CHAPTER 71

Rapid Decontamination of Chemical Warfare Agents

RICHARD K. GORDON AND EDWARD D. CLARKSON

I. INTRODUCTION

Chemical warfare agents are some of the most lethal and sinister substances manufactured (organophosphates) and the most psychologically threatening (vesicants). Organophosphate nerve agents include tabun (GA), sarin (GB), and soman (GD) and the low volatile compound VX, some of which were incorporated into munitions during World War II. Sarin was used by terrorists in the mid-1990s in Japan. The chemical nerve agents irreversibly inhibit the enzyme acetylcholinesterase (AChE) in both the central nervous system (CNS) and peripheral nervous system (PNS). When AChE is inhibited, it cannot hydrolyze the neurotransmitter acetylcholine (ACh). Excess endogenous ACh produces characteristic signs of nerve agent poisoning and cholinergic overload, such as hypersecretion and respiratory distress. When the nerve agent accesses the CNS, symptoms such as convulsions appear and lead to coma and death. In contrast, vesicants, such as sulfur mustard used in World War I and the Iran–Iraq War in the 1980s, are known not for lethality, but for the resulting burns and blisters that incapacitate military personnel. In contrast to nerve agents, where symptoms are observed in minutes, sulfur mustard presents clinical symptoms hours after exposure, although tissue damage by alkylation is rapid. Sulfur mustard alkylates peptides, proteins, and nucleic acids, and perturbs other cellular components. It is documented that to reduce the effects of chemical warfare agents, decontamination of the skin soon after exposure is the best post-medical countermeasure against chemical warfare agents. Thus, the decontamination product should be lightweight for incorporation into the limited space in a soldier's pack and easily used in the field under harsh and likely confusing conditions. Personal wipes, pads, and sponges have been developed to facilitate this goal. These products should exhibit long-term stability and be nontoxic and environmentally friendly. In addition to removing these toxic agents from skin, increased effectiveness can be obtained by detoxification of the chemical agents both on and in the skin and upon removal to protect the environment. Detoxification of the agent will also prevent secondary contamination of medical personnel or a soldier's buddy. The different approaches to solving field decontamination and detoxification are explored in this chapter. The importance of continued improvement of personal decontamination products cannot be underestimated when one considers today's constant threat of chemical warfare, terrorist acts, and pesticide spills.

II. THE NATURE OF HUMAN SKIN

Human skin, the largest human organ, developed as a physical barrier to the environment (to keep things out) but also maintains the aqueous nature of the human body (to keep things in). Mammalian skin consists of three major layers: stratum corneum, epidermis, and dermis. The stratum corneum, the thin outer layer of keratin-filled dead cells (corneocytes) bounded by densely crosslinked protein and embedded in crystalline lamellar lipids, represents the major barrier protecting the body from loss of internal components and entry of undesirable external materials. The layer underneath the stratum corneum, the epidermis, contains cells that differentiate from viable keratinocytes to corneocytes during their migration from the dermis to the stratum corneum. It also contains a large number of specialized dendritic cells. Smaller amounts of specialized cells are integral to the epidermis, including the pigmentation melanocytes, the immunological Langerhans cells, and the sensory Merkel cells. Throughout the epidermis sebaceous glands, sweat glands, and hair can be found. The next inward layer, the dermis, contains hair follicles with associated sebaceous glands, ecrine sweat glands and ducts, dendritic cells, and a vascular network including subepidermal capillaries, vascular plexi associated with the sweat glands, and dermal papillae associated with the hair follicles. Capillaries are responsible for transporting any chemicals that enter the skin systemically. Recent reviews of the skin structure and permeation are available for further reading (Menon, 2002; Hadgraft and Lane, 2005; Godin and Touitou, 2007; Wester and Maibach, 2000).

The stratum corneum, composed of keratinized dead cells that are continually being replaced, is the first major barrier to chemical agents. The barrier qualities of the stratum corneum depend on a number of factors, including its location on the body, which affects thickness, and how much hair is present. Thus, hair follicles and sweat glands can either provide channels through the stratum corneum, and thereby bypass its barrier attributes, or at least provide increased surface area for penetration of compounds, since a number of compounds were shown to penetrate faster in hair follicle-rich areas (Illel *et al.*, 1991). Maibach studied three radiolabeled pesticides – parathion, malathion, and carbaryl – for their permeability at 13 different anatomical sites in humans (Maibach *et al.*, 1971). Variations in percutaneous penetration were observed; higher penetration of the pesticides occurred at the abdomen and dorsum of the hand.

The lipid matrix is another feature important for barrier function in the epidermis. The arrangement of lamellar-like sheets yields a barrier to hydrophilic compounds and transcutaneous water transport. Extraction of those lipids from skin with organic solvents reduces barrier function (Hadgraft, 2001). The lamellae, which have few phospholipids as they are catabolized, ultimately contain mainly ceramides, cholesterol, and fatty acids (Wertz and Downing, 1989; Bouwstra and Ponec, 2006). The resulting matrix is composed of nonpolar compounds enriched in cholesterol that are adapted to protect from water loss. While extraction of these lipids may increase the penetration of aqueous moieties, in the case of organophosphates (note the organic-like nature of the chemical warfare agents, as described below), the hydrophobic nature of skin likely facilitates partition of these chemical agents through the lipid matrix, which then enter the subepidermal capillaries for dissemination throughout the body. Further insight into the barrier properties of skin can be observed in disease states including psoriasis, where there is an increase in epidermal cell replication yielding an irregularly stacked stratum corneum and abnormal capillaries in the dermis. This leads to an increase in drug penetration such as hydrocortisone (Kranz *et al.*, 1977). No studies have evaluated pesticide or chemical warfare agent penetration in psoriasis.

