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REVIEW OF TOXICOLOGICAL DATA REGARDING CONTACT HAZARDS OF CHEMICAL AGENTS

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August 2006

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13. SUPPLEMENTARY NOTES					
14. ABSTRACT "Contact hazard" is a complex function of: (1) agent percutaneous (PC) toxicity; (2) agent bioavailability; and (3) the exposure scenario. While visibly wet, agent-contaminated surfaces present an obvious contact hazard, chemical exposure becomes increasingly more risky when there is no visible sign or detection of agent contamination – particularly if the surface has been theoretically decontaminated. The inability to detect contamination does NOT indicate that there is no hazard. The larger body of data clearly indicates that "contact hazard" cannot be separated from "agent fate". Merely determining the amount of agent retained in a surface does not determine the "contact hazard" that may be present, because it does not address the bioavailability of the agent. The potential toxicological hazards of liquid/vapor agent-contaminated/decontaminated surfaces and materials have not been adequately characterized. Some agent data are sparse, and animal-to-human extrapolation has been more qualitative than quantitative. The existing human toxicity estimates for PC exposure are fraught with uncertainty, and their error bars are large. More research is necessary to develop reliable human toxicity estimates without which, it is impossible to determine contact hazard.					
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PREFACE

The work reported herein was performed at the request of the Program Manager, Operational Toxicology, Joint Science and Technology Office, in support of the Low Level CWA Research Program. This work started in April 2004 and was completed in March 2006. This report presents results of a review of the contact hazard data and human toxicity estimates for selected persistent agents, with recommendations for further studies.

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REVIEW OF TOXICOLOGICAL DATA REGARDING CONTACT HAZARDS OF CHEMICAL AGENTS

1 BACKGROUND

Manthei *et al.* (1983) stated, "Surfaces that have been contaminated with liquid chemical warfare agents and then decontaminated (chemically or physically) must not be presumed to be clean and safe to touch... these surfaces may still emit agent vapors and also may produce physiological responses when contacted by an individual. In the absence of detectable liquid residual agent on the surface, this latter situation has been given the name contact hazard."

The work presented herein was funded to review the toxicological data on "Contact Hazard". This study has focused on the more persistent agents known to exhibit percutaneous (PC) toxicity [*i.e.*, mustard (HD), lewisite (L), VX, soman (GD), cyclosarin (GF), and thickened (T) agents (Table 1)] and will provide: (1) determination of the extent of the salient data; (2) review, analysis, and modeling of those data (where possible); and (3) delineation and characterization of the data gaps in a comprehensive technical report. The ultimate, but not direct result, of this study—with input and prioritization from the user community, will be a program to fill the toxicological data gaps.

Table 1. Physical Properties of Selected Persistent Agents*

Agent	Physical State	Boiling Point	Freezing (FP) or Melting Point (MP)	Vapor Pressure (torr)	Volatility (mg/m ³)
GD	liquid	198 °C	-42 °C (MP)	4.01 x 10 ⁻¹ @ 25 °C	3.93 x 10 ³ @ 25 °C
GF	liquid	228 °C	-30 to -50 °C (FP)	9.27 x 10 ⁻² @ 25 °C	8.98 x 10 ² @ 25 °C
VX	liquid	292 °C	< -51 °C and -39 to -60 °C (FP)	8.78 x 10 ⁻⁴ @ 25 °C	1.26 x 10 ¹ @ 25 °C
HD	oily liquid	218 °C	14.45 °C (FP)	1.06 x 10 ⁻¹ @ 25 °C	9.06 x 10 ² @ 25 °C
L	liquid	196 °C	-44.7 to 1.8 °C (FP)	3.46 x 10 ¹ @ 25 °C	3.86 x 10 ³ @ 25 °C
HT	liquid	not constant	13 °C (MP)	7.7 x 10 ⁻² @ 25 °C	7.83 x 10 ² @ 25 °C

*adapted from Abercrombie (2003)

More than 200 documents—classified and unclassified, were reviewed for relevant contact hazard toxicity data. The studies included field trials (with agent contamination of large areas of terrain), laboratory tests with contaminated surfaces, and field and laboratory tests with humans and animals. Many of the studies cannot be duplicated, and despite their faults (which include conclusions based upon human toxicity estimates that are no longer accepted), they are invaluable. Fewer than half of the studies actually contained any toxicity data, but several are included in this report because they contained concepts important to better understanding the hazards of contacting surfaces that have been contaminated by chemical agents.

2 INTRODUCTION

"All contact hazard scenarios have in common the starting point that a toxic material must be delivered on surfaces by an agent delivery system." Although surfaces have been decontaminated, agent can reappear as it desorbs from substrates, and the persistence and magnitude of any potential residual hazard must be ascertained (Carlton, 1990).

2.1 Defining “Contact Hazard”.

Implicit in the notion of contact hazard is the fact that the agent is somewhat persistent. The Department of the Army (1963) defined persistency as “an expression of the duration of effectiveness of a war gas, which is dependent on physical and chemical properties of the gas, weather, methods of dissemination, and condition of terrain”.

2.1.1 Current Definitions.

The most commonly used definition of “Contact Hazard” was given by Klein (1983).

“Given a surface that has been contaminated with a liquid chemical agent and that surface undergoes a process after which the agent no longer can be detected as a liquid, contact hazard is that situation in which a toxicological hazard can result if an individual then touches that surface with the bare skin.”

Schwope *et al.* (1985) amplified the definition to include soldier contact—not just bare skin, and pointed out that agent contamination could not be detected by liquid chemical detectors. Hence, personnel would not be able to assess the degree of hazard associated with decontaminated equipment, or as re-stated by Carlon (1990), the ability to detect vapor will not suffice to warn troops of a contact hazard. Both authors stressed that the degree of physiological response from a contact hazard is often greater than that predicted from detectable vapor coming off the surface or the PC toxicity vapor data.

Later, Klein (1989) described contact hazard as a process dependent upon the diffusion of agent “sorbed” into a surface—meaning either adsorbed or absorbed. “The Residual Agent and Contact Hazard Workshop” (Stuempfle and Klein, 1988) limited the definition of contact hazard to the hazard presented by residual chemical agent in surfaces following removal or decontamination of the BULK surface contamination. (The subsequent phenomenon is that chemical agent in the sub-surface can diffuse to the surface and become a hazard.)

In 1990, Carlon stated that Klein’s (1983) definition of contact hazard was too narrow for several reasons: (1) “...what solid particles such as agents of biological origin (ABO) are contacted on surfaces?; (2) What if toxic vapors are contacted percutaneously, rather than by the respiratory route?; (3) What if the skin is clothed?; (4) What if contamination is first picked up on the fingers and then transferred to the skin, or to the mouth where it is ingested?; (5) What about the inhalation threat from particulates that are reaerosolized from surfaces?; (6) What about secondary vapor threat from contaminated surfaces as they weather, especially in enclosed areas?; (7) What about the threats from liquid/solid mixes such as “dusty” agents that use solid carriers in which liquids are absorbed from improved dissemination?”

In a study designed, but never executed, to evaluate materials for residual hazards after decontamination, Brimhall (1991) included chemical warfare (CW) agent-contaminated surfaces as contact hazards—as well as contact with objects that have been contaminated and then decontaminated, but which still have residual agent present. The latter hazard arises because rubber, plastic, paint, crevices, and porous surfaces absorb chemical agent and then release it after the surface of the equipment has been decontaminated. When the object is held in contact with the skin, some of the agent can migrate (diffuse) to the surface of the object and transfer to the skin. The hazard is a function of contact time, amount of agent, and agent migration rates. As stated by Armour and Sturgeon (1992), the extent of the contact hazard depends on the initial degree of contamination, the meteorological conditions, the surface type, and the time between the attack and the resumption of operations. More recently, Chinn (2000) defined contact hazard based upon the level of surface contamination—50 mg/m² for GD and 100 mg/m² for HD. This was based upon a contamination density of 10 g/m² and 99.5 and 99.0% evaporation for HD and GD, respectively.

2.1.2 Historical Definitions.

Contact Hazard.

The Chemical Warfare Board (CWB) (1944) perceived contact hazard as the CW hazard to troops unloading supplies on the beach but remaining on the beach for only comparatively short

(unspecified) periods. The definition used during the San Jose Project [(SJPR), (1945a,b,c,d,e,f,g,h)] was somewhat variable, but contact hazard was generally defined as the danger of developing casualty-producing or disabling lesions from vesicant agents picked up on clothing or bare skin while troops traversed 100 yd, making “short-period” contact¹ in the prone position with visibly contaminated ground and/or undergrowth.

Traversing Hazard.

Similarly, traversing hazard (Chemical Warfare Board, 1944) was defined as troops crossing a contaminated area in good boots within a 15-minute time period. Again, the definition used for the SJPR was somewhat variable, but in general, the traversing hazard was the danger of developing disabling or casualty-producing lesions from liquid vesicant picked up on the clothing or bare skin—apart from any hazard produced by vesicant vapor, while troops attempted to avoid areas of visible liquid contamination while alternately walking and crawling across terrain contaminated with liquid vesicant. A similar definition was employed in the SJPR (1945a,b,c,d,e,f,g,h).

Occupying Hazard.

The Chemical Warfare Board (1944) characterized occupying hazard as the danger of exposure to troops occupying an area for at least 4 hr, during which portions of the body, clothed or unclothed, were in contact with the ground surface. Likewise, the definition used by the SJPR (1945a,b,c,d,e,f,g,h) was not consistent throughout the project, but it was generally defined as the potential danger of developing disabling or casualty-producing lesions from vesicant vapor and/or from liquid vesicant picked up by the clothing or bare skin when troops continuously occupied, for at least 24 hr, a jungle area contaminated with a vesicant. The troops ate, slept, worked in, and patrolled the area during this period, while exercising normal anti-gas precautions and avoiding contact with areas of visible liquid.

Casualty.

The above definitions are put into better perspective when one considers the historical definitions of “casualty”. Rothschild (1937) defined casualty as an individual killed or so injured as to require treatment beyond the simplest first aid measures—one who must be evacuated. The Project Coordination Staff (1946) defined disability as follows:

Totally disabled—incapable of performing any military duties; incapable of usefully remaining in the field, regardless of the seriousness of the military situation.

Partially disabled—capable of performing limited military duties; includes many individuals who would be evacuated in accordance with British or United States practice but who in a critical situation could be retained in the lines as capable of contributing to an action, particularly to static defense.

Injured, without disability—capable of performing full military duties; injuries such that military effectiveness is interfered with only slightly or not at all.

In SJPR-31 (1945a) injuries without disability were described as small, pin-point vesicles on the hands and/or forearms, discrete vesicles surrounded by erythema and/or erythema and edema. Although the above casualty definitions of the 1940s are not obviously at odds with today’s standards, in some cases, the perception of what constituted a casualty appears to be markedly different. The following is an illustrative example of “partially disabled”, excerpted directly from the Project Coordination Staff (1946) report.

“Case 3. Severe Partial Disability; hot and humid weather. A masked observer clothed in unimpregnated tropical battle dress was exposed in a field test to a dosage of approximately 610 mg min/cu m (time: 55 min; temperature 82 °F; relative humidity: 89%). He did not exercise during the exposure.

¹ “Short period” contact was not defined, and contact differed from study to study.

“Subjective symptoms: Vomited 5 hr after exposure. At 24 hr, he was still nauseated, with arms and scrotum painful and burning. He was unable to sleep on the 2d day because of pain in the penis and scrotum. The pain persisted, and there was burning over most of the body. Admitted to the hospital on the 11th day.

“Objective injuries: Twenty-four hours after exposure there was general erythema over the entire body and the scrotum was edematous. The scrotal edema had increased to the penis, wrists, and elbow folds were also involved at 2 days, the buttocks and the backs of the knees at 3 days. By the 5th day there was extensive dry scarring and scabbing of the scrotum. Raw areas appeared on the scrotum and glans penis on the 7th day. When hospitalized on the 11th day the glans was markedly erythematous and slightly excoriated; the raw area of the scrotum had almost completely healed but there was severe and extensive desquamation over the whole body. By the 13th day the scrotal edema had disappeared but the glans was still swollen and completely denuded. By the 22nd day the glans was well scabbed with clean, healthy skin beneath. The observer was discharged from the hospital but kept under observation and treatment for an additional 8 days.

“Practical assessment: On the 1st day able to march 6 miles full equipment and complete an assault course only with difficulty. On the 2d day marching was very uncomfortable and he was unable to wear full equipment or get over obstacles on the assault course, even with the aid of a suspensory bandage. On the 10th day he carried out the standard march but was unable to run around the assault course.

“Casualty status: Partially disabled from 5 hr to 31 days after exposure, severely so from the 10th to 22nd days.”

In a field test with dogs exposed to vesicants, Armstrong (1927) defined “light casualty” as visible general effects from the gas for a period of not longer than 120 hr. “Moderate casualties” showed effects for more than 120 hr and less than 21 days. “Severe casualties” were affected for 21 days or longer. In later studies (Armstrong *et al.* 1928a,b), “light casualty” was re-defined as not showing general effects for longer than 96 hr.

2.1.3 Scope and Definition of Present Study.

For the purposes of the documents reviewed for this report, “surfaces” included contaminated soil and vegetation, as well as man-made materials. “Surfaces” were not limited to those that had been decontaminated or were no longer visibly wet—particularly with regard to soil and vegetation. Similarly, studies that included vapor hazards from surface contamination were also included. If a persistent agent produces sufficient agent vapor to trigger a detector or produce toxic effects a contact hazard is likely to exist. Indeed, the Project Coordination Staff (1946) stated that the hazard from contact with liquid H to troops traversing or occupying an area, cannot be divorced from vapor hazard, since where there is liquid contamination there will be vapor, and under some conditions, the hazard from vapor may outweigh that from liquid, and *vice versa*.

It is difficult to separate out traversing and occupying hazards from contact hazard—particularly within the current context, so for purposes of this study, no distinction has been made between them, but emphasis has been placed on physical contact with a contaminated or formerly contaminated surface/material. The efficacy of personal protective equipment (PPE) was not evaluated; however, it was noted that the “better” protective ensembles afforded significant protection in highly contaminated areas (SJPR 1945a,b,c,d,e,f,g,h). Similarly, the intent of this review was not to evaluate the efficacy of various methods of decontamination.

2.2 Modeling and Estimating “Contact Hazard”.

There are several different theories and models for what constitutes a contact hazard. It is not clear that any one theory or model is applicable to all situations, but there are two primary models of

contact hazard (Manthei *et al.*, 1988). In the first (the diffusion/vapor transfer model), agent is assumed to have been sorbed into a material, and then it diffuses back to the surface from which it evaporates and is absorbed as vapor by a contacting surface (e.g., skin). In the second (diffusion/surface distribution transfer model), it is postulated that the surface of the contaminated material can be compared to a pseudo-liquid, and agent transfers across the interface to the contacting surface. In the latter case, the rate of transfer is faster than that for vapor.

The diffusion/vapor transfer model was proposed first and was the driver for determining acceptable levels of decontamination (or acceptable levels of contamination—how “dirty” is clean enough?). In fact, the levels of acceptable decontamination were defined by head-space analysis of decontaminated materials and dominated the experimental data for a number of years (Hall *et al.*, 1989). However, it was subsequently realized that a flowing airstream may not be as efficient a sink for desorbing agent as a contacting surface and that the vapor hazard did not relate to the amount of agent remaining in painted surfaces. The latter mechanism has since been substantiated, and analysis of the data indicates that both mechanisms may play roles in contact hazard—dependent upon the agent and the surface.

Klein (1987) stated that the agent flux from a surface is no measure of the contamination in the surface or of the hazard to the soldier. He stated further that meaningful interpretation of the degree of hazard associated with a surface requires that the contamination history and physical properties of the surface are well known, and this information is more important than the data collected from the surface itself. Carlon (1990) stated that the data suggest that the hazard from a liquid-free surface can exceed the hazard that would be predicted based on directly measured vapor evolution rates.

3 RELEVANT CONTACT HAZARD DATA

It should be noted that many of the historical studies—in the field and in the laboratory were done with contamination densities that were considerably higher than the current standard of 10 g/m²—the levels of contamination are not realistic by today's standards. These studies are included for completeness, but the findings should be put into the proper perspective with regard to severity of effects and agent persistence.

3.1 Human.

Some of the very earliest studies were done by Marshall *et al.* (1918) at American University. Although much of the study entailed observing the effects of vapor off-gassing in mice and dogs, soil samples were evaluated for vesicant potential in dogs and humans. Contamination densities ranged from about 14 to 270 g/m²! It was concluded that an unprotected man “would be quite liable” to a skin burn within 4 days post-contamination and that within 24-hr post-contamination an unprotected man would become a casualty within 1-2 hr.

The human studies employed thousands of volunteers [there were 1800 in the Bloom and Savit (1945) study alone] and were mainly done in the 1940s. They often focused around refining the offensive use of vesicants or correlating physiological responses with various detector methods. Their purpose was not really to delineate the magnitude and duration of the contact hazard, and many of the “exposures” were done in full personal protective equipment (PPE). These studies were typically more qualitative than quantitative, and the time-course of the investigation of the hazard was relatively brief—especially with regard to the known persistence of some of the agents. It should be noted that the perception of hazard in these studies may not actually parallel the standards and definitions of today [“Historical Definitions”]. Also, some descriptions of hazard *versus* safety are based upon a well-defined scenario or one-time contact, which may have limited applicability to the needs of today.

Anderson (1943) applied contaminated soil to the forearms of volunteers to determine the duration of time soil could be “safely” occupied. However, “safe occupation” was not well defined. Anderson did observe that the physiological data were worse than predicted by vapor pressure data, and that the vapor hazard fluctuated with the temperature and time of day and that “hazard” was often a function of the meteorological conditions. He also noted that the duration of vesicant activity in soil is decreased by hot weather—although hot weather does enhance vapor evolution and the concomitant vapor effects. Small drops were less effective than large drops; heavier contamination was more

effective, and rain reduced effectiveness. Persistence was more dependent on type of surface than contamination density.

Gorton (1944) published a summary of C.D.R.E. (India) Report No. 265, "The Decontamination of Areas Contaminated with Mustard Gas under Field Conditions in India". The study consisted of a series of mustard contaminations of open desert, decontamination of some areas, and physiological exposures of protected and unprotected troops. It was shown that treatment of contaminated surfaces with bleach prevented vapor evolution but did not reduce the occupational hazard. The traversing hazard was slightly reduced. It was stated that certain severe casualties from a high dosage ($Ct \sim 220 \text{ mg min/m}^3$) were incapacitated for 1-5 weeks, "almost entirely by reason of the great vulnerability of the genital areas under Indian conditions".

Bloom and Savit (1945) contaminated different fabrics, woods, painted surfaces, plastics, rubber and earth with various sulfur mustards or HT and then partially decontaminated the materials with chemicals or aeration. The irritant responses in 1800 volunteers exposed to the contaminated swatches were measured against chemical detectors. Perhaps the most relevant finding was that all the test materials did not behave alike, and it was not possible to predict the irritant response on the basis of the detector tests.

The SJPR performed a major series of jungle studies with vesicants in the 1940s (Figure 1). The underlying objectives of the studies were framed from an offensive perspective rather than the determination of the magnitude and duration of the human contact hazard. Typically bombs with various chargings were used to determine: (1) the areas covered by vapor dosages of 200 mg min/m^3 and 1000 mg min/m^3 for specified time periods after bombing, (2) traversing hazard for troops wearing Full British Protection 1.5 hr after bombing, (3) occupying hazard for troops wearing Full British Protection, and (4) contact hazard for troops wearing Full British Protection at seven and 13 days after bombing and Class III Protection 13 days after bombing. The procedures were similar for each test, but the classification of clothing varied somewhat; the clothing are tabulated in Table 2. It is interesting to note that although the men simulated war-time maneuvers, such as marching creeping, and crawling, they avoided obvious areas of contamination. As previously stated, the San Jose technical staff defined *contact hazard* "as the danger of developing casualty-producing or disabling lesions from vesicant agents picked up on the clothing or bare skin while troops in the prone position made short-period unavoidable contact with contaminated ground and/or undergrowth 100 yd over typical visibly contaminated terrain." The data are summarized in Table 3. The procedure outlined for each test remained similar throughout. Specifically, observers donned in a particular class of protective suit and carrying their rifles, assessed contact hazard at specified time periods from 0.5 to 144 hr post contamination. To simulate war-time actual events, men were instructed to fall prone to the ground and advance by creeping, crawling and/or a 4-hr march for a predetermined time period or distance to assess (1) the effects of skin moisture (*i.e.*, sweat and mud-stain) after contamination and (2) the location and severity of lesions as a function of activity.

² 200 mg min/m^3 was estimated to produce temporary blindness and 1000 mg min/m^3 was estimated to produce casualties in 50-100% of masked troops from systemic effects and burns.

Table 2. PPE Ensembles in SJPR Studies

Date of Report	Class 1	Full British Protection	Class 2	Class 3
April 1945	2-piece fatigues ^a		2-piece fatigues ^a	
	long underpants ^b		short underpants ^b	
	long-sleeve undershirt ^b		long-sleeve undershirt ^b	
	socks, leggings ^b		socks, leggings ^b	
	gloves ^b		gloves ^b	
	hood ^b		hood ^b	
	shoes ^c		shoes ^c	
	gas mask		gas mask	
May 1945		2-piece fatigues ¹		fatigues ³
		hood, socks ⁴		long underpants ⁴
		long underpants ⁴		socks ²
		short-sleeve undershirt ⁴		leggings ²
		leggings ²		shoes ²
		M5 ointment		helmet liners; webbing
		shoes ⁵		gas mask or eye shield
		gas mask		
June 1945		2-piece fatigues ¹		fatigues ²
		long underpants ⁴		short drawers ⁴
		short-sleeve undershirt ⁴		long-sleeved undershirt ⁴
		socks ⁴		socks
		shoes ⁵		shoes ²
		leggings ²		
		M5 ointment		gas mask or eye shield
		hood ⁵		
July 1945	2-piece fatigues ¹	2-piece fatigues ¹		fatigues ²
	long-underpants ⁴	long underpants ⁴		socks ²
	long-sleeve undershirt ⁴	short-sleeve undershirt ⁴		shoes ²
	socks	socks		gas mask
	shoes ⁵	shoes ¹		
		M5 ointment ¹		
	leggings ⁴	leggings ²		
		hood ¹		
	gas mask	gas mask		

^a HBT, CC2-impregnated

^b CC2-impregnated

^c Impregnated

¹ CC2-impregnated

² Non-impregnated

³ HBT, non-impregnated

⁴ CC2-impregnated

⁵ Impregnated

Table 2., cont. PPE Ensembles in SJPR Studies

Date of Report	Class 1	Full British Protection	Class 2	Class 3
Aug 1945	2-piece fatigues ⁱ	2-piece fatigues ⁱ		fatigues ⁱⁱ
	long-limb underpants ⁱⁱⁱ	long underpants ⁱ		socks ⁱⁱ
	long-sleeve undershirt ⁱⁱⁱ	short-sleeve undershirt ⁱⁱⁱ		shoes ⁱⁱ
	leggings ⁱⁱⁱ	socks ⁱⁱⁱ		gas mask or eye shields
	shoes ⁱⁱⁱ			
	hood ⁱⁱⁱ			
	gas mask			
Sept 1945		2-piece fatigues ⁱ		fatigues ⁱⁱ
		long underpants ⁱⁱⁱ		socks ⁱⁱ
		short-sleeve undershirt ⁱⁱⁱ		shoes ⁱⁱ
		socks		gas mask
		leggings ⁱⁱ		
		M5 ointment		
		shoes ⁱ		
		hood ⁱ		
gas mask				

ⁱ HBT, CC2 impregnated
ⁱⁱ Non-impregnated
ⁱⁱⁱ Impregnated

Table 3. Data Summary from San Jose Project Reports

Report	Date	Contamination	Hazard		
			Traversal	Occupation	Contact
SJPR-81	16 Apr 1945	H-filled M47A2 bombs; contamination density equivalent to 625 lb of H per artillery square	---	---	at 6 hr after bombing men wearing Class I sustained blisters on elbows and knees after momentary contact in prone position adjacent to site of bomb burst; similar lesions produced 12 hr after bombing for men wearing Class II
					none at 60 hr after bombing for men in any type of clothing, but bare skin blistered by contact with contaminated terrain 100 hr after bombing
SJPR-31	14 May 1945	200, 4.2 in. ML mortar bombs each charged with 3.75 lb of HT; contamination density equivalent to 80 lb per artillery square (12 tons per square mile)	none, if visible liquid avoided, for troops wearing: (a) Full British 1.5 hr after contamination or (b) troops wearing non-impregnated clothing 48 hr after contamination	absent on target area, if visible liquid avoided, for (a) troops wearing Full British during the 24-hr period commencing 1.5 hr after contamination and (b) for troops wearing non-impregnated clothing during the 24-hr period commencing 48 hr after contamination	absent on the target area for (a) troops wearing Full British, 24 hr after contamination and (b) troops wearing non-impregnated clothing, 60 hr after contamination
			present on target area 48 hr after contamination for troops wearing non-impregnated clothing if areas of visible liquid not avoided		

* An artillery square was an area of 100 yd x 100 yd.

Table 3., cont. Data Summary from San Jose Project Reports

Report	Date	Contamination	Hazard		
			Traversal	Occupation	Contact
SJPR-39	12 Jun 1945	nine E27R1 clusters each containing five 50-lb LC50 bombs charged with 47 lb of HT dropped in or near a jungle target of 16 artillery squares	no hazard during daylight 1.5 hr after bombing for troops wearing Full British—if areas of visible liquid avoided	none for troops wearing Full British during 24-hr period commencing 1.5 hr after contamination—if visible liquid avoided	on target for troops wearing Full British 32 hr after bombing
					on target for troops wearing Class III 17 days after bombing; absent at 27 days
SJPR-72	14 Jul 1945	36 British, 500 lb, Mark II bombs charged with HT thickened with methyl methacrylate dropped from an altitude of 2500 ft; only 16 bombs impacted target area	absent during daylight hours for troops wearing Full British 1.75 hr after contamination—if areas of visible liquid avoided	absent for troops wearing Full British during 24 hr commencing 1.75 hr after contamination	none at 20 days post-contamination for troops wearing Class I
					none at 23 days post-contamination for troops wearing Class III
SJPR-45	28 Jul 1945	two B-24 bombers, flying 100 ft above the canopy, dropped 24 E2741 clusters of LC 50-lb A/C bombs charged HT along the fringe of a simulated airstrip	---	--	area useable 1 hr post bombing for troops working in area 4 hr, wearing Class I, rubber boots, and gloves
					no lesions of "casualty significance" at 8 days post-contamination for troops moving through area for 2 hr in Class I, w/ eyeshields, but not masks
					casualty-level lesions likely up to 8 days post-contamination w/ unimpregnated clothing
					none at 14 days post-contamination for troops working in area wearing Class III and impregnated gloves

* After dark, contaminated areas were not visible and could not be avoided.

Table 3., cont. Data Summary from San Jose Project Reports

Report	Date	Contamination	Hazard		
			Traversal	Occupation	Contact
SJPR-73	28 Jul 1945	36 British bombs, charged HT dropped from altitude of 8000 ft over jungle; contamination density ~ two bombs per artillery square (~54 tons per square mile)	none during daylight for troops wearing Full British at 1.5 hr after bombing—if areas of visible liquid avoided	none for troops wearing Full British during 24-hr period commencing 1.5 hr after contamination—if visible liquid avoided	for troops wearing Full British 7 days after bombing, but not 13 days after bombing
					none for troops wearing Class III, 13 days after bombing
SJPR-62	31 Aug 1945	160 rounds of HT charged bombs used to produce contamination density of 140 lb of HT per artillery square (21.5 tons of HT per square mile)	in the impact area for 8 hr post-contamination for troops wearing Class III with masks—even though they attempted to avoid areas of visible liquid	none at 26 hr post-contamination, for troops occupying area for 24 hr, wearing Class III with eyeshields—if areas of visible liquid avoided	none at 2 days after contamination at impact sites for troops wearing either Class I or Full British
					none at 3 days after contamination for troops wearing Class III with masks
SJPR-34	Sep 1945	seventy-six E27R1 clusters of Canadian LC 50-pound A/C bombs charged Levinstein H (5 bombs per cluster) dropped from 500 ft onto 16-artillery square target	none at 1.75 hr post-contamination, for troops equipped with Full British, traversing four artillery square area contaminated with 10 clusters—if areas of visible liquid avoided	none at 1.75 hr post-contamination for troops wearing Full British, occupying area for 24 hr—if visible liquid avoided	present on target area for troops wearing Full British, 6 days after bombing, but was
					absent 14 days after bombing for troops wearing Class III



Figure 1. Observers Crawling through Mustard-Contaminated Jungle at San Jose

Fritz (1944) reported on a comprehensive study to “improve and develop methods, techniques and systems for the decontamination of military materiel contaminated with old or new war gases”. The “military materiel” consisted of aircraft or stripped-down portions thereof; the vesicants were H and L. A number of trials were done; only the more salient are included here. Several tests were performed to assess in-flight cockpit hazard and the efficacy of flight for decontaminating contaminated aircraft. In other tests contaminated bits of materiel were placed onto the arms of volunteers to test for vesicancy; human tests were done only when the DB-3 cup test indicated that the amount of vesicant was not sufficient to cause severe burns. It was observed that decontamination could be effected by a stream of hot air blown through the airplane and that during flight a dangerous vapor concentration could build up in an airplane with exterior contamination. The data indicate that cockpit levels were high enough to have caused burns in unprotected individuals under moderate temperatures. Cracks and crevices on the exterior of the airplane were not decontaminated by flight or washing and were deemed to be a hazard for mechanics. At least one of the physiological burn tests (Table 4) indicated that the DB-3 methodology did not predict the potential contact hazard.

Table 4. Physiological Burn Tests (30-Min Exposure) in Men Using Panels from A-35-A Airplane after a 2-Hr Flight (Fritz, 1944)

Subject	Panel #1 (DB-3 = 0 @ 35 °C)		Subject	Panel #2 (DB-3 = 0.5 @ 35 °C)		Subject	Panel #3 (DB-3 = 1.0 @ 35 °C)	
	24 hrs*	48 hrs*		24 hrs*	48 hrs*		24 hrs*	48 hrs*
1	1	1	10	1	1	19	1	2
2	1	1	11	1	1	20	---	3
3**	5	5	12	---	1	21	1	1
4	1	1	13	---	1	22	1	1
5	1	2	14	1	1	23	1	3
6	1	1	15	---	2	24	1	2
7	2	2	16	---	1	25	1	2
8	1	2	17	---	2	26	---	2
9	2	2	18	---	2	27	2	2
			---			28	2	2
avg.	1.2	1.5	avg.	1	1.3	avg.	2.4	2.0

* observation time post-exposure

** unusually sensitive; omitted from averages

1 = slight redness; 2 = moderate redness; 3 = raised redness; 4 = raised redness + incipient blisters; 5 = big blisters

According to Klein (1989), during the 1970s several research groups in Europe used mustard to contaminate test plates painted with a US military standard paint. The agent was permitted to sorb into the paint for a fixed time, and then the surface of the plate was decontaminated. The flux of HD vapors was measured, and the cleaned surface was evaluated by affixing the plates to the arms of volunteers. It was observed that there was a poor correlation between the measured flux and the degree of irritation produced by the plate, so studies were undertaken in the US to reconcile the data. Salient data were reviewed and toxicological data were generated by Manthei *et al.* [(1988) see below]. One goal of the effort was to compare the severity of injury resulting from direct (rabbit) skin contact of an HD-contaminated plate *versus* indirect exposure from vapor emanating from a plate affixed 1 cm above the skin surface. Although the data were not presented by Klein (1989), it was concluded that the vapor transport mechanism was disproved by Manthei *et al.* (1988) [see below]; however, it should be pointed out that Klein defined contact hazard as “contact”—rather than indirect exposure *via* vapor. Of note was the observation that uniform pressure of test plates on skin is necessary for direct comparability of results, and this could not be effected. Manthei *et al.* (1988) [see below] also used dental dam to develop a sorption model. Klein observed that there was no correlation between dental dam and human skin under similar conditions.

3.2 Animal.

Animal Data Summary.

Some of the earliest mustard persistency studies were done by Armstrong (1927) and Armstrong *et al.* (1928a,b,c) at what is now the U.S. Army Edgewood Area of Aberdeen Proving Ground, MD. Mustard contamination was effected by static firing of different types of shells. Persistency was determined by placing rats or staking dogs in the shell area post-contamination to determine the vapor hazard or placing contaminated soil³ onto the shaved abdomens of guinea pigs or rabbits to determine persistency in and vesicancy of the soil. Armstrong (1927) and Armstrong *et al.* (1928c) found that L persisted in the soil for five days when shells were fired with a 21 g booster and 12 days when shells were fired with a 112 g booster. The HS fired with a 112 g booster persisted for less than an hour. Four hours after contaminating a 2463 ft² circular area with eleven 155mm shells filled with 9.7 lb of HS, Armstrong *et al.* (1928a) staked five dogs⁴ in the shell area for an 18-hr exposure. All were affected; two

³ Collected at specified times, at depths up to 0.75 in., and placed into stoppered bottles

⁴ Impounded animals weighing 15-45 lb, with bellies shaved.

died; only one was considered to be a "light casualty". The soil samples were vesicant on the shaved bellies of the rabbits up to and including the eighth day after the shells were exploded, and it was noted that rabbits are 1/10th as sensitive as humans to mustard⁵. Subsequently, Armstrong *et al.* (1928b) investigated persistency after statically firing low, medium, and high booster shells, containing from 1 to 29 lb of HS. At 1 hr post-contamination, two dogs were staked in the high booster shell bank for 4 hr; one was classed as a moderate casualty, and the other was classed as a severe casualty (no dogs were placed in the low and medium booster shell areas). Soil samples from the low and medium boosters was vesicant up to and including the ninth day, while the soil from high booster shells was not vesicant even 1 hr after the shell burst.

Armstrong *et al.* (1928c) compared the efficacy and persistency of HS, L, and Methylchloroarsine (MD2). Exposures of dogs⁵ staked in the shell areas for four and 24 hr post-contamination indicated that all three compounds were harmful in the vapor phase for at least 21 hr. Exposures of rats indicated that all the compounds persisted in harmful amounts for at least 48 hr. It was also noted that the arsenical compounds were more persistent than mustard, and the shell craters were still vesicant three months later. Rain started shortly after one "shoot" and persisted for several days. The HS-contaminated soil was not vesicant to rabbits, and it was postulated that the rain had hydrolyzed it or washed it away.

In a study to determine the length of time mustard persists in an area after shells have been discharged, Eldridge and Wells (1926) detonated 11 shells containing 9.74 lb of mustard in a circular area of approximately 480 ft² (approximately 0.22 lb of mustard/ft²). Four days later, two dogs with shaved chests and abdomens were each staked in a shell crater for 5 hr. At the end of the exposure, both animals were symptomatic of mustard vapor intoxication. One died 2.5 days later, and the other died 3.5 days post-exposure. Eight days after the initial detonation, two more dogs were staked in two different craters for 5 hr. One animal was severely burned and died 4.5 days later. The other showed only mild effects, and it was noted that the eyes were clear, which would tend to indicate that the residual vapor was minimal.

The CWB (1944) performed a complex study at Dry Tortugas, FL. The site was selected for its similarity to Pacific atolls and consisted of rock coral, bone coral, coral sand, and debris from other marine life. The intent of the study was to determine: (a) the penetration of H in coral, (b) the persistency of H in coral, (c) the effectiveness of H-vapor concentrations produced by contamination of coral, (d) the effect of sea water on H-contaminated coral, (e) the technique of using hand tools for clearing H-contaminated coral sand, (f) the effectiveness of different bleach formulations for decontamination, (g) comparison of effects of coral sand and bleach on H, and (h) traversing, contact, and occupation hazards of H-contaminated coral sand. Liquid H was sprayed in concentrations of 50, 100, or 300 g/m². Maximum penetration was observed to occur approximately 1 hr post-contamination, reaching a depth of 2 in. with the bulk of the agent being retained in the top 1/2 inch. It was originally planned to use volunteer personnel to determine the hazard, but, "Due to inability to secure such personnel, the presence or absence of these hazards had to be deduced for correlation of chemical analysis, confined surface vapor tests, spotted dick tests, and biological tests on rabbits." The animals were exposed for 4 hr—either staked to the contaminated terrain or suspended in cages over the contaminated areas; the hair over the contact area was previously shaved. In interpreting final results, humans were considered to be twice as sensitive to H as rabbits. This synopsis will focus on the data most pertinent to persistence and contact hazard.⁶ The details of the tests are somewhat sketchy. Negative reactions were designated "0"; mild reactions were designated "1"; moderate reactions were designated "3"; marked reactions included vesication and were designated "3"; extreme reactions also included vesication and were designated "4". No detailed description of the skin reactions was provided. For the penetration of H in coral: (a) 1/4 rabbits exposed to H vapor and liquid from mine craters 10 days after contamination, died within 96 hr, while the remaining three showed moderate to extreme reactions; (b) 2/4 rabbits exposed to H vapor and liquid 12 days after contamination exhibited marked reactions. Similar testing was performed on beach

⁵ See Appendix A; the disparity in rabbit-human sensitivity may have been underestimated

⁶ See Appendix B for a detailed statistical analysis of the rabbit toxicity data.

sand. Five hours after contamination, 4-hr exposures of rabbits to a contamination density of 300 g/m² of Levinstein H—producing post-contamination vapor and vapor/liquid contact, caused an “extreme reaction”; but, by the end of the first day, similar exposures produced only “moderate” reactions. On the second day post-contamination, the reactions had diminished to “mild”; by the seventh day there were no reactions at all. The rest of the data are given in Tables 5-7. It was found by the authors of the present report (see Appendix B) that the route of exposure (ocular *versus* percutaneous), the type of terrain (sand *versus* coral), the duration of agent aging prior to rabbit exposure, and several interactions between these factors are statistically significant. Of particular significance is that rabbits suffered skin burns in the absence of ocular effects, thus indicating that the eye is not necessarily a good detector for contact hazard. Also, the significant interaction of terrain type with the route of exposure⁷ prevents the general application of the CWB report findings to other exposure scenarios—contact hazard needs to be investigated on a case-by-case basis. The CWB concluded that unprotected troops would sustain casualties from vapor up to 12 hr post contamination and that lethal levels of liquid would be encountered for 3-5 hr post contamination. It was estimated that a casualty-level contact hazard would exist for poorly protected personnel up to 3-4 days—even if bomb/mine craters were avoided, and that the craters were dangerous for up to 14 days. An occupation hazard for unprotected personnel was estimated to persist for ≥2 weeks, and with larger contamination densities the hazard duration increased. Given that rabbits may be more than two-fold less sensitive to H than humans (Eyster and Maver, 1920; Henry 1991), the conclusions of this study might well underestimate the human toxic effects (Appendix A).

Table 5. Effects in Rabbits of Levinstein and “Stripped” H^a on Coral Sand (Chemical Warfare Board, 1944)

Time post-contamination (days)	Initial Contamination Density (g/m ²)							
	50		100		300		300 ^{RHS}	
	Depth of Sample (In.)							
	0-0.5 ^b	0.5-2 ^c	0-0.5 ^b	0.5-2 ^c	0-0.5 ^b	0.5-2 ^c	0-0.5 ^b	0.5-2 ^c
1	1	0	2	1	1	0	3	0
2	1	0	1	0	3	1	3	0
3	—	—	—	—	1	1	1	1

^a Levinstein H—unless designated RHS (stripped mustard)

^b sand scraped off and residual extracted for skin test

^c top 0.5 in of sand removed; rabbit placed in contact with exposed surface

Table 6. Persistency of Levinstein and “Stripped” H^a on Coral Sand—Effects in Rabbits (Chemical Warfare Board, 1944)

Time post-contamination (days)	Initial Contamination Density (g/m ²)							
	50		100		300		300 ^{RHS}	
	eye ^b (vapor)	skin ^c (liq & vapor)	eye ^b (vapor)	skin ^c (liq & vapor)	eye ^b (vapor)	skin ^c (liq & vapor)	eye ^b (vapor)	skin ^c (liq & vapor)
1	0	1	0	2	4	1	4	3
2	0	1	0	1	3	3	3	3
3	0	0	0	0	0	1	0	0
5	0	0	0	0	0	0	1	1

^a Levinstein H—unless designated RHS (stripped mustard)

^b vapor effects determined by staking rabbits in cages 1 ft above contaminated square meter areas

^c rabbits staked to contaminated area

⁷ It was found (see Appendix B) that for eye exposures, vapor off-gassing from sand is more likely to produce a toxic response than off-gassing from coral. However, the reverse is true for direct skin exposures (direct contact with coral is more likely to produce a response than contact with sand).

Table 7. Persistency of Leivinstein and "Stripped" H^a on Coral Sand and Ordinary Beach Sand (Chemical Warfare Board, 1944)

Time post-contamination (days)	Initial Contamination Density (g/m ²)									
	50		100		300			300 ^{RHS}		
	coral sand				beach sand		coral sand			
	eye ^b (vapor)	skin ^c (liq & vapor)	eye ^b (vapor)	skin ^c (liq & vapor)	eye ^b (vapor)	skin ^c (liq & vapor)	eye ^b (vapor)	skin ^c (liq & vapor)	eye ^b (vapor)	skin ^c (liq & vapor)
5 hr	0	—	0	—	4	—	4	—	4	—
1	0	1	0	2	3	4	2	4	2	4
2	0	2	0	2	2	3	1	3	4	3
4	0	0	0	—	—	—	—	—	—	—
7	—	—	0	0	0	0	0	0	0	0

^a Leivinstein H—unless designated RHS (stripped mustard)

^b vapor effects determined by staking rabbits in cages 1 ft above contaminated square meter areas

^c rabbits staked to contaminated area

In the late 1950's and early 1960s, Reich did some of the more seminal contact hazard studies on VX. They were performed on Carroll Island at the Edgewood Area of Aberdeen Proving Ground. Persistency was defined as "an expression of the duration of effectiveness of a war gas, which is dependent on physical and chemical properties of the gas, weather, methods of dissemination, and condition of terrain." The first study (Reich, 1959a) investigated the persistence of V-agent on 23 soil plots, eight sand plots, and nine sod plots. Testing on sod was discontinued when it was observed that the persistency of VX was approximately the same in all three media. Macroburettes, microburettes, or a spinning-tip apparatus were used to apply the agent to test plots in the desired particle sizes and contamination densities. Contamination densities are given in Table 8. At prescribed time intervals after the dispersion of agent, rabbits (clipped on their bellies to produce approximately 50 cm² of bare skin)

Table 8. VX Contamination Densities and Particle Sizes (Reich 1959a)

Terrain	Contamination Density (g/m ²)	Particle Size (mmd: μ)
Soil	0.3 to 31.5	200-4100
Sand	6.5 to 20	200-3600
Sodded	0.3 to 17.2	185-450

were staked⁸ to the contaminated ground for 1 hr. As per lethality data and cholinesterase depression⁹ surface contamination disappeared within days. When agent was disseminated in densities below 7 g/m² and animals were exposed 24 hr post-contamination for 1 hr, there were few fatalities. In sand plots contaminated to ~ 6.5 to 20 g/m² (August to October) all animals died when exposed at 1 and 24 hr post-contamination. Chemical analysis of 0.5-in. deep sand plugs detected residual agent at 1 week, but there were no deaths. Persistence of V-agent in dry sand was greater than in soil or grass sod, which is somewhat paradoxical because agent can be extracted from soil or grass sod for longer periods of time

⁸ The animals were restrained to preclude oral ingestion from contaminated terrain.

⁹ Raw data for cholinesterase inhibition were not given in the report, but they were found in an archived laboratory notebook.

than from sand. Some of the trials on sod were difficult to interpret because half of them were conducted in the winter. However, there are indications that the authors may have been in error about this finding (see Appendix C). In a different set of sod trials aimed at determining transferability of agent to clothing, a test site at Carroll Island was contaminated with densities ranging from approximately 1-4 g/m². Some of the agent was dispersed as 500 μ shattered droplets; the rest was disseminated as 3000 μ free-falling droplets. The persistency was determined under warm, moderately wet weather and is given in Table 9. Several critical observations were made with regard to different soil types and the hazard posed by the residual agent: (a) VX was less persistent on grassy terrain and soil than on sand; (b) residual contamination in the top 0.5-in. layer of soil did not agree with the results of the animal exposures and does not reflect the amount of agent available for transfer; (c) contamination on the surface declined more rapidly and was more adversely affected by weather than the contamination in the top 0.5-in. layer; (d) VX was chemically detectable for weeks after dissemination, but the surface contamination persisted for only a few days; and (e) in soil, the decline in residual contamination was initially rapid and then leveled off asymptotically, with the initial rate of decline being proportional to the contamination density—regardless of the original contamination density, several days after contamination, the hazard was negligible. Reich concluded that if animal data can be assumed to be indicative of occupational hazard (i.e., hazard incident to sitting or lying down in the area for several hours without benefit of protective equipment), the expected duration of such hazard could be from less than 1 day to several days, depending upon the density of contamination. For densities below 2.5 g/m², no appreciable number of fatalities might be expected to result from PC effects suffered by troops entering and occupying the area in somewhat less than 24 hr following contamination. For high densities (10 to 30 g/m²), the area might be safe for continuous occupation sometime after 2 days, but less than a week following contamination. The sod and soil data are summarized in Tables 9 and 10.

Table 9. VX Sod Trials under Warm Conditions (Reich, 1959a)

Trial	Initial Contamination Density (g/m ²)	Particle Size	Residual Surface Contamination (g/m ²)			Rabbit Mortality		
			1 Hr	1 Day	2 Days	1 Hr	1 Day	2 Days
1	8.5	small	1.950	0.111	0.007	---	---	0/2
2	13.9		2.690	0.128	0.008		1/2	
3	6.9		1.300	0.052	0.004		0/2	---
4	3.6		0.890	0.036	0.003			
5	2.2		0.230	---	0.001			
6	10.0	large	1.700	0.080	0	---	0/2	---
7	12.9		2.900	0.116				
8	8.2		0.980	0.054				
9	3.4		1.380	0.010				
10	1.0		0.050	0				

trials 1-5: average temperature 80 °F; 0.26 in rain between 1-hr and 1-day sampling; trace precipitation between 1-day and 2-day sampling with moist terrain during 2-day sampling
trials 6-10: average temperature 77 °F; 1.3 in rain between 1-hr and 1day sampling with moist terrain during 1-day sampling; trace precipitation between 1-day and 2-day sampling

Table 10. VX Soil Trials (Reich, 1959a)

Residual Contamination Density* (g/m ²)	Deaths at Specified Times After Initial Contamination					
	1 Hr		1 Day		1 Week	
< 1.0	10/18	55%	2/18	11%	5/28**	18
1.1-2.0	5/6	83%	0/4	0%	0/4	0%
2.1-3.0	2/2	100%	3/8	38%	1/2	50%
3.1-4.0	6/6		2/8	25%	---	---
4.1-5.0	2/2		---	---	1/2	50%
5.1-6.0			---	---	---	---
6.1-7.0			---	---	---	---
>7.0	6/6	1/6	17%	---	---	
total	35/44	80%	8/44	18%	7/36	19%

*DB3 analysis of soil plugs

**ground temperature >99 °F; other inconsistencies due to high or low temperatures

Reich (1961)¹⁰ compiled and summarized results from a comprehensive study designed to: (1) determine the persistency of VX on materials of construction; (2) evaluate chemical and flame decontamination of VX-contaminated terrain and materials; (3) compare the pick-up of American, British, and Canadian simulants with pick-up of VX from terrain; (4) compare the pick-up of VX on different kinds of cloth; (5) measure the pick-up on troops traversing terrain contaminated with VX; (6) determine the pick-up of VX from snow-covered terrain, and (7) investigate the evaporation rates and dosage production of VX from terrain and materials. The toxicological data will be emphasized herein. Toxicological tests on materials of construction (Table 11) were of 1 hr duration and were conducted on restrained, masked rabbits, with depilated undersides. The exposure paradigm was to expose two animals on test strips contaminated with the lowest density of VX. If no animals died, other pairs of animals were exposed to surfaces contaminated to a higher density. Exposures were continued in this manner until no deaths

Table 11. Persistency of VX on Materials of Construction (Reich, 1961)

Z (days)	Materials* on Which Deaths Occurred at Specified Contamination Densities (g/m ²)								
	Large Particles**					Small Particles***			
	0.7	2.2	3.0	5.8	7.3	5	9	16	45
1 hr	all	all	all	all	all	all	all	all	all
1	APSW	APSW	APSW	APSW	APSW	CP	CP	CP	all
2	P	P	P	P	P	P	P	P	CP
3	P	P	P	P	P	none	P	P	P
4	---	---	---	---	---	---	P	P	P
5	---	---	---	---	---	---	P	P	P
6	none	none	---	---	P	---	---	---	---
7	---	---	none	none	none	---	none	none	P
8	---	---	---	---	---	---	---	---	none

*A = asphalt; C = concrete; P = "painted" wood (type of paint not specified); S = steel; W = unpainted wood

**2.3 mm drops; agent 95% pure

***0.6 mm drops; 75% pure

¹⁰ Methods of contamination were not explicitly stated.

occurred. It was concluded that the persistency of VX on surfaces was more a function of the surface than the contamination density, and large droplets of agent were more persistent than small droplets—on all surfaces except concrete. Based on cholinesterase (ChE) data, which were not given, it was further concluded that at moderate temperatures, VX disseminated in densities of about 5 g/m² could be expected to persist as a contact hazard up to 2 days on concrete and steel, up to 3 days on wood and asphalt, and up to 6 days on painted wood. Toxicological tests for decontamination procedures evaluated chemical (Table 12) and flame decontamination procedures. For the chemical decontaminations the materials were contaminated and then decontaminated 30 min later. The decontaminant was allowed to react for 30 min, and then two restrained rabbits, with depilated undersides, were placed on each surface for 20 min. It appears that the decontaminant was not rinsed off prior to exposing the rabbits, because it was stated that all rabbits suffered edema, erythema, and ulceration from skin exposure to bleach. Bleach slurry was observed to be superior to water and the hypochlorite solution. The toxicological data for flame decontamination were not shown, but it was stated that flame was effective against VX disseminated on vegetated terrain in a density of 5 g/m², but flame did not eliminate the hazards from VX disseminated at a density of 100 g/m²; the mortality fraction was 1/2. Evaporation rate and dosage production were determined in an elaborate set of annulus trials using dogs, mice and rabbits. The annulus shape was selected so that no correction for wind direction would be required when vapor sampling was done at the center of the annulus. The annular areas consisted of grass-covered terrain, bare soil, steel, and concrete. Dogs and rabbits were placed at ground level; mice were suspended in cages at 1, 3, and 5.5 ft. Some of the rabbits were masked to preclude vapor inhalation. The animals were exposed for 12 hr in the center of the annulus; survivors were observed for an additional 12 hr and ChE activity was determined in dogs and rabbits. Bubbler samples for vapor analysis were taken every hour. It was reported that the evaporation rate and dosage production of liquid VX on surfaces were greatly dependent on the type of surface contaminated, as well as on meteorological conditions. The measured vapor dosages were highest from metal surfaces. The recovery of vapor from tall grass was greater than from short grass and bare soil. Very low vapor dosages were obtained from contaminated concrete. In most of the trials, the vapor produced lethality in masked and unmasked animals. LC_{t50s} and probit slopes were not calculated because air moves vertically as well as horizontally, so the animals were most likely exposed to fluctuating vapor concentrations, and for exposures of more than 1 hr, the bubblers indicated that the concentration profile over the exposure was not constant (as in chamber tests), and we do not know how the rapidly fluctuating concentrations affect toxicological parameters of interest (LC_{t50}, probit slope, toxic load exponent, and concentration-time profile).

Table 12. VX Decontamination Studies (Reich, 1961)

Decontaminant	Contamination Density (g/m ²)	Surfaces on Which Indicated Deaths Occurred		
		0/2	1/2	2/2
STB Slurry	3.9	soil, sand, sod, concrete, steel, paint	asphalt, wood*	(none)
	100	soil	wood	sand, sod, asphalt, steel, paint
Ca(ClO) ₂ solution	3.1	soil, sand, sod, steel, wood	paint	asphalt, concrete
H ₂ O	3.5	soil, concrete, steel	sand, sod, wood	asphalt, paint

* No toxic signs were observed; death was attributed to heat prostration.

The Reich data are summarized in Tables 13-21. The entire set of Reich's data is given in Appendix C, which also contains a statistical evaluation of those data (performed for this report). Reich used a statistically poor experimental design, and his findings should be treated with caution and considered to be qualitative (at best). For the most part, the statistical evaluation in Appendix C confirmed Reich's findings in the 1959a and 1961 reports—with some important exceptions: (a) the

persistence of VX in sand may be a greater PC exposure threat than was originally concluded by the Traversal Program; (b) larger VX droplets are less persistent than smaller droplets; and (c) rabbit cholinesterase data from exposed rabbits were nearly worthless due to the large fluctuations of cholinesterase values in the control rabbits. The possible greater persistency of smaller droplets *versus* larger droplets appears somewhat counter-intuitive, since on smooth surfaces (such as glass) smaller droplets evaporate more quickly due to their larger surface-area-to-volume ratios in comparison to larger droplets. However, for the porous substrates tested, the larger area-to-volume ratios of the smaller droplets will have more rapid wicking action and liquid absorption by the substrate. So, in this instance, it could be that the wicking mechanism on the porous substrates is predominant over the evaporation mechanism, resulting in smaller droplets being more persistent than larger droplets.

Bryant (1959) cited some research¹¹ that was somewhat contrary to the Reich reports, stating that soil rapidly neutralizes V-agents, and ground vegetation may absorb them so quickly that "depilated animals are unaffected when brought into contact with it quite soon after the contamination is laid".

Hott and Alexander (1960) performed several VX decontamination studies on Carroll Island. The materials studied were 1 m² areas of soil, grass sod, roofing cement (plastic); old concrete, sand, painted¹² plywood, unpainted plywood and sheet iron. Contamination densities were either 5 g/m² (2.5 mm drops; 66% pure for chemical decontamination; 76.7% pure for flame decontamination) or 100 g/m² (contamination was done with a sprinkling can; agent purity unknown). One half hour post-contamination, some of the surfaces were chemically decontaminated with either super tropical bleach (STB; 40-60 slurry) or 5% calcium hypochlorite (HTH) and water—both at the rate of 1.89 L/m². Some of the sod surfaces were immediately subjected to flame decontamination by spraying 0.95 L/m² gasoline onto the area with subsequent ignition or directing the ignited contents of a portable flame-thrower on the 2 m² of contaminated sod. Toxicity was evaluated with white rabbits clipped in their ventral area, 1 day prior to testing. On the day of testing, the animals were masked and restrained on open frames so that the clipped area pressed against the decontaminated surfaces for a 1-hr exposure. (The area of skin in contact with the surface varied with the weight of the rabbit.) Decontamination efficiency was estimated by comparing the observed toxic responses with a graph relating mg/kg of VX applied to rabbit skin *versus* observed time to death *versus* weight of the rabbit and estimated amount of skin in contact with residual agent. The results are given in Table 22. It was observed that when control animals were exposed to the chemical decontaminant STB slurry, severe, erythema, edema, and ulcerations to the skin resulted. Also, a depilatory action occurred. No other decontaminants produced any damage to the skin. Water was the poorest of the chemical decontaminants, but in comparison to the surfaces without decontamination, water removed or destroyed two-thirds of the effective agent.

