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**Low-Level Chemical Agent Toxicology:
Examination of Surface Contamination on Rats
after Whole-Body Exposure to Lethal Levels
of VX Agent Vapor**

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14. ABSTRACT (LIMIT 200 WORDS) The objective was to define safe handling procedures for animals contaminated with VX vapor. Efficacy of four decontaminants was tested on rats after whole-body exposure to lethal levels of VX vapor. The decontaminant requirements were, at minimum, that it must (1) be rapidly effective with low toxicity; (2) create nontoxic byproducts; (3) cause minimal irritation; and (4) have rapid application and removal properties. Given the desired properties, four liquid-based decontamination solutions were identified: dilute bleach (~0.5%), soapy water, Canadian reactive skin decontamination lotion (RSDL), and an organophosphorus hydrolase (OPH). After VX exposure, rat bodies were decontaminated by wetting with the candidate solution, allowing a contact time of 10 min, and then rinsing with water. VX contamination levels were determined by extraction of the euthanized animals with isopropyl alcohol and analysis by gas chromatography–mass spectrometry. VX contamination results were as follows: 65.3 µg/rat for the no-decontamination (control) group, 21.8 µg/rat for dilute bleach, 27.0 µg/rat for OPH, 15.6 µg/rat for soapy water, and 3.2 µg/rat for RSDL. Overall, RSDL performed the best in minimizing VX contamination. Using this initial data, procedures were developed and tested that allow for the safe handling of animals after whole-body exposure to VX.													
15. SUBJECT TERMS <table border="0" style="width: 100%;"> <tr> <td>Inhalation decontamination</td> <td>Whole body</td> </tr> <tr> <td>Organophosphorus hydrolase</td> <td>Soapy water</td> </tr> <tr> <td>Canadian reactive skin decontamination lotion (RSDL)</td> <td>Dilute bleach</td> </tr> <tr> <td>O-Ethyl-S-(2-diisopropylaminoethyl) methyl phosphonothiolate (VX)</td> <td>Rats</td> </tr> </table>						Inhalation decontamination	Whole body	Organophosphorus hydrolase	Soapy water	Canadian reactive skin decontamination lotion (RSDL)	Dilute bleach	O-Ethyl-S-(2-diisopropylaminoethyl) methyl phosphonothiolate (VX)	Rats
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PREFACE

The work described in this report was authorized under project no. 206023, Low Level Toxicology. The work was started in November 2004 and completed in May 2005. The experimental data are contained in ECBC Laboratory Notebook no. 04-0202. Raw data and the final report from this study are stored in the Toxicology Archives at U.S. Army Combat Capabilities Development Command Chemical Biological Center (CCDC CBC; Aberdeen Proving Ground, MD), which was previously known as the U.S. Army Edgewood Chemical Biological Center (ECBC).

In conducting this study, investigators adhered to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication no. 86-23, 1985, as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, Washington, DC). These investigations were also performed in accordance with the requirements of Army Regulation (AR) 70-18, *Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs*, and the ECBC Institutional Animal Care and Use Committee (IACUC), which oversees the use of laboratory animals. This project's assigned IACUC protocol no. 05-359, was approved on 21 November 2004.

All animals were cared for as stated in this research protocol and as specified in NIH Publication no. 85-23, 1985 (or updates). Records are maintained in official ECBC Laboratory Notebooks in the Life Sciences Official Archives and/or in the CCDC CBC Technical Library. Studies were conducted under and in compliance with good laboratory practice standards and were reviewed periodically by the quality assurance coordinator or the coordinator's designee.

The performance of this study was consistent with the objectives and standards in *Good Laboratory Practices for Non-Clinical Laboratory Studies* (21 CFR 58, Food and Drug Administration, U.S. Department of Health and Human Services, April 1988).

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CONTENTS

	PREFACE	iii
1.	INTRODUCTION	1
2.	MATERIALS AND METHODS.....	3
2.1	Chemicals.....	3
2.2	Decontamination Candidates	6
2.3	Inhalation Chamber.....	7
2.3.1	Vapor Generation.....	7
2.3.2	Sampling System: Sorbent Tubes	8
2.4	Experimental Design.....	9
2.4.1	Initial Evaluation of the Four Candidates	9
2.4.2	Agent Transfer to Gloves.....	10
2.4.3	Refinement of Decontamination Procedures	10
2.5	Animal Model	10
2.6	Methods.....	11
2.6.1	Decontamination Procedures for Live Animals.....	11
2.6.2	VX Assay: Quantification of VX from Rat Surface Contamination	14
2.7	Data Analysis	15
3.	RESULTS	15
3.1	Comparison of Candidate Decontaminants	15
3.2	Transfer of VX to Gloves	16
4.	DISCUSSION	18
5.	CONCLUSIONS.....	20
	REFERENCES	21
	BIBLIOGRAPHY	23
	ACRONYMS AND ABBREVIATIONS	27
	APPENDIX: SIGMASTAT STATISTICAL ANALYSIS PRINTOUTS.....	29

FIGURES

1.	Structure of VX.....	1
2.	Structure of GVX (<i>O</i> -ethyl methylphosphonofluoridate), the G-analogue of VX	3
3.	The 1000 L Rochester-style exposure chamber	7
4.	VX vapor generation using a saturator cell.....	8
5.	Initial restraint system.....	12
6.	Improved restraint system, view 1	13
7.	Improved restraint system, view 2	13
8.	Decontamination procedure for live rats after VX exposure	14
9.	Comparison of four VX decontaminants after rat whole-body exposure	16
10.	Residual VX in water rinse of decontaminated rats.....	16
11.	Glove-transfer comparison of soap and water- and RSDL-decontaminated rats	17

TABLES

1.	Physical and Chemical Properties of VX.....	2
2.	Impurities Present in VX	4
3.	Results for the Candidate Decontaminants after Whole-Body VX Exposure with Euthanized Rats	5
4.	Transfer to Glove after Decontamination Experiment.....	18

LOW-LEVEL CHEMICAL AGENT TOXICOLOGY: EXAMINATION OF SURFACE CONTAMINATION ON RATS AFTER WHOLE-BODY EXPOSURE TO LETHAL LEVELS OF VX AGENT VAPOR

1. INTRODUCTION

O-Ethyl-*S*-(2-diisopropylaminoethyl) methyl phosphonothiolate (VX or EA 1701; Figure 1) is an extremely potent acetylcholinesterase inhibitor by all routes of exposure. This organophosphorus compound (OP) is substantially more toxic than other related OP nerve agents such as isopropyl methylphosphonofluoridate (GB or sarin), ethyl *N,N*-dimethylphosphoramidocyanidate (GA or tabun), and pinacolyl methyl phosphonofluoridate (GD or soman) (Somani and Romano, 2001), especially after percutaneous exposure. Most of the known toxic effects of VX in vivo are derived from studies in which VX was administered subcutaneously, percutaneously, intravenously, or as an inhaled aerosol (van der Schans et al., 2003; Bide and Risk, 2000; Craig et al., 1977; Gupta et al., 1991; Rickett et al., 1986). Related VX work includes two studies on the toxic effects of chemically neutralized VX in rats (Muse et al., 2002; Manthei et al., 1990). However, few studies exist in which reliable toxicity estimates in animals have been established for VX administered as a vapor (Hartmann, 2002).

Contributing to this lack of information is the difficulty in producing stable vapor concentrations in a controlled environment due to the very low vapor pressure of VX. The vapor pressure of VX is 0.0007 mmHg at 25 °C (Table 1) as compared to GB, which has a vapor pressure of 2.1 mmHg at 20 °C (Gupta, 2009). After VX is disseminated as a vapor, it is deposited on all surfaces, including the fur and skin of any test animals. It is very resistant to decontamination by simple purges with clean air. Overall, safe handling of animals after lethal-level exposure is one of the major considerations for conducting whole-body VX exposure studies, and it is of critical concern to researchers and animal technicians. Purging the test chamber with clean air to lessen the contamination of the test subjects is not a viable solution for VX-exposed subjects. This necessitates the decontamination of test animals after exposure.

