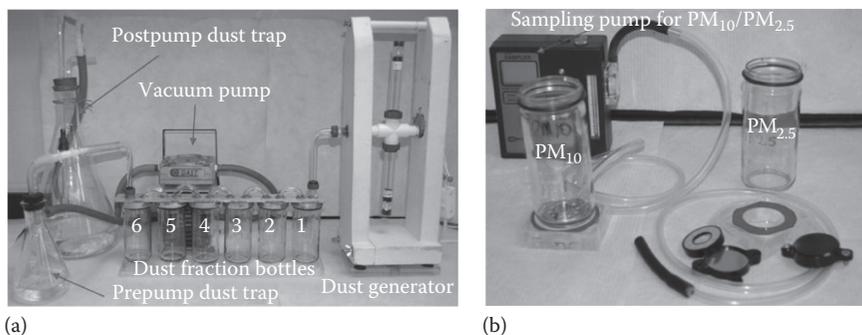


Figure 5.16 Dust generator/dust fraction collector assembly (a) with dust fraction particle size decreasing from bottle #1 to bottle #6. The PM₁₀ and PM_{2.5} dust samplers (b) were placed at the bottle #4 position in the dust fraction collector.

generator first suspends soil particles in the air and a vacuum pump then moves dust particles through the dust fraction collector bottles #1, #2, #3, #4, #5, and #6. The particle size of the dust fractions decreases from bottle #1 to bottle #6. The largest sand particles remain as residual in the dust generator. Water is added to the bottom of bottle #5 and #6 to prevent the smallest ricin/soil dust particles from escaping from the dust fraction collector. Past the dust fraction collector bottles, a prepump Erlenmeyer water dust trap and a postpump Erlenmeyer water dust trap were used to further ensure that no ricin particles enter the laboratory air. As a further safety measure, the dust generator/dust collector assembly was operated inside a fume hood. Very small dust particles of $<10\ \mu\text{m}$ (PM_{10}) or $<2.5\ \mu\text{m}$ ($\text{PM}_{2.5}$) diameter can be inhaled into the lung and are the most dangerous. Samplers for PM_{10} and $\text{PM}_{2.5}$ particles were placed at the bottle #4 position in the dust fraction collector to measure the actual size of the transported dust particles (Figure 5.16b). The flow rate of the PM_{10} and $\text{PM}_{2.5}$ sampling pump is one-fifth the flow rate of the dust generator/dust collector assembly. Hence, the PM_{10} and $\text{PM}_{2.5}$ samplers determine dust particle size and only collect about one-fifth of the quantity of moving dust. Dried ricin powder was mixed with samples of sand and clay soils (Figure 5.17), samples were placed in dust generator, and dust fractions collected. Most of the sand particles (residual) were too large to move out of the dust generator (Figure 5.17a). Most of the ricin was collected in trap1 and trap2. At a ricin-contaminated site, the ricin left in the coarser fractions (i.e., trap1, trap2) would repeatedly act as a ricin source that could be resuspended in air many times in

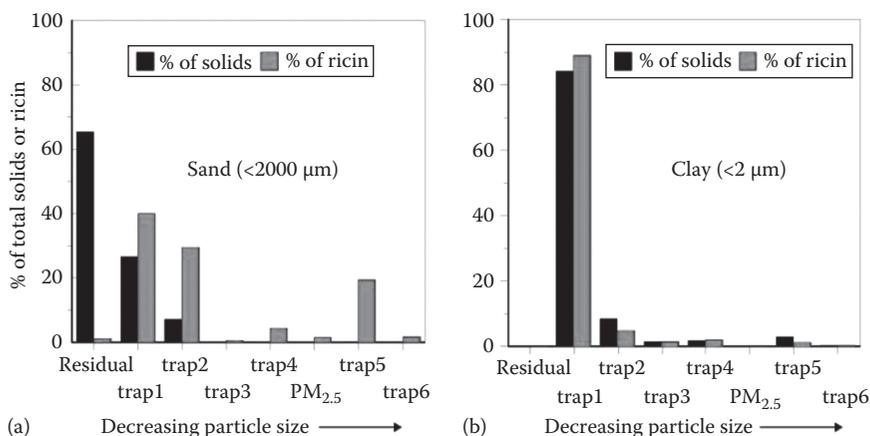


Figure 5.17 Movement and concentration of ricin in soil dust from (a) sand- and (b) clay-textured soil samples. Dust fractions were collected using a dust generator/fraction collector assembly. Ricin and soil particles collected in the $\text{PM}_{2.5}$ sampler were $<2.5\ \mu\text{m}$ in diameter.