In some later studies, Hott and Alexander (1965) investigated the residual contact and vapor hazards from VX-contaminated painted steel, painted wood, and Navy canvas, after decontamination with either simulated sea water and/or calcium hypochlorite. The wood and steel panels painted with one coat of red primer followed by two coats of standard Navy exterior enamel. Prior to contamination, the panels were sprayed with sea water and allowed to dry; they were then contaminated with 50 μm particles at the desired density. Ten minutes post-contamination, the surfaces were decontaminated. New Zealand White rabbits of both genders, clipped in the ventral area 24 hr before exposure, were used for contact testing; they were fitted with hoods to prevent oral ingestion. Male white mice were used for vapor studies. When no deaths occurred, blood samples were taken for ChE determinations. The rabbits were used as a bioassay to estimate the residual contamination. Mortality data were not given nor was the basis for estimating the residual contamination.

¹¹ The data were attributed to WS Ladell in Porton Note 40; the report could not be located.

¹² Type of paint was not specified.

Table 13. VX IH Data: Annulus Trial 2*—Short, Moderately Dense Grass [10.3 g/m²; 2.9 mm particle] (Reich, 1961)

Species	Agent	Exposure Type	Total vapor dosages (mg/m ³) and mortality at different times following ground contamination																	
			1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	15hr	19hr	23hr	wind (mph)	temp (°C)	
mouse (1 ft)	VX	WB	1.26	2.73	3.52	3.85	3.92	3.96	4.01	4.07	4.1									
0/10			0/10	1/10	1/10	2/10	2/10	4/10	7/10	7/10	10/10									
0.6			1.35	1.7	1.86	1.87	1.9	1.91	1.92	1.92	1.93	1.94			1.98	2.05	2.35	1-	13.5-	
0/10			0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10			7/10	8/10	8/10	4.2	17
0.26			0.58	0.68	0.75	0.76	0.79	0.79	0.79	0.79	0.79	0.79	0.79			0.81	0.88	1.07		
0/10			0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10			1/10	1/10	1/10		
rabbit (ground)			1.40	2.87	3.69	4.03	4.19	4.29	4.37	4.41	4.46	4.47	4.48		4.61	4.73	4.9			
0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6		0/6	1/6	1/6	1-	-1.8	
dog (ground)			1.40	2.87	3.69	4.03												1-	1.5	
0/2	0/2	1/2	2/2															3.6		

* 9/29/58

Table 14. VX IH Data: Annulus Trial 3*—Damp Terrain Covered with 8 to 10 in. Grass [9.5 g/m²; 6 mm particle] (Reich, 1961)

Species	Agent	Exposure Type	Total vapor dosages (mg/m ³) and mortality at different times following ground contamination																		
			1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	15hr	19hr	23hr	wind (mph)	temp (°C)		
mouse (1 ft)	VX	WB	3.15	7.69																	
0/10			9/10	10/10																	
3.87			8.26	11.44	12.4																
0/10			3/10	9/10	10/10																
1.12			2.44	3.59	4.05	4.47	4.56	4.66	4.7												
0/10			0/10	2/10	3/10	8/10	8/10	9/10	9/10												
dog (ground)			2.87	6.73	9.12																
0/2	1/2	2/2																			
rabbit masked (ground)		PC vapor	2.87	6.73	9.12	10.09	10.91	11.26	11.54	11.66	11.71										
0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	2/3	3/3											
rabbit unmasked (ground)		WB	2.87	6.73	9.12	10.09	10.91	11.26	11.54	11.66	11.71										
0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/3	2/3	3/3										

* 8/16/60

Table 15. VX IH Data: Annulus Trial 5*—Terrain Scraped Free of Vegetation [9.8 g/m²; 6 mm particle] (Reich 1961)

Species	Agent	Exposure Type	Total vapor dosages (mg/m ³) and mortality at different times following ground contamination													temp (°C)												
			1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	15hr		19hr	23hr	wind (mph)									
mouse (1 ft)	VX	WB	0.17	0.6	0.68	0.73	0.76	0.78	0.79	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80								
mouse (3 ft)			0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10		
mouse (5.5 ft)			0.08	0.35	0.42	0.45	0.47	0.48	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	
dog (ground)			0.05	0.16	0.19	0.20	0.21	0.21	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	
rabbit masked (ground)			0.18	0.62	0.76	0.80	0.84	0.86	0.87	0.87	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	
rabbit unmasked (ground)			0/2	0/2	0/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
			0.18	0.62	0.76	0.80	0.84	0.86	0.87	0.87	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	
			0/3	0/3	1/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3
			0.18	0.62	0.76	0.80	0.84	0.86	0.87	0.87	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	
			0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

* 8/15/60

Table 16. VX IH Data: Annulus Trial 4*—Damp Terrain Covered with 8 to 10 in. Grass [9.8 g/m²; 6 mm particle] (Reich, 1961)

Species	Agent	Exposure Type	Total vapor dosages (mg/m ³) and mortality at different times following ground contamination													temp (°C)												
			1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	15hr		19hr	23hr	wind (mph)									
mouse (1 ft)	VX	WB	1.94	4.3	5.52																							
mouse (3 ft)			3/10	4/10	10/10																							
mouse (5.5 ft)			1.33	2.6	3.52																							
dog (ground)			6/10	9/10	10/10																							
rabbit masked (ground)			0.4	0.97	1.35	1.58	1.77	1.87	1.93	1.97	1.99	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
rabbit unmasked (ground)			2/10	3/10	3/10	4/10	6/10	7/10	7/10	8/10	8/10	9/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
			1.68	3.71	4.83	5.4	6.09	6.47	6.68	6.83	6.88	6.93	6.95	6.95	6.95	6.96	6.96	6.96	6.96	6.96	6.96	6.96	6.96	6.96	6.96	6.96	6.96	
			0/2	0/2	0/2	0/2	1/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
			1.68	3.71	4.83	5.4	6.09	6.47	6.68	6.83	6.88	6.93	6.95	6.95	6.95	6.96	6.96	6.96	6.96	6.96	6.96	6.96	6.96	6.96	6.96	6.96	6.96	
			1/3	1/3	1/3	1/3	1/3	1/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3

* 8/16/60

Table 17. VX IH Data: Annulus Trial 6*—Terrain Scraped Free of Vegetation [11.2 g/m²; 6 mm particle] (Reich, 1961)

Species	Agent	Exposure Type	Total vapor dosages (mg/m ³) and mortality at different times following ground contamination												wind (mph)	temp (°C)			
			1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr			15hr	19hr	23hr
mouse (1 ft)	VX	WB	0.40	0.92	1.36	1.58	1.78	1.88	1.93	1.96	1.97	1.98	1.99	1.99					
mouse (3 ft)			0/10	0/10	0/10	0/10	4/10	6/10	6/10	8/10	8/10	8/10	8/10	8/10	8/10				
mouse (5.5 ft)			0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	4/10	4/10	5/10	6/10	6/10				
dog (ground)			0.14	0.28	0.39	0.47	0.56	0.61	0.63	0.64	0.64	0.64	0.64	0.64	0.64				
rabbit masked (ground)	PC vapor	WB	0.48	0.93	1.31	1.67	1.77	1.88	1.93	1.97	2.01	2.02	2.02	2.02					
rabbit masked (ground)			0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2					
rabbit unmasked (ground)			0.48	0.93	1.31	1.67	1.77	1.88	1.93	1.97	2.01	2.02	2.02	2.02	2.02				
			1/3	1/3	1/3	1/3	1/3	1/3	1/3	1/3	1/3	2/3	2/3	2/3					
			0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3		

*8/17/60

Table 18. VX IH Data: Annulus Trial 7*—Sheet Metal [9 g/m²; 6 mm particle] (Reich, 1961)

Species	Agent	Exposure Type	Total vapor dosages (mg/m ³) and mortality at different times following ground contamination												wind (mph)	temp (°C)			
			1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr			15hr	19hr	23hr
mouse (1 ft)	VX	WB	2.54	9.5	15.4	17.46													
mouse (3 ft)			0/10	0/10	9/10	10/10													
mouse (5.5 ft)			1.83	4.93	8.56	10.25													
dog (ground)			0/10	0/10	6/10	10/10													
rabbit masked (ground)	PC vapor	WB	0.89	2.28	3.67	4.21	4.73												
rabbit masked (ground)			0/10	0/10	1/10	7/10	10/10												
rabbit unmasked (ground)			2.57	8.14															
			0/2	2/2															
			2.57	8.14	14.91	17.33	18.18	18.23	18.28	18.32	18.36	18.4	18.43	18.43					
			0/3	0/3	0/3	1/3	1/3	1/3	1/3	1/3	1/3	2/3	2/3	2/3					
			2.57	8.14															
			0/3	3/3															

*8/15/60

22.2-
40.6

1.4-
6.1

Table 19. VX IH Data: Annulus Trial 8*—Sheet Metal [11.6 g/m²; 6 mm particle] (Reich, 1961)

Species	Agent	Exposure Type	Total vapor dosages (mg/m ³) and mortality at different times following ground contamination												temp (°C)											
			1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr		15hr	19hr	23hr	wind (mph)							
mouse (1 ft)	VX	WB	1.95	5.48	10.37																					
mouse (3 ft)			7/10	9/10	10/10																					
mouse (5.5 ft)			1.24	2.6	5.54	6.86																				
dog (ground)	PC vapor	WB	0.52	1.09	2.2	3.13																				
rabbit masked (ground)			0/10	1/10	1/10	1/10																				
rabbit unmasked (ground)			1.94	4.45	10.24	16.56	22.22	24.4	25.2																	
			0/2	0/2	1/2	2/2																				
			1.94	4.45	10.24	16.56	22.22	24.4	25.2																	
			0/3	0/3	0/3	0/3	0/3	0/3	0/3																	
			1.94	4.45	10.24	16.56	22.22	24.4	25.2																	
			0/3	0/3	0/3	1/3	2/3	2/3	3/3																	

* 8/17/60

Table 20. VX IH Data: Annulus Trial 9*—Concrete [3.7 g/m²; 6 mm particle] (Reich, 1961)

Species	Agent	Exposure Type	Total vapor dosages (mg/m ³) and mortality at different times following ground contamination												temp (°C)											
			1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr		15hr	19hr	23hr	wind (mph)							
mouse (1 ft)	VX	WB	0.02	0.05	0.08	0.09	0.12	0.15	0.17	0.19	0.02	0.22	0.23	0.24												
mouse (3 ft)			0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10											
mouse (5.5 ft)			0.02	0.05	0.08	0.10	0.13	0.15	0.16	0.17	0.19	0.21	0.21	0.22	0.24											
dog (ground)	PC vapor	WB	0.02	0.04	0.06	0.08	0.10	0.12	0.13	0.14	0.15	0.16	0.18	0.19												
rabbit masked (ground)			0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10											
rabbit unmasked (ground)			0.03	0.07	0.09	0.13	0.16	0.18	0.21	0.22	0.24	0.25	0.27	0.28												
			0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2												
			0.03	0.07	0.09	0.13	0.16	0.18	0.21	0.22	0.24	0.25	0.27	0.28												
			0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3												
			0.03	0.07	0.09	0.13	0.16	0.18	0.21	0.22	0.24	0.25	0.27	0.28												
			0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3												

* 9/21/60

Table 21. VX IH Data: Annulus Trial 10*—Concrete [5.7 g/m³; 6 mm particle] (Reich, 1961)

Species	Agent	Exposure Type	Total vapor dosages (mg/m ³) and mortality at different times following ground contamination													wind (mph)	temp (°C)				
			1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	15hr			19hr	23hr		
mouse (1 ft)	VX	WB	0.08	0.18	0.31	0.5	0.66	0.77	0.86	0.93	1.01	1.08	1.14	1.14							
			0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10	1/10						
mouse (3 ft)			0.05	0.13	0.21	0.29	0.38	0.43	0.50	0.55	0.61	0.67	0.72	0.72	0.78						
			0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10	2/10						
mouse (5.5 ft)			0.06	0.14	0.22	0.33	0.4	0.47	0.52	0.54	0.58	0.62	0.67	0.72	0.72						
			0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10						
dog (ground)			0.08	0.22	0.41	0.65	0.79	0.88	0.94	1.01	1.07	1.13	1.18	1.18	1.24						
			0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2						
rabbit masked (ground)			0.08	0.22	0.41	0.65	0.79	0.88	0.94	1.01	1.07	1.13	1.18	1.18	1.24						
			0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3						
rabbit unmasked (ground)			0.08	0.22	0.41	0.65	0.79	0.88	0.94	1.01	1.07	1.13	1.18	1.18	1.24						
			0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3						

* 9/21/60

Table 22. Decontamination Efficacy (Hott and Alexander, 1960)

Species	Agent	Surface	Decon	Contam (g/m ²)	t (hr)	Temp (°F)	Mortality
rabbit	VX	Soil	STB		1	71-88	0/2
		Steel					
		Concrete					
		Sand					
		Sod					
		Paint					
		Wood*					
		Tar*					
		Soil	HTH	5			0/2
		Steel					2/2
		Concrete					0/2
		Sand					1/2
		Sod					0/2
		Paint					2/2
		Wood					0/2
		Tar					2/2
		Soil	Water				0/2
		Steel					1/2
		Concrete					2/2
		Sand					1/2
		Sod					2/2
		Paint					0/2
		Wood					2/2
		Tar					0/2
		Soil	STB	100			2/2
		Steel					1/2
		Concrete					2/2
		Sand					0/2
		Sod					0/2
		Paint					1/2
Wood	2/2						
Tar	0/2						
Sod	Gas	5	0/2				
	Napalm		0/2				
	Gas	100	1/2				
	Napalm		2/2				

t = exposure duration

* death due to heat prostration

The purpose of a 1964 study by Koblin *et al.* was to determine the degree of risk from surfaces and material that had been declared "clean". The surfaces used were painted steel, painted wood paneling, and water-proofed canvas. The decontamination procedures were geared to efficiently washing down contaminated ships. Fifteen minutes after decontamination, two rabbits, with ventral sides clipped, were pressed against the surface for 1 hr—unless they died sooner. If no deaths occurred, additional rabbits were tested at 90 and 180 min post-decontamination. Cholinesterase activity was determined after the rabbits were removed from the surface, but those data were not given. The vapor hazard to mice was determined if there were no rabbit deaths. In these tests, mice were placed in boxes 1 in. above the panels for 1 hr. In both cases, each time animals were tested, a sample of the surface was removed for chemical analysis. Vapor concentrations were determined with bubblers. Contact hazard was less for the canvas than for the painted steel and metal surfaces. Surface contamination could not

be detected with rollers, and it was concluded that contact hazard resulted from agent within the paint and a residual contamination density of 0.1 g/m² would not represent a hazard to a man dressed in two layers of clothing over a period of at least 1 hour. Although this statement is apparently supported by the data, the basis of this determination was not stated. Similarly, many of the tables in the document give "effective contamination" after decontamination, and the basis for these values is not explicitly given. (It is inferred that it may be based on ChE determinations and/or deaths, but insufficient information is given to verify/evaluate the potential contamination densities.) The data are given in Table 23.

Table 23. Exposure to Decontaminated Surfaces (Koblin et al, 1964)

Species	Agent	Material	Exposure Type	L (g/m ²)	t (hr)	Z (hr)	Mortality (time of exposure)
rabbit ^a	VX ^a	Canvas	PC	1.3	1	+3	2/2 (15 min) 2/2 (90 min)
		Metal		1.0			
		Wood		2.0			
	VX ^b	Canvas		2.3			
		Metal		2.6			
		Wood		0.8			
	VX ^c	Canvas		7.8		+0.25	0/2 (15 min)
		Metal		10.9			2/2 (15 min) 1/2 (90 min)
		Wood		9.8			0/2 (15 min)
	VX ^d	Canvas		7.3		2/2 (15 min) 1/2 (90 min)	
		Metal		5.6		2/2 (15 min) 1/2 (90 min)	
		Wood		9.7		2/2 (15 min) 2/2 (90 min)	
	VX ^e	Metal		1.8		0/2 (15 min)	
		Wood		1.5			
		Metal		4.4			
		Wood		3.6			
	VX ^f	Metal		3.8		2/2 (15 min)	
		Wood		3.3			
mouse ^b	VX ^e	Metal	IH	7.0	1	+0.25	7/10 (15 min)
		Wood		4.0			9/10 (15 min)
	VX ^g	Canvas		7.3			0/10 (90 min)
	Metal	4.4					
	VX ^h	Wood		3.6			0/10 (15 min)
		Metal		3.8			
		Metal		3.3			

Z = time zero for contamination; L = liquid; V = vapor; t = exposure duration

^a clipped white rabbits

^b white mice

^c Trial 1: no decontaminant was sprayed on panels

^d Trial 2: decontaminated panels with 60 gal/m² of ocean water at 30 psi

^e Trial 3: decontaminated panels with 60 gal/m² of bleach solution and ocean water after 10 min

^f Trial 4: decontaminated panels with 3 ten-minute applications of 60 gal/m² ocean water

^g Trial 5: decontaminated panels with 3 ten-minute applications of 60 gal/m² bleach solution

^h Trial 6: decontaminated panels with 3 ten-minute applications of 80 gal/m² ocean water, 60 gal/m² bleach solution and 60 gal/m² ocean water.

Manthei *et al.* (1983, 1985, 1986, and 1988) performed some of the more seminal studies on contact hazard. In large measure, the studies were designed to test the two models for contact hazard—vapor *versus* pseudo-liquid partitioning. The theory was that if partitioning did not occur the wound resulting from direct contact would be of equal or similar severity to that produced by vapor exposure. Manthei *et al.* also hypothesized that a vapor-induced wound would cover a larger area and be less severe than a wound induced by direct contact—in which the agent would be concentrated in a smaller area. In all four studies, factorial experimental designs were used, but experimental logistics precluded randomization of the experimental runs. Detailed statistics, such as analysis of variance (ANOVA) were precluded by the limitations of the software available at the time and the sheer volume of data. [Indeed, performing ANOVAs on all of these datasets for this report is not practical; however, ANOVAs were performed on some of the data from the 1988 study (Appendix D).] If randomization and ANOVAs had been used, the statistical mathematics would have amplified the findings and impact of these studies (see Appendix D). However, as long as these facts are considered when reviewing the data from these studies, useful conclusions can still be drawn. Additionally, none of the four studies made a serious attempt to translate the observed dose-response curves in the test animals into a corresponding expected human dose-response curve. Instead, the animals were primarily CW agent detectors, with the degree/severity of the toxicological response being the “signal”. The scoring system used by Manthei *et al.* (1983, 1986, 1988) is given in Table 25. For these three studies, the same types of alkyd and polyurethane paint were used; however, the cure times for the paint—prior to agent contamination, varied among the studies. Also, in the 1983 and 1988 studies, the alkyd paint was exposed to 40 hr of sunlight during the curing process. Table 24 provides a summary of the curing conditions for these three studies. It is noted that care must be exercised in reaching any conclusion about the superiority of one type of paint over the other. As noted by Manthei *et al.* (1983), this is particularly true for painted surfaces that have not been completely cured prior to contamination. The studies will be presented and discussed individually.

Table 24. Scoring System for Skin Reactions (Manthei *et al.*, 1983, 1986, 1988)

Erythema and Eschar Formation	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formations (injuries in depth)	4
Edema Formation	Value
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Table 25. Summary of Paints and Agents Studied by Manthei *et al.* (1983, 1985, 1988)

Year	Paint	Curing	Agent(s)	“Doses” (mg)
1983	polyurethane	30 days	HD, THD	0.5, 5.0, 25.0
	alkyd	90 days w/40 hr sun		
1985	polyurethane	1 yr	VX, TVX	
	alkyd	1 yr w/40 hr sun		
1988	polyurethane	1 yr	HD	
	alkyd	1 yr		

In Manthei *et al.* (1983) HD or THD was deposited as a 25.0, 5.0, or 0.5 mg drop onto 1 x 2 inch metal plates coated with either alkyd or polyurethane paint. With some exceptions (as noted) the plates were "aged" for 30 min post-contamination, and then rinsed with a solvent¹³ to simulate/effect decontamination, aged¹⁴ again, and then placed either (a) directly onto the clipped dorsal skin of a rabbit or (b) put into a template and affixed to the rabbit such that the plate was 1 cm above the skin.¹⁵ Some of the plates were chemically analyzed for residual mustard following decontamination or when they were removed from the rabbits. Control test plates were contaminated as above and were not solvent rinsed. The plates were left in place for 15 or 60 min. Skin irritation was evaluated at 24, 48, and 72 hr according to 16 CFR 1500.41 and the Draize technique (Table 25). The values assigned to the two types of skin reaction are given below. A total score (the primary irritation index, PII) is calculated using Equation 1, with S being the individual erythema/eschar or edema values (using the scale in Table 25) at 24 and 72 hr post-exposure. Final scores (PII) were based upon the average of six rabbits per group, with a maximum possible score of eight. A score of ≥ 5.0 was interpreted as indicating primary skin irritation;

$$PII = \frac{\{S_{[erh,24hr]} + S_{[erh,72hr]}\}}{2} + \frac{\{S_{[edm,24hr]} + S_{[edm,72hr]}\}}{2} \quad (1)$$

2.0-4.99 was interpreted as moderate skin irritation; 0.01-1.99 was interpreted as mild skin irritation. At 72 hr post-exposure, the diameters of the lesions were measured and averaged for each group of six test animals. HD did not spread over the surface alkyd-painted plates—as it did on the polyurethane-painted plates (Table 26), and the plates were not dry after the 30-min aging; the polyurethane plates were described as "dry", "damp", or "damp-wet". As indicated in Tables 27 and 28, there was little difference in the toxic responses between polyurethane- and alkyd-painted plates not rinsed with isopropyl alcohol (IPA), and at the higher contamination densities there was little difference between direct and vapor contact. IPA was more efficient in removing HD from polyurethane paint than alkyd paint, and on the latter surface direct contact was more significant than vapor contact at the lower contamination densities. Unfortunately the experimental design was not balanced with regard to testing the different paint surfaces or fielded decontaminants. There were three test conditions in which toxic effects were observed and no HD could be detected. Studies were also done with thickened mustard (THD). When deposited on metal plates THD did not spread as did HD. The degree of spread was estimated as 5-7% *versus* 45% for polyurethane-painted plates and only 5% on alkyd-painted plates.

Table 26. Spread and Residual HD on Different Paint Surfaces (Manthei *et al.*, 1983)

Paint	HD (mg)	Mean Area Covered (%)	Range (%)
polyurethane	25.0	45.0	15-80
alkyd		5.0	—
polyurethane	5.0	15.3	5-30
alkyd		2-3	—
polyurethane	0.5	4.5	3-5
alkyd		<1	—

¹³ No measurements were made to account for agent evaporation off the plates, and the amount of agent removed by the solvent was not measured.

¹⁴ Aging durations were varied and are noted in tables.

¹⁵ Both preparations were occluded to prevent agent loss.

Table 27. Summary Skin Irritation Scores for HD (Manthei *et al.*, 1983)

Paint	Paradigm	Skin Irritation Score					
		25.0 mg HD		5.0 mg HD		0.5 mg HD	
		Vapor	Direct	Vapor	Direct	Vapor	Direct
polyurethane alkyd	30-min age; no rinse; 60-min exposure	7.46	7.95	7.25	7.45	3.13	7.21
		7.46	7.29	7.25	7.38	5.71	7.29
polyurethane alkyd	30-min age; IPA rinse; 60-min exposure	0.92	2.75	0	0	0	0
		6.59	6.88	6.12	7.54	2.40	6.59
polyurethane	no rinse; 60-min age; 60-min exposure	—	—	—	—	4.84	4.71
	no rinse; 180-min age; 60-min exposure	—	—	—	—	0.09	4.79
alkyd	30-min age; IPA rinse; 15-min age; 15-min exposure	5.08	7.42	2.88	6.0	0	5.38
	30-min age; IPA rinse; 15-min age; 60-min exposure	5.50	7.33	3.09	6.75	0.55	7.09
	30-min age; IPA rinse; 5-hr age; 15-min exposure	1.25	6.66	0	4.80	0	1.13
	30-min age; IPA rinse; 5-hr age; 60-min exposure	5.42	7.34	0.30	6.54		6.42

The THD findings (Tables 29 and 30) paralleled those for HD; however, the irritation scores were slightly less and the damage areas were somewhat larger. Manthei *et al.* (1983) concluded that both the vapor and pseudo-liquid models were necessary to describe the observed toxic effects.

Table 28. 72-Hr Damage Area—HD-Contaminated Plates (Manthei et al., 1983)

Paint	Paradigm	HD (mg)	Contact	Damage Area (in. ²)			Residual (mg)	
				Eschar	Erythema	Edema		
polyurethane alkyd	30-min age; no rinse; 60-min exposure	25.0	direct	1.33	5.46	8.75	---	
				1.29	4.74	> 4.74		
			vapor	3.71	7.63	10.54		
				2.97	4.97	> 4.97		
		5.0	direct	1.14	3.50	5.38		
				1.15	2.67	> 2.67		
			vapor	2.25	5.88	6.88		
				2.02	3.82	> 3.82		
		0.5	direct	0.12	0.64	0.69		
0.31	0.74			> 0.74				
vapor	0.63		1.83	2.00				
	0.25		2.05	> 2.05				
polyurethane alkyd	30-min age; IPA rinse; no age; 60-min exposure	25.0	direct	0	0	0	0	
				0.310	1.719	2.552		0.6375
			vapor	0	0	0		0
				0.802	4.729	5.641		0.5088
		5.0	direct	0	0	0.0		0
				0.063	0.898	1.346		0.5715
			vapor	0	0	0.0		0
				0.065	2.797	3.365		0.3962
		0.5	direct	0	0	0.0		0
				0.045	0.419	0.495		0.0275
			vapor	0	0	0.0		0
				0.013	0.819	0.694		0.0603
alkyd	30-min age; IPA rinse; 15-min age; 60-min exposure	25.0	direct	0.211	1.125	1.781	0	
			vapor	1.242	3.761	3.448		
		5.0	direct	0.091	0.610	0.818	---	
			vapor	0	0.632	0.519		
		0.5	direct	0.063	0.331	0.331		
			vapor	0	0.039	0.00		
	30-min age; IPA rinse; 15-min age; 15-min contact	25.0	direct	0.245	0.581	0.821	0.6375	
			vapor	0.229	1.432	1.391	0.5088	
		5.0	direct	0.024	0.355	0.355	0.5715	
			vapor	0	0.724	0.594	0.3962	
		0.5	direct	0.016	0.115	0.132	0.0275	
			vapor	0	0	0	0.0603	
	30-min age; IPA rinse; 5-hr age; 60-min contact	25.0	direct	0.407	0.729	1.469	---	
			vapor	0.438	1.750	2.313		
		5.0	direct	0.104	0.250	0.274		
			vapor	0	0	0		
		0.5	direct	0.066	0.157	0.157		
			vapor	0	0	0		
30-min age; IPA rinse; 5-hr age; 15-min contact		25.0	direct	0.108	0.305	0.370		
			vapor	0.00	0.313	0.500		
	5.0	direct	0.025	0.135	0.156			
		vapor	0	0	0			
0.5	direct	0.005	0.011	0.011				
	vapor	0	0	0				
polyurethane	180-min age; no rinse; 60-min contact	0.5	direct	0	0.206	0.206	0.181	
			vapor	0	0	0	0.0293	

Table 29. Studies with THD (Manthei et al., 1983)

Paint	Paradigm	Skin Irritation Score	
		25.0 mg THD	
		Vapor	Direct
polyurethane	30-min age; no rinse; 60-min exposure	7.04	7.50
alkyd		7.08	7.67
polyurethane	30-min age; acetone rinse; 15-min age; 60-min exposure	0	0
alkyd		6.88	4.75
polyurethane	30-min age; acetone rinse; 5-hr age; 15-min exposure	0	0
alkyd		0	4.04
polyurethane	30-min age; acetone rinse; 5-hr age; 60-min exposure	0	0
alkyd		2.50	6.33

Table 30. 72-Hr Damage Area—THD-Contaminated Plates (Manthei et al., 1983)

Paint	Paradigm	THD (mg)	Contact	Damage Area (in. ²)		
				Eschar	Erythema	Edema
polyurethane	30-min age; no rinse; 60-min exposure	25.0	direct	1.96	4.40	7.50
alkyd				1.73	3.87	6.67
polyurethane			vapor	1.96	5.02	9.02
alkyd				1.67	4.37	5.77
polyurethane	30-min age; acetone rinse; 15-min age; 60-min exposure		direct	0	0	0
alkyd				0.261	1.225	1.370
polyurethane			vapor	0	0	0
alkyd				0.42	1.677	1.833
polyurethane	30-min age; acetone rinse; 15-min age; 15-min exposure	direct	0	0	0	
alkyd			0.065	0.242	0.440	
polyurethane		vapor	0	0	0	
alkyd			0	0	0	
polyurethane	30-min age; acetone rinse; 5-hr age; 60-min exposure	direct	0	0	0	
alkyd			0	0.594	0.724	
polyurethane		vapor	0	0	0	
alkyd			0	0.469	0.219	

Manthei et al. (1985) was also designed to test the two contact hazard models, and the experimental paradigm was virtually identical to that used above. It was hypothesized that if the number of deaths, toxic signs, and/or whole blood cholinesterase inhibition were greater for direct contact—rather than vapor contact, there was strong support for the liquid-vapor transport theory. However, if the results were the same for direct and vapor contact, then the hazard would be more related to vapor transport. The test agents were VX and thickened VX (TVX). The rabbits were observed for toxic signs during and after exposure. The spread of VX was not consistently greater on the polyurethane-painted plates—as had been observed above for HD, the spread of TVX was less than that for VX (Table 31). Contrary to what was observed for HD, VX dried somewhat more on the alkyd than the polyurethane plates; TVX did not dry on either paint. After rinsing with IPA, significantly more agent was retained on alkyd than polyurethane paint. Manthei et al. (1985) noted that based upon the amount of VX remaining on the plates, the toxic responses would have been expected to be worse for the alkyd plates, and this was not necessarily the case. In fact, for vapor contact, the alkyd surfaces produced less severe effects. It was concluded (for vapor contact) that the agent contained in the alkyd paint was not able to flux from the surface at a sufficient rate to present the same level of hazard as direct contact or the 1 cm air barrier was sufficient to significantly reduce the vapor hazard and that both transfer models—vapor and pseudo-liquid were involved in this exposure paradigm.

Table 31. Spread of VX and TVX on Different Paint Surfaces (Manthei et al., 1985)

Paint	Agent	Agent Applied (mg)	Mean Area Covered (%)
polyurethane	VX	25.0	86
alkyd			91
polyurethane		5.0	58
alkyd			51
polyurethane		0.5	25
alkyd			11
polyurethane	TVX	25.0	20
alkyd			15

Table 32. Contact Hazard of Polyurethane and Alkyd Paint (Manthei et al., 1985)

Paint	Paradigm	VX (mg)	Contact	# Responding		% ChE Inhibition (whole blood)	
				Toxic Signs	Death		
polyurethane	30-min age; IPA rinse; 15-min exposure	25.0	direct	6/6	0/6	56.3	
alkyd							75.2
polyurethane			vapor	1/6		3.8	
alkyd				3/6		54.4	
polyurethane		5.0	direct	4/6			43.4
alkyd				5/6		82.2	
polyurethane			vapor	0/6		1.1	
alkyd						44.7	
polyurethane		0.5	direct	2/6			9.2
alkyd						37.7	
polyurethane			vapor	0/6		0.2	
alkyd						12.6	
polyurethane	30-min age; IPA rinse; 5-hr age; 15-min exposure	25.0	direct	3/6	0/6	71.9	
alkyd						6/6	73.5
polyurethane			vapor	0/6		66.2	
alkyd				1/6		53.4	
polyurethane		5.0	direct	5/6			58.6
alkyd				6/6		63.4	
polyurethane			vapor			23.6	
alkyd						24.9	
polyurethane		0.5	direct	0/6			66.2
alkyd						8.8	
polyurethane			vapor			14.6	
alkyd						5.8	

Table 33. Residual Agent on Test Plates—Control and 60-Min Exposures (Manthei et al., 1985)

Paint	Agent	"Dose" (mg)	Residual Agent (mg)															
			30-min age no rinse				30-min age w/ rinse				30-min age w/ rinse + 15-min age				30-min age w/ rinse + 5-hr age			
			control	exposed		control	exposed		control	exposed		control	exposed		control	exposed		
				direct	vapor		direct	vapor		direct	vapor		direct	vapor		direct	vapor	
polyurethane	VX	25.0	21.485	2.232	9.414	0.413	0.165	0.247	0.275	0.310	0.297	0.236	0.129	0.140				
		5.0	4.652	0.985	3.981	0.181	0.124	0.148	0.213	0.070	0.164	0.168	0.110	0.070				
		0.5	0.416	0.089	0.383	0.005	0.002	0.006	0.002	0.002	0.012	0.003	0.006	0.003				
alkyd	TVX	25.0	20.022	2.051	16.578	0.008	0.009	0.009	0.016	0.016	0.016	0.021	0.021	0.018				
		25.0	17.439	10.313	14.200	1.684	1.346	1.710	1.662	1.572	1.638	1.679	1.585	1.314				
	5.0	4.155	4.594	5.012	0.850	0.877	0.870	0.860	0.737	0.819	0.658	0.705	0.568					
	0.5	0.384	0.292	0.318	0.171	0.162	0.182	0.112	0.125	0.132	0.094	0.076	0.095					
	TVX	25.0	18.833	6.336	15.266	0.199	0.230	0.240	0.212	0.186	0.205	0.090	0.098	0.115				

Table 34. Residual Agent on Test Plates—Control and 15-Min Exposures (Manthei et al., 1985)

Paint	Agent	"Dose" (mg)	Residual Agent (mg)											
			30-min age w/ rinse + 15-min age				30-min age w/ rinse + 5-hr age							
			control	exposed		control	exposed		control	exposed		control	exposed	
				direct	vapor		direct	vapor		direct	vapor		direct	vapor
polyurethane	VX	25.0	0.301	0.415	0.385	0.132	0.129	0.140						
		5.0	0.254	0.244	0.201	0.082	0.110	0.070						
		0.5	0.005	0.001	0.005	0.001	0.006	0.003						
alkyd	TVX	25.0	0.012	0.007	0.017	0.012	0.014	0.017						
		25.0	2.376	1.769	2.015	1.314	1.330	1.382						
		5.0	1.020	0.845	0.946	0.621	0.571	0.573						
	TVX	0.5	0.156	0.091	0.127	0.083	0.086	0.080						
	TVX	25.0	0.095	0.086	0.083	0.099	0.093	0.098						

Table 35. Contact Hazard of VX on Polyurethane and Alkyd Paint (Manthei *et al.*, 1985)

Paint	Paradigm	VX (mg)	Contact	# Responding		% ChE Inhibition (whole blood)	
				Toxic Signs	Death		
polyurethane	30-min age; no rinse; 60-min exposure	25.0	direct	6/6	6/6	100	
alkyd						99.9	
polyurethane			vapor		0/6	77.6	
alkyd						9.4	
polyurethane		5.0	direct	6/6	6/6	100	
alkyd						99.5	
polyurethane			vapor		0/6	40.5	
alkyd						22.6	
polyurethane		0.5	direct	6/6	6/6	100	
alkyd						56.0	
polyurethane			vapor		0/6	12.4	
alkyd						12.8	
polyurethane	30-min age; no rinse; 60-min exposure	25.0	direct	6/6	0/6	47.8	
alkyd						74.7	
polyurethane			vapor			4/6	24.1
alkyd							9.0
polyurethane		5.0	direct	6/6		6/6	49.8
alkyd							73.6
polyurethane			vapor			6/6	40.1
alkyd							16.3
polyurethane		0.5	direct	6/6		2/6	6.1
alkyd							28.7
polyurethane			vapor			1/6	23.0
alkyd							18.2
polyurethane	30-min age; IPA rinse; 15-min age; 60-min exposure	25.0	direct	6/6	0/6	71.0	
alkyd						75.2	
polyurethane			vapor			3/6	7.0
alkyd							54.2
polyurethane		5.0	direct	6/6		5/6	11.3
alkyd							82.2
polyurethane			vapor			0/6	11.4
alkyd							44.7
polyurethane		0.5	direct	6/6		1/6	4.6
alkyd							82.2
polyurethane			vapor			0/6	12.0
alkyd							12.6
polyurethane	30-min age; IPA rinse; 5-hr age; 60-min exposure	25.0	direct	6/6	0/6	25.2	
alkyd						60.2	
polyurethane			vapor			0/6	10.8
alkyd							8.8
polyurethane		5.0	direct	6/6		2/6	18.6
alkyd							60.3
polyurethane			vapor			1/6	0.9
alkyd							9.4
polyurethane		0.5	direct	6/6		0/6	0
alkyd							19.1
polyurethane			vapor			0/6	6.0
alkyd							12.6

Table 36. Contact Hazard of TVX on Painted Steel (Manthei et al., 1985)

Paint	Paradigm	TVX (mg)	Contact	# Responding		% ChE Inhibition (whole blood)
				Toxic Signs	Death	
polyurethane alkyd	30-min age; no rinse; 60-min exposure	25.0	direct	6/6	6/6	99.6
polyurethane alkyd			vapor	5/6	0/6	100.0
polyurethane alkyd	30-min age; acetone rinse; 60-min exposure	25.0	direct	0/6	0/6	33.2
polyurethane alkyd			vapor			12.0
polyurethane alkyd	30-min age; acetone rinse; 15-min age; 60-min exposure	25.0	direct	0/6	0/6	0
polyurethane alkyd			vapor			2.0
polyurethane alkyd	30-min age; acetone rinse; 15-min age; 60-min exposure	25.0	direct	0/6	0/6	12.6
polyurethane alkyd			vapor	1/6		7.3
polyurethane alkyd	30-min age; acetone rinse; 15-min age; 15-min exposure	25.0	direct	0/6	0/6	5.2
polyurethane alkyd			vapor	1/6		0
polyurethane alkyd	30-min age; acetone rinse; 5-hr age; 60-min exposure	25.0	direct	0/6	0/6	7.4
polyurethane alkyd			vapor			2.8
polyurethane alkyd	30-min age; acetone rinse; 5-hr age; 15-min exposure	25.0	direct	0/6	0/6	6.9
polyurethane alkyd			vapor			10.5
polyurethane alkyd	30-min age; acetone rinse; 5-hr age; 15-min exposure	25.0	direct	0/6	0/6	28.0
polyurethane alkyd			vapor			2.6
polyurethane alkyd	30-min age; acetone rinse; 5-hr age; 15-min exposure	25.0	direct	0/6	0/6	11.0
polyurethane alkyd			vapor			0.8

Manthei et al. (1986) performed tests similar to above using concrete, Plexiglas™, and XM40 nylon carrier test plates. The underlying hypothesis was that if animals were exposed to agent vapor and to direct contact with surfaces contaminated with HD, the toxicological response would be more severe if it involved partitioning of a pseudo-liquid from direct contact. The rationale was to select a low sorbency material, a high sorbency material, and a cloth. If both conditions produced equivalent responses, then it was mediated by vapor.¹⁶ Test plates measured 1 x 2 in. and were contaminated with single drops of HD weighing 25, 5, or 0.5 mg. All plates were aged for 30 min; some were decontaminated with IPA. It was stated that there was very little spread of HD on the plates, and it did not dry. Exposure durations were either 5 or 60 min. Skin irritation was scored as above (Table 25). Following removal from the animals all of the plates were analyzed for residual HD. Extraction of HD—following a 24-hr soak in diethyl phthalate averaged >100% for 25-mg contamination, ~ 93% for 5-mg contamination, and ~ 61% for 0.5-mg contamination. Toxicity and HD recovery data for Plexiglas™ are given in Table 37. Contrary

¹⁶ Manthei et al. (1983) showed that both conditions could apply for the experimental paradigms used therein.

Table 37. HD on Plexiglas™—Direct and Vapor Contact (Manthei et al., 1986)

Contact Exposure		HD (mg)											
		25.0				5.0				0.5			
Route	Conditions	Recovery (mg) [%]	Edema (in. ²)	Irritation Index	Recovery (mg) [%]	Edema (in. ²)	Irritation Index	Recovery (mg) [%]	Edema (in. ²)	Irritation Index	Recovery (mg) [%]	Edema (in. ²)	Irritation Index
direct	30-min age; no rinse;	15.3**	13.96	7.75	3.4**	4.13	7.92	0.136**	2.38	7.75	0.136**	2.38	7.75
	60-min exposure	[61.3]			[68.3]			[27.2%]					
vapor		12.2**	10.54	7.33	4.0**	8.67	6.92	0.096**	3.69	6.54	0.096**	3.69	6.54
		[48.9]			[79.7]			[19.1%]					
direct	30-min age; IPA rinse;	below	0	0	below	0	0	below	0	0	below	0	0
vapor	60-min exposure	detection			detection			detection			detection		
		limits*			limits*			limits*			limits*		

* < 0.0016 mg/sample; **24-hr soak

Table 38. HD on Concrete—Direct and Vapor Contact (Manthei et al., 1986)

Contact Exposure		HD (mg)											
		25.0				5.0				0.5			
Route	Conditions	Recovery [%]	Edema (in. ²)	Irritation Index	Recovery [%]	Edema (in. ²)	Irritation Index	Recovery [%]	Edema (in. ²)	Irritation Index	Recovery [%]	Edema (in. ²)	Irritation Index
direct	30-min age; no rinse; 60-min exposure	15.2 ^a	3.13	7.59	11.4% ^a	3.17	7.91	7.7%	0.54	7.75	7.7%	0.54	7.75
vapor		15.1 ^a	7.38	7.79	59.0% ^b	5.14	7.71	32.0% ^b	0.41	6.25	6.7%	0.41	6.25
direct	30-min age; IPA rinse; 60-min exposure	46.7 ^c	3.64	7.83	13.0% ^a	1.23	7.92	26.3%	0.42	7.09	26.3%	0.42	7.09
vapor		51.8 ^c	5.76	7.79	55.5% ^b	3.64	7.67	28.8%	0.20	4.58	28.8%	0.20	4.58
direct	30-min age; IPA rinse; 15-min age; 60-min exposure	24.6 ^c	2.28	7.84	20.0%	1.00	7.92	18.5%	0.35	7.92	18.5%	0.35	7.92
vapor		29.4 ^c	4.63	7.67	24.5%	5.19	7.71	18.7%	0.57	5.71	18.7%	0.57	5.71
direct	30-min age; IPA rinse; 15-min age; 15-min exposure	28.8 ^b	2.09	7.42	22.7% ^b	1.10	8.00	16.0%	0.18	6.58	16.0%	0.18	6.58
vapor		35.6 ^b	6.54	7.84	24.1% ^b	3.87	7.42	21.0%	0.10	2.00	21.0%	0.10	2.00
direct	30-min age; IPA rinse; 5-hr age; 60-min exposure	11.5 ^b	2.62	7.75	8.8%	1.14	7.75	4.3%	0.33	5.84	4.3%	0.33	5.84
vapor		14.9 ^b	5.76	6.79	10.9% ^b	1.28	6.12	4.5%	0	0	4.5%	0	0
direct	30-min age; IPA rinse; 5-hr age; 15-min exposure	11.8 ^b	1.09	7.55	8.3%	0.17	7.25	11.9%	0	0	11.9%	0	0
vapor		10.4 ^b	0.74	7.42	8.5%	0	0	13.0%	0	0	13.0%	0	0

^a72-hr solvent soak; ^b168-hr solvent soak; ^c192-hr solvent soak

Table 39. HD on XM40 Nylon—Direct and Vapor Contact (Manthei et al., 1986)

Route	Paradigm	HD (mg)			5.0			0.5		
		25.0			Recovery (mg) [%]	Edema (in. ²)	Irritation Index	Recovery (mg) [%]	Edema (in. ²)	Irritation Index
direct	30-min age; IPA rinse; 60-min exposure	below detection limits*	0.024	6.25	below detection limits*	0.93	2.25	below detection limits*	0	0.42
vapor		0	0.5	0	0	0	0			
direct	30-min age; IPA rinse; 15-min age 60-min exposure	(0.0029) [0.015]	0.01	0.76	(0.0029) [0.062]	0	0.17	(0.0029) [0.583]	0	0
vapor		(0.0048) [0.022]	0	0	(0.0024) [0.050]	0	0	0	0	

* < 0.0016 mg/sample

to Plexiglas™, HD rapidly soaked into the concrete; similar to Plexiglas™, there was little spreading. The amount of HD recoverable from concrete was a function of the length of time it was soaked in DEP—the longer the soak, the higher the recovery; crushing the concrete after the longer soak doubled the amount of agent recovered (Table 38). Tests with XM40 fabric (tests were limited to decontaminated fabric) indicated that it was more effective as a contact hazard than a vapor hazard (Table 39). It was concluded that: (a) HD sorbs slowly into Plexiglas™ and can be totally removed with solvent so that a contact hazard no longer exists; (b) HD quickly sorbs into concrete and rinsing with or soaking (for days) in solvent does not remove all of the trapped agent, and a direct contact hazard exists; (c) XM40 nylon carrier cloth slowly sorbed HD, and after 30-min contact, the solvent rinse removed nearly 100% of the agent; damage to skin was evident only at the high dose (25 mg) by direct contact, and the damage was confined to a very small area.

The purpose of Manthei *et al.* (1988) was to determine if the degree of HD-induced skin irritation in rabbits could be used to predict the dose of HD that had produced the injury. The study was predicated upon previous work (see above), which demonstrated that both the vapor and pseudo-liquid models were involved in contact hazard, and—all things being equal, direct contact produced a more severe injury than vapor.¹⁷ The project was divided into three phases: (1) determination of the accuracy of the agent delivery systems and analytical procedures; (2) study of skin irritation in rabbits and swine following 60-min exposures to known levels of agent contamination; and (3) study of skin irritation in rabbits and agent absorption by dental dam (investigated as a possible surrogate skin) from unknown levels of agent desorbing from different surfaces for a particular decontamination scenario.¹⁸ [The experimental data for the third phase were subjected to an extensive analysis of variance for this report (Appendix D)]. Agent was applied as single, discrete droplets. Delivery of very small amounts of agent was determined to be less accurate than delivery of larger amounts. For the direct contact portion of phase two, agent was deposited directly on the clipped skin of the animals, and following exposure, the animals were blotted if visible liquid was observed on the skin. As indicated in Tables 40 and 41 the swine were less sensitive than the rabbits. In the third phase, two physiological responses and three physical measurements were quantified (Appendix D). The physiological responses were (1) the intensity of HD-induced injury resulting from “contact” with decontaminated painted metal coupons and (2) the size of the injured area. The physical measurements were (a) the amount of HD absorbed by dental dam under conditions identical to which the test rabbits were subjected; (b) the amount of HD retained by the painted metal coupons after “exposure” to either unpainted stainless steel (control), rabbit skin, or dental dam; and (c) the amount of distributed “spread” of HD on the coupons (Tables 42 to 44). Two of the major conclusions made by Manthei *et al.* (1988) were that dental dam was a good experimental substitute for skin, and that the type of paint can have a major effect on the subsequent damage from PC exposure to HD on painted metal surfaces (post-decontamination). Findings from the subsequent analysis of the dataset (Appendix D) were in agreement with these original conclusions, except that dental dam was not unconditionally a good substitute.

¹⁷ It was hypothesized that with direct contact the agent was concentrated on a smaller area of skin; therefore, the dose per unit area of skin was higher.

¹⁸ Agent contamination of painted metal coupons, 30 min of aging, agent decontamination with IPA rinse, different periods of post-decontamination aging, and then 60 min of exposure of skin/dental dam to metal coupon.

Table 40. Skin Irritation in Rabbits* Following 60-Min Exposure to HD (Manthei et al. 1988)

HD (mg)	Exposure	Mean Area of Skin Damage						Irritation Index
		24 Hr			72 Hr			
		erythema	eschar	edema	erythema	eschar	edema	
0.010	direct	0.065	---	0.065	0.038	0.0079	0.038	5.83
0.032		0.089		0.089	0.035		0.035	6.42
0.100		0.188		0.188	0.091	0.0287	0.091	6.92
0.320		0.313		0.781	0.229	0.046	0.360	7.84
1.000		0.922		2.417	1.041	0.206	1.365	7.67
3.200		1.479		4.44	1.234	0.531	1.89	7.84
0.010	vapor	0	---	0	0	0	0	0
0.032		0.467		0.467	0.25	0	0.25	0.42
0.100		3.177		4.48	2.677	1.04	4.84	7.21
0.320		4.604		6.19	4.719	2.167	5.79	7.5
1.000		3.67		8.23	4.81	2.92	5.15	7.25
3.200		4.25		7.40	6.06	3.09	6.48	7.25

*3 rabbits per value

Table 41. Skin Irritation in Swine [60-Min Exposure] (Manthei et al. 1988)

HD (mg)	Exposure	Mean Area of Skin Damage						Irritation Index							
		24 Hr			72 Hr										
		erythema	eschar	edema	erythema	eschar	edema								
0.010	direct	0.0039	0.0013	0	0.0039	0.0013	0	2.33							
0.032		0.0156	0.0039	0.0156	0.0156	0.0039		4.50							
0.100		0.0353	0.0104	0.0353	0.0678	0.0068	0.0235	5.50							
0.320		0.0796	0.047	0.0796		0.0392	0.0625	5.34							
1.000		0.1615	0.1094	0.2135	0.2083	0.1719	0.4740	7.84							
3.200		0.3906	0.3906	0.5833	0.4219	0.4219	0.667	7.34							
0.010	vapor	0	0	0	0	0	0	0							
0.032		---	0	0	0	0	0	0							
0.100															
0.320									1.313	0.33	2.271	1.698	0.083	2.38	4.75
1.000									3.0104	1.6404	5.083	3.1797	1.8958	5.4818	7.33
3.200									2.750	1.943	4.938	3.141	2.031	5.900	7.67

Table 42. Skin Irritation and Edema in Rabbits and Comparison of HD Recovery from Painted Plates and Dental Dam
 [30-Min Age, IPA Rinse, 60-Min Exposure] (Manthel et al., 1988)

Paint	HD (mg)	Contact	Irritation Score	Edema (in. ²)	Spread (% of plate)		Dryness		HD Recovered (mg)					
					Dental Dam	Rabbit	Dental Dam	Rabbit	Painted Plate		Dental Dam	p/ Contact w/ Painted Plate		
									p/ Contact w/ Dental Dam	p/ Contact w/ Rabbit				
alkyd*	10	control	---	---										
		direct	7.82	1.06	2.0	2.0								
		vapor	4.13	1.31										
	2	control	---	---										
		direct	7.63	0.49	0.5	0.5	wet	wet						
		vapor	0.75	0.14										
	0.5	control	---	---										
		direct	6.87	0.21	0.25	0.25								
		vapor	0	0										
poly-urethane*	10	control	---	---	25.8	19.8	damp	damp						
		direct	8.00	2.67	25.6	22.0	-wet	wet						
		vapor	7.57	3.92	19.6	26.3								
	2	control	---	---	13.9	11.9	dry-	damp-						
		direct	7.82	2.06	12.0	10.4	damp-	wet						
		vapor	5.85	2.55	11.1	12.4								
	0.5	control	---	---	3.4	4.5								
		direct	6.7	0.27	5.6	3.6	dry	dry						
		vapor	0.06	0.09	5.0									

Table 43. Skin Irritation and Edema in Rabbits and Comparison of HD Recovery from Painted Plates and Dental Dam [30-Min Age, IPA Rinse, 15-Min Age, 60-Min Exposure] (Manthei et al., 1988)

Paint	HD (mg)	Contact	Irritation Score	Edema (in. ²)	Spread (% of plate)		Dryness		HD Recovered (mg)			
					Dental Dam	Rabbit	Dental Dam	Rabbit	p/ Contact w/ Dental Dam	Painted Plate p/ Contact w/ Rabbit	Dental Dam p/ Contact w/ Painted Plate	
alkyd*	10	control	---	---	2.31	2.0			0.1900 ± 0.0160	0.1769 ± 0.0110	---	
		direct	7.63	0.64	2.19	2.25			0.1413 ± 0.0109	0.1388 ± 0.0148	0.0568 ± 0.0052	
		vapor	3.23	1.00					0.1600 ± 0.0131	0.1650 ± 0.0240	0.0398 ± 0.0026	
	2	control	---	---	0.5	0.5	wet	wet	0.0659 ± 0.0087	0.0550 ± 0.0054	---	
		direct	7.63	0.38					0.0456 ± 0.0072	0.0538 ± 0.0093	0.0205 ± 0.0029	
		vapor	0.06	0.03					0.0575 ± 0.0111	0.0503 ± 0.0041	0.0138 ± 0.0007	
	0.5	control	---	---	0.25	0.25			0.0243 ± 0.0035	0.0176 ± 0.0044	---	
		direct	6.38	0.12					0.0121 ± 0.0076	0.0158 ± 0.0034	0.0087 ± 0.0012	
		vapor	0	0					0.0198 ± 0.0027	0.0188 ± 0.0031	0.0062 ± 0.0007	
	poly-urethane*	10	control	---	---	29.4	27.0			0.2019 ± 0.0245	0.2081 ± 0.0684	---
			direct	8.00	2.21	25.9	21.3	damp-wet	damp-wet	0.0244 ± 0.0050	0.0120 ± 0.0029	0.1128 ± 0.0492
			vapor	7.04	2.92	27.5	3.8			0.0356 ± 0.0145	0.0086 ± 0.0023	0.1163 ± 0.0336
2		control	---	---	13.0	13.5			0.0634 ± 0.0244	0.0934 ± 0.0456	---	
		direct	8.00	1.17	11.3	12.1	damp-wet	damp-wet	0.0106 ± 0.0029	0.0041 ± 0.0016	0.0210 ± 0.0095	
		vapor	2.76	1.06	14.0	4.0			0.0156 ± 0.0042	0.0044 ± 0.0006	0.0310 ± 0.0096	
0.5	control	---	---	6.6				0.0043 ± 0.0014	0.0056 ± 0.0016	---		
	direct	3.73	0.16	5.6	3.8	dry	dry	0.0036 ± 0.0007	0.0016 ± 0.0005	0.0020 ± 0.0009		
	vapor	0	0	5.6	4.4	damp-wet	damp-wet	0.0048 ± 0.0029	0.0016 ± 0.0004	0.0053 ± 0.0058		

Table 44. Skin Irritation and Edema in Rabbits and Comparison of HD Recovery from Painted Plates and Dental Dam [30-Min Age, IPA Rinse, 5-Hr Age, 60-Min Exposure] (Manthei et al., 1988)

Paint	HD (mg)	Contact	Irritation Score	Edema (in. ²)	Spread (% of plate)			Dryness		HD Recovered (mg)			
					Dental Dam	Rabbit	Dental Dam	Rabbit	p/ Contact w/ Dental Dam	Painted Plate	p/ Contact w/ Rabbit	Dental Dam	p/ Contact w/ Painted Plate
alkyd*	10	control	---	---	2.06					0.0988 ± 0.0088	0.0900 ± 0.0100	---	
		direct	7.51	0.28	2.25					0.1056 ± 0.0174	0.0800 ± 0.0071	0.0100 ± 0.0007	
		vapor	0	0	2.13					0.0988 ± 0.0109	0.0763 ± 0.0079	0.0065 ± 0.0009	
	2	control	---	---						0.0350 ± 0.0063	0.0316 ± 0.0064	---	
		direct	5.51	0.06	0.5		wet	wet		0.0359 ± 0.0044	0.0114 ± 0.0022	0.0048 ± 0.0007	
		vapor	0	0						0.0366 ± 0.0027	0.0105 ± 0.0015	0.0024 ± 0.0007	
	0.5	control	---	---						0.0082 ± 0.0027	0.0113 ± 0.0023	---	
		direct	4.07	0.26	0.25					0.0100 ± 0.0009	0.0094 ± 0.0022	0.0016 ± 0.0003	
		vapor	0	0						0.0089 ± 0.0027	0.0100 ± 0.0005	0.0061 ± 0.0013	
poly-urethane*	10	control	---	---	22.8					0.0163 ± 0.0035	0.0137 ± 0.0044	---	
		direct	1.91	0.30	23.8		damp-wet	damp-wet		0.0175 ± 0.0070	0.0095 ± 0.0014	0.0035 ± 0.00008	
		vapor	0	0	30.0					0.0171 ± 0.0051	0.0101 ± 0.0018	0.0025 ± 0.000070	
	2	control	---	---						0.0094 ± 0.0031	0.0077 ± 0.0020	---	
		direct	1.69	0.18	14.0		dry-damp	dry-damp		0.0095 ± 0.0009	0.0044 ± 0.0006	0.0022 ± 0.0011	
		vapor	0	0	13.8					0.0108 ± 0.0023	0.0054 ± 0.0009	0.0021 ± 0.00021	
	0.5	control	---	---	5.5					0.0048 ± 0.0010	0.0021 ± 0.0004	---	
		direct	0.75	0.018	4.8		dry	dry		0.0031 ± 0.0008	0.0014 ± 0.0004	0.0013 ± 0.0005	
		vapor	0	0	5.0					0.0055 ± 0.0012	0.0035 ± 0.0006	0.0017 ± 0.0004	

3.3 Other Studies—Simulants and Pick-Up.

Although many of the following studies are not toxicity data on contact hazard, brief discussions are included because they are relevant to planning the “way forward” to filling toxicity voids in contact hazard data—particularly if simulants will be required for some of the testing.

