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Testing and Evaluation

The testing and evaluation of equipment, methodologies, and toxicological factors are critical for the development of CB defensive strategies. Testing must be done on several different levels, ranging from rigorous tests of components of protective equipment to assessments of current doctrine and training by means of simulations and exercises (including war games).

TOXICOLOGICAL TESTING

In keeping with the principles and spirit of the nonproliferation agreements entered into by the United States, U.S. policy prohibits most tests using real agents and studies with human volunteers (except with surrogate agents). Therefore, most human and animal studies are done with simulants, which may not be adequate surrogates for potential threat agents (Rhodes et al., 1998). In fact, the airborne behavior of simulants and real agents differ significantly, as do their dermal penetration and metabolic effects. These differences make estimating absorbed doses and toxic effects extremely difficult. Thus, based on simulant challenge data, it is extremely difficult to determine whether specific pieces of equipment meet requirements.

Although fewer studies have been done on dermal penetration than on the inhalation or ingestion of agents, the current clothing R&D program is predicated on the need to avoid a percutaneous challenge from agents. Current efforts to develop a new protective ensemble (i.e., JSLIST) and chemically protective undergarments are based on the principle of

preventing CB agents from contacting the skin. Although dermal contact is an obvious danger for blister-type agents, such as nitrogen and sulfur mustards, the percutaneous threat of other agents (e.g., G or V agents or biological agents) has not been established. The available data on percutaneous absorption and the physiological effects of agents absorbed percutaneously are very limited.

For the most part, percutaneous threats from chemical agents have been defined using data from animal studies. Percutaneous threats from biological agents have been defined mostly anecdotally; however, there are situations in which skin contact with biological agents has been shown to have adverse effects (Johnson, 1990; LeDuc, 1989; Mikolich and Boyce, 1990). Because the requirements for protection against percutaneous threats are based on such sketchy data, the goals, requirements, and results of the R&D programs are necessarily based on uncertainties. Strategies for rigorous testing of dermatological exposures will be necessary in two important areas: (1) quantifying skin uptake and resultant toxicology, and (2) determining the efficacy of skin decontamination.

Tests of percutaneous toxicology can be interpreted using well defined models. DPK models are based on relationships among the amount of agent presented to the skin surface, the amount of drug (or toxic chemical) absorbed into the body, and the rate of contamination. Dermatopharmacodynamic (DPD) models determine the CB effects of the absorbed dose (Gupta et al., 1993; Marzulli and Maibach, 1991; Zhai and Maibach, 1996). DPK models describe agent uptake as a function of dose and time; while, DPD models are used to evaluate the relationship between the concentration at the effector or target site and the biologic effect. DPK models may provide kinetic details and suggest mechanisms that could supplement traditional clinical studies. Therefore, DPK models can be used to evaluate both percutaneous absorption (Gupta et al., 1993; Shah et al., 1991, 1993; Zhai and Maibach, 1996) and dermal decontamination (Wester and Maibach, 1999b).

Evaluation of Percutaneous Penetration

DPD models have been used to ascertain bioavailability from percutaneous exposure. Bioavailability can be defined as the rate and extent to which the administered toxic agent is absorbed via the skin and becomes available at the site of chemical action and/or reaches the general circulation. Thus, the approach based on these models can be used to evaluate the absorption and toxicity of agents during dermal exposures, the effectiveness of decontamination, and the degree of protection provided by protective treatments, such as applications of barrier creams.

DPD models can be used to estimate the movement of chemicals into

the first organ system (skin), and quantities can be determined indirectly by noninvasive bioengineering techniques (e.g., colorimetry, transepidermal water loss, laser doppler velocimetry, etc.) (Berardesca et al., 1995a, 1995b; Elsner et al., 1994; Frosch et al., 1993a; McDougal, 1991, 1998; Wester and Maibach, 1999c; Wilhelm et al., 1997).

The following 10 factors should be included in rigorous evaluations of percutaneous absorption (Wester and Maibach, 1983):

- vehicle release
- absorption kinetics
- excretion kinetics
- cellular and tissue distribution
- substantivity
- wash and rub resistance
- volatility
- binding
- anatomical pathways
- cutaneous metabolism

For more detailed scientific information on these 10 factors, see Appendix E.

Vehicle Release

Percutaneous absorption of a drug from a vehicle (i.e., mechanism of transport) depends on the partition of the chemical between the vehicle and the skin and the solubility of the drug in the vehicle (e.g., isopropyl alcohol, dimethyl sulfoxide). Solubility, concentration, and pH of the drug can influence interactions among the vehicle, the active chemical, and the skin. Vehicles sometimes contain agents, such as urea, that can enhance percutaneous absorption. In some cases, the vehicle itself may enhance absorption or change skin integrity. An occlusive vehicle, for example, could alter skin hydration.