subsequent wind gusts. Pure $<2.5 \mu\text{m}$ particles of ricin were collected in the $\text{PM}_{2.5}$ sampler and even more respirable-size ricin particles were collected in trap5 and trap6. Ricin mixed with a sandy soil can be moved and respirable ricin particles can be concentrated in soil dust. In contrast, ricin was much less effectively moved or concentrated in dust from the clay soil (Figure 5.17b). The primary particles in a clay are $<2 \mu\text{m}$, but most primary particles form into larger silt-size ($<50 \mu\text{m}$) aggregates. Clay particles contain adsorbed water that acts to aggregate the particles together. About 90% of the clay soil and ricin particles were collected in trap1 and trap2 and very little moved as respirable-size particles. Negligible amounts of soil and ricin were collected in the $\text{PM}_{2.5}$ sampler. The proportion of clay soil material to ricin collected in trap5 was greater than clay soil/ricin sample before operation of the dust generator/dust collector. Hence, the clay soil trap5 dust was less dangerous than the clay soil/ricin sample before operation of the dust generator. Dust from a sandy soil contaminated with ricin would be very hazardous, but dust from a clayey soil contaminated with ricin would be much less hazardous.

Surfactants are often applied to road surfaces to control dust generated at construction sites. Samples of the commercial surfactants, polyacrylamide (PAM), Haul Road Dust Control (HRDC), Chemloc 101 (Chemloc), and Soiltac were purchased and applied to samples of Brownfield loamy fine sand soil contaminated with aqueous ricin from castor seed extract. Brownfield loamy fine sand soil (85% sand, 2% silt, and 13% clay) samples treated with castor seed extract and the commercial surfactants were air-dried and used in the dust generator/dust fraction collector assembly (Figure 5.16). The total weight of fine ricin dust in trap3, trap4, trap5, and trap6 was measured for each treatment and graphed (Figure 5.18). The Brownfield soil sample treated only with aqueous castor seed extract yielded $\sim 0.85 \text{ mg}$ ricin dust. This is much less fine ricin dust than was generated from soils mixed with dry ricin powder. Adsorption of dissolved ricin to soil particles greatly limits dust formation. Hence, rainfall would greatly reduce ricin dust from a contaminated soil. The Brownfield soil sample treated with PAM and castor extract formed much less ricin dust than the sample treated only with castor extract. The HRDC, Chemloc, and Soiltac surfactants were less effective than PAM (Zartman et al., 2005).

5.2.10 *Rotifer use to measure ricin toxicity*

Marine rotifers (*Brachionus plicatilis*) are small aquatic animals that are often used in toxicology testing (Snell, 2005). In toxicology testing, toxins or poisons are added to the aqueous media of rotifers. Rotifer populations were grown and maintained in an aerated bucket maintained at a temperature of $\sim 25^\circ\text{C}$ under moderate lighting in simulated seawater

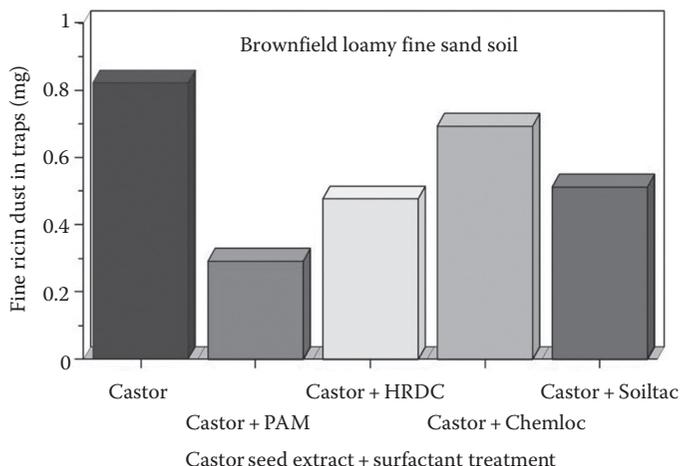


Figure 5.18 Dust control surfactant effects in limiting the generation of fine ricin dust particles and transport of ricin in soil dust.