Aging contributes to decreased lipid barrier protection, decreased intercellular cohesion and increased absorption of toxic material. This barrier is also complicated by environmental effects such as exposure to sun, disease, and other aging processes that include many changes to the structure of the skin. Examples of such changes are decreased amounts of collagen, loss of melanocytes, decreased number of glands and hair follicles, reduced blood flow (Yates and Hiley, 1979), and the loss of lipid content in the stratum corneum (Elias and Ghadially, 2002). Another study found that 11 of 14 pesticides showed different rates of skin penetration in aged rats compared to young rats (Shah *et al.*, 1987). Generally, decreased absorption occurred in studies of aged skin (Fisher *et al.*, 1992; Farage *et al.*, 2007).

Percutaneous absorption *in vivo* leads to the occurrence of chemical or drug delivery to the microcirculation in the dermis. The period of time that it takes for entrance to the blood supply and circulation throughout the body depends on the diffusion parameters and the interaction with the lipid matrix (Roberts, 1997). Thus, chemicals exhibiting a longer lag time through the skin should be less toxic if quickly removed compared to rapidly penetrating compounds. Another aspect of percutaneous absorption is whether there are single or multiple exposures to the chemical. Some chemicals, such as azone (1-dodecylazacylohepan-2-one), alter the organization of the skin so that there is an increase of absorption or synergistic effect observed with each exposure (Ademola *et al.*, 1993). Chemicals that do not alter the skin's structure would not be likely to increase their bioavailability and absorption, but rather provide an additive response (Bucks *et al.*, 1985).

III. ORGANOPHOSPHATE NERVE AGENTS

Nerve agents are among the most toxic of the known chemical agents. Nerve agents are organophosphates (OPs) that bind irreversibly to acetylcholinesterase (AChE) (Taylor *et al.*, 1999), and to the bioscavenger butyrylcholinesterase (BChE) (Wolfe *et al.*, 1992) in both the peripheral and central nervous system. AChE is responsible for terminating the action of the neurotransmitter acetylcholine by hydrolysis. OP-inhibited AChE results in an excess of acetylcholine and the overstimulation of muscarinic and nicotinic receptors. Characteristic signs of nerve agent poisoning and cholinergic overload include hypersecretion and respiratory distress. When the nerve agent is transported past the blood–brain barrier, convulsions can lead to coma and death. OPs pose a hazard in both their vapor and liquid states. Notably, AChE inhibitors are used as both a therapy for treating glaucoma, myasthenia gravis, Alzheimer's disease or atropine poisoning and for more sinister reasons, e.g. as pesticides to kill insects and as chemical warfare agents by terrorists and in warfare to kill humans (Sidell, 1977; Leikin *et al.*, 2002; Martin and Lobert, 2003).

The G-nerve agents include GA (tabun, ethyl *N*,*N*-dimethyl-phosphoramidocyanidate), GB (sarin, isopropyl-methylphosphonofluoridate), GD (soman, 1,2,2-trimethylpropyl methylphosphonofluoridate), and VX (*o*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothiolate). The V-type nerve agents are several orders of magnitude less volatile than the G-type agents and act primarily as a liquid via the percutaneous route; for example, VX is several orders of magnitude more lethal percutaneously than sarin (Reutter, 1999).

Log P data (octanol:water partition coefficients and a reflection of lipid solubility) of nerve agents were used to both predict absorption through the skin and determine the distribution of OP compounds in tissues, and then correlated with toxicity as measured by the onset of fasciculation in

guinea pigs. An excellent correlation ($r = 0.95$) was established between the measured log P value and the rate of onset of local fasciculations, reflecting absorption in the skin and penetration to blood and dissemination to muscle tissue throughout the animal (Czerwinski *et al.*, 2006).

Maxwell and Lenz (1992) reported that, in general, AChE and BChE are more reactive with cationic nerve agents such as VX, while neutral agents that contain less than two bulky groups (e.g. GA, GB, and GD) were equally reactive with the cholinesterase enzymes. Since AChE has a smaller active site than BChE, the size and ionic character of the active sites determine the specificity of these esterases for the agents. The estimated ranked percutaneous nerve agent LD_{50}s are VX > GD > GA > GB, which reflect their volatility. The ranked volatility for these agents is VX < GA < GD < GB.

Using parathion as a model simulant for the nerve agent VX, the *in vitro* percutaneous absorption through unprotected human skin and clothed and uniformed skin was determined. The percent parathion dose absorbed through the unprotected skin was significantly greater than that observed through dry uniformed skin, while absorption was higher through the wet (sweat) uniform. These results suggested that military uniforms and public clothing provide protection to this stimulant and by analogy to VX; but absorption through cloth and skin quantitatively occurred more readily with wet clothing than dry. Thus, even with clothing, immediate responses and decontamination of skin and clothing are required (Wester *et al.*, 2000).

In conclusion, due to the extreme toxicity of nerve agents, the search for medical decontamination countermeasures to OPs is of paramount importance. Rapid removal from the skin would prevent penetration to the general circulation and the resulting decrements of cholinergic toxicity, which ultimately leads to seizure and/or death in untreated individuals. In the development of medical decontamination countermeasures to nerve agent poisoning, it is acknowledged that different nerve agent administration routes are likely to have different requirements for effective treatment. There is a limited window of opportunity for decontamination treatment following agent exposure. The signs of poisoning develop within minutes, and if decontamination is delayed, it is likely that toxic levels of the nerve agents will be disseminated via the blood stream after the agent has been absorbed. Decontamination will prevent continued absorption of the agent, reducing the need for further medical management (Table 71.1) (Hamilton *et al.*, 2004; Clarkson *et al.*, 2004).

