

AD _____

Award Number: DAMD17-99-D-0010

TITLE: A Medical Research and Evaluation Facility (MREF) and
Studies to Support the Medical Chemical Defense Program

PRINCIPAL INVESTIGATOR: Carl T. Olson, D.V.M., Ph.D.
James E. Estep, D.V.M., Ph.D.

CONTRACTING ORGANIZATION: Battelle Memorial Institute
Columbus, Ohio 43201-2693

REPORT DATE: July 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20040922 044

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE July 2004	3. REPORT TYPE AND DATES COVERED Annual (1 Jun 03-31 May 04)	
4. TITLE AND SUBTITLE A Medical Research and Evaluation Facility (MREF) and Studies to Support the Medical Chemical Defense Program			5. FUNDING NUMBERS DAMD17-99-D-0010	
6. AUTHOR(S) Carl T. Olson, D.V.M., Ph.D. James E. Estep, D.V.M., Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Battelle Memorial Institute Columbus, Ohio 43201-2693 E-Mail: olsonc@battelle.org / estep@battelle.org			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES Original contains color plates. All DTIC reproductions will be in black and white.				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) For Task 0001, Battelle's Medical Research and Evaluation Facility successfully functioned in compliance with local, state, and federal government regulations while developing and improving medical countermeasures against relevant chemical agents. In Task 0002, 594 total compounds/combinations have been tested in the mouse ear assay. In Task 0003, penetration cells, M8 paper, rabbits and guinea pigs are used to test topical skin protectants (TSP). Gene array analyses of mouse skin exposed to HD have been performed for Task 0007. In Task 0008, clinical evaluations of Reactive Skin Decontamination Lotion have been completed and a report is being prepared. Effects of VR in cynomolgus monkeys have been studied and differences from the response of rhesus monkeys investigated in Task 0009. Evaluation of decontamination efficacy of a number of compounds against HD was investigated in Task 0010. Task 0012 studies the healing of HD burns in swine and evaluates various treatments. Substance P gene expression was used to evaluate protective countermeasures against HD in Task 0013. SERPACWA and combinations of TSPs were evaluated against VX and HD in guinea pigs in Task 0015. Efficacy of four oximes, in conjunction with ATR, was investigated as treatment for exposure to cholinesterase inhibitors in Task 0016. Task 0017 evaluated various decontaminants for the Joint Services Family of Decontaminant Systems. Human BuChE was evaluated in Task 0018 for treatment of anticholinesterase intoxication. Various sorbent decontamination systems were compared in Task 0019, and decontamination solutions for use on remains was completed in Task 0020. Tasks 0021 and 0023 were not funded. Task 0022 evaluated the synthesis of radiolabelled GD, and Task 0024 is determining the toxicity of various oximes in mice.				
14. SUBJECT TERMS Chemical Defense, medical countermeasures, animal models, protection, autoinjector, treatment, HD, GD, VX, topical skin protectant, penetration cell, RSDL, M8			15. NUMBER OF PAGES 40	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

_____ Where copyrighted material is quoted, permission has been obtained to use such material.

_____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

_____ Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council (National Academy Press, 1996).

_____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal law 45 CFR 46.

_____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institute of Health.

_____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

_____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.



Program Manager Signature Date

 7/20/04

PI - Signature Date

TABLE OF CONTENTS

	Page
Cover	Cover
SF298	
FOREWORD	
INTRODUCTION.....	1
BODY	1
Key Research Accomplishments.....	1
Task 0001 – Maintain the Medical Research and Evaluation Facility (MREF) as a Functional Chemical Surety Materiel (CSM) Laboratory.....	1
Task 0002 – Evaluation of Pretreatment and Treatment Compounds for Topical HD Exposure Using the Mouse Ear Model	1
Task 0003 – Efficacy rTSP DTN Screening	5
Task 0007 – Gene Array Analysis	8
Task 0008 – Canadian Reactive Skin Decontamination Lotion (RSDL)	8
Task 0009 – Russian V-Agent (VR) and VX Effects in Monkeys	8
Task 0010 – Evaluation of Decontamination Procedures for Chemical Warfare Agent Exposure to Skin	10
Task 0012 – The Efficacy of Candidate Regimens in Promoting Improved Healing of Cutaneous Sulfur Mustard Burns in Weanling Swine	10
Task 0013 - Localization of Substance P Gene Expression for Evaluating Protective Countermeasures	11

TABLE OF CONTENTS
(Continued)

	Page
Task 0015 – Evaluation of SERPACWA and Dilute Bleach Decontaminant Against Novel Agents	11
Task 0016 – Efficacy of Oximes Against Organophosphorus Compounds in Non-Human Primates.....	11
Task 0017 – Joint Service Family of Decontamination systems (JSFDS) Block III .	12
Task 0018 – Efficacy of Human Butyrylcholinesterase for Soman Poisoning	13
Task 0019 – Government Testing of the Sorbent Decontamination System (SDS) in Accordance with the Decision Tree Network	14
Task 0020 – Evaluation of Decontamination Solutions for Use on Remains.....	14
Task 0021 – Midazolam Study in Primates	14
Task 0022 – Study to Determine the Maximum Attainable Specific Activity of [Methyl- ¹⁴ C] Dimethyl Methylphosphonate in 100-200 mCi Quantities and Synthesize 0.5 g of [Methyl- ¹⁴ C] GD with the Maximum Attainable Specific Activity Determined in Phase I	14
Task 0023 – Efficacy Testing of Cholinesterase Reactivators in the Rabbit	15
Task 0024 – Oxime Toxicity in Mice	15
REPORTABLE OUTCOMES.....	15
CONCLUSIONS.....	17
REFERENCES	17
APPENDIX A. MANUSCRIPT/PRESENTATION ABSTRACTS	A-1

INTRODUCTION

This contract provides the necessary personnel, facilities/equipment, and supplies to perform research, development, testing, and evaluation (RDT&E) using chemical agents. The RDT&E program is to provide information on basic, applied, and developmental biomedical questions critical to providing improved medical countermeasures against existing chemical agents and emerging threats. RDT&E efforts involve animal models, alternatives to animal models, and comprehensive benchtop procedures utilizing chemical agents (CA), RDT&E dilute solutions of CA, and other hazardous chemicals. Our facility provides adequate space for studies in multiple animal species, *in vitro* models including isolated organ systems and cell cultures, and benchtop analytical and medicinal chemistry procedures.

BODY

KEY RESEARCH ACCOMPLISHMENTS

Task 0001 – Maintain the Medical Research and Evaluation Facility (MREF) as a Functional Chemical Surety Materiel (CSM) Laboratory

The MREF's CA laboratories and facilities were maintained and functioned in compliance with government regulations. Inventories of CA were performed and usage reports were maintained, and three task orders were proposed and/or developed. In addition to staff training and development on-site, MREF staff attended three scientific conferences or training courses. Details on travel and program issues can be found in the quarterly reports provided to the Contracting Officer. The MREF has successfully passed inspections or certifications by the USDA, Ohio EPA, Madison County (OH) Health Department, Battelle's IACUC, ISO 9001 Registrar, Association for Assessment and Accreditation of Laboratory Animal Care (AALAC International), SCOB and IG.

Task 0002 – Evaluation of Pretreatment and Treatment Compounds for Topical HD Exposure Using the Mouse Ear Model

Task 0002, a follow on to Task 95-43 under Contract No. DAMD17-89-C-9050, was approved on October 8, 1999. Testing was initiated on October 13, 1999. This report covers the period of June 1, 2003 to May 31, 2004.

The equivalent of 594 compounds, of the proposed 574, have been tested.

Studies were initiated April 21, 2003 to evaluate paired combinations of the nine compounds listed below. Final results of all combination studies are listed in the table below. Both Day 7 and Day 14 ear tissue Draize scores are presented for these compounds given as a single treatment 10 minutes after a 0.08 mg HD challenge.

#36 = DMSA

#37 = DMPS

#1308 = 4-methyl-2-mercaptopyridine-1-oxide
 #2082 = dexamethasone
 #2086 = indomethacin
 #2525 = BAL
 #2842 = hydrocortisone
 #2980 = octyl homovanillamide
 #3537 = capsaicin

ICD#	Compound Name	Pass/Fail Draize Scoring Day 7	Pass/Fail Draize Scoring Day 14
2525	BAL		
37	DMPS		
2980	Octyl homovanillamide		
2525 + 37			
2525 + 2980			
37 + 2980			
2086	Indomethacin	F	
37	DMPS		
2842	Hydrocortisone	F	
2086 + 37		F	
2086 + 2842		F	
37 + 2842		F	
2525	BAL		
2842	Hydrocortisone	F	
3537	8-Methyl-N-vanillyl-6-nonenamide		
2525 + 2842			
2525 + 3537			
2842 + 3537			
2525	BAL	F	F
36	DMSA	F	F
2082	Dexamethasone	F	F
2525 + 36			F
2525 + 2082			
36 + 2082		F	F
2842	Hydrocortisone	F	F
1308	4-Methyl-2-mercaptopyridine-1-oxide		F
36	DMSA	F	F
2842 + 1308			
2842 + 36		F	F
1308 + 36			F
2525	BAL	F	F
2086	Indomethacin	F	F
1308	4-Methyl-2-mercaptopyridine-1-oxide		
2525 + 2086			
2525 + 1308			F
2086 + 1308			
2980	Octyl homovanillamide		
2086	Indomethacin		F
36	DMSA	F	F
2980 + 2086			
2980 + 36			