Absorption Kinetics

Absorption kinetics vary according to a number of factors: (1) skin application site, (2) individual variations, (3) skin condition, (4) occlusion, (5) chemical concentration and surface area, and (6) number of applications. Percutaneous absorption in humans and animals varies with the anatomical site to which the compound is applied. Even if application conditions remain the same (e.g., application site, compound, concentration, dose, vehicle), absorption can vary by several-fold because of individual variations.

Any change in skin condition, especially changes in the barrier function of the stratum corneum, whether natural or inflicted, may alter percutaneous absorption. Skin condition also changes with age; the stratum corneum of preterm infants is not fully developed and, therefore, is more permeable than in a fully developed infant. Damage, disease, and occlusion (overhydration) may also increase absorption levels. Percutaneous absorption is often increased when the application site is occluded. Chemical concentration and surface area are also critical parameters in determining the amount of absorption. As the concentration of the applied dose increases, the total amount absorbed increases. As the surface area of the applied dose increases, the total amount of absorption also increases.

If any topically administered compound is applied more than once a day, the topical exposure may be chronic. Absorption from one application of a high concentration may be greater than the same concentration applied in equally divided doses. The mechanisms controlling this are not yet understood.

Excretion Kinetics

A potentially toxic chemical will be more or less damaging depending on the rate of excretion or retention in the body. For instance, although lindane and hexachlorophene are not well absorbed, their potential for toxicity is enhanced by their slow excretion and storage in lipid compartments. In general, water-soluble compounds are rapidly excreted and are generally less toxic.

Cellular and Tissue Distribution

The concentration of chemical in the skin is usually highest near the surface and lowest in the dermis. Differences in percutaneous absorption depend not only on the thickness, surface area, and number of cell layers in the stratum corneum, but also on lipid composition and concentration distribution of the chemical in the skin layers.

Substantivity

Substantivity is a measure of the portion of the applied dose that binds to the skin surface and may eventually be lost by skin exfoliation. When radioisotopes are used, substantivity can be monitored by surface counting; otherwise, skin stripping cellophane tape can be used.

Wash and Rub Resistance

A compound applied to skin surface can be partially removed by washing or rubbing. Mechanical stress on the skin, such as friction from clothing, may alter both the distribution of the applied dose and percutaneous absorption.

Volatility

Volatility refers to the partition of a chemical between its vehicle on the skin surface and the surrounding air (an important factor for mosquito repellents). Accurate determinations of percutaneous absorption *in vitro* or *in vivo* with animal and human models require simulations of air flow with volatile chemicals.

Binding

Chemicals may bind to the stratum corneum (i.e., substantivity) or to other tissue compartments (e.g., viable epidermis, dermis, fat, or appendages). The rate and extent of binding have only been documented for a few compounds, but the methodology appears to be adequate. Toxic agents presumably bind to several tissue compartments. Chemical defensive agents might saturate binding sites to decrease the toxicity of the attacking agent.

Anatomic Pathways

Penetration occurs throughout the stratum corneum. Empirically, it is known that hairy areas (terminal or vellus hairs) are more permeable than glabrous sites (e.g., the retroauricular area, face, scalp, and axilla are more permeable than the forearm). Understanding the mechanisms controlling these differences might provide insights for chemical defense.

Cutaneous Metabolism

The skin is an extremely active metabolic organ that contains numerous chemical-metabolizing enzymes. Metabolism of a chemical in skin may alter the pharmacological and/or toxicological effect on the system. When studying the availability of topically administered drugs or environmental contaminants, one must consider the metabolizing ability of the skin, which may affect the bioavailability of the drug during the first

passage through the skin. For example, hydrocortisone can be metabolized to cortisone and dihydrodiols can be metabolized to epoxide diols, which are more potent carcinogens than the original chemicals.

Evaluation of Barrier Creams

Recently, DPD models and noninvasive bioengineering techniques have been adapted to quantify the efficacy of protective or decontaminating barrier creams. These tests provide accurate, reproducible, and objective observations that can reveal subtle differences before visual clinical signs (e.g., blisters) appear (Berardesca et al., 1995a, 1995b; Elsner et al., 1994; Frosch et al., 1993a; Wilhelm et al., 1997). These tests are considered to be more humane than traditional tests because, by the time a blister develops after vesicant exposure, critical biological events have occurred. Data on the efficacy of barrier creams from recent experiments are summarized in Table 6-1. The *in vivo* and *in vitro* methods used to evaluate barrier creams are provided in Appendix C.