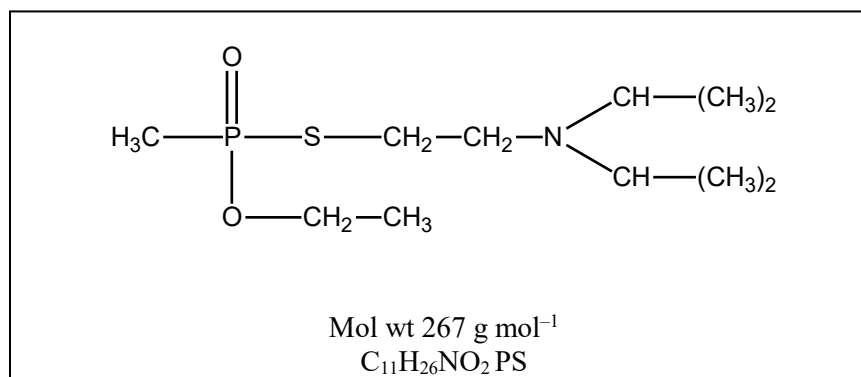


Figure 1. Structure of VX. Chemical Abstracts Service (CAS) registry numbers 50782-69-9, 51848-47-6, 53800-40-1, and 70938-84-0.

Table 1. Physical and Chemical Properties of VX*

Boiling point at 760 mmHg	568 °F (298 °C)
Vapor pressure	0.0007 mmHg at 25 °C
Vapor density (air, 1 STP)	9.2 at 25 °C
Solubility (g per 100 g of solvent)	30 at 25 °C in water; soluble in organic solvents
Freezing/melting point (°C)	-39 °C
Liquid density (g/mL)	1.006 at 20 °C
Volatility (mg/m ³)	10.5 at 25 °C

*From Gupta, 2009.

Various decontamination methods are available for VX, including reactive substances that chemically modify the agent and nonreactive substances that simply remove the agent from the surface. Reactive treatments include both liquid and solid formulations. Proven liquid decontaminants include 5% bleach, aqueous caustic solutions such as 10% aqueous sodium hydroxide, and Canadian reactive skin decontamination lotion (RSDL). Effective reactive solids that have been used in the past include Dutch powder and certain organic resins. Nonreactive substances may also be either liquids such as water, surfactants, and organic solvents, or solids such as Fuller's earth and activated charcoal. The type of decontaminant used depends on the scenario.

The optimum decontaminant for VX vapor whole-body animal studies should, at minimum, be rapid, effective, nontoxic, minimally irritating to the animals, easily applied, and easily removed, and it should have no toxic byproducts. As a result of these requirements, solid decontamination systems, whether reactive or nonreactive, are not a viable option. They would be difficult to apply and remove from animal fur. Nonreactive powders could generate additional hazards after adsorbing VX. These contaminated powders could create a dusty agent problem that would be more difficult to contain than the original liquid. Although 5% bleach and caustic solutions are rapid and effective, they would cause stress and undue harm to the test animals.

Given the desired properties for animal studies, several candidate liquid-based decontamination solutions were identified, including dilute bleach (~0.5%), soapy water, RSDL, and organophosphorus hydrolase (OPH). Dilute bleach, 0.5% sodium hypochlorite, is one of the standard personnel decontamination solutions used for most chemical warfare agents (CWAs) by the U.S. Army (Hurst, 1997). Some studies have indicated that water or soapy water is effective in removing CWA contamination, especially in cases of mass casualty decontamination (Somani and Romano, 2001; van Hooideonk et al., 1983). The effectiveness of the Canadian RSDL, which consists of an oxime in a water-soluble polyethylene glycol-based liquid, has been demonstrated with nerve agents and vesicants (Sawyer et al., 1991). RSDL has been accepted by the Canadian Army as a primary personnel decontamination product and was approved in 2005 by the U.S. Food and Drug Administration. OPH has also demonstrated efficacy in the destruction of organophosphorus-based nerve agents (Cheng et al., 1998). Solutions of OPH at around pH 8 would be compatible with animal surface-decontamination procedures.

Of the two main objectives, the first was to quantify the VX surface contamination after whole-body exposure to lethal VX levels. The second objective was to establish procedures to reduce the contamination level such that the rats could be handled safely after exposure. Successful completion of these objectives serves to provide for a safe working

environment for future VX vapor-exposure studies, assist with identification of experimental effects that could impact operational readiness, and provide a basis for predictions useful for military operational risk management decisions. In addition, this report presents data comparing four decontaminants under VX vapor-exposure conditions that have direct operational value.

2. MATERIALS AND METHODS

2.1 Chemicals

Neat VX was used for all vapor exposures. The structure of GVX is shown in Figure 2, and its corresponding physical and chemical properties are listed in Table 2. VX was received from the Chemical Transfer Facility at U.S. Army Edgewood Chemical Biological Center (ECBC; now the U.S. Army Combat Capabilities Development Command Chemical Biological Center; Aberdeen Proving Ground, MD) in individually sealed, 5 mL ampoules (lot no. VX-U-1243-CTF-N) and certified as Chemical Agent Standard Analytical Reference Material (CASARM). Seven iterations of a ^{31}P NMR analysis were performed in accordance with an established method (Brickhouse et al., 1997) to certify the purity of the material as 93.6 ± 0.5 mol % pure. Impurities and their respective percentages are shown in Table 3. Triethylphosphate (99.9%; catalog no. 240893; Sigma-Aldrich; St. Louis, MO) was used as the internal standard for the VX purity assays. All external standards for VX vapor quantitation were prepared daily with isopropanol (IPA) solvent (purity, >99%; Burdick & Jackson; Muskegon, MI). The internal standard for the extraction studies was *O*-($^2\text{H}_5$ -ethyl)-*S*-(2-diisopropylamino ethyl) methyl phosphonothiolate ($^2\text{H}_5$ -VX), with purity of >97%, which was procured from ECBC. Gases such as helium and nitrogen (minimum purity, 99.999%) were obtained from Messer, Inc. (Chattanooga, TN).

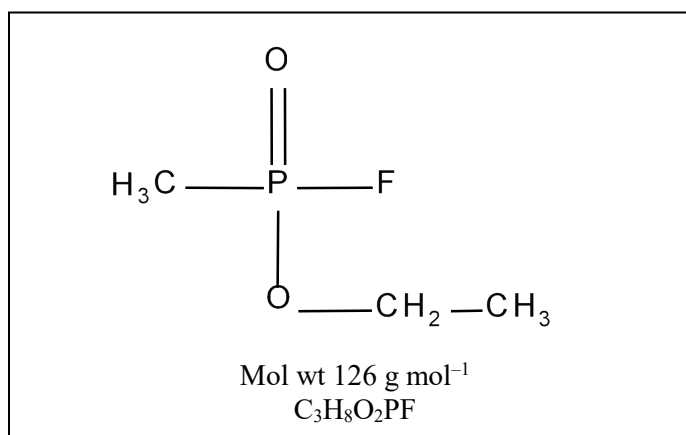


Figure 2. Structure of GVX (*O*-ethyl methylphosphonofluoridate), the G-analogue of VX. CAS registry number 673-97-2.

Table 2. Impurities Present in VX*

Compound	Mol %
VX	93.6
Diisopropylaminoethane thiol	2.1
HCN/H ⁺	1.2
Diethyl methylphosphonate	1.0
Diethyl dimethyldiphosphonate (VX pyro)	0.8
Phosphonic acids and esters (δ 20–39)	0.4
Other ¹ H impurities	0.31
Unsymmetrical VX pyro	0.11
Chloroform	0.1
Other phosphorus impurities	0.27

*Lot no. VX-U-1243-CTF-N.

Table 3. Results for the Candidate Decontaminants
after Whole-Body VX Exposure with Euthanized Rats