TABLE 71.1. VX applied to pig skin (ear)

Decontamination	
Delay[a] (min)	**Signs**
0	–
15 (no decontamination)	+++
15 (decontamination)	+

[a]Delay in decontamination
Hamilton *et al.* (2004)

IV. VESICATING AGENTS (DISTILLED SULFUR MUSTARD, HD; IMPURE SULFUR MUSTARD, H; LEWISITE, L)

Sulfur mustard (HD), a synthetic vesicating agent, was a major chemical warfare agent during World War I and continues to be a modern-day threat (Reutter, 1999; Ghanei and Harandi, 2007; Bismuth *et al.*, 2004). Sulfur mustard's simple and cheap chemical synthesis makes it readily accessible to terrorists and use by the military. Sulfur mustard is an alkylating agent that causes its damage by disrupting nucleic acids and proteins, impairing cell homeostasis and eventually causing cell death, although the significance of the multiple pathways is unclear (Smith *et al.*, 1995). Whole-body exposure results in cutaneous (liquefaction necrosis of the epidermis), respiratory (injures the laryngeal and tracheobronchial mucosa), and ocular effects (severe conjunctivitis). In contrast to H agents, there is no delay with lewisite, which produces immediate burning of the skin and eyes. Compared with the G-nerve agents, sulfur mustard has a relatively low acute lethal toxicity, that is, its toxicity as an incapacitating agent is of much greater concern than its capacity to kill. Furthermore, mustard is persistent in the soil and other materials for hours to weeks (Devereaux *et al.*, 2002).

The skin is an important port of entry for vesicating agents. The agent's lipophilic nature, and the propensity of skin to exclude aqueous compounds but not lipophilic substances, make the skin an unwitting transport system. An increase in ambient temperature causes increased penetration (which was used effectively in World War I, where it was disseminated at night and warmed in the early morning sun). It has been estimated that 80% of liquid mustard evaporates, and 20% penetrates the skin. Of the 20%, 12% is retained in the skin matrix, while 8% is absorbed systemically, so only large dosages of mustard will produce significant systemic toxicities (Cullumbine, 1947; Dacre and Goldman, 1996).

Mustard skin lesions first present erythema followed by blisters (Somani and Babu, 1989). Erythema usually begins 2–24 h after contact, followed by acute itching, which diminishes as the characteristic blisters appear. Blisters initially appear 18 h after contamination as small vesicles within the area of erythema, which then coalesce to form the characteristic pendulous blisters containing large volumes of clear but yellow fluid. Blisters are not painful *per se* but they may be uncomfortable and may feel tense. Warm, moist areas such as genitalia and axilla are more likely to exhibit bullous lesions. By 48 h post-exposure, blistering is clearly evident and a new round of blisters appears. Due to the disruption of the skin layer, the large blisters break leading to erosions and full thickness skin loss and

ulceration, necrosis, and 72 h post-exposure, formation of an eschar. The eschar sloughs in a 4–6 day time period, finally leaving a pigmented scar (Reid *et al.*, 2000, 2007). The burn caused by blister agents is much slower to heal in comparison with a thermal burn, likely due to the multiple mechanisms by which the agent affects biological tissue, as known from World War I and reestablished in Iranian casualties. The site of healed mustard burns is hypersensitive to mechanical trauma. In a comparison of cutaneous lesions in 500 mustard-exposed Iranian veterans and 500 unexposed veterans, a correlation was observed between exposure and skin lesions such as severe dry skin, hyper- and hypopigmentation, local hair loss, eczema, and chronic urticaria. Histopathological examination of skin biopsies has revealed nonspecific findings including epidermal atrophy, keratosis, and basal membrane hyperpigmentation (Balali-Mood and Hefanzi, 2006).

V. MODEL SYSTEMS TO MEASURE ABSORPTION, REMOVAL, AND DECONTAMINATION

A. Rats

There are many different animal models that have been used to assess the percutaneous absorption of toxic chemicals. There is little question that while *in vivo* human studies are best for predicting the absorption of percutaneous applied chemical warfare agents, ethics preclude conducting such studies. Rats have been widely used in the study of skin contamination, wounds, and healing and the efficacy of different decontamination modalities (Wester and Maibach, 2000; Shah *et al.*, 1987; Baynes *et al.*, 1997).

B. Guinea Pigs

While rats are often selected for their availability, low cost, small size, and thorough biological characterization, they are not the ideal chemical warfare agent model because they contain a high amount of carboxylesterase, a potential hydrolytic enzyme for OPs (Sweeney and Maxwell, 2003). Unlike rats, humans have small amounts of this enzyme relative to acetylcholinesterase and butyrylcholinesterase. To overcome this limitation, the guinea pig, which exhibits low carboxylesterase, has been developed as a model for chemical warfare agent exposure (Fonnum *et al.*, 1985). Guinea pigs have been evaluated for skin damage due to burns, often used as a wound healing model for sulfur mustard (Ramos *et al.*, 2008), and for skin irritation to toxic industrial chemicals (Kennedy, 2007; Weaver *et al.*, 2003). Guinea pigs have also been used to study absorption of chemical warfare agents through the skin (Dalton *et al.*, 2006; Wormser *et al.*, 2002), uptake of radioactive sulfur mustard through guinea pig skin (Logan *et al.*, 1999), as animal models for pretreatment regimens to protect against chemical warfare agents (Wetherell *et al.*, 2006), and as a model of cholinesterase activity assessments (Haigh *et al.*, 2005) to GD exposure and OP-induced seizure (Harrison *et al.*, 2004).