TEST EQUIPMENT

Because the United States has moved to a joint service environment and adheres to the CWC and the BWC, testing has become a complicated issue in two respects. First, simulant agents that mimic the chemical and physical properties of real agents must be used. Second, responsibilities must be distributed across services. For example, the Marine Corps is responsible for testing JSLIST but does not “own” the expertise for performing these tests; the toxicological expertise resides in the Army at SBCCOM Soldier Systems Center. The U.S. Army Chemical School (now relocated to Ft. Leonard Wood) has the capability to conduct exercises using real chemical agents, but such experiments are limited to training exercises. Army facilities cannot be used for testing components of PPE or ensembles, per se (DoD, 1999).

PPE components and ensembles have been tested by contract personnel at Dugway Proving Ground in Utah using simulants. The Dugway facilities have excellent capabilities (1) for testing with CB simulants, such as methyl salicylate (MES), which is environmentally benign and relatively nontoxic; (2) for large-scale field testing of the integrity, degree of protection, and decontamination of PPE; and (3) for modeling exercises using various exposure scenarios. Unfortunately, although the capability for performing quantitative tests is available, no mechanism has been established for coordinating the toxicological, human factors, and exposure assessments of the studies.

The lack of coordination becomes apparent in a review of the studies

TABLE 6-1 Efficacy of Barrier Creams

Models		
<i>In Vitro</i>	<i>In Vivo</i> Animals or Human	Irritants or Allergens
	Guinea pigs	n-Hexane, trichloroethylene, and toluene
	Guinea pigs	cutting oil
	Humans with a history of allergic reactions to test allergens	epoxy resin, glyceryl monothioglycolate, frullania, and tansy
	Humans who had positive patch tests to toxicodendron extract	toxicodendron extract
	Guinea pigs and humans	sodium lauryl sulfate, sodium hydroxide, toluene, and lactic acid
Human skin		dyes (eosin, methylviolet, oil red O)
	Machinists	Castrol oil
	Humans with a history of allergic reaction to poison ivy/oak	urushiol
	Nickel-sensitive patients	nickel disc
	Humans	dyes (methylene blue and oil red O)

Barrier Cream	Efficacy	References
3 water-miscible creams	Had limited protective effects.	Mahmoud and Lachapelle, 1985
2 barrier creams	Exacerbated the irritation.	Goh, 1991a, 1991b
1 barrier cream	Minimized the development of allergic contact dermatitis.	McClain and Storrs, 1992
various barrier preparations	Most provided good protective effects.	Grevelink et al., 1992
several barrier creams	Some suppressed irritation, some failed, and some caused severe irritation.	Frosch et al., 1993b, 1993c, 1993d; Frosch and Kurte, 1994
16 barrier creams	Various protection effects.	Treffel et al., 1994
1 barrier cream and 1 afterwork emollient	Had no significant effect against dermatitis from cutting fluid.	Goh and Gan, 1994
quaternium-18 bentonite (Q18B) lotion	Significantly reduced reactions.	Marks et al., 1995
ethylenediamine-tetraacetate (EDTA) gels	Significantly reduced the amount of nickel in the epidermis <i>in vitro</i> , and significantly reduced positive reactions <i>in vivo</i> .	Fullerton and Menne, 1995
3 barrier creams	Two creams were effective, one increased the cumulative amount of dye.	Zhai and Maibach, 1996

TABLE 6-1 Efficacy of Barrier Creams (continued)

Models		
<i>In Vitro</i>	<i>In Vivo</i> Animals or Human	Irritants or Allergens
	Humans	water
	Humans	10% sodium lauryl sulfate, 1% NaOH, 30% lactic acid, and undiluted toluene
	Humans	toluene
	Humans	toluene and NaOH
	Guinea pigs	sulphur mustard
	Humans	self-application of barrier cream
Human skin		[³⁵ S]-SLS
	Humans	sodium lauryl sulfate, ammonium hydroxide (NH ₄ OH), urea, Rhus

Barrier Cream	Efficacy	References
2 barrier creams and a moisturizer	Various protection effects.	Olivarius et al., 1996
4 barrier creams and white petrolatum	Had different protective effects but all products were very effective against SLS.	Schl�ater-Wigger and Elsner, 1996
several barrier creams	All markedly reduced the effect of repetitive toluene contact.	Grunewald et al., 1996
several barrier creams	None prevented the skin erythema induced by toluene. One barrier cream, as well as petrolatum and a fatty cream, protected the skin significantly against NaOH.	Treffel and Gabard, 1996
povidone iodine (PI) ointment	Showed powerful protective effect.	Wormser et al., 1997
oil-in-water emulsion	Self-application was incomplete.	Wigger-Alberti et al., 1997
3 quaternium-18, bentonite (Q18B), gels	Protection effects were 88%, 81%, and 65%, respectively.	Zhai et al., 1999
several protectants	Most suppressed the SLS irritation and Rhus allergic reaction, but did not suppress NH ₄ OH and urea irritation.	Zhai et al., 1998

referred to as the Man-in-Simulant Test (MIST) Program. Despite its shortcomings (described below), MIST is an extremely valuable program that has the potential to test complete and partial PPE ensembles under controlled field conditions. A series of tests in which individuals were exposed to MES at a steady-state concentration of 100 mg/m³ for a period of two hours provided a useful “reality check” (NRC, 1997b). As is often necessary in field-testing situations, however, compromises had to be made. In the MIST ensemble testing, the PPE components were worn and compared with standard issue uniforms. As the test progressed, some of the PPE were damaged. Of these, some were repaired and reworn, while others were worn without repair. Although this is probably what would happen in real-world use, the initial protocol was degraded, thus compromising the use of the data to predict what would happen if PPE were used in a contaminated environment during an actual field deployment.