Rat Treatment and No.	Experiment Date	Rat Weight (g)	Calculated Surface Area, $9.1 \times \text{Weight}^{0.66}$ (cm ²)	Total VX/A* (μg)	Total VX/B* (μg)	Sum A + B (μg)	Sum/Area (μg/cm ²)
Control							
13	1/12/2005	236	335.1	61.65	11.04	72.69	0.21693
14	1/12/2005	183	283.3	58.74	19.62	78.36	0.27659
18	1/12/2005	236	335.1	50.01	24.66	74.67	0.22283
47	1/27/2005	279	374.2	100.4	14.71	115.11	0.30759
48	1/27/2005	284	378.6	49.79	15.72	65.51	0.17301
41	1/27/2005	256	353.6	55.22	19.34	74.56	0.21088
36	1/27/2005	325	413.9	21.38	11.14	32.52	0.07857
38	1/27/2005	304	396.0	35.34	11.89	47.23	0.11926
39	1/27/2005	295	388.3	18.54	8.62	27.16	0.06995
			(No decon)	Control average		65.31	0.18618
					SD	26.78	0.08333
					RSD	41.00	44.76
Bleach							
6	1/12/2005	218	318.0	19.34	5.44	24.78	0.07793
17	1/12/2005	220	319.9	27.22	11.49	38.71	0.12100
20	1/12/2005	243	341.6	14.52	4.7	19.22	0.05626
25	1/20/2005	308	399.5	7.38	5.84	13.22	0.03309
31	1/20/2005	289	383.0	5.28	2.8	8.08	0.02109
35	1/20/2005	226	325.7	6.17	2.46	8.63	0.02650
43	1/27/2005	337	423.9	20	6.31	26.31	0.06206
45	1/27/2005	287	381.3	14.32	6.77	21.09	0.05531
40	1/27/2005	298	390.9	25.06	11.57	36.63	0.09372
				Bleach average		21.85	0.06077
					SD	11.05	0.03274
					RSD	50.59	53.87
Soap							
15	1/12/2005	243	341.6	15.36	6.38	21.74	0.06364
11	1/12/2005	228	327.6	10.86	4.93	15.79	0.04821
9	1/12/2005	233	332.3	11.42	6.74	18.16	0.05465
27	1/20/2005	282	376.9	5.28	2.8	8.08	0.02144
21	1/20/2005	278	373.3	6.17	2.46	8.63	0.02312
34	1/20/2005	224	323.7	5.84	4.1	9.94	0.03070
37	1/27/2005	307	398.6	10.6	3.46	14.06	0.03527
44	1/27/2005	299	391.7	17.9	5.14	23.04	0.05882
50	1/27/2005	280	375.1	14.08	6.43	20.51	0.05468
				Soap average		15.55	0.04339
					SD	5.74	0.01599
					RSD	36.89	36.85

(continued)

Table 3. Results for the Candidate Decontaminants
after Whole-Body VX Exposure with Euthanized Rats (Continued)

Rat Treatment and No.	Experiment Date	Rat Weight (g)	Calculated Surface Area, $9.1 \times \text{Weight}^{0.66}$ (cm ²)	Total VX/A* (μg)	Total VX/B* (μg)	Sum A + B (μg)	Sum/Area (μg/cm ²)
OPH							
16	1/12/2005	230	329.4	22.87	7.44	30.31	0.09200
12	1/12/2005	227	326.6	25.64	7.99	33.63	0.10297
10	1/12/2005	226	325.7	29.05	4.61	33.66	0.10336
32	1/20/2005	276	371.6	4.35	1.5	5.85	0.01574
23	1/20/2005	298	390.9	6.64	1.5	8.14	0.02083
29	1/20/2005	319	408.8	7.77	2.66	10.43	0.02551
51	2/2/2005	346	431.4	59.89	16.8	76.69	0.17779
59	2/2/2005	300	392.6	19.87	5.74	25.61	0.06523
52	2/2/2005	301	393.5	14.59	4.09	18.68	0.04748
OPH average						27.00	0.07232
SD						21.56	0.05259
RSD						79.84	72.71
RSDL							
7	1/12/2005	231	330.4	0	0	0	0.00000
8	1/12/2005	250	348.1	0	0	0	0.00000
19	1/12/2005	226	325.7	0	0	0	0.00000
30	1/20/2005	310	401.2	0	0	0	0.00000
22	1/20/2005	304	396.0	0	0	0	0.00000
28	1/20/2005	335	422.3	0	0	0	0.00000
53	2/2/2005	333	420.6	0	7.15	7.15	0.01700
57	2/2/2005	330	418.1	0	16.14	16.14	0.03860
58	2/2/2005	342	428.1	0	5.84	5.84	0.01364
RSDL average						3.24	0.00769
SD						5.61	0.01338
RSD						173.21	173.92

SD, standard deviation; RSD, relative standard deviation.

* To extract VX from euthanized rat fur, each rat was dipped sequentially in two beakers (A and B). Each beaker contained 500 mL of IPA. Rats were kept in each beaker for 10 min. Each beaker was then separately analyzed for the amount of VX it contained.

2.2 Decontamination Candidates

The four decontamination solutions that were evaluated along with controls were soapy water, 0.5% bleach, RSDL, and OPH. No decontamination solution was used in the control test. All decontamination candidates were aqueous-based liquids that were prepared immediately before use, except for the RSDL, which was obtained in 21 mL pouches with a sponge applicator or in 500 mL bottles.

RSDL in 21 mL sponge pouches was procured from O'Dell Engineering/E-Z-EM Canada (Cambridge, ONT). Household bleach (analyzed ~5%) was diluted 1:10 each day with deionized water to generate the 0.5% decontamination solution. Soapy water was generated fresh

daily from a 1:10 dilution in deionized water of Johnson's Head-to-Toe baby wash (Johnson&Johnson; New Brunswick, NJ). OPH was provided by Dr. V. K. Rastogi (ECBC). All other chemicals, solvents, and gases were used as obtained with no further purification.

2.3 Inhalation Chamber

Whole-body vapor exposures were conducted in a 1000 L dynamic airflow inhalation chamber (Figure 3). The hexagonal Rochester-style chamber is constructed of stainless steel with Plexiglas windows on each of its six sides. The interior of the exposure chamber was maintained under negative pressure (0.25 in.H₂O) as indicated by a calibrated Magnehelic gauge (Dwyer Instruments; Michigan City, IN). Room air was drawn through the exposure chamber (at 570–580 L/min) and measured at the chamber outlet with a calibrated thermoanemometer (model 8565; Alnor; Skokie, IL). Temperature and humidity were recorded for every exposure.



Figure 3. The 1000 L Rochester-style exposure chamber.

2.3.1 Vapor Generation

The vapor-generation system was located at the chamber inlet and was contained within a stainless steel box maintained under negative pressure. Saturated VX vapor streams (0.05–5 mg/m³) were generated by a continuous flow of nitrogen carrier gas (8–202 mL/min) through a glass vessel functioning as a multipass saturator cell (Glassblowers.com; Turnersville, NJ) that contained 1 mL of liquid VX (Figure 4). The main body of the saturator cell consisted of

a 100 mm long, 25 mm outer diameter (o.d.) cylindrical glass tube with two vertical 7 mm o.d. tubes (inlet and outlet) at each end (Figure 4). The main body of the saturator cell contained a porous, hollow, ceramic cylinder, which served to absorb the liquid VX and thereby increase the contact area between the liquid VX and the nitrogen carrier gas. The saturator cell was fabricated from Alundum fused alumina (Norton Co.; Worcester, MA) to allow nitrogen gas to make three passes along the surface of the wetted ceramic cylinder before exiting the outlet arm of the saturator cell. The saturator cell body was immersed in a Thermo NESLAB constant-temperature bath (Thermo Fisher Scientific; Portsmouth, NH) containing mineral oil so that a combination of nitrogen gas flow rate and temperature could be used to regulate the amount of VX vapor entering the inhalation chamber. The bath was maintained at 30–90 °C, depending upon the required VX concentration, and the outlet arm of the saturator cell was wrapped in heat tape and maintained at 90 °C. It was necessary to maintain a continuous flow of VX vapor through the chamber to preserve the passivation of the chamber. This allowed for generation and maintenance of stable chamber concentrations.

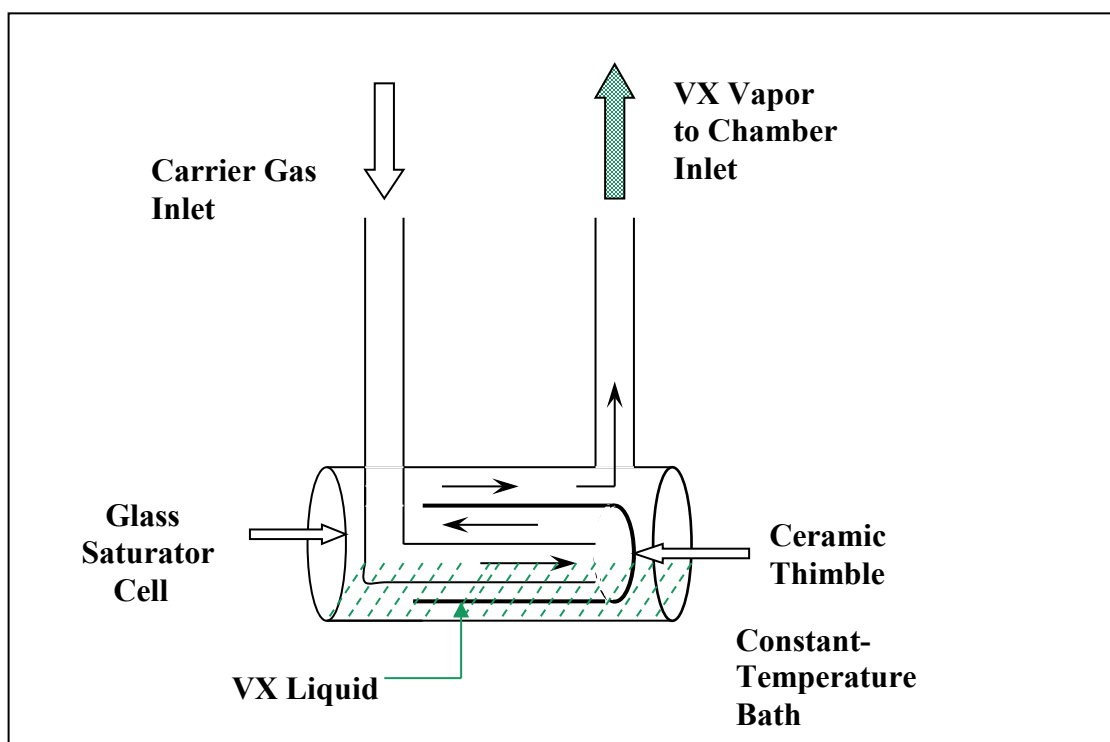


Figure 4. VX vapor generation using a saturator cell.