ICD#	Compound Name	Pass/Fail Draize Scoring Day 7	Pass/Fail Draize Scoring Day 14
2086 + 36		F	F
2980	Octyl homovanillamide		
1308	4-Methyl-2-mercaptopyridine-1-oxide		
2082	Dexamethasone		
2980 + 1308			
2980 + 2082			
1308 + 2082			
2082	Dexamethasone	F	F
37	DMPS		F
3537	8-Methyl-N-vanillyl-6-nonenamide		
2082 + 37			
2082 + 3537			
37 + 3537			
2980	Octyl homovanillamide		
2842	Hydrocortisone	F	F
3537	8-Methyl-N-vanillyl-6-nonenamide		
2082	Dexamethasone	F	F
2980 + 2842			F
2980 + 3537			F
2842 + 2082		F	F
1308	4-Methyl-2-mercaptopyridine-1-oxide		F
3537	8-Methyl-N-vanillyl-6-nonenamide		
2086	Indomethacin	F	F
1308 + 3537			F
1308 + 2086			
3537 + 2086			F
1308 (1.2 mg)	4-Methyl-2-mercaptopyridine-1-oxide		
1308 (0.6 mg)			F
1308 (0.3 mg)			
1308 (0.15 mg)			
2086	Indomethacin	F	F
2086 + 1308 (1.2 mg)			
2086 + 1308 (0.6 mg)			
2086 + 1308 (0.3 mg)			
2086 + 1308 (0.15 mg)			
37	DMPS		
1308	4-Methyl-2-mercaptopyridine-1-oxide		F
3537	8-Methyl-N-vanillyl-6-nonenamide		
2086	Indomethacin	F	F
37 + 1308			F
37 + 3537		F	F
37 + 2086			F
37 IP pre	DMPS IP pre-treatment	F	F
(37 IP + 1308) pre			
(37 IP + 1308 + 2086) pre			
(37 IP + 1308 + 2086) post		F	F
(37 IP + 1308) post		F	F
37 IP post	DMPS IP post-treatment	F	F

From this testing, it was evident that several effective pretreatments were not effective as treatments (ICD #36, 2082, 2086, 2842). Compounds #1308, 2525, 2980 and 3537 generally were effective when tested alone or in combinations when compared to the HD-only control. For #1308, 2980 and 3537, none of the combination therapies provided significantly greater reduction in HD injury than did the compound alone. Combination therapies with #36 and 37 provided significantly greater reduction in HD injury only when #1308, 2980, or 3537 was included in the combination. The conclusions derived from this testing were that:

- a. No individual compound or combination tested appeared more effective than #1308 alone.
- b. No combination therapy provided significantly greater reduction in HD injury than either individual component.

Another study was conducted in which a maximum volume of the highest concentration of four compounds (#1308, 2086, 2525, and 3537) was applied to both ears of study mice. This provided toxicity data, tabulated below, for these compounds prior to conducting additional combination experiments.

ICD#	Normal dose	Dose applied	Day 14 #alive/total#	Day 14 motor test #passed/total#	Avg body wt change from Day 0-14	Clinical observations
1308	0.6 mg	6 mg	10/10	10/10	+ 4.2 g	Normal
(mercapto)		3 mg	10/10	10/10	+ 6.8 g	Normal
		1.5 mg	10/10	10/10	+ 5.9 g	Normal
2086	0.34 mg	4 mg	0/10	--	--	See footnote #1
(indo)		2 mg	1/10	1/1	+ 0.9 g	See footnote #2
		1 mg	5/10	4/5	+ 4.6 g	See footnote #3
2525	6.25 mg	37.5 mg	10/10	10/10	+ 3.1 g	Erythema Day 1-2
(BAL)		25 mg	10/10	10/10	+ 4.3 g	Erythema Day 1-2
		12.5 mg	10/10	10/10	+ 5.8 g	Erythema Day 1-2
3537	0.25 mg	4 mg	10/10	10/10	+ 4.9 g	Ears held erect through Day 4-5
(capsaicin)		2 mg	10/10	10/10	+ 6.2 g	Ears held erect through Day 4-5
		1 mg	10/10	10/10	+ 6.3 g	Ears held erect through Day 4-5
EtOH	--	--	10/10	10/10	+ 7.2 g	Normal

Footnote #1: 3 died Day 2
 4 died Day 3
 1 died Day 4
 2 died Day 6
 Inactive, not eating, no stool, ruffled fur, dehydrated, lethargic prior to death.

Footnote #2: 1 died Day 2
 6 died Day 3
 2 died Day 5
 1 survivor returned to normal on Day 9.

Footnote #3: 2 died Day 2
 3 died Day 5
 3 survivors normal
 2 survivors returned to normal on Day 4.

Molecular Biomarkers

Task 0002 funds were allocated to investigate alterations in molecular biomarker gene expression in the mouse ear vesicant model with treatment compounds. The Study Director for this portion of Task 0002 is Carol L. Sabourin, Ph.D. The results of the study were accepted for publication in *J Toxicol Cutaneous Ocul Toxicol*.

In early 2004, an estimate of cost to complete this task resulted in a decrease of \$85K to the negotiated cost. *In vivo* work was completed in April 2004. The project duration has been extended until November 2004, which will provide time to complete final testing requested by the client and to close out this project.

Abstracts of two manuscripts are included in Appendix A.

Two posters were presented at the U.S. Army Medical Defense Bioscience Review, May 2004. Abstracts are included in Appendix A of this report.

Task 0003 – Efficacy rTSP DTN Screening

Work was completed prior to 2003 for Modules 3 (Penetration Cell Liquid Test), and 5 (Rabbit Lesion Area Ratio [LAR] Test). Battelle has received no indication that further work on this task will be requested. A Draft Report will be prepared.

Module 1 (Penetration Cell Vapor Test)

- Of the nine candidates challenged with chemical agent vapor, three (ICD 3970, 3973, and 3996) passed the screen with fewer than 2/5 trials per candidate allowing more than 1 µg of HD to penetrate over a 20-hr test, and three (ICD 3965, 3970, and 3973) passed the screen against GD.

Module 2 (M8 Paper Test)

- Of the 17 ICD candidates challenged with liquid HD, the following 16 passed the screen, allowing breakthrough in fewer than 2/9 trials over a 6-hr test:

4020	4080
4029	4081
4050	4082
4052	4083
4076	4084
4077	4085
4078	4086
4079	4087

Module 5 (Rabbit GD and VX Liquid Survivability Test)

- A dose-lethality response curve was prepared for the standard and the leading candidates, ICD 3834, 4028, and 4029. The results, shown in Figure 1, indicate that all three of the candidates were as effective as the standard against VX.

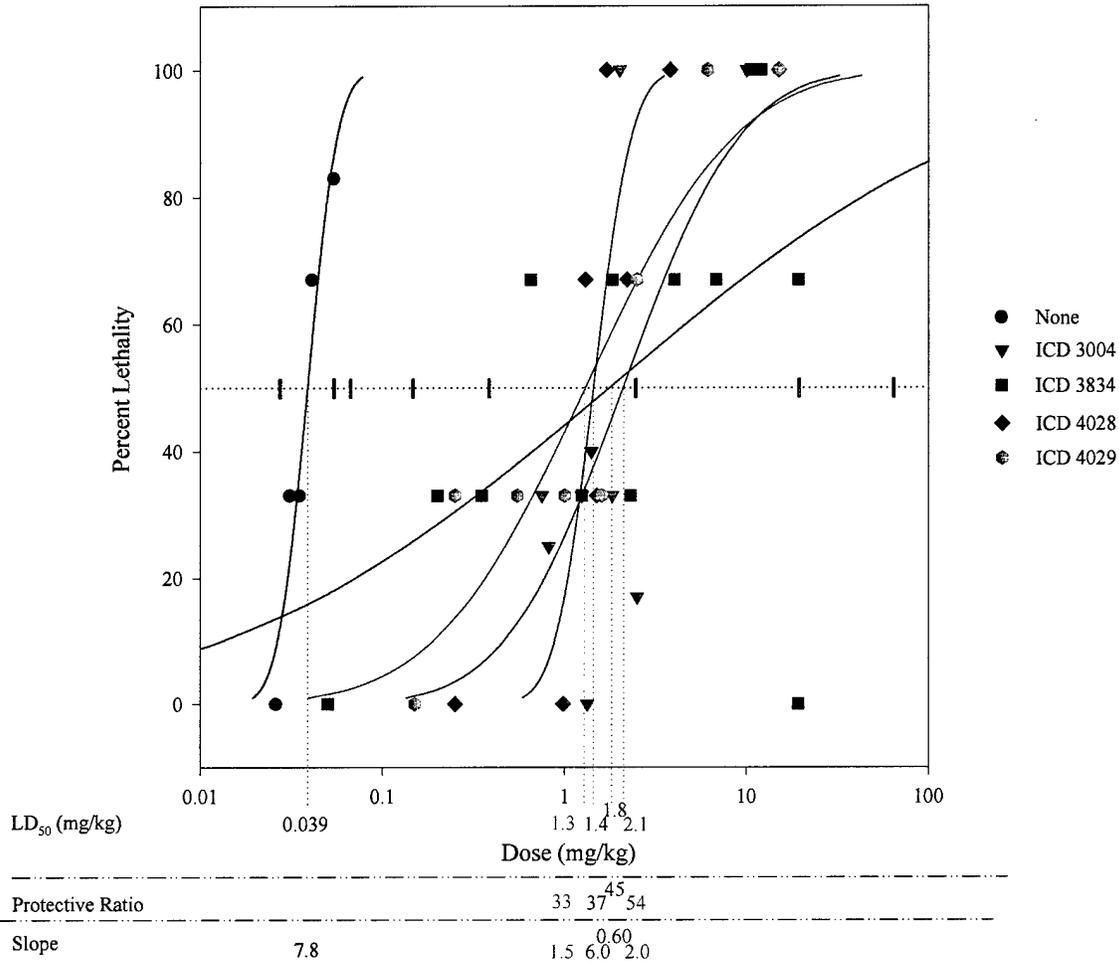


Figure 1. Liquid Percutaneous VX Dose – Lethal Response of Rabbits

Module 7 (Rabbit GD Vapor Survivability Test)

- Of the six candidates challenged with GD vapor, four (ICD 4020, 4029, 4051, and 4052) were found to be significantly better than no pretreatment, whereas ICD 4028 and 4050 were statistically equivalent to no pretreatment. Relative to the standard, ICD 3004, all except ICD 4028 were superior.
- Re-formulations of four of those candidates were tested as well, and ICD 4020, 4029, and 4052 were found to be significantly better than no pretreatment, whereas ICD 4050 was statistically equivalent to no pretreatment. Relative to the standard, all were superior.