For evaluating the decontamination of guinea pig skin, typically, sedated and shaved guinea pigs were cutaneously exposed to neat soman on their sides. One minute after exposure, a sponge wrapped around a pair of forceps was moved across the guinea pig's side; then the forceps were rotated 180 degrees, so that the clean surface of the sponge was pointed at the animal. Three more passes were taken from the rear towards the front. An identical procedure was used when the protocol required an additional second sponge to decontaminate the animal. Similarly, guinea pigs were used for decontamination of sulfur mustard. In this case, 24 h post-neat HD exposure and decontamination, animals were injected with trypan blue and then euthanized. The skin covering the backs of the animals was removed. In addition, skin punches were taken from each of the exposure sites (control, exposed, and decontaminated sites) (Gordon *et al.*, 1999; Gordon and Doctor, 2003).

C. Swine

Pig skin has long been a valuable model for human skin (Meyer *et al.*, 1978; Riviere and Monteiro-Riviere, 1991) since pig skin expresses a sparse hair covering, epidermis, and a similar arrangement of dermal collagen and elastic fibers to human skin. Again, many investigations have used the porcine skin model to study cutaneous toxicology of sulfur mustard (Gold *et al.*, 1994). Pig skin, because of its similarity to human skin with respect to hair covering, apocrine sweat glands, and other morphological similarities (Reifenrath *et al.*, 1991), is an attractive model for cutaneous absorption and toxicology studies of OP nerve agents (Hamilton *et al.*, 2004). Cutaneous absorption studies show that pig skin permeability, compared to rat and rabbit, most closely resembles that of human skin (Bartek *et al.*, 1972) with a variety of test agents. Therefore the pig represents a good model to assess the effects of introduction of extraneous material or chemicals on the early events of exposure. The downside is that pigs are large animals, difficult to house, are more costly, and require special cages to maintain them in comparison to rodents.

VI. DECONTAMINATION REQUIREMENTS

Medical decontamination executes removal and/or neutralization of chemical warfare agents, which, upon penetration of the skin, produces vesication, or for OPs, penetrates to the systemic circulation and inactivates ChEs. The most important process for the exposed soldier or civilian is to remove the chemical agent from the skin as quickly as possible. The soldier, under harsh conditions, must use the

product quickly to minimize transdermal penetration. A decontaminant that inactivates the chemical agent prevents its penetration through the skin and potentially protects a medical worker or buddy from suffering a second hand exposure.

Other criteria for the decontaminating system and reagents are that they are as universal as possible and protect against the various classes of chemical agents (as well as radiochemicals and biological agents, although the latter compounds will not be discussed). In other words, the soldier has a limited amount of space and weight to carry, and cannot carry multiple decontamination schemes. Furthermore, it is unlikely that a soldier would be able to determine the type of agent with which he is contaminated in the absence of symptoms.

In addition, proven efficacy of a decontamination product would have to meet Federal Drug Administration (FDA) guidelines and approval, assuring safety of the product for the soldier. The product should be environmentally safe to use by itself and render the chemical/biological agent environmentally safe, to prevent cross-contamination. Logistics would preclude decontamination products that require freezing or refrigeration, since they would not be available in the field. Lastly, the product needs to be simple and easy to use by the soldier under stressful conditions. Complicated decontamination procedures are unacceptable, since they increase the probability of failure during the stressful decontamination procedure. Products like the M291 kit (see below) are used by simply wiping the contaminated skin, and do not require significant training. However, the M291 kit has some drawbacks, such as a black offensive dust that is precluded from the eye. Thus, a decontaminating product should not have offensive odor (as some potential mercapto compounds exhibit) (Shi *et al.*, 2008) and be nonirritating and nonallergenic, or the product will be hesitantly used.

Methods for decontamination, neutralization, and removal of chemicals, such as OP and organosulfur compounds, herbicides and insecticides, are known in the literature (Hurst, 1977; Houston and Hendrickson, 2005; Rosenberg, 2005; Baker, 2004). The compositions and devices utilized for medical purposes are markedly different from nonmedical devices; the latter are not compatible with the skin or other sensitive tissues, having undesirable properties such as corrosiveness, flammability, toxicity, difficulty in making and storing, two component system composition, and limited shelf-life. For example, DS2, a standard decontamination agent, is comprised of 70% diethylenetriamine, 28% ethylene glycol monomethyl ether, and 2% NaOH by weight (Modec, 2003). Although DS2 is effective, it is corrosive upon exposure to air. DS2 and any matter resulting from its use are classified and regulated as hazardous material. After an application, the DS2 must stand for 30 min before rinsing the treated area with water. Additionally, DS2 comprises a teratogen. Clearly, this is not a better method for neutralizing, detoxifying, decontaminating, and cleaning personnel exposed to chemical warfare compounds.

VII. DECONTAMINATION SCHEMES

OP nerve agents are a serious threat to military and civilian personnel. Another serious problem that may be encountered while caring for personnel contaminated with OP nerve agents is the possibility that there will be cross-contamination to the medical personnel treating affected personnel. During combat or terrorist acts, individuals might be exposed to chemical toxins before they don their protective gear. Several schemes, each exhibiting their own advantages and disadvantages as medical decontaminants, are described below. While the ideal candidate does not exist, the final product must be fieldable for the individual – that is, for personal use in a rapid, deployable manner. Simple materials such as bleach and complex products such as OP-degrading immobilized enzymes sponges are described. Inexpensive and readily available household materials were tested for pesticide decontamination of fabric materials over 20 years ago (Easter and De Jonge, 1985). The household products provided only marginal decontamination efficacy.

A. Classical Liquid–Sodium Hypochlorite (Bleach)

Decontamination methods employing hypochlorite formulations have some corrosive and toxic side effects. A Chlorox® (hypochlorite) solution is composed of household bleach, which is about 5% sodium hypochlorite. Thus, for a 0.5% solution, bleach is mixed with 9 parts water, although even this diluted solution is contraindicated for use in or on a number of anatomical areas including the eye.