2.3.2 Sampling System: Sorbent Tubes

The solid sorbent tube sampling system consisted of a 20:35 mesh Tenax TA fast flow sorbent tube (part no. AO-06-2717) and an ACEM-900 thermal desorption unit (TDU; Dynatherm Analytical Instruments; Kelton, PA) coupled to a gas chromatography (CG) system equipped with flame photometric detection (FPD). Samples were drawn from the middle of the

exposure chamber by inserting a rod containing a sampling tube through small access ports located on the walls of the chamber. The rod was hooked to a vacuum line that drew a sample through the tube at a rate of 3–5 L/min for 1–9 min, depending on the chamber concentration. Sample flow rates were controlled with calibrated mass flow controllers (Matheson; Montgomeryville, PA) and were verified before and after sampling with a calibrated flowmeter (DryCal, Bios International; Pompton Plains, NJ) connected in-line with the sample stream. The sample tube was transferred to the TDU and prepared for injection onto an Rtx-5 column (15 m × 0.32 mm × 0.5 µm; Restek; State College, PA). Temperature and flow programming within the TDU desorbed VX from the sorbent tube directly onto the GC column. Detection was performed with FPD in the phosphorous mode.

The sampling system was calibrated by direct injection of external standards onto the sorbent tubes before insertion into the TDU and analysis with GC/FPD. In this way, injected VX standards were put through the same sampling scheme as the chamber samples. A linear regression fit (correlation coefficient [r^2] = 0.999) of the standard data was used to calculate the VX concentration of each chamber sample.

Concentration uniformity was checked at several locations throughout the chamber, including areas directly above the animal cages. At higher generated agent concentrations, vacuum pumps were used to draw air through glass fiber filter pads at high flow rates to test for the presence of aerosols. Analysis of the glass fiber pads required IPA desorption and liquid extract injection onto a 20:35 mesh Tenax TA fast flow sorbent tube. The sorbent tube was thermally desorbed and analyzed by GC/FPD.

2.4 Experimental Design

The study was organized into three phases: (1) initial evaluation of the four candidates, (2) agent transfer to gloves, and (3) refinement of decontamination procedures. In the first two phases, euthanized rats were used; this minimizes handling hazards for the researchers and minimizes pain and discomfort for the rats. In the third phase, live animals were used to develop and test decontamination procedures under actual exposure conditions. All exposures were performed at VX concentrations of between 0.5 and 5.0 mg/m³ for 10 or 60 min.

2.4.1 Initial Evaluation of the Four Candidates

Decontamination evaluation. Rats were euthanized and then exposed in 3 groups of 18 animals. Rat carcasses were handled with tongs whenever possible during experiment 1. Gloves used to contact surfaces that had not undergone decontamination, including rat carcasses, were considered contaminated and were handled as specified in the agent-use standing operating procedure.

Within an approved agent hood, rats were decontaminated with one of the four candidate solutions. Following decontamination, residual VX was extracted by dipping the rat in 500 mL of IPA for 10 min. Immediately after the first extraction, the rats underwent a second IPA residual VX extraction using 500 mL of fresh IPA for an additional 10 min. The individual IPA samples were then analyzed using GC with mass spectrometric detection (MSD) to quantify the amount of VX in the IPA, which represented the residual VX contamination on the surface of the rat. The amounts of VX in the first and second IPA extraction solutions were summed to give

a total amount of VX on the rat (Table 3). These values were used to determine the two best candidates, RSDL and soapy water, which were then used for the remaining tests.

2.4.2 Agent Transfer to Gloves

Transfer to gloves hazard test. Following euthanasia, exposure, and decontamination using each of the best two treatments as determined in Section 2.4.1, personnel wearing two pairs of protective gloves handled each rat. Gloves were changed after handling each rat. Handling the rats simulated typical post-exposure procedures such as collection of blood samples. After each rat was handled for 3 min, it was placed in an individual, sealed container for vapor hazard testing. The outer and inner pairs of gloves were removed, and residual VX was extracted by pouring 250 mL of IPA into each inside-out glove, twisting shut the glove opening, shaking the glove, and finally, collecting the IPA in a jar for analysis by GC with mass spectrometry (MSD).

The post-exposure vapor hazard testing of the rats was assessed by taking air samples from within the sealed container that held the treated rat carcass for 60–120 min using Tenax TA sorbent tubes (depot area air monitoring system [DAAMS] tubes) and analysis by GC/FPD.

2.4.3 Refinement of Decontamination Procedures

Live animal tests. A group of six unexposed rats were used to simulate actual exposures to develop safe procedures for transport and application of the decontaminants following actual exposures. These rats were euthanized after development of these procedures.

Plastic storage containers with lids were used to transport exposure cages with rats to an approved surety hood for decontamination. The distance between exposure chamber and surety hood was short, and any additional stress on the rats was considered minimal. After transport to the surety hood, rats were quickly removed from the exposure cages and placed in individual plastic containers with lids (12 × 6 × 6 in.) until decontaminant solution could be applied. The reason for quick transfer from exposure cage to holding cage was to reduce additional contact and exposure of rats to the VX-contaminated exposure cages. After all of the rats were decontaminated, the plastic transport, exposure cages, and individual holding containers were immersed in 5% bleach solution and decontaminated. At the conclusion of each VX experiment (24 h post exposure), all VX-exposed rats were euthanized, dipped in 5% bleach solution, and placed in a biohazard bag for 24 h before disposal.

From the time the exposure chamber door was opened and during subsequent transport and decontamination in a surety hood, all study participants were required personal protection equipment (PPE) to include M40 masks and butyl rubber gloves.

2.5 Animal Model

Sexually mature male Sprague-Dawley rats (Charles River Laboratories; Wilmington, MA) weighing between 180 and 300 g were used in this study. Upon arrival, the animals were identified by marking the tails. Rats were housed individually in plastic shoebox cages. Animals were housed in an Association for Assessment and Accreditation of Laboratory

Animal Care-accredited facility. The animals were quarantined for a minimum of 5 days after arrival. Ambient conditions were maintained at 70 ± 5 °F and 30–70% relative humidity with a 12:12 hour light–dark cycle. Rats were provided with certified laboratory rat chow and filtered house water ad libitum, except during exposure. All experiments and procedures were approved by the ECBC Institutional Animal Care and Use Committee and were conducted in accordance with the requirements of Army Regulation 70-18 and the National Research Council's *Guide for the Care and Use of Laboratory Animals*.

2.6 Methods

The methods consisted of procedures for decontaminating the exposed animals and analysis for quantifying residual VX.

2.6.1 Decontamination Procedures for Live Animals

The test animals in the first and second phases of the protocol were euthanized before VX exposure. After exposure, each rat was decontaminated by pouring the candidate decontaminant over the carcass to wet all surfaces. The carcass was allowed to remain for 10 min before it was rinsed off with deionized water, with the exception of RSDL use. The RSDL was applied by a combination of pouring any free liquid in the pouch onto the surface of the rat and then using the impregnated sponge to spread the thick liquid over the remainder of the carcass.