Module 9 (Guinea Pig GD Liquid Survivability Test)

- A dose-lethality response curve was determined for the standard and leading candidates, ICD 3834, 4028, and 4029. The results indicated that ICD 3834 and 4029 were significantly more effective than the standard against GD, whereas ICD 4028 was equivalent to the standard. See Figure 2 below.

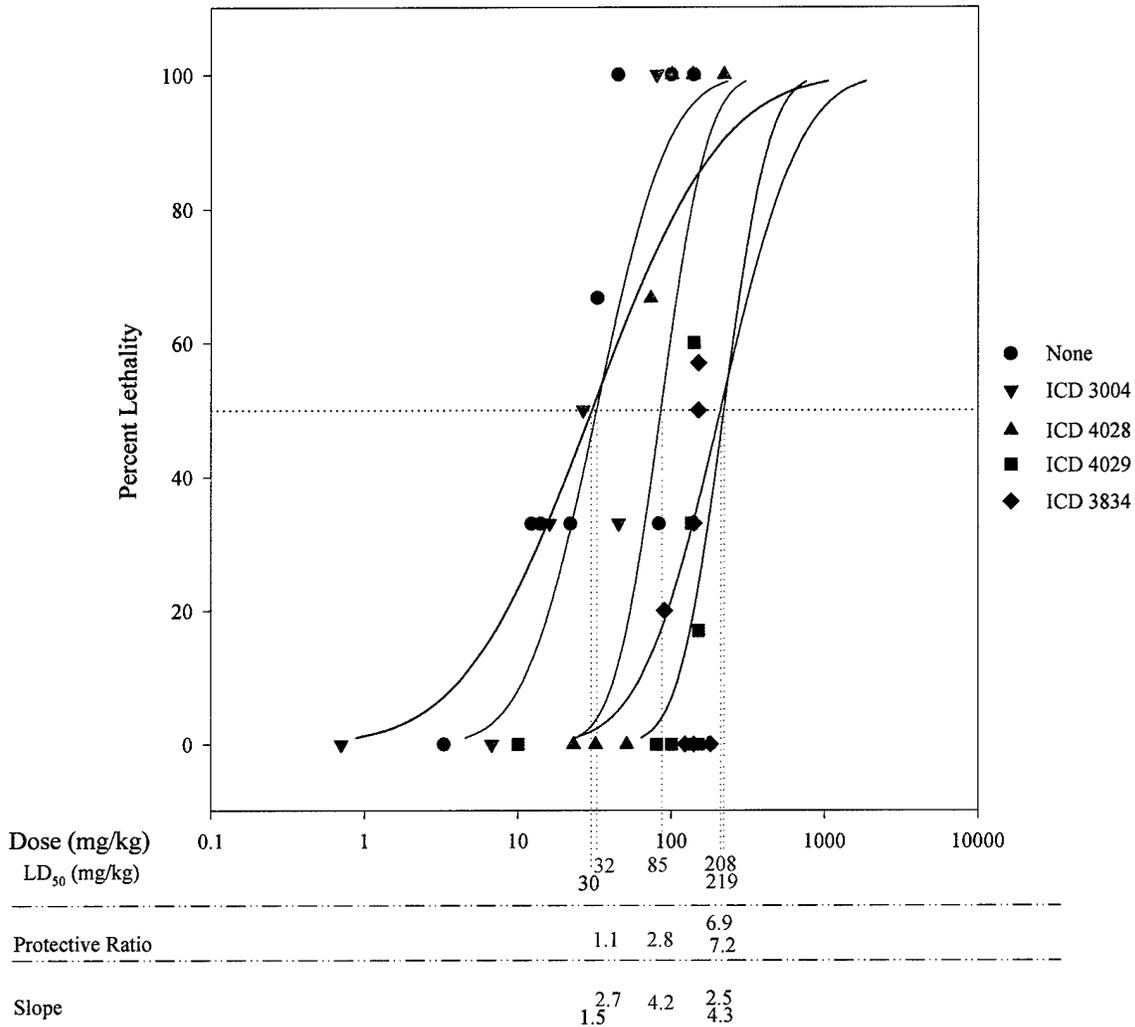


Figure 2. Liquid GD Percutaneous Dose – Lethality Response of GPs

- Results from a dose optimization study indicated that 100 mg/kg of liquid GD over a 2-hr topical exposure period allows discrimination among candidate aTSPs for protective efficacy.
- Of the seven candidates challenged with GD liquid, six, ICD 3834, 4020, 4029, 4050, 4051, and 4052, were significantly better than no pretreatment, whereas ICD 4028 was statistically equivalent to no pretreatment. Relative to the standard, ICD 3004, only 4020 and 4029 were superior.

Task 0007 – Gene Array Analysis (1176 cDNA Blot)

Dr. James Dillman requested that studies with the 5002 gene microarray continue for HD-treated ears/control ears and for two drug-HD-treated ear/control ear studies using five animals per group and three groups. He also requested that studies that were using animals at three HD doses and five different time points be discontinued. All tissue samples from the Phase 3 drug studies have been sent to Dr. Dillman at USAMRICD. The Final Report was submitted in May 2004 and the study file closed.

One manuscript resulting from this task was accepted for publication in *Toxicology*. One presentation was prepared for the 43rd Annual Meeting of the Society of Toxicology held in Baltimore, MD in March 2004, and another poster was presented at the U.S. Army Medical Defense Bioscience Review in Hunt Valley, MD, May 2004. Abstracts are in Appendix A.

Task 0008 – Canadian Reactive Skin Decontamination Lotion (RSDL)

The Final Reports for clinical studies conducted at HillTop Research, Inc. have been received and forwarded to the Chemical/Biological Medical Systems (CBMS) Project Management Office. The Draft Final Report for the clinical work was approved. The Final Report for the clinical work is being completed. The Task Summary report also is being prepared.

A poster was presented at the annual meeting of the Society of Toxicology. A copy of the abstract is included in Appendix A of this report.

Task 0009 - Russian V-Agent (VR) and VX Effects in Monkeys

The objectives of this task were to determine in a nonhuman primate model the toxicity of Russian V-agent (VR) in relation to that of VX, and to determine the efficacy of pyridostigmine bromide (PB) pretreatment and atropine (ATR)/ pralidoxime chloride (2-PAM) treatment of intoxication with VR or VX. This task also was to develop a new animal model and to compare results from cynomolgus monkeys (*Macaca fascicularis*) to those obtained earlier in rhesus monkeys (*Macaca mulatta*).

Since the best available data on effects of nerve agents in rhesus monkeys are for soman (GD), the median lethal dose (MLD) of intramuscularly (im) administered GD was estimated in cynomolgus monkeys. The MLD with ATR and 2-PAM treatment also was estimated. The effect on the GD MLD of adding PB pretreatment to the ATR/2-PAM therapy was determined next. A study to predict the im dose of PB necessary to create 20-30 percent red blood cell (RBC) acetylcholinesterase inhibition (AChE-I) at approximately 1 hr following injection also was needed. Correlation of blood cholinesterase (ChE) activity measurements using Hitachi and Roche instruments, and of ChE activity measurements performed at USAMRICD and at Battelle was accomplished.

The PB dose estimated to produce 25 percent whole blood ChE inhibition in cynomolgus monkeys was 57 µg/kg. In rhesus monkeys, the PB dose estimated to produce 25 percent

inhibition of RBC ChE activity is approximately 25 µg/kg. Because of these results, the *in vitro* response to PB of blood samples (n = 3) from five different primate groups (humans, Chinese-origin rhesus, Indian-origin rhesus, African green, and cynomolgus monkeys) was analyzed. Baseline whole blood ChE activity levels were similar in Indian rhesus, Chinese rhesus, cynomolgus and African green monkeys, but were significantly less than the human level. Plasma ChE activity levels were similar in human beings, Indian rhesus, Chinese rhesus, and cynomolgus, but these were significantly greater than that in African green monkeys. RBC AChE activity levels were more variable among the groups. These were highest in humans, lowest in cynomolgus, and mid-level in Chinese rhesus, Indian rhesus, and African green monkeys. All species exhibited a strong inhibitory response to PB. In whole blood, the dose-response trend was significantly steeper for African green monkeys than for humans, Indian rhesus, Chinese rhesus, and cynomolgus monkeys. Baseline ChE and AChE activity, and the *in vitro* inhibitory response to PB, differed among primate groups; in particular, baseline plasma ChE and whole blood ChE inhibition measured in African green monkeys differed from those in other primates.