Undiluted bleach is not used because it is toxic to the skin and may create more damage than no decontaminant. Hairless guinea pigs were exposed to sulfur mustard in wounds and the surrounding intact skin, and then decontaminated with water, 0.5 or 2.5% bleach. No significant differences were observed among wounds decontaminated with the three solutions. Unexpectedly, the skin surrounding nondecontaminated (but exposed) control animals showed the least visual pathology. The lesions observed after decontamination might be due to the mechanical flushing of sulfur mustard onto the perilesional skin, by chemical damage of the skin induced by the solution, enhanced penetration of the agent, or interaction of sulfur mustard with the decontaminating solutions (Gold *et al.*, 1994). In a study evaluating decontamination of GB from skin, it was observed that rabbits receiving GB had no convulsions or deaths without decontamination. In contrast, when decontaminated with 5% bleach, there were increased symptoms and death, suggesting that 5% bleach perturbed the protective barrier of the skin or facilitated GB transport through the skin (Kondritzer *et al.*, 1959). Since diluted bleach

(0.5%) is a nonirritant to human skin, it is preferred (Racioppi *et al.*, 1994).

In contrast, the effectiveness of diluted bleach has been demonstrated. This study measured the rate of sulfur mustard disappearance from the skin after topical application of the vesicant, which rapidly penetrates the skin due to its hydrophobicity. Three swabbing treatments of undiluted HD-exposed skin with gauze pads soaked in 0.5% hypochlorite caused 68% reduction in skin HD content and a 64% reduction when hypochlorite was replaced by water (Wormser *et al.*, 2002). The effectiveness of 0.5% hypochlorite with water for decontaminating sulfur mustard on guinea pigs was also evaluated. However, the gauze pads soaked with the bleach contained microgram quantities of HD when water was used but no detectable HD levels when 0.5% bleach was used. Thus, the neutralizing effect of 0.5% bleach occurred after the agent was removed from the skin and away from the lipophilic structures of the skin where the 0.5% hypochlorite could react with and reduce levels of the agent.

Similar to bleach's oxidative iodine properties, topical povidone–iodine at 15 and 30 min post-exposure to sulfur mustard exhibited protective effects. Severity of the dermal parameters, acute inflammation and dermal necrosis was significantly reduced, and reduced skin damage was observed in areas adjacent to treated sites (Brodsky and Wormser, 2007).

B. Powder Decontamination Material: M291 Skin Decontamination Kit

The current individual product provided to the US soldier for use in the field is the M291 personal skin decontamination kit (Figure 71.1). There are three main components incorporated into individual pouches: a fiber pad (six to a pouch), an absorbent activated charcoal, and a reactive resin (Ambergard XE-555). Each component serves a unique purpose. First, the cotton pad provides structural integrity for use on a finger. Second, the carbon incorporated into the pad absorbs organic material such as OPs and HD. Third, the ion-exchange resin binds chemical agents and very slowly detoxifies them. The soldier takes the M291 pad and rubs the area that needs to be decontaminated. The goal is to rapidly bind chemical agents still on the surface of the skin and prevent their penetration through the stratum corneum. The M291 kit is precluded from use in the eye because its particulate nature is irritating; but can otherwise be used on the face and around wounds. Much of the black powder from the M291 kit remains on the skin, and has been a deterrent to its use. However, the black powder provides the soldier with an indication that the material still remains and should be brushed off for maximum effectiveness.

Efficacy of the M291 kit has been evaluated in a number of animal models for OP poisoning (DO49 Technical Report, 1987). In an early report, rabbit skin was shaved (to mimic human skin without fur) and then the skin exposed to

FIGURE 71.1. M291 kit (www.defenselink.mil).

GD and VX for 2 min. Decontamination with the M291 kit yielded higher LD_{50}s (of more than ten- and 20-fold, respectively) (Hobson *et al.*, 1985), in comparison to control (not decontaminated) animals. In another study, with rabbits under similar conditions, the penetration of the organophosphate VX was measured by red blood cell AChE inhibition. The M291 kit increased the amount of VX required to inhibit AChE by 50% (Joiner *et al.*, 1988). Thus, the M291 kit neutralized and/or removed VX so that less penetrated through the skin to be systemically delivered as indicated by RBC AChE inhibition. In another animal model, the guinea pig, decontamination with the M291 kit after 1 min of neat exposure to GD increased the LD_{50} from 9.9 to 17.7 mg/kg, yielding a protective ratio of 1.8. Some of the differences in protection can be related to the animal model (rabbit in comparison to the guinea pig as described above) and likely methods of decontamination (number of wipes with the M291 pad). The M291 kit is also efficacious in rabbits against the vesicating agents HD and L. Shaved dorsal skin of rabbits was exposed to neat HD and then decontaminated after 1 min. Vesicant-induced histopathology determined after tissue staining was reduced over 20-fold compared to nondecontaminated control rabbits.

C. Liquid Decontamination Material – Sandia Foam

Sandia National Laboratories decontaminating foam (licensed to Modec Inc., Denver, Colorado) is a solution (MDF-100) composed of two parts: part a: a solution of 6.6% *N*,*N*,*N*,*N*′′,*N*′-penta-methyl,-*N*′-tallow alkyl 1,3-propanamine diammonium; 2.6% tallow pentamethyl propane quaternary ammonium compounds; benzyl-C12-18 alkyl dimethyl; 1% isopropyl alcohol, and part b: a solution of 8% hydrogen peroxide. Mixing the two parts results in a foam-like product which lasts for up to 30 min (Figure 71.2).