The object of Reich (1959b) was to estimate the quantity of V-agent that could be picked up on the clothing of troops traversing contaminated areas of dense grass. The study employed (1) troops crawling through simulant¹⁹-contaminated terrain (to simulate traversing open terrain under fire at night); (2) rollers pushed over simulant-contaminated, and (3) rollers pushed over VX-contaminated terrain. Ground contamination was effected by either the “spinning tip apparatus” (for small particles) or the “multijet dispenser” (for large particles). Contamination density and particle size distribution were estimated with sampling pans and M6 paper. The quantity of simulant picked up on the test subjects was directly proportional to the contamination density and averaged 1% of the contamination density per unit length of the traversal path and was relatively independent of particle size. However, in “man versus roller” trials—the smaller particles were somewhat more transferable to rollers; this was also observed with rollers traversing agent-contaminated terrain. Comparison of transferability of agent to simulant indicated that the transferability of agent decreased with increasing contamination density, and the quantity of agent picked up on a roller traversing contaminated areas 1 day after dispersion was approximately 4% of that picked up when traversal occurred within 1 hr of contamination.

Reich (1960) used rollers to estimate the pick-up of VX from three different types of terrain: (1) normal or relatively dry; (2) rain-soaked, and (3) rain-soaked immediately after contamination. The total pick-up of VX was highest from terrain rain-soaked prior to contamination and lowest when heavy rain fell after contamination and before “traversal”. Pick-up from normal terrain was about 2/3 of that from prior rain-soaked terrain. “Wetness” of the terrain markedly affected cloth penetration on the rollers. About 35% of the agent penetrated to the inner layer of the roller with wet terrain; where as only 1% penetrated with dry terrain.

Reich (1961) reported on the pick-up of simulants on short grass traversed by a 24-in. wide cloth-covered roller around which two layers of laundered, bleached cotton sateen were wrapped. The data indicated that none of the simulants was picked up or penetrated fabric to the same degree as VX. The data are given in Table 45 and are presented to amplify this point. Similar findings have been reported by Fish (1959), and this type of data has been a mainstay of much of the “contact hazard” research. Also, many such studies—including this, were poorly controlled with respect to the condition of the terrain (e.g., temperature, moisture content of soil, etc.). Reich (1961) also presented data for pick-up on troops traversing VX-contaminated terrain. The volunteers wore masks and specially-designed protective garments under the outer test clothing. Contamination sites were selected for the nature of their vegetation and included short grass, tall grass, and shrubbery. The men crawled through the grass-covered areas and walked through shrubbery in a crouching manner. When grass-covered terrain was traversed a cloth covered roller was rolled alongside crawling subjects for comparison of pick-up. The traversal distance was 25 m. Pre-tests were done with simulant contamination (DBP). It was observed that the quantity of VX picked-up was terrain-dependent, and simulant pick-up was greater than agent pick-up (Table 46). Given the fairly constant ratio for man/roller pick-up on grass, Reich concluded that the roller provided a good estimate of the pick-up for man. Pick-up for different body regions is given in Table 47. Tests were also performed on virgin snow-covered terrain (~6-10 in. deep). Twenty-five-meter test strips were contaminated to a density of approximately 5 g/m² VX in 0.6 mm MMD drops. The strips were traversed by rollers at 10 min, 30 min, 1 hr, 3 hr, 1 day, and 2 days after contamination. The persistency of VX on snow-covered terrain was observed to be greater than on other types of terrain, and pick-up of VX increased with time and then decreased. It was hypothesized that the initial increase results from droplets diffusing into the surrounding snow, thereby increasing the volume and surface of contaminated snow. The subsequent decline in pick-up was attributed to excessive dilution.

¹⁹ Dibutyl phthalate dyed with FD&C No. 32 Red.

Table 45. Comparison of Simulant versus VX Pick-Up on Rollers (Reich, 1961)

Contaminant	Contam Density (g/m ²) [std dev]	Traversal Time	Pick-Up on Roller Cloth*							
			Outer		Inner		Total		Ratio**	
			Dye	Chem	Dye	Chem	Dye	Chem	Dye	Chem
			(mg)		(mg)		(mg)		(mg)	
DBP	1.57 [0.82]	10	644	690	0	55	644	745	2.6	3.1
		30	416	496		37	416	533		
		60	263	287		2	263	316		
DEP	1.61 [1.08]	10	1258	1009	0	73	1258	1082	10.4	8.7
		30	1532	1238		109	1532	1347		
		60	2740	2125	14	78	2754	2203		
TBP	2.47 [1.50]	10	244	165	0	2	244	167	0.5	0.3
		30	85	44		0	85	44		
		60	58	32		58	32			
VX	2.97 [2.50]	10	---	371	---	3	---	374	---	---
		30	---	288	---	2	---	290		
		60	---	310	---	4	---	314		

*simulants dyed; pick-up on cloth analyzed chemically by dye content; VX determined by DB3

**pick-up expressed as a ration of average total pick-up of simulant to the pick-up of agent per g/m² of contamination

Table 46. Pick-up on Troops Traversing Contaminated Terrain (Reich, 1961)

Terrain Cover	Trial	Agent	Density (g/m ²)	Traversal Time* (min)	Avg. Man Pick-up (mg)	Roller Pick-up (mg)	Man/Roller Ratio	Pick-up Factor (mg/m/g/m ²)
sparse, short grass	1	DBP	0.9	22	235	139	1.7	10.4
	2		0.3	22	97	74	1.3	12.9
	3	VX	2.4	30	146	95	1.5	2.4
	4		1.7	41	112	72	1.6	2.6
moderately-dense, short grass	1	VX	1.0	15	197	203	1.0	7.9
	2			39	127	88	1.4	5.1
	3		0.9	51	143	89	1.6	6.4
	4		2.3	20	502	367	1.4	8.7
	5		2.7	36	345	262	1.3	5.1
	6		2.9	50	209	223	0.9	2.9
dense, tall grass	1	DBP	1.5	25	1202	916	1.3	32
	2		1.3	24	1787	1554	1.1	55
	3		1.4	28	1946	1697	1.2	56
	4	VX	0.4	32	501	413		50
	5		0.6	41	366	321	1.1	24
	6		0.7	54	418	406	1.0	
shrubbery	1	DBP	0.7	45	110	---	---	6.3
	2		0.8	30	129			6.4
	3	VX	0.4	45	29			2.0
	4		0.7	30	80			4.6

*time elapsing between start of ground contamination and traversal

Table 47. Distribution of Agent on Troops Traversing Contaminated Terrain (Reich, 1961)

Location	Type of Terrain			
	Grass			Shrubby
	Sparse, Short	Moderately-Dense Short	Dense, Tall	
(Avg % Total Pick-up)				
jacket	26	28	28	12
trousers	35	43	33	28
gloves	11	10	13	13
boots	16	11	12	33
mask	2	1	3	2
rifle	9	6	10	8
helmet & misc.	1	1	1	4

4 TOXICITY AND CONTACT HAZARD

The purpose of this document was to review the existing toxicological data on contact hazard. However, there is an underlying issue that cannot be ignored—independent of whatever toxicity data exist for evaluating “contact hazard”, the problem cannot be grasped without a firm understanding of the underlying PC toxicity of the chemical agents in question. Such an understanding requires adequate human and animal PC toxicity data (see below) for liquid and for vapor phases (Manthei *et al.* 1983, 1985, 1986, 1988).

4.1 Percutaneous Liquid Toxicity.

There is low confidence in the human nerve agent toxicity estimates for PC liquid exposure (Reutter and Wade, 1994; COT, 1997; Reutter *et al.*, 2003), and the potency of the agents may be underestimated.²⁰ This must be rectified before “contact hazard” can begin to be understood. Indeed, Elskamp *et al.* (1973) stated that to evaluate contact hazard, it is necessary to know the minimum amount of agent producing an effect on human skin. However, human testing is no longer done, and the existing human data for nerve agents are sparse and limited to rather mild effects²¹ in relatively healthy, young male volunteers. In contrast, animal studies are largely limited to severe and lethal effects, and the existing animal studies were not properly designed to provide data for extrapolation to humans.

The situation is further complicated by the fact that modeling human toxicity estimates is especially difficult for PC exposures. There are huge species differences in skin, and there are body region differences within species (Wester and Noonan, 1980). The effects of these differences on PC absorption span orders of magnitude. Hence, effective toxic doses can vary markedly from one body region to the next and can be a function of anything affecting skin permeability—the physical properties of the agent, heat, humidity, perspiration, *etc.* Effective dosages in humans are also a function of the presence or absence of clothing (or hair).²² Toxic effects in animals can be a function of the presence or

²⁰ A meta-analysis was performed on the intravenous (IV) and PC data for nerve agents in multiple animal species to develop a model for estimating human toxicity. The effort provided a good statistical basis to indicate that many of the human toxicity estimates are too high—they underestimate the potency to humans.

²¹ In Cullumbine *et al.* (1954) one individual died and another had very severe effects; many other subjects were exposed to these and higher doses and suffered only mild effects. The cause of these apparently untoward responses can only be speculated.

²² Depending upon the agent and exposure scenario hair and/or clothing can be protective; however, they can also trap the agent and in the case of volatile agents, effectively increase the delivered dose.

absence of fur, as well as the manner in which the fur was removed (Muir and Callaway, 1951). [Unlike humans, most laboratory species have fur and do not perspire.]

The PC liquid database for HD is relatively extensive (Reutter and Wade, 1994), but the data are limited to local effects from exposure of discrete areas. These data may be excellent for formulating human estimates for the less-than-lethal effects of HD, but they will not suffice for what will be required in the determination of contact hazard when no liquid or vapor can be detected, and/or the agent has sorbed into a surface. The data required for understanding contact hazard will include toxicity testing in animals, and it will be necessary to relate animal studies to the known human database. As noted above, there are extraordinary differences between human and animal skin. These differences are further amplified with regard to vesicants. Animals do not blister like humans; many species do not blister at all. As a result, the animal data for vesicants are largely limited to severe and lethal effects—systemic effects, whereas the most likely concerns with “contact hazard” are local effects.

The sensitivity of animals—relative to humans, has not been systematically researched for any of the chemical agents. Since animal data are required for modeling human toxicity, understanding the relative sensitivity of humans and various test species is critical to establishing robust human toxicity estimates. Cullumbine *et al.* (1954) investigated several G-agents in humans and rabbits. However, there were some flaws in the design of the study, and the number of rabbits was fairly limited. The limited non-lethal mustard data in animals are difficult to compare to human data because of the lack of vesicancy of mustard in non-human species. Some species comparison studies were done by Marshall and Williams (1918), with mustard solutions in paraffin oil. There were insufficient rabbit data ($n = 2$) to state anything about the sensitivity of rabbits relative to other species. The dog was the most sensitive species, and was perhaps slightly more sensitive than humans in a similar study (Marshall *et al.*, 1918). The least sensitive species was the monkey (Marshall and Williams, 1918), and there were several orders of magnitude of difference in the human response to different percentages of mustard in solution (Marshall *et al.*, 1918).²³ McMaster *et al.* (1945) stated that the skin of rabbits is less sensitive to mustard than human skin; however, they found that rabbit skin was more easily irritated than human skin—by chemical decontaminants or experimental manipulation. Their animal-of-choice was the white pig, which was stated to react the most similarly to humans. Some metric needs to be established for extrapolating animal data to humans.

4.2 Percutaneous Vapor Toxicity.

The issues underlying the human estimates for PC vapor toxicity are very similar to those underlying PC liquid toxicity. There have not been any systematic investigations of comparative human-animal toxicity. Only one vesicant study was found in which humans and an animal species (rabbit) were simultaneously and similarly tested (Eyster and Maver, 1920), and rigorous statistical analysis of those data (Appendix A) indicates that rabbits are significantly less sensitive than humans to the non-systemic effects of mustard. No comparative human-animal studies were found for nerve agents, and it is difficult to do a retrospective comparison because the PC vapor studies in animals typically gassed the animals until death, while the limited non-lethal human studies involved gassing for a set duration. Median effective dosages will differ between studies that gas until an effect is observed *versus* studies that gas for a set duration and then wait to observe a possible effect. The former will produce greater median effective dosages, because there is usually a delay between receiving an effective dosage and the appearance of the effect in question. Furthermore, no type of benchmark has been used to correlate the existing PC vapor data, in a manner similar to the PC to IV ratio used for PC liquid exposures (Section 6). Until recently, little interest has been demonstrated in modeling potential trends in mammalian PC vapor toxicity to improve extrapolation from this dataset to develop human estimates.

Sommerville (2004)²⁴ performed a statistical review of the existing PC vapor lethality data for mammalian whole-body exposures. A total of 34 EC_{50s} (severe effects and lethality) representing seven

²³ Sulzberger *et al.* (1947) cite several other studies confirming this observation.

²⁴ Sommerville, D.R. *Review and Statistical Analysis of Mammalian Nerve Agent Percutaneous Vapor Lethality Data*, U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, unpublished work, 2004.

organophosphorus nerve agents (GA, GB, GD, GF, EA1356, VE and VX) and six species (mouse, guinea pig, rabbit, monkey, dog and goat) were analyzed. Using linear regression, three statistical trends were identified:

- (1) On an absolute dosage basis larger species are less sensitive to PC vapor exposures than smaller species. This is probably due to differing surface area to volume ratios of large animals *versus* small animals.
- (2) PC vapor potency increases as volatility decreases.
- (3) The intrinsic toxicity of an agent—as represented by the IV toxicity, is the most important factor for scaling PC vapor lethality, followed by equal contributions from species body mass and agent volatility.

Unfortunately, the quality of the dataset analyzed by Sommerville was such that the human estimates derived from his analysis have large error bars associated with them (roughly plus or minus a factor of two). Additional mammalian data are required to improve the precision of the estimates.

It is unlikely that for contact hazard scenarios of interest the whole body will be exposed. The existing mammalian nerve agent PC vapor database does not contain data on partial body exposures (*i.e.*, arm or leg only). It is possible to mathematically scale toxicity estimates from a whole body basis to a partial body basis, but actual experimental data would be preferable.

5 STATE OF TOXICITY DATA FOR CONTACT HAZARD AND RECOMMENDATIONS FOR FUTURE STUDIES

Arguably, the most seminal contact hazard studies are those of Manthei *et al.* (1983, 1985, 1986, 1988); all underscore the statement, "Surfaces that have been contaminated with liquid chemical warfare agents and then decontaminated (chemically or physically) must not be presumed to be clean and safe to touch—these surfaces may still emit agent vapors and also may produce physiological responses when contacted by an individual. In the absence of detectable liquid residual agent on the surface, this latter situation has been given the name contact hazard" (Manthei *et al.*, 1983). The "seriousness of the hazard" is a function of the material, the initial dose, the time between contamination and decontamination, the type and amount of decontaminant used, and the meteorological conditions (Elskamp *et al.*, 1973). As early as 1918, Marshall *et al.* observed that—depending upon meteorological conditions the vapor concentration above a mustard-contaminated plot could be somewhat higher several hours post-contamination than it was immediately following contamination. Anderson (1943) made similar observations, and this has been re-confirmed in recent studies done in the Czech Republic with HD, GD, and VX (Davis and Fagan, 2000)—depending upon the surface, meteorological conditions or other factors can cause agent to diffuse to the surface, thereby increasing the hazard.

Baldauf (1988) stated that vapor flux from a surface was dependent upon the original contamination density and that surface contamination cannot be determined by measuring flux unless the initial density and time post-contamination are known. He further stated that agent on a surface can exist as (1) neat liquid, (2) neat liquid trapped (absorbed) within surface pores, (3) agent dissolved in surface layers (paint, oils, moisture), (4) agent physically adsorbed onto surface, and (5) agent chemically adsorbed.

According to Carlon (1990), studies have shown that alkyd paints sorb more liquid and then desorb more vapor than urethane paints. Hence, urethane paints have been selected for military use. However, the data of Manthei *et al.* (1983, 1985, 1988) indicate that the picture is decidedly more complicated than merely selecting one type of paint. Bioavailability is a key issue and it is not a straightforward problem.

From the perspective of toxicological implications and contact hazard, the data of Manthei *et al.* (1983, 1985, 1986, 1988) indicate that two mechanisms are involved in contact hazard—vapor and/or pseudo-liquid partitioning. Moreover, the mechanism for a given exposure scenario is likely a function of both the agent and surface. The Manthei *et al.* (1983, 1985, 1986, 1988) data indicate several common themes. First, it is clear that physiological injury can occur from both types of exposure—simple vapor transport and/or pseudo-liquid partitioning between a contaminated surface and exposed skin. Second, in likelihood and intensity of injury—all other factors being equal, direct contact is generally more effective

than vapor contact in producing physiological effects; however HD vapor-induced burns are typically larger than those produced by direct contact. [This is demonstrated in Figure 2 by the comparison of the intensity of injury measurements (PII) for the PC exposure of rabbits to HD from either decontaminated painted metal (Manthei *et al.*, 1983, 1988) or concrete (Manthei *et al.*, 1986).] Third, when large amounts of residual agent are involved (all other factors being equal), the size of vesicant injuries is greater from vapor contact than from direct contact. [However, smaller injuries (from smaller amounts of agent), from direct contact are greater in size (Figures 3 and 4)]. In general, direct contact produces smaller but more intense injuries, while vapor contact creates larger and less intense injuries (Figures 5 and 6) proportional to the amount of agent that was absorbed by the surface prior to decontamination. Fourth, the degree of absorption appears to be specific to the type of agent and surface involved. [For instance, in Manthei *et al.* (1986), it was found that Plexiglas™ and (to a lesser extent) nylon carrier cloth do not readily absorb HD, and the injuries to rabbits were either nonexistent (Plexiglas™) or negligible (carrier cloth) after exposure to the respective decontaminated surface. It was also found that polymer-thickened HD (Manthei *et al.*, 1983) and VX (Manthei *et al.*, 1985) are not readily absorbed by painted metal surfaces, thus posing a minimal contact hazard post-decontamination, with the one exception being THD on alkyd-painted steel (enough residual agent was present post-decontamination to cause significant injury to rabbits).] Fifth, the exact hazard is dependent on the agent desorption properties of the surface. (In the final analysis, it is how the agent absorption and desorption properties of the surface interact with each other that determines the actual contact hazard). Sixth, the interaction between agent desorption and absorption and its impact on the contact hazard is complex—depending on many factors and factor interactions. However, there is one significant problem with these studies: there was no mass balance for the agent and statements regarding residual amounts should, perhaps, be viewed qualitatively—rather than quantitatively.

Many of the variables investigated by Manthei *et al.* (1983, 1985, 1986, 1988) were related to the desorption/absorption processes: (a) amount of initial agent contamination; (b) duration of agent aging pre- and post-decontamination; (c) duration of skin-surface contact, type of exposure (direct *versus* vapor); and (d) the type of agent and surface involved. Because of the number of factors involved, Manthei *et al.* (1983, 1985, 1986, 1988) were correct in using a factorial experimental design approach, but Manthei *et al.* were aimed at proving the principle—rather than presenting the most complete picture that rigorous statistical design and analysis would have afforded. For this report, the 1988 Manthei *et al.* study was subjected to rigorous statistical analysis to “make sense” of the findings. In the third phase of that study two physiological responses and three physical measurements were quantified (Appendix D). The physiological responses were (1) the intensity of HD-induced injury resulting from “contact” with decontaminated painted metal coupons and (2) the size of the injured area. The physical measurements were (a) the amount of HD absorbed by dental dam under conditions identical to which the test rabbits were subjected; (b) the amount of HD retained by the painted metal coupons after “exposure” to either unpainted stainless steel (control), rabbit skin, or dental dam; and (c) the amount of HD spread on the coupons (Tables 42 to 44). Multiple ANOVAs were performed upon physiological measurements and the first two physical measurements to determine how they were affected by: (i) the amount of HD initially loaded on the metal coupons (0.5, 2 or 10 mg); (ii) the type of paint used on the coupons (alkyd *versus* polyurethane); (iii) the type of contact (vapor or direct) between coupon and test surface; and (iv) the aging duration between coupon decontamination and exposure of the coupon to a test surface (0, 15 or 300 min). Table 48 presents a comparison of some of the major findings of the ANOVA done for this report with those originally reported by Manthei *et al.* (1988) for the third phase (more details are in Appendix D). Of particular interest to future contact hazard studies is the assertion of Manthei *et al.* (1988) that dental dam would make a very good substitute for actual skin, which, if true, would greatly lessen the need for using animals. However, rigorous analysis of the data (Appendix D) determined that dental dam can serve as a suitable substitute only if it has been properly calibrated against actual skin for the exposure conditions of interest. Differences were found between how much agent was absorbed by dental dam and rabbit skin (either as inferred by the amount of agent remaining in the metal coupons after exposure or by direct measurement of the dental dam); the exact difference was a function of the exposure conditions. Manthei *et al.* (1988) did not systematically explore how the size and intensity of the injury varied as a function of the physiological and physical factors they measured. An ANOVA (Appendix D) revealed that the size of the injury was not dependent on the type of contact or on any of its interactions with the other factors. This is in contrast to what was found in Manthei *et al.* (1983, 1986),

with the difference probably resulting from the greater agent loadings investigated in the two earlier studies (Appendix D). Instead, the type of paint played a major role (with polyurethane paint producing larger damage areas than alkyd paint), and this is probably due to the greater spreading of HD on polyurethane paint in comparison to alkyd paint [Tables 42 to 44]. This is in contrast to the intensity of injury being heavily dependent on the type of contact (with direct contact producing greater damage than vapor contact). Furthermore, the intensity of injury is not dependent on the main effect of type of paint; instead, it is dependent on the interaction of the type of paint with the type of contact (and to a lesser extent on the interaction of the type of paint with the aging duration).

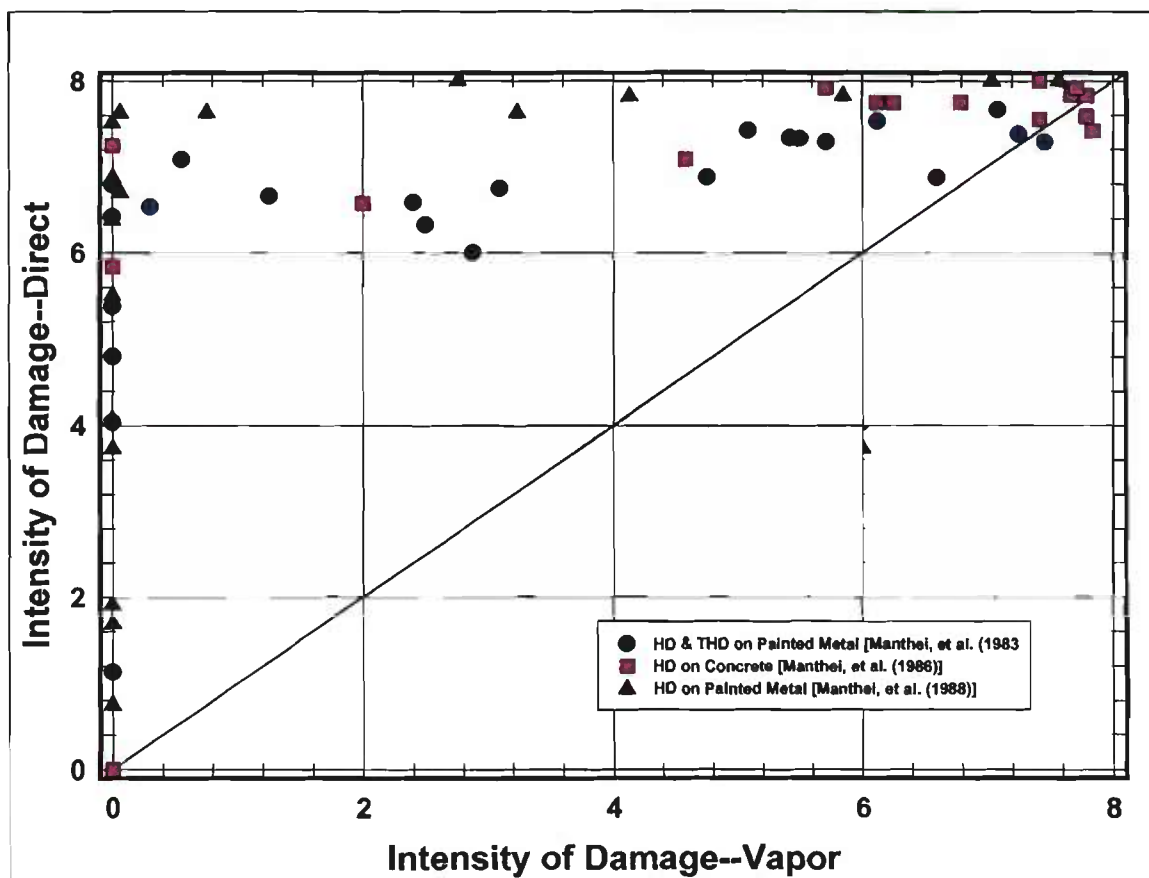


Figure 2. Comparison of Average Primary Irritation Index Values from Direct and Vapor Contact or Rabbit PC Exposures to HD in Decontaminated Painted Metal and Concrete Surfaces from Manthei *et al.* (1983, 1986 & 1988)

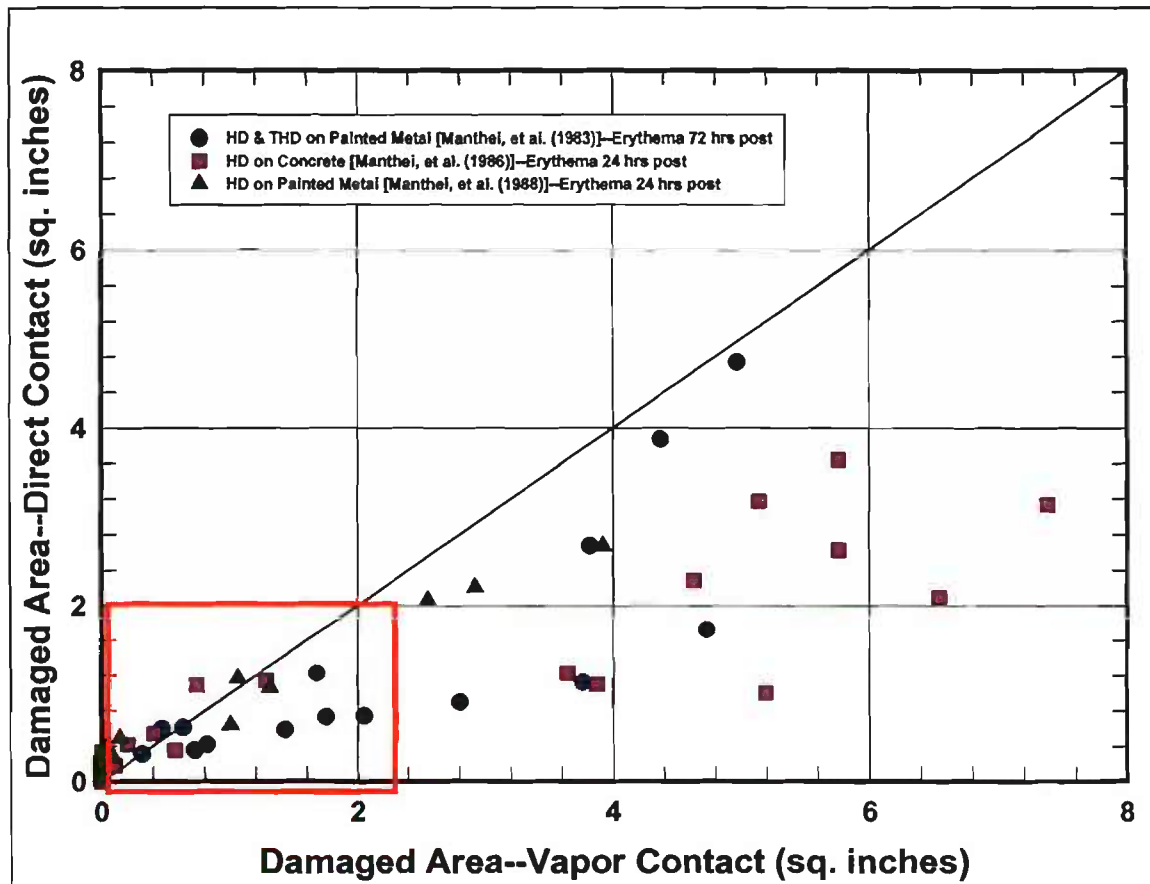


Figure 3. Size Comparison of Skin Damaged Areas (Erythema) from Direct and Vapor Contact for Rabbit PC Exposures to HD in Decontaminated Painted Metal and Concrete Surfaces from Manthei *et al.* (1983, 1986 & 1988)

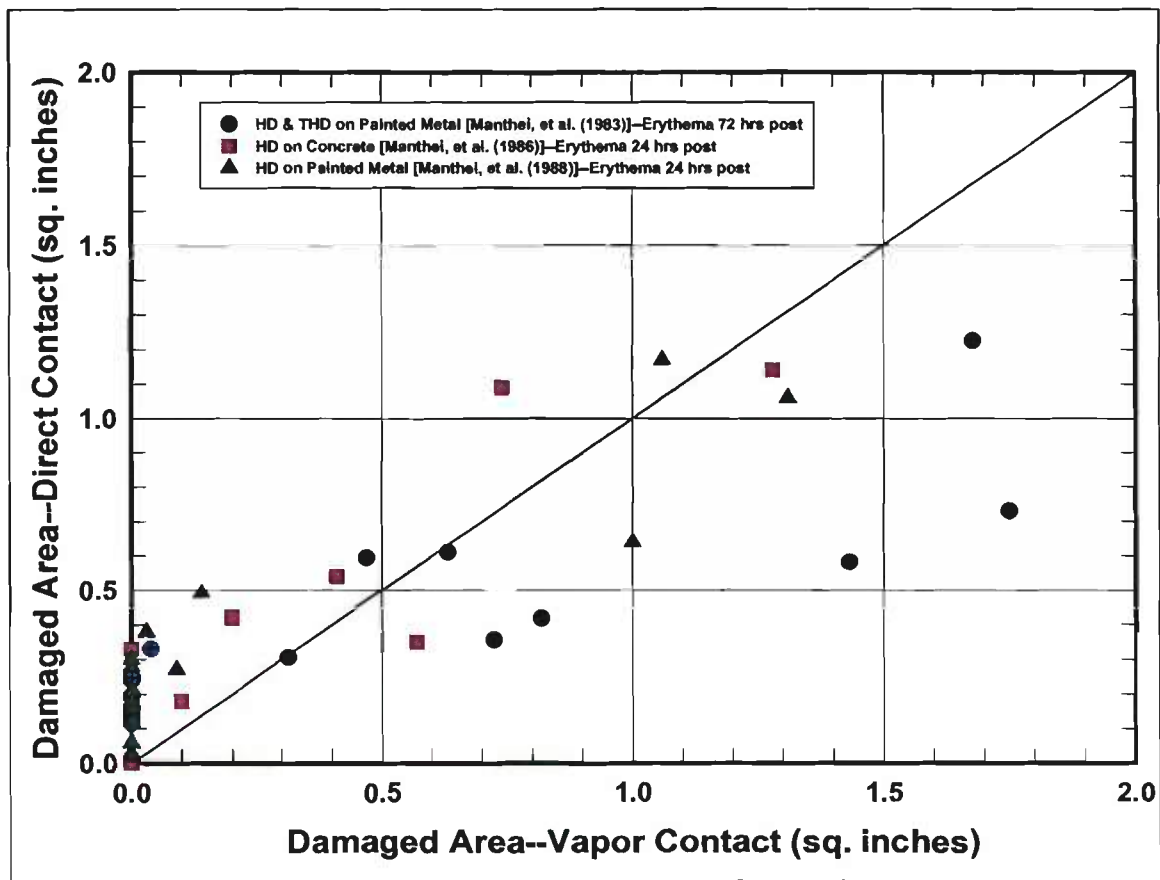


Figure 4. Enlarged View of Highlighted Section of Figure 3

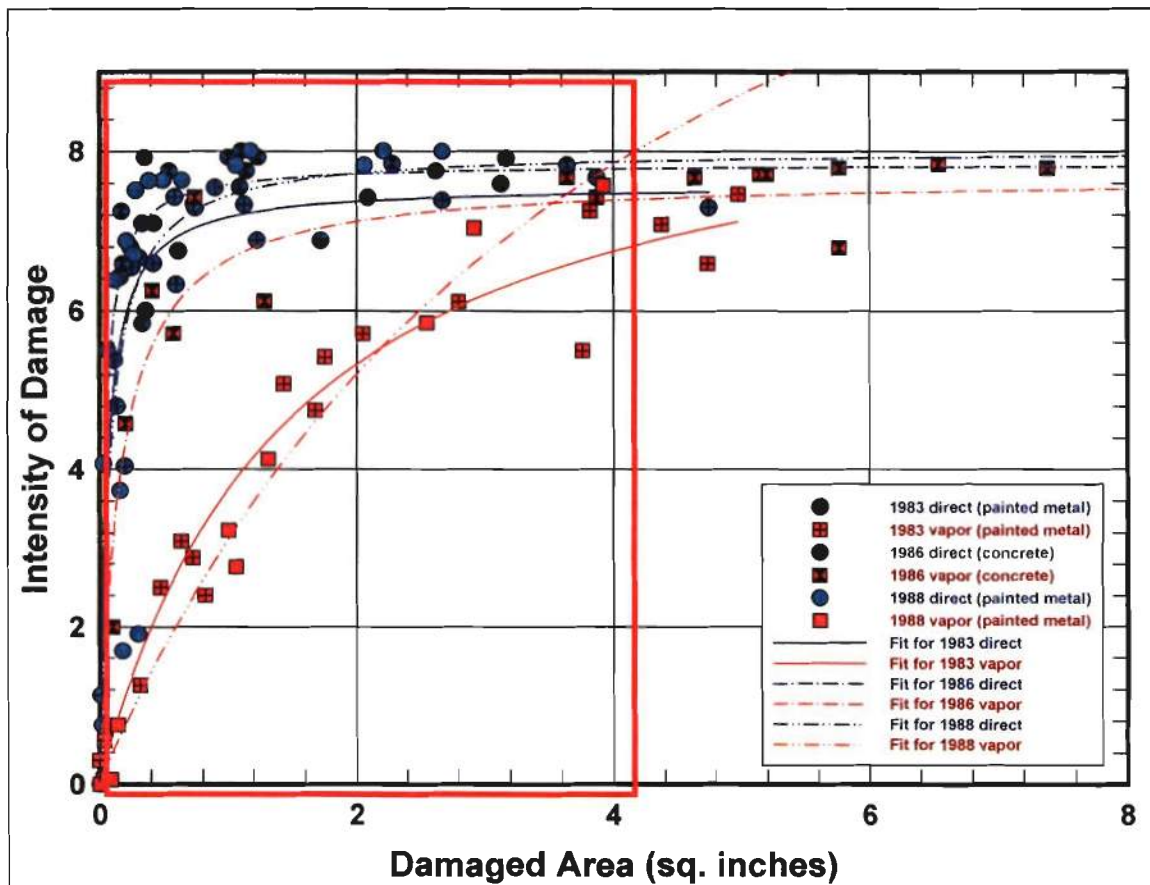


Figure 5. Size Comparison of Damaged Areas (Erythema) from Direct and Vapor Contact for Rabbit PC Exposures to HD in Decontaminated Painted Metal and Concrete Surfaces from Manthei *et al.* (1983, 1986 & 1988)

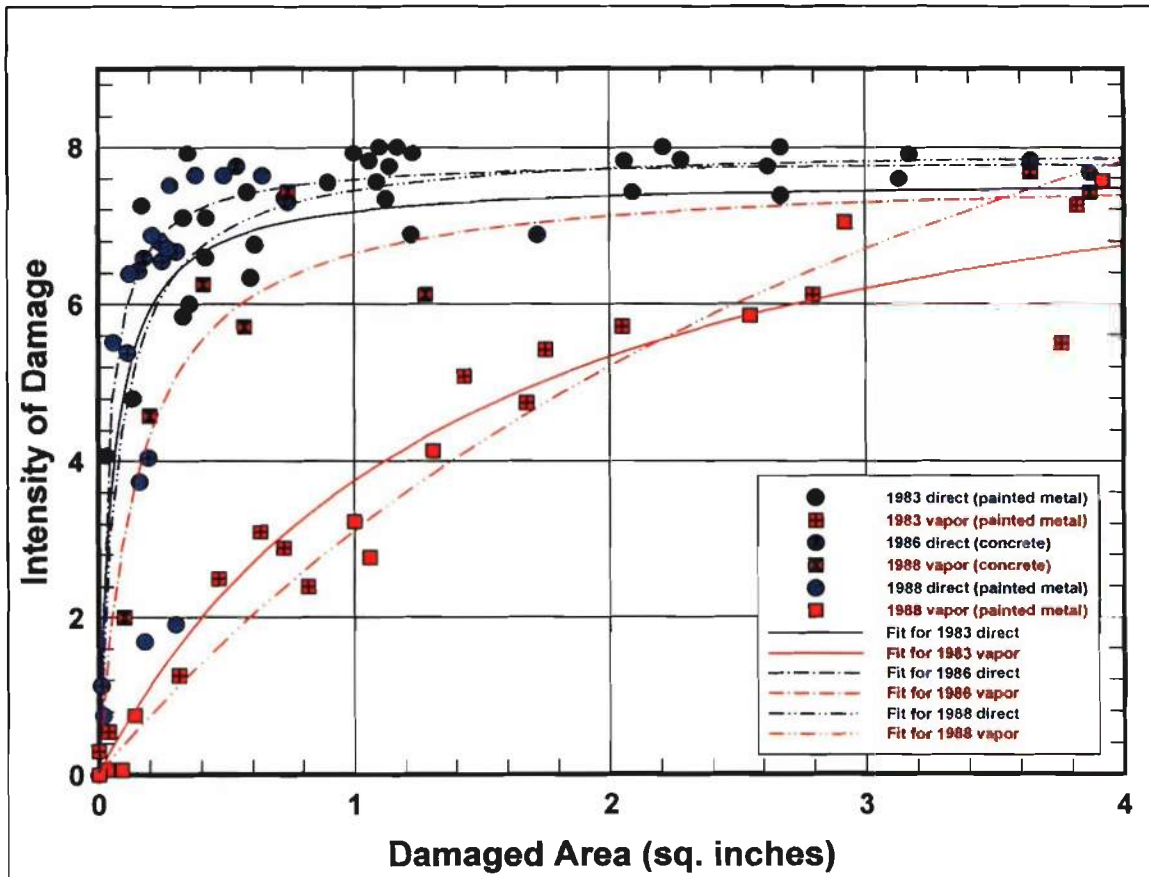


Figure 6. Enlarged View of Section of Figure 5

Table 48. Comparison of Manthei *et al.* (1988) Findings versus Sommerville (Appendix D) Findings

Finding	Manthei <i>et al.</i> (1988)	Sommerville
Area of Agent Damage on Rabbit Skin		
Effect of Paint Type	Contact with decontaminated polyurethane produced larger areas of damage than contact with alkyd	Direct effect of paint type significant; significant interactions between paint type and other factors (agent loaded and aging); observed difference between paint types decreased with decreased agent loaded or increased aging
Effect of Contact Type	Only one observation based on limited review of data (trials with zero aging); vapor contact produced larger damage area than direct contact but only with agent loading of 10 mg	ANOVA of all data showed contact type and interactions with other factors not significant; agent loading greater than 10 mg not investigated; linear regression analysis of area of damage (direct <i>versus</i> vapor) showed vapor contact produced larger damage areas at higher HD loadings; at lower loadings direct contact produced larger damage areas
Effect of Agent Amount Loaded and Aging Time Between Decontamination and Skin Contact	Observed great reduction in damage area when aging increased from 0 to 15 minutes; area of damage increased with agent loaded	Based on ANOVA of total dataset, both agent loaded and aging have significant effects; with about the same influence as paint type
Comparison of Significant Factors for Intensity and Area of Damage	No comparison made	Factors affecting size and intensity of damage differ; intensity of damage is dependent on contact type, but is not dependent on paint type; size of damage dependent on paint type, but not dependent on contact type; intensity and size of damage about equally dependent on the agent loaded and aging duration
Usefulness of Dental Dam as a Rabbit Skin Simulant	Dental dam is a very good substitute for skin	Dental dam can be good skin simulant, but only if properly calibrated for exposure conditions (e.g., paint, agent loaded, aging duration, contact type); there are absorption differences between dental dam and rabbit skin; exact difference will be a function of exposure conditions

Table 48., cont. Comparison of Manthei *et al.* (1988) Findings versus Sommerville (Appendix D) Findings

Finding	Manthei <i>et al.</i> (1988)	Sommerville
Agent Transfer—Agent Recovery from Test Coupons and Dental Dam for Dental Dam Trials		
Effect of Paint Type	For all aging durations (0, 15, 300 min), alkyd retained more agent	ANOVA for entire test coupon dataset, showed alkyd retained more agent than polyurethane; however, ANOVA for agent recovery dental dam found main effect of paint type not significant; only interactions with aging and agent loaded were significant; discrepancy between the two types of agent recovery data is dependent on paint type
Effect of Interaction of Paint Type and Aging Duration	For all aging durations (0, 15, 300 min), alkyd retained more agent than polyurethane	Manthei <i>et al.</i> incorrect about lack of interaction between aging and paint type; ANOVA shows significant interaction; no significant difference between amount of agent recovered from alkyd and polyurethane with 300-min aging
Effect of Interaction of Paint Type and Contact Type	Direct contact with alkyd transfers more agent than vapor contact (based on amount of agent recovered from dental dam); reverse true for polyurethane	ANOVAs on test coupon and dental dam agent recovery data did not confirm observations of Manthei <i>et al.</i> regarding interaction between paint type and contact type
Effect of Contact Type	Direct contact transfers more agent from test coupon to dental dam than vapor contact	Confirmed via ANOVA of agent retention data from test coupons; ANOVA of agent recovery data from dental dam did not find contact type to be significant; there is discrepancy between the two types of agent recovery data (test coupon and dental dam) in their dependence on contact type
Effect of Aging Between Decontamination and Skin Contact	Considerable reduction of agent retained by test coupons after 15-min aging compared to zero aging	Confirmed by ANOVA; however significant individual interactions of aging with contact type, paint type, and agent loaded (interaction with paint type most influential)
Effect of Agent Amount Loaded	(1) No observation on direct effect of agent loaded; based on agent recovery data from test coupons, (2) significant interaction between agent loaded and paint type	Confirmed by ANOVA; also amount of agent has no significant effect on amount retained by alkyd, with reverse true for polyurethane; interaction between agent loaded and paint type much weaker agent recovery data from dental dam
Agent Transfer/Recovery from Test Coupons for Skin Trials	Very few observations	Comparison of agent recovery from dental dam and rabbit skin shows for skin trials that most important factors/interactions are paint type, paint, and interaction with aging; also important with dental dam but not to same degree; account for 88% of total variance for skin trials and 57% for dental dam trials; another difference between the two groups of agent recovery data is that there is a larger variance in skin trials (with a statistical significance of 99.5%)

Table 48., cont. Comparison of Findings of Manthei *et al.* (1988) versus Sommerville (Appendix D) Findings

Finding	Manthei <i>et al.</i> (1988)	Sommerville
Agent Transfer—Control Coupons for Dental Dam and Rabbit Skin Trials		
Effect of Type of Paint—Agent Absorption	Limited observations; polyurethane retained more agent than alkyd for loading of two and 10 mg; reverse true for loading of 0.5 mg	ANOVA showed paint type not significant for amount of agent initially absorbed; interaction of paint type with agent loaded was significant; the only statistically significant difference between alkyd and polyurethane paint occurred at agent loading of 0.5 mg; alkyd paint absorbs more agent
Effect of Type of Paint and Agent Amount Loaded	No observations made	Agent desorbed more readily from polyurethane particularly with increased aging; difference between paint types not statistically significant at zero aging; effect of paint type significantly less than effect of aging duration and agent loaded on agent retention
Controls for Dental Dam versus Rabbit Skin	No observations made	ANOVA of control coupon data found no significant difference in amount of agent recovered from controls
Agent Transfer—General Comments		
Painted Surface to Another Surface	Good agreement among test plates and amount of HD left after contacting dental dam or skin	Significant difference between amount of HD left on test plates after contacting dental dam versus skin
Painted Surface to Dental Dam	Amount of HD transferred to dental dam and recovered by chemical analysis assumed to be equal to amount transferred to skin	ANOVA on agent recovery data from painted metal showed dental dam absorbs less than skin; however difference depends on other factors, such as paint type and agent loaded
Intensity of Agent Damage on Rabbit Skin		
Effect of Paint Type	No observations made	Direct effect of paint type not significant; significant interaction between paint type and contact type (vapor versus direct), as well as between paint type and aging
Effect of Contact Type	Based on analysis of rabbit exposures with no aging between agent rinse and skin contact, intensity of injury less with vapor than direct contact; difference more pronounced for alkyd than for polyurethane	Based on ANOVA of total dataset, direct contact produced more intense injury than vapor contact; type of contact most influential factor or interaction on the intensity of agent damage
Effect of Agent Amount Loaded and Aging	Observed great reduction in intensity of damage when aging increased from zero to 15 min; greater damage with greater agent loaded	Based on ANOVA of total dataset, both agent loaded and aging are significant effects, but neither as influential as contact type

6 SUMMARY, CONCLUSIONS, AND THE WAY FORWARD

The toxicological data for contact hazard are seriously inadequate. This statement is applicable to the data for contact hazard *per se* and the data necessary to provide reliable human toxicity estimates of the hazards resulting from direct or indirect contact with surfaces that have been contaminated.

Many of the existing studies were designed to address questions that are not necessarily relevant today. The human contact hazard data were not generated from the perspective of determining the mechanism of agent "contact"—vapor versus pseudo-liquid transfer of sorbed agent, nor were they done from the perspective of determining the duration and severity of the hazard. However, the studies do indicate that a hazard can persist for days, weeks, and longer. The following observations are made:

- (1) Simulant and roller pick-up studies—compared with live agent and/or human traversal indicate poor correlation between the former and the latter.
- (2) Many of the animal studies failed to follow proper statistical practices in the design and execution of the experiments. Common errors included (but were not limited to): lack of proper randomization procedures; frequent confounding of the main factor effects (due to poor design or lack of randomization); and frequent use of designs with incomplete crossing of factors. Thus, the findings of these studies must be viewed with some skepticism.
- (3) Bioavailability must be systematically investigated; it cannot be assumed that the amount of recoverable agent equates with the toxic hazard.

As stated by Manthei *et al.* (1983), "One major problem that has faced researchers investigating contact hazard has been comparing their results with those reported in the literature. For painted surfaces, it is very important to know the history and physical condition of the paint before making assessments of the data collected from the surfaces. It is known that alkyd paints require extensive exposure to the atmosphere, frequently on the order of 6 months to a year, until the paint has cured completely. Even after a complete cure is accomplished, the paint is very sorptive for liquids deposited on the surface. Similarly, urethane type paints can vary in their sorptive properties depending on the cure history of the paint. In addition to the cure state of the paint film, other key paint film properties include paint thickness, surface roughness, surface cleanliness, and freedom from cracks and crevices. Without this information, results of one set of experiments can not be directly compared with those obtained by others. At a very low contamination level, it might be possible for the transport of HD vapor to be almost undetected on the rabbit skin. This would make it difficult to select the correct mechanism."

The larger body of data clearly indicates that "contact hazard" cannot be separated from "agent fate". Merely determining the amount of agent retained in a surface does not determine the "contact hazard" that may be present, because it does not address the bioavailability of the agent. This is clearly demonstrated by the work of Manthei *et al.* (1983, 1985, 1986, 1988) in which paints containing more agent were not necessarily more of a hazard, and the desorption of agent from paints can present a hazard during the desorption process—even following decontamination. Further, the size of the contaminating drops affects toxicity and persistency: toxicity is a function of drop size *per se*, and drop size affects persistency, hence the amount of agent available to elicit a toxic response. Further, the agent absorption, adsorption, and desorption properties of the contaminated surface determine the actual contact hazard, and these are intimately related to the "fate" of the agent on the surface. Finally, the inability to detect contamination does NOT indicate that there is no hazard—as eloquently demonstrated by Manthei *et al.* (1983, 1985, 1986, 1988).

The requisite toxicological testing will consist of PC (liquid and vapor) and IV administration of selected agents. The doses need to range from those producing mild effects through lethality. Dosemetrics must be included, and the studies must be designed for purposes of extrapolating the data to humans. This will require blood sampling for regenerated agent, ChE inhibition *etc.*, and these requirements limit the species that can be used—adequate body surface area, for PC testing; sufficient blood volume for monitoring agent and effects.

Why is IV testing necessary? A huge number of factors affect PC toxicity. Toxic agents generally elicit the greatest effect and produce the most rapid response *via* the IV route, while the PC route is often the least effective route of exposure (Klaassen *et al.*, 1991). When these factors can be readily separated, but it is not possible to quantitatively determine the exact impact of each factor, a semi-empirical modeling approach may be used to model toxicity. When ready separation of factors is not possible, empirical methods can be used (*e.g.*, the cumulative impact of the physical properties of an agent on toxicity and potency might be evaluated *via* the choice of the agent itself—GB *versus* VX, rather than trying to isolate the toxic contribution of each physical property). When the latter is the case, IV toxicity can be used to bound PC toxicity. Given this, an agent cannot be more potent/efficacious percutaneously than *via* IV exposure (Silver *et al.*, 1952), so IV potency provides the lower bound for

establishing PC potency, and the PC/IV ratio is a convenient measure of PC potency.²⁵ The smallest possible value for the PC/IV ratio is one—indicating equal potency by both routes of exposure. This approach is strengthened by the fact that experimentally derived median effective dosages (ED₅₀s) for IV administration are more precise and reproducible than those for PC administration because there are fewer experimental variables associated with IV exposure.²⁶ This approach is not new. There is considerable historical precedent for evaluating nerve agent PC toxicity using the ratio of PC to IV ED₅₀s (Callahan, 1962; Feinsilver, 1960; Frankel and Wiles, 1960; Manthei *et al.*, 1976; Marzulli, 1955; Wiles, 1962, 1969; Wiles *et al.* 1966, 1971, 1974). However, the method apparently was never fully extended to the development of human PC nerve agent toxicity estimates—particularly for severe effects and lethality. This may well have been a function of the fact that IV exposure is unlikely in a battlefield environment.²⁷

When defensible human toxicity estimates for IV exposure are not available, they must be developed before the PC/IV model can be used for human estimates for PC exposure. The foundation for developing human IV estimates was laid by Sommerville (2004)^{28,29} who modeled lethality for IV exposure in non-anesthetized mammalian species as a function of species body mass for G- and V-type agents. An allometric scaling equation for mammalian IV toxicity was derived, and human IV toxicity estimates for 12 agents were calculated. It was found that (in general) on per mg/kg basis, larger mammalian species are more susceptible to nerve agent poisoning *via* the IV route than smaller mammalian species. Even when human IV toxicity estimates are available, selection of the appropriate PC/IV ratio value for a particular exposure scenario is not trivial. There can be a wide range in PC/IV ratios among mammalian species. Different PC/IV ratios may be warranted for different agent/exposure scenarios. For example, the lowest ratio could provide a “worst-case” estimate, while the median ratio would provide more of a “best guess”. This methodology is employed as a meta-analysis—no single species is used to model PC nerve agent toxicity for humans. The whole available mammalian PC to IV ratio dataset is considered. It should be noted that this approach assumes that the allometric relationship is valid for the agent in question. Such an assumption should be verified for any new nerve agent by performing IV lethality studies on a minimum of two or three mammalian species—preferably non-rodent³⁰ and encompassing as large a range of species body mass values as possible.