The MLDs for GD without any treatment and with ATR/2-PAM treatment were not statistically different than those established in earlier studies for rhesus monkeys, however the protective ratio (PR; the MLD of animals with therapy divided by the MLD of untreated animals) provided by ATR/2-PAM was virtually 1, i.e., there essentially was no benefit of this treatment. The PR provided by pretreatment with PB and ATR/2-PAM therapy was less than 5 in cynomolgus monkeys, significantly less than that in rhesus monkeys of earlier studies. The estimated MLD of VX in cynomolgus monkeys was about twice the MLD in rhesus monkeys, while the MLD of VR was about the same as VX in rhesus.

Because of the differences in response to therapy, ATR pharmacokinetic (PK) studies were performed. The maximum serum ATR concentration attained was higher in cynomolgus monkeys than in rhesus monkeys, the maximum concentration was attained much faster in cynomolgus monkeys than in rhesus monkeys, and ATR was eliminated from the serum faster than in rhesus monkeys.

An additional 12 cynomolgus monkeys were tested to determine whether administration of the 0.4 mg/kg ATR dose in two 0.2 mg/kg injections would provide better protection from GD than a single 0.4 mg/kg ATR injection. Three of eight animals scheduled to receive the second ATR dose 40 min after GD challenge survived long enough to be administered the second 0.2 mg/kg ATR injection. Because of the early deaths, ATR injections were re-scheduled for 1 min and 10 min following GD challenge for the last four animals. Three of these four animals survived long enough to be administered the second dose. The split ATR dose provided significantly greater protection against significantly greater GD challenge doses, despite the loss of half the animals prior to administration of the second ATR injection.

The results obtained with cynomolgus monkeys are different in many respects from those obtained with rhesus monkeys. The ChE inhibition in blood following PB injection differed, as did the ATR PK, the response to chemical agents, and the response to therapy following injection of chemical agents. Experimentation under this task ceased, and remaining animals were transferred to Task 0016, "Efficacy of Oximes Against Organophosphorus Compounds in Nonhuman Primates." A Final Report was written, submitted, and accepted by USAMRMC.

Task 0010 – Evaluation of Decontamination Procedures for Chemical Warfare Agent Exposure to Skin

In the rabbit, relative erythema was not a sensitive enough endpoint to distinguish among candidate personal skin decontaminants (PSDs) against a topical 1- μ L challenge of sulfur mustard. However, using edema lesion area as an endpoint for evaluating PSD efficacy, several PSDs proved significantly effective and superior to the currently fielded M291 Skin Decon Kit, as shown in Figure 3 below.

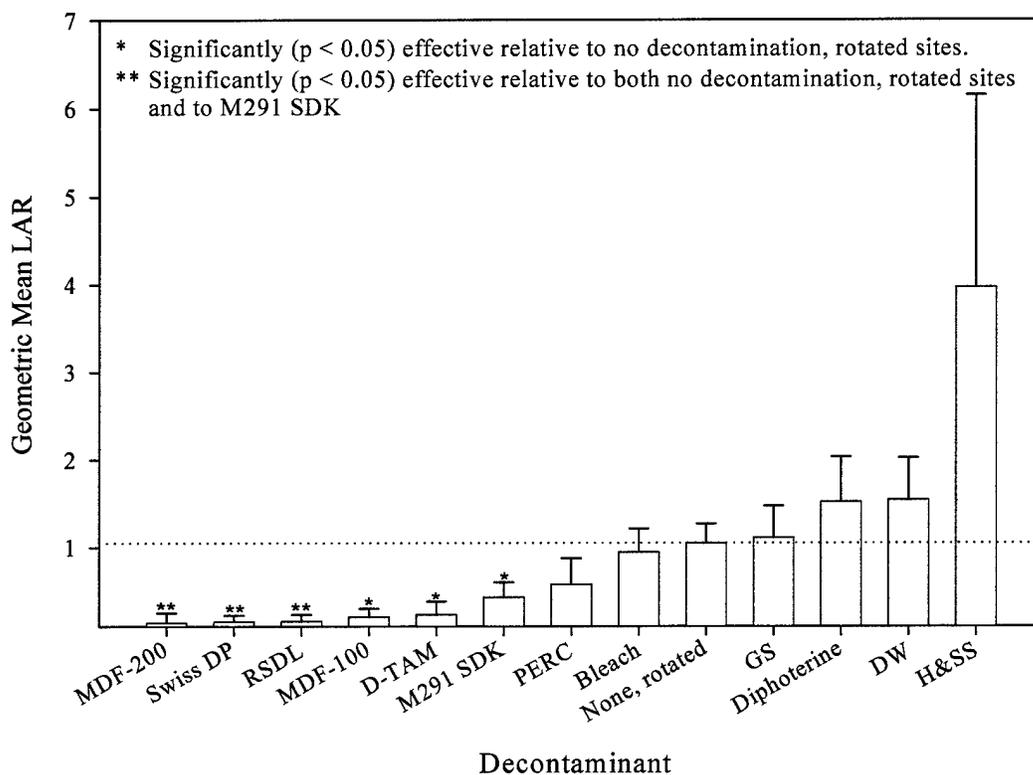


Figure 3. Geometric Mean Lesion Area Ratio and Upper 95% Confidence Limit in Rabbits Challenged Topically with 1000 nL of HD and Treated with 12 Candidate Personal Skin Decontaminants (Data Pooled Across Two Module 2 Studies)

All laboratory work for this task is complete. A Draft Report was sent to the Army in July 2003. A Final Report was submitted in November 2003, and accepted by USAMRMC.

Task 0012 - The Efficacy of Candidate Regimens in Promoting Improved Healing of Cutaneous Sulfur Mustard Burns in Weanling Swine

Part A, an HD dose ranging study to determine parameters that produce epidermal only and superficial dermal lesions was completed. An Interim Report was submitted to USAMRICD in June 2003. Dr. John Graham met with Dr. Frances Reid in July 2003 to review the data, and exposure times for the validation study, Part B, were selected. Part B was initiated in August 2003, and the *in vivo* portion of the validation study completed in September. A Draft Final Report is being prepared.

**Task 0013 - Localization of Substance P Gene Expression
for Evaluating Protective Countermeasures**

A Draft Final Report, "Task 0013, Localization of Substance P Gene Expression for Evaluating Protective Countermeasures Against Sulfur Mustard", was sent to Drs. James Dillman and David Lenz (COR) in September 2003. The Final Report was accepted in March 2004. A manuscript describing results of this task was accepted for publication in *Toxicology*. Manuscript and poster abstracts are located in Appendix A of this report.

**Task 0015 – Evaluation of SERPACWA and
Dilute Bleach Decontaminant Against Novel Agents**

This task was expanded in scope to include testing of combinations of SERPACWA and the leading candidate active topical skin protectants (aTSP), ICD 3834 and ICD 4028, against challenges with VX and HD in both liquid (subscript "L") and "derivatized" (subscript "D") forms as shown in the following table.

Dose Response Endpoint:	Challenge Material			
	VX _D	VX _L	HD _D	HD _L
Pretreatment	24-hr lethality		24-hr lesion	
None	+	+	+	+
SERPACWA	+	+	+	+
ICD 3834	+	+	+	+
ICD 4028	+	+	+	+

Percent lethality of hairless guinea pigs was used as the endpoint for VX, and lesion area ratios on the backs of hairless guinea pigs was used as the endpoint for HD. A Draft Final Report for the original statement of work, and one for the add-on work, is being prepared.

**Task 0016 - Efficacy of Oximes Against Organophosphorus Compounds in
Non-Human Primates**

This study was to estimate the median lethal dose (MLD) of two anticholinesterase compounds (CMP 2 and CMP 4) when injected intramuscularly (im) in male cynomolgus monkeys (*Macaca fascicularis*). It also was to estimate the treatment efficacy of pyridostigmine bromide (PB) injected prior to challenge in conjunction with atropine (ATR), one of several oximes, and diazepam (DIAZ) injected sequentially starting 1 min following challenge with CMP 2. The MLD of CMP 2 is 11.5 µg/kg (9.4-14.1), and the MLD of CMP 4 is 4.5 µg/kg (3.7-5.6). The MLDs of these compounds without therapy are statistically the same as those in rhesus monkeys (*Macaca mulatta*), as determined in Task 97-50.

Eight animals have been treated with 2-PAM as oxime, two following 8X CMP 2 MLD, two following 6X MLD, two at 4X MLD, and two at 2X MLD. Seventeen monkeys have been treated with ICD 585 as oxime, five following 10X CMP 2 MLD, four following 8X MLD, two at 6X MLD, four at 4X MLD, and two at 2X CMP 2 MLD. Fifteen nonhuman primates (NHP) have been treated with ICD 039 as oxime, seven following 10X CMP 2 MLD, four following 8X

MLD, two at 6X MLD, and two at 4X MLD. Eleven NHP have been treated with HI6 DMS as oxime, five following 10X CMP 2 MLD, four at 8X MLD, and two at 6X CMP 2 MLD.