In the third phase of the protocol, decontamination procedures were developed to accommodate working with live VX-contaminated rats. Several methods of decontaminating live animals were evaluated. Different factors affected the design of the decontamination procedures. Most importantly, the mobility of the rats needed to be controlled to prevent VX dissemination via contaminated fluids (i.e., saliva, urine, and blood) and solids (i.e., fur and feces). Also, all of the decontamination processes that were considered involved the animals becoming wet with decontaminant and rinse water. A rat's natural reaction to wetness is shaking, which could indiscriminately distribute VX liquid before the animal is decontaminated.

Mobility control was achieved by placing the rat in a tube with enough holes to be able to distribute decontaminant and subsequent rinse water. Initially, restraints were constructed from a semi-rigid galvanized hardware cloth (20–23 gauge, 4 hole mesh per inch) cut into 7.5×9.0 in. rectangles that could be rolled into a 9 in. long cylinder having a diameter of approximately 2 in. (Figure 5). The cylinder shape was retained by placing plastic or metal rings around the exterior of the hardware cloth. Once the rat climbed into the cylinder, the ends were blocked by additional wire mesh or metal rods. The problem with this initial design was the presence of sharp points where the mesh was cut. The points could be minimized through grinding with abrasives, but any sharp points that remained could compromise personal protective equipment such as gloves.

An improved cylinder was achieved using polyvinyl chloride (PVC; 2 in. schedule 40) tubes cut to 9 in. lengths. The tubes were drilled with multiple $3/8$ in. holes and two $3/8$ in. channels to allow the decontamination solution to easily contact the animal surface (Figures 6 and 7). The tube ends were blocked with PVC tube butt ends that were drilled with

several 3/8 in. holes to allow for the flow of liquid and air. Ambulatory rats would readily enter the tube if the rats were held by the tail and their head and front feet were at the opening of the tube.

Figure 8 demonstrates how the tubes were dipped into RSDL and rinse water. Animals were dipped quickly but completely to obtain RSDL contact with all surfaces. Excess RSDL was allowed to drain back into the large graduated plastic cylinder. After draining, the tubes with the RSDL-coated rats were placed into a plastic tub for 10 min before the rats in the tubes were rinsed with tap water. Rats dipped in this manner showed no residual VX, as judged by extraction of the euthanized rats or by air monitoring to the worker population limit for VX.

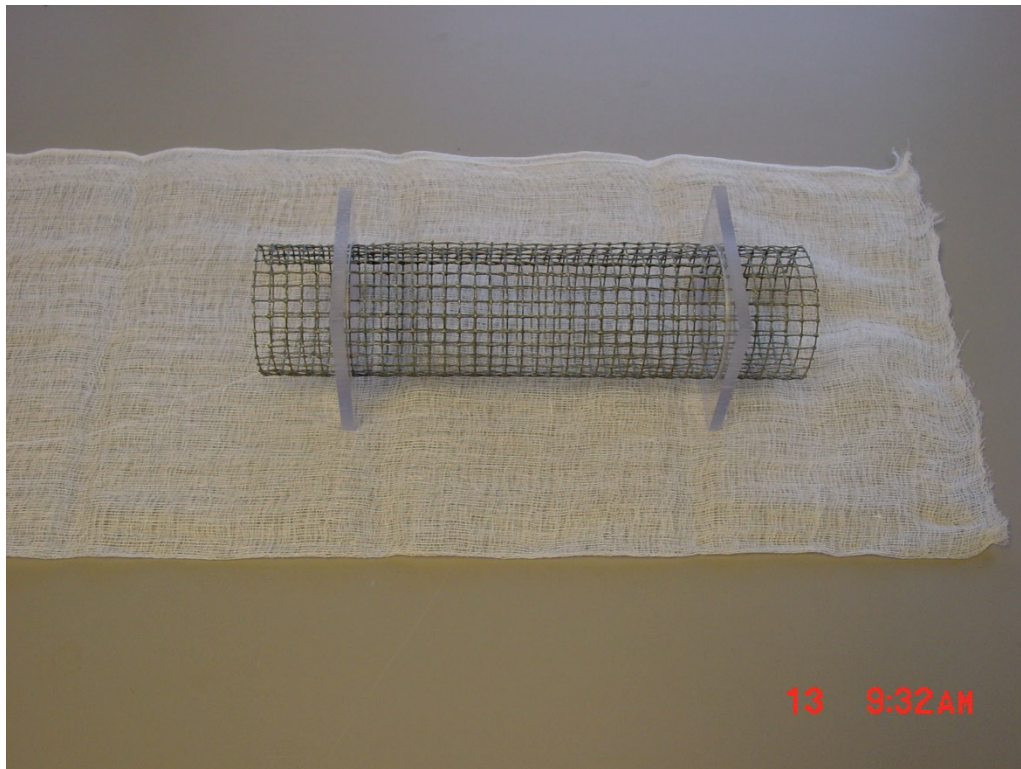


Figure 5. Initial restraint system.



Figure 6. Improved restraint system, view 1.



Figure 7. Improved restraint system, view 2.

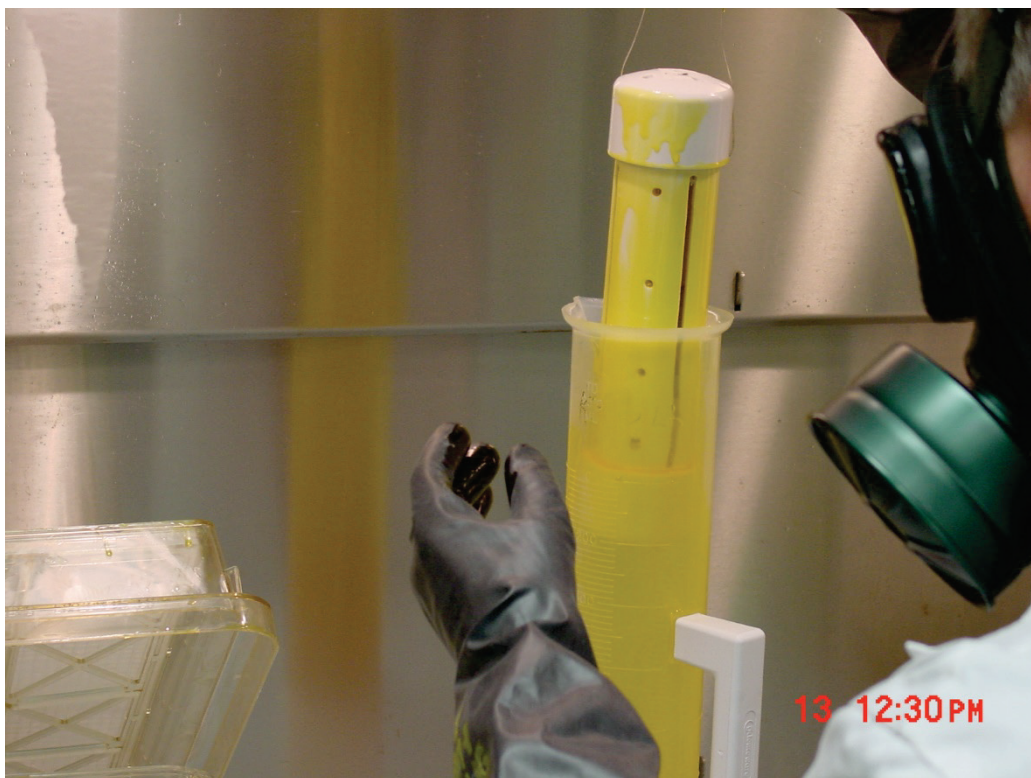


Figure 8. Decontamination procedure for live rats after VX exposure.

2.6.2 VX Assay: Quantification of VX from Rat Surface Contamination

Following sample collection from either the contaminated rats or the protective gloves that were used to handle the post-exposed rats, samples were prepared as follows. A known aliquot of IPA sample dip (or diluted IPA sample dip) was added to a 2.0 mL microvial containing 0.5 g of granular sodium sulfate (Na_2SO_4). Internal standard ($^2\text{H}_5\text{-VX}$) was added, and the vial was vortexed and centrifuged. The supernatant was removed and transferred to a GC autosampler vial for analysis.

The VX was then quantified. A 50 μL volume was autoinjected into the large-volume Programmed Temperature Vaporization (PTV) injector port (Agilent Technologies; Wilmington, DE). The following parameters were used: initial temperature, 0 $^\circ\text{C}$; initial time, 6.1 min; final temperature, 250 $^\circ\text{C}$; rate, 720 $^\circ\text{C}/\text{min}$ (maximum ballistic heating as listed in the Agilent manual); vent time, 6.00 min; vent flow, 300 mL/min; purge flow, 50 mL/min; and purge time, 9.7 min.