The necessary studies required to fill the void surrounding “contact hazard” will require toxicological testing in multiple species—species appropriate for the agents in question. The question is what are the appropriate species? Based on studies of PC absorption (Wester and Noonan, 1980), the rat and rabbit are not good predictive models of PC absorption in humans—their rate of dermal absorption is considerably higher than that of humans; swine and monkeys may be better predictors.

²⁵ One of the earliest proponents of this approach was Marzulli (1955).

²⁶ IV toxicity has also been used as a benchmark for inhalation toxicity (Silver, 1953 and Harvey, *et al.*, 1970).

²⁷ In a review of two summary documents (Department of the Army, 1974a,b) on CW agents, human lethality estimates for only two nerve agents (GB and VX) were found.

²⁸ Sommerville, D.R., Review and Statistical Analysis of Mammalian (Non-Anesthetized) Nerve Agent Intravenous Lethality Data: Part I—Review of US Studies, U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, 2006 (in preparation). UNCLASSIFIED.

²⁹ Sommerville, DR, Review and Statistical Analysis of Mammalian (Non-Anesthetized) Nerve Agent Intravenous Lethality Data: Part II—Comparison Between US and Foreign Studies, U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, 2006 (in preparation). SECRET.

³⁰ Rodents—particularly mice and rats- are problematic for modeling nerve agent IV toxicity because they are relatively resistant to these agents. This resistance is due the organophosphate (OP) scavenging properties of carboxylesterases (CaE) in their plasma and organs (Cerasoli, *et al.*, 2002; Maxwell *et al.*, 1987; Maxwell, 1992a,b; Maxwell and Brecht, 2001; Lancios, 2002; Mioduszewski *et al.*, 2001). However, CaE-induced resistance to nerve agents is not universal. Maxwell (1992a,b) reported that CaE is less protective against GA and VX than other OP nerve agents. Sommerville (2004a,b) empirically confirmed this and also found that CaE affords less protection for VE and EA5365 than for many other agents. The practical effect of CaE protection is to lower the estimate of the allometric scaling exponent (Sommerville, 2004a,b); if this allometric exponent is not properly adjusted, IV toxicity for larger animals will be overestimated.

Swine are somewhat refractory to the effects of nerve agents—their LD₅₀s are higher than those of many other species (Reutter *et al.*, 2003).

However the question at hand is not PC absorption *per se*, but relevant toxicological models—which include: (a) the species used; (b) the type of exposure; (c) the type of data obtained; and (d) the mathematical and statistical treatment of the data. Appropriate species are (1) not refractory to nerve agents (*e.g.*, rodents), (2) can be used for PC and IV exposures, and (3) are large enough for repeated blood samples. What constitutes an appropriate animal model also includes the extent of the knowledgebase for the species—with regard to available data on the compound of interest and similar compounds. PC exposures cannot be safely executed in small species and such species may not provide sufficient blood samples. Despite their stated limitations, rabbits and swine fulfill the criteria for species suitable for PC and IV exposure and from which sufficient blood samples can be drawn. In addition, the nerve agent databases on these two species are quite extensive. The known limitations of these species can be overcome, in part, by the modeling of the toxicological data. In short, the conundrum devolves to the infamous quote from George Box (1987), “Remember that all models are wrong; the practical question is how wrong do they have to be to not be useful.” The way forward to comprehending contact hazard is to better understand the PC toxicity of the chemical agents and to establish reliable human toxicity estimates.

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APPENDIX A

MINITAB™ ANALYSIS OF RABBIT AND HUMAN MUSTARD PC VAPOR TOXICITY DATA FROM EYSTER AND MAVER (1920)

A1 INTRODUCTION

Quantal human and rabbit mustard (HD) percutaneous (PC) vapor toxicity data from Eyster and Maver (1920)¹ were reviewed and analyzed using modern statistical software.² The purpose of this analysis was to estimate the relative difference between humans and rabbits in their sensitivity to exposure to HD vapor, and to estimate the EC_{t50} (skin reaction) for rabbits.

Eyster and Maver (1920) exposed rabbits and humans to HD vapor *via* a vapor cup apparatus (2 cm diameter). For rabbits, skin (shaved) and eyes¹ were exposed; exposure durations ranged from 15 to 60 min; vapor concentrations ranged from 10 to 1860 mg/m³. Tests were also performed with skin covered with either dry or wet wool. For rabbits, the effects on the skin were ranked qualitatively on a scale from 0 (no effect) to 3 (severe reaction). For the human subjects, exposure durations were limited to just 30 min, with vapor concentrations ranging from 1 to 51 mg/m³. Only bare skin was exposed—no runs were performed with either dry or wet wool. The effects on the skin were not ranked quantitatively; instead a qualitative description of the observed effect was recorded. No guide was provided by Eyster and Maver to equate the skin reaction levels between rabbit and human, so HD PC vapor toxicity can only be compared *via* the ratio of EC_{t50} values (skin reaction) for the two species.

The original quantal rabbit data were subjected to several types of statistical analysis (probit analysis³ and binary and ordinal logistic regression^{4,5}) using MINITAB™.² EC_{t50} (skin reaction) and probit slope values were calculated separately for rabbit and human, and the ratio of EC_{t50} values (with confidence limits) was estimated. The time dependence of HD PC vapor toxicity in the rabbit was also investigated to see if Haber's law (EC_{t50} stays constant with respect to exposure time) was a valid model for explaining the data.

A1.1 Analysis Background Information.

Date of Analysis: 6 December 2002

Analyst: Douglas R. Sommerville, PE, Edgewood CB Center, APG, MD

Statistical Analysis Performed Using MINITAB™, v. 13.32

Analyst comments within the MINITAB™ printouts shown below are preceded by [DRS].

A1.2 Nomenclature.

ANOVA	Analysis of Variance
Clothing	0 for bare skin; 1 for dry wool; and 2 for wet wool
Conc	HD (mustard) concentration (mg/m ³)
Ct	Concentration x time (mg-min/m ³)
EC _{t50}	Effective Concentration-time for 50% of exposed individuals (mg-min/m ³)
EC ₅₀	Effective Concentration for 50% of exposed individuals (mg/m ³)
Groups	H0: Human bare skin R0: Rabbit bare skin R1: Rabbit with dry wool R2: Rabbit with wet wool
GroupsA	R0: Rabbit bare skin

¹ Only the skin data were included in this analysis.

	RC: Rabbit with either dry or wet wool
k0, k1, etc	Fitted Coefficients from linear regression analysis
logEC	Log base 10 of EC ₅₀
logC	Log base 10 of vapor concentration
log(t)	Log base 10 of exposure time
logECt	Log base 10 of ECt ₅₀
Number	Number of subjects in test group
Residual	Residual from linear regression fit (predicted LD50 minus actual LD50)
Score	Severity of skin reaction in rabbits (from 0 (no effect) to 3 (severe effect))
Skin	Number of individuals experiencing a skin reaction
Species	Self-explanatory
St. Resid	Standardized residual
time	Exposure time (minutes)
Z	Normit

A2 DATA PREPARATION

The following quantal data were extracted for analysis from Eyster and Maver.

Data Display

Row	Species	Groups	Conc	time	Ct	Score	Clothing	Skin
1	Human	H0	1	30	30	*	0	1
2	Human	H0	6	30	180	*	0	1
3	Human	H0	7	30	210	*	0	1
4	Human	H0	9	30	270	*	0	1
5	Human	H0	12	30	360	*	0	0
6	Human	H0	12	30	360	*	0	1
7	Human	H0	17	30	510	*	0	1
8	Human	H0	18	30	540	*	0	1
9	Human	H0	20	30	600	*	0	1
10	Human	H0	21	30	630	*	0	1
11	Human	H0	22	30	660	*	0	1
12	Human	H0	24	30	720	*	0	1
13	Human	H0	26	30	780	*	0	1
14	Human	H0	34	30	1020	*	0	1
15	Human	H0	40	30	1200	*	0	1
16	Human	H0	40	30	1200	*	0	1
17	Human	H0	44	30	1320	*	0	0
18	Human	H0	51	30	1530	*	0	1
19	Human	H0	76	30	2280	*	0	1
20	Rabbit	R0	1860	15	27900	1	0	1
21	Rabbit	R1	1860	15	27900	1	1	1
22	Rabbit	R0	10	30	300	0	0	0
23	Rabbit	R0	18	30	540	0	0	0
24	Rabbit	R0	25	30	750	1	0	1
25	Rabbit	R0	40	30	1200	0	0	0
26	Rabbit	R0	47	30	1410	1	0	1
27	Rabbit	R0	54	30	1620	0	0	0
28	Rabbit	R0	60	30	1800	0	0	0
29	Rabbit	R0	100	30	3000	0	0	0
30	Rabbit	R0	120	30	3600	0	0	0
31	Rabbit	R0	160	30	4800	1	0	1
32	Rabbit	R0	160	30	4800	2	0	1
33	Rabbit	R0	200	30	6000	0	0	0
34	Rabbit	R0	220	30	6600	2	0	1
35	Rabbit	R0	220	30	6600	2	0	1
36	Rabbit	R0	240	30	7200	2	0	1

37	Rabbit	R0	280	30	8400	2	0	1
38	Rabbit	R0	400	30	12000	2	0	1
39	Rabbit	R0	590	30	17700	2	0	1
40	Rabbit	R0	720	30	21600	3	0	1
41	Rabbit	R0	900	30	27000	3	0	1
42	Rabbit	R0	1000	30	30000	3	0	1
43	Rabbit	R0	1020	30	30600	3	0	1
44	Rabbit	R0	1179	30	35370	2	0	1
45	Rabbit	R0	1190	30	35700	3	0	1
46	Rabbit	R0	1200	30	36000	3	0	1
47	Rabbit	R0	1830	30	54900	3	0	1
48	Rabbit	R1	18	30	540	0	1	0
49	Rabbit	R1	60	30	1800	0	1	0
50	Rabbit	R1	63	30	1890	0	1	0
51	Rabbit	R1	100	30	3000	0	1	0
52	Rabbit	R1	120	30	3600	0	1	0
53	Rabbit	R1	160	30	4800	1	1	1
54	Rabbit	R1	160	30	4800	2	1	1
55	Rabbit	R1	200	30	6000	0	1	0
56	Rabbit	R1	220	30	6600	2	1	1
57	Rabbit	R1	240	30	7200	1	1	1
58	Rabbit	R1	280	30	8400	2	1	1
59	Rabbit	R1	900	30	27000	3	1	1
60	Rabbit	R1	1190	30	35700	3	1	1
61	Rabbit	R1	1200	30	36000	3	1	1
62	Rabbit	R2	1179	30	35370	0	2	0
63	Rabbit	R2	25	30	750	1	2	1
64	Rabbit	R2	40	30	1200	0	2	0
65	Rabbit	R2	47	30	1410	0	2	0
66	Rabbit	R2	54	30	1620	0	2	0
67	Rabbit	R2	160	30	4800	1	2	1
68	Rabbit	R2	220	30	6600	2	2	1
69	Rabbit	R2	400	30	12000	2	2	1
70	Rabbit	R2	590	30	17700	0	2	0
71	Rabbit	R2	680	30	20400	2	2	1
72	Rabbit	R2	720	30	21600	3	2	1
73	Rabbit	R2	790	30	23700	2	2	1
74	Rabbit	R2	1000	30	30000	2	2	1
75	Rabbit	R2	1020	30	30600	2	2	1
76	Rabbit	R0	1600	45	72000	2	0	1
77	Rabbit	R1	1600	45	72000	2	1	1
78	Rabbit	R0	30	60	1800	0	0	0
79	Rabbit	R0	40	60	2400	0	0	0
80	Rabbit	R0	510	60	30600	3	0	1
81	Rabbit	R0	790	60	47400	2	0	1
82	Rabbit	R1	30	60	1800	0	1	0
83	Rabbit	R1	40	60	2400	0	1	0
84	Rabbit	R2	510	60	30600	0	2	0
85	Rabbit	R2	790	60	47400	2	2	1

A3 DATA ANALYSIS

A3.1 Ordinal Logistic Regression Analysis of Score versus logCt and GroupsA for Rabbit.

An ordinal logistic regression was performed on the rabbit data to determine the effect of clothing on the toxicity of HD PC vapor on rabbits. Ordinal regression allows the use of the qualitative scores assigned by Eyster and Maver to rank the severity of skin reaction on rabbits (0 = no effect; 1 = very slight effect; 2 = moderate effect; 3 = severe lesion). The following are the results of the initial analysis.

MINITAB Printout of Results of Analysis

Ordinal Logistic Regression: Score versus logCt, GroupsA

Link Function: Normit

Response Information

Variable	Value	Count
Score	0	24
	1	9
	2	21
	3	12
	Total	66

Factor Information

Factor	Levels	Values
GroupsA	2	R0 RC

66 cases were used

Logistic Regression Table

Predictor	Coef	SE Coef	Z	P
Const(1)	6.398	1.177	5.43	0.000
Const(2)	6.985	1.215	5.75	0.000
Const(3)	8.464	1.326	6.38	0.000
logCt	-1.8723	0.3106	-6.03	0.000
GroupsA				
RC	0.5533	0.2961	1.87	0.062

[DRS] Clothing (as represented by the term GroupsA) is borderline significant (P-value of 0.062).

Log-likelihood = -63.275

Test that all slopes are zero: G = 46.881, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	152.189	166	0.771
Deviance	117.185	166	0.998

Measures of Association:

(Between the Response Variable and Predicted Probabilities)

Pairs	Number	Percent	Summary Measures
Concordant	1318	84.6%	Somers' D 0.70
Discordant	233	15.0%	Goodman-Kruskal Gamma 0.70
Ties	6	0.4%	Kendall's Tau-a 0.51
Total	1557	100.0%	

If a binary logistic regression is performed on the rabbit data (0 = no effect; 1 = any skin reaction), then clothing becomes a non-significant factor. Only ordinal logistic regression had the strength (in this instance) to detect the effect of clothing.

The effect of exposure duration on the toxicity was also investigated via ordinal logistic regression by substituting logC and log(t) into the model for logCt. It was found that there was not enough statistical

evidence to reject the null hypothesis that the toxicity obeys Haber's Law (ECt_{50} is constant with respect to t). Thus, for all subsequent work, Haber's Law was assumed.

A3.2 Probit Analysis of Skin versus logCt and Groups for Human and Rabbit.

A probit analysis was performed on the combined rabbit and human dataset. The factor Groups was used to divided the dataset into four groups: human (bare skin)—H0, rabbit (bare skin)—R0, rabbit (skin covered with dry wool)—R1, and rabbit (skin covered with wet wool)—R2.

MINITAB Printout of Results of Analysis

Probit Analysis: Skin versus Ct, Groups

Distribution: Lognormal base 10

Response Information

Variable	Value	Count
Skin	1	59 (Event)
	0	26
	Total	85

Factor Information

Factor	Levels	Values
Groups	4	H0 R0 R1 R2

Estimation Method: Maximum Likelihood

Regression Table

Variable	Coef	Standard Error	Z	P
Constant	-2.4561	0.9004	-2.73	0.006
Ct	1.3948	0.3130	4.46	0.000
Groups				
R0	-2.2514	0.5867	-3.84	0.000
R1	-2.6491	0.6352	-4.17	0.000
R2	-2.7558	0.6814	-4.04	0.000
Natural Response	0.000			

Test for equal slopes: Chi-Square = 9.3638, DF = 3, P-Value = 0.025
Log-Likelihood = -37.205

Multiple degree of freedom test

Term	Chi-Square	DF	P
Groups	19.788	3	0.000

Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	92.184	71	0.046
Deviance	71.638	71	0.456
Hosmer-Lemeshow	4.675	8	0.792

Table of Observed and Expected Frequencies:
 (See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

Value	Group										Total	
	1	2	3	4	5	6	7	8	9	10		
1												
Obs	2	2	4	6	6	7	7	9	8	8	59	
Exp	1.4	3.1	3.9	5.6	6.0	7.5	7.0	8.3	7.6	8.7		
0												
Obs	6	7	4	3	2	2	1	0	0	1	26	
Exp	6.6	5.9	4.1	3.4	2.0	1.5	1.0	0.7	0.4	0.3		
Total	8	9	8	9	8	9	8	9	8	9	85	

Groups = H0

Tolerance Distribution

Parameter Estimates

Parameter	Estimate	Standard Error	95.0% Normal CI	
			Lower	Upper
Location	1.7609	0.3396	1.0953	2.4265
Scale	0.7170	0.1609	0.4618	1.1131

Characteristics of Distribution

	Estimate	Standard Error	95.0% Normal CI	
			Lower	Upper
Mean(MTTF)	225.2535	154.0603	58.9521	860.6845
Standard Deviation	850.6388	909.3335	104.6671	6913.218
Interquartile Range(IQR)	156.6369	104.4939	42.3692	579.0797

	Estimate	Standard Error	95.0% Fiducial CI	
			Lower	Upper
Median	57.6609	45.0864	6.2761	214.3527
First Quartile(Q1)	18.9367	17.8183	1.0467	81.2497
Third Quartile(Q3)	175.5736	119.3810	32.6981	650.8222

Table of Percentiles

Percent	Percentile	Standard Error	95.0% Fiducial CI Lower	95.0% Fiducial CI Upper
50	57.6609	45.0864	6.2761	214.3527

Groups = R0

Tolerance Distribution

Parameter Estimates

Parameter	Estimate	Standard Error	95.0% Normal CI	
			Lower	Upper
Location	3.3750	0.2062	2.9709	3.7792
Scale	0.7170	0.1609	0.4618	1.1131

Characteristics of Distribution

	Estimate	Standard Error	95.0% Normal CI	
			Lower	Upper
Mean(MTTF)	9263.966	6099.317	2549.019	33668.28
Standard Deviation	34984.09	42628.98	3211.147	381136.8
Interquartile Range(IQR)	6441.983	3139.350	2478.600	16742.98

	Estimate	Standard Error	95.0% Fiducial CI	
			Lower	Upper
Median	2371.411	1125.933	720.4283	5839.905
First Quartile(Q1)	778.8053	464.4899	131.6609	2020.099
Third Quartile(Q3)	7220.788	3387.165	2909.564	22873.67

Table of Percentiles

Percent	Percentile	Standard Error	95.0% Fiducial CI	
			Lower	Upper
50	2371.411	1125.933	720.4283	5839.905

Groups = R1

Tolerance Distribution

Parameter Estimates

Parameter	Estimate	Standard Error	95.0% Normal CI	
			Lower	Upper
Location	3.6602	0.2448	3.1805	4.1399
Scale	0.7170	0.1609	0.4618	1.1131

Characteristics of Distribution

	Estimate	Standard Error	95.0% Normal CI	
			Lower	Upper
Mean(MTTF)	17863.05	14401.87	3678.617	86741.47
Standard Deviation	67457.35	91365.10	4744.069	959196.4
Interquartile Range(IQR)	12421.62	7784.684	3636.860	42425.80

	Estimate	Standard Error	95.0% Fiducial CI	
			Lower	Upper
Median	4572.624	2576.952	1288.161	15091.84
First Quartile(Q1)	1501.716	946.2784	271.1132	4533.092
Third Quartile(Q3)	13923.34	8389.035	4614.685	66640.38

Table of Percentiles

Percent	Percentile	Standard Error	95.0% Fiducial CI	
			Lower	Upper
50	4572.624	2576.952	1288.161	15091.84

Groups = R2

Tolerance Distribution

Parameter Estimates

Parameter	Estimate	Standard Error	95.0% Normal CI	
			Lower	Upper
Location	3.7367	0.2511	3.2445	4.2288
Scale	0.7170	0.1609	0.4618	1.1131

Characteristics of Distribution

	Estimate	Standard Error	95.0% Normal CI	
			Lower	Upper
Mean(MTTF)	21304.43	15996.31	4890.466	92808.94
Standard Deviation	80453.25	102967.3	6548.499	988428.8
Interquartile Range(IQR)	14814.69	8854.586	4591.328	47802.09

	Estimate	Standard Error	95.0% Fiducial CI	
			Lower	Upper
Median	5453.556	3153.261	1352.138	17082.99
First Quartile(Q1)	1791.026	1209.247	262.9341	5553.553
Third Quartile(Q3)	16605.72	9649.514	5336.092	68474.50

Table of Percentiles

Percent	Percentile	Standard Error	95.0% Fiducial CI	
			Lower	Upper
50	5453.556	3153.261	1352.138	17082.99

Table of Relative Potency

Factor: Groups

Comparison	Relative Potency	95.0% Fiducial CI	
		Lower	Upper
H0 VS R0	41.1268	8.5363	366.3611
H0 VS R1	79.3020	14.5450	993.5333
H0 VS R2	94.5798	17.1537	1000.944
R0 VS R1	1.9282	0.4348	10.6278
R0 VS R2	2.2997	0.4800	11.4376
R1 VS R2	1.1927	0.1888	6.2948

=====

The results of the above probit analysis are summarized in the following table.

Table. Results of Probit Analysis of Eyster and Maver HD PC Vapor Toxicity Data for Humans and Rabbits at Moderate Temperatures

Group	Species	Clothing	HD PC Vapor ECT ₅₀ (Skin Reaction) Estimates and Fiducial Intervals			Ratio of ECT ₅₀ (Skin Reaction) Values (Rabbit/Human)		
			Fit	Lower Limit (95%)	Upper Limit (95%)	Fit	Lower Conf. Limit (95%)	Upper Conf. Limit (95%)
H0	Human	none	58	6.3	214			
R0	Rabbit	none	2400	720	5800	41	8.5	366
R1	Rabbit	dry wool	4600	1300	15000	79	15	994
R2	Rabbit	wet wool	5500	1400	17000	95	17	1001

It was found from the analysis that the null hypothesis [no difference among the four groups (H0, R0, R1 and R2)] can be rejected with 95% confidence. In particular, the ratio of ECT₅₀ (rabbit/human) is statistically different from a value of one, for any rabbit category compared to human (bare skin). However, none of the individual rabbit ECT₅₀ values are statistically different from the other rabbit values. However, as noted in Section A3.1, an ordinal logistic regression did detect a difference (with slight statistical significance) due to the presence or absence of clothing on the rabbit.

It should be noted that the above ECT₅₀ values are for exposures to HD vapor *via* a vapor cup. For whole body exposures, the ECT₅₀ values will probably be a factor of 3 to 4 lower (for rabbit and human).

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APPENDIX B

MINITAB™ ANALYSIS OF RABBIT PERCUTANEOUS MUSTARD VAPOR TOXICITY DATA FROM THE CHEMICAL WARFARE BOARD REPORT ON PROJECT 433 (1944)

B1 INTRODUCTION

Quantal rabbit mustard (H) percutaneous (PC) toxicity data from the Chemical Warfare Board (CWB) 1944 report on Project No. 433 (*Effect of Sands on Chemical Agent H*) were reviewed and analyzed using modern statistical software (MINITAB™, version 14). The purpose of this analysis of the CWB (1944) data was to investigate the extent of the contact hazard from H-contaminated sand and coral. [See Section 3.2 (main body of report) for a more detailed description of this work.]

The CWB (1944) report used rabbits as biosensors. They were either staked to the ground or suspended in cages one foot above ground. For PC exposures the skin was shaved; in some cases agent was extracted from soil and then applied to skin [Table 5 (main body of report)]. All of the data were collected from 23 March to 19 April 1944 at Garden Key, Dry Tortugas, FL. The meteorological conditions were fairly constant over this period, with the air temperatures generally ranging from 74 to 85°F, and the ground temperatures ranging from 74 to 98°F (dry surface) and 74 to 92°F (wet surfaces). It was assumed, in this statistical re-analysis, that the indirect effect from variation in meteorological conditions on toxic responses in the rabbits was minor in comparison to the effect from other factors.

B1.1 Analysis Background Information.

Date of Analysis: 9 June 2005

Analyst: Douglas R. Sommerville, PE, Edgewood CB Center, APG, MD

Statistical Analysis Performed Using MINITAB™, v. 14

Analyst comments within the MINITAB™ printouts shown below are preceded by [DRS].

B1.2 Nomenclature.

Agent	Type of mustard agent used to contaminate sand levinH—Levinstein H strippedH—Stripped mustard
Aging	Duration (in days) between contamination of terrain and rabbit exposure
AgingB	Equals 1 for Aging from 0 to 1.99 Equals 2 for Aging from 2.0 to 2.99 Equals 3 for Aging from 3.0 to 3.99 Equals 4 for Aging greater than or equal to 4
ANOVA	Analysis of Variance
Binary	No effect (Binary equals zero) vs. any effect (Binary equals one)
BinaryS	No effect (Binary equals zero) vs. any effect (Binary equals one) with data from Depth equal to “deep” excluded
Density	Initial agent mass density on contaminated surface (in g/m ²).
Depth	Depth of contaminated surface that rabbit was exposed to Shallow—Rabbit exposed to sand sample from upper surface Deep—Rabbit exposed to sand sample obtained from one-half to 2 in. below the upper surface
ED _{xx}	Initial contamination density that will produce XX% effects in exposed rabbits
EPRO	Predicted event probability from final model fit
logA	Logarithm (base 10) of Aging
logD	Logarithm (base 10) of Density
noSand	Equals one for if Type is either bone or rock coral; zero otherwise

Route	Route of exposure Eyes—Exposure of eyes to vapor off-gassing from contaminated surface from a distance of 1 ft below the rabbit Skin—Vapor and liquid exposure of shaved skin due to direct contact of rabbit with contaminated surface
Sand	Indicator variable for Sand vs. Coral yes—either beach or coral sand no—either bone or rock coral unknown—type of terrain was not recorded (it was either sand or coral)
Score	Observed severity of toxic effect to either eye or skin 0—no effect 1—mild reaction 2—moderate reaction 3—marked reaction (vesication) 4—extreme reaction (vesication)
SPRE	Standardized Pearson residual
Table	Number of Table in Chemical Warfare Board (CWB) (1944)
Table7	Equals one if data are from Table 7 of CWB (1944); and zero otherwise
Type	Type of terrain Bcoral—bone coral Bsand—beach sand Csand—coral sand Rcoral—rock coral unknown—type of terrain was not recorded (it was either sand or coral)
unkSand	Equals one if Type equals "unknown"; zero otherwise
yesSand	Equals one if Type is either beach or coral sand; zero otherwise
Z	Normit

B2 DATA PREPARATION

The following quantal data for PC exposures of rabbits to H data were extracted from Tables 2, 3, 5, 6, and 7 of the CWB (1944) report. Each row (163 rows total) represents the test conditions and observed results for one exposed rabbit. There is no indication as to whether the runs in the study were properly randomized. However, incomplete crossing of the test parameters in the experimental design is not that great. Thus, investigation of interactions between the various factors is possible.

One problem with the way the data were recorded is that the type of terrain (sand or coral) was used in the taking of the quantal data in Table 7 of CWB (1944). This was accounted for in the subsequent analysis by using indicator variables to distinguish between what was known for terrain type (beach and coral sand; and bone and rock coral) and was unknown (data from Table 7).

B2.1 Listing of Quantal Data.

Row	Table	Type	Route	Aging	Density	Depth	Agent	Score	Binary
1	2	Csand	Skin	1.0000	50	deep	levinH	0	0
2	2	Csand	Skin	1.0000	100	deep	levinH	1	1
3	2	Csand	Skin	1.0000	300	deep	levinH	0	0
4	2	Csand	Skin	1.0000	300	deep	strippedH	0	0
5	2	Csand	Skin	2.0000	50	deep	levinH	0	0
6	2	Csand	Skin	2.0000	100	deep	levinH	0	0
7	2	Csand	Skin	2.0000	300	deep	levinH	1	1
8	2	Csand	Skin	2.0000	300	deep	strippedH	0	0
9	2	Csand	Skin	3.0000	300	deep	levinH	1	1
10	2	Csand	Skin	3.0000	300	deep	strippedH	1	1
11	2	Csand	Skin	1.0000	50	shallow	levinH	1	1
12	2	Csand	Skin	1.0000	100	shallow	levinH	2	1
13	2	Csand	Skin	1.0000	300	shallow	levinH	1	1

14	2	Csand	Skin	1.0000	300	shallow	strippedH	3	1
15	2	Csand	Skin	2.0000	50	shallow	levinH	1	1
16	2	Csand	Skin	2.0000	100	shallow	levinH	1	1
17	2	Csand	Skin	2.0000	300	shallow	levinH	3	1
18	2	Csand	Skin	2.0000	300	shallow	strippedH	3	1
19	2	Csand	Skin	3.0000	300	shallow	levinH	1	1
20	2	Csand	Skin	3.0000	300	shallow	strippedH	1	1
21	3a	Csand	Skin	1.0000	50	shallow	levinH	1	1
22	3a	Csand	Skin	1.0000	100	shallow	levinH	2	1
23	3a	Csand	Skin	1.0000	300	shallow	levinH	1	1
24	3a	Csand	Skin	1.0000	300	shallow	strippedH	3	1

Row	Table	Type	Route	Aging	Density	Depth	Agent	Score	Binary
25	3a	Csand	Skin	2.0000	50	shallow	levinH	1	1
26	3a	Csand	Skin	2.0000	100	shallow	levinH	1	1
27	3a	Csand	Skin	2.0000	300	shallow	levinH	3	1
28	3a	Csand	Skin	2.0000	300	shallow	strippedH	3	1
29	3a	Csand	Skin	3.0000	50	shallow	levinH	0	0
30	3a	Csand	Skin	3.0000	100	shallow	levinH	0	0
31	3a	Csand	Skin	3.0000	300	shallow	levinH	1	1
32	3a	Csand	Skin	3.0000	300	shallow	strippedH	0	0
33	3a	Csand	Skin	5.0000	50	shallow	levinH	0	0
34	3a	Csand	Skin	5.0000	100	shallow	levinH	0	0
35	3a	Csand	Skin	5.0000	300	shallow	levinH	0	0
36	3a	Csand	Skin	5.0000	300	shallow	strippedH	1	1
37	3b	Bsand	Skin	1.0000	300	shallow	levinH	4	1
38	3b	Bsand	Skin	2.0000	300	shallow	levinH	3	1
39	3b	Bsand	Skin	7.0000	300	shallow	levinH	0	0
40	3b	Csand	Skin	1.0000	50	shallow	levinH	1	1
41	3b	Csand	Skin	1.0000	100	shallow	levinH	2	1
42	3b	Csand	Skin	1.0000	300	shallow	levinH	4	1
43	3b	Csand	Skin	1.0000	300	shallow	strippedH	4	1
44	3b	Csand	Skin	2.0000	50	shallow	levinH	2	1
45	3b	Csand	Skin	2.0000	100	shallow	levinH	2	1
46	3b	Csand	Skin	2.0000	300	shallow	levinH	3	1
47	3b	Csand	Skin	2.0000	300	shallow	strippedH	3	1
48	3b	Csand	Skin	4.0000	50	shallow	levinH	0	0
49	3b	Csand	Skin	7.0000	100	shallow	levinH	0	0
50	3b	Csand	Skin	7.0000	300	shallow	levinH	0	0
51	3b	Csand	Skin	7.0000	300	shallow	strippedH	0	0
52	5	Bcoral	Skin	1.0000	50	shallow	levinH	1	1
53	5	Bcoral	Skin	1.0000	100	shallow	levinH	1	1
54	5	Bcoral	Skin	1.0000	300	shallow	levinH	2	1
55	5	Bcoral	Skin	1.0000	300	shallow	strippedH	2	1
56	5	Bcoral	Skin	2.0000	50	shallow	levinH	2	1
57	5	Bcoral	Skin	2.0000	100	shallow	levinH	2	1
58	5	Bcoral	Skin	2.0000	300	shallow	levinH	2	1
59	5	Bcoral	Skin	2.0000	300	shallow	strippedH	1	1
60	5	Bcoral	Skin	3.0000	50	shallow	levinH	0	0
61	5	Bcoral	Skin	3.0000	100	shallow	levinH	1	1
62	5	Bcoral	Skin	3.0000	300	shallow	levinH	2	1
63	5	Bcoral	Skin	3.0000	300	shallow	strippedH	1	1
64	6	Rcoral	Skin	1.0000	50	shallow	levinH	2	1
65	6	Rcoral	Skin	1.0000	100	shallow	levinH	2	1
66	6	Rcoral	Skin	1.0000	300	shallow	levinH	2	1
67	6	Rcoral	Skin	1.0000	300	shallow	strippedH	0	0
68	6	Rcoral	Skin	2.0000	50	shallow	levinH	1	1
69	6	Rcoral	Skin	2.0000	100	shallow	levinH	2	1
70	6	Rcoral	Skin	2.0000	300	shallow	levinH	2	1
71	6	Rcoral	Skin	2.0000	300	shallow	strippedH	2	1
72	6	Rcoral	Skin	3.0000	50	shallow	levinH	1	1

73	6	Rcoral	Skin	3.0000	100	shallow	levinH	1	1
74	6	Rcoral	Skin	3.0000	300	shallow	levinH	2	1
75	6	Rcoral	Skin	3.0000	300	shallow	strippedH	1	1
76	7	Unknown	Skin	1.0000	100	shallow	levinH	1	1
77	7	Unknown	Skin	1.0000	100	shallow	levinH	2	1
78	7	Unknown	Skin	1.0000	100	shallow	levinH	4	1
79	7	Unknown	Skin	1.0000	100	shallow	levinH	4	1
80	7	Unknown	Skin	2.0000	100	shallow	levinH	3	1
81	7	Unknown	Skin	2.1250	100	shallow	levinH	2	1
82	7	Unknown	Skin	2.1250	100	shallow	levinH	3	1
83	7	Unknown	Skin	2.3330	100	shallow	levinH	2	1
84	7	Unknown	Skin	2.3330	100	shallow	levinH	3	1
85	7	Unknown	Skin	4.0830	100	shallow	levinH	0	0

Row	Table	Type	Route	Aging	Density	Depth	Agent	Score	Binary
86	7	Unknown	Skin	7.0000	100	shallow	levinH	0	0
87	7	Unknown	Skin	7.0000	100	shallow	levinH	0	0
88	7	Unknown	Skin	7.0000	100	shallow	levinH	0	0
89	7	Unknown	Skin	7.2500	100	shallow	levinH	0	0
90	3a	Csand	Eye	1.0000	50	shallow	levinH	0	0
91	3a	Csand	Eye	1.0000	100	shallow	levinH	0	0
92	3a	Csand	Eye	1.0000	300	shallow	levinH	4	1
93	3a	Csand	Eye	1.0000	300	shallow	strippedH	4	1
94	3a	Csand	Eye	2.0000	50	shallow	levinH	0	0
95	3a	Csand	Eye	2.0000	100	shallow	levinH	0	0
96	3a	Csand	Eye	2.0000	300	shallow	levinH	3	1
97	3a	Csand	Eye	2.0000	300	shallow	strippedH	3	1
98	3a	Csand	Eye	3.0000	50	shallow	levinH	0	0
99	3a	Csand	Eye	3.0000	100	shallow	levinH	0	0
100	3a	Csand	Eye	3.0000	300	shallow	levinH	0	0
101	3a	Csand	Eye	3.0000	300	shallow	strippedH	0	0
102	3a	Csand	Eye	5.0000	50	shallow	levinH	0	0
103	3a	Csand	Eye	5.0000	100	shallow	levinH	0	0
104	3a	Csand	Eye	5.0000	300	shallow	levinH	0	0
105	3a	Csand	Eye	5.0000	300	shallow	strippedH	1	1
106	3b	Bsand	Eye	0.2083	300	shallow	levinH	4	1
107	3b	Bsand	Eye	1.0000	300	shallow	levinH	3	1
108	3b	Bsand	Eye	2.0000	300	shallow	levinH	2	1
109	3b	Bsand	Eye	7.0000	300	shallow	levinH	0	0
110	3b	Csand	Eye	0.2083	50	shallow	levinH	0	0
111	3b	Csand	Eye	0.2083	100	shallow	levinH	0	0
112	3b	Csand	Eye	0.2083	300	shallow	levinH	4	1
113	3b	Csand	Eye	0.2083	300	shallow	strippedH	4	1
114	3b	Csand	Eye	1.0000	50	shallow	levinH	0	0
115	3b	Csand	Eye	1.0000	100	shallow	levinH	0	0
116	3b	Csand	Eye	1.0000	300	shallow	levinH	2	1
117	3b	Csand	Eye	1.0000	300	shallow	strippedH	2	1
118	3b	Csand	Eye	2.0000	50	shallow	levinH	0	0
119	3b	Csand	Eye	2.0000	100	shallow	levinH	0	0
120	3b	Csand	Eye	2.0000	300	shallow	levinH	1	1
121	3b	Csand	Eye	2.0000	300	shallow	strippedH	4	1
122	3b	Csand	Eye	4.0000	50	shallow	levinH	0	0
123	3b	Csand	Eye	7.0000	100	shallow	levinH	0	0
124	3b	Csand	Eye	7.0000	300	shallow	levinH	0	0
125	3b	Csand	Eye	7.0000	300	shallow	strippedH	0	0
126	5	Bcoral	Eye	1.0000	50	shallow	levinH	0	0
127	5	Bcoral	Eye	1.0000	100	shallow	levinH	0	0
128	5	Bcoral	Eye	1.0000	300	shallow	levinH	0	0
129	5	Bcoral	Eye	1.0000	300	shallow	strippedH	0	0
130	5	Bcoral	Eye	2.0000	50	shallow	levinH	0	0
131	5	Bcoral	Eye	2.0000	100	shallow	levinH	0	0

132	5	Bcoral	Eye	2.0000	300	shallow	levinH	1	1
133	5	Bcoral	Eye	2.0000	300	shallow	strippedH	0	0
134	5	Bcoral	Eye	3.0000	50	shallow	levinH	0	0
135	5	Bcoral	Eye	3.0000	100	shallow	levinH	0	0
136	5	Bcoral	Eye	3.0000	300	shallow	levinH	0	0
137	5	Bcoral	Eye	3.0000	300	shallow	strippedH	0	0
138	6	Rcoral	Eye	1.0000	50	shallow	levinH	0	0
139	6	Rcoral	Eye	1.0000	100	shallow	levinH	0	0
140	6	Rcoral	Eye	1.0000	300	shallow	levinH	0	0
141	6	Rcoral	Eye	1.0000	300	shallow	strippedH	0	0
142	6	Rcoral	Eye	2.0000	50	shallow	levinH	0	0
143	6	Rcoral	Eye	2.0000	100	shallow	levinH	0	0
144	6	Rcoral	Eye	2.0000	300	shallow	levinH	0	0
145	6	Rcoral	Eye	2.0000	300	shallow	strippedH	0	0
146	6	Rcoral	Eye	3.0000	50	shallow	levinH	0	0

Row	Table	Type	Route	Aging	Density	Depth	Agent	Score	Binary
147	6	Rcoral	Eye	3.0000	100	shallow	levinH	0	0
148	6	Rcoral	Eye	3.0000	300	shallow	levinH	1	1
149	6	Rcoral	Eye	3.0000	300	shallow	strippedH	0	0
150	7	Unknown	Eye	1.0000	100	shallow	levinH	0	0
151	7	Unknown	Eye	1.0000	100	shallow	levinH	0	0
152	7	Unknown	Eye	1.0000	100	shallow	levinH	3	1
153	7	Unknown	Eye	1.0000	100	shallow	levinH	2	1
154	7	Unknown	Eye	2.0000	100	shallow	levinH	2	1
155	7	Unknown	Eye	2.1250	100	shallow	levinH	0	0
156	7	Unknown	Eye	2.1250	100	shallow	levinH	4	1
157	7	Unknown	Eye	2.3330	100	shallow	levinH	0	0
158	7	Unknown	Eye	2.3330	100	shallow	levinH	1	1
159	7	Unknown	Eye	4.0830	100	shallow	levinH	0	0
160	7	Unknown	Eye	7.0000	100	shallow	levinH	0	0
161	7	Unknown	Eye	7.0000	100	shallow	levinH	0	0
162	7	Unknown	Eye	7.0000	100	shallow	levinH	0	0
163	7	Unknown	Eye	7.2500	100	shallow	levinH	0	0

B2.2 Tabulated Statistics: BinaryS as a Function of Several Parameters.

The following is a tabulation of the number of rabbits affected *per* total number exposed for the test conditions for those runs conducted with Depth = "shallow".

Tabulated statistics: BinaryS as a Function of Density, Aging and Route

Results for Route = Eye:

	Rows: Density Columns: Aging											
	0.21	1.00	2.00	2.13	2.33	3.00	4.00	4.08	5.00	7.00	7.25	All
50	0	0	0	*	*	0	0	*	0	*	*	0
	1	4	4	0	0	3	1	0	1	0	0	14
100	0	2	1	1	1	0	*	0	0	0	0	5
	1	8	5	2	2	3	0	1	1	4	1	28
300	3	5	6	*	*	1	*	*	1	0	*	16
	3	9	9	0	0	6	0	0	2	3	0	32
All	3	7	7	1	1	1	0	0	1	0	0	21
	5	21	18	2	2	12	1	1	4	7	1	74

Results for Route = Skin:

Rows: Density Columns: Aging

	0.21	1.00	2.00	2.13	2.33	3.00	4.00	4.08	5.00	7.00	7.25	All
50	* 0	5 5	5 5	* 0	* 0	1 3	0 1	* 0	0 1	* 0	* 0	11 15
100	* 0	9 9	6 6	2 2	2 2	2 3	* 0	0 1	0 1	0 4	0 1	21 29
300	* 0	10 11	11 11	* 0	* 0	7 8	* 0	* 0	1 2	0 3	* 0	29 35
All	* 0	24 25	22 22	2 2	2 2	10 14	0 1	0 1	1 4	0 7	0 1	61 79

Cell Contents: BinaryS : Number of Rabbits showing any effect
Total number of rabbits exposed

Tabulated statistics: BinaryS as a Function of Density, AgingB, Route and Sand

Results for Sand = yes:

Rows: Density Columns: AgingB / Route

	1		2		3		4		All
	Eye	Skin	Eye	Skin	Eye	Skin	Eye	Skin	All
50	0 3	3 3	0 2	3 3	0 1	0 1	0 2	0 2	6 17
100	0 3	3 3	0 2	3 3	0 1	0 1	0 2	0 2	6 17
300	8 8	7 7	5 5	7 7	0 2	3 4	1 5	1 5	32 43
All	8 14	13 13	5 9	13 13	0 4	3 6	1 9	1 9	44 77

Results for Sand = no:

Rows: Density Columns: AgingB / Route

	1		2		3		4		All
	Eye	Skin	Eye	Skin	Eye	Skin	Eye	Skin	All
50	0 2	2 2	0 2	2 2	0 2	1 2	* 0	* 0	5 12
100	0 2	2 2	0 2	2 2	0 2	2 2	* 0	* 0	6 12
300	0 4	3 4	1 4	4 4	1 4	4 4	* 0	* 0	13 24
All	0 8	7 8	1 8	8 8	1 8	7 8	* 0	* 0	24 48

Results for Sand = unknown: Rows: Density Columns: AgingB / Route

	1		2		3		4		All
	Eye	Skin	Eye	Skin	Eye	Skin	Eye	Skin	All
50	* 0	* 0	* 0	* 0	* 0	* 0	* 0	* 0	* 0

100	2	4	3	5	*	*	0	0	14
	4	4	5	5	0	0	5	5	28
300	*	*	*	*	*	*	*	*	*
	0	0	0	0	0	0	0	0	0
All	2	4	3	5	*	*	0	0	14
	4	4	5	5	0	0	5	5	28

Cell Contents: BinaryS : Number of Rabbits showing any effect
Total number of rabbits exposed

Rabbit 67 was later identified as an outlier and was removed from the dataset for the final analyses. Number 67 was the only rabbit not to be affected by skin exposure to H-contaminated terrain after only 1 or 2 days of weathering (out of 47 rabbits exposed). For all runs after 4 days or more of weathering (Aging >= 4 days), there was no difference in the quantal data between eye and skin exposures.

B3 DATA ANALYSIS

A series of statistical analyses were conducted on the CWB dataset to address the following questions concerning toxic response in the rabbit from PC exposure to H-contaminated terrain (either sand or coral):

- (1) Does the toxicity vary as a function of depth beneath the surface of the contaminated terrain?
- (2) Does the toxicity vary as a function of the agent used?
- (3) Does the toxicity vary as a function of the type of sand/coral contaminated?
- (4) Which route of exposure is the most sensitive detector for identifying the presence of contaminated terrain (eyes or skin) under the conditions used in the study? For eye exposures, the rabbit was suspended 1 foot above the contaminated surface; while for skin exposures, the shaved skin of the rabbit was placed in contact with the contaminated surface.
- (5) How does the toxicity vary as a function of weathering duration (Aging)?
- (6) How does the toxicity vary as a function of the initial agent contamination mass density?
- (7) Are there any statistically significant interactions between the various factors that were investigated?

The binary response data (Binary) were analyzed using the binary logistic regression routine (using a probit-link function) in MINITAB® (version 14). This routine is an extension of the one-factor probit analysis. Finney (1971), Fox (1997), and Hosmer and Lemeshow (1989) provide background information. Equation [1] is the basic model that was used (modified, as needed, by dropping non-significant terms and the addition of significant interactions):

$$Y_N = (Y_P - 5) = k_o + k_D \log_{10} D + k_A \log_{10} A + k_{depth} (\text{Depth}) + k_{agent} (\text{Agent}) + k_{route} (\text{Route}) + \sum_i^2 k_{type,i} (\text{Sand}) \quad [1]$$

where Y_N is a normit, Y_P is a probit, the ks are fitted coefficients, and the other parameters are defined in Section B1.2. Mathematically in Equation [1], the parameters logD and logA are covariates; Depth, Agent and Route are two-level factors; and Sand is a three level factor. It was determined that there was no significant difference between the two types of coral (bone and rock), nor between the two types of sand (beach and coral). Only between sand and coral (as represented by the factor Sand) was any significant difference found. Y_N equals -1, 0 and 1 at the 16, 50 and 84% response levels, respectively.

Effective H (initial) contamination density estimates as a function of XX percent lethality can be calculated by solving for D in Equation [1] and using the appropriate value for Y_N :

$$\log_{10} D_{xx} = \frac{\left[Y_N - k_o - k_A \log_{10} A - k_{depth} (\text{Depth}) - k_{agent} (\text{Agent}) - k_{route} (\text{Route}) - \sum_i^2 k_{type,i} (\text{Sand})_i \right]}{(k_d)} \quad [2]$$

Confidence limits on logD estimates were calculated using methods from Mood *et al.* (1974) and Barry (1978) (see previous example of application in Sommerville (2004)). Barry (1978) gives the standard error of a ratio, (a/b) , which is based upon the propagation of error formula for a ratio:

$$\text{std err of } \left(\frac{a}{b} \right) = \left(\frac{a}{b} \right) \sqrt{\left(\frac{\text{var}(a)}{a^2} \right) + \left(\frac{\text{var}(b)}{b^2} \right) - (2) \left(\frac{\text{cov}(a,b)}{ab} \right)} \quad [3]$$

where $\text{var}(a)$, $\text{var}(b)$, and $\text{cov}(a,b)$ are the variance of the quantities, a and b , and their covariance, respectively. The 95% confidence limits for the ratio will equal $(a/b) \pm (1.96)(\text{std err})$. The following relations from Mood, *et al.* (1974) were also used to get the necessary information for determining the limits for logD:

$$\text{var}(a \pm b) = \text{var}(a) + \text{var}(b) \pm (2)\text{cov}(a, b) \quad [4]$$

$$\text{cov}(a \pm b, c) = \text{cov}(a, c) \pm \text{cov}(b, c) \quad [5]$$

where $\text{cov}(a \pm b, c)$ is the covariance of the quantity, $(a \pm b)$, with a third quantity, c . [An example of how Equations [3] to [5] are used in the present analysis is shown in Section B4.3.]

B4 RESULTS

The results of two analyses are shown below. The first (Section B4.1) was made to determine the effect of depth of contamination in terrain (Depth) on the PC toxicity to exposed rabbits, using the response variable, Binary. The second (in Section B4.2) was made on just binary response data (BinaryS) taken from exposures where Depth equals "shallow". The results from the second analysis are shown in greater detail in Figures B1 through B4 and Tables B1 and B2.

B4.1 Binary Logistic Regression Analysis of Binary vs. logD, logA, Depth, Agent, Route and Type.

It was found that the factors of Agent and Type were not statistically significant, so these factors were dropped from this analysis and the subsequent analysis (Section B4.2). The covariates, noSand and unkSand, were substituted for Type. The following is the best model fit to the quantal data.

MINITAB Printout of Results of Analysis

Binary Logistic Regression: Binary vs. logD, logA, Depth, noSand, unkSand, Route, Route*noSand and Route*logA

Link Function: Normit

Response Information

Variable	Value	Count	
Binary	1	86	(Event)
	0	76	
	Total	162	

Factor Information

Factor	Levels	Values
Route	2	Eye, Skin
Depth	2	deep, shallow

Logistic Regression Table

Predictor	Coef	SE Coef	Z	P
Constant	-9.44296	1.88659	-5.01	0.000
logD	3.07404	0.676474	4.54	0.000
logA	-1.46391	0.524565	-2.79	0.005
noSand	-1.33013	0.496595	-2.68	0.007
unkSand	0.838082	0.419951	2.00	0.046
Route				
Skin	3.38959	0.721305	4.70	0.000
Route*noSand				
Skin	2.92272	0.895081	3.27	0.001
Route*logA				
Skin	-4.41677	1.24014	-3.56	0.000
Depth				
shallow	2.54826	0.692344	3.68	0.000

Log-Likelihood = -48.213

Test that all slopes are zero: G = 127.536, DF = 8, P-Value = 0.000

Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	76.4047	63	0.120
Deviance	60.7492	63	0.557
Hosmer-Lemeshow	9.7010	8	0.287

Measures of Association:

(Between the Response Variable and Predicted Probabilities)

Pairs	Number	Percent	Summary Measures	
Concordant	6170	94.4	Somers' D	0.89
Discordant	345	5.3	Goodman-Kruskal Gamma	0.89
Ties	21	0.3	Kendall's Tau-a	0.45
Total	6536	100.0		

Variance-Covariance Matrix of Model Fit

Constant	logD	logA	noSand	unkSand	Route (skin)	Route* noSand (skin)	Route* logA (skin)	Depth (shallow)
3.55923	-1.18477	0.133797	0.117591	-0.361888	-0.875074	-0.382197	1.11824	-0.814722
-1.18477	0.45762	-0.077374	-0.066571	0.132257	0.222860	0.203851	-0.28193	0.151306
0.13380	-0.07737	0.275169	0.018855	-0.043671	0.027676	-0.046631	-0.20876	-0.022285
0.11759	-0.06657	0.018855	0.246607	0.026134	0.024825	-0.256857	0.02136	-0.029033
-0.36189	0.13226	-0.043671	0.026134	0.176359	0.091446	0.043133	-0.09686	0.022353
-0.87507	0.22286	0.027676	0.024825	0.091446	0.520281	-0.061484	-0.75400	0.300659
-0.38220	0.20385	-0.046631	-0.256857	0.043133	-0.061484	0.801171	-0.03975	-0.025139
1.11824	-0.28193	-0.208759	0.021365	-0.096856	-0.754004	-0.039752	1.53795	-0.407472
-0.81472	0.15131	-0.022285	-0.029033	0.022353	0.300659	-0.025139	-0.40747	0.479340

Calculation of Ratio of ED₅₀s (deep/shallow)

a ==> fitted coefficient for Depth = shallow
b ==> fitted coefficient for Depth = deep (= zero)
c ==> fitted coefficient for logD

$$\log(\text{ED}_{50}(\text{sh})) - \log(\text{ED}_{50}(\text{dp})) = \frac{(a-b)}{c} = \frac{[(2.54826) - 0]}{(3.07404)} = (0.828961)$$

Var(a-b) = (0.479340)
Cov(a-b, c) = (0.151306)
Var(c) = (0.45762)

$$\text{std err of } \left(\frac{(a-b)}{c} \right) = (0.828961) \sqrt{\left(\frac{(0.479340)}{(2.54826)^2} \right) + \left(\frac{(0.45762)}{(3.07404)^2} \right) - (2) \left(\frac{(0.151306)}{(2.54826)(3.07404)} \right)}$$

$$= (0.240)$$

95% Confidence Limits = ±(1.96)(0.240) or (0.470)
(0.359) ≤ true value of log Ratio ≤ (1.299)
(2.3) ≤ true value of Ratio ≤ (19.9)
Ratio estimate of ED₅₀'s (deep/shallow) = 10^(0.828961) = 6.7

Based upon the above, the factor Depth is statistically significant, with the probability of a toxic response increasing for exposure to the top layer of contaminated vs. contaminated sand taken from a depth of one half to 2 in. below the top surface. There is a factor of 7 difference between the ED₅₀s for shallow and deep sand samples (although the 95% confidence limits range from 2.3 to 20). This supports the original findings of the CWB (1944).

A review of the goodness-of-fit tests indicated some lack of model fit. Upon further review, it was decided to drop all data from the dataset where Depth equaled "deep" and to drop Depth from the model for the next analysis. The new quantal variable was BinaryS.

B4.2 Binary Logistic Regression Analysis of BinaryS vs. logD, logA, Sand, Route, Sand*Route and logA*Route.

One complication in the final analysis was the unknown type of terrain used for the data recorded in Table 7 of the CWB report. Either coral or sand was used, but no definitive statement was made as to which it was. The factor Sand is, thus, represented by two covariates, noSand and unkSand. The former equals one if the terrain was coral; otherwise, it is zero. The latter equals one if the terrain is unknown; otherwise, it is zero. The following is the best model fit to the quantal data.