Results of probit model fitting of the lethality data at various times are presented in the table below. Protective ratios (PR; the MLD of animals with therapy divided by the MLD of untreated animals) and statistical evaluation of therapies compared with no treatment also are presented in table format. Statistical evaluation of quality of life, based on clinical signs of intoxication, is being performed to determine if significant differences exist.

**Separate Slopes Probit Model Fitted to Dose-Lethality Data for
Five Treatment Groups of Monkeys Challenged with Compound 2**

Treatment Group	Nos. of Animals	24-hr LD ₅₀ (ug/kg)	48-hr LD ₅₀ (ug/kg)	7-Day LD ₅₀ (ug/kg)
None	8	11.6	11.6	11.6
2-PAM	8	69.0	53.9	46.0
HI-6	11	108	108	79.7
ICD-039	15	115	115	96.6
ICD-585	17	87.8	75.9	65.4

Protective Ratios of Oximes Relative to the Untreated Group of Monkeys

Treatment Group	24-hr Lethality		48-hr Lethality		7-Day Lethality	
	Protective Ratio (95% Confidence Interval)	P-value for Comparing Ratio to 1.0 #	Protective Ratio (95% Confidence Interval)	P-value for Comparing Ratio to 1.0 #	Protective Ratio (95% Confidence Interval)	P-value for Comparing Ratio to 1.0 #
2-PAM	5.4 (2.6, 11.2)	<.0001	4.3 (2.1, 8.7)	<.0001	3.4 (1.6, 6.9)	0.0010
HI-6	9.4 (5.0, 17.7)	<.0001	9.4 (5.1, 17.3)	<.0001	4.8 (2.4, 9.7)	<.0001
ICD-039	8.4 (4.6, 15.4)	<.0001	8.5 (4.7, 15.1)	<.0001	7.8 (4.4, 13.7)	<.0001
ICD-585	6.8 (3.7, 12.3)	<.0001	6.1 (3.4, 11.1)	<.0001	5.6 (3.2, 9.9)	<.0001

The LD₅₀ for the oxime is significantly greater than that of the untreated group if the protective ratio is significantly greater than one (unity) at the p<0.05 level.

**Task 0017 - Joint Service Family of
Decontamination Systems (JSFDS) Block III**

Modules SD110 and 120 for *in vitro* chemistry testing have been completed and the data compiled. Modules SD210 and SD220 for the *in vitro* removal testing have been completed. The Draft Summary Report has been completed. Modules SD160, SD180, and SD260,

biological agent testing, have been completed. The Draft Summary Report has been completed. The SD310 *in vivo* HD lesion area ratio testing was completed, and the Draft Final Report was submitted in March 2003. The laboratory testing for SD370 was completed, and the Draft Final Report is in the final stages of preparation. Based upon the results of previous work completed under this task, SD320 testing will not be conducted. Results of testing were presented to the Source Selection Evaluation Board. Summary Reports of testing of each product submitted were prepared, and the draft reports are being reviewed by the client.

The scope of work for this task was expanded and a revised proposal was submitted in December 2003. The execution of new work was begun in January 2004. This work will continue testing with RSDL. The scope of this additional work includes the development and validation of an analytical method to extract diacetyl monoxime (DAM) from blood in support of future clinical studies. The work was initiated and will continue through next quarter. The additional work also included coordination with HillTop Research, Inc. to develop a draft protocol for a clinical study entitled, "A Study to Assess the Absorption and Safety of 2,3-Butanedione Monoxime in Topically Applied RSDL." The draft protocol was sent for review by HillTop in January 2004. A meeting to discuss the draft protocol was held in March 2004, and the protocol was revised and sent to the Chemical/Biological Medical Systems (CBMS) Project Office. CBMS communicated changes to the draft, and a draft final protocol, informed consent form, and advertisement are in preparation for submission to the following in sequence: HillTop, Army, Battelle. The Investigator's Brochure has been revised and a draft was sent to CBMS in March 2004. The brochure is in the process of being finalized.

Task 0018 – Efficacy of Human Butyrylcholinesterase for Soman Poisoning

Objectives of this study were to 1) estimate the effect of human butyrylcholinesterase (HuBuChE) injected intramuscularly (im) on blood cholinesterase activity over time in cynomolgus monkeys (*Macaca fascicularis*), and 2) determine the effectiveness of injected human BuChE in preventing lethal intoxication of cynomolgus monkeys by soman (GD). Six male, cynomolgus monkeys were dosed intramuscularly with HuBuChE. Three received approximately 5.2 mg/kg body weight and three received approximately 8.65 mg/kg. Blood samples were taken periodically over 8 days and sent to USAMRICD for ChE analyses and kinetic modeling.

Six naïve monkeys then were injected im with approximately 24.1 mg/kg of HuBuChE and following blood ChE analyses approximately 10 hr later, injected im with approximately 1.5 X GD median lethal dose (MLD). No signs of intoxication were observed, and following blood ChE analyses approximately 1 hr 45 min after GD injection, the same animals were injected with approximately 2 X GD MLD. Again, no signs of intoxication were observed, and following further blood ChE analyses approximately 2 hr later, the monkeys were injected again with 2 X GD MLD. One animal exhibited typical signs of intoxication, including tremors, excessive salivation/bronchial discharge, convulsions and prostration starting at approximately 4 min after the third GD injection. The animal exhibited signs throughout the day and was found dead the following morning. A second monkey exhibited similar signs of intoxication, and this animal was euthanatized on the second day. The four remaining animals did not exhibit signs of

intoxication other than a transient inappetence. These four animals were sent to Walter Reed Army Institute of Research, and remain in good health.

Results of this task were presented in a poster at the 2004 U.S. Army Medical Defense Bioscience Review. A Final Report is being prepared.

Task 0019 - Government Testing of the Sorbent Decontamination System (SDS) in Accordance with the Decision Tree Network

After completing the initial modules and reviewing the data, it was recommended that before further testing was conducted the decontamination mitt be redesigned to better disseminate the sorbent material. This recommendation was accepted and the materials were remanufactured. The replacement material was received and testing was completed. The results were presented to the Army in July 2003. The laboratory testing for Module SD310 was completed and data were compiled and reported. Module SD320 testing was completed.

The Module SD370 protocol was written and approved by Battelle's IACUC. Approval from the USAMRMC Animal Care and Use Review Office has been received.

Laboratory testing on subcontracts with Springborn Laboratories (SD420 and SD440) and Sitek Research Laboratories (SD410) has been completed. SD420 and SD440 work was initiated in March 2003 and was completed in June 2003. The SD410 cytotoxicity work was completed and the Draft Report was submitted in March 2003.

Task 0020 - Evaluation of Decontamination Solutions for Use on Remains

This task was awarded, incrementally funded, and the laboratory work completed. The Final Report was submitted and accepted and the task closed out.

Task 0021 - Midazolam Study in Primates

This task was cancelled.

Task 0022 - Study to Determine the Maximum Attainable Specific Activity of [Methyl-¹⁴C] Dimethyl Methylphosphonate in 100-200 mCi Quantities and Synthesize 0.5 g of [Methyl-¹⁴C] GD with the Maximum Attainable Specific Activity Determined in Phase I

The evaluation of the synthesis of [¹⁴C] Dimethyl Methylphosphonate has been completed. A letter report indicating the synthesis was successful was prepared. A Draft Final Report was submitted in March 2004.

Task 0023 – Efficacy Testing of Cholinesterase Reactivators in the Rabbit

This task was cancelled.

Task 0024 - Oxime Toxicity in Mice

Phase 1, 24-hr range-finding and intramuscular (im) median lethal dose (MLD) studies were conducted for 7 oximes (2PAM/Cl, HI-6 dimethane sulfonate, HI-6 dichloride, ICD #039, 585, 692, and 2445). Probit dose-response models were fitted to the data to estimate MLDs. Phase 2A was conducted to determine toxicity of a single im dose of each oxime using 14-day clinical observations, periodic body weights, motor incapacitation testing, and gross necropsies. Phase 2B determined toxicity of a single im dose of each oxime in conjunction with atropine and Phase 2C determined toxicity of a single im dose of each oxime in conjunction with atropine and a pre-treatment with pyridostigmine bromide. Tissues will be sent to USAMRICD. All *in vivo* work was accomplished before the end of May 2004.

REPORTABLE OUTCOMES

Presentations were prepared for the 43rd annual meeting of the Society of Toxicology, Baltimore, MD in March 2004. A copy of abstracts are included in Appendix A of this report.

- Sabourin CL, Rogers JV, Choi YW, Kiser RC, Casillas RP, Babin MC, Schlager JJ. Time- and dose-dependent analysis of gene expression in sulfur mustard-exposed mice. *The Toxicologist* 78, 1891, 2004.
- Rogers JV, Choi YW, Kiser RC, Casillas RP, Babin MC, Schlager JJ, Sabourin CL. Gene expression in mice exposed to low and high levels of sulfur mustard. *The Toxicologist* 78: 1733, 2004.
- Tonucci DA, Masachi S, Lockhart L, Millward M, Liu D, Clawson R, Murphy V, O'Dell P, Lanouette, MC, Hayes T, Sabourin C. Clinical safety of Reactive Skin Decontamination Lotion (RSDL). *The Toxicologist* 78: 1724, 2004).
- Casbohm SL, Rogers JV, Stonerock MK, Martin JL, Ricketts-Kaminsky KM, Babin MC, Casillas RP, Sabourin CL. Localization of substance P gene expression for evaluating protective countermeasures against sulfur mustard. *The Toxicologist* 78: 1730, 2004.