The mixture was tested in the guinea pig model, where fur was shaved on the side of the animal one day before exposing the skin of the anesthetized animals to neat GD or VX (Lukey *et al.*, 2004). The animals were decontaminated 1 min later with sterile gauze soaked in the combined solution in a defined manner: the contaminated side was wiped across the exposure site in the direction of the shaved fur once and then rotated so that a clean surface of the gauze could be used to wipe the skin for three additional passes. Next, the OP-exposed area was similarly dried with a second piece of gauze. The exposed area was wiped a total of eight times. Twenty-four hours later, survival was determined. Control non-decontaminated animals yielded an LD_{50} of 11.3 mg/kg for the OP GD, while animals decontaminated with the mixture yielded an LD_{50} of 400 mg/kg, a 35-fold protective ratio. For the OP VX, cutaneous neat exposure and decontamination with Sandia foam yielded an LD_{50} of 10.1 mg/kg compared to the control animals' LD_{50} of 0.14 mg/kg, a 72-fold protective ratio.

FIGURE 71.2. Sandia foam.

Despite its efficacy, Sandia foam has a number of drawbacks for field use by the soldier. First, it must be stored as separate components, which would require a rapid, personal, on-site mixing chamber for combining the two solutions. Second, the presence of hydrogen peroxide, a strong oxidizing agent, precludes its use near the eye, and would create much discomfort if used in a wound (Watt *et al.*, 2004). To partially address these concerns, Sandia developed the formulation DF-200, which in part contains less hydrogen peroxide and surfactant.

D. Liquid Decontamination Material – Diphotérine®

Diphotérine is a product for chemical spatters on the eye and skin. Prevor Laboratory in France manufactures this odorless, colorless liquid dispensed as an eye wash or skin decontamination spray. It is composed of an aqueous solution to wash many chemical families and pull hydrophilic chemical agents away from the surface of tissues, an amphoteric solution that acts on acids and bases and restores the tissue physiological pH, and a hypertonic solution that stops penetration of corrosive chemicals into tissues. The pH is slightly alkaline (pH 7.2–7.7) and is sterile. Although not classified as such in the USA, it is classified as a medical device in Europe, Canada, Australia, and Brazil (www.prevor.com).

Diphotérine's action on more than 600 chemical compounds was reviewed (Hall *et al.*, 2002). These chemicals included acids, alkalis, oxidizing and reducing agents, irritants, lacrimators, solvents, alkylating agents, and radionuclides. In the literature, there is one abstract describing the decontamination of sulfur mustard (Gerasimo *et al.*, 2000). In this report, radiolabeled sulfur mustard was placed on human skin for 5 min *in vitro*. The skin was then treated with Diphotérine, water and soap, or saline at different time periods after sulfur mustard exposure and Diphotérine was reported to be significantly better at removing sulfur mustard. No reports could be found in the literature for organophosphate decontamination, although Hall *et al.* (2002) also state that Diphotérine is suitable for decontamination of OP pesticides.

The bulk of evaluation of Diphotérine has occurred in the European workplace. There, it has been reported not to be irritating to normal human eyes or skin, and is essentially

nontoxic in guinea pigs and does not sensitize their skin (Mathieu *et al.*, 2007). The product has prevented or decreased severity of chemical eye/skin burns with 96% sulfuric acid, 100% acrylic acid, 50% acrylamide, solid sodium hydroxide flakes, and dimethylethylamine; no eye/skin burns developed and there was no necessity for further medical or surgical burn treatment in a German metallurgy facility where 24 workers were exposed to weak or strong acids and bases and obtained immediate Diphotérine decontamination (Nehles *et al.*, 2006). Clearly, Diphotérine's potential is intriguing and needs to be critically evaluated for decontamination and detoxification of chemical warfare agents.

E. Liquid and Sponges: Reactive Skin Decontamination Lotion

Reactive skin decontamination lotion (RSDL) was developed for cutaneous decontamination of chemical warfare agents after exposure to a chemical warfare agent (Figure 71.3). It was developed for topical use by the Defense Research Establishment in Suffield, Canada, with broad spectrum decontamination properties for chemical agent cutaneous threats. The RSDL solution is composed of 1.25 M potassium 2,3-butanedione monoximate in polyethylene glycol monoethyl ethers of average molecular weight 550 daltons ($MPEG_{550}$) with 10% w/w water (pH 10.6). The pads consist of a sponge-like plastic foam, Opcell®, which is lightweight and easy to store. It is used instead of a cotton pad, and the Opcell holds more of the decontaminating solution for spreading on the skin.

The toxicological profile of this formulation was determined prior to FDA (Food and Drug Administration) clearance by the US military (Tonucci *et al.*, 2004). The Army tested the product's safety by conducting skin irritation, sensitization, and photoirritation studies in more than 300 people. It also tested its effectiveness by treating animals that had been exposed to chemical agents. On March 28, 2003, the product was approved by the FDA to remove or neutralize chemical warfare agents and T-2 fungal toxin from the skin (but not other biological threat agents or radiological contaminants).

The efficacy of reactive skin decontamination lotion has been demonstrated. The high efficacy of this lotion in inactivating OPs was measured in rats. In addition, primary cultures of chick embryo neurons were used to test the efficacy of the RSDL. By relating the anticholinesterase activity in these cultures of the OP/RSDL mixture to that of pure OP standards, a sensitive measure of the value of the RSD in inactivating tabun, sarin, soman, and VX was obtained. Experiments with all four nerve agents in this *in vitro* system provided a good correlation with the *in vivo* data, and also indicated that the inactivation process was time and agent dependent and also related to the ratio of OP to RSDL. RSDL is also effective in decontaminating GD and VX cutaneously in exposed guinea pigs (Table 2, Gordon, unpublished observations).

FIGURE 71.3. RSDL reactive skin decontamination lotion (www.nbcdefence.net).