MINITAB Printout of Results of Analysis

Binary Logistic Regression: BinaryS vs. logD, logA, noSand, unkSand, Route, Route*noSand and Route*logA

Link Function: Normit

Response Information

Variable	Value	Count	
BinaryS	1	82	(Event)
	0	70	
	Total	152	

Factor Information

Factor	Levels	Values
Route	2	Eye, Skin

Logistic Regression Table

Predictor	Coef	SE Coef	Z	P
Constant	-10.9926	2.95658	-3.72	0.000
logD	4.84678	1.26315	3.84	0.000
logA	-1.80751	0.621636	-2.91	0.004
noSand	-1.51444	0.537809	-2.82	0.005
unkSand	1.52817	0.657721	2.32	0.020
Route				
Skin	7.57428	2.00119	3.78	0.000
Route*noSand				
Skin	3.58635	1.15610	3.10	0.002
Route*logA				
Skin	-12.2338	3.69693	-3.31	0.001

Log-Likelihood = -31.158

Test that all slopes are zero: G = 147.452, DF = 7, P-Value = 0.000

Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	26.9827	57	1.000
Deviance	29.4119	57	0.999
Hosmer-Lemeshow	7.7758	8	0.456

Measures of Association:

(Between the Response Variable and Predicted Probabilities)

Pairs	Number	Percent	Summary Measures	
Concordant	5596	97.5	Somers' D	0.95
Discordant	126	2.2	Goodman-Kruskal Gamma	0.96
Ties	18	0.3	Kendall's Tau-a	0.48
Total	5740	100.0		

Variance-Covariance Matrix of Model Fit

	Constant	logD	logA	noSand	unkSand	Route (skin)	Route* noSand (skin)	Route* logA (skin)
Constant	8.74134	-3.71151	0.593301	0.307963	-1.48524	-4.10081	-1.54780	6.1921
logD	-3.71151	1.59555	-0.294772	-0.170414	0.60745	1.71109	0.69905	-2.6012
logA	0.59330	-0.29477	0.386432	0.054527	-0.12952	-0.22680	-0.15462	0.1603
noSand	0.30796	-0.17041	0.054527	0.289238	0.01095	-0.08087	-0.33505	0.2035
unkSand	-1.48524	0.60745	-0.129516	0.010949	0.43260	0.75934	0.21859	-1.1091
Route	-4.10081	1.71109	-0.226795	-0.080865	0.75934	4.00475	0.53906	-7.0058
Route*noSand	-1.54780	0.69905	-0.154620	-0.335048	0.21859	0.53906	1.33656	-1.2722
Route*logA	6.19209	-2.60123	0.160279	0.203547	-1.10907	-7.00577	-1.27221	13.6673

The lack of model fit that was encountered when trying to fit data from “deep” and “shallow” values for Depth was eliminated when only “shallow” data were used. There are two significant factor interactions: Route*noSand and Route*logA. Other functions of Density and Aging were investigated, but the logarithm functions, logD and logA, were found to provide the best model fit. The route of exposure (Eye vs. Skin) has a very large impact on the probability of effect, with the greater probability occurring with skin route. However, the difference between the two routes changes with aging duration (as indicated by the significant factor interaction of Route*logA). Since no quantal data were collected on coral after 3 days of aging (Section B2), any extrapolation from the above model fit for exposure to contaminated coral beyond 3 days should be viewed with caution.

Estimates of the median and 16% effective H (initial) contamination density from the above model fit are listed in Table B1. Ratio of ED₅₀ estimates are listed in Table B2 for comparison of Eye to Skin (for each type of terrain) and Sand to Coral (for each route of exposure). Values from these tables are also shown in Figures B1 to B4.

Based on the model fit both route of exposure (Route) and type of contaminated terrain (noSand and unkSand) are statistically significant factors, and there is a significant interaction between the two factors. Also, the logarithms of the initial contamination density (logD) and aging duration (logA) are important covariates. There is a significant interaction between logA and Route.

Table B1. Effective H (Initial) Contamination Density Estimates for Rabbit PC Exposures from Model Fit of Section B4.2 as Function of Terrain and Exposure Route

Terrain	Route	Aging (Days)	Median Effective H (Initial) Contamination Density (g/m ²)			Effective (16% Level) H (Initial) Contamination Density (g/m ²)		
			Fit	Lower Limit (95%)	Upper Limit (95%)	Fit	Lower Limit (95%)	Upper Limit (95%)
Sand	Eye	1	185	136	254	116	75	179
		2	240	184	314	150	104	215
		3	279	209	373	174	122	248
		4	311	225	430	194	135	279
		5	338	236	484	211	144	309
		6	362	244	535	225	151	337
		7	383	251	584	239	156	364
Coral	Eye	1	381	242	599	237	155	363
		2	493	308	790	307	205	461
		3	573	344	956	357	233	549
		4	638	369	1105	398	251	630
		5	694	387	1242	432	265	705
		6	742	402	1370	463	276	776
		7	786	415	1491	490	285	843
Sand	Skin	1	5.1	1.2	22.2	3.2	0.6	15.6
		2	37.8	19.7	72.4	23.6	10.8	51.3
		3	122	79	190	76.3	45.4	128.1
		4	281	151	525	176	94	327
		5	537	227	1274	335	146	767
		6	911	309	2686	568	203	1591
		7	1424	399	5087	888	264	2987
Coral	Skin	1	1.9	0.4	10.1	1.2	0.2	7.1
		2	14.1	5.6	35.6	8.8	3.1	25.1
		3	45.7	22.4	93.5	28.5	12.8	63.5
		4	105	48	232	65.6	28.7	149.6
		5	201	77	524	125	48	326
		6	341	109	1061	212	70	646
		7	532	144	1960	332	93	1178

Table B2. Ratios (Eye to Skin and Sand to Coral) of Median Effective H (Initial) Contamination Density Estimates for Rabbit PC Exposures from Model Fit of Section B4.2

Terrain	Route	Aging (Days)	Ratio of ED ₅₀ Estimates		
			Fit	Lower Limit (95%)	Upper Limit (95%)
Sand	Eye to Skin	1	36.5	8.25	162
		2	6.35	3.16	12.8
		3	2.28	1.37	3.80
		4	1.10	0.57	2.15
		5	0.63	0.26	1.53
		6	0.40	0.13	1.19
		7	0.27	0.07	0.97
Coral	Eye to Skin	1	201	32.8	1227
		2	34.9	11.4	107.2
		3	12.5	5.0	31.7
		4	6.07	2.32	15.84
		5	3.45	1.17	10.19
		6	2.18	0.64	7.43
		7	1.48	0.38	5.81
Sand to Coral	Skin	1 to 7	0.49	0.28	0.84
Sand to Coral	Skin	1 to 7	2.68	1.17	6.14

In general, direct PC exposure to contaminated terrain is a more sensitive bioindicator than eye exposures to agent vapor off-gassing from contaminated terrain (from a distance of 1 ft). The difference is greater at the shorter aging durations, and at the longer durations the difference eventually disappears. In fact, at the shorter aging durations the estimated ED₅₀ (skin) is actually lower than the estimated ED₀₁ (eyes) for exposure to either contaminated coral or sand (see Figures B2 and B3). Thus, it is possible for a rabbit not to experience any eye effects due to vapor contact from H-contaminated terrain yet still be affected from direct skin contact.

For sand there is no statistically significant difference between the ED₅₀s for eye and skin exposures after 3-4 days of weathering. For coral it takes 5-6 days to find no significant difference between the ED₅₀s of eye and skin exposures (Figure B4).

For eye exposures vapor off-gassing from sand is more likely to produce a toxic response than off-gassing from coral. However, the reverse is true for direct skin exposures (direct contact with coral is more likely to produce a response than contact with sand). [It should be noted that, in general, for H exposure, the eye is a more sensitive target organ than skin. The disparity observed in the present study was likely a function of the fact that the eyes of the rabbits were subjected to vapor contact, while the skin was subjected to direct liquid-pseudo-liquid contact.]

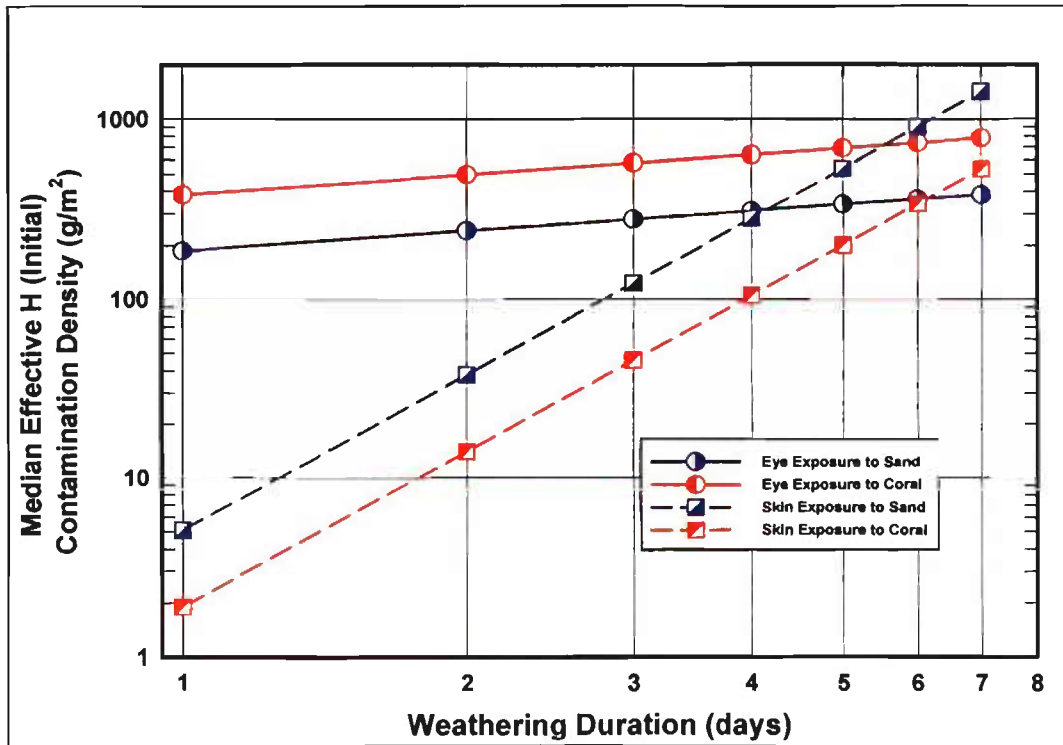


Figure B1. Estimates of Median Effective H (Initial) Contamination Density for PC Eye and Skin Exposure of Rabbits to Contaminated Terrain from Model Fit of Section B4.2

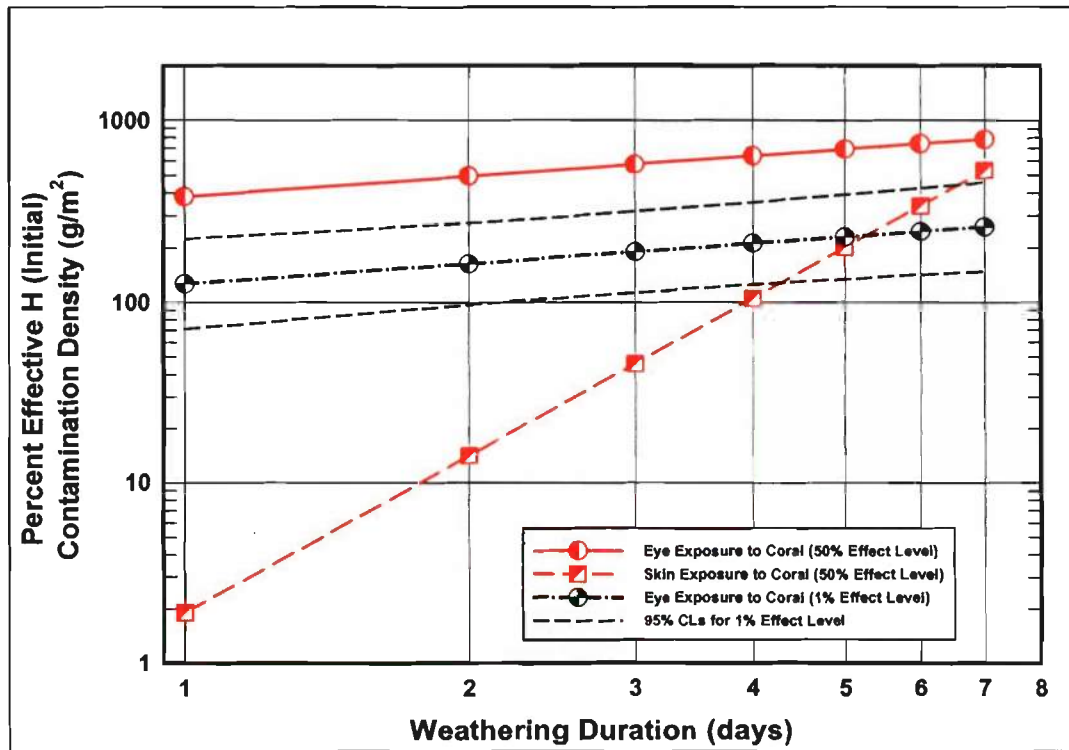


Figure B2. Comparison of Estimated H (Initial) ED₅₀s for PC Eye and Skin Exposures (and ED₀₁ for Eye Exposure) of Rabbits to Contaminated Coral from Model Fit of Section B4.2

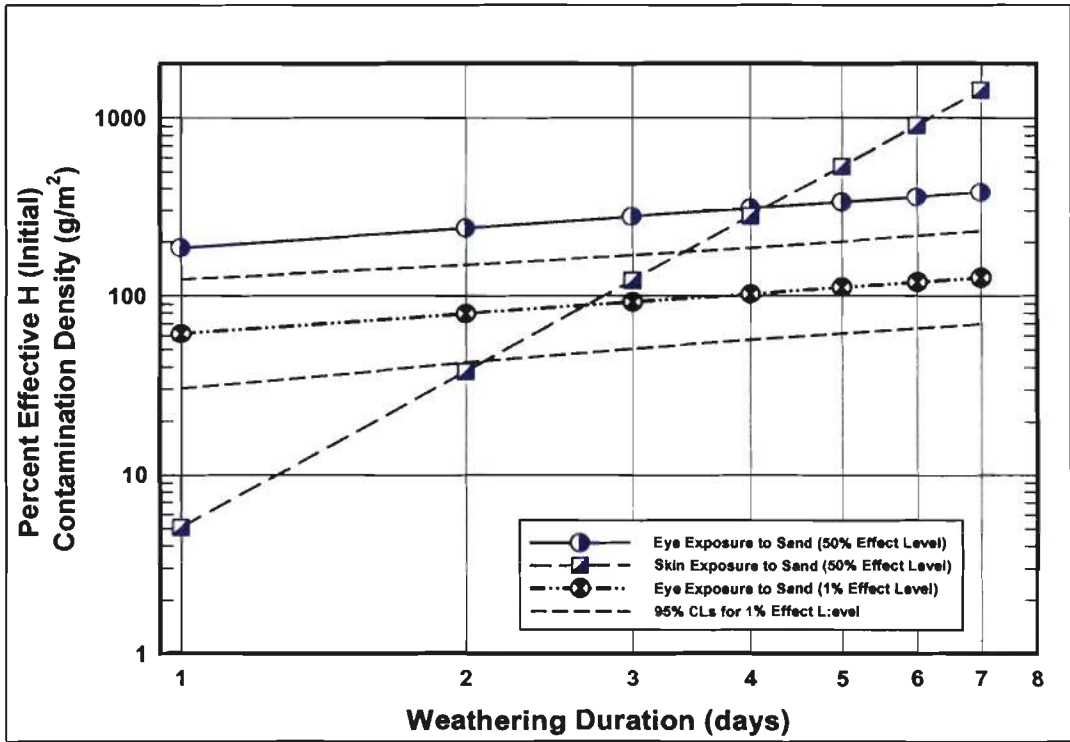


Figure B3. Comparison of Estimated H (Initial) ED₅₀s for PC Eye and Skin Exposures (and ED₀₁ for Eye Exposure) of Rabbits to Contaminated Sand from Model Fit of Section B4.2

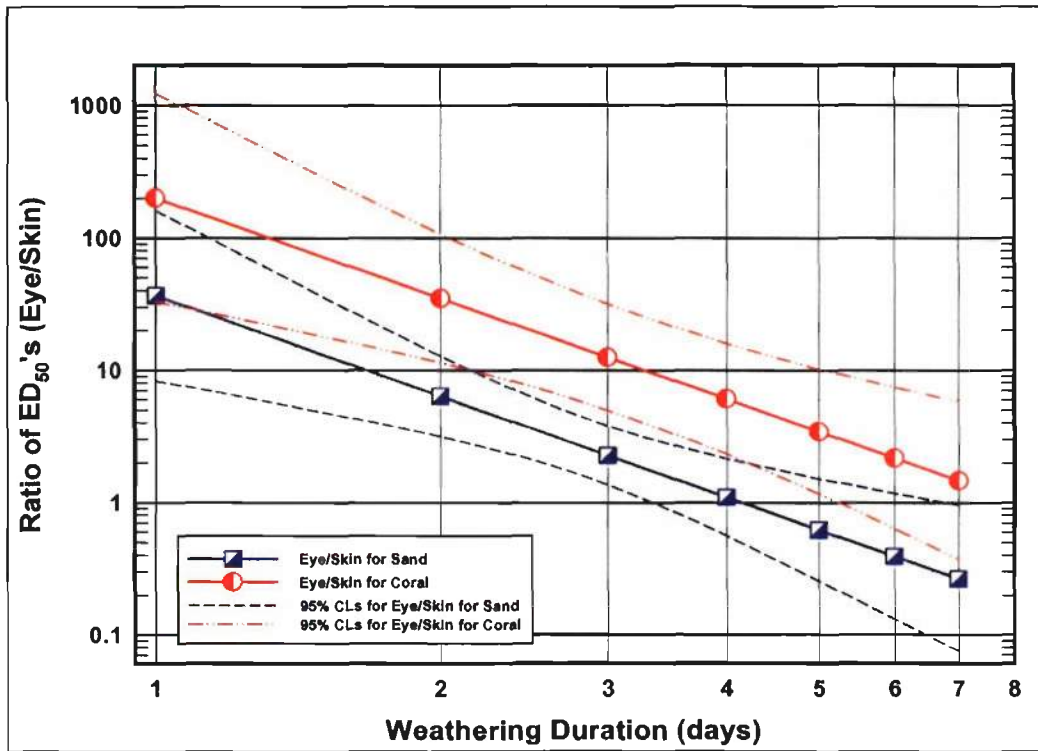


Figure B4. Comparison of Ratios of H (Initial) ED₅₀s (Eye to Skin) from PC Exposures of Rabbits to Contaminated Terrain from Model Fit of Section B4.2

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APPENDIX C

STATISTICAL ANALYSIS OF RABBIT VX PERCUTANEOUS LETHALITY DATA FROM THE CHEMICAL WARFARE LABORATORIES TRAVERSAL PROGRAM

C1 INTRODUCTION

In the late 1950s and early 1960s, the U.S. Army Chemical Warfare Laboratory implemented the Traversal Program, the primary objective of which was to estimate potential casualties arising from traversal and occupational hazards associated with V-agent contaminated surfaces. There were three phases of the program: Phase A—Persistence and Decontamination (determining the persistence of V-agents in the field); Phase B—Pick-up (determining the degree of pick-up by personnel traversing or occupying areas V-agent contaminated terrain); and Phase C—Casualty Production (estimating the pick-up effectiveness in casualty production).

In Phase A rabbit bioassay tests were conducted (in conjunction with other measurements, e.g., chemical analysis of soil samples) to evaluate VX persistence on various types of terrain: sand, soil and grass sod. The abdominal fur of the rabbits was clipped, and the rabbits were then placed on contaminated test plots for 1 hr and then removed for observation. The quantity of transferable contaminant was indicated by the observed toxic effects (lethality and cholinesterase depression). Three other factors were controlled and varied: the initial VX mass density in the contaminated plot; the time period between plot contamination, and the exposure of the test rabbits (or weathering duration); and exposure of the plot to weathering effects. Densities from 0.3 to 31.5 g/m² and drop diameters of 200 to 4100 μ were investigated, and the period between contamination and rabbit exposure ranged from 1 hr to 2 weeks.

Reich (1959) reported several specific and general findings from the rabbit bioassay tests on contaminated terrain and chemical analysis of the contaminated material:

Soil Plots Specific Findings

- (1) Traces of VX were found several weeks after the agent was dispersed.
- (2) Decline in residual VX contamination was initially very rapid and then leveled off almost asymptotically with respect to time.
- (3) Initial rate of decline appeared to be directly proportional to the density of the dispersed agent.
- (4) High air temperatures and rainfall appeared to reduce agent persistence.
- (5) All the above effects appear to be independent of the particle size.
- (6) For initial agent densities below 7 g/m², only a few rabbit fatalities were encountered in post-contamination exposures from 1 to 24 hr. For higher contamination densities, deaths might be expected to occur in post-contamination exposures up to several days.
- (7) The quantity of agent recovered (via chemical extraction) from the top 0.5-in. layer of soil cannot be directly correlated to the number of rabbit lethality; therefore, it does not reflect the amount of agent available for transfer. The VX contamination on the surface declines much more rapidly and is more adversely affected by weather than is the contamination of the top 0.5-in. layer of soil. This observation helps to explain the fact that the number of fatalities is more dependent on weathering duration than agent amount left in the top 0.5-in. soil layer.

Sand Plots Specific Findings

(1) All rabbits exposed after either 1 hr or 24 hr of plot weathering died. However, after weathering of 7 days and more, no deaths were recorded, despite the presence of residual VX in the top 0.5-in. sand layer.

(2) Curves of residual VX concentration vs. weathering duration for soil and sand are of the same shape.

Sod Plots Specific Findings

(1) In a general manner, the persistency of VX in soil, sand and sodded terrain is roughly of the same magnitude.

(2) An inverse relationship between persistence and weathering duration can be inferred from all of the results.

General Findings

(1) The persistence of VX is effectively only a matter of days.

(2) VX mass densities below 2.5 g/m^2 are not likely to produce appreciable number of fatalities due to PC effects suffered by troops entering and occupying the contaminated area in somewhat less than 24 hr after contamination.

(3) For higher VX mass densities ($10\text{-}30 \text{ g/m}^2$), the area might be safe for occupation sometime after 2 days and less than a week following contamination.

C2 DATA SUMMARY

For this report, only the rabbit bioassay data were reviewed. The raw bioassay data were reported by Koblin *et al.* (1957) and Reich (1959) from the data recorded in Alexander's laboratory notebook. All three sources were reviewed.

For each individual exposure group (initial contamination density and weathering duration combination), two rabbits were exposed percutaneously to the contaminated plot for 1 hr. Lethality data are available for 127 groups. Cholinesterase depression data were not available for all of the trials and were found to be unpredictable. A review of the cholinesterase data from 97 control rabbits found wide variation in measured percent cholinesterase with the lower 95% confidence limit roughly equaling 30% of the pre-exposure measurement—an exposed rabbit would have to have a post-exposure cholinesterase measurement below this value in order to just be outside the random noise. Thus, only lethality data were considered for this analysis. The lethality data are listed in Section C2.2.

C2.1 Nomenclature.

Date	Date that plot was initially contaminated
Deaths	Number of rabbit deaths
Density	Initial ground contamination density of liquid VX in grams/square meter
logD	Logarithm base 10 of Density
Month	Month in which plot was initially contaminated
Number	Number of rabbits exposed
Plot	Plot designation for trial
RConc	Residual VX contamination (from soil plugs) at time of rabbit exposure in grams/square meter
SGroup	Equals one if drop diameter is larger than 1000μ ; zero otherwise
Shade	Plot was shaded from the weather and sun after contamination
Size	Diameter of VX droplets used to contaminate plot in microns
SPRE	Standardized Pearson residual
EPRO	Predicted event probabilities from final model fit
Summer	Equals "yes" (or one) if plot was initially contaminated in either the month of June, July, August or September; otherwise, equals "no" (or zero).

Type Type of terrain: Soil, Sand or Sod (Grass)
 Type2 Type of terrain: Sand (Type2 equals zero) vs. Soil or Sod (Type2 equals one)
 WtGroup Grouping of Wtime values. Equals "short" if Wtime equals 1 hr, "medium" for Wtime
 values from 1 to 3 days, and "long" if Wtime is 4 days or longer.
 Wtime Plot weathering duration between plot contamination and rabbit exposure in days

C2.2 Data Display.

Row	Date	Plot	Summer	Type	Shade	Density	RConc	Size	Wtime	Deaths	Number
1	3/12/1957	2B	no	Soil	yes	4.0	0.93	1450	0.042	1	2
2	3/12/1957	2B	no	Soil	yes	4.0	2.11	1450	1.000	0	2
3	3/12/1957	2B	no	Soil	yes	4.0	1.49	1450	7.000	0	2
4	3/12/1958	3C	no	Soil	yes	0.4	0.74	780	0.042	0	2
5	3/12/1958	3C	no	Soil	yes	0.4	0.29	780	1.000	0	2
6	3/18/1957	1G	no	Soil	yes	20.0	2.88	3250	0.042	2	2
7	3/18/1957	1G	no	Soil	yes	20.0	4.00	3250	1.000	2	2
8	3/18/1957	1G	no	Soil	yes	20.0	2.89	3250	7.000	1	2
9	3/18/1957	1G	no	Soil	yes	20.0	2.85	3250	14.000	0	2
10	3/18/1957	2D	no	Soil	yes	31.5	3.87	3480	0.042	2	2
11	3/18/1957	2D	no	Soil	yes	31.5	2.98	3480	1.000	2	2
12	3/18/1957	2D	no	Soil	yes	31.5	4.48	3480	7.000	1	2
13	3/18/1957	2D	no	Soil	yes	31.5	3.96	3480	14.000	0	2
14	3/18/1957	3A	no	Soil	yes	20.0	2.88	3400	0.042	2	2
15	3/18/1957	3A	no	Soil	yes	20.0	4.00	3400	1.000	1	2
16	3/18/1957	3A	no	Soil	yes	20.0	2.89	3400	7.000	0	2
17	3/18/1957	3A	no	Soil	yes	20.0	2.85	3400	14.000	0	2
18	4/22/1957	2C	no	Soil	yes	1.8	1.14	200	0.042	2	2
19	4/22/1957	2C	no	Soil	yes	1.8	1.84	200	1.000	0	2
20	4/22/1957	2C	no	Soil	yes	1.8	0.30	200	7.000	0	2
21	4/30/1957	3E	no	Soil	yes	0.5	0.31	200	0.042	2	2
22	4/30/1957	3E	no	Soil	yes	0.5	0.11	200	1.000	1	2
23	4/30/1957	3E	no	Soil	yes	0.5	0.39	200	7.000	0	2
24	4/30/1957	3F	no	Soil	yes	0.5	0.20	200	0.042	2	2
25	4/30/1957	3F	no	Soil	yes	0.5	0.17	200	1.000	0	2
26	4/30/1957	3F	no	Soil	yes	0.5	0.24	200	7.000	0	2
27	5/6/1957	1J	no	Soil	yes	1.0	0.36	200	0.042	2	2
28	5/6/1957	1J	no	Soil	yes	1.0	0.68	200	1.000	0	2
29	5/6/1957	1J	no	Soil	yes	1.0	0.37	200	7.000	0	2
30	5/12/1957	3C	no	Soil	yes	0.4	****	780	0.042	0	2
31	5/12/1957	3C	no	Soil	yes	0.4	****	780	1.000	0	2
32	5/14/1957	3B	no	Soil	yes	0.3	0.20	200	0.042	0	2
33	5/14/1957	3B	no	Soil	yes	0.3	0.20	200	1.000	0	2
34	5/14/1957	3B	no	Soil	yes	0.3	0.07	200	7.000	0	2
35	5/14/1957	2J	no	soil	yes	0.3	0.50	200	0.042	1	2
36	5/14/1957	2J	no	Soil	yes	0.3	0.41	200	1.000	0	2
37	5/14/1957	2J	no	Soil	yes	0.3	0.11	200	7.000	0	2
38	6/4/1957	3I	yes	Soil	yes	1.7	1.35	470	0.042	2	2
39	6/4/1957	3I	yes	Soil	yes	1.7	1.55	470	1.000	0	2
40	6/4/1957	3I	yes	Soil	yes	1.7	0.07	470	7.000	0	2
41	6/10/1957	1H	yes	Soil	yes	6.9	4.21	230	0.042	2	2
42	6/10/1957	1H	yes	Soil	yes	6.9	3.78	230	1.000	0	2
43	6/10/1957	1H	yes	Soil	yes	6.9	0.53	230	7.000	0	2
44	6/10/1957	2H	yes	Soil	yes	4.6	5.32	220	0.042	2	2
45	6/10/1957	2H	yes	Soil	yes	4.6	3.33	220	1.000	0	2
46	6/10/1957	2H	yes	Soil	yes	4.6	1.06	220	7.000	0	2
47	6/24/1957	1E	yes	Soil	no	20.0	54.00	3700	0.042	2	2
48	6/24/1957	1E	yes	Soil	no	20.0	9.17	3700	1.000	0	2
49	6/24/1957	2G	yes	Soil	no	20.0	49.40	3720	0.042	2	2
50	6/24/1957	2G	yes	Soil	no	20.0	7.70	3720	1.000	0	2
51	6/24/1957	3G	yes	Soil	no	20.0	47.00	4140	0.042	2	2
52	6/24/1957	3G	yes	Soil	no	20.0	15.00	4140	1.000	1	2

53	7/2/1957	1B	yes	Soil	no	4.8	6.45	225	0.042	2	2
54	7/2/1957	1B	yes	Soil	no	4.8	3.85	225	1.000	0	2
55	7/2/1957	1B	yes	Soil	no	4.8	0.20	225	7.000	0	2
56	7/2/1957	1D	yes	Soil	no	3.5	3.85	225	0.042	2	2
57	7/2/1957	1D	yes	Soil	no	3.5	2.73	225	1.000	0	2
58	7/2/1957	1D	yes	Soil	no	3.5	0.14	225	7.000	0	2
59	7/9/1957	2I	yes	Soil	no	2.9	1.22	225	0.042	1	2

Row	Date	Plot	Summer	Type	Shade	Density	RConc	Size	Wtime	Deaths	Number
60	7/9/1957	2I	yes	Soil	no	2.9	0.73	225	1.000	1	2
61	7/9/1957	2I	yes	Soil	no	2.9	0.09	225	7.000	2	2
62	7/9/1957	2E	yes	Soil	no	1.6	0.63	225	0.042	2	2
63	7/9/1957	2E	yes	Soil	no	1.6	0.34	225	1.000	0	2
64	7/9/1957	2E	yes	Soil	no	1.6	0.01	225	7.000	1	2
65	7/9/1957	3D	yes	Soil	no	0.6	0.61	225	0.042	0	2
66	7/9/1957	3D	yes	Soil	no	0.6	0.58	225	1.000	0	2
67	7/9/1957	3D	yes	Soil	no	0.6	0.10	225	7.000	2	2
68	8/27/1957	2C	yes	Sand	no	20.0	29.50	3600	0.042	2	2
69	8/27/1957	2C	yes	Sand	no	20.0	16.70	3600	1.000	2	2
70	8/27/1957	2C	yes	Sand	no	20.0	0.80	3600	7.000	0	2
71	8/27/1957	2E	yes	Sand	yes	20.0	28.40	3800	0.042	2	2
72	8/27/1957	2E	yes	Sand	yes	20.0	21.60	3800	1.000	2	2
73	8/27/1957	2E	yes	Sand	yes	20.0	0.90	3800	7.000	0	2
74	8/27/1957	1C	yes	Sand	no	20.0	26.70	3600	0.042	2	2
75	8/27/1957	1C	yes	Sand	no	20.0	11.70	3600	1.000	2	2
76	8/27/1957	1C	yes	Sand	no	20.0	0.35	3600	7.000	0	2
77	8/27/1957	1E	yes	Sand	yes	20.0	4.90	3500	0.042	2	2
78	8/27/1957	1E	yes	Sand	yes	20.0	3.10	3500	1.000	2	2
79	8/27/1957	1E	yes	Sand	yes	20.0	0.05	3500	7.000	0	2
80	9/18/1957	1H	yes	Sand	no	11.9	3.00	225	0.042	2	2
81	9/18/1957	1H	yes	Sand	no	11.9	0.25	225	1.000	2	2
82	9/18/1957	1H	yes	Sand	no	11.9	0.18	225	7.000	0	2
83	9/18/1957	1J	yes	Sand	yes	9.1	2.60	225	0.042	2	2
84	9/18/1957	1J	yes	Sand	yes	9.1	1.30	225	1.000	2	2
85	9/18/1957	1J	yes	Sand	yes	9.1	0.08	225	7.000	0	2
86	9/23/1957	2H	yes	Sand	no	13.9	5.90	400	0.042	2	2
87	9/23/1957	2H	yes	Sand	no	13.9	3.80	400	1.000	2	2
88	9/23/1957	2H	yes	Sand	no	13.9	0.08	400	7.000	0	2
89	9/23/1957	2J	yes	Sand	yes	6.5	2.30	400	0.042	2	2
90	9/23/1957	2J	yes	Sand	yes	6.5	0.98	400	1.000	2	2
91	9/23/1957	2J	yes	Sand	yes	6.5	0.02	400	7.000	0	2
92	1/7/1958	1	no	Sod	no	4.9	1.94	300	0.042	2	2
93	1/7/1958	1	no	Sod	no	4.9	1.67	300	1.000	2	2
94	1/7/1958	1	no	Sod	no	4.9	0.25	300	8.000	0	2
95	1/21/1958	2	no	Sod	no	0.4	0.12	380	0.042	0	2
96	1/21/1958	2	no	Sod	no	0.4	0.03	380	1.000	1	2
97	1/21/1958	3	no	Sod	no	0.3	0.06	395	0.042	1	2
98	1/21/1958	3	no	Sod	no	0.3	0.01	395	1.000	1	2
99	6/3/1958	2	yes	Sod	no	11.3	2.30	250	0.042	1	2
100	6/3/1958	2	yes	Sod	no	11.3	0.06	250	1.000	0	2
101	6/3/1958	2	yes	Sod	no	11.3	0.03	250	3.000	0	2
102	6/3/1958	1	yes	Sod	no	7.5	1.37	250	0.042	2	2
103	6/3/1958	1	yes	Sod	no	7.5	0.07	250	1.000	1	2
104	6/3/1958	1	yes	Sod	no	7.5	0.03	250	3.000	0	2
105	6/16/1958	3	yes	Sod	no	3.5	0.40	185	0.042	1	2
106	6/16/1958	3	yes	Sod	no	3.5	0.01	185	1.000	0	2
107	6/16/1958	4	yes	Sod	no	2.2	0.29	260	0.042	0	2
108	6/16/1958	4	yes	Sod	no	2.2	0.01	260	1.000	0	2
109	6/30/1958	5	yes	Sod	no	12.6	2.25	450	0.042	2	2
110	6/30/1958	5	yes	Sod	no	12.6	0.15	450	1.000	2	2
111	6/30/1958	5	yes	Sod	no	12.6	0.10	450	2.000	1	2
112	6/30/1958	5	yes	Sod	no	12.6	0.09	450	3.000	2	2

113	6/30/1958	6	yes	Sod	no	17.2	2.17	185	0.042	2	2
114	6/30/1958	6	yes	Sod	no	17.2	0.19	185	1.000	2	2
115	6/30/1958	6	yes	Sod	no	17.2	0.17	185	2.000	1	2
116	6/30/1958	6	yes	Sod	no	17.2	0.08	185	3.000	2	2
117	7/30/1958	5	yes	Sod	yes	2.2	0.23	500	1.000	0	2
118	7/30/1958	4	yes	Sod	yes	3.6	0.89	500	1.000	0	2
119	7/30/1958	3	yes	Sod	yes	6.9	1.30	500	1.000	0	2
120	7/30/1958	2	yes	Sod	yes	13.9	2.70	500	1.000	1	2
Row	Date	Plot	Summer	Type	Shade	Density	RConc	Size	Wtime	Deaths	Number
121	7/30/1958	1	yes	Sod	no	8.5	2.00	500	2.000	0	2
122	7/30/1958	2	yes	Sod	no	13.9	2.70	500	2.000	0	2
123	8/12/1958	9	yes	Sod	yes	3.4	1.40	3000	1.000	0	2
124	8/12/1958	8	yes	Sod	yes	8.2	1.00	3000	1.000	0	2
125	8/12/1958	7	yes	Sod	yes	12.9	2.90	3000	1.000	0	2
126	8/12/1958	6	yes	Sod	yes	10.0	1.70	3000	1.000	0	2

=====

Reich (1959) noted that some of the rabbit deaths were most likely due to high air and ground temperatures (in excess of 99°F) instead of agent effects—in particular, 5/6 rabbits from Runs 61, 64, and 67. The three soil plots were all contaminated on 9 July 1957, and the rabbits were exposed after 7 days of weathering. This analysis has determined that these deaths were statistical outliers, which is consistent with the belief that these rabbits died from heat stress, and the results from these five rabbits were not used in the subsequent analysis.

Residual concentration values were not available for Runs 30 and 31. Residual concentration value for Run 20 was taken the day after the rabbit exposure, and for Run 94, the value is an average of the values from the day before and 6 days after the rabbit exposure. All suspect values are in bold.

C2.3 Run Factor Characterization.

The following is a breakdown, by various factor categories, of the runs in this study. The number of runs (with two rabbits per run) per category coordinate is listed in the following tables. The purpose of this review is to determine how well balanced the experimental design was in this study.

C2.3.1 Tabulated Statistics: Summer and Type.

Summer (yes for run starting in June to September; no otherwise)
Type (Soil type)

	Summer	Sand	Sod	Soil	All
no		0	7	37	44
yes		24	28	30	82
All		24	35	67	126

C2.3.2 Tabulated Statistics: Shade and Sgroup vs. Type and Summer.

Summer (yes for run starting in June to September; no otherwise)
Type (Soil type)
Shade (yes for plot shaded from weather; no otherwise)
Sgroup (one for droplet diameter larger than 1000 μ ; zero otherwise)

Results for Summer = no

<u>Shade</u>	<u>Sand</u>	<u>Sod</u>	<u>Soil</u>	<u>All</u>
no	0	7	0	7
yes	0	0	37	37
All	0	7	37	44

Results for Summer = yes

<u>Shade</u>	<u>Sand</u>	<u>Sod</u>	<u>Soil</u>	<u>All</u>	<u>Shade Total</u>
no	12	20	21	53	60
yes	12	8	9	29	66
All	24	28	30	82	126

<u>SGroup</u>	<u>Sand</u>	<u>Sod</u>	<u>Soil</u>	<u>All</u>
0	0	7	22	29
1	0	0	15	15
All	0	7	37	44

<u>SGroup</u>	<u>Sand</u>	<u>Sod</u>	<u>Soil</u>	<u>All</u>	<u>Sgroup Total</u>
0	12	24	24	60	89
1	12	4	6	22	37
All	24	28	30	82	126

C2.3.3 Tabulated Statistics: Wtime and WtGroup vs. Type and Summer.

Summer (yes for run starting in June to September; no otherwise)

Type (Soil type)

Wtime (weathering duration of contaminated plot in days)

Results for Summer = no

<u>Wtime</u>	<u>Sand</u>	<u>Sod</u>	<u>Soil</u>	<u>All</u>
0.042	0	3	12	15
1.000	0	3	12	15
2.000	0	0	0	0
3.000	0	0	0	0
7.000	0	0	10	10
8.000	0	1	0	1
14.000	0	0	3	3
All	0	7	37	44

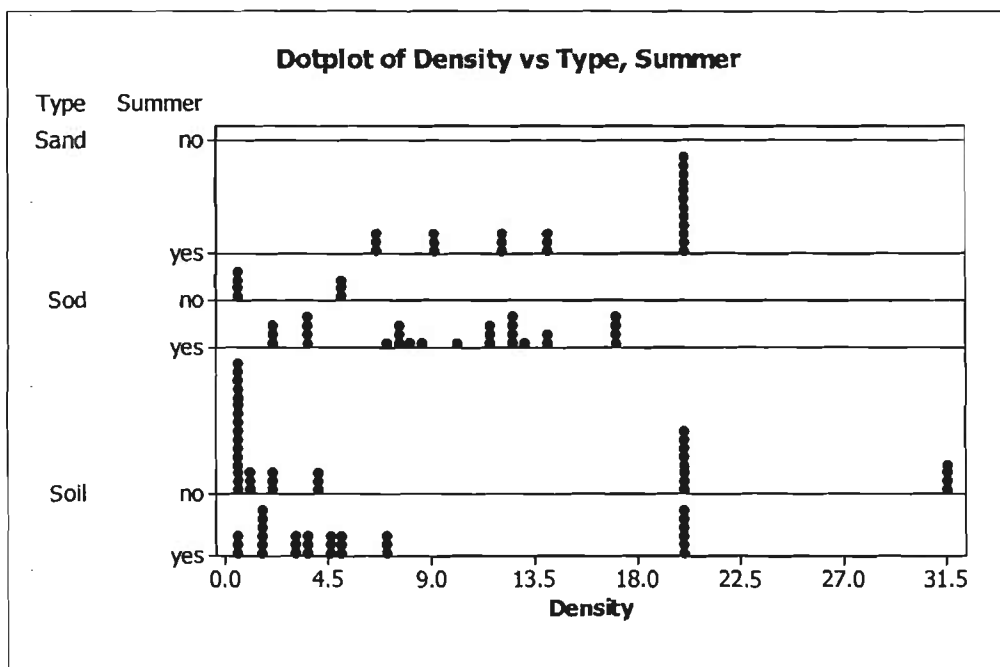
Results for Summer = yes

<u>Wtime</u>	<u>Sand</u>	<u>Sod</u>	<u>Soil</u>	<u>All</u>	<u>Wtime Total</u>
0.042	8	6	11	25	40
1.000	8	14	11	33	48
2.000	0	4	0	4	4
3.000	0	4	0	4	4
7.000	8	0	8	16	26
8.000	0	0	0	0	1
14.000	0	0	0	0	3
All	24	28	30	82	126

<u>WtGroup</u>	<u>Sand</u>	<u>Sod</u>	<u>Soil</u>	<u>All</u>
short	0	3	12	15
medium	0	3	12	15
long	0	1	13	14
All	0	7	37	44

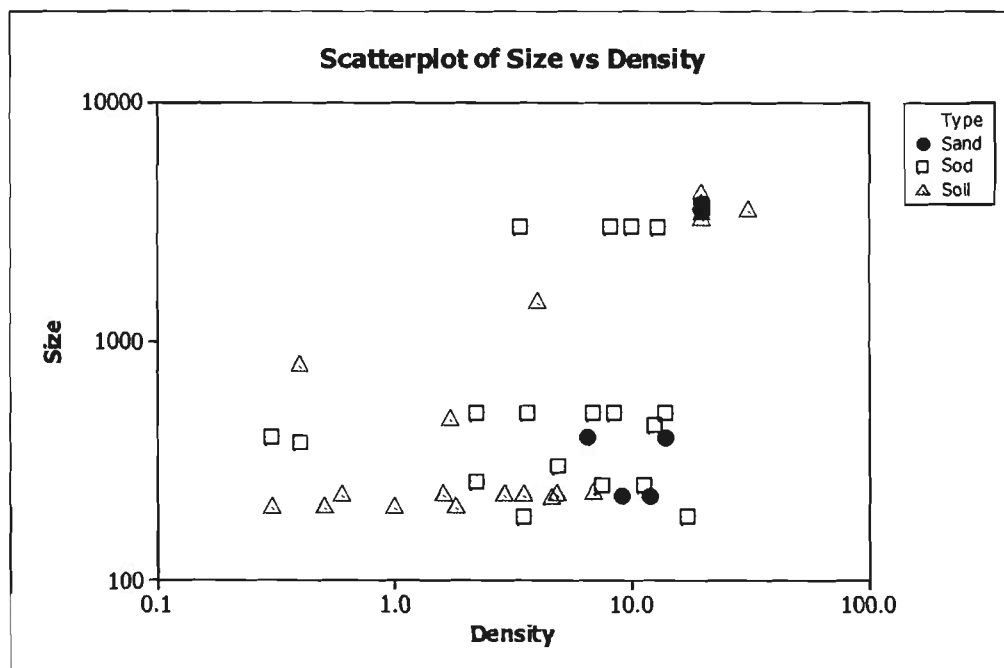
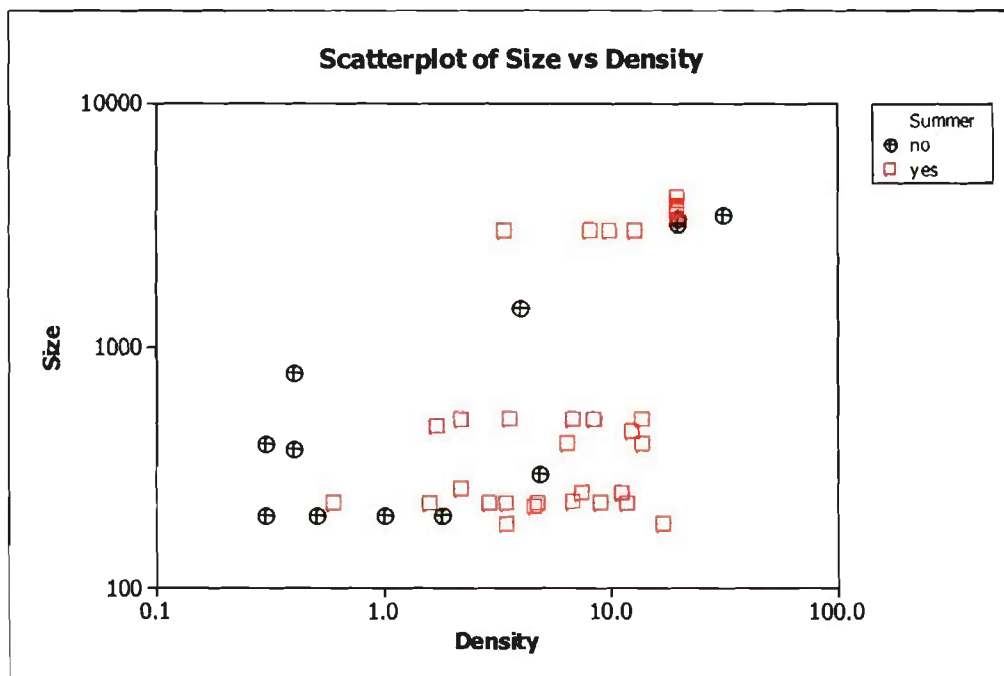
<u>WtGroup</u>	<u>Sand</u>	<u>Sod</u>	<u>Soil</u>	<u>All</u>	<u>WtGroup Total</u>
short	8	6	11	25	40
medium	8	22	11	41	56
long	8	0	8	16	30
All	24	28	30	82	126

C2.3.4 Dotplot of Density vs. Type and Summer.



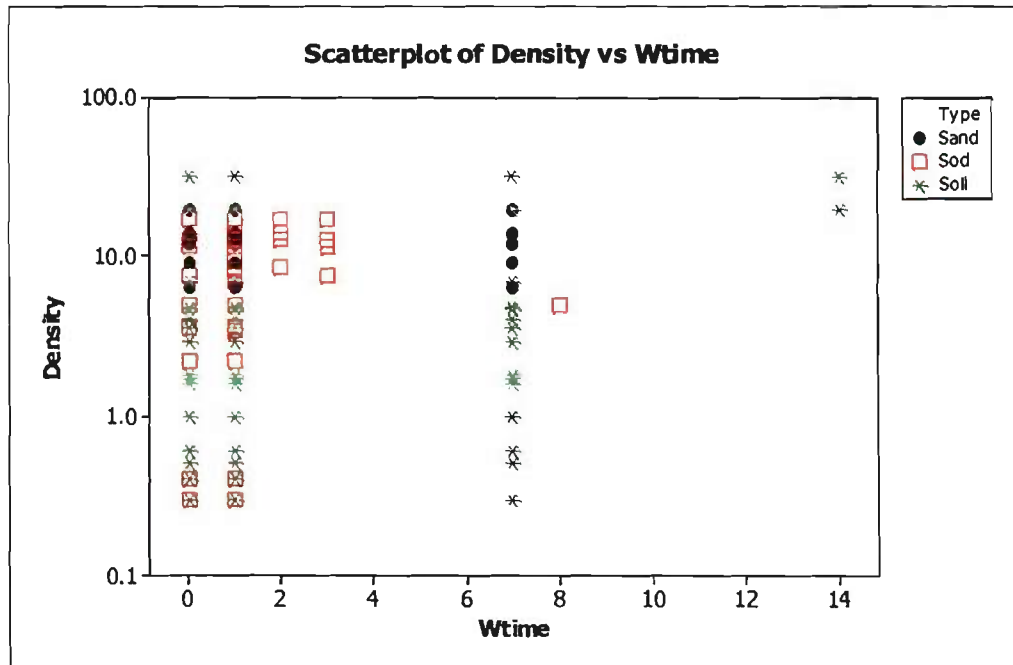
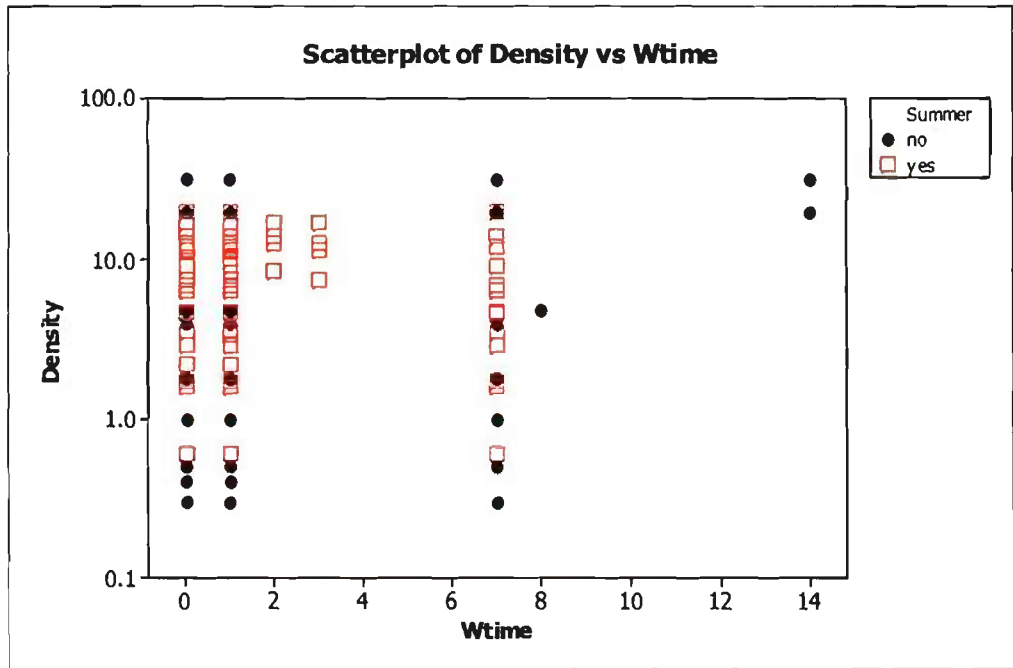
KEY: Each dot=one run
 Summer=yes for run starting in June to September; otherwise no
 Type=terrain in plot (sand, soil, sod)
 Density=initial liquid VX contamination (grams/square meter)

C2.3.5 Scatterplot of Size vs. Density (with Summer and Type Subgroups).



KEY: Size=VX droplet diameter (microns)
 Summer=yes for run starting in June to September; otherwise no
 Type=terrain in plot (sand, soil or sod)
 Density=initial liquid VX contamination (grams/square meter)

C2.3.6 Scatterplot of Density vs. Wtime (with Summer and Type Subgroups).



KEY: Wtime=weathering duration prior to rabbit exposure (days)
 Summer=yes for run starting in June to September; otherwise no
 Type=terrain in plot (sand, soil, sod)
 Density=initial liquid VX contamination (grams/square meter)

C2.3.7 Analysis of Variance (ANOVA) of logD and logS vs. Shade.

LogD—logarithm based 10 of Density (initial liquid VX contamination density in grams/square meter)

LogS—logarithm based 10 of Size (VX droplet diameter used on plots in microns)

Shade (yes for plot shaded from weather; no otherwise)

One-way ANOVA: logS vs. Shade

Source	DF	SS	MS	F	P
Shade	1	1.093	1.093	4.27	0.041
Error	124	31.721	0.256		
Total	125	32.814			

S = 0.5058 R-Sq = 3.33% R-Sq(adj) = 2.55%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
no	60	2.6602	0.4740	(-----*-----)
yes	66	2.8466	0.5330	(-----*-----)

-----+-----+-----+-----+-----+
2.64 2.76 2.88 3.00

Pooled StDev = 0.5058

One-way ANOVA: logD vs. Shade

Source	DF	SS	MS	F	P
Shade	1	1.401	1.401	3.72	0.056
Error	124	46.688	0.377		
Total	125	48.089			

S = 0.6136 R-Sq = 2.91% R-Sq(adj) = 2.13%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
no	60	0.7747	0.5329	(-----*-----)
yes	66	0.5635	0.6786	(-----*-----)

-----+-----+-----+-----+-----+
0.45 0.60 0.75 0.90

Pooled StDev = 0.6136

Experimental run values for logS and logD are correlated with Shade, with 95.9 and 94.4% statistical significance.

C2.3.8 Analysis of Variance of logD and logS vs. Summer.

LogD—logarithm based 10 of Density (initial liquid VX contamination density in grams/square meter)

LogS—logarithm based 10 of Size (VX droplet diameter used on plots in microns)

Summer (yes for run starting in June to September; no otherwise)

One-way ANOVA: logS vs. Summer

Source	DF	SS	MS	F	P
Summer	1	0.055	0.055	0.21	0.650
Error	124	32.759	0.264		
Total	125	32.814			

S = 0.5140 R-Sq = 0.17% R-Sq(adj) = 0.00%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev	CI
no	44	2.7862	0.5229	(-----*-----)
yes	82	2.7426	0.5092	(-----*-----)

-----+-----+-----+-----+-----
2.640 2.720 2.800 2.880

Pooled StDev = 0.5140

One-way ANOVA: logD vs. Summer

Source	DF	SS	MS	F	P
Summer	1	9.568	9.568	30.80	0.000
Error	124	38.522	0.311		
Total	125	48.089			

S = 0.5574 R-Sq = 19.90% R-Sq(adj) = 19.25%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev	CI
no	44	0.2879	0.7666	(-----*-----)
yes	82	0.8659	0.4045	(-----*-----)

-----+-----+-----+-----+-----
0.25 0.50 0.75 1.00

Pooled StDev = 0.5574

Experimental run values for logS are not correlated with Summer. However, run values for logD are correlated with Summer with over 99.9% statistical significance.

C2.3.9 Breakdown of Lethality Data by Weathering Duration and Terrain Type.

The following is a breakdown of the number of rabbit deaths per terrain type and weathering duration (WtGroup). The vast majority of deaths (discounting deaths due to high air temperatures) occurred with WtGroup equal to short and medium. Only two deaths (out of 59 total exposures) occurred for WtGroup equal to long.

WtGroup	short	medium	long	All	
Sand	16	16	0	32	<== Deaths
	16	16	16	48	<== Number
Soil	35	8	2	45	<== Deaths
	46	46	41	133	<== Number
Sod	11	16	0	27	<== Deaths
	18	50	2	70	<== Number
All	62	40	2	104	<== Deaths
	80	112	59	251	<== Number

C3 EXPERIMENTAL DESIGN IN ORIGINAL STUDY

A statistically proper experimental design was not used by the Traversal Program researchers. As a result, any conclusion drawn from a statistical analysis of the data set needs to be interpreted with caution. The following is a list of the major design problems.

(1) Lack of randomization of trials runs—All of the runs with soil plots were performed first, followed next by the sand plots, and ending with all of the sod plots. Due to the importance of weathering on VX persistence, this is a major oversight. Runs on soil, sand and sod plots should have been conducted simultaneously to avoid the potential confounding of an unknown systemic error with terrain type.

(2) Incomplete crossing in the experimental design—Neither a full nor a standard fractional factorial design was used in this study. Due to the lack of balance in the design, it was not possible (in many cases) to determine if significant factor interactions exist or whether one factor or another was responsible for an observed effect (due to the confounding of the two factors). Imbalances were found among the following groups of factors.