The following manuscripts were submitted for publication. Abstracts of these manuscripts are included in Appendix A of this report.

- Casbohm SL, JV Rogers, MK Stonerock, JL Martin, KM Ricketts-Kaminsky, MC Babin, RP Casillas, CL Sabourin. Localization of substance P gene expression for evaluating protective countermeasures against sulfur mustard. *Toxicology*, accepted.
- Sabourin CLK, JV Rogers, YW Choi, RC Kiser, RP Casillas, MC Babin, JJ Schlager. Time- and dose-dependent analysis of gene expression in sulfur mustard-exposed mice. Submitted.

- Rogers JV, YW Choi, RC Kiser, MC Babin, RP Casillas, JJ Schlager, CLK Sabourin. Microarray analysis of gene expression in murine skin exposed to sulfur mustard. Submitted.
- Sabourin CLK, YW Choi, MK Stonerock, JD Waugh, RC Kiser, MM Danne, KL Buxton, RP Casillas, MC Babin, JJ Schlager. Expression profiling of sulfur mustard exposure in murine skin: chemokines, cytokines, and growth factors. In: Valdes JJ and Sekowski JW (eds), *Toxicogenomics and Proteomics*. IOS Press NATO Science Series, Series I: Life and Behavioural Sciences 2004, Vol. 356, pp 109-116.
- Babin, MC, MY Gazaway, N Krogel, LW Mitcheltree, KM Ricketts, K Skvorak, RE Sweeney, I Koplovitz, RC Kiser, DM Moore, and RP Casillas. A 7-day mouse model to assess protection from sulfur mustard (SM) skin injury. *J Toxicol Cutaneous Ocul Toxicol*, Vol. 22, No. 4, pp. 231-242, 2003.
- Sabourin CLK, JV Rogers, MK Stonerock, NA Niemuth, RC Kiser, SL Casbohm, MC Babin, JJ Schlager, and RP Casillas. Modulation of cytokine gene expression by anti-inflammatory agents following in vivo sulfur mustard injury. *J Toxicol Cutaneous Ocular Toxicol*, In press 2004.

Presentations were prepared for the U.S. Army Medical Defense Bioscience Review in Hunt Valley, MD, May 17-21, 2004. Abstracts of these presentations are included in Appendix A of this report.

- Sabourin CL, Rogers JV, Choi YW, Kiser RC, Casillas RP, Babin MC, Schlager JJ. Time- and dose-dependent analysis of gene expression in sulfur mustard-exposed mice.
- Rogers JV, Choi YW, Kiser RC, Casillas RP, Babin MC, Schlager JJ, Sabourin CL. Gene expression in mice exposed to low and high levels of sulfur mustard.
- Casbohm SL, Rogers JV, Stonerock MK, Martin JL, Ricketts-Kaminsky KM, Babin MC, Casillas RP, Sabourin CL. Localization of substance P gene expression for evaluating protective countermeasures against sulfur mustard
- Kiser RC, Moore DM, Niemuth NA, Biddle BM, Shumaker SM, Babin MC, Casillas RP, Koplovitz I, Smith, WJ. Combination treatments against cutaneous sulfur mustard exposure in the mouse ear vesicant model (MEVM).
- Kiser RC, Moore DM, Niemuth NA, Biddle BM, Shumaker SM, Babin MC, Koplovitz I, Smith WJ. Toxicity of candidate antivesicant compounds.
- Hoffman TL, Snider TH, Matthews MC, Graham JS, Doxzon BF, Lumpkin HL, Stevenson RS, Hanssen KA, Deckert RR, Braue EH, Jr. Assessment of active topical skin protectants against challenges with sulfur mustard, soman, or VX.
- Snider TH, Jarvis RJ, Matthews MC, Braue EH, Jr. Assessment of personal skin decontaminants against topical challenges with sulfur mustard.

- Wilhelm CM, Snider TH, Matthews MC, Maxwell DM. Efficacy of SERPACWA and personal skin decontaminants against topical VX challenges.
- Reid FM, Graham JS, Niemuth NA, Matthews MC, Hoffman T, Vasconcelos D. Development of a weanling swine model for sulfur mustard-induced superficial dermal injury.
- Reid FM, Graham JS, Niemuth NA, Matthews MC, Hoffman TL, Vasconcelos D. Histopathologic characterization of a superficial dermal sulfur mustard-induced lesion in the weanling swine model.
- Lenz DE, Clark CR, Capacio BR, Luo C, Saxena A, Doctor BP, Olson CT. Protection against soman poisoning by human butyrylcholinesterase in cynomolgus monkeys.

CONCLUSIONS

During the fourth year of the contract, the MREF met the requirements to sustain a chemical agent laboratory, passed all external organization inspections, and conducted critical research in support of the Medical Chemical Defense Research Program for the U.S. Army Medical Research and Materiel Command.

REFERENCES

Not applicable.

APPENDICES

Appendix A. Manuscript/Presentation Abstracts

APPENDIX A. MANUSCRIPT/PRESENTATION ABSTRACTS

Time- and Dose-Dependent Analysis of Gene Expression in Sulfur Mustard-Exposed Mice

CL Sabourin¹, JV Rogers¹, YW Choi¹, RC Kiser¹, RP Casillas¹,
MC Babin¹, and JJ Schlager²

¹Battelle Memorial Institute, Medical Research & Evaluation Facility,
Columbus, OH 43201-2693

²Pharmacology Division, US Army Medical Research Institute of Chemical Defense,
Aberdeen Proving Ground, MD

ABSTRACT

The chemical warfare agent sulfur mustard (SM) produces blister formation with a severe inflammatory reaction in skin of exposed individuals. The development of efficacious countermeasures against SM vesication requires an understanding of the cellular and molecular mechanism of SM-induced tissue injury. This study examined SM-induced alterations in gene expression using microarrays (5002 genes) to identify transcriptional events associated with SM skin injury. Mice (n=3) were exposed topically to SM (0.04, 0.08, and 0.16 mg) on the inner surface of the right ear and skin tissues were harvested at 1.5, 3, 6, and 12 h. Genes were selected based on the three mice in the same dose group demonstrating a ≥ 2 fold increase or decrease in gene expression for the SM-exposed tissue when compared to the methylene chloride vehicle control ear at all 3 doses and 4 time points. At the 0.04 mg SM dose, the genes observed were primarily involved in inflammation, apoptosis, and cell cycle regulation. Exposure to 0.08 mg SM increased the expression of genes related to inflammation and cell cycle regulation. Exposure to 0.16 mg SM led to a total of six genes that were changed at all observed time periods; however these genes do not appear to be directly influential in biological mechanisms such as inflammation, apoptosis, and cell cycle regulation as was observed at the lower SM doses of 0.04 and 0.08 mg. These functional categories have been observed in previous studies utilizing both *in vivo* and *in vitro* model systems of SM-induced dermal injury, suggesting that molecular mechanisms associated with inflammation, apoptosis, and cell cycle regulation may be appropriate targets for developing prophylactic/therapeutic treatments for SM skin injury.

This work was conducted under the U.S. Army Medical Research and Materiel Command Contract. DAMD17-99-D-0010, Task Order 0007.

Gene Expression in Mice Exposed to Low and High Levels of Sulfur Mustard

JV Rogers¹, YW Choi¹, RC Kiser¹, RP Casillas¹, MC Babin¹,
JJ Schlager², and CL Sabourin¹

¹Battelle Memorial Institute, Medical Research & Evaluation Facility,
Columbus, OH 43201-2693

²Pharmacology Division, US Army Medical Research Institute of Chemical Defense,
Aberdeen Proving Ground, MD 21010-5400

ABSTRACT

Sulfur mustard [bis-(2-chloroethyl)-sulfide; SM] exposure leads to blister formation in skin of exposed individuals. This study examined SM-induced changes in gene expression using cDNA microarrays in skin from mice cutaneously exposed to SM. Ear skin from five mice, paired as SM-exposed right ear and vehicle control left ear at six dose levels (0.005, 0.01, 0.02, 0.04, 0.08, and 0.16 mg), was harvested at 24 h. Alterations in gene expression were analyzed using cDNA microarrays containing 1,176 genes. Genes were selected on the basis of all mice (N=5) in the same dose group demonstrating a ≥ 2 -fold increase or decrease in gene expression in SM-exposed tissue compared to control skin at all six SM doses. When comparing skin exposed at all six SM doses with controls, a total of 19 genes within apoptosis, transcription factors, cell cycle, inflammation, and oncogenes and tumor suppressors categories were found to be up-regulated; no genes were down-regulated. A comparison of skin exposed to low (0.005 and 0.01 mg) and high (0.08 and 0.16 mg) doses of SM showed differences in the number and category of genes that were up- or down-regulated. Low level SM primarily altered transcription factors and repressors as well as genes involved in the cell cycle (e.g., *cdc25B*; cyclins) and apoptosis (e.g., *GADD45*; TNF receptor ligands); whereas high level SM also altered genes related to cytokines, chemokines, growth factors and hormones (*IL-1* α , *MCP-1*, *MIP-1* α and *MIP-2*). The results of this study provide a further understanding of the molecular responses to cutaneous SM exposure, and enable the identification of potential diagnostic markers and therapeutic targets for treating exposure to low and high levels of SM.

This work was conducted under the U.S. Army Medical Research and Materiel Command under Contract No. DAMD17-99-D-0010, Task Order 0007.