The GC system (model 6890; Agilent Technologies) included a Restek Rtx-1701 column (30 m $\times$ 0.32 mm $\times$ 1.0 μm film thickness) with a flow rate of 3 mL/min (63 cm/s). The GC oven program was as follows: initial temperature, 50 $^\circ\text{C}$ for 10.3 min; to 80 $^\circ\text{C}$ at 50 $^\circ\text{C}/\text{min}$ (0 min hold); to 210 $^\circ\text{C}$ at 15 $^\circ\text{C}/\text{min}$ (0 min hold); and finally, to 280 $^\circ\text{C}$ at 50 $^\circ\text{C}/\text{min}$; which was held for 3.0 min. MSD (model 5793; Agilent Technologies) was performed by chemical ionization with ammonia reagent gas in the positive ion mode using the mass-to-charge ratio (m/z) 268/273 (MH^+) ion ratio ($\text{VX}/^2\text{H}_5\text{-VX}$) for quantification and m/z 269 (VX) and

274 ($^2\text{H}_5\text{-VX}$) ions as qualifiers. Linear internal standard calibration curves for VX were generated from 10 to 1000 pg using standards in IPA. The Agilent software provided with the MSD system was used to process and analyze the data. The software allowed for automatic analysis of the internal standard method based on the analyte area ratios of the peaks at their respective retention times.

2.7 Data Analysis

A statistics program such as Minitab, version 13 or later (Minitab; State College, PA), or SigmaStat, version 3.1 or later (Systat Software; San Jose, CA) was used for the analysis of the data to compare treatments. Comparison of decontamination methods was made by a one-factor analysis of variance followed by a Tukey's multiple-comparison test to determine significant differences between pairs of decontaminations. For the transfer to glove study and the live rat decontamination study, a t test was used to compare the two decontamination methods with respect to the amounts of VX left on the gloves and the rats, respectively. Statistical significance was defined as $p < 0.05$.

3. RESULTS

3.1 Comparison of Candidate Decontaminants

The four candidate decontaminants were compared against each other and against control exposed rats. The results are presented in Table 3 and Figure 9. Agent could be recovered in some cases from the water rinse that was used to remove the decontaminant. Results for this water rinse presented in Figure 10 are semiquantitative because the exact volume of the rinse was not measured, although in all cases, the volume was close to 1 L. The statistical analysis is presented in the Appendix.

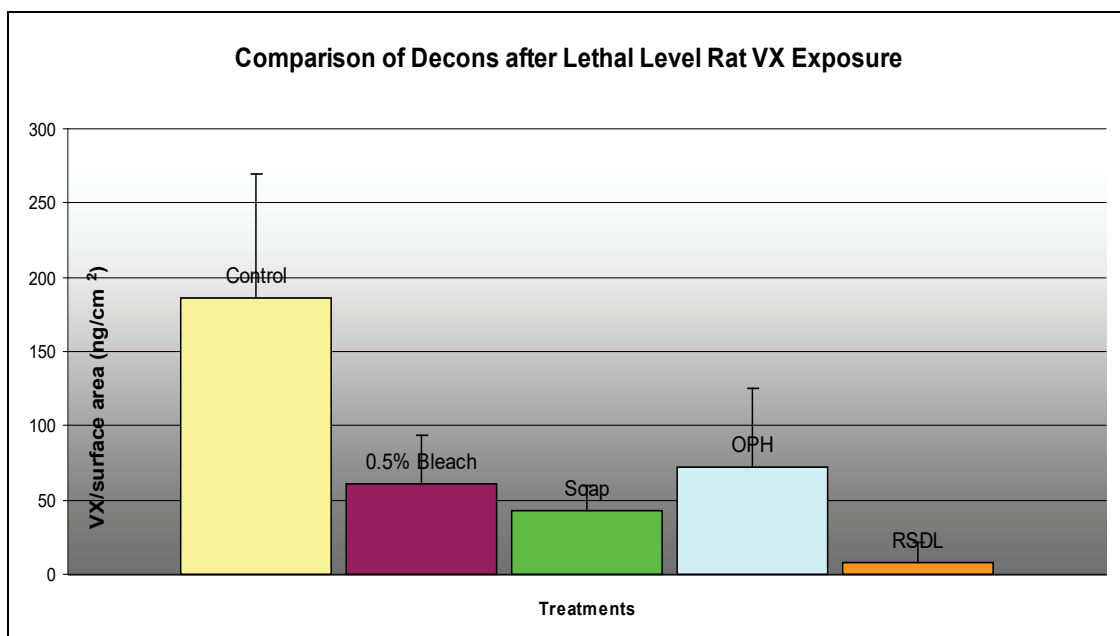


Figure 9. Comparison of four VX decontaminants after rat whole-body exposure.

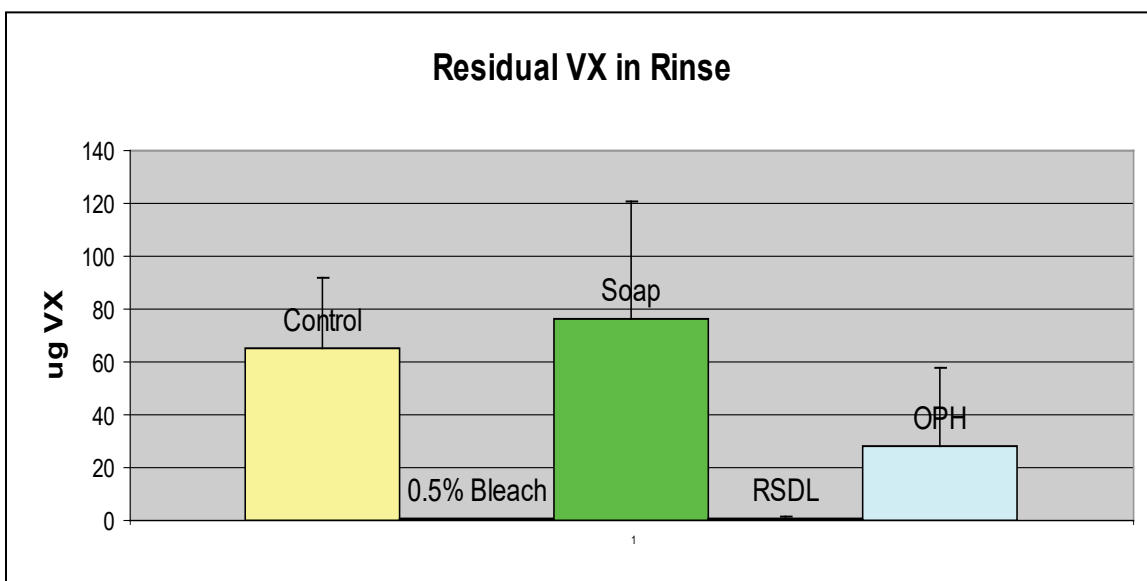


Figure 10. Residual VX in water rinse of decontaminated rats.

3.2 Transfer of VX to Gloves

The two best candidates from the first experiment were used to determine the amount of VX that could be transferred to gloves when handling the decontaminated animals. The results are summarized in Table 4 and Figure 11. RSDL was clearly superior to soap and water. The positive results seen in some cases with RSDL were attributed to inadequate coverage of the animals with the decontamination solution.

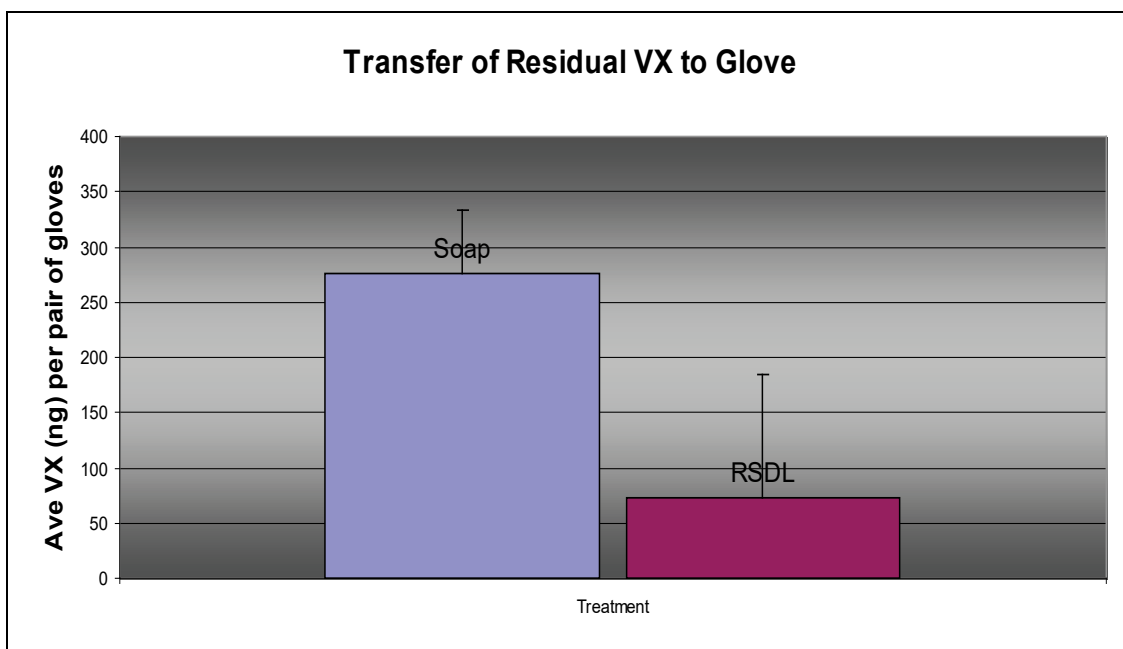


Figure 11. Glove-transfer comparison of soap and water- and RSDL-decontaminated rats.