The product was also compared to Fuller's earth in a pig model. The potency of the RSDL/sponge was statistically better than Fuller's earth against skin injury induced by sulfur mustard, observed 3 days post-exposure. RSDL was more efficient than Fuller's earth in reducing the formation of perinuclear vacuoles and inflammation processes in the epidermis and dermis. The potencies of the RSDL/sponge and Fuller's earth were similar to severe inhibition of plasma cholinesterases induced by VX poisoning. Both systems completely prevented cholinesterase inhibition, which indirectly indicates a prevention of toxic absorption through the skin (Taysse *et al.*, 2007).

However, there are some caveats for the use of RSDL. The application of RSDL directly to open wounds impaired wound strength and decreased collagen content in the early phases of wound healing. This may have clinical implications for the treatment and outcomes of chemical casualty combat trauma (Walters *et al.*, 2007). RSDL is also reported to be flammable.

F. Polyurethane Sponge

At the Walter Reed Army Institute of Research, an enzyme immobilized polyurethane foam sponge to decontaminate the skin of chemical warfare agents in a wide variety of

environmental conditions is being developed (Munnecke, 1979; Wood *et al*., 1982; Havens and Rase, 1993) (Figure 71.4). A porous polyurethane foam formed *in situ* from water-miscible hydrophilic urethane prepolymers has been combined with enzymes such as ChEs, producing immobilized enzyme sponges (Gordon *et al.*, 1999; Gordon and Doctor, 2003; Ember, 1997; Medlin, 1998). In this method, the enzyme becomes an integral part of the solid support. Some of the advantages of this technique include retention of similar kinetic characteristics as the soluble form of the enzyme. Most important, the immobilized enzyme retains high activity after prolonged storage, and it is resistant to the detrimental effects of low and high temperatures and long exposure to the environment. In addition, the enzymes are covalently attached to the polyurethane so they will not leach from this polymer support.

In order to increase the OP/enzyme stoichiometry, polyurethane immobilized enzymes were combined with oximes (enzyme reactivators such as HI-6; Peter *et al.*, 2007) so that the catalytic activity of OP-inhibited AChE (or BChE) is rapidly and continuously restored before irreversible aging of the enzyme–OP complex can occur. The OP is continuously detoxified. Thus, a reusable immobilized enzyme sponge of ChEs and oximes for OP decontamination is the envisioned product. Next, it was demonstrated that the OPs diisopropyl fluorophosphate (DPF) or 7-(methyl-ethoxyphosphinyloxy)-1-methylquinolinium iodide (MEPQ) inhibited the activity of ChE sponges, as was observed for non-immobilized ChE in solution. The oxime HI-6 restored activity of the AChE sponge until the molar concentration of MEPQ reached approximately 1,000 times that of the cholinesterase active site. However, the AChE sponge could be recycled many times by rinsing the sponge with HI-6 in the absence of OP. In this case, most of the original ChE activity could then be restored to the sponge. Therefore, the bioscavenger approach can be used externally: the sponge would soak up OP decontaminating the OP contaminated skin (Caranto *et al.*, 1994). Then the ChE sponge and oxime would detoxify the OP in the sponge. We have found that the ability of the immobilized enzymes and HI-6 to detoxify the OP MEPQ was dependent upon the efficiency of the sponge in decontaminating particular surfaces including plastic or guinea pig skin.

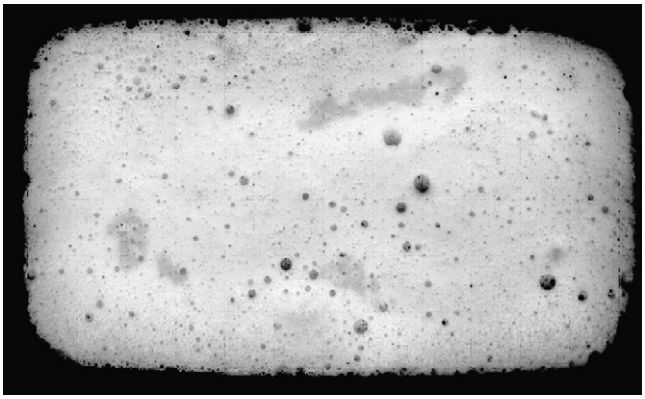

FIGURE 71.4. Polyurethane sponge.

Characteristics of polyurethane immobilized enzymes are as follows. The longevity of sponges composed of immobilized ChEs is greater than 5 years at room temperature (not shown). The immobilized enzymes are also very stable in aqueous environments. One significant difference and advantage the immobilized enzymes have compared to the soluble ChEs is that immobilized enzymes do not dissociate (leach) from the sponge. Therefore, the immobilized enzymes can be left in the liquid or other environments. For instance, the AChE activity in the immobilized sponge was stable for more than 60 days in continuous immersion in aqueous samples including Allegheny River fresh water or brackish water (Gordon *et al.*, 2002). Since the results were identical for autoclaved and untreated water, the immobilized enzymes were also resistant to microbial-induced proteolytic degradation. Also note that the same sponge was assayed multiple times over many days, so it is evident that the immobilization process confers dramatic stability to covalently coupled ChEs.

OP removal by the sponge and formulation is as follows. The capacity of sponges to remove GD or other OPs from guinea pig skin was determined using a back-titration method, where removed OP in the sponge was added to a known amount of ChE. Inhibition of the exogenously added ChE permits quantitation of the OP concentration. From these studies, it was determined that additional components when added to the sponge improved the sponge's efficacy by leaching out the chemical warfare agents. In this case, we settled on tetraglyme, which has a propensity to dissolve organic-like materials, including OPs and sulfur mustard (see below). In part, the inability of sponge–tetraglyme to remove all the GD likely reflects the rapid penetration of GD through skin and that tetraglyme cannot extract this fraction. It also points to the requirement for as rapid decontamination as possible. These results clearly demonstrate that the sponge not only removed OP from the skin of the guinea pigs, but in the presence of oxime effectively and completely detoxified the OPs within hours. Thus, these sponges would not pose any additional cross-contamination hazard.