(Hoff and Snell, 2008). Microalgae (*Nannochloropsis*) were used to feed the rotifers. Rotifers will consume particles of food or debris of $<5 \mu\text{m}$ in diameter. Carmine consists of small red particles that can be ingested by rotifers. Under a microscope, ingested red carmine particles are easily identified within the gut of healthy rotifers (Figure 5.19a). Only healthy, living rotifers can ingest carmine and carmine can be added near the end of experiments to distinguish living from dead rotifers. Simulated

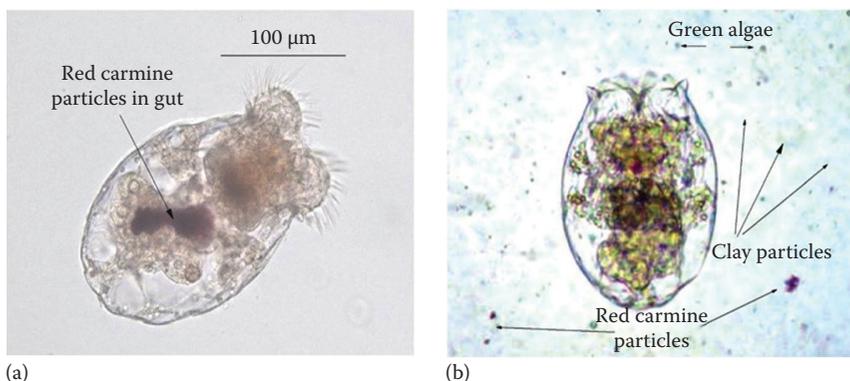


Figure 5.19 Marine rotifers (*Brachionus plicatilis*) used to examine ricin toxicity. Healthy rotifer (a) consumed red carmine particles. Montmorillonite addition (b) protected rotifer from lethal ricin dose.

seawater with and without ricin from castor seed extract was added to culture plate wells containing 10–100 rotifers. Preliminary experiments determined that an 18–24 hour incubation time at 25°C was needed to insure enough time for ricin toxicity to develop. Ricin from fresh castor seed extracts consistently killed rotifers, but ricin purified using dialysis and $(\text{NH}_4)_2\text{SO}_4$ precipitation was not consistently toxic. It is not clear why the purified ricin was not reliably toxic to rotifers. The ricin purification method might denature ricin to ricin toxoid. Ricin toxoid is a non-toxic or weakly toxic form of ricin that reacts with antibodies like ricin (Pradhan et al., 2008). Immunochemical measurement techniques, such as enzyme-linked immunosorbent assay (ELISA), cannot distinguish ricin from ricin toxoid. Alternatively, rotifers might need to ingest particles of castor seeds containing ricin to effectively absorb the toxin. In adsorption experiments, montmorillonite effectively adsorbs ricin and might be used to prevent toxicity to rotifers. Rotifers were added to culture plate wells containing simulated seawater and a lethal ricin concentration (~400 µg/mL) derived from a fresh castor seed extract. After an 18–24-hour incubation, 95%–100% of the rotifers were dead. In culture plate wells with 1 mg/mL montmorillonite in addition to the lethal ricin concentration, 95%–100% of rotifers were healthy and alive after 18–24 hours (Figure 5.19b). Red carmine, algae, and montmorillonite particles are visible within the rotifer gut and outside in the background (Figure 5.19b). Montmorillonite effectively adsorbed ricin and prevented toxicity to rotifers. This suggests that montmorillonite could be used as an antidote to oral ricin poisoning.

5.2.11 *Ricin and activated carbon*

Activated carbon is commonly recommended as an antidote to oral ricin poisoning, but it is a poor ricin sorbent based on adsorption isotherms (Figure 5.20). Activated carbon is very effective in removing nonpolar compounds, such as gasoline and pesticides, from water, but activated carbon has a problem with preloading (Manahan, 2004). In preloading, dissolved natural organic matter in wastewater can load up activated carbon surfaces and hinder pesticide sorption. Preloading can also be described as competitive adsorption where many different compounds compete for the same adsorption sites. Calcium humate (i.e., soil organic matter) is a polar material and a much better ricin sorbent than decolorizing activated carbon and coconut activated carbon was even less effective (Figure 5.20). Ricin and other proteins are polar compounds that do not readily adsorb to nonpolar activated carbon surfaces. Montmorillonite (or bentonite) is used as a food additive, and food-grade bentonite would be a much better antidote to oral ricin poisoning than activated carbon.