Table 4. Transfer to Glove after Decontamination Experiment

Rat Treatment	Rat No.	VX (μg)		
		Left Glove	Right Glove	Sum
RSDL	60	0	0	0
	61	0	0	0
	62	0	0	0
	63	0	0	0
	64	0	0	0
	65	0.0671	0	0.0671
	98	0.0675	0.1531	0.2206
	99	0.1876	0.1142	0.3018
	100	0	0.0642	0.0642
Average	0.072633			
SD	0.112301			
%RSD	154.6129			
Soap/H ₂ O	69	0.1061	0.0943	0.2004
	70	0.1434	0.1638	0.3072
	71	0.1278	0.1603	0.2881
	72	0.0935	0.0908	0.1843
	73	0.1468	0.1701	0.3169
	74	0.1113	0.249	0.3603
	75	0.1202	0.1344	0.2546
	76	0.0906	0.1551	0.2457
	77	0.1878	0.1326	0.3204
Average	0.275322			
SD	0.058488			
%RSD	21.24344			

SD, standard deviation; RSD, relative standard deviation.

4. DISCUSSION

In published reports, Levitin and coworkers have stated that the critical level of decontamination needed after nerve-agent vapor exposure is simple removal of the subject from the contaminated atmosphere (Levitin et al., 2003). This course of action may be sufficient for volatile nerve agents such as GB. For agents such as VX, the rate of evaporation is very slow, which leads to persistent contamination of surfaces including skin, hair, and fur. However, few studies have been published on vaporous VX exposure. This is probably because of the difficulty in generating only vapor, as well as the assumption that liquid VX (either from an aerosol or on a surface) would be the most likely source of exposure. After vapor-only exposure, contamination would need to be at significant levels and also be transferable for decontamination to be necessary.

In our study, the recovery of VX from control rats (rats that were not decontaminated) was 65.3 ± 26.8 μg per animal. This is a significant level for a rat, given that the dermal LD_{50} (median lethal dose; amount of toxic agent that is sufficient to kill 50% of a population) in rats is 100 $\mu\text{g}/\text{kg}$ (Somani and Romano, 2001). Therefore, 65.3 μg represents approximately 2.45 times the LD_{50} (average weight of the control group was 0.266 kg). Control rats were not studied in the glove-transfer test. However, after the soapy water-decontaminated rats were handled for 3 min, approximately 0.275 μg per rat was transferred. This was 1.8% (percent transfer = $0.275 \times 100/15.55$) of the average VX remaining after decontamination. In the case of RSDL, 2.2% was transferred to the gloves (percent transfer = $0.0726 \times 100/3.24$). Assuming a 2.0% transfer of VX to the gloves, then approximately 1.3 $\mu\text{g}/\text{rat}$ (or 0.02×65.31 $\mu\text{g}/\text{rat}$) would probably have been observed for control animals.

Data derived from a landmark study dealing with percutaneous exposure of VX on human subjects (Sim, 1962) can help place the possible transfer hazard for humans in perspective. In this study, human subjects underwent percutaneous exposure to various parts of the body with differing microgram amounts of VX. Relevant to this study, two of the most likely areas for percutaneous exposure were the hand (palm, 20 $\mu\text{g}/\text{kg}$; dorsum, 30 $\mu\text{g}/\text{kg}$) and the cheek (5 $\mu\text{g}/\text{kg}$). Clinical signs of toxicity and changes in blood cholinesterase (ChE) levels were recorded for a minimum of 24 h. At these levels of exposure, the maximum ChE decrease in red blood cells (RBCs) occurred within 12 h. The ChE levels at exposure sites on the palmar portion of the hand were 91% and on the dorsum were 94% of baseline (palmar range, 83–98%; dorsum range, 81–104%). Of the eight subjects receiving a 5 $\mu\text{g}/\text{kg}$ percutaneous dose of VX on the cheek, the average decrease in RBC ChE was 29% of baseline (range, 5–50%). Several subjects (two of eight) experienced minor signs of toxicity (headache, nightmares, dizziness, and localized sweating) after palmar exposure of the hand. No subjects experienced any signs after exposure to the dorsum of hand. Of those receiving a cheek exposure, four of eight subjects showed moderate signs of toxicity (nausea, vomiting, and weakness) and had decreased RBC ChE levels that ranged from 5 to 31% of baseline. One of eight subjects with a cheek exposure showed minor signs of toxicity.

The clinical signs of toxicity and the dosage amounts used by Sims were compared with the residual VX concentrations found on the test rats and the gloves. Control rats yielded an average of 65.31 μg of VX per rat, which for a 70 kg person would be 0.933 $\mu\text{g}/\text{kg}$ of VX if all of the VX had transferred. Given a 2% transfer amount, the potential dose from handling a single rat would be approximately 0.02 $\mu\text{g}/\text{kg}$ of VX. In the case where only one animal was handled, the contamination potential appears to be relatively small. However, whole-body studies typically have used 20 rats per test exposure, which generates a possible cumulative exposure per experiment of 20–30 μg of VX on the exterior gloves. VX penetration through the gloves and breakthrough, which are necessary for actual exposure, are dependent on several factors such as glove material, glove thickness, contamination location on the glove, time, and temperature. The contamination would be concentrated on specific parts of the glove that contact the rats, such that local VX densities would be higher on the palm than on the back of the glove. High localized VX densities give rise to greater penetration rates and greater danger in the event of a torn glove.

Decontamination requires a reaction between the agent and the active moiety of the decontaminant. The reaction rate depends on the reactant concentrations, the reaction activation energy, the temperature, and the reaction matrix. Molecules of decontamination must come close to VX and have sufficient energy to react. In our work, the reactions of VX with dilute bleach (0.5%) and OPH enzyme did not perform as well as those with RSDL, probably for several reasons. First, there was a relatively short contact/reaction time with the contaminated surfaces (10 min). This meant that the reactive components of the decontamination solution had to rapidly contact the VX on the skin and fur surfaces. Both dilute bleach and OPH are aqueous based. The VX on the rat surface would not have been very soluble in the simple aqueous medium, especially at basic pH values. RSDL is a polyethylene glycol-based product that would dissolve VX, allow reactants to meet, and thereby cause the nucleophilic substitution reaction with the oxime to proceed rapidly. Soapy water did not decontaminate the VX; however, the surfactant present in the aqueous solution was capable of dissolving the VX to remove it from the rat surface. The bleach solution activity probably could have been enhanced by adding a surfactant to assist in VX dissolution.

5. CONCLUSIONS

The results of the experiments demonstrated that significant VX surface contamination occurred during whole-body exposures at lethal levels, and this contamination was controlled or eliminated by certain decontamination procedures. Of the decontaminants tested, RSDL provided superior performance that was limited only by how thoroughly it was applied. Soapy water lessened the surface contamination significantly; however, the resulting effluent was contaminated, and caution is required when disposing of it.