Clinical Safety of Reactive Skin Decontamination Lotion

DA Tonucci¹, S Masaschi¹, M Millward¹, L Lockhart¹, D Liu², R Clawson², V Murphy³,
P O'Dell⁴, M Lanouette⁵, TL Hayes⁶, and CL Sabourin⁶

¹Hill Top Research, Inc, Cincinnati, OH, ²Chemical Biological Medical Systems Project
Management Office, Ft Detrick, MD, ³MarCorSysCom, Quantico, VA, ⁴O'Dell Engineering,
Cambridge Ontario, Canadian Department of National Defence
Ottawa, Ontario,
⁶Battelle, Columbus, OH

ABSTRACT

A clinical program was designed to assess the dermal safety of a new personal skin decontaminant system. Reactive Skin Decontamination Lotion (RSDL) is a liquid, reactive lotion which removes from the skin and destroys chemical warfare agents and toxins. The clinical program included: a 21-day cumulative dermal irritation in 30 subjects, a Repeat Insult Patch Test (RIPT; Jordan/King modified Draize design) in 200 subjects and a photo-irritancy/allergenicity study in 30 subjects. For cumulative irritancy 25 µL of RSDL applied to the kit applicator (1 cm punch of applicator sponge) was patched occlusively for 21 consecutive days. Results from the study indicated that RSDL was of low irritancy potential versus the positive (0.5 M sodium lauryl sulphate) and negative (normal saline) controls. For allergenicity, 25 µL of RSDL applied to the kit applicator and patched 9 times, with continuous exposure, for a 3-week induction phase, followed by a 2-week rest period where subjects received no exposure to RSDL and then a single challenge application at a naïve site. RSDL was not allergenic in the RIPT as indicated by low erythema scores reported during the challenge phase. Finally, RSDL was tested for phototoxicity by comparing the skin reaction to a single exposure to RSDL with or without UVA/UVB exposure. Photoallergenicity was determined in a similar manner as in the RIPT with the exception that subjects were exposed to UVB radiation after patch removal during induction and challenge. RSDL was found not phototoxic, nor photoallergenic. These studies indicate that RSDL has a low risk for the development of dermal toxicity.

Supported by RSDL/Foreign Comparative Testing Program and conducted under USAMRMC Contract No. DAMD17-99-D-0010.

Localization of Substance P Gene Expression for Evaluating Protective Countermeasures Against Sulfur Mustard

SL Casbohm¹, JV Rogers¹, MK Stonerock¹, JL Martin², KM Ricketts-Kaminsky²,
MC Babin¹, RP Casillas¹, and CL Sabourin¹.

¹Medical Research & Evaluation Facility, Battelle Memorial Institute, Columbus, OH and ²US
Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD

ABSTRACT

Sulfur mustard [bis(2-chloroethyl)sulfide; SM] is a chemical warfare agent that produces edema and blister formation with a severe inflammatory reaction. The neuropeptide Substance P (SP) contributes to skin inflammation. The mouse ear vesicant model for cutaneous SM injury has been used to evaluate pharmacological agents for countering SM injury. The vanilloid, olvanil has been shown to reduce SM-induced edema and mRNA expression of cytokines and chemokines, suggesting that blocking the inflammatory effects of SP may provide protection against SM-induced dermal injury. This study examined SP expression in skin exposed to SM. Mice were exposed topically to SM (0.16 mg) on the inner surface of the right ear, with or without olvanil pretreatment, and tissues were collected at 1, 10, 30, 60, and 360 min following SM exposure. SP mRNA was localized in skin using *in situ* hybridization. In naïve skin, mRNA localization was associated with blood vessels and sebaceous glands. In SM-exposed samples, hybridization was also detected in perivascular dermal cells. SP protein expression was identified immunohistochemically in the ear skin of naïve, SM-, olvanil/SM-, and vehicle-treated mice. For the inner surface of the ear, the mean number of SP-positive perivascular cells in the dermis of olvanil-treated/SM-exposed ears was significantly lower than that of SM-exposed ears at both 60 and 360 min. In the outer surface of the ear, the mean number of SP-positive perivascular cells in the dermis of olvanil-treated/SM-exposed ears was significantly lower than that of SM-exposed ears at 360 min. These findings suggest that drugs targeting pro-inflammatory neuropeptides may reduce the severity of SM-induced cutaneous damage. Supported by the U.S. Army Medical Research and Materiel Command under Contract No. DAMD17-99-D-0010, Task Order 0013.

SUBMITTED

**Microarray Analysis of Gene Expression in Murine
Skin Exposed to Sulfur Mustard**

JV Rogers,¹ YW Choi,¹ RC Kiser,¹ MC Babin,¹ RP Casillas,¹
JJ Schlager,^{2,3} and CLK Sabourin,^{1,*}

¹Battelle Memorial Institute, Medical Research and Evaluation Facility
Columbus, OH 43201

²US Army Medical Research Institute of Chemical Defense, Pharmacology Division,
Aberdeen Proving Ground, MD 21010

³Present Address: Air Force Research Laboratory, Operational Toxicology Branch
(AFRL/HEST), Wright-Patterson AFB, OH 45433

ABSTRACT

The chemical warfare agent sulfur mustard [bis-(2-chloroethyl)-sulfide; SM] produces a delayed inflammatory response followed by blister formation in skin of exposed individuals. Studies are underway evaluating the efficacy of pharmacological compounds to protect against SM skin injury. Microarray analysis provides the opportunity to identify multiple transcriptional biomarkers associated with SM exposure. This study examined SM-induced changes in gene expression in skin from mice cutaneously exposed to SM using cDNA microarrays. Ear skin from five mice, paired as SM-exposed right ear and unexposed left ear at six dose levels (0.005, 0.01, 0.02, 0.04, 0.08, and 0.16 mg), was harvested at 24 hr post-exposure. SM-induced gene expression was analyzed using cDNA microarrays that included 1,176 genes. Genes were selected on the basis of all mice (N=5) in the same dose group demonstrating a ≥ 2 -fold increase or decrease in gene expression for the SM-exposed tissue compared to the control ear tissue at all six SM doses. When skin exposed to all six concentrations of SM was compared to controls, a total of 19 genes within apoptosis, transcription factors, cell cycle, inflammation, and oncogenes and tumor suppressors categories were found to be up-regulated; no genes were observed to be down-regulated. Differences in the number and category of genes that were up- or down-regulated in skin exposed to low (0.005-0.01 mg) and high (0.08-0.16 mg) doses of SM were also observed. The results of this study provide a further understanding of the molecular responses to cutaneous SM exposure, and enable the identification of potential diagnostic markers and therapeutic targets for treating SM injury.

KEYWORDS: Sulfur Mustard (SM); Skin; Inflammation; Microarray; Gene Expression; Mouse

**Expression Profiling of Sulfur Mustard Exposure in Murine Skin:
Chemokines, Cytokines, and Growth Factors**

CLK Sabourin¹, YW Choi¹, MK Stonerock¹, JD Waugh¹, RC Kiser¹, MM Danne¹,
KL Buxton¹, RP Casillas¹, MC Babin¹, and JJ Schlager²

¹Battelle Memorial Institute, Medical Research and Evaluation Facility, Columbus, OH, USA;

²US Army Medical Research Institute of Chemical Defense, Pharmacology Division, Aberdeen Proving Ground, MD, USA

ABSTRACT

The chemical warfare agent sulfur mustard (SM) produces a delayed inflammatory response followed by blister formation in skin of exposed individuals. Severity and time to onset of inflammation and tissue damage vary greatly and depend on various factors such as dose, ambient temperature, and exposure site. Gene arrays provide the opportunity to identify multiple transcriptional events associated with low-level exposure SM. This study examined SM-induced changes in gene expression in skin from mice cutaneously exposed to SM using cDNA arrays. Skin from 5 mice, paired as SM-exposed right ear and unexposed left ear at six dose levels (0.005, 0.01, 0.02, 0.04, 0.08, and 0.16 mg) was harvested at 24 hours post-exposure. The lowest concentration (0.005 mg) produced no apparent tissue damage. The highest SM dose (0.16 mg) produced severe injury characterized by edema, dermal infiltration of inflammatory cells, premature death of basal layer epidermal cells, and epidermal-dermal separation. We analyzed SM-induced genes using cDNA microarray technology, which included 1,176 genes. The genes were classified in six categories. Chemokines, cytokines and growth factors represented a large number of the genes altered with SM exposure. A number of these genes not known to be associated with SM toxicity were identified. The results provide not only a new molecular basis for understanding the response to SM, but also a useful resource for future development of diagnostic markers and therapeutic targets for SM injury.

A 7-Day Mouse Model to Assess Protection from Sulfur Mustard (SM) Skin Injury

MC Babin^{1,2*}, KM Ricketts², RC Kiser¹, MY Gazaway², N Krogel², LW Mitcheltree²,
DM Moore¹, K Skvorak², RE Sweeney², I Koplovitz², and RP Casillas¹

¹Battelle Memorial Institute

Medical Research and Evaluation Facility, Columbus, OH.

²U.S. Army Medical Research Institute of Chemical Defense

Aberdeen Proving Ground, MD.