(a) Summer and Type—No runs were conducted in the non-summer runs with sand plots. Thus, the possible interaction between Summer and Type cannot be properly investigated.

(b) Shade with Type and Summer—For the non-summer runs, all of the non-shaded trials were on Sod plots, while the shaded trials were all on Soil plots.

(c) Density and Size with Shade—Analysis of variance was performed separately for logD and logS on Shade. It was found that there was a statistically significant shift in the mean values of logD and logS as a function of Shade. The droplet diameters (Size) were smaller for the non-shaded plots. The reverse was true for the contamination densities (Density), with lower densities being found in the shaded plots.

(d) Density with Type and Summer—For the Sod plots in the non-summer trials, no Density values greater 4.9 g/m² were investigated. For Sand plots (none of which were performed in non-summer months), no Density values lower than 5 g/m² were investigated.

(e) Size and Density (with Type and Summer)—Overall, droplet diameter (Size) was only slightly positively correlated (r² of 33.5%) with initial ground contamination (Density) on a log-log scale. However, the correlation between these two factors on a log-log scale was higher for non-summer trials (r² of 70.2%) than for summer trials (r² of 30.9%). For Type, the values were r² values were 57.9% (Soil trials), 70.4% (Sand trials), and 0% (Sod trials).

(f) Wtime with Type—For the 35 Sod runs, only one run (in the non-summer months) involved a weathering duration longer than 3 days.

C4 REANALYSIS OF TRAVERSAL PROGRAM DATA

The binary response data (rabbit lethality) were analyzed using the binary logistic regression routine (using a probit-link function) in MINITAB™ (version 14). This routine is an extension of the one-factor probit analysis. Finney (1971) and Fox (1997) provide background information. Many different model fits were investigated, and the following is the final model fit:

$$\begin{aligned}
 Y_N = (Y_P - 5) = & (3.010) + (-1.812)(WtG_{med}) + (-4.721)(WtG_{long}) \\
 & + (2.355)(\log_{10} D) + (-1.961)(Type2) \\
 & + (-1.874)(Summer) + (-1.145)(SGroup)
 \end{aligned}
 \tag{1}$$

where Y_N is a normit, Y_P is a probit, WtG_{med} equals one if $WtGroup$ equals “medium” and zero otherwise, WtG_{long} equals one if $WtGroup$ equals “long” and zero otherwise, the k s are fitted coefficients, and the other parameters are defined in Section C2.1. Y_N equals -1 , 0 and 1 at the 16, 50, and 84% response levels, respectively. There was no statistically significant lack of fit (Section C4.2).

The effect of the initial contamination density (Density) was modeled as a linear function and a logarithmic function ($\log D$); a logarithmic function was found to work best. For weathering duration ($Wtime$), linear and logarithmic functions were found to work equally well in fitting the quantal data. Since the vast majority of runs (114 out of 126) were made at just three weathering durations (1 hr, 1 day, and 7 days), there was insufficient coverage of separate $Wtime$ values to accurately determine which function of $Wtime$ (linear and logarithmic) would work the best in fitting the data. Hence, it was decided to convert $Wtime$ into a three level factor, $WtGroup$, with values of “short” (1 hr), “medium” (1-3 days), and “long” (>3 days). The resulting model fit with $WtGroup$ was better than using either function of $Wtime$.

The parameter, $WtGroup$, also proved useful in the analysis of the Sand plot quantal data. The breakdown of the sand quantal dataset is problematic when analyzed *via* multifactor probit analysis. All of the rabbits exposed to Sand plots at $Wtime$ equal to 1 hr and 1 day died, and none of the rabbits exposed at $Wtime$ equal to 7 days died. Thus, if the sand dataset is analyzed by itself, no meaningful estimation would be possible of the median effective values of $\log D$ at each of the three separate values for $Wtime$. However, by combining the sand plot data with those of soil and sod, a meaningful analysis is possible for the sand data, but the results will be highly dependent on how $Wtime$ is handled in the model. The approach that introduces the least amount of bias is to model $Wtime$ as a qualitative factor rather than as a quantitative function (linear, logarithmic, etc.). This would not have been an issue had the original researchers conducted additional sand plot runs at lower Density values at $Wtime$ values of 1 hr and 1 day until no rabbit deaths were observed.

Early model fits indicated no statistical difference between soil and sod plots; so, for terrain type the two level factor ($Type2$ —soil/sod vs. sand) was used instead of the three-level factor ($Type$ —soil, sod and sand). The effect of droplet diameter was modeled *via* the use of a two level factor, $SGroup$ (zero for droplet diameters $<1000\mu$, and one otherwise), instead of a covariate. This was done to avoid over fitting the data, particularly because of the slight correlation between droplet diameter ($Size$) and initial contamination density (D) (Section C3). Residual VX ground contamination ($RConc$) (based on the chemical analysis of soil plugs) at the time of rabbit exposure was found not to be statistically significant. The shading of contaminated plots ($Shade$) was not used in the model due to its being correlated with other factors in the model ($\log D$, $SGroup$ and $Type2$). Also, Factor interactions were avoided because of the large degree of incomplete crossing in the experimental design (Section C3).

Lethal VX (initial) contamination density estimates as a function of XX percent lethality can be calculated by solving for D in Equation [1] and using the appropriate value for Y_N :

$$\log_{10} D_{xx} = \frac{\left[(3.010) + Y_N + (-1.812)(WtG_{med}) + (-4.721)(WtG_{long}) \right. \\ \left. + (-1.961)(Type2) + (-1.874)(Summer) + (-1.145)(SGroup) \right]}{(2.355)} \quad [2]$$

Confidence limits on logD estimates can be calculated using methods from Mood *et al.* (1974) and Barry (1978). Barry (1978) gives the standard error of a ratio, (a/b) , which is based upon the propagation of error formula for a ratio:

$$\text{std err of } \left(\frac{a}{b}\right) = \left(\frac{a}{b}\right) \sqrt{\left(\frac{\text{var}(a)}{a^2}\right) + \left(\frac{\text{var}(b)}{b^2}\right) - (2)\left(\frac{\text{cov}(a,b)}{ab}\right)} \quad [3]$$

where $\text{var}(a)$, $\text{var}(b)$, and $\text{cov}(a,b)$ are the variance of the quantities, a and b , and their covariance, respectively. The 95% confidence limits for the ratio will equal $(a/b) \pm (1.96)(\text{std err})$. The following relations from Mood, *et al.* (1974) were also used to get the necessary information for determining the limits for logD:

$$\text{var}(a \pm b) = \text{var}(a) + \text{var}(b) \pm (2)\text{cov}(a, b) \quad [4]$$

$$\text{cov}(a \pm b, c) = \text{cov}(a, c) \pm \text{cov}(b, c) \quad [5]$$

where $\text{cov}(a \pm b, c)$ is the covariance of the quantity, $(a \pm b)$, with a third quantity, c . An example of how Equations [3] to [5] are used in the present analysis is shown in Section C4.3.

A listing of the final model predictions for each quantal data point is presented in Section C4.1, the MINITAB™ printout of the final model fit is listed in Section C4.2, and plots and tabulated values of the model fit (from Equation [1]) are shown in Section C4.3.

C4.1 Model Prediction Display.

Row	Date	Summer	Type2	logD	WtGroup	SGroup	Deaths	Number	SPRE	EPRO
1	3/12/1957	no	SoilSod	0.60206	short	1	1	2	-2.06288	0.906877
2	3/12/1957	no	SoilSod	0.60206	medium	1	0	2	-1.01462	0.312106
3	3/12/1957	no	SoilSod	0.60206	long	1	0	2	-0.02601	0.000337
4	3/12/1958	no	SoilSod	-0.39794	short	0	0	6	-3.06388	0.544485
5	3/12/1958	no	SoilSod	-0.39794	medium	0	1	6	1.55625	0.044573
6	3/18/1957	no	SoilSod	1.30103	short	1	4	4	0.07811	0.998500
7	3/18/1957	no	SoilSod	1.30103	medium	1	3	4	-0.86568	0.876169
8	3/18/1957	no	SoilSod	1.30103	long	1	1	8	1.50055	0.039740
10	3/18/1957	no	SoilSod	1.49831	short	1	2	2	0.02450	0.999701
11	3/18/1957	no	SoilSod	1.49831	medium	1	2	2	0.34759	0.947449
12	3/18/1957	no	SoilSod	1.49831	long	1	1	4	1.21581	0.098672
18	4/22/1957	no	SoilSod	0.25527	short	0	2	2	0.33010	0.950521
19	4/22/1957	no	SoilSod	0.25527	medium	0	0	2	-1.30301	0.435760
20	4/22/1957	no	SoilSod	0.25527	long	0	0	2	-0.04630	0.001065
21	4/30/1957	no	SoilSod	-0.30103	short	0	4	4	1.64825	0.633051
22	4/30/1957	no	SoilSod	-0.30103	medium	0	1	4	1.48089	0.070549
23	4/30/1957	no	SoilSod	-0.30103	long	0	0	4	-0.00486	0.000006
27	5/6/1957	no	SoilSod	0.00000	short	0	2	2	0.60578	0.852867
28	5/6/1957	no	SoilSod	0.00000	medium	0	0	2	-0.78624	0.222778
29	5/6/1957	no	SoilSod	0.00000	long	0	0	2	-0.01550	0.000120
32	5/14/1957	no	SoilSod	-0.52288	short	0	2	6	-0.54228	0.427608
33	5/14/1957	no	SoilSod	-0.52288	medium	0	1	6	2.47115	0.023069
34	5/14/1957	no	SoilSod	-0.52288	long	0	0	4	-0.00137	0.000000
38	6/4/1957	yes	SoilSod	0.23045	short	0	2	2	1.84389	0.388897
39	6/4/1957	yes	SoilSod	0.23045	medium	0	0	2	-0.19431	0.018137
40	6/4/1957	yes	SoilSod	0.23045	long	0	0	2	-0.00075	0.000000
41	6/10/1957	yes	SoilSod	0.83885	short	0	2	2	0.54898	0.875025

42	6/10/1957	yes	SoilSod	0.83885	medium	0	0	4	-1.21449	0.254246
43	6/10/1957	yes	SoilSod	0.83885	long	0	0	2	-0.01887	0.000178
44	6/10/1957	yes	SoilSod	0.66276	short	0	2	2	0.79803	0.769076
45	6/10/1957	yes	SoilSod	0.66276	medium	0	0	2	-0.58312	0.140997
46	6/10/1957	yes	SoilSod	0.66276	long	0	0	2	-0.00821	0.000034
47	6/24/1957	yes	SoilSod	1.30103	short	1	6	6	1.16171	0.863031
48	6/24/1957	yes	SoilSod	1.30103	medium	1	1	6	-0.47133	0.236496
53	7/2/1957	yes	SoilSod	0.68124	short	0	2	2	0.76851	0.782108
54	7/2/1957	yes	SoilSod	0.68124	medium	0	0	2	-0.60698	0.150960
55	7/2/1957	yes	SoilSod	0.68124	long	0	0	2	-0.00899	0.000040
56	7/2/1957	yes	SoilSod	0.54407	short	0	3	4	0.33759	0.675919
57	7/2/1957	yes	SoilSod	0.54407	medium	0	0	4	-0.64125	0.087655
58	7/2/1957	yes	SoilSod	0.54407	long	0	0	2	-0.00447	0.000010
59	7/9/1957	yes	SoilSod	0.46240	short	0	1	2	-0.31108	0.6004110
60	7/9/1957	yes	SoilSod	0.46240	medium	0	1	2	2.63882	0.060852
62	7/9/1957	yes	SoilSod	0.20412	short	0	2	2	1.94028	0.365350
63	7/9/1957	yes	SoilSod	0.20412	medium	0	0	2	-0.17956	0.015548
64	7/9/1957	yes	SoilSod	0.20412	long	0	0	1	-0.00045	0.000000
65	7/9/1957	yes	SoilSod	-0.22185	short	0	0	2	-0.46070	0.088947
66	7/9/1957	yes	SoilSod	-0.22185	medium	0	0	2	-0.03989	0.0000792
68	8/27/1957	yes	Sand	1.30103	short	1	8	8	0.09624	0.998875
69	8/27/1957	yes	Sand	1.30103	medium	1	8	8	1.42377	0.893124
70	8/27/1957	yes	Sand	1.30103	long	1	0	8	-0.77487	0.047812
80	9/18/1957	yes	Sand	1.07555	short	0	2	2	0.01562	0.999878
81	9/18/1957	yes	Sand	1.07555	medium	0	2	2	0.26429	0.968353
82	9/18/1957	yes	Sand	1.07555	long	0	0	2	-0.63833	0.146252
83	9/18/1957	yes	Sand	0.95904	short	0	2	2	0.02626	0.999656
84	9/18/1957	yes	Sand	0.95904	medium	0	2	2	0.36326	0.943263

Row	Date	Summer	Type2	logD	WtGroup	SGroup	Deaths	Number	SPRE	EPRO
85	9/18/1957	yes	Sand	0.95904	long	0	0	2	-0.48172	0.092256
86	9/23/1957	yes	Sand	1.14301	short	0	2	2	0.01138	0.999935
87	9/23/1957	yes	Sand	1.14301	medium	0	2	2	0.21734	0.978100
88	9/23/1957	yes	Sand	1.14301	long	0	0	2	-0.74597	0.185723
89	9/23/1957	yes	Sand	0.81291	short	0	2	2	0.04799	0.998857
90	9/23/1957	yes	Sand	0.81291	medium	0	2	2	0.52457	0.892266
91	9/23/1957	yes	Sand	0.81291	long	0	0	2	-0.32960	0.047352
92	1/7/1958	no	SoilSod	0.69020	short	0	2	2	0.08729	0.996253
93	1/7/1958	no	SoilSod	0.69020	medium	0	2	2	0.73684	0.805775
94	1/7/1958	no	SoilSod	0.69020	long	0	0	2	-0.20927	0.020313
99	6/3/1958	yes	SoilSod	1.05308	short	0	1	2	-3.01962	0.951031
100	6/3/1958	yes	SoilSod	1.05308	medium	0	0	4	-1.85638	0.437733
102	6/3/1958	yes	SoilSod	0.87506	short	0	2	2	0.50584	0.891723
103	6/3/1958	yes	SoilSod	0.87506	medium	0	1	4	-0.14960	0.282336
107	6/16/1958	yes	SoilSod	0.34242	short	0	0	2	-1.44433	0.492612
108	6/16/1958	yes	SoilSod	0.34242	medium	0	0	4	-0.38334	0.033612
109	6/30/1958	yes	SoilSod	1.10037	short	0	2	2	0.28932	0.961327
110	6/30/1958	yes	SoilSod	1.10037	medium	0	5	6	1.87446	0.481912
113	6/30/1958	yes	SoilSod	1.23553	short	0	2	2	0.19734	0.981446
114	6/30/1958	yes	SoilSod	1.23553	medium	0	5	6	1.25939	0.607541
118	7/30/1958	yes	SoilSod	0.55630	medium	0	0	2	-0.45867	0.092332
120	7/30/1958	yes	SoilSod	1.14301	medium	0	1	4	-1.15465	0.521956
121	7/30/1958	yes	SoilSod	0.92942	medium	0	0	2	-1.00687	0.327108
123	8/12/1958	yes	SoilSod	0.53148	medium	1	0	2	-0.10826	0.005707
124	8/12/1958	yes	SoilSod	0.91381	medium	1	0	2	-0.34038	0.051610
125	8/12/1958	yes	SoilSod	1.11059	medium	1	0	2	-0.54877	0.121793
126	8/12/1958	yes	SoilSod	1.00000	medium	1	0	2	-0.42303	0.076864

Some individual runs were deleted from the above printout since the parameter values for the deleted runs were identical to those of other runs. For example, Runs 6 and 14 have identical values for

logD, Wtime, Type2, Summer and SGroup; therefore, MINITAB™ prints out the standardized Pearson residual (SPRE) and estimated event probability (EPRO) at the first instance of a particular parameter value grouping (in this case, Run 6) but not at subsequent listing of runs executed at the same coordinates (Run 14 in this instance). The number of deaths and exposed rabbits (Number) for Runs 6 and 14 are combined together and listed under Run 6; the two deaths out of two rabbits exposed at Runs 6 and 14 are combined into four deaths out of four exposed.

C4.2 MINITAB™ Printout of Final Model Fit.

Binary Logistic Regression: Deaths, Number vs. logD, WtGroup, Type2, Summer and SGroup

Link Function: Normit

Response Information

Variable	Value	Count
Deaths	Success	104
	Failure	143
Number	Total	247

Factor Information

Factor	Levels	Values
WtGroup	3	short, medium, long
Type2	2	Sand, SoilSod
Summer	2	no, yes

Logistic Regression Table

Predictor	Coef	SE Coef	Z	P
Constant	3.00974	0.565765	5.32	0.000
logD	2.35481	0.378122	6.23	0.000
WtGroup				
medium	-1.81166	0.295552	-6.13	0.000
long	-4.72142	0.572306	-8.25	0.000
Type2				
SoilSod	-1.96093	0.435782	-4.50	0.000
Summer				
yes	-1.87367	0.393647	-4.76	0.000
Sgroup	-1.14478	0.370180	-3.09	0.002

Tests for terms with more than 1 degree of freedom

Term	Chi-Square	DF	P
WtGroup	71.7710	2	0.000

Log-Likelihood = -77.336

Test that all slopes are zero: G = 181.558, DF = 6, P-Value = 0.000

Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	73.6966	75	0.521
Deviance	75.8900	75	0.450
Hosmer-Lemeshow	12.0889	8	0.147

Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

Value	Group										Total
	1	2	3	4	5	6	7	8	9	10	
Success											
Obs	0	2	3	1	4	10	15	29	22	18	104
Exp	0.0	0.7	1.3	2.6	6.4	11.9	14.9	26.0	23.2	18.0	
Failure											
Obs	25	26	21	23	20	16	9	1	2	0	143
Exp	25.0	27.3	22.7	21.4	17.6	14.1	9.1	4.0	0.8	0.0	
Total	25	28	24	24	24	26	24	30	24	18	247

Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs	Number	Percent	Summary Measures	
Concordant	13864	93.2	Somers' D	0.87
Discordant	942	6.3	Goodman-Kruskal Gamma	0.87
Ties	66	0.4	Kendall's Tau-a	0.43
Total	14872	100.0		

Variance-Covariance Matrix [V]

	Constant	logD	WtGroup		SoilSod	Summer (yes)	SGroup (1)
			Medium	Long			
Constant	0.320089	0.075784	-0.0673042	-0.199582	-0.219594	-0.132156	-0.074419
logD	0.075784	0.142976	-0.0560136	-0.114017	-0.036353	-0.110766	-0.091560
Medium	-0.067304	-0.056014	0.0873510	0.092628	0.023584	0.030745	0.024686
Long	-0.199582	-0.114017	0.0926276	0.327534	0.127676	0.102537	0.042827
SoilSod	-0.219594	-0.036353	0.0235836	0.127676	0.189906	0.062493	0.035870
Summer	-0.132156	-0.110766	0.0307453	0.102537	0.062493	0.154958	0.078051
SGroup	-0.074419	-0.091560	0.0246856	0.042827	0.035870	0.078051	0.137034

C4.2.1 Sample Calculation of Confidence Limits for Estimated Fit for LD_{xx}.

The following are the limit calculations for the median lethality estimate (LD₅₀) using Equation [2] for the case where WtGroup = Short; on a Sand plot, Summer (yes); and SGroup equals one (large drop), using Equations [3] to [5] with values from matrix V above:

$$\text{numerator of [2]} = a = (0.00871) \qquad \text{denominator of [2]} = b = (2.355)$$

Using Equation [4]:

$$\begin{aligned} \text{var}(a) &= (0.320089) + (0.154958) + (0.137034) + 2\{(-0.132156) + (-0.074419) + (0.078051)\} \\ &= (0.3551) \end{aligned}$$

$$\text{var}(\log D) = \text{var}(b) = (0.1430)$$

Using Equation [5]:

$$\text{cov}(a,b) = \{(0.075784) + (-0.110766) + (-0.091560)\} \times (-1) = (0.1265)$$

Using Equation [3]:

$$\text{std err of } \left(\frac{a}{b}\right) = \left(\frac{(0.00871)}{(2.355)}\right) \sqrt{\left(\frac{(0.3551)}{(0.00871)^2}\right) + \left(\frac{(0.1430)}{(2.355)^2}\right) - (2)\left(\frac{(0.1265)}{(0.00871)(2.355)}\right)} = (0.2527)$$

Using the standard error, the 95% confidence limits are calculated:

$$\begin{aligned} [\text{est. of } \log_{10} D_{50} - (1.96)(0.2527)] &\leq \text{true } \log_{10} D_{50} \leq [\text{est. of } \log_{10} D_{50} + (1.96)(0.2527)] \\ [-0.4916] &\leq \text{true } \log_{10} D_{50} \leq [0.4990] \end{aligned}$$

C4.2.2 ANOVA of Residuals from Final Model Fit.

The standardized Pearson residuals (SPRE) were analyzed to check for lack of model fit. A series of ANOVAs were performed of SPRE as a function of Wtime, Type, Shade, Sgroup, Summer, Density and Month. In none of the ANOVAs was it found that the SPRE was a statistically significant function of any of these parameters. The lowest p-value (or greatest statistical significant) was found with Type (p-value equals 0.255).

C4.3 Display of Final Model Fit.

In Tables C1 and C2, the estimated median (LD_{50}) and 5% (LD_{05}) lethality initial VX contamination densities, respectively, are listed as a function of weathering duration, terrain type, season, and drop size. Associated 95% confidence limits are also shown. The ratio of the upper and lower limit is an indicator of the precision of the estimate—the greater the ratio value, the more variance associated with the estimate. Some representative values from these two tables are shown in Figures C1 to C3. Sample widths of the 95% confidence limits are shown in Figure C4.

The precision of the final model fits (as shown in Tables C1 and C2) are a reflection of the parameter space that experimental data were actually collected over. The largest variances in model fits (as reflected by the larger upper/lower ratio values in the Tables C1 and C2) are found with the sand plots for non-summer months. In essence, the model estimates for this group represents an extrapolation of the model fit beyond the parameter space of the experiment, since no experimental data were collected on sand plots during non-summer months. Conversely, the estimates for soil/sod plots as a group have less variance than those for sand plots.

Table C1. Estimates of Median Lethal VX (Initial) Contamination Density from Model Fit (Equation [1])

Weathering Duration	Terrain Type	Summer	Drop Size	LD ₅₀ (g/m ²)	95% Confidence Limits		Ratio Upper/Lower
					Lower	Upper	
Short	Sand	no	Small	0.053	0.017	0.17	10.0
Short	Sand	no	Large	0.16	0.046	0.56	12.1
Short	Sand	yes	Small	0.33	0.12	0.90	7.5
Short	Sand	yes	Large	1.0	0.3	3.2	9.8
Short	SoilSod	no	Small	0.36	0.22	0.58	2.6
Short	SoilSod	no	Large	1.1	0.6	2.2	3.9
Short	SoilSod	yes	Small	2.2	1.4	3.5	2.4
Short	SoilSod	yes	Large	6.9	3.4	13.7	4.0
Medium	Sand	no	Small	0.31	0.11	0.87	7.9
Medium	Sand	no	Large	0.95	0.32	2.8	8.6
Medium	Sand	yes	Small	1.9	0.85	4.4	5.2
Medium	Sand	yes	Large	5.9	2.4	14.6	6.1
Medium	SoilSod	no	Small	2.1	1.2	3.8	2.9
Medium	SoilSod	no	Large	6.5	3.5	11.8	3.3
Medium	SoilSod	yes	Small	13.2	8.9	19.5	2.2
Medium	SoilSod	yes	Large	40.3	23.5	69.1	2.9
Long	Sand	no	Small	5.3	2.0	14.4	7.3
Long	Sand	no	Large	16.3	6.7	39.9	6.0
Long	Sand	yes	Small	33.3	13.5	82.1	8.1
Long	Sand	yes	Large	102.0	44.8	232.4	5.2
Long	SoilSod	no	Small	36.3	11.3	116.3	10.3
Long	SoilSod	no	Large	111.1	37.7	327.8	8.7
Long	SoilSod	yes	Small	226.6	67.9	755.9	11.1
Long	SoilSod	yes	Large	694.1	220.7	2183.6	9.9

Table C2. Estimates of Five Percent Lethal VX (Initial) Contamination Density from Model Fit (Equation [1])

Weathering Duration	Terrain Type	Summer	Drop Size	LD ₅₀ (g/m ²)	95% Confidence Limits		Ratio Upper/Lower
					Lower	Upper	
Short	Sand	no	Small	0.011	0.0025	0.045	18.5
Short	Sand	no	Large	0.032	0.0067	0.16	23.2
Short	Sand	yes	Small	0.066	0.017	0.25	14.7
Short	Sand	yes	Large	0.20	0.046	0.89	19.5
Short	SoilSod	no	Small	0.072	0.033	0.16	4.8
Short	SoilSod	no	Large	0.22	0.083	0.59	7.1
Short	SoilSod	yes	Small	0.45	0.21	1.0	4.5
Short	SoilSod	yes	Large	1.4	0.52	3.7	7.1
Medium	Sand	no	Small	0.06	0.018	0.22	12.5
Medium	Sand	no	Large	0.19	0.050	0.73	14.6
Medium	Sand	yes	Small	0.39	0.13	1.2	8.9
Medium	Sand	yes	Large	1.2	0.36	3.9	11.0
Medium	SoilSod	no	Small	0.42	0.23	0.78	3.4
Medium	SoilSod	no	Large	1.3	0.60	2.8	4.6
Medium	SoilSod	yes	Small	2.6	1.6	4.3	2.7
Medium	SoilSod	yes	Large	8.1	4.0	16.3	4.1
Long	Sand	no	Small	1.1	0.41	2.8	6.7
Long	Sand	no	Large	3.3	1.3	8.1	6.2
Long	Sand	yes	Small	6.7	2.9	15.6	5.4
Long	Sand	yes	Large	20.4	8.9	47.0	5.3
Long	SoilSod	no	Small	7.3	2.9	18.3	6.3
Long	SoilSod	no	Large	22.2	9.2	53.8	5.8
Long	SoilSod	yes	Small	45.4	17.3	119.1	6.9
Long	SoilSod	yes	Large	139.0	53.6	360.0	6.7

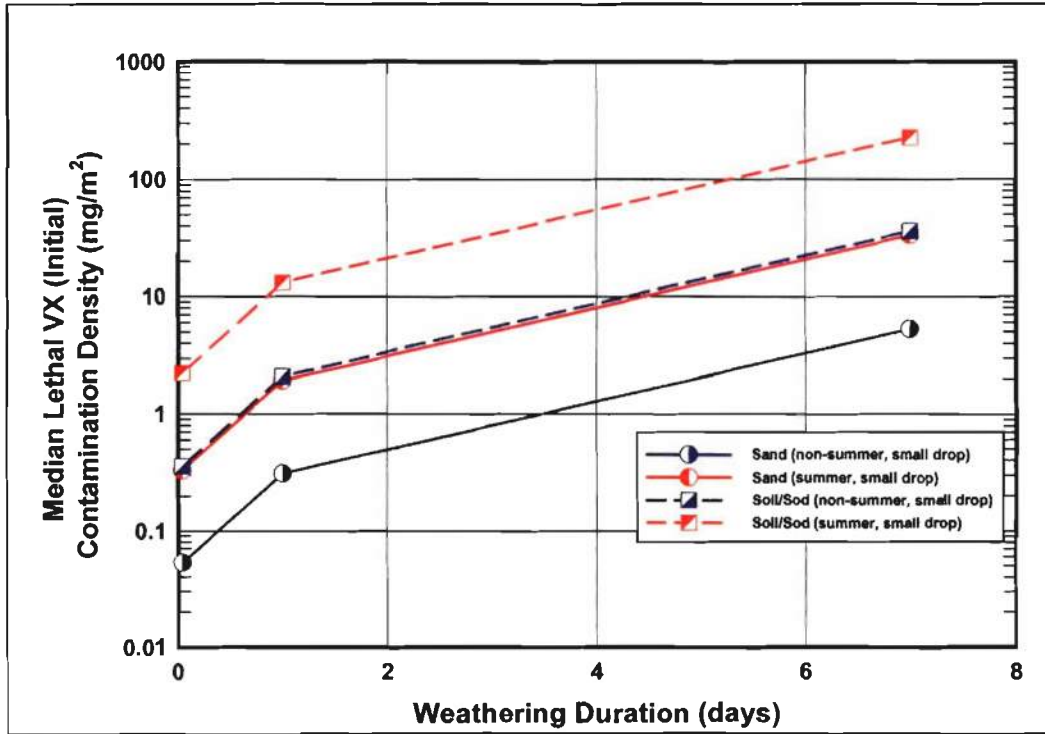


Figure C1. PC Exposure of Rabbits to Contaminated Terrain—Median Lethal VX (Initial) Contamination Density vs. Weathering Duration Prior to Exposure (Small Drops)

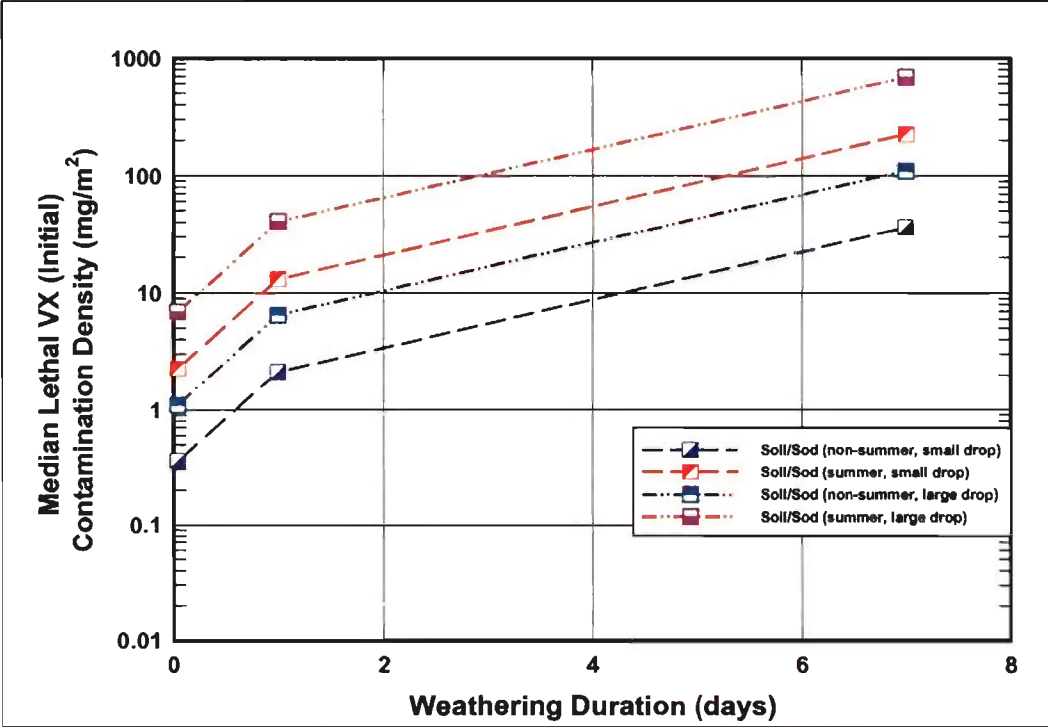


Figure C2. PC Exposure of Rabbits to Contaminated Soil/Sod--Median Lethal VX (Initial) Contamination Density vs. Weathering Duration Prior to Exposure (Small and Large Drops)

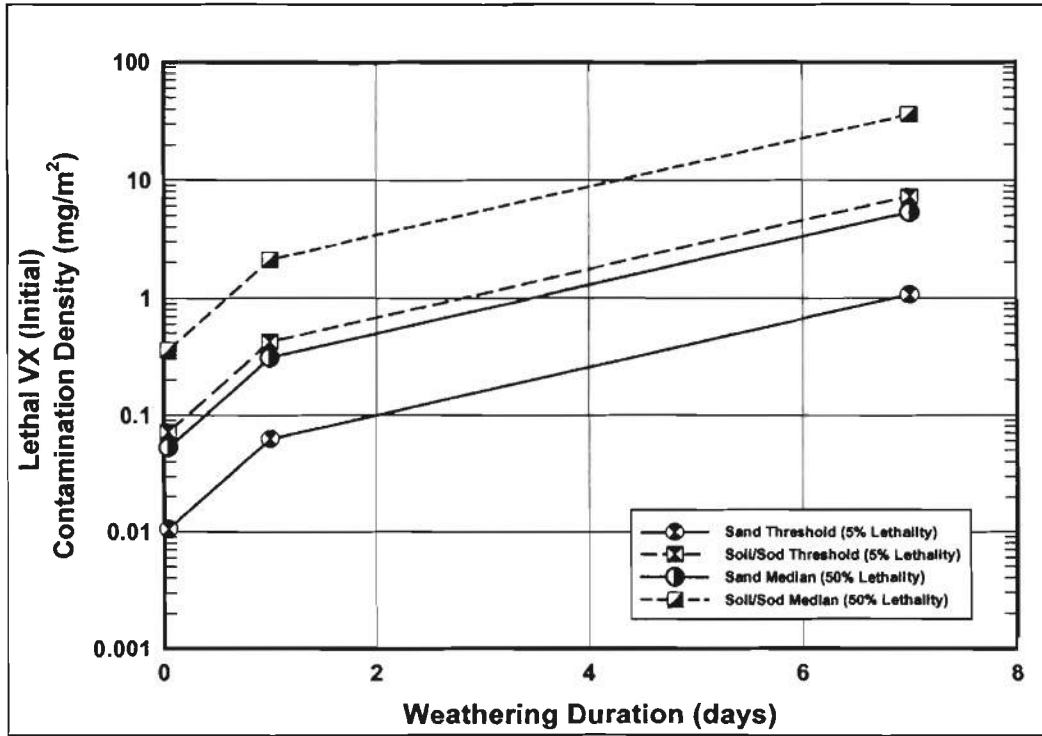


Figure C3. Rabbit PC Exposure to Contaminated Terrain (Non-Summer)--Median & Threshold Lethal VX (Initial) Contamination Density vs. Weathering Duration Prior to Exposure (Small Drops)

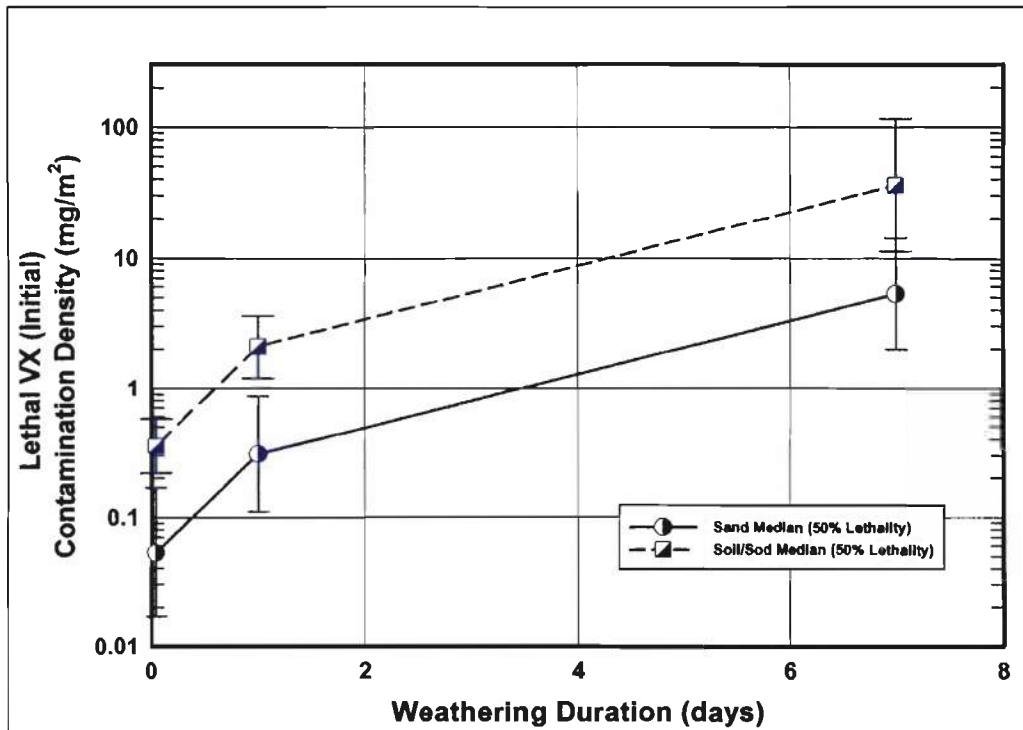


Figure C4. Rabbit PC Exposure to Contaminated Terrain (Non-Summer)—Median Lethal VX (Initial) Contamination Density vs. Weathering Duration Prior to Exposure (Small Drops)—Comparison of 95% Confidence Limits

C5 DISCUSSION

The statistical reanalysis of the Traversal Program VX PC rabbit exposure data has confirmed many of the findings of the Traversal Program. However, there are important contradictions between the original findings of the Traversal Program and those of the current reanalysis. Both are discussed in greater detail below. Due to the lack of randomization and poor experimental design, all conclusions must be interpreted with caution.

C5.1 Terrain Effects on VX Persistence.

The Traversal Program had originally concluded that the persistency of VX in soil, sand, and sodded terrain were roughly of the same magnitude. The current reanalysis of the data found that VX in the soil/sod plots was a factor of 6.8 less persistent than the sand plots, which does not contradict the original Traversal finding. However, it is still possible that the difference between VX persistence in sand and soil/sod is greater than factor of 10 for two reasons. First, there were no runs involving weathering durations of 1 hr and 1 day where any rabbit survived exposure. Second (which is related to the first), the lowest initial VX density on sand used in the study was 6.5 mg/m². The estimated LD₅₀s for sand for short and medium weathering durations (Table C1) are all below 6.5 mg/m². Additional runs with lower initial VX densities should have been conducted to better define the LD₅₀s on sand.

A statistical exercise was conducted to gauge the possible effects of additional runs on sand plots. Two fictional runs of two rabbits each were added to the dataset (one run each at WtGroup values of short and medium) at the following test coordinates: logD = -0.5; sand; summer = yes; and Sgroup = 0. A value of -0.5 was chosen for logD, since this was roughly the lowest value used for the soil/sod plots. If the quantal result should be four deaths out of four exposed, the ratios of the LD₅₀s of soil/sod and sand would equal about a factor of 20. Conversely, no deaths out of four exposed would produce a difference of about a factor of five. The use of only four more rabbits could have had a major impact on the findings of the Traversal Program findings with regard to differences among soil types on VX persistency.

C5.2 Meteorological Effects on VX Persistence.

The Traversal Program had originally concluded that the persistency of VX decreases with higher air temperatures and the presence of rainfall. This was confirmed for high air temperatures in the current reanalysis for soil and sod plots, but this could not be confirmed in sand plots, since no runs were conducted on sand plots in non-summer runs. The impact of rainfall could not be adequately estimated in the current reanalysis due to correlation of which plots were shaded from the elements (Shade) with other factors being investigated (logD, SGroup and Type2—Section C4). As a result, no definite conclusion can be made on the impact of rainfall on VX persistence.

C5.3 Effect of Weathering Duration on VX Persistence.

The Traversal Program had originally concluded that the persistency of VX decreases with longer weathering durations, and this was confirmed for all terrain types in the current reanalysis of the dataset. However, a better understanding of this relationship could have been obtained had more runs been conducted (on all types of terrain types) at some weathering duration (WtGroup) value intermediate between 1 and 7 days (ex. 3 or 4 days). This is of particular importance with the sand plots, where no deaths were observed in any of the runs at WtGroup = 7 days.

C5.4 Effect of Droplet Size on VX Persistence.

The Traversal Program had originally concluded that the persistency of VX was independent of the VX droplet size used in the initial contamination of the terrain plots. However, the poor experimental design that was used in the Program (Sections C2 and C3) does not readily allow any sort of proper conclusion to be made on the impact of droplet size. The results of the current reanalysis of the dataset show that VX persistence is an inverse function of the droplet size, with smaller sizes being more persistent. Yet, even this finding must be viewed with caution due to the same poor experimental design.

If this finding is truly valid, smaller droplets are probably more persistent because they are more quickly absorbed (*via* wicking) into the terrain, and this mechanism has a greater influence on persistence than the higher evaporation rate of smaller droplets in comparison to larger droplets. Thus, agent loss due to evaporation would be less than would have occurred when larger droplets are used.

C5.5 General Observations on VX Persistence on Sand Plots.

Of the three terrain types investigated by the Traversal Program (soil, sod and sand), the data collected by the Program sheds the least understanding on the persistence of VX on sand. Unfortunately, the original researchers do not appear to acknowledge the existence of this data gap by their treatment of sand plots as being roughly equivalent to soil and sod plots in terms of VX persistency. Testing on sand plots was not as thorough as was done on the soil and sod plots, probably because the researchers thought that there was no significant difference.

Results of the current reanalysis of the dataset indicate that sand plots could possibly be a more substantial threat than soil or sod plots than was originally concluded by the Traversal Program. In non-summer months, significant numbers of deaths could occur in rabbits due to PC exposure from VX contaminated sand plots after a weathering duration of 7 days of plots with initial VX densities of from 1 to 10 mg/m² (Figures C3 and C4). However, additional animal testing is required to verify this finding.

C5.6 General Observations on Use of Other Toxicological Endpoints.

Pre and post-exposure cholinesterase data were collected on the rabbits used in this study, but the data were of limited use due to the large fluctuation in cholinesterase values in the control rabbits (Section C2). Any exposed rabbit not having cholinesterase depression greater than 70% could not be statistically concluded to have been affected by VX exposure, which was erroneously assumed by the original researchers.

Having quantal data on other toxicological endpoints would have been useful in the current reanalysis, since a more precise ordinal logistical regression analysis could have then been performed on the dataset (instead of the binary logistical regression analysis that was actually used) (Sommerville, 2004). The noting of other non-lethal endpoints (twitching, convulsions, *etc.*) in surviving rabbits should be done in any future studies.

C5.7 General Observations on Impact of Experimental Design.

The manner in which the values of the test coordinates were balanced (Section C2.3) by the Traversal Program and the experimental design that they used should be used as examples on how not to design a future experimental study. None of their conclusions or findings can be statistically defended with any confidence. In fact, important findings (Section C5.5 for an example) were possibly overlooked due to their poor experimental design.

C6 CONCLUSIONS

The findings of the Traversal Program on the persistence of VX liquid in contaminated ground should be treated with caution and should be considered to be of qualitative value, at best, due to the poor experimental design and balance of test coordinates used in their study. A statistical reanalysis of their dataset has found (within the limits imposed by the poor quality of the initial experimental design) several details that are at odds with the original conclusions of the Traversal Program:

(1) The persistency of VX in sand may be a greater PC exposure threat that was originally concluded by the Traversal Program.

(2) Larger VX droplets are less persistent than smaller droplets (Section C5.4).

(3) Rabbit cholinesterase data from exposed rabbits were nearly worthless due to the large fluctuations of cholinesterase values in the control rabbits.

(4) No definitive conclusions can be made on the impact of rainfall on VX persistence due to the poor experimental design that was used. However, VX persistence is less in summer months (June, July, August, and September) than other months of the year.

(5) After 1 day of weathering, the persistence of VX in the various terrain plots decreased rapidly. Only two rabbit deaths (ignoring the five deaths attributed to high air temperatures by the original researchers) were recorded due to PC exposure to VX contaminated terrain on plots that had been weathered for 7 days or longer. Since there were very few trials conducted between 1 and 7 days of weathering, the exact point where the persistence has fallen to effectively zero cannot be readily determined. Also, the exact function of persistence on weathering duration (*e.g.*, linear, logarithmic, *etc.*) cannot be determined due to effectively limited number of individual weathering duration values used in the original study (essentially 1 hr, 1 day, and 7 days).

Table C3. Soil Data for PC Effects of VX in Rabbits(Reich, 9 Feb 1959)

Surface [Dispenser]	Init Contam (g/m ²) MMD [μ]	Resid Contam (g/m ²)	Z (days)	Deaths	ChE Activity* (%)	Ground Temp (°F)	Air Temp (°F)	Rain (in.)	Date	
soil (shaded) [microburette]	(4) [1450]	0.93	1 hr	1/2	---	41	50-55	---	3/12/57	
		2.11	1	0/2	85	66			3/13/56	
		1.13	2	---	---	---			3/14/57	
		1.76	3				0.9	3/15/57		
		---	6	0/2	75	---	0.2	3/18/57		
		1.49	7				0.1	3/19/57		
		1.98	9	---	---	---	---	3/21/57		
	0.28	14	0.1				3/26/57			
	(0.4) [780]	0.74	1 hr	0/2	---	41	50-55	---	3/12/57	
		0.29	1	---	---	---			---	3/13/56
		0.63	2						100	66
		0.50	3	---	---	---	0.9	3/15/57		
		---	6				0.2	3/18/57		
		0.20	7	---	---	---	0.1	3/19/57		
---		9	---				3/21/57			
0	14	0.1	3/26/57							
soil (shaded) [macroburette]	(20) [3250]	2.88	1 hr	2/2	---	41	45-50	0.2	3/18/57	
		4.00	1	---		38		0.1	3/19/57	
		3.05	2			---		---	---	3/20/57
		3.12	3	---	---			3/21/57		
		2.89	4	1/2	30	41		---	3/22/57	
			7					3/25/57		
		---	8	---	---	---		0.1	3/26/57	
		1.44	10					---	3/28/57	
		---	11	0/2	50	35		0.1	3/29/57	
		2.85	14					0.9	4/1/57	
		---	17	---	---	---		0.2	4/4/57	
	18		0.2				4/5/57			
	0.14	21	---	---	---	0.3	4/8/57			
	0.03	23				---	4/10/57			
	(31.5) [3480]	3.87	1 hr	2/2	---	41	45-50	0.2	3/18/57	
		2.98	1	---		38		0.1	3/19/57	
		9.90	2			---		---	3/20/57	
		12.52	3	1/2	90	41		---	3/21/57	
		6.48	4					---	3/22/57	
		4.48	7	---	---	---		---	3/25/57	
		---	8					0.1	3/26/57	
4.03		10	0/2	40	35	---		3/28/57		
---		11				0.1		3/29/57		
3.96		14	---	---	---	0.9		4/1/57		
---	17	---				---	---	0.3	4/4/57	
	18		0.2	4/5/57						
1.96	21	0.3	4/8/57							

*in survivors; Z=time zero for contamination

Table C3, cont.

Soil Data for PC Effects of VX in Rabbits (Reich, 9 Feb 1959)

Surface [Dispenser]	Init Contam (g/m ²) MMD [μ]	Resid Contam (g/m ²)	Z (days)	Deaths	CHE Activity* (%)	Ground Temp (°F)	Air Temp (°F)	Rain (in.)	Date			
soil (shaded) [spinning tip]	(0.5) [750]	0.84	1 hr	---	---		45-70	0.3	4/3/57			
		0.20	1					0.2	4/4/57			
		---	2-45					1.7	4/5/57			
		0.07	47					---	5/20/57			
	(1.8) [<200]	1.14	1 hr	2/2	---	75	65-70	---	4/22/57			
		1.84	1	0/2					4/23/57			
		---	7	95					78	4/49/57		
		0.29	8	---	---	---	55-60	---	4/30/57			
		---	14					0.1	5/6/57			
		0.51	15					---	5/7/57			
		0.38	21					0.1	5/13/57			
		---	22					---	5/14/57			
		---	26					1.0	5/18/57			
		0.15	28				---	5/20/57				
		---	33				0.3	5/25/57				
		0.3	37				---	5/29/57				
		(0.5) [<200]	0.31				1 hr	2/2	---	---	55-60	---
	0.11		1				1/2	75	5/1/57			
	---		6				---	---	0.1			5/6/57
	0.39		7				0/2	100	78	---	5/7/57	
	0.38		13	---	---	---	65-70	0.1	5/13/57			
	---		14					---	5/14/57			
	---		18					1.0	5/18/57			
	0.21		20					---	5/20/57			
	---		25				0.3	5/25/57				
	0.06		29				---	5/29/57				
	(0.5) [<200]	0.20	1 hr				2/2	---	---	55-60	---	4/30/57
		0.17	1	0/2	85	5/1/57						
		---	6	---	---	0.1	5/6/57					
		0.24	7	0/2	70	78	---	5/7/57				
		0.29	13	---	---	---	65-70	0.1	5/13/57			
		---	14					---	5/14/57			
		---	18				---	5/18/57				
	0.04	20	---				5/20/57					
	(1.0) [<200]	0.36	1 hr	2/2	---	65	60-70	0.1	5/6/57			
		0.68	1	0/2	35			78	---	5/7/57		
		0.37	7		65			83	0.1	5/13/57		
		---	8	---	---	---		---	---	5/14/57		
		---	12						1.0	5/18/57		
		0.16	14						---	5/20/57		
---		19	0.3						5/25/57			
0.04	23	---	5/29/57									

*in survivors; Z=time zero for contamination

Table C3, cont.

Soil Data for PC Effects of VX in Rabbits (Reich, 9 Feb 1959)

Surface [Dispenser]	Init Contam (g/m ²) MMD [μ]	Resid Contam (g/m ²)	Z (days)	Deaths	ChE Activity* (%)	Ground Temp (°F)	Air Temp (°F)	Rain (in.)	Date	
soil (shaded) [spinning tip]	(0.3) [<200]	0.50	1 hr	1/2	20	---	65-70	0.1	5/14/57	
		0.41	1	0/2	80			---	5/15/57	
		---	4	---	---			1.0	5/18/57	
		0.11	7	0/2	100			---	5/20/57	
		---	11-25	---	---		75-80	2.4	5/25-6/18/57	
		0.23	41					---	6/24/57	
		---	42-74					2.4	6/25-7/27/57	
		0.06	76					---	7/29/57	
	---	81	0.6	8/3/57						
	0.01	85	---	8/7/57						
	(0.3) [<200]	0.20	1 hr	0/2	20	---	65-70	0.1	5/14/57	
		---	1		85			---	5/15/57	
		---	4	---	---			1.0	5/18/57	
		0.07	7	0/2	85			---	5/21/57	
		---	11	---	---			65-70	0.3	5/25/57
		0.008	15						---	5/29/57
		---	19						0.6	6/2/57
		---	22						1.3	6/5/57
	---	25	0.3	6/8/57						
	0.13	41	---	6/24/57						
	(1.7) [470]	1.35	1 hr	2/2	---	---	65-70	---	6/4/57	
		1.55	1	0/2	30			1.3	6/5/57	
		---	4	---	---			0.2	6/8/57	
		0.07	7	0/2	100		90	80	---	6/11/57
		0.11	13	---	---		---		---	6/17/57
		0.13	20						---	6/24/57
		---	21					0.8	6/25/57	
		---	25	---	---		---	75-80	0.6	6/29/57
		---	34						---	7/8/57
		0	48						---	7/22/57
		---	49	---	---		---	75-80	0.3	7/23/57
		---	53						0.1	7/27/57
	0.07	55	---			7/29/57				
	---	60	0.6	8/3/57						
	0.04	64	---	8/7/57						
	(4.6) [220]	5.32	1 hr	2/2	---	81	80	---	6/10/57	
		3.33	1	0/2	80	90		---	6/11/57	
		1.06	7		40			---	6/17/57	
		0.43	15	---	---	---	80	0.8	6/25/57	
		---	19					---	6/29/57	
---		28	0.6					7/8/57		
0.04		42	---	---	---	75-80	---	7/22/57		
---		43					0.3	7/23/57		
---		47					0.1	7/27/57		
0.15		49	---	---	---	75-80	---	7/29/57		
---	54	0.6					8/3/57			
0.05	58	---					8/7/57			

*in survivors; Z=time zero for contamination

Table C3, cont.

Soil Data for PC Effects of VX in Rabbits (Reich, 9 Feb 1959)

Surface [Dispenser]	Init Contam (g/m ²) MMD [μ]	Resid Contam (g/m ²)	Z (days)	Deaths	ChE Activity* (%)	Ground Temp (°F)	Air Temp (°F)	Rain (in.)	Date		
soil (shaded) [spinning tip]	(6.9) [230]	4.21	1 hr	2/2	---	81	80	---	6/10/57		
		3.78	1	0/2	80	90			6/11/57		
		0.53	7		90	6/17/57					
				0.45	15				0.8	6/25/57	
				---	19				0.6	6/29/57	
				---	28					7/8/57	
				0.18	42				---	7/22/57	
				---	43	---	---		0.3	7/23/57	
				---	47				0.1	7/27/57	
				0.19	49				---	7/29/57	
				---	54				0.6	8/3/57	
				0.1	58				---	8/7/57	
soil [macroburette]	(20) [3700]	54.0	1 hr	2/2	---	96	80	---	6/24/57		
		9.17	1	0/2	85	98			0.8	6/25/57	
		0.51	3						---	6/27/57	
				---	5				0.6	6/29/57	
				0.26	7				---	7/1/57	
				0.12	14				0.6	7/8/57	
				0.13	17				---	7/11/57	
				0.06	22	---	---		---	7/16/57	
				0.11	28				---	7/22/57	
				---	29				0.3	7/23/57	
				---	33				0.1	7/27/57	
				0.04	35				---	7/29/57	
			---	40				0.6	8/3/57		
			0.02	44				---	8/7/57		
		(20) [3720]	49.4	1 hr	2/2	---	96	80	---	6/24/57	
			7.7	1	0/2	20	98			0.8	6/25/57
			1.3	3						---	6/27/57
				---	5				0.6	6/29/57	
				0.27	7				---	7/1/57	
				0	14				0.6	7/8/57	
				0.15	17				---	7/11/57	
				0.04	22	---	---		---	7/16/57	
				0	28				---	7/22/57	
				---	29				0.3	7/23/57	
			---	33				0.1	7/27/57		
			0.03	35				---	7/29/57		
		---	40				0.6	8/3/57			
		0	44				---	8/7/57			

*in survivors; Z=time zero for contamination

Table C3, cont.