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ACRONYMS AND ABBREVIATIONS

CAS	Chemical Abstracts Service
CASARM	Chemical Agent Standard Analytical Reference Material
ChE	cholinesterase
CWA	chemical warfare agent
DAAMS	depot area air monitoring system
GA	tabun; ethyl <i>N,N</i> -dimethylphosphoramidocyanidate
GB	sarin; isopropyl methylphosphonofluoridate
GC	gas chromatography
GVX	G-analogue of VX; <i>O</i> -ethyl methylphosphonofluoridate
FPD	flame photometric detection
GD	soman
IPA	isopropanol, 2-hydroxypropane
LD ₅₀	median lethal dose: the amount of a toxic agent that is sufficient to kill 50% of a population of animals usually within a certain time
MS	mass spectrometry
MSD	mass spectrometric detection
o.d.	outer diameter
OP	organophosphorus
OPH	organophosphorus hydrolase
PVC	polyvinyl chloride
r^2	coefficient of determination
RBC	red blood cell
RSD	relative standard deviation
RSDL	reactive skin decontamination lotion
SD	standard deviation
SE	standard error
TDU	thermal desorption unit
VX	EA 1701; <i>O</i> -ethyl- <i>S</i> -(2-diisopropylaminoethyl) methyl phosphonothiolate
VX pyro	diethyl dimethyldiphosphonate
wt	weight

Blank

APPENDIX

SIGMASTAT STATISTICAL ANALYSIS PRINTOUTS

Phase 1		Data Table for Statistical Analysis				
Column No.	1	2	3	4	5	
	Control	Bleach	Soap	OPH	RSDL	
Total VX µg/cm ²	0.216926	0.077925	0.063638	0.092003	0	
	0.276589	0.120999	0.048206	0.102969	0	
	0.222835	0.056262	0.054654	0.103362	0	
	0.30759	0.033094	0.021439	0.015744	0	
	0.173012	0.021095	0.023115	0.020826	0	
	0.210876	0.026501	0.030703	0.025512	0	
	0.078572	0.062065	0.035272	0.17779	0.017	
	0.119256	0.055314	0.058816	0.065233	0.038605	
	0.069953	0.093716	0.054676	0.047477	0.013643	
	Phase 2		Soap/Water			
Column No.	12	13	14	15		
Soap/Water	Rat	Left Glove	Right Glove	Sum		
µg VX	69	0.1061	0.0943	0.2004		
	70	0.1434	0.1638	0.3072		
	71	0.1278	0.1603	0.2881		
	72	0.0935	0.0908	0.1843		
	73	0.1468	0.1701	0.3169		
	74	0.1113	0.249	0.3603		
	75	0.1202	0.1344	0.2546		
	76	0.0906	0.1551	0.2457		
	77	0.1878	0.1326	0.3204		
	RSDL					
Column No.	17	18	19	20		
RSDL	Rat	Left Glove	Right Glove	Sum		
µg VX	60	0	0	0		
	61	0	0	0		
	62	0	0	0		
	63	0	0	0		
	64	0	0	0		
	65	0.0671	0	0.0671		
	98	0.0675	0.1531	0.2206		
	99	0.1876	0.1142	0.3018		
	100	0	0.0642	0.0642		

Descriptive Statistics: Monday, July 18, 2005, 11:02:05 AM

Data source: Data 2 in VX LCt Decon 01

Column	Size	Missing	Mean	SD	SE	C.I. of Mean
Control	9	0	0.186	0.0833	0.0278	0.0641
Bleach	9	0	0.0608	0.0327	0.0109	0.0252
Soapy	9	0	0.0434	0.0160	0.00533	0.0123
OPH	9	0	0.0723	0.0526	0.0175	0.0404
RSDL	9	0	0.00769	0.0134	0.00446	0.0103
Rat	9	0	73.000	2.739	0.913	2.105
Left glove	9	0	0.125	0.0306	0.0102	0.0235
Right glove	9	0	0.150	0.0470	0.0157	0.0361
Sum	9	0	0.275	0.0585	0.0195	0.0450
Rat	9	0	74.667	18.317	6.106	14.079
Left glove	9	0	0.0358	0.0640	0.0213	0.0492
Right glove	9	0	0.0368	0.0596	0.0199	0.0458
Sum	9	0	0.0726	0.112	0.0374	0.0863

Column	Range	Max	Min	Median	25%	75%
Control	0.238	0.308	0.0700	0.211	0.109	0.236
Bleach	0.0999	0.121	0.0211	0.0563	0.0314	0.0819
Soapy	0.0422	0.0636	0.0214	0.0482	0.0288	0.0557
OPH	0.162	0.178	0.0157	0.0652	0.0243	0.103
RSDL	0.0386	0.0386	0.000	0.000	0.000	0.0145
Rat	8.000	77.000	69.000	73.000	70.750	75.250
Left glove	0.0972	0.188	0.0906	0.120	0.103	0.144
Right glove	0.158	0.249	0.0908	0.155	0.123	0.165
Sum	0.176	0.360	0.184	0.288	0.234	0.318
Rat	40.000	100.000	60.000	64.000	61.750	98.250
Left glove	0.188	0.188	0.000	0.000	0.000	0.0672
Right glove	0.153	0.153	0.000	0.000	0.000	0.0767
Sum	0.302	0.302	0.000	0.000	0.000	0.105

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	Sum	Sum of Squares
Control	-0.138	-1.106	0.172	0.546	1.676	0.368
Bleach	0.619	-0.171	0.151	0.685	0.547	0.0418
Soapy	-0.257	-1.748	0.204	0.327	0.391	0.0190
OPH	0.888	0.617	0.166	0.586	0.651	0.0692
RSDL	1.865	3.302	0.384	<0.001	0.0692	0.00197
Rat	0.000	-1.200	0.101	0.807	657.000	48021.000
Left glove	0.987	0.976	0.134	0.768	1.127	0.149
Right glove	0.913	1.912	0.224	0.213	1.350	0.220
Sum	-0.320	-0.876	0.152	0.681	2.478	0.710
Rat	0.835	-1.702	0.368	<0.001	672.000	52860.000
Left glove	2.019	4.122	0.379	<0.001	0.322	0.0443
Right glove	1.334	0.323	0.398	<0.001	0.331	0.0406
Sum	1.516	1.115	0.297	0.021	0.654	0.148

One Way Analysis of Variance

Monday, July 18, 2005, 9:41:40 AM

Data source: Data 2 in VX LCt Decon 01

Normality Test: Failed ($P < 0.050$)

Test execution ended by user request, ANOVA on ranks begun.

Kruskal-Wallis One-Way Analysis of Variance on Ranks

Monday, July 18, 2005, 9:41:40 AM

Data source: Data 2 in VX LCt Decon 01

Group	N	Missing	Median	25%	75%
Col 1	11	2	0.211	0.109	0.236
Col 2	11	2	0.0563	0.0314	0.0819
Col 3	11	2	0.0482	0.0288	0.0557
Col 4	11	2	0.0652	0.0243	0.103
Col 5	11	2	0.000	0.000	0.0145

$H = 29.343$ with 4 degrees of freedom ($P = <0.001$).

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$),

To isolate the group or groups that differ from the others, use a multiple comparison procedure.

All pairwise multiple comparison procedures (Tukey test):

Comparison	Diff of Ranks	q	$P < 0.05$
Col 1 vs Col 5	297.000	7.538	Yes
Col 1 vs Col 3	174.000	4.416	Yes
Col 1 vs Col 2	131.000	3.325	No
Col 1 vs Col 4	128.000	3.249	Do Not Test
Col 4 vs Col 5	169.000	4.289	Yes
Col 4 vs Col 3	46.000	1.167	No
Col 4 vs Col 2	3.000	0.0761	Do Not Test
Col 2 vs Col 5	166.000	4.213	Yes
Col 2 vs Col 3	43.000	1.091	Do Not Test
Col 3 vs Col 5	123.000	3.122	No

Note: The multiple comparisons on ranks do not include an adjustment for ties.

A result of "Do Not Test" occurs for a comparison when no significant difference is found between the two rank sums that enclose that comparison. For example, if you had four rank sums sorted in order, and found no significant difference between rank sums 4 versus 2, then you would not test 4 versus 3 and 3 versus 2, but still test 4 versus 1 and 3 versus 1 (4 vs 3 and 3 vs 2 are enclosed by 4 vs 2: 4 3 2 1). Note that not testing the enclosed rank sums is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the rank sums, even though one may appear to exist.

APPENDIX ABBREVIATIONS

ANOVA	analysis of variance
C.I.	confidence interval
Col	column
decon	decontamination
diff	difference
K-S	Kolmogorov-Smirnov
LCt	lethal concentration
OPH	organophosphorus hydrolase
<i>P</i> , Prob	probability
RSDL	reactive skin decontamination lotion
VX	<i>O</i> -ethyl- <i>S</i> -(2-diisopropylaminoethyl) methyl phosphonothiolate

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