Another problem with the MIST study was that the comparability of MES to H or V agents was not taken fully into account. Thus, the MIST review committee judged that the MIST data might be used qualitatively to rank some types of PPE, but that the data could not be used to make quantitative assessments because the information obtained using the passive dosimeters (i.e., samplers) could not be correlated to the amount of H or V agents that contacted the skin.

Based on the assumption that MES was a reasonable surrogate for H agents, the test data showed the following results:

- Under the most favorable conditions (PPE in excellent condition), the complete ensemble provided protection against a challenge of $\leq 3,000$ mg-min/m³.
- Damage to the ensemble during use degraded performance to a challenge capability of ≤ 500 mg-min/m³.
- Ensembles that were damaged, repaired, or reworn provided protection against a challenge of ≤ 500 mg-min/m³.
- The areas of greatest vulnerability in an intact PPE are seals and closures.
- The passive dosimeters used to test other components of the protective equipment did not function reliably in the mask.

Because of problems in the experimental design, the data on protection afforded by PPE ensembles could only be used in a qualitative way. Nevertheless, they can still be very useful. For example, the data showed that, within the limits of statistical error, the BDO did not afford significantly greater protection than chemical protective undergarments. This apparent anomaly is attributable to the large differences among ensemble components.

The MIST study might be considered a pilot study for more definitive future studies. The data from the MIST study could be used to design a stronger statistical study based on the basic science aspects of using simulants, such as MES, to determine whether or not PPE ensembles provide adequate protection against challenges with CB agents (or suitable simulants). To facilitate the selection of suppliers of materials, closures, and other parts of the PPE ensemble, the tests should be designed to compare the performance of various ensembles (or components). At a minimum, better methods for validating the use of simulants will be necessary so that results can be used quantitatively. This may require better coordination among groups at SBCCOM and Dugway Proving Ground.

Current mask filters are extremely efficient and afford adequate protection under expected challenge conditions. The point of failure in respiratory protection is the mask seal, not the filter cartridge. The MIST Program did not test masks because the passive monitors used to detect MES and the mask systems were incompatible. This problem has not yet been resolved.

PREDICTIVE MODELS AND SIMULATIONS

Modeling and simulation are often used in place of prototyping to predict the operational characteristics of protective systems. Current models, however, may not be robust or reliable enough to use for making crucial decisions. A major problem is the lack of basic science information, such as the persistence of agents in various environments and under various conditions, rates of deposition, uptake and metabolism in living human skin, and rates of penetration under realistic conditions.

EXERCISES AND SYSTEMS EVALUATIONS

Various types of exercises are used to evaluate the ability of deployed and deploying forces to operate in a CB environment. Computer exercises and war games can be used to predict the likely behavior and effects of operating in a CB environment. The accuracy of the predictions depends on the quality of the data used in the computer model parameter estimates. Computerized war games and scenarios, such as CB2010 (a simulation of the effects of a "low-tech" CB attack on a U.S. force during deployment from a base in CONUS in the year 2010) predicted that a CB attack would significantly impact force projection capabilities, especially the speed of deployment and the effectiveness of forces. A subsequent, more realistic war game with a similar scenario was "played" during a deployment exercise at Pope Air Force Base and Fort Bragg. The actual effects of spraying a simulated thickened mustard on mission-critical

equipment and service areas from a crop-duster-type aircraft demonstrated that the computer simulation might have been optimistic. The impacts were more protracted and additional problems were identified.

FINDINGS AND RECOMMENDATIONS

Finding. Testing of dermatological threat agents has not been consistent. The available data are not sufficiently precise to make an accurate evaluation of potential percutaneous threats from agents other than blister agents or irritants.

Recommendation. Tests of dermatological threat agents should be conducted to establish the level of protection necessary to provide adequate margins of safety and to establish quantitative criteria for evaluating the performance of protective equipment, such as gloves, undergarments, and overgarments.

Finding. Mask testing under the MIST program was unreliable because the passive dosimeters did not function satisfactorily.

Recommendation. Active samplers or improved passive samplers for mask testing using simulants should be developed and made available for tests of the joint service lightweight integrated suit technology (JSLIST) ensemble.