Soil Data for PC Effects of VX in Rabbits (Reich, 9 Feb 1959)

Surface [Dispenser]	Init Contam (g/m ²) MMD [μ]	Resid Contam (g/m ²)	Z (days)	Deaths	ChE Activity* (%)	Ground Temp (°F)	Air Temp (°F)	Rain (in.)	Date		
soil [macrourette]	(20) [4140]	47.0	1 hr	2/2	---	96	80	---	6/24/57		
		15.0	1	1/2	35	98		0.8	6/25/57		
		0.41	3					---	6/27/57		
		---	5					0.6	6/29/57		
		0.16	7				75-80	---	7/1/57		
		0.08	14					0.6	7/8/57		
		0.09	17					---	7/11/57		
		0.11	22					---	7/16/57		
		0	28	---	---	---		---	7/22/57		
		---	29					0.3	7/23/57		
		---	33					0.1	7/27/57		
		0.08	35					---	7/29/57		
		---	40					0.6	8/3/57		
		0.19	44					---	8/7/57		
soil [spinning tip]	(4.8) [225]	6.45	1 hr	2/2	---	88	75-80	---	7/2/57		
		3.85	1	0/2	85			---	7/3/57		
		0.8	6	---	---	---		0.6	7/8/57		
		0.2	7	0/2	85	96		---	7/9/57		
		0.09	14					---	7/16/57		
		0.07	20					---	7/22/57		
		---	21					0.3	7/23/57		
		---	25	---	---	---		0.1	7/27/57		
		0.03	27					---	7/29/57		
		---	32					0.6	8/3/57		
		0.06	36					---	8/7/57		
		(3.5) [225]	3.85	1 hr	2/2	---		88	75-80	---	7/2/57
			2.73	1	0/2	85				---	7/3/57
			0.29	6	---	---		---		0.6	7/8/57
	0.14		7	0/2	85	96	---	7/9/57			
	0.02		14				---	7/16/57			
	0.13		20				---	7/22/57			
	---		21				0.3	7/23/57			
	---		25	---	---	---	0.1	7/27/57			
	0.02		27				---	7/29/57			
	---		32				0.6	8/3/57			
	0.04		36				---	8/7/57			
	(2.9) [225]		1.22	1 hr	1/2	30	96	80		---	7/9/57
			0.73	1	1/2	65	97			---	7/10/57
			0.19	2	---	---	---			---	7/11/57
		0.09	7	2/2		99	---		7/16/57		
		0.11	13				---		7/22/57		
		---	14				0.3		7/23/57		
---		18				0.1	7/27/57				
0.08		20				---	7/29/57				
---		25				0.6	8/3/57				
0		29				---	8/7/57				

*in survivors; Z=time zero for contamination

Table C3, cont.

Soil Data for PC Effects of VX in Rabbits (Reich, 9 Feb 1959)

Surface [Dispenser]	Init Contam (g/m ²) MMD [μ]	Resid Contam (g/m ²)	Z (days)	Deaths	ChE Activity* (%)	Ground Temp (°F)	Air Temp (°F)	Rain (in.)	Date
soil [spinning tip]	(1.6) [225]	0.63	1 hr	2/2	---	96	80	---	7/9/57
		0.34	1	0/2	100	87			7/10/57
		0.04	2	---	---	---			7/11/57
		0	7	1/2	60	99			7/16/57
		0	13						7/22/57
		---	14						7/23/57
		---	18	---	---	---			0.3 7/27/57
		0	20						0.1 7/27/57
		0	20						---
	(0.6) [225]	0.61	1 hr	0/2	65	96	80	---	7/9/57
		0.58	1		100	87			7/10/57
		0.18	2	---	---	---			7/11/57
		0.10	7	2/2		99			7/16/57
		0	13						7/22/57
		---	14						7/23/57
		---	18	---	---	---			0.3 7/27/57
		0.05	20						0.1 7/29/57
		---	25						---
0.06	29				0.6 8/7/57				

*in survivors; Z=time zero for contamination

Table C4. Sand Data for PC Effects of VX in Rabbits (Reich, 9 Feb 1959)

Surface [Dispenser]	Init Contam (g/m ²) MMD [μ]	Resid Contam (g/m ²)	Z (days)	Deaths	ChE Activity* (%)	Ground Temp (°F)	Air Temp (°F)	Rain (in.)	Date	
sand [macrourette]	(20.0) [3600]	26.7	1 hr	2/2	---	85	70-75	---	8/27/57	
		11.7	1			83			8/28/57	
		9.2	2	---	---	8/29/57				
		0.14	3	---	---	8/30/57				
		0.35	7	0/2	80	84			9/3/57	
		---	11	---	---	---			0.3	9/7/57
		---	13	---	---	---			0.2	9/9/57
		0	14	---	---	---			2.5	9/10/57
0	15	---	---	---	---	9/11/57				
sand (shaded) [macrourette]	(20.0) [3800]	28.4	1 hr	2/2	---	85	70-75	---	8/27/57	
		21.6	1			83			8/28/57	
		7.5	2	---	---	8/29/57				
		6.1	3	---	---	8/30/57				
		0.9	7	0/2	35	84			9/3/57	
		---	11	---	---	---			0.3	9/7/57
		---	13	---	---	---			0.2	9/9/57
		---	14	---	---	---			2.5	9/10/57
0.2	15	---	---	---	---	9/11/57				
sand [macrourette]	(20.0) [3600]	26.7	1 hr	2/2	---	85	70-75	---	8/27/57	
		11.7	1			83			8/28/57	
		9.2	2	---	---	8/29/57				
		0.14	3	---	---	8/30/57				
		0.35	7	0/2	90	84			9/3/57	
		---	11	---	---	---			0.3	9/7/57
		---	13	---	---	---			0.2	9/9/57
		---	14	---	---	---			2.5	9/10/57
0	15	---	---	---	---	9/11/57				
sand (shaded) [macrourette]	(20.0) [3500]	4.9	1 hr	2/2	---	85	85	---	8/27/57	
		3.1	1			83	83		8/28/57	
		5.2	2	---	---	8/29/57				
		3.9	3	---	---	8/30/57				
		0.05	7	0/2	90	84	84		9/3/57	
		---	11	---	---	---	---		0.3	9/7/57
		---	13	---	---	---	---		0.2	9/9/57
		---	14	---	---	---	---		2.5	9/10/57
0.27	15	---	---	---	---	9/11/57				
sand [spinning tip]	(11.9) [225]	3.0	1 hr	2/2	---	77	75	---	9/18/57	
		0.25	1			79			9/19/57	
		5.1	2	---	---	9/20/57				
		---	3	---	---	0.1			9/21/57	
		0.35	5	---	---	---	50-65	---	9/23/57	
		0.41	6	---	---	---			9/24/57	
		0.18	7	0/2	90	69			9/25/57	
		0	14	---	---	---			10/2/57	

*in survivors; Z=time zero for contamination

Table C4, cont.

Sand Data for PC Effects of VX in Rabbits (Reich, 9 Feb 1959)

Surface [Dispenser]	Init Contam (g/m ²) MMD [μ]	Resid Contam (g/m ²)	Z (days)	Deaths	ChE Activity* (%)	Ground Temp (°F)	Air Temp (°F)	Rain (in.)	Date			
sand (shaded) [spinning tip]	(9.1) [225]	2.6	1 hr	2/2	---	77	75	---	9/18/57			
		1.3	1			79			9/19/57			
		3.9	2	---		9/20/57						
		---	3	---		---	0.1	9/21/57				
		0.4	5			---	---	9/23/57				
		0.22	6			---	---	9/24/57				
		0.08	7			0/2	50	69	50-65	---	9/25/57	
		0	14	---		---	---	---	---	10/2/57		
sand [spinning tip]	(13.9) [400]	5.9	1 hr	2/2	---	70	50-60	---	9/23/57			
		3.8	1			76			9/24/57			
		0.8	2	---		---			---	---	9/25/57	
		0.9	3			---			---	9/26/57		
		0.3	4			0/2			55	66	9/27/57	
		0.08	7	0/2		55			66	66	9/30/57	
		---	13	---		---			---	---	0.8	10/6/57
		---	14			---			---	---	0.2	10/7/57
0.05	15	---	---		---	0.1	10/8/57					
sand (shaded) [spinning tip]	(6.5) [400]	2.3	1 hr	2/2	---	70	50-60	---	9/23/57			
		0.98	1			76			9/24/57			
		0.30	2	---		---			---	---	9/25/57	
		0.12	3			---			---	9/26/57		
		---	4			0/2			20	66	66	9/27/57
		0.02	7	0/2		20			66	66	9/30/57	
		---	13	---		---			---	---	0.8	10/6/57
		---	14			---			---	---	0.2	10/7/57
0.05	15	---	---		---	0.1	10/8/57					

Table C5. Sod Data for PC Effects of VX in Rabbits (Reich, 9 Feb 1959)

Surface [Dispenser]	Init Contam (g/m ²) MMD [μ]	Resid Contam (g/m ²)	Z (days)	Deaths	ChE Activity* (%)	Ground Temp (°F)	Air Temp (°F)	Rain (in.)	Date	
sod [spinning tip]	(4.9) [300]	1.94	1 hr	2/2	---	35	25-35	0.5	1/7/58	
		1.67	1			27		1/8/58		
		1.06	2	28		---		1/9/58		
		0.51	3	33		1/10/58				
		0.35	7	0/2		90		39	1.4	1/14/58
		---	8	---		---		44	0.5	1/15/58
		0.14	14	---		---		36	0.1	1/21/58
	(0.4) [380]	0.12	1 hr	0/2	65	36	35	0.2	1/21/58	
		0.03	1	1/2		43		0.6	1/22/58	
		0.02	2	---		31		---	1/23/58	
	(0.3) [395]	0.06	1 hr	1/2	---	30	35	0.2	1/21/58	
		0.01	1			100		43	0.6	1/22/58
			2	---		---		31	---	1/23/58
	(11.3) [250]	2.3	1 hr	1/2	---	70	65	---	6/3/58	
		0.06	1	0/1		25			87	6/4/58
		0.04	2	---		---			---	6/5/58
		0.03	3	0/2		---			80	6/6/58
	(7.5) [250]	1.37	1 hr	2/2	---	70	65	---	6/3/58	
		0.07	1	1/2		30			87	6/4/58
		0.06	2	---		---			---	6/5/58
		0.03	3	0/2		---			80	6/6/58
	(3.5) [185]	0.4	1 hr	1/2	25	70	65	---	6/16/58	
		0.01	1	0/2		30			83	6/17/58
			2	---		---			---	---
	(2.2) [260]	0.29	1 hr	0/2	25	70	65	---	6/16/58	
		0.01	1			30			83	6/17/58
		0.002	2			---			---	---
	(12.6) [450]	2.25	1 hr	2/2	---	90	80	---	6/30/58	
		0.15	1			95			7/1/58	
		0.10	2	1/2		75			97	7/2/58
		0.09	3	2/2		---			100	7/3/58
	(17.2) 185	2.17	1 hr	2/2	---	90	80	---	6/30/58	
		0.19	1			95			7/1/58	
		0.17	2	1/2		70			97	7/2/58
		0.08	3	2/2		---			100	7/3/58

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APPENDIX D

STATISTICAL ANALYSIS OF MUSTARD CONTACT HAZARD DATA FROM MANTHEI *ET AL.* (1988)

D1 INTRODUCTION

Data associated with the mustard contact hazard study conducted by Manthei *et al.* (1988) (Section 3.2) were reviewed and analyzed using modern statistical software (MINITAB™, version 14). The primary purpose of this study was to determine the degree of skin irritation in rabbits resulting from either direct or vapor contact with a mustard (HD)-contaminated surface that had been decontaminated with solvent. Though a balanced, factorial experimental design was used by Manthei *et al.* (1988), no formal statistical analysis was conducted on the data collected using this design. Such an analysis (on a limited basis) is provided in this appendix.

Analysis of variance (ANOVA) was used to examine the following five groups of data from Manthei *et al.* (1988):

1. Primary Irritation Index values from mustard induced injury to skin of the test rabbits
2. Size of surface injury in test rabbit
3. Amount of HD absorbed by dental dam under conditions identical to what the test rabbits were subjected to
4. Amount of HD recovered from the test metal coupons after either dental dam or test rabbit had been exposed to the coupon
5. Amount of HD recovered from control metal coupons that neither dental dam or test rabbit had been exposed to

For each exposure (of either dental dam or test rabbit) the following general procedure was used:

1. A metal coupon (painted with either an alkyd or polyurethane paint (Paint)) was contaminated with either of three amounts (Loading) of liquid HD (0.5, 2, and 10 mg)
2. The liquid HD was left on the coupon for 30 min, after which the coupon was rinsed (or decontaminated) with a liquid solvent
3. After decontamination, the coupon was allowed to age (Aging) for either of three durations (0, 15, or 300 min)
4. After aging, the coupon was then applied for 60 min to either dental dam or test rabbit (using either of two forms of contact (Contact)—direct or vapor) or held back as a control.
5. Coupon was removed from test subject.

From the above procedure used in the main part of the original study, five different factors were varied: Paint (2 levels), Loading (3 levels), Aging (3 levels), Contact (2 levels), and Mode (control, dental dam or test rabbit). Per each test condition, eight dental dam samples or test rabbits were used. So, 864 metal coupons ($2 \times 3 \times 3 \times 2 \times 3 \times 8$) were used under 108 test conditions ($2 \times 3 \times 3 \times 2 \times 3$).

Unfortunately, Manthei *et al.* did not properly randomize the order of the runs, though they did randomize which rabbits were assigned to each test condition. For instance, all of the dental dam runs were performed before runs involving rabbits and within the groups of dental dam and rabbit, all of the controls were done first, followed by the direct contact runs, and then the vapor contact runs. There are two major reasons that a proper ANOVA cannot be performed on the individual data points. First, the effect of any systemic error on the final ANOVA results cannot be entirely ruled out. Second, estimates of the variance will be artificially reduced. (This is particularly true because all eight replicates of the same test condition were run simultaneously.) In short, the variance was artificially reduced, so some factors or interactions could be incorrectly identified as being statistically significant when in reality they were not.

To alleviate the effects of non-randomization of run order, ANOVAs were performed using only the average response from each test condition group of eight test subjects. Furthermore, factors or

interactions were considered statistically significant only if their p-values were ≤ 0.01 —instead of the more typical criterion of a value of 0.05. Also, the relative strength of each factor and interaction in influencing a change in the measured response of interest was evaluated by examination of the variance components of the factors and interactions. Changes in factor levels that produce large changes in the response correspond to factors and interactions having large variance component values.

Analysis Background Information

Date of Analysis: 19 July 2005

Analyst: Douglas R. Sommerville, PE, Edgewood CB Center, APG, MD

Statistical Analysis Performed Using MINITAB™, v. 14

Analyst comments within the MINITAB™ printouts shown below are preceded by [DRS].

Nomenclature

Aging	Duration (in minutes) between decontamination of the test coupon and exposure of either rabbit or dental dam. For control coupons, it is the duration (in minutes) between decontamination of the test coupon and chemical analysis of the coupon to measure amount of residual agent Three levels used: 0, 15, and 300 min
AmntAvg	Group geometric mean of amount of agent removed from the eight test coupons of one test condition after exposure of test subjects is completed for vapor and direct contact exposures
ANOVA	Analysis of Variance
CntrlAvg	Group geometric mean of amount of agent removed from the eight control test coupons of one test condition
Contact	Type of contact between test coupon and subject (either dental dam or rabbit) Control—test coupon is not contacted with a test subject Direct—test coupon is applied directly to the surface of the test subject Vapor—test coupon is suspended one cm from the subject using a holder attached to the subject
DMass	Amount of agent mass absorbed by the dental dam (in μg)
DlogLR	logLR values from just the dental dam exposures
Earea	Effective area of injury on test rabbit (in in.^2) Shallow—Rabbit exposed to sand sample from upper surface Deep—Rabbit exposed to sand sample obtained from 0.5 to 2 in. below the upper surface
Loading	Amount of agent initially deposited on the test coupon (in mg) Three levels used: 0.5, 2, and 10 mg
logA	Logarithm (base 10) of Aging
logCntl	Logarithm (base 10) of CntrlAvg
logDM	Logarithm (base 10) of Dmass
logLR	Logarithm (base 10) of LossRat
logRcv	Logarithm (base 10) of Rcvred
logSpk	Logarithm (base 10) of Spike
LossRat	Agent loss ratio equals the ratio of AmntAvg and its corresponding CntrlAvg
Mode	Type of test surface being exposed, or in the case of the control coupons, what test subject group were the controls generated in conjunction with. dental—dental dam rabbit—test rabbit
Paint	Type of paint used to coat the test coupon alkyd—alkyd type paint poly—polyurethane type paint
PII	Average Primary Irritation Index for a test group of eight rabbits
Rcvrd	Amount (in μg) from spiked dental dam for recovery study
RlogLR	logLR values from just the rabbit exposures
Spike	Amount (in μg) used to spike dental dam for recovery study

D2 DATA PREPARATION

The group averages as originally reported Manthei *et al.* were not suitable for analysis. Arithmetic means were recorded, but geometric means are needed for the mass recovery values to perform the ANOVA using a logarithmic transformation on the amount of mass recovered or absorbed, so data were extracted from the original laboratory notebook, and geometric means were calculated for use in this statistical analysis.

The values for the amount of HD recovered from the dental dam were adjusted *per* the results of the precision study performed by Manthei *et al.* (the results listed in their Table 4). At low amounts of contamination, 100% recovery of HD from dental dam was not achieved. The percent recovery ranged from 81.7 to 97.5%. Using the original data from the laboratory notebook (see below), a calibration curve was calculated. This curve was then used to adjust all of the recorded individual HD mass recoveries from the dental dam (for those recoveries below 213.1 μg). The correlation coefficient squared for Equation [D1] was 96.0%, with an F-statistic value of 822.

$$\log\text{Rcv} = (-0.2096) + (1.0900)\log\text{Spk} \quad [\text{D1}]$$

D2.1 Listing of Experimental Data.

D2.1.1 Original Experimental Data from Laboratory Notebook used to Generate Table 4 in Manthei *et al.* (1988).

Row	Spike	Rcvrd
1	10	8.8
2	10	4.2
3	10	6.4
4	10	10.2
5	10	9.0
6	10	7.2
7	10	10.2
8	10	6.8
9	10	9.4
10	10	11.8
11	10	7.6
12	10	6.4
13	32	25.0
14	32	26.0
15	32	30.0
16	32	23.0
17	32	25.0
18	32	21.4
19	32	17.0
20	32	19.0
21	32	23.0
22	32	32.0
23	32	28.4
24	32	36.0
25	100	92.5
26	100	92.5
27	100	87.5
28	100	85.0
29	100	95.0
30	100	107.5
31	100	112.5
32	100	95.0
33	100	97.5
34	100	97.5

35 100 102.5
 36 100 105.0

D2.1.2 Control Coupon Data.

Row	Mode	Paint	Loading	Aging	CntlAvg	logCntl
1	dental	alkyd	0.5	0	31.03	1.49178
2	dental	alkyd	2.0	0	106.22	2.02621
3	dental	alkyd	10.0	0	303.92	2.48276
4	dental	poly	0.5	0	8.15	0.91116
5	dental	poly	2.0	0	161.51	2.20820
6	dental	poly	10.0	0	365.24	2.56258
7	dental	alkyd	0.5	15	24.09	1.38184
8	dental	alkyd	2.0	15	65.45	1.81591
9	dental	alkyd	10.0	15	189.43	2.27745
10	dental	poly	0.5	15	4.05	0.60746
11	dental	poly	2.0	15	59.68	1.77583
12	dental	poly	10.0	15	200.55	2.30222
13	dental	alkyd	0.5	300	7.68	0.88536
14	dental	alkyd	2.0	300	34.48	1.53757
15	dental	alkyd	10.0	300	98.41	1.99304
16	dental	poly	0.5	300	4.70	0.67210
17	dental	poly	2.0	300	8.97	0.95279
18	dental	poly	10.0	300	15.88	1.20085
19	rabbit	alkyd	0.5	0	29.41	1.46850
20	rabbit	alkyd	2.0	0	90.80	1.95809
21	rabbit	alkyd	10.0	0	322.13	2.50803
22	rabbit	poly	0.5	0	16.64	1.22115
23	rabbit	poly	2.0	0	109.72	2.04029
24	rabbit	poly	10.0	0	401.17	2.60333
25	rabbit	alkyd	0.5	15	16.98	1.22994
26	rabbit	alkyd	2.0	15	54.78	1.73862
27	rabbit	alkyd	10.0	15	176.57	2.24692
28	rabbit	poly	0.5	15	5.38	0.73078
29	rabbit	poly	2.0	15	84.88	1.92881
30	rabbit	poly	10.0	15	199.11	2.29909
31	rabbit	alkyd	0.5	300	11.06	1.04376
32	rabbit	alkyd	2.0	300	31.02	1.49164
33	rabbit	alkyd	10.0	300	89.50	1.95182
34	rabbit	poly	0.5	300	2.05	0.31175
35	rabbit	poly	2.0	300	7.51	0.87564
36	rabbit	poly	10.0	300	13.11	1.11760

D2.1.3 HD Recovery Data from Test Coupons after Direct and Vapor Contact with Test Subject.

Row	Mode	Paint	Contact	Loading	Aging	CntrlAvg	AmntAvg	LossRat	logLR
1	dental	alkyd	direct	0.5	0	31.03	15.25	0.4915	-0.30848
2	dental	alkyd	direct	2.0	0	106.22	50.08	0.4715	-0.32652
3	dental	alkyd	direct	10.0	0	303.92	132.99	0.4376	-0.35892
4	dental	alkyd	vapor	0.5	0	31.03	23.83	0.7680	-0.11464
5	dental	alkyd	vapor	2.0	0	106.22	64.52	0.6074	-0.21653
6	dental	alkyd	vapor	10.0	0	303.92	211.75	0.6967	-0.15695
7	dental	poly	direct	0.5	0	8.15	1.83	0.2246	-0.64859
8	dental	poly	direct	2.0	0	161.51	12.27	0.0759	-1.11976
9	dental	poly	direct	10.0	0	365.24	24.13	0.0661	-1.17980
10	dental	poly	vapor	0.5	0	8.15	4.13	0.5064	-0.29551
11	dental	poly	vapor	2.0	0	161.51	26.62	0.1648	-0.78304
12	dental	poly	vapor	10.0	0	365.24	73.44	0.2011	-0.69659
13	dental	alkyd	direct	0.5	15	24.09	7.52	0.3122	-0.50557
14	dental	alkyd	direct	2.0	15	65.45	45.15	0.6899	-0.16121
15	dental	alkyd	direct	10.0	15	189.43	140.86	0.7436	-0.12866

16	dental	alkyd	vapor	0.5	15	24.09	19.67	0.8165	-0.08804
17	dental	alkyd	vapor	2.0	15	65.45	56.55	0.8640	-0.06349
18	dental	alkyd	vapor	10.0	15	189.43	159.57	0.8424	-0.07448
19	dental	poly	direct	0.5	15	4.05	3.54	0.8731	-0.05894

Row	Mode	Paint	Contact	Loading	Aging	CntrlAvg	AmntAvg	LossRat	logLR
20	dental	poly	direct	2.0	15	59.68	10.30	0.1726	-0.76296
21	dental	poly	direct	10.0	15	200.55	23.87	0.1190	-0.92445
22	dental	poly	vapor	0.5	15	4.05	3.92	0.9682	-0.01403
23	dental	poly	vapor	2.0	15	59.68	15.10	0.2530	-0.59688
24	dental	poly	vapor	10.0	15	200.55	33.26	0.1658	-0.78042
25	dental	alkyd	direct	0.5	300	7.68	9.92	1.2919	0.11123
26	dental	alkyd	direct	2.0	300	34.48	35.72	1.0357	0.01523
27	dental	alkyd	direct	10.0	300	98.41	104.45	1.0613	0.02584
28	dental	alkyd	vapor	0.5	300	7.68	8.54	1.1129	0.04646
29	dental	alkyd	vapor	2.0	300	34.48	36.48	1.0577	0.02436
30	dental	alkyd	vapor	10.0	300	98.41	98.24	0.9983	-0.00074
31	dental	poly	direct	0.5	300	4.70	3.01	0.6393	-0.19430
32	dental	poly	direct	2.0	300	8.97	9.47	1.0556	0.02350
33	dental	poly	direct	10.0	300	15.88	16.27	1.0243	0.01043
34	dental	poly	vapor	0.5	300	4.70	5.40	1.1470	0.05956
35	dental	poly	vapor	2.0	300	8.97	10.59	1.1813	0.07236
36	dental	poly	vapor	10.0	300	15.88	16.49	1.0382	0.01628
37	rabbit	alkyd	direct	0.5	0	90.80	60.83	0.6699	-0.17399
38	rabbit	alkyd	direct	2.0	0	322.13	232.55	0.7219	-0.14152
39	rabbit	alkyd	direct	10.0	0	29.41	18.31	0.6226	-0.20579
40	rabbit	alkyd	vapor	0.5	0	322.13	203.09	0.6305	-0.20031
41	rabbit	alkyd	vapor	2.0	0	29.41	23.28	0.7917	-0.10144
42	rabbit	alkyd	vapor	10.0	0	90.80	75.37	0.8301	-0.08087
43	rabbit	poly	direct	0.5	0	109.72	3.85	0.0351	-1.45469
44	rabbit	poly	direct	2.0	0	401.17	15.45	0.0385	-1.41454
45	rabbit	poly	direct	10.0	0	16.64	1.59	0.0955	-1.02000
46	rabbit	poly	vapor	0.5	0	401.17	21.57	0.0538	-1.26922
47	rabbit	poly	vapor	2.0	0	16.64	2.49	0.1496	-0.82507
48	rabbit	poly	vapor	10.0	0	109.72	5.00	0.0456	-1.34104
49	rabbit	alkyd	direct	0.5	15	54.78	50.16	0.9157	-0.03825
50	rabbit	alkyd	direct	2.0	15	176.57	138.06	0.7819	-0.10685
51	rabbit	alkyd	direct	10.0	15	16.98	15.39	0.9066	-0.04258
52	rabbit	alkyd	vapor	0.5	15	176.57	163.52	0.9261	-0.03334
53	rabbit	alkyd	vapor	2.0	15	16.98	18.59	1.0947	0.03930
54	rabbit	alkyd	vapor	10.0	15	54.78	53.05	0.9684	-0.01395
55	rabbit	poly	direct	0.5	15	84.88	4.40	0.0518	-1.28567
56	rabbit	poly	direct	2.0	15	199.11	11.71	0.0588	-1.23062
57	rabbit	poly	direct	10.0	15	5.38	1.58	0.2929	-0.53328
58	rabbit	poly	vapor	0.5	15	199.11	8.36	0.0420	-1.37675
59	rabbit	poly	vapor	2.0	15	5.38	1.54	0.2857	-0.54409
60	rabbit	poly	vapor	10.0	15	84.88	3.82	0.0451	-1.34582
61	rabbit	alkyd	direct	0.5	300	31.02	10.41	0.3354	-0.47444
62	rabbit	alkyd	direct	2.0	300	89.50	79.74	0.8910	-0.05012
63	rabbit	alkyd	direct	10.0	300	11.06	9.14	0.8261	-0.08297
64	rabbit	alkyd	vapor	0.5	300	89.50	75.88	0.8478	-0.07171
65	rabbit	alkyd	vapor	2.0	300	11.06	9.99	0.9028	-0.04441
66	rabbit	alkyd	vapor	10.0	300	31.02	11.20	0.3612	-0.44225
67	rabbit	poly	direct	0.5	300	7.51	5.27	0.7020	-0.15366
68	rabbit	poly	direct	2.0	300	13.11	9.41	0.7175	-0.14418
69	rabbit	poly	direct	10.0	300	2.05	1.31	0.6402	-0.19368
70	rabbit	poly	vapor	0.5	300	13.11	9.97	0.7609	-0.11867
71	rabbit	poly	vapor	2.0	300	2.05	3.42	1.6666	0.22183
72	rabbit	poly	vapor	10.0	300	7.51	4.40	0.5856	-0.23240

D2.1.4 HD Recovery Data from Dental Dam, and PII and Earea Data for Rabbits after Contact with Test Coupons.

Row	Paint	Contact	Loading	Aging	DMass	logDM	PII	Earea
1	alkyd	direct	0.5	0	18.15	1.25891	6.87	0.21
2	alkyd	direct	2.0	0	49.54	1.69497	7.63	0.49
3	alkyd	direct	10.0	0	111.11	2.04574	7.82	1.06
4	alkyd	vapor	0.5	0	11.04	1.04303	0.00	0.00
5	alkyd	vapor	2.0	0	23.00	1.36182	0.75	0.14
6	alkyd	vapor	10.0	0	62.75	1.79762	4.13	1.31
7	poly	direct	0.5	0	18.86	1.27565	6.70	0.27
8	poly	direct	2.0	0	158.71	2.20060	7.82	2.06
9	poly	direct	10.0	0	444.63	2.64800	8.00	2.67
10	poly	vapor	0.5	0	10.58	1.02442	0.06	0.09
11	poly	vapor	2.0	0	104.42	2.01880	5.85	2.55
12	poly	vapor	10.0	0	249.35	2.39682	7.57	3.92
13	alkyd	direct	0.5	15	11.21	1.04975	6.38	0.12
14	alkyd	direct	2.0	15	24.66	1.39201	7.63	0.38
15	alkyd	direct	10.0	15	63.06	1.79978	7.63	0.64
16	alkyd	vapor	0.5	15	8.29	0.91856	0.00	0.00
17	alkyd	vapor	2.0	15	17.22	1.23604	0.06	0.03
18	alkyd	vapor	10.0	15	45.58	1.65877	3.23	1.00
19	poly	direct	0.5	15	2.70	0.43172	3.73	0.16
20	poly	direct	2.0	15	22.83	1.35852	8.00	1.17
21	poly	direct	10.0	15	108.32	2.03472	8.00	2.21
22	poly	vapor	0.5	15	5.22	0.71760	0.00	0.00
23	poly	vapor	2.0	15	34.58	1.53877	2.76	1.06
24	poly	vapor	10.0	15	117.56	2.07028	7.04	2.92
25	alkyd	direct	0.5	300	2.37	0.37385	4.07	0.03
26	alkyd	direct	2.0	300	6.43	0.80805	5.51	0.06
27	alkyd	direct	10.0	300	12.79	1.10695	7.51	0.28
28	alkyd	vapor	0.5	300	8.08	0.90755	0.00	0.00
29	alkyd	vapor	2.0	300	3.27	0.51480	0.00	0.00
30	alkyd	vapor	10.0	300	8.61	0.93479	0.00	0.00
31	poly	direct	0.5	300	1.84	0.26489	0.75	0.02
32	poly	direct	2.0	300	2.82	0.45068	1.69	0.18
33	poly	direct	10.0	300	4.76	0.67730	1.91	0.30
34	poly	vapor	0.5	300	2.49	0.39604	0.00	0.00
35	poly	vapor	2.0	300	3.00	0.47641	0.00	0.00
36	poly	vapor	10.0	300	3.30	0.51915	0.00	0.00

D3 DATA ANALYSIS

A series of ANOVAs was conducted on the data listed in Section 2 using the following basic models:

- (1) logCntrl as a function of Loading, Paint, Aging, Mode and all of their interactions
 - (a) Do the two different types of paint absorb differing amounts of agent?
 - (b) Is there a systemic difference between the control coupons for the dental dam and test rabbit phases of the study?
- (2) logLR as a function of Loading, Paint, Contact, Aging, Mode and all of their interactions
 - (a) Is there a difference between the test rabbits and the dental dam in the amount of agent left on the coupon after exposure? Larger amounts of residual agent on the coupon correspond to less amount of agent being transferred to the test subject.
 - (b) Is there a difference between vapor and liquid contact?
- (3) DlogLR as a function of Loading, Paint, Contact, Aging, and all of their interactions
 A repeat of paragraph (2) above, except focused on the data from the coupons on the dental dams.

- (4) RlogLR as a function of Loading, Paint, Contact, Aging, and all of their interactions
A repeat of paragraph (2) above, except focused on the data from the coupons on the rabbits.
- (5) logDM as a function of Loading, Paint, Contact, Aging, and all of their interactions
If dental dam absorbs proportional amounts of agent as test rabbits (see paragraph (2) above), then this ANOVA can be used in conjunction with (6) and (7) below to roughly correlate agent amount with degree of injury
- (6) PII as a function of Loading, Paint, Contact, Aging, and all of their interactions
- (7) Earea as a function of Loading, Paint, Contact, Aging, and all of their interactions

In order to have enough degrees of freedom for each base model, the highest order interaction (either four-way or five-way) was not included in the model in the initial analysis. After the initial attempt, statistically insignificant factors and interactions were dropped one at a time from the model (in order of higher to lower p-values) until only significant factors and interactions remained. The balanced ANOVA routine in MINITAB™ was used for all ANOVA calculations. The MINITAB™ printouts for the results of the seven ANOVAs are shown below. Also in a separate section Earea is compared graphically to PII.

D4 RESULTS

D4.1 ANOVA of logCntl as a Function of Loading, Paint, Aging, Mode and Their Interactions.

ANOVA: logCntl vs. Paint, Loading, Aging

Factor	Type	Levels	Values
Paint	fixed	2	alkyd, poly
Loading	fixed	3	0.5, 2.0, 10.0
Aging	fixed	3	0, 15, 300

Analysis of Variance for logCntl

Source	DF	SS	MS	F	P
Paint	1	0.75331	0.75331	72.90	0.000
Loading	2	7.83752	3.91876	379.23	0.000
Aging	2	3.85761	1.92880	186.66	0.000
Paint*Loading	2	0.22921	0.11460	11.09	0.001
Paint*Aging	2	0.53649	0.26825	25.96	0.000
Loading*Aging	4	0.28014	0.07004	6.78	0.002
Paint*Loading*Aging	4	0.33523	0.08381	8.11	0.001
Error	18	0.18600	0.01033		
Total	35	14.01550			

S = 0.101653 R-Sq = 98.67% R-Sq(adj) = 97.42%

Source	Variance component	Error term	Expected Mean Sq. for each Term (using restricted model)	Percent of Variance Components to Total
1 Paint	0.04128	8	(8) + 18 Q[1]	6.4
2 Loading	0.32570	8	(8) + 12 Q[2]	50.2
3 Aging	0.15987	8	(8) + 12 Q[3]	24.6
4 Paint*Loading	0.01738	8	(8) + 6 Q[4]	2.7
5 Paint*Aging	0.04299	8	(8) + 6 Q[5]	6.6
6 Loading*Aging	0.01493	8	(8) + 4 Q[6]	2.3
7 Paint*Loading*Aging	0.03674	8	(8) + 2 Q[7]	5.7
8 Error	0.01033	(8)		1.6

[DRS] Sample Calculation

Var Component for Paint = Q[1]

$Q[1] = (MS[1] - MS[Error]) / 18 = (0.75331 - 0.01033) / 18 = 0.04128$

Percent of Q[1] to Total = $(0.04128) / (0.04128 + 0.032570 + 0.15987 + 0.01738)$

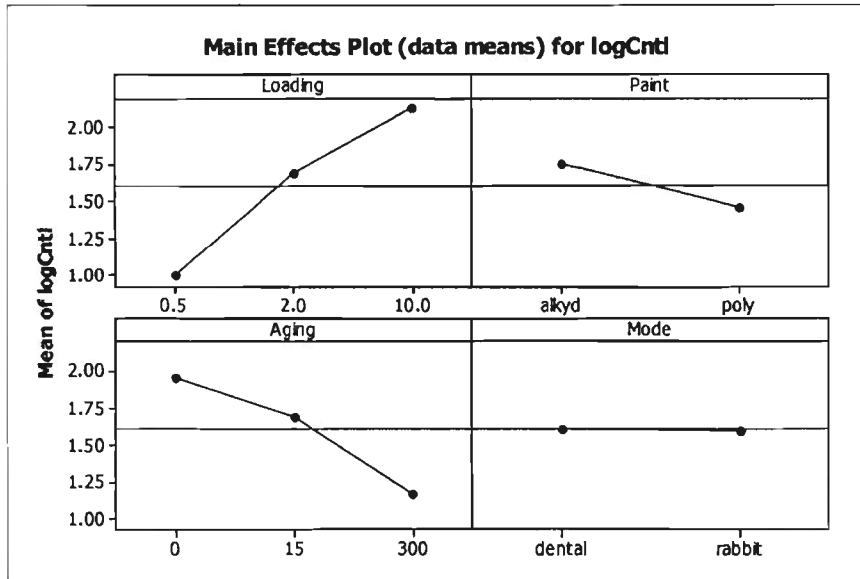


Figure D1. Main Effects Plot for LogCntl as a Function of Loading, Paint, Aging and Mode

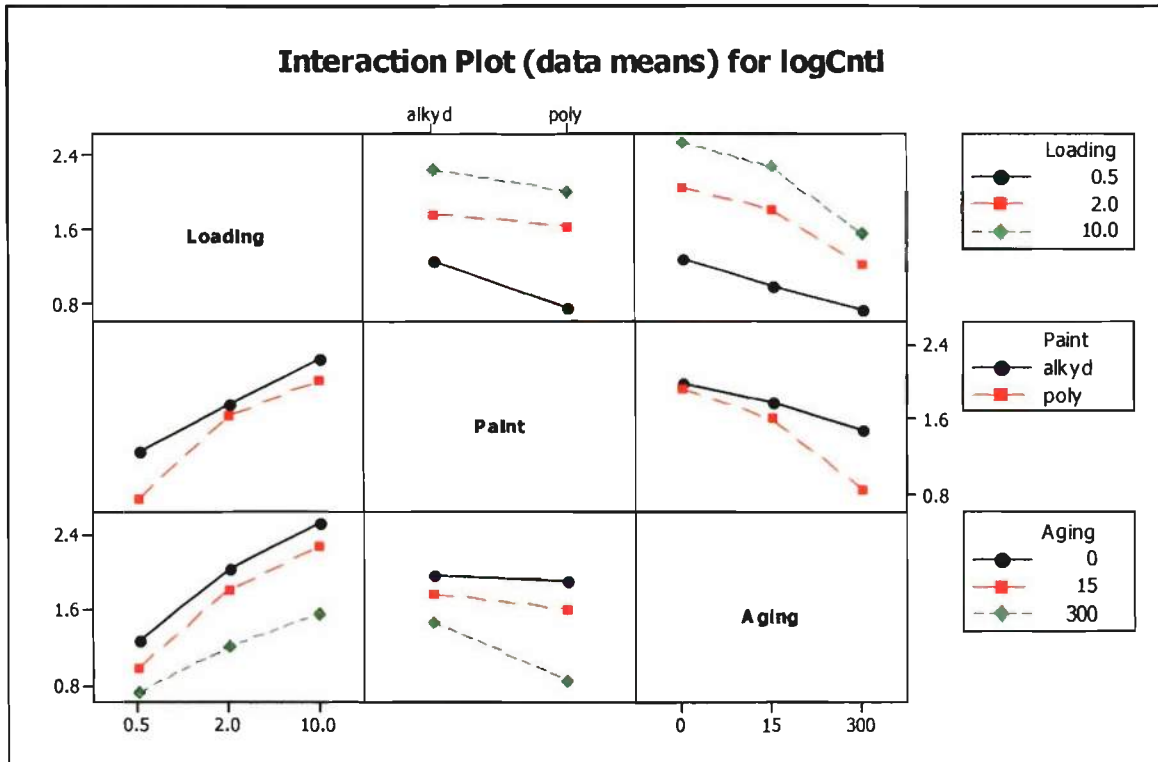


Figure D2. Interaction Plot for LogCntl as a Function of Loading, Paint, and Aging

Mode and all its interactions with the other three factors were found to be statistically insignificant. This indicates that there was no difference between the control coupons produced during the rabbit trials and the control coupons for the dental dam trials. Of the remaining three factors, all are statistically significant at the 99% significance level, as well as all of their interactions. The influence of the factors/interactions on the response rank as follows (based on the values of their variance component) in order of decreasing influence: Loading > Aging > Paint*Aging > Paint > Paint*Loading*Aging > Paint*Loading > Loading*Aging. The first two factors account for 75% of the total variance. Figures D1 and D2 illustrate the main effects and interactions for this data set.

The interaction of Paint*Aging is the most influential interaction, and examination of the plot for this interaction in Figure D2 demonstrates the reason. When Aging equals zero, there is no significant difference between alkyd and polyurethane painted surfaces (Paint is not significant at Aging equal to zero) in the amount of agent absorbed by these two paints during 30 min of contact between agent and paint. However, for longer Aging periods, less agent (with statistical significance) is recovered from the polyurethane painted coupons. This is in agreement with what Manthei *et al.* had qualitatively originally noted about the 15- and 300-min Aging durations. One can conclude from the significant interaction of Paint*Aging that mustard leaves polyurethane paint at a faster rate than from alkyd paint. Thus, there is the potential for mustard off-gassing from polyurethane paint causing more intense injury than off-gassing from alkyd paint for exposures occurring within 15 min of decontamination. This is investigated further in Sections D4.6 and D4.7.

D4.2 ANOVA of logLR as a Function of Loading, Paint, Contact, Aging, Mode and Their Interactions.

ANOVA: logLR vs. Paint, Aging, Mode

Factor	Type	Levels	Values
Paint	fixed	2	alkyd, poly
Aging	fixed	3	0, 15, 300
Mode	fixed	2	dental, rabbit

Analysis of Variance for logLR

Source	DF	SS	MS	F	P
Paint	1	4.2964	4.2964	85.18	0.000
Aging	2	3.6414	1.8207	36.10	0.000
Mode	1	0.5641	0.5641	11.18	0.001
Paint*Aging	2	2.4241	1.2121	24.03	0.000
Paint*Mode	1	0.5696	0.5696	11.29	0.001
Aging*Mode	2	0.0033	0.0016	0.03	0.968
Paint*Aging*Mode	2	0.5450	0.2725	5.40	0.007
Error	60	3.0263	0.0504		
Total	71	15.0702			

S = 0.224585 R-Sq = 79.92% R-Sq(adj) = 76.24%

Source	Variance component	Error term	Expected Mean Sq. for each Term (using restricted model)	Percent of Variance Components to Total
1 Paint	0.11801	9	(9) + 36 Q[1]	28.1
2 Aging	0.07387	9	(9) + 24 Q[2]	17.6
3 Mode	0.01434	9	(9) + 36 Q[3]	3.4
4 Paint*Aging	0.09702	9	(9) + 12 Q[4]	23.1
5 Paint*Mode	0.02898	9	(9) + 18 Q[5]	6.9
6 Aging*Mode	0	9	(9) + 12 Q[6]	0.0
7 Paint*Aging*Mode	0.03743	9	(9) + 6 Q[7]	8.9
8 Error	0.05044		(9)	12.0

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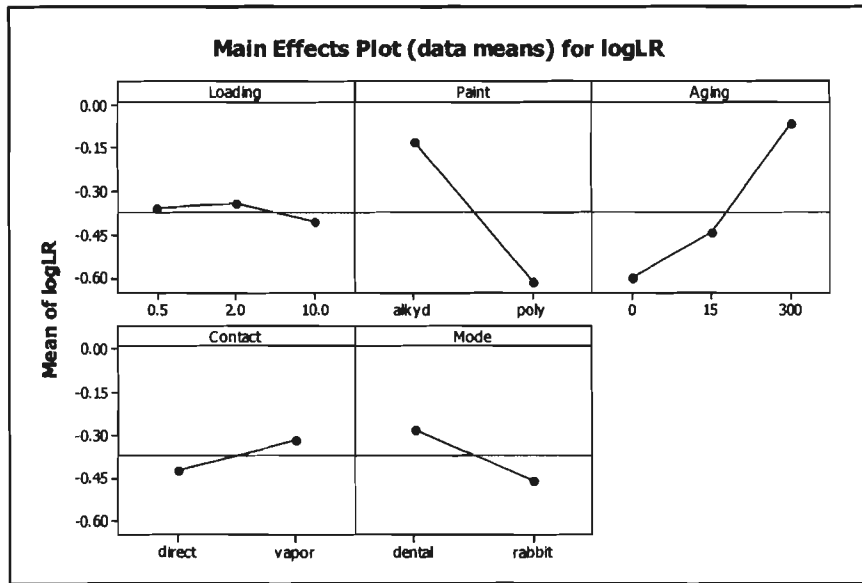


Figure D3. Main Effects Plot for LogLR as a Function of Loading, Paint, Contact, Aging, and Mode

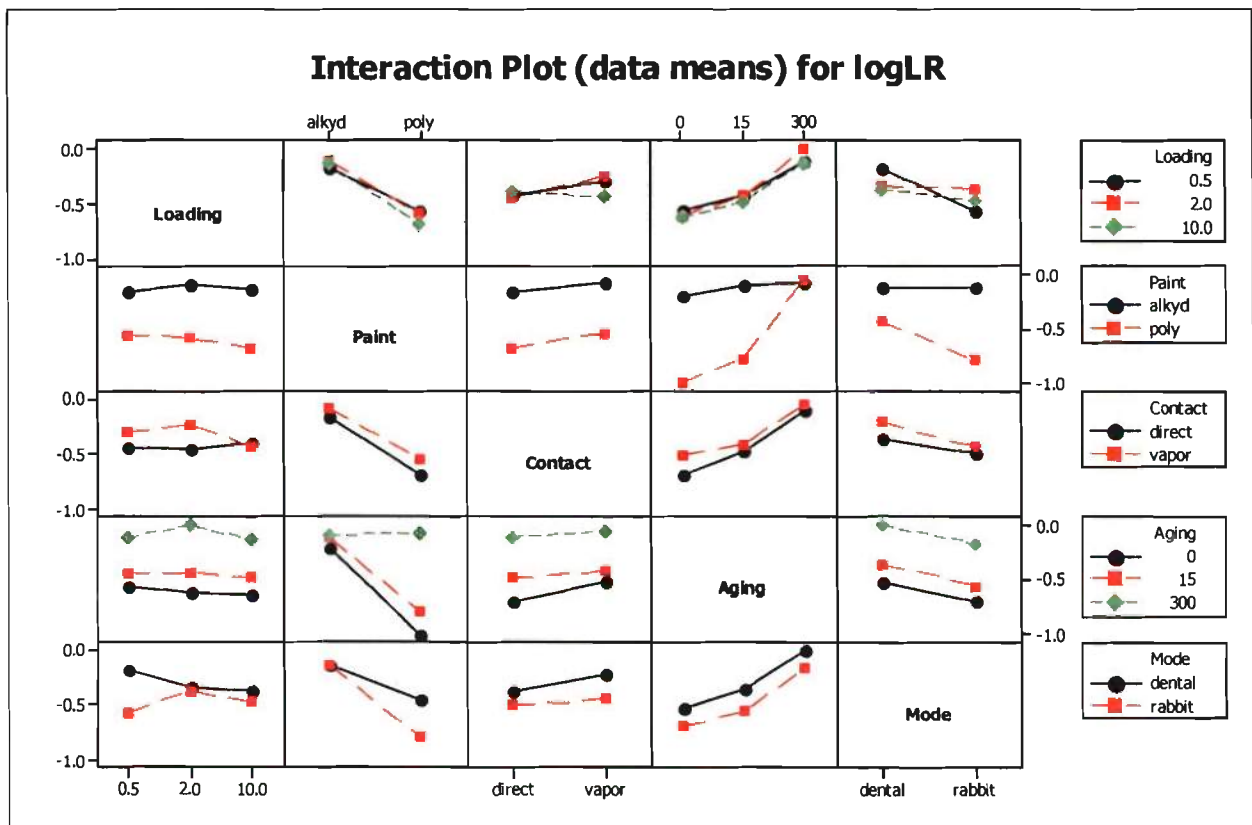


Figure D4. Interaction Plot for LogLR as a Function of Loading, Paint, Contact, Aging, and Mode

Loading and all its interactions with the other factors were found to be not statistically significant. The same was true for Contact and all its interactions as well. The p-value for the main effect for Contact equals 0.045—not quite low enough to be considered statistically significant by the standards being used in this appendix (Section D1). Of the remaining three factors, all are statistically significant at the 99% significance level, as well as all of their interactions (except for Aging*Mode). The influence of the factors/interactions on the response rank as follows (based on the values of their variance component) in order of decreasing influence: Paint > Paint*Aging > Aging > Paint*Aging*Mode > Paint *Mode > Mode. The first three factors/interactions account for 69% of the total variance. Figures D3 and D4 illustrate the main effects and interactions for this data set.

There is a statistically significant difference between the amount of agent left on coupons for the rabbit trials vs. the coupons for the dental dam trials, as indicated by the significant terms of Paint*Aging*Mode, Paint*Mode, and Mode. However, the interactions of Mode with Paint and with Paint*Aging are more influential than the main effect of Mode. This indicates that if dental dam is to be used as a simulant for rabbit skin it needs to be calibrated against the specific type of paint and aging durations that will be used. In general, more agent was recovered from the coupons in the dental dam studies (which corresponds to less agent being absorbed by the dental dam relative to the rabbit skin).

D4.3 ANOVA of DlogLR as a Function of Loading, Paint, Contact, Aging and Their Interactions.

ANOVA: DlogLR vs. Loading, Paint, Contact, Aging

Factor	Type	Levels	Values
Loading	fixed	3	0.5, 2.0, 10.0
Paint	fixed	2	alkyd, poly
Contact	fixed	2	direct, vapor
Aging	fixed	3	0, 15, 300

Analysis of Variance for DlogLR

Source	DF	SS	MS	F	P
Loading	2	0.24116	0.12058	14.45	0.000
Paint	1	0.86863	0.86863	104.11	0.000
Contact	1	0.22241	0.22241	26.66	0.000
Aging	2	1.78992	0.89496	107.27	0.000
Loading*Paint	2	0.31602	0.15801	18.94	0.000
Loading*Aging	4	0.15267	0.03817	4.57	0.013
Paint*Aging	2	0.38463	0.19231	23.05	0.000
Contact*Aging	2	0.08795	0.04397	5.27	0.018
Loading*Paint*Aging	4	0.39942	0.09986	11.97	0.000
Error	15	0.12515	0.00834		
Total	35	4.58796			

S = 0.0913423 R-Sq = 97.27% R-Sq(adj) = 93.64%

Source	Variance component	Error term	Expected Mean Sq. for each Term (using restricted model)	Percent of Variance Components to Total
1 Loading	0.00935	10	(10) + 12 Q[1]	3.5
2 Paint	0.04779	10	(10) + 18 Q[2]	18.0
3 Contact	0.01189	10	(10) + 18 Q[3]	4.5
4 Aging	0.07389	10	(10) + 12 Q[4]	27.8
5 Loading*Paint	0.02495	10	(10) + 6 Q[5]	9.4
6 Loading*Aging	0.00746	10	(10) + 4 Q[6]	2.8
7 Paint*Aging	0.03066	10	(10) + 6 Q[7]	11.5
8 Contact*Aging	0.00594	10	(10) + 6 Q[8]	2.2
9 Loading*Paint*Aging	0.04576	10	(10) + 2 Q[9]	17.2
10 Error	0.00834	(10)		3.1

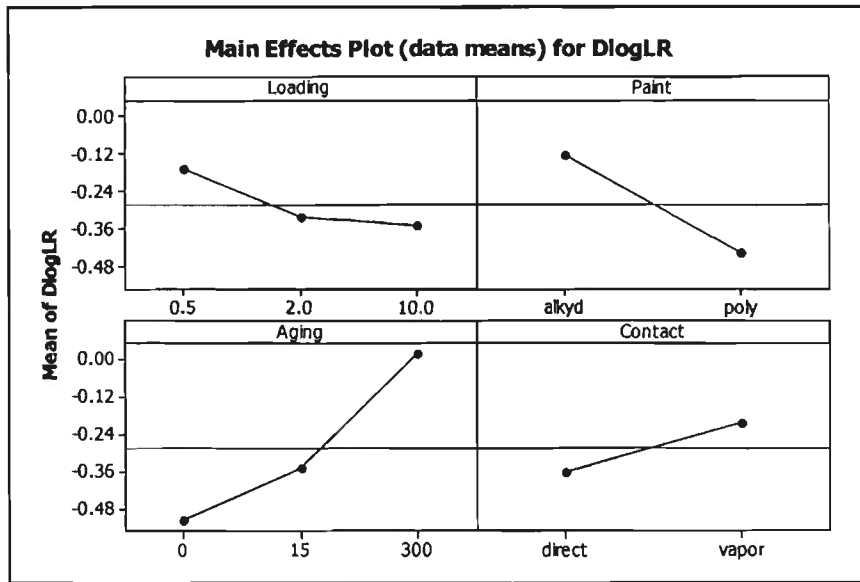


Figure D5. Main Effects Plot for DLogLR as a Function of Loading, Paint, Contact, and Aging

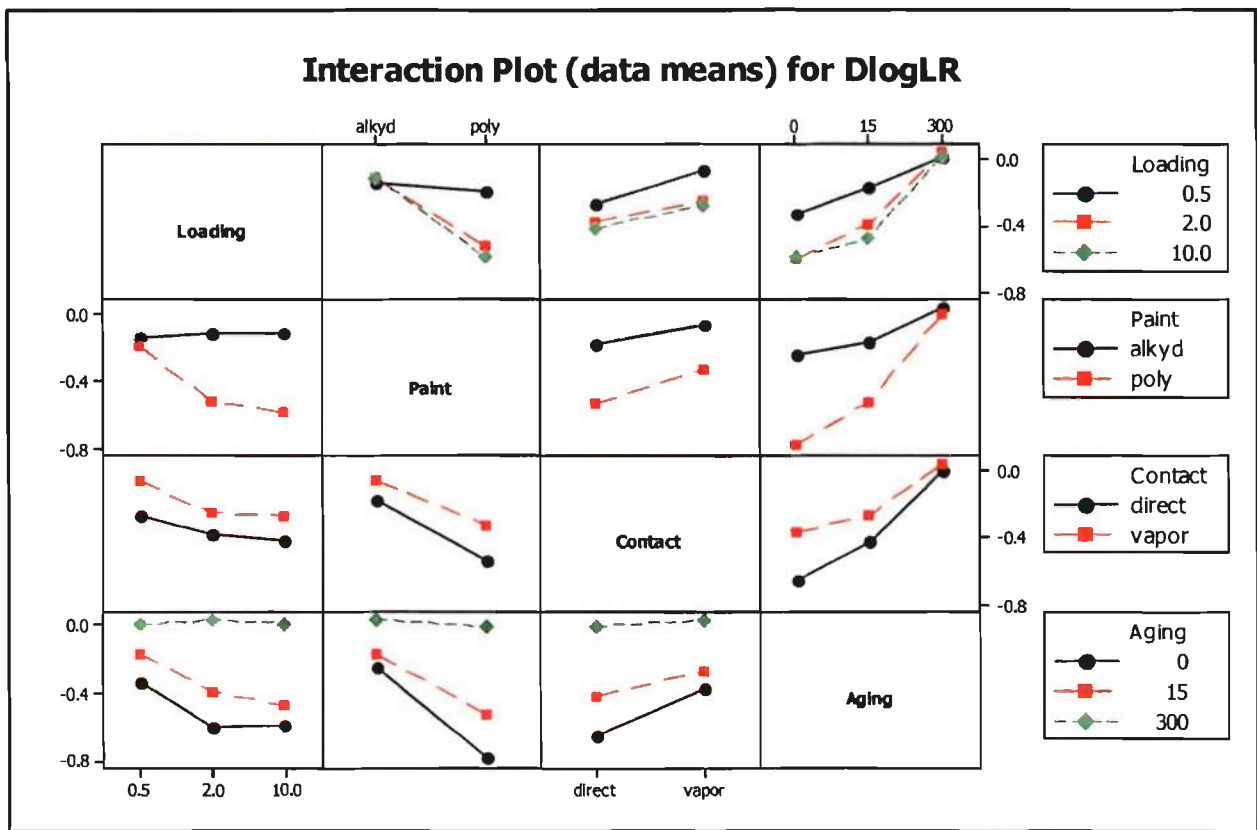


Figure D6. Interaction Plot for DLogLR as a Function of Loading, Paint, Contact, and Aging

All of the main effects—four of the six possible two-way interactions and one three-way interaction, were found to be statistically significant. The influence of the factors/interactions on the response rank as follows (based on the values of their variance component) in order of decreasing influence: Aging > Paint > Loading*Paint*Aging > Paint*Aging > Loading*Paint > Contact > Loading > Loading*Aging > Contact*Aging. The first four factors/interactions account for 75% of the total variance. Figures D5 and D6 illustrate the main effects and interactions for this data set.