The protective ratios of the sponge are as follows. It proved to be impossible to modify the prepolymer since currently there is no formulation with an increased hydrophobic nature that might be expected to absorb the OP more effectively. Instead, we utilized additives described above to provide the additional ability to remove soman from the skin, protecting guinea pigs significantly better than the M291 kit (Table 71.2). A comparison with RSDL is also shown. Compared to LD_{50} values of 9.9 and 17.7 mg/kg for untreated animals (not decontaminated) and the M291 kit, respectively, the sponge provided an LD_{50} of 290. This combination is also effective against VX contaminated guinea pigs: the sponge increased the LD_{50} from 0.14 mg/kg to 21, yielding a protective ratio of 150. RSDL provided a protective ratio of 137.

TABLE 71.2. Decontamination of OP-exposed guinea pigs

OP/treatment	LD_{50} (mg/kg)	PR
Soman		
M291 kit	17.7	1.8
RSDL	240	24
Sponge	290	29
None	9.9	n/a
VX		
M291 kit	0.14	n/a
RSDL	19.1	137
Sponge	21.0	150
None	0.14	n/a

PR – protective ratio

Sulfur mustard decontamination and formulation are as follows. The sponge was used to wipe guinea pig skin contaminated with neat sulfur mustard. The following day, the animals were injected with trypan blue. Those areas representing vesicant injury take up the dye. It was observed that the neat HD-exposed tissue (positive control) has a significant dye uptake, while the area decontaminated by the sponge has only a slight uptake of the dye. The control has no dye uptake. In addition, the amount of HD taken up and removed by the sponge was measured over time. While neat mustard remained after 15 min in water, the corresponding amounts are destroyed in the matrix of the sponge and additives. Histopathology of the HD-exposed skin specimens after 24 h demonstrated microvesicles, coagulation at the dermal interface, and in the most severe cases, dermal coagulation. Overall, sponge decontamination of the HD-exposed area exhibited characteristics associated with reduced exposure (microvesicles). Thus, these sponges could reduce the damage that HD produced. ChEs may provide sinks as alkylation sites for the sulfur mustard, and account for some of the reduced toxicity of the agent upon sponge decontamination. Another feature demonstrated was that the tetraglyme leaches from skin not only OPs, but also the organic-like sulfur mustard, thereby reducing its ability to alkylate skin proteins. Finally, the formulation of the sponge was modified to include nucleophilic additives to act as a reactive moiety for the sulfur mustard in place of skin proteins. Taken together, the polyurethane sponge was shown to decontaminate and detoxify guinea pig skin exposed to two classes of chemical warfare agents: OPs such as GD and alkylating compounds such as HD.

G. Immobilized Enzyme Badges

The sponge can incorporate a detection system for OPs and alkylating agents – a unique attribute not present in current decontamination methods (Gordon *et al.*, 2002). The immobilized enzymes provide a detector and a rapid field system capable of identifying the type of OP. In addition to OP detection, a coupled enzyme reaction provides a rapid colorimetric or electrochemical indication of mustard. With the constant threat of chemical warfare or terrorist acts, the development of alternative means of protecting and decontaminating individuals from exposure to CWA agents is critical.

Like Diphotérine, which has been evaluated only for sulfur mustard decontamination, the polyurethane enzyme immobilized sponge is a novel technology that has demonstrated efficacy for OP and sulfur mustard cutaneous poisoning, but is only now undergoing evaluation for decontamination of biological warfare agents (T-2 mycotoxin, botulinum toxin) and radionucleotides (Gordon *et al.*, 2006). Another advantage of ChEs over general reacting additives is that the ChEs are the direct target for current or future warfare agents, and therefore should not require major reformulation. These new technologies likely will provide more efficacious solutions for the soldier in the future.

VIII. CONCLUDING REMARKS AND FUTURE DIRECTION

OP nerve agents are a serious threat to military and civilian personnel. These agents are some of the most potent toxic agents and are specific inhibitors of ChEs. OP nerve agents can exist as a vapor and be inhaled; as a liquid they can contaminate skin, or can be ingested if food or water is contaminated. Vesicating agents such as sulfur mustard cause irreversible cell damage as a result of rapid alkylation, and were an agent of terror during World War I and more recently in Iran and Iraq. Another serious problem that may be encountered while caring for personnel contaminated with chemical warfare agents is the possibility that there will be cross-contamination to the medical personnel. In addition, during combat or terrorist acts, individuals might be exposed to chemical toxins before they don their protective gear. Decontamination post-exposure has the potential to be an important, integral, and therefore necessary step for medical countermeasures against chemical warfare agents. The products described here must meet several criteria to be effective personal decontaminants and detoxifiers of chemical warfare agents for the soldier, although there is room for improvement, thus novel technologies have been discussed. However, any product must be lightweight for individual use, yet be shelf-stable under environmental conditions found in the field. The importance for readily available and rapid use of a decontamination and detoxification product is due to the rapid damage caused by CWAs – OPs penetrate skin in less than 5 min and mustard produces irreversible cell damage as a result of alkylation equally rapidly. The product should also be environmentally friendly. In the future, one product should incorporate chemical, biological, and radiological decontamination and,

when possible, detoxification. With the constant threat of chemical warfare, terrorist acts, or spillage of pesticides, the development of alternative means of protecting and decontaminating individuals from exposure is critical.

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