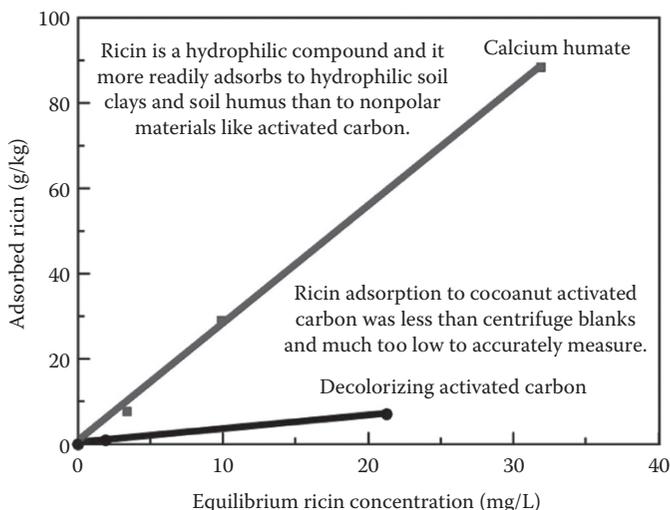


Figure 5.20 Activated carbon is a poor sorbent for ricin, but it is widely recommended as an antidote to oral ricin ingestion. Silicate clays, such as montmorillonite, and soil organic matter (e.g., calcium humate) are much more effective ricin sorbents than activated carbon.

5.3 Palliative measures to limit ricin contamination/toxicity

Ricin intoxication usually occurs through ingestion or inhalation. Conventional treatment for ricin ingested is to treat the patient with activated charcoal (Franz and Jaaks, 1997; Despott and Cachia, 2004; CDC, 2014). Since ricin is such a large polar molecule, we know that charcoal is not an effective way to treat ricin ingestion (USAMARIID, 2011). Our work (Figures 5.2, 5.3, and 5.20) has shown that expanding 2:1 clays such as montmorillonite would be much more effective than charcoal. Ricin inhalation is the most toxic form of exposure. Ricin is too large to pass through the skin and oral toxicity is 1000 times less than inhaled ricin. When it is suspected or known that ricin powder has been dispersed inside buildings, increasing the moisture content of the air to 100% humidity using a humidifier would be the most effective first step for response. Personnel in full protective gear could setup humidifiers and return after the building has been humidified with less restrictive protective clothing. Cooling the room would enhance condensation, but air drafts from an air conditioner might suspend ricin particles in the air. Air conditioners might be used after the room has been humidified. Ricin will absorb moisture from the air and increase condensation of water to contaminated surfaces. Condensed water on interior surfaces

would act to dissolve, aggregate, and attach ricin to surfaces. This would largely eliminate the risk of inhaling ricin dust. Potential ricin-contaminated surfaces could then be safely wiped with bleach solutions to remove any ricin.

Steps to safely decontaminate dispersed ricin powder:

1. Increase relative humidity or apply water to eliminate ricin dust.
2. After water application or humidification, wipe contaminated surfaces with a bleach solution.
3. A montmorillonite gel applied to wipes could adsorb ricin and mitigate toxicity.

5.4 *Conclusions*

Ricin sorbs to a variety of materials, but smectite minerals, such as montmorillonite, sorb the most ricin. Ricin sorption is pH dependent with the greatest sorption to soil clays in acidic solutions. Ricin adsorption isotherms to montmorillonite have a Langmuir form, which suggests that ricin adsorbs to surfaces and more is adsorbed to high-surface area materials. Ricin adsorption occurs rapidly to montmorillonite and high ionic strength suppresses adsorption. Ricin adsorption expands the interlayers and shifts the basal spacing of montmorillonite. Very little ricin sorbs to activated carbon, which suggests that montmorillonite rather than activated carbon should be recommended as an antidote to oral ricin ingestion. Small amounts of ricin are sorbed by fruits and vegetables, but the consumption of a large quantity of contaminated fruits and vegetables would be required for a toxic oral dose. Adsorption isotherms and breakthrough curves indicate that ricin sorption to clays greatly limits ricin movement in soils and the potential for groundwater or surface water contamination is low except for very sandy soils. Inhaled ricin is the most toxic form and wind generated dust in soils can produce respirable-size ($<10\ \mu\text{m}$, $<2.5\ \mu\text{m}$) ricin particles. Ricin in the dust generated from dry ricin powder mixed with soils can be greatly concentrated in respirable-size dust particles, particularly for sandy soils. Dust control agents applied to ricin-contaminated soils can significantly reduce the formation of respirable ricin particles.

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