As was found with the previous ANOVA for logLR, Aging, Paint and Aging*Paint are important terms in modeling the amount of agent recovered from test coupons after contact with a test surface (either dental dam or rabbit skin). The comparison between the ANOVAs for DlogLR and RlogLR are discussed in the next section.

D4.4 ANOVA of RlogLR as a Function of Loading, Paint, Contact, Aging and Their Interactions.

ANOVA: RlogLR vs. Loading, Paint, Contact, Aging

Factor	Type	Levels	Values
Loading	fixed	3	0.5, 2.0, 10.0
Paint	fixed	2	alkyd, poly
Contact	fixed	2	direct, vapor
Aging	fixed	3	0, 15, 300

Analysis of Variance for RlogLR

Source	DF	SS	MS	F	P
Loading	2	0.22223	0.11111	3.48	0.047
Paint	1	3.99737	3.99737	125.07	0.000
Contact	1	0.02595	0.02595	0.81	0.376
Aging	2	1.85477	0.92738	29.02	0.000
Loading*Contact	2	0.43431	0.21715	6.79	0.004
Paint*Aging	2	2.58453	1.29227	40.43	0.000
Error	25	0.79904	0.03196		
Total	35	9.91820			

S = 0.178778 R-Sq = 91.94% R-Sq(adj) = 88.72%

Source	Variance component	Error term	Expected Mean Sq. for each Term (using restricted model)	Percent of Variance Components to Total
1 Loading	0.00660	7	(7) + 12 Q[1]	1.2
2 Paint	0.22030	7	(7) + 18 Q[2]	38.4
3 Contact	0	7	(7) + 18 Q[3]	0.0
4 Aging	0.07462	7	(7) + 12 Q[4]	13.0
5 Loading*Contact	0.03087	7	(7) + 6 Q[5]	5.4
6 Paint*Aging	0.21005	7	(7) + 6 Q[6]	36.6
7 Error	0.03196		(7)	5.6

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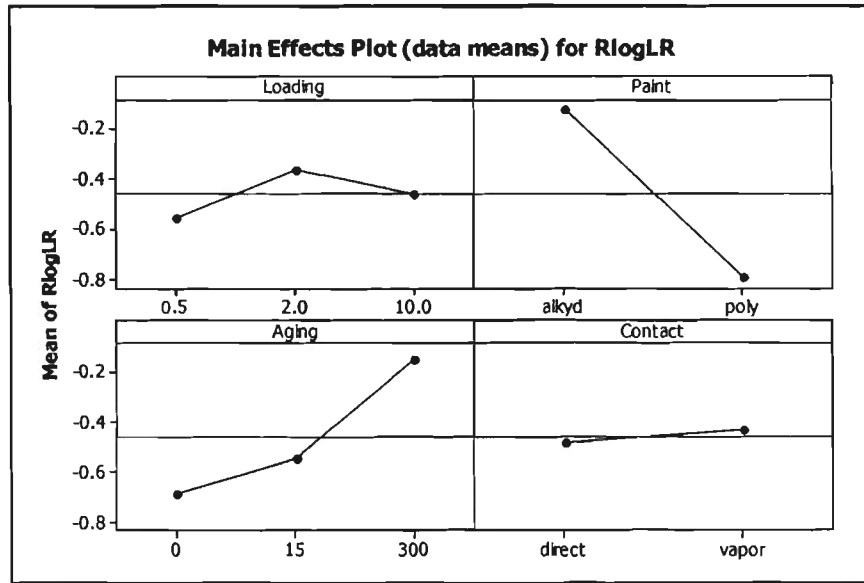


Figure D7. Main Effects Plot for RLogLR as a Function of Loading, Paint, Contact, and Aging

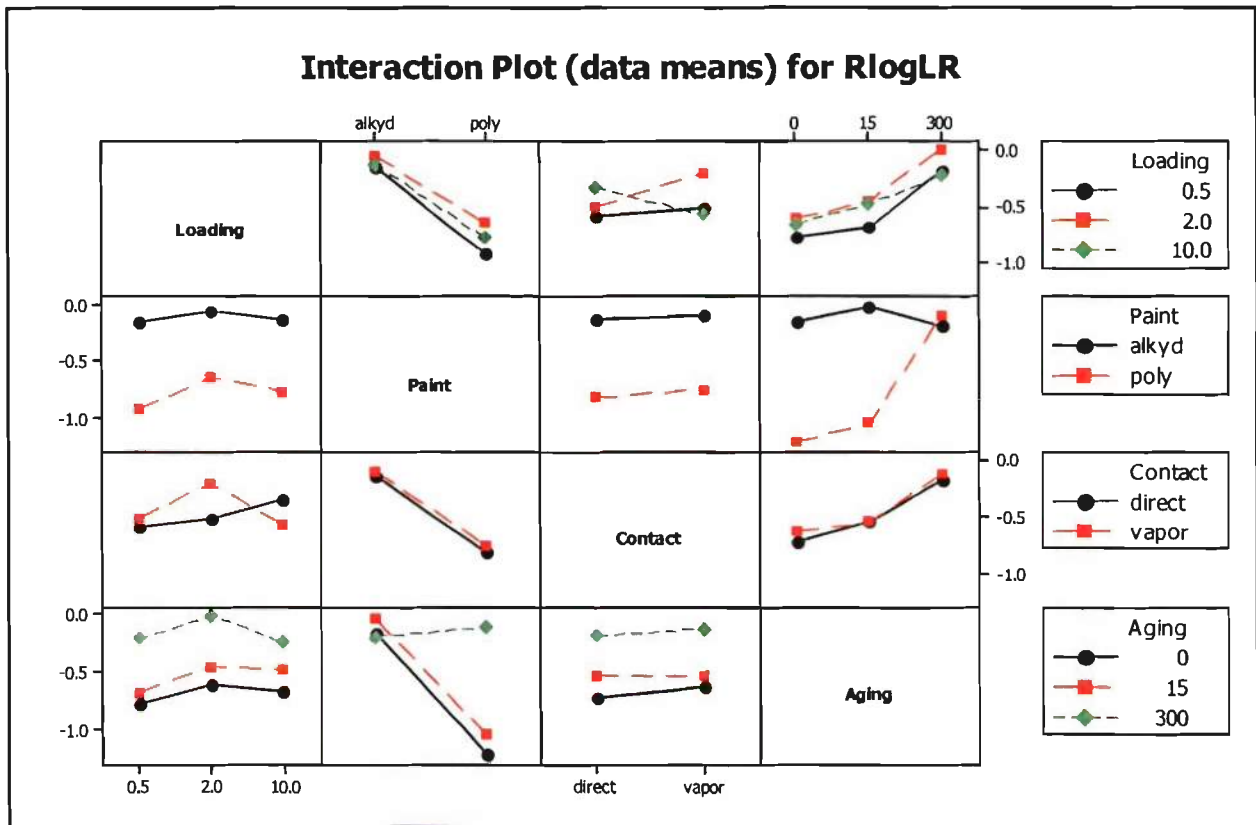


Figure D8. Interaction Plot for RLogLR as a Function of Loading, Paint, Contact, and Aging

Three of the four main effects (Loading, Paint and Aging) and only two of the six possible two-way interactions were found to be statistically significant. The influence of the factors/interactions on the response rank as follows (based on the values of their variance component) in order of decreasing influence: Paint > Paint*Aging > Aging > Loading*Contact > Loading. The first two factors/interactions account for 75% of the total variance, and the first three account for 88%. Figures D7 and D8 illustrate the main effects and interactions for this data set.

As was found with the previous ANOVAs for logLR (Section D4.2) and DlogLR (Section D4.3), Aging, Paint and Aging*Paint are important terms in modeling the amount of agent recovered from test coupons after contact with a test surface (either dental dam or rabbit skin). However, there are some important differences among the three ANOVAs. First, the overall standard deviation was greater in RlogLR ($S = 0.1788$) vs. than in DlogLR ($S = .00913$). The difference is statistically significant (F value = $(0.1787/0.0913)^2$ or 3.83, with (25 numerator, 15 denominator) degrees of freedom, for a p -value of 0.005). This could be due to more variability in the rabbits than in the dental dam with respect to absorption of agent vapor from the test coupons. Second, Loading*Paint*Aging was the third most influential term for DlogLR, but it was not even statistically significant for RlogLR. Third, Contact was significant for DlogLR, but not for RlogLR. The comparison of the ANOVAs for DlogLR and RlogLR reinforces the findings of the ANOVA for logLR—dental dam can be used as simulant for rabbit skin, but it needs to be calibrated under the same conditions expected with the rabbit skin.

D4.5 ANOVA of logDM as a Function of Loading, Paint, Contact, Aging and Their Interactions.

ANOVA: logDM vs. Loading, Paint, Contact, Aging

Factor	Type	Levels	Values
Loading	fixed	3	0.5, 2.0, 10.0
Paint	fixed	2	alkyd, poly
Aging	fixed	3	0, 15, 300

Analysis of Variance for logDM

Source	DF	SS	MS	F	P
Loading	2	4.1978	2.0989	64.90	0.000
Paint	1	0.0099	0.0099	0.31	0.585
Aging	2	7.6572	3.8286	118.39	0.000
Loading*Paint	2	0.3364	0.1682	5.20	0.014
Loading*Aging	4	0.8513	0.2128	6.58	0.001
Paint*Aging	2	0.7446	0.3723	11.51	0.000
Error	22	0.7115	0.0323		
Total	35	14.5087			

$S = 0.179830$ $R-Sq = 95.10\%$ $R-Sq(adj) = 92.20\%$

Source	Variance component	Error term	Expected Mean Sq. for each Term (using restricted model)	Percent of Variance Components to Total
1 Loading	0.17221	7	(7) + 12 Q[1]	26.7
2 Paint	0	7	(7) + 18 Q[2]	0.0
3 Aging	0.31636	7	(7) + 12 Q[3]	49.0
4 Loading*Paint	0.02264	7	(7) + 6 Q[4]	3.5
5 Loading*Aging	0.04512	7	(7) + 4 Q[5]	7.0
6 Paint*Aging	0.05666	7	(7) + 6 Q[6]	8.8
7 Error	0.03234	(7)	(7)	5.0

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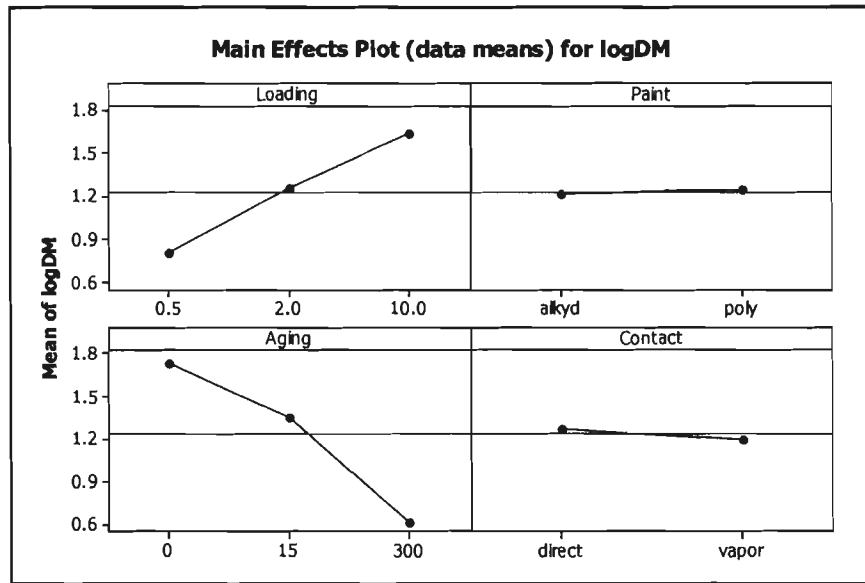


Figure D9. Main Effects Plot for logDM as a Function of Loading, Paint, Contact, and Aging

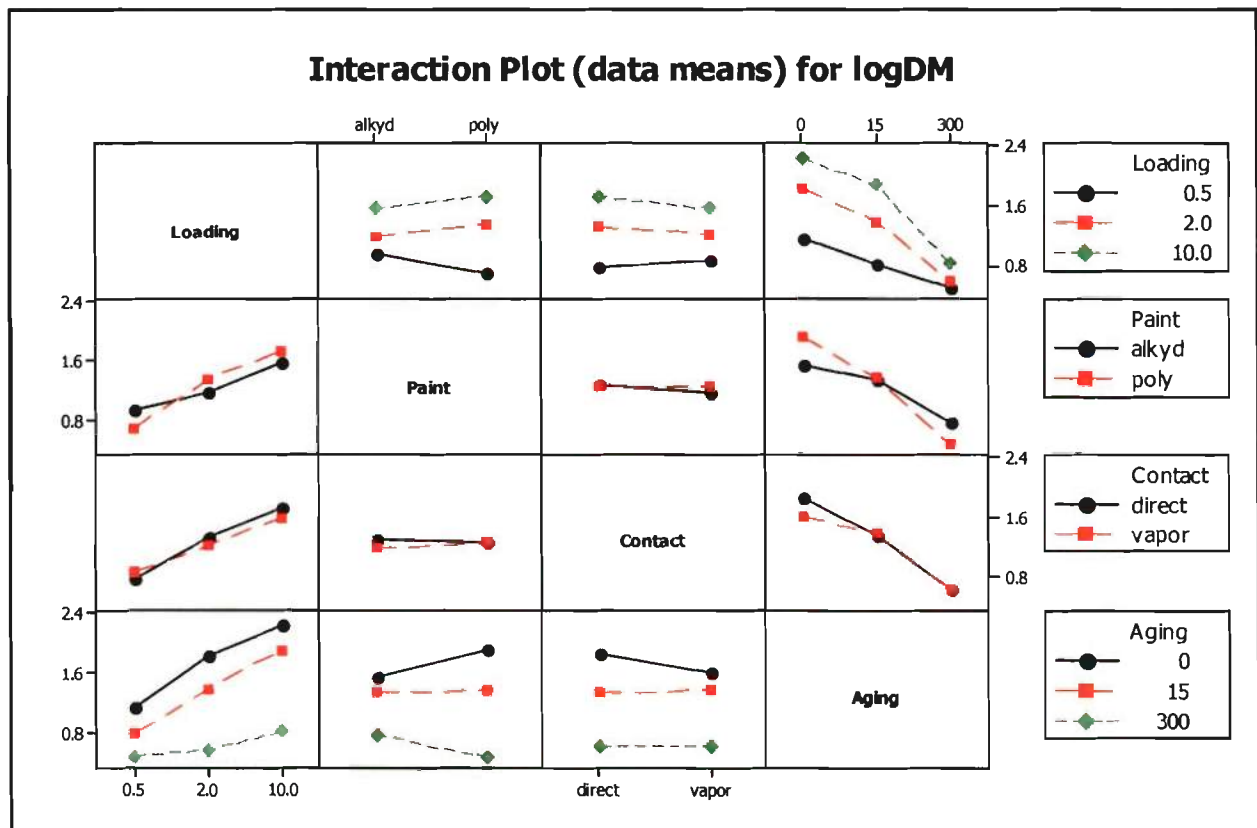


Figure D10. Interaction Plot for logDM as a Function of Loading, Paint, Contact, and Aging

Neither Contact nor any of its interactions was found to be statistically significant with respect to the amount of agent absorbed by the dental dam (logDM). Only the main effects of Loading and Aging are statistically significant, along with three two-way interactions. The influence of the factors/interactions on the response rank as follows (based on the values of their variance component) in order of decreasing influence: Aging > Loading > Paint*Aging > Loading*Aging > Loading*Paint. The first two factors/interactions account for 75% of the total variance, and the first three account for 85%. Figures D9 and D10 illustrate the main effects and interactions for this data set.

The results for this ANOVA are similar to that for DlogLR (Section D4.3), except that Loading is more important for logDM than it was for DlogLR. Also, Paint, by itself, is not statistically significant for logDM (only interactions of Paint with Aging and Loading). For DlogLR, Paint, by itself, was statistically significant and influential. A better comparison between DlogR and logDM would have been possible had the solvent rinse of the test coupons (after the coupons had been allowed to age after the initial contamination) been analyzed for the amount of HD present. The amount of agent recovered from the initial solvent rinse--coupled with the three other amounts that were measured (amount of agent initially deposited on coupon {Loading}, amount of agent extracted from dental dam post-exposure {logDM}, and amount of agent extracted from coupons post-exposure {DlogR}), would permit the calculation of an agent mass balance. This could then be used as a check on the accuracy of logDM and DlogR.

D4.6 ANOVA of PII as a Function of Loading, Paint, Contact, Aging and Their Interactions.

ANOVA: PII vs. Loading, Paint, Contact, Aging

Factor	Type	Levels	Values
Loading	fixed	3	0.5, 2.0, 10.0
Paint	fixed	2	alkyd, poly
Contact	fixed	2	direct, vapor
Aging	fixed	3	0, 15, 300

Analysis of Variance for PII

Source	DF	SS	MS	F	P
Loading	2	49.185	24.593	13.32	0.000
Paint	1	0.012	0.012	0.01	0.936
Contact	1	161.290	161.290	87.35	0.000
Aging	2	80.850	40.425	21.89	0.000
Paint*Contact	1	24.272	24.272	13.14	0.001
Paint*Aging	2	21.730	10.865	5.88	0.008
Error	26	48.011	1.847		
Total	35	385.351			

s = 1.35888 R-Sq = 87.54% R-Sq(adj) = 83.23%

Source	Variance component	Error term	Expected Mean Sq. for each Term (using restricted model)	Percent of Variance Components to Total
1 Loading	1.89550	7	(7) + 12 Q[1]	9.6
2 Paint	0	7	(7) + 18 Q[2]	0.0
3 Contact	8.85794	7	(7) + 18 Q[3]	44.8
4 Aging	3.21483	7	(7) + 12 Q[4]	16.2
5 Paint*Contact	2.49167	7	(7) + 9 Q[5]	12.6
6 Paint*Aging	1.50300	7	(7) + 6 Q[6]	7.6
7 Error	1.847		(7)	9.3

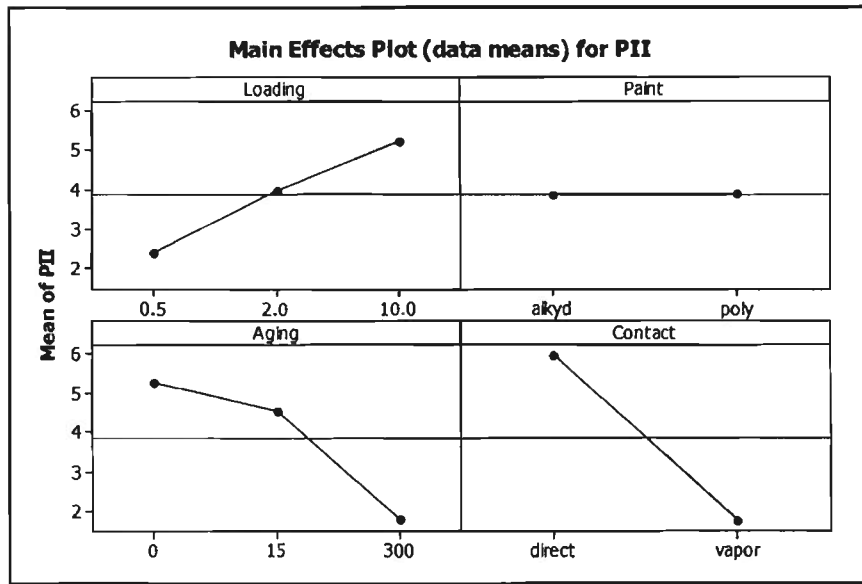


Figure D11. Main Effects Plot for PII as a Function of Loading, Paint, Contact, and Aging

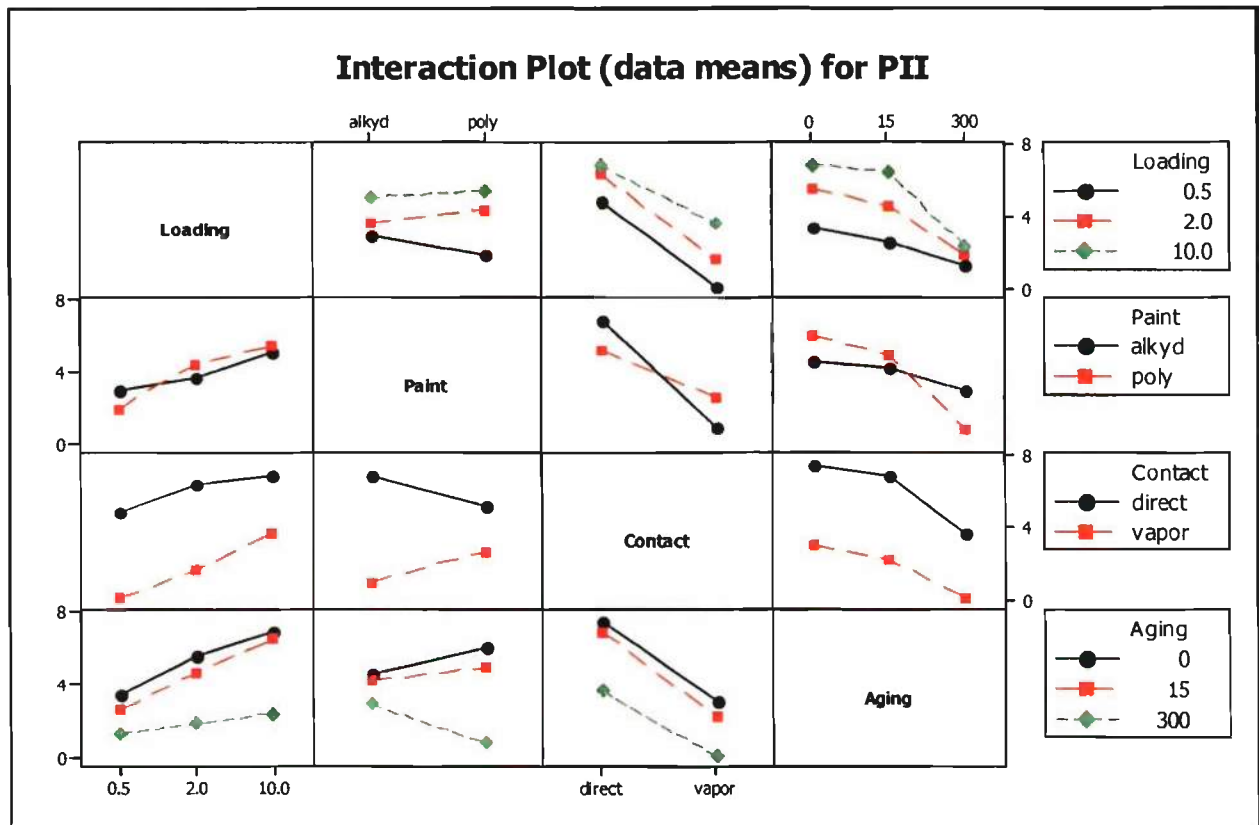


Figure D12. Interaction Plot for PII as a Function of Loading, Paint, Contact, and Aging

Only the main effects of Loading, Contact and Aging are statistically significant, along with two two-way interactions (both of which involve Paint). The influence of the factors/interactions on the response rank as follows (based on the values of their variance component) in order of decreasing influence: Contact > Aging > Paint*Contact > Loading > Paint*Aging. The first three factors/interactions account for 73% of the total variance. Figures D11 and D12 illustrate the main effects and interactions for this data set.

Direct contact produces more severe injuries than vapor contact, as represented by the term, Contact. Contact has the largest influence on the response, PII, among all the significant factors/interactions. A distance second and third are Aging and Paint*Contact. The Paint*Contact interaction manifests itself as follows. Direct contact with alkyd paint coupons produced larger PII values (and more injury severity) than direct contact with polyurethane paint coupons. However, the opposite is true for vapor contact—polyurethane paint coupons produces higher PII values than alkyd paint coupons. Another significant interaction, Paint*Aging, finds that the PII for contact with alkyd paint coupons stays relatively constant with respect to aging duration (Aging), but for polyurethane paint coupons, PII values are high for short aging duration, then rapidly decrease as the aging duration increases.

D4.7 ANOVA of Earea as a Function of Loading, Paint, Contact, Aging and Their Interactions.

ANOVA: Earea vs. Loading, Paint, Aging

Factor	Type	Levels	Values
Loading	fixed	3	0.5, 2.0, 10.0
Paint	fixed	2	alkyd, poly
Aging	fixed	3	0, 15, 300

Analysis of Variance for Earea

Source	DF	SS	MS	F	P
Loading	2	9.9076	4.9538	57.94	0.000
Paint	1	5.3130	5.3130	62.14	0.000
Aging	2	8.2447	4.1223	48.21	0.000
Loading*Paint	2	2.5896	1.2948	15.14	0.000
Loading*Aging	4	4.3606	1.0902	12.75	0.000
Paint*Aging	2	2.8838	1.4419	16.86	0.000
Loading*Paint*Aging	4	1.5086	0.3772	4.41	0.012
Error	18	1.5391	0.0855		
Total	35	36.3470			

S = 0.292409 R-Sq = 95.77% R-Sq(adj) = 91.77%

Source	Variance component	Error term	Expected Mean Sq. for each Term (using restricted model)	Percent of Variance Components to Total
1 Loading	0.40569	8	(8) + 12 Q[1]	20.9
2 Paint	0.29042	8	(8) + 18 Q[2]	15.0
3 Aging	0.33640	8	(8) + 12 Q[3]	17.3
4 Loading*Paint	0.20155	8	(8) + 6 Q[4]	10.4
5 Loading*Aging	0.25118	8	(8) + 4 Q[5]	12.9
6 Paint*Aging	0.22607	8	(8) + 6 Q[6]	11.6
7 Loading*Paint*Aging	0.14585	8	(8) + 2 Q[7]	7.5
8 Error	0.08550	(8)		4.4

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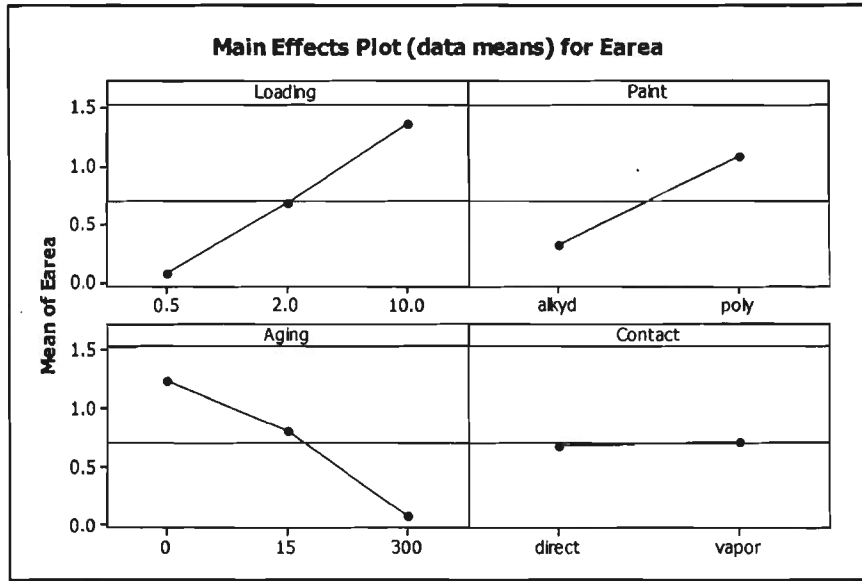


Figure D13. Main Effects Plot for Earea as a Function of Loading, Paint, Contact, and Aging

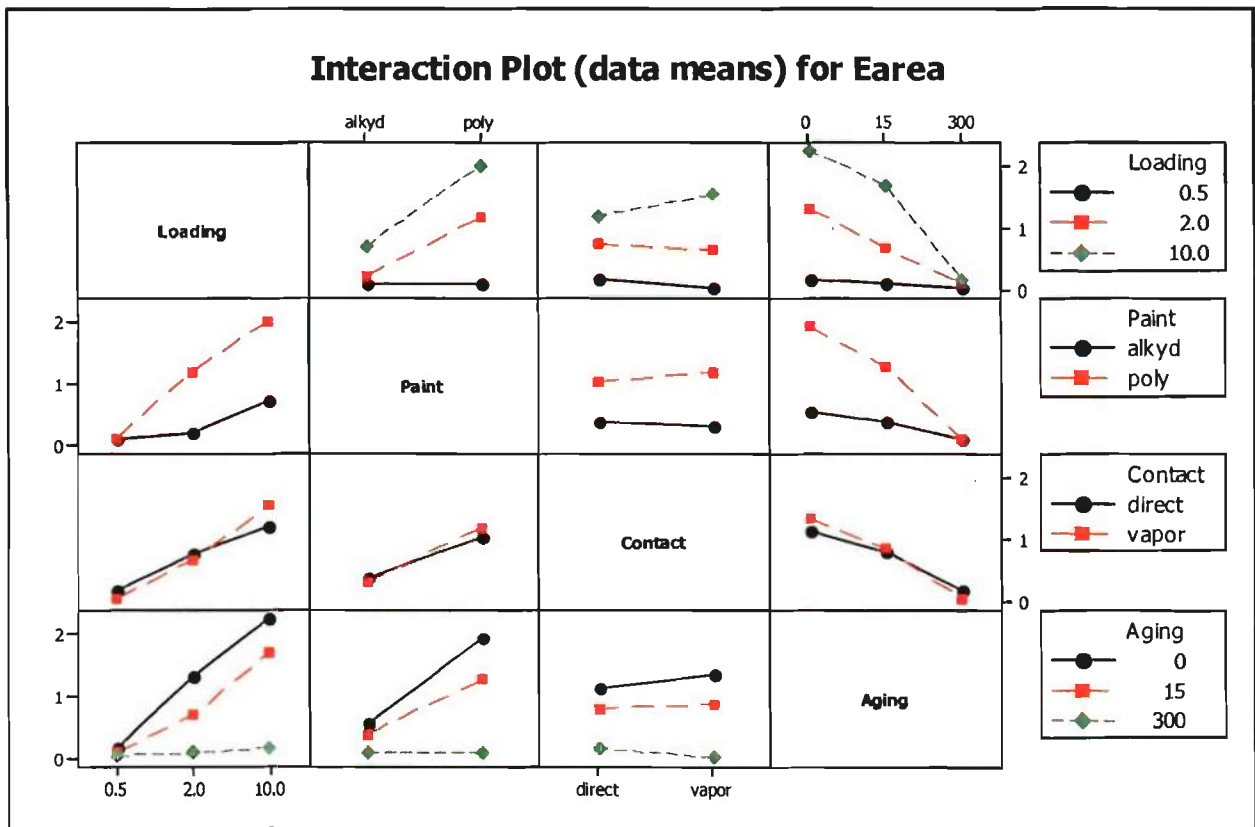


Figure D14. Interaction Plot for Earea as a Function of Loading, Paint, Contact, and Aging

Contact and all of its interactions are not statistically significant. The other three factors, Loading, Paint and Aging are statistically significant, along with all of their two-way interactions and the one three-way interaction. The influence of the factors/interactions on the response rank as follows (based on the values of their variance component) in order of decreasing influence: Loading > Aging > Paint > Loading*Aging > Paint*Aging > Loading*Paint > Loading*Paint*Aging. The first five factors/interactions account for 78% of the total variance. Figures D13 and D14 illustrate the main effects and interactions for this data set.

Unlike the severity of injury on rabbit skin (as represented by PII), the size of the injured area is not heavily dependent on just one factor (Contact in the case of the AVOVA for PII). Instead, most of the factors/interactions (excluding Contact and any of its interactions) have relatively the same influence on the response, Earea. So, the severity of the injury depends on Contact, while the size of the injury depends on everything other than Contact. Another difference between the ANOVAs for PII and Earea is the nature of the interaction of Paint*Aging. For PII, the response is roughly equal between alkyd and polyurethane paint coupons for Aging equal to 0 and 15 min. Then, at 300 min, alkyd produces a more severe injury than polyurethane. For Earea, the response is roughly equal between alkyd and polyurethane paint coupons for Aging equal to 300 min. At the two shorter durations (0 and 15 min), polyurethane produces a larger injured area than alkyd paint.

D4.8 Comparison of Earea and PII.

Before reaching any final conclusions on how Earea and PII vary as a function of Loading, Aging, Contact, Paint and their interactions, the relationship between Earea and PII was investigated. It was found that there is a difference in how Earea varies with PII due to the factor, Contact (Figure D15).

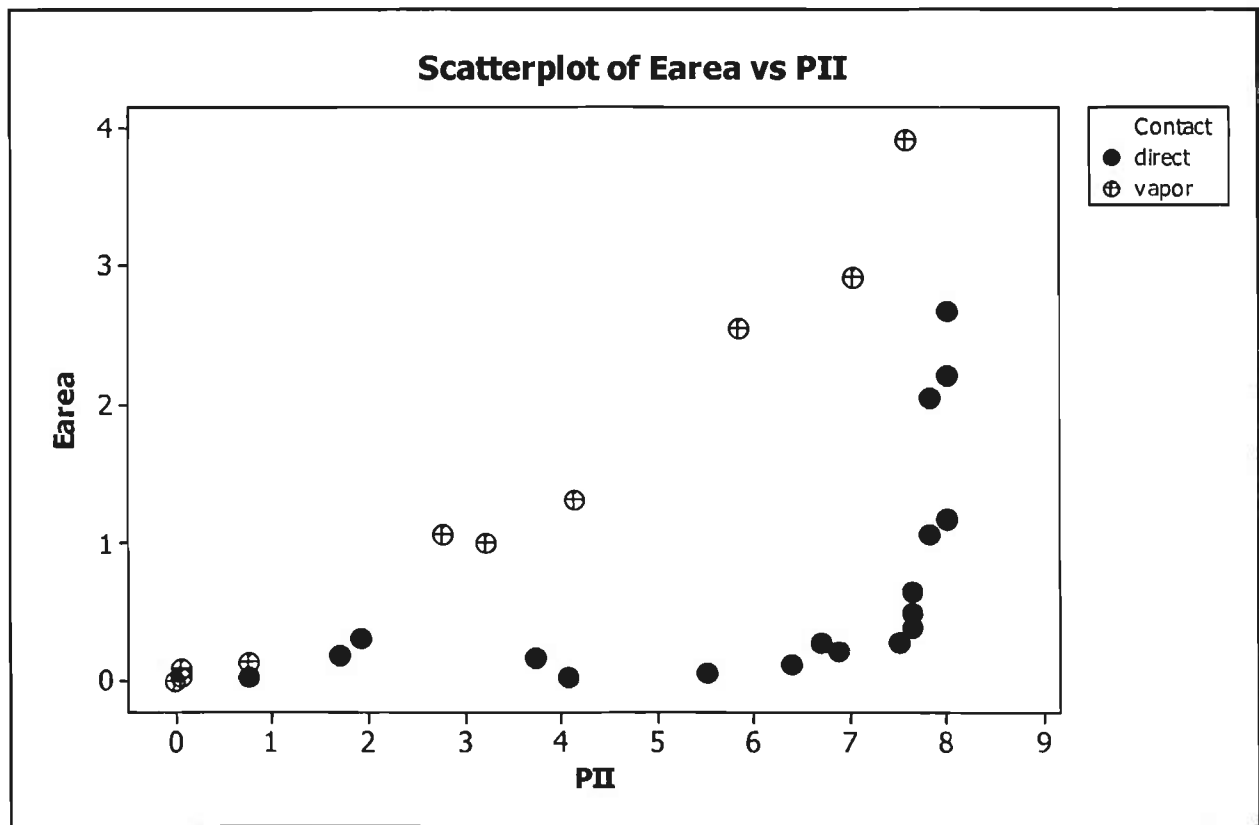


Figure D15. Area of HD-Induced Damage (Earea) vs. Intensity of Damage (PII) from Manthei *et al.* (1988)

For vapor contact, the relationship between Earea and PII is more linear than that for direct contact. Also, for some set value of intensity (PII), the size of the damage area (Earea) will be larger for vapor contact than for direct contact. However, to arrive at the same degree of damage intensity, vapor contact requires more agent than direct contact. Thus, a pair-wise comparison was performed in which Earea for direct contact at every Loading, Aging and Paint test coordinate was plotted vs. its exact counterpart Earea for vapor contact (Figure D16).

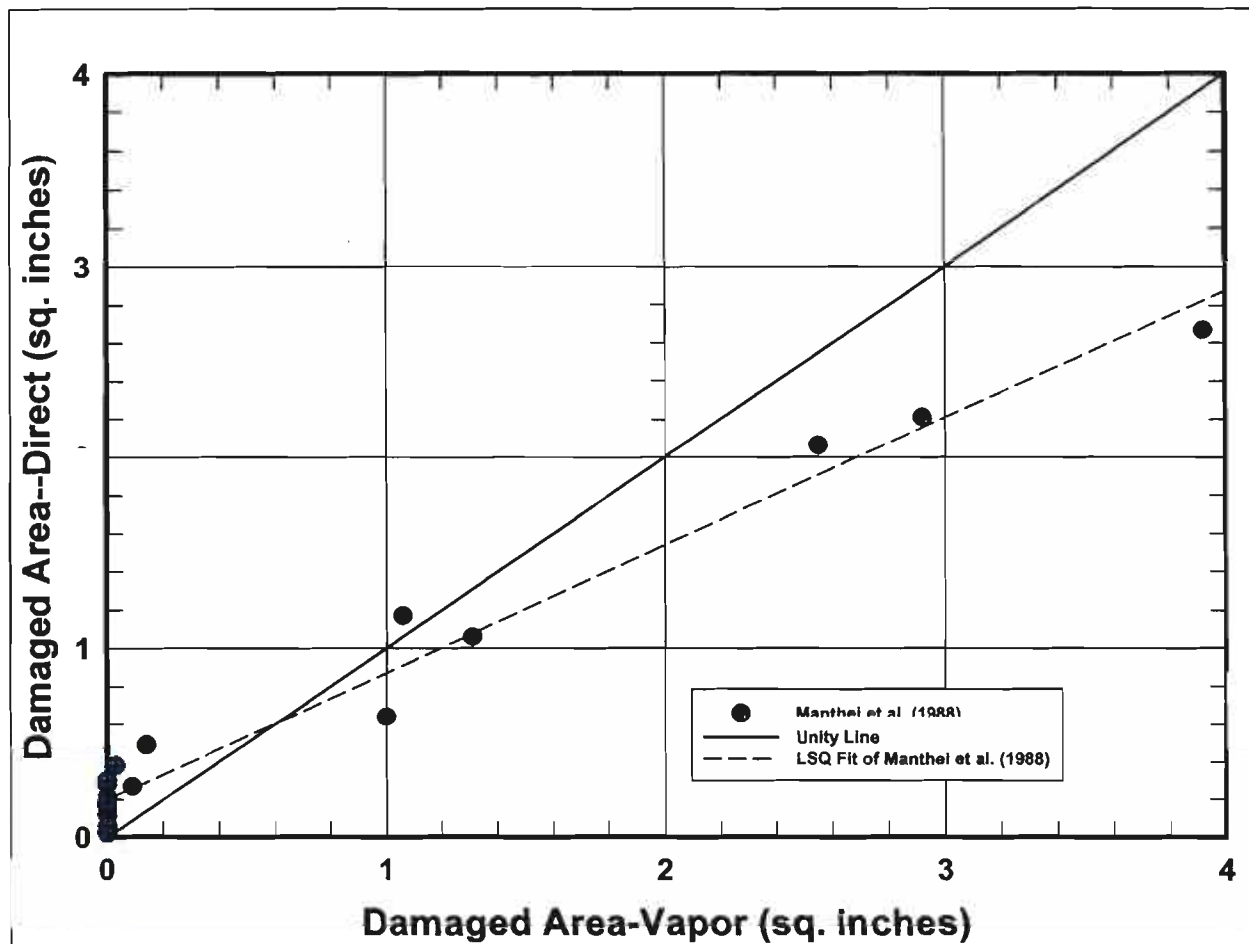


Figure D16. Area of HD-Induced Damage for Direct Contact vs. Vapor Contact for Equal Exposure Conditions from Manthei et al. (1988)

For smaller damaged areas (below about 0.5 to 1 in.²), direct contact produces larger damaged areas than vapor contact. For the larger areas, vapor contact produces larger damaged areas than direct contact. This trend (see least square fit of data in Figure 16) suggests an interaction between the type of contact and size of damaged area. However, from the viewpoint of the ANOVA (Section D4.7), only 5 out of 18 direct-vapor pairs are located below the unity line (or Earea(vapor) > Earea(direct)). Based on a two-tailed paired t-test, the p value for this outcome only equals 0.096; thus, the null hypothesis (no difference between the two types of contact) cannot be rejected (based on the 95% confidence criteria). This could explain why the ANOVA failed to find that Contact was a statistically significant factor. Had larger agent loading values been investigated, then based on the observed trend, more area pairs with Earea(vapor) > Earea(direct) would probably had been observed, thereby possibly changing the outcome of the ANOVA.

D5 CONCLUSIONS

The following is a comparison on a per subject basis of the original findings of Manthei *et al.* (1988) and the current review of this dataset.

D5.1 Agent Transfer—Control Coupons for Dental Dam and Rabbit Skin Trials.

D5.1.1 Effect of Type of Paint—Initial Agent Absorption.

Manthei *et al.* (1988): Limited observations were made on the data from the aging duration (period between coupon decontamination and extraction of agent from coupon) equal to zero. It was found that polyurethane control coupons retained more agent than alkyd control coupons for the initial agent loading values of 2 and 10 mg. The reverse was true for the initial loading of 0.5 mg.

Sommerville (2005): An ANOVA was performed on just the control coupon data taken with an aging duration of zero (not included in this report). It was found that the type of paint was an insignificant factor on the amount of agent initially absorbed, but the interaction of the type of paint with the initial amount of agent loading on the coupon was statistically significant. This partially supports the observation of Manthei *et al.*, with the exception that the only statistically significant difference between alkyd and polyurethane paint occurs at the initial agent loading of 0.5 mg. Alkyd paint is able to absorb more agent at this loading. For the other two loading values there is no significant difference between the paints. The only other significant factor/interaction was the much more influential factor: the amount of initial agent loading.

D5.1.2 Effect of Type of Paint and Initial Agent Loading—Overall.

Manthei *et al.* (1988): No overall observations were made.

Sommerville (2005): Agent desorbed more readily from polyurethane paint control coupons than from alkyd paint control coupons, particularly as the aging duration was increased. The difference between the two different types of paint was not statistically significant at an aging duration of zero. However, the effect of the type of paint was significantly less than the effect that aging duration and initial aging loading had on agent retention.

D5.1.3 Effect of Study Phase (Controls for Dental Dam vs. Rabbit Skin).

Manthei *et al.* (1988): No overall observations were made.

Sommerville (2005): An ANOVA of the control coupon data found that there was no statistically significant difference in the amount of agent recovered from the control coupons from the dental dam and rabbit skin studies.

D5.2 Agent Transfer—Agent Recovery from Test Coupons and Dental Dam for Dental Dam Trials.

D5.2.1 Effect of Type of Paint.

Manthei *et al.* (1988): For all three aging durations investigated (0, 15, and 300 min), alkyd paint retained more agent than the polyurethane paint.

Sommerville (2005): Based on an ANOVA for the whole test coupon dataset, alkyd paint retained more agent than the polyurethane paint, in general. However, the ANOVA of the agent recovery data from the dental dam found that the main effect of type of paint was not statistically significant. Instead, only the interactions paint with the aging duration and amount of initial agent loading on the coupon were statistically significant. There is a discrepancy between the two types of agent recovery data (test coupon and dental dam) in their dependence on the type of paint.

D5.2.2 Effect of the Interaction of the Type of Paint and the Aging Duration.

Manthei *et al.* (1988): For all three aging durations investigated (0, 15, and 300 min), alkyd paint retained more agent than the polyurethane paint.

Sommerville (2005): Manthei *et al.* are incorrect about the lack of interaction between the aging duration and the type of paint. A statistically significant interaction was discovered *via* ANOVA of the agent recovery data from test coupons. At an aging duration of 300 min, there is no statistically significant difference between the amount of agent recovered from alkyd and polyurethane paint.

D5.2.3 Effect of the Interaction of the Type of Paint and Type of Contact.

Manthei *et al.* (1988): Direct contact with alkyd paint transfers more agent than vapor contact (based on the amount of agent recovered from the dental dam). The reverse is true for polyurethane paint.

Sommerville (2005): ANOVAs on the test coupon and dental dam agent recovery data did not confirm the comments of Manthei *et al.* regarding the interaction between type of paint and type of contact.

D5.2.4 Effect of Type of Contact.

Manthei *et al.* (1988): Direct contact transfers more agent from test coupon to dental dam than does vapor contact.

Sommerville (2005): The above statement is only confirmed *via* an ANOVA of the agent retention data from the test coupons. The ANOVA of the agent recovery data from the dental dam did not find the type of contact to be statistically significant. There is a discrepancy between the two types of agent recovery data (test coupon and dental dam) in their dependence on the type of contact.

D5.2.5 Effect of Aging Duration Between Decontamination of Coupon and Contact with Skin.

Manthei *et al.* (1988): After an aging duration of 15 min a considerable reduction of agent retained by the test coupons compared to an aging duration of zero was observed.

Sommerville (2005): The above observation was confirmed by ANOVA of the test coupon data. However, there are also statistically significant individual interactions of aging duration with the type of contact, the type of paint, and the initial amount of agent loading on the coupon (with the interaction with the type of paint being the influential).

D5.2.6 Effect of Initial Agent Loading on Coupon.

Manthei *et al.* (1988): No observation on the direct effect of initial agent loading is made. However, a significant interaction exists between agent loading and the type of paint based on the agent recovery data from the test coupons.

Sommerville (2005): ANOVA of the test coupon data confirms the above observation. Also, the amount of initial agent loading has no significant effect on the amount of agent retained by the test alkyd coupons, with the reverse being true for polyurethane paint. However, this interaction between agent loading and type of paint is much weaker with the agent recovery data from the dental dam.

D5.3 Agent Transfer—Agent Recovery from Test Coupons for Rabbit Skin Trials.

Manthei *et al.* (1988): Very little is said about the agent recovery data from the test coupons for the rabbit skin trials.

Sommerville (2005): Comparison of the agent recovery data from the dental dam and rabbit skin trials yields interesting observations. For the rabbit skin trials, the most important factors/interactions are the type of paint, its interaction with the aging duration, and the aging duration. These factors/interactions are also important in the dental dam trials but not to the same degree—the factors/interactions account for 88% of the total variance for the rabbit skin trials and 57% for the dental dam trials. Another key difference between the two groups of test coupon agent recovery data is that there is a larger variance in the rabbit skin trials (with a statistical significance of 99.5%).

D5.4 Agent Transfer—General Comments.

D5.4.1 Painted Surface to Another Surface.

Manthei et al. (1988): There was good agreement among the test plates and the amount of HD left on them after contacting dental dam or rabbit skin.

Sommerville (2005): There is a statistically significant difference between the amounts of HD left on the test plates after being in contact with dental dam vs. contact with rabbit skin.

D5.4.2 Painted Surface to Dental Dam.

Manthei et al. (1988): The amount of HD transferred to the dam and recovered by chemical analysis is assumed to be near the amount transferred to rabbit skin.

Sommerville (2005): The ANOVA on the agent recovery data from the painted metal coupons found that the dental dam is absorbing less than the rabbit skin in general. However, this difference is dependent on other factors, such as the type of paint and the initial agent loading on the test coupons.

D5.5 Intensity of Agent Damage on Rabbit Skin.

D5.5.1 Effect of Type of Paint.

Manthei et al. (1988): No observations made.

Sommerville (2005): The direct effect of paint type was found to be statistically insignificant. However, there was a significant interaction between paint type and the type of contact (vapor vs. direct), as well as between paint type and the aging duration (the period between the rinsing of the contaminated test coupon and its application to the rabbit skin).

D5.5.2 Effect of Type of Contact.

Manthei et al. (1988): Based on analysis of the rabbit exposures involving no aging duration between agent rinse and skin contact, intensity of injury was less with vapor contact than with direct contact. The difference was between the two was more pronounced for alkyd than for polyurethane paint surfaces.

Sommerville (2005): Based on ANOVA of the total dataset, direct contact was found to produce more intense injury than vapor contact. Also, the type of contact was the most influential factor or interaction on the intensity of agent damage. The interaction discussed above (type of paint and type of contact) was confirmed by ANOVA for the whole dataset.

D5.5.3 Effect of Initial Agent Loading on Coupon and Aging Duration Between Decontamination of Coupon and Contact with Skin.

Manthei et al. (1988): Observed that there was a great reduction in the intensity of damage when the aging duration is increased from 0 to 15 min. As the initial loading increases in value, the greater the intensity of damage becomes.

Sommerville (2005): Based on ANOVA of total dataset, both the initial agent loading and aging duration are statistically significant effects, but neither is as influential as the type of contact.

D5.6 Area of Agent Damage on Rabbit Skin.

D5.6.1 Effect of Type of Paint.

Manthei et al. (1988): Contact with decontaminated polyurethane paint coupons produced larger areas of damage than did contact with alkyd paint coupons.

Sommerville (2005): The direct effect of paint type was found to be statistically significant. However, there were significant interactions between paint type and other factors (initial agent loading on

coupon and aging duration), as well. The observed difference between the two types of paint decreases as either the initial agent loading decreases or the aging duration increases.

D5.6.2 Effect of Type of Contact.

Manthei *et al.* (1988): Only one observation was made—based on a limited review of the data (trials with aging duration of zero), vapor contact produces a larger damaged area than direct contact but only at an initial agent loading of 10 mg.

Sommerville (2005): An ANOVA of the whole dataset found that the type of contact was not a statistically significant factor, nor were any of its interactions with other factors. This finding could be due to the fact that agent loading values greater than 10 mg were not investigated (Section D4.8). A linear regression analysis of size of damaged area (direct vs. vapor) found that vapor contact produces larger damaged areas only as the size of the damaged area (from vapor and direct) in general increases. For damaged areas smaller than about 0.5 to 1 in.², direct contact produces the larger damaged area.

D5.6.3 Effect of Initial Agent Loading on Coupon and Aging Duration Between Decontamination of Coupon and Contact with Skin.

Manthei *et al.* (1988): Observed that there was a great reduction in the size of skin damage when the aging duration is increased from 0 to 15 min. As the initial loading increases in value, the greater the size of damage becomes.

Sommerville (2005): Based on ANOVA of total dataset, both the initial agent loading and aging duration are statistically significant effects. Both of these factors have about the same influence as the type of paint.

D5.7 Comparison of Significant Factors for Intensity and Size of Agent Damage on Rabbit Skin.

Manthei *et al.* (1988): no comparison was made.

Sommerville (2005): The intensity of damage differs from the size of damage in terms of what factors they are dependent on. The intensity of damage is heavily dependent on the type of contact, but it is not dependent on the type of paint. The size of damage is dependent on the type of paint, but it is not dependent on the type of contact. However, the intensity and size of damage are about equally dependent on the amount of initial agent loading and on the aging duration.

D5.8 Usefulness of Dental Dam as a Rabbit Skin Simulant.

Manthei *et al.* (1988): Dental dam is a very good substitute for skin.

Sommerville (2005): Dental dam can make a very good simulant for skin, but only if the dental dam is properly calibrated for the exposure conditions of interest (*e.g.*, type of paint, initial agent loading, aging duration, and the type of contact). There are differences between dental dam and rabbit skin in how much agent they absorb, and the exact difference will be a function of the exposure conditions (see previous discussion).

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DEPARTMENT OF THE ARMY
US ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
EDGEWOOD CHEMICAL BIOLOGICAL CENTER
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REPLY TO
ATTENTION OF

JAN 06 2017

RDCB-DSR-S

MEMORANDUM THRU Director, Edgewood Chemical Biological Center, (RDCB-D/Dr. Joseph L. Corriveau), 5183 Blackhawk Road, Aberdeen Proving Ground, Maryland 21010-5424

FOR Defense Technical Information Center (DTIC), 8725 John J. Kingman Road, Ft Belvoir, VA 22060-6218

SUBJECT: Request for Change in Distribution

1. This action is in response to an Edgewood Chemical Biological Center (ECBC) Internal Request for a Change in Distribution for the attached listed documents:
2. The listed documents have current distribution statements or classifications which limit their release. ECBC Subject Matter Experts have reviewed the documents and deemed them suitable for the change in distribution to read "Approved for public release; distribution unlimited."
3. The point of contact is Adana Eilo, ECBC Security Specialist, (410) 436-2063 or adana.l.eilo.civ@mail.mil.

Encl


RONALD L. STAFFORD
Security Manager

ECBC Documents for Downgrading/Change in Distribution

1. Callahan, J.F. *The Relation between Skin Thickness and the Penetration Rate of VX through Skin*. In *Research Program of the Field Toxicology Branch*; CRDL TM 20-27; Callahan, JF, *et al.* Eds.; Directorate of Medical Research, U.S. Army Chemical Research and Development Laboratories, U.S. Army Chemical Center: Edgewood Arsenal, MD, 1962; UNCLASSIFIED Report. **CBRNIAC-CB-118810 Dist. E.**
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2. Callahan, J.F.; Cresthull, P.; Christensen, M.K.; Crook, J.W.; Wiles J.S.; Owens, E.; Hart, J.; Worden, F.X. *Intravenous Toxicity of VX in Marzulli et al. Biological Studies on VX during Fiscal Year 1958*; CWL Special Publication 2-18; U.S. Army Chemical Warfare Laboratories: U.S. Army Chemical Center, 1959; UNCLASSIFIED Report **AD0313760 Dist. C.**
Recommended for public release.
3. Frankel, H.M.; Wiles, J.S. *Lethality of VX in Rats at High and Low Temperatures*; CRDLR-3023; U.S. Army Chemical Research, and Development Laboratories: Edgewood Arsenal, MD, 1960; UNCLASSIFIED Report **AD0243462 Dist C.**
Recommended for public release.
4. Marzulli, F.N. *A Comparison of Toxic Properties of the V Agents with GB*; MLSR-75; U.S. Army Chemical Corp Medical Laboratories: U.S. Army Chemical Center, MD, 1955; UNCLASSIFIED Report **AD0090916 Dist. C.**
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