ABSTRACT

The mouse ear vesicant model (MEVM) is a screening tool used to identify protective compounds against acute sulfur mustard (SM)-induced skin injury. It provides endpoints of edema and histopathology 24 h following a topical SM exposure to assess protection against inflammation and tissue damage. To further evaluate successful compounds, the MEVM was modified for use as a 7-day model. Dose response studies were conducted with SM to select an optimal challenge dose for the new model. Due to severity of SM-induced tissue damage by Day 7, edema and histopathology were determined unreliable endpoints. Therefore, a modified Draize scoring system (no damage to extensive necrosis) was incorporated as an endpoint to evaluate tissue damage out to Day 7. To aid in optimal SM dose selection, retro synthetic capsaicin (RSCAP), a protective compound in the MEVM, was evaluated as a treatment 15 min before exposure to 0.06, 0.08, and 0.16 mg SM. RSCAP provided similar significant protection at Day 7 against the 0.06 (42% reduction) and 0.08 mg doses (32% reduction) but was not effective against the severely necrotizing 0.16 mg SM dose. Based on these results, an optimum SM dose of 0.08 mg was selected. RSCAP and two pharmacologically inactive analogs were tested as topical treatments 15 min prior to SM challenge. RSCAP significantly reduced injury, whereas the inactive analogs had no protective effect. RSCAP also significantly reduced SM injury when administered topically 10 min after SM challenge. These data support the use of the 7-day mouse ear vesicant treatment model (MEVTM) in evaluating candidate antivesicant compounds.

**Alterations of Gene Expression in Sulfur Mustard-exposed
Skin Topically-treated with Vanilloids**

CLK Sabourin,^{1,*} JV Rogers,¹ MK Stonerock,¹ NA Niemuth,¹ RC Kiser,¹ SL Casbohm,¹
MC Babin,^{1,2} JJ Schlager,³ and RP Casillas,¹

ABSTRACT

Sulfur mustard [bis(2-chloroethyl)sulfide, SM] is a chemical warfare agent that penetrates the skin rapidly and causes extensive blistering. Using the mouse ear vesicant model (MEVM), we evaluated the effect of topically applied anti-inflammatory agents (octyl homovanillamide and heptyl isovanillamide) on ear edema formation and gene expression following SM exposure. Relative ear weight and real-time reverse transcriptase polymerase chain reaction of GM-CSF, IL-1 β , and IL-6 were used to evaluate the effects of octyl homovanillamide and heptyl isovanillamide. Both vanilloids significantly reduced SM-induced edema. At the single dose and number of animals/group tested, octyl homovanillamide produced a trend of reduced mRNA levels; however, the reduction was not significant for GM-CSF, IL-1 β , or IL-6. Heptyl isovanillamide significantly reduced ($P \leq 0.05$) GM-CSF, IL-1 β , and IL-6 mRNA levels. These results show that octyl homovanillamide and heptyl isovanillamide reduce skin edema and heptyl isovanillamide significantly reduced cytokine mRNA expression following SM exposure. In addition to measuring edema formation, monitoring expression of biomarkers such as GM-CSF, IL-1 β , and IL-6 may also serve to evaluate therapeutic treatments against SM-induced dermal injury.

Modulation of Cytokine Gene Expression by Anti-inflammatory Agents Following *In Vivo* Sulfur Mustard Injury

CL Sabourin¹, MK Stonerock¹, NA Niemuth¹, RC Kiser¹, SL Casbohm¹, RP Casillas¹, MC Babin¹, and JJ Schlager².

¹Battelle Memorial Institute, Medical Research and Evaluation Facility, Columbus, OH, USA;
²US Army Medical Research Institute of Chemical Defense, Pharmacology Division, Aberdeen Proving Ground, MD, USA

ABSTRACT

Sulfur mustard [bis(2-chloroethyl)sulfide, SM] is a chemical warfare agent that penetrates the skin rapidly and causes extensive blistering after a latent period. We have used the mouse ear vesicant model for cutaneous SM injury quantitation to evaluate pharmacological agents in a number of drug classes, including anti-inflammatory drugs. Topically applied anti-inflammatory agents, including indomethacin and vanilloids, have been shown to reduce SM-induced skin inflammation and tissue damage. We previously identified an early increase in the *in vivo* expression of the inflammatory cytokines GM-CSF, IL-1 β , and IL-6 following murine cutaneous exposure to SM. The goal of this study was to determine the effect of topically administered anti-inflammatory agents in reversing inflammatory mediator gene expression following SM-induced injury. Alterations in mouse (n=6) cutaneous GM-CSF, IL-1 β , and IL-6 gene expression from ears with and without pre-treatment with indomethacin, heptylisovanillamide, and octyl homovanillamide were examined using real time quantitative RT-PCR. Indomethacin pretreatment produced a significant reduction in the SM-mediated increase of IL-1 β and IL-6 mRNA levels. Heptylisovanillamide pretreatment produced significant reductions of GM-CSF, IL-1 β , and IL-6 mRNA levels. Octyl homovanillamide produced an apparent reduction of cytokine mRNA levels; however, the reduction did not reach statistical significance at the present animals numbers. SM-induced inflammation was significantly modulated by all three anti-inflammatory agents as determined by reduction in tissue edema (n=10). The alteration in cytokine gene expression suggests that these drugs play a role in modulating SM-induced injury by reducing inflammatory pathways.

Supported by the US Army Medical Research and Materiel Command under Contract No. DAMD17-99-D-0010, Task Order 0002.

**Combination Treatments Against Cutaneous Sulfur Mustard
Exposure in the Mouse Ear Vesicant Model (MEVM)**

RC Kiser¹, DM Moore¹, NA Niemuth¹, BM Biddle¹, MC Babin¹, RP Casillas¹,
I Koplovitz², and WJ Smith²

¹Battelle Memorial Institute, Medical Research and Evaluation Facility,
Columbus, OH 43201-2693

²U.S. Army Medical Research Institute of Chemical Defense,
Aberdeen Proving Ground, MD 21010-5400

ABSTRACT

The mouse ear vesicant model (MEVM) has been used as a screening tool at Battelle to identify compounds protective against sulfur mustard (SM)-induced cutaneous injury. The original MEVM provided a quantitative edema response and histopathological endpoints as measurements of inflammation and tissue damage at 24 h following a topical SM (0.16 mg) exposure. Compound effectiveness was defined as a reduction in these endpoints. To further evaluate lead candidate compounds, the MEVM more recently was modified for use as a 7-day model. At the same time, a modified Draize scoring system of 0-4 (from no damage to extensive necrosis) was incorporated as the primary endpoint to quickly and easily evaluate and grade the extent of ear tissue damage up to Day 7 and/or Day 14. Nine lead candidate compounds were selected to be evaluated as single +10 min post-treatments after a 0.08 mg SM challenge. These compounds were tested alone and in combination with each other, with ear tissues being scored on Day 7 and additionally on Day 14. Compounds and/or combinations of compounds were determined to either pass or fail based on Draize ear tissue scores being statistically better than those observed for the SM no treatment control group. Day 7 Draize scores were significantly reduced when 4 compounds (4-Methyl-2-mercaptopyridine-1-oxide (ICD# 1308), BAL (ICD# 2525), octyl homovanillamide (ICD# 2980), and 8-Methyl-N-vanillyl-6-nonenamide or capsaicin (ICD# 3537) were tested alone and in combination with 7 or 8 of the other compounds. Even at Day 14, some tissue scores were significantly reduced when treated with 1308, 2980 and 3537 alone or in combinations with the other compounds. In many cases, however, the combination treatments were not significantly more effective than the individual treatments.

This work was supported by the U.S. Army Medical Research and Materiel Command under Contract DAMD17-99-D-0010, Task Order 0002.

Toxicity of Candidate Antivesicant Compounds

RC Kiser¹, DM Moore¹, NA Niemuth¹, BM Biddle¹, MC Babin¹, RP Casillas¹,
I Koplovitz², and WJ Smith²

¹Battelle Memorial Institute, Medical Research and Evaluation Facility,
Columbus, OH 43201-2693

²U.S. Army Medical Research Institute of Chemical Defense,
Aberdeen Proving Ground, MD 21010-5400

ABSTRACT

Since 1997, Battelle's Medical Research and Evaluation Facility has supported the U.S. Army Medical Research Institute of Chemical Defense program in evaluating candidate drugs for topical pretreatment and therapeutic protection against sulfur mustard (SM) injury using the mouse ear vesicant model (MEVM). Several compounds have proven to reduce tissue damage when tested as pre- and/or post-treatments against SM. In order to gain some additional information on the toxicity potential of four of these compounds, a study was conducted applying various doses of each compound to both ears and observing the mice for 14 days. Clinical observations and body weights over time were recorded. A simple motor incapacitation test was also administered. The motor incapacitation test consisted of placing a mouse on the top of a 5"x 5" screen that was subsequently inverted. Mice climbing back to the top of the 5"x 5" screen grid within 1 minute of inversion passed the performance test. Mice which fell or remained on the bottom of the grid for the one minute test period fail. The four compounds tested were 4-Methyl-2-mercaptopyridine-1-oxide (ICD# 1308), indomethacin (ICD# 2086), BAL (ICD# 2525), and 8-Methyl-N-vanillyl-6-nonenamide or capsaicin (ICD# 3537). Lethality was observed only at the high doses of ICD #2086. A dose response in average body weight changes from Day 0 to 14 was observed for the other three compounds. Body weight gains were significantly more variable at the high doses of these compounds. Motor incapacitation testing demonstrated no evidence of gross behavioral deficit for any of the compounds tested.

This work was supported by the U.S. Army Medical Research and Materiel Command under Contract DAMD17-99-D-0010, Task Order 0002.

Assessment of Active Topical Skin Protectants Against Challenges with Sulfur Mustard, Soman, or VX

TL Hoffman¹, TH Snider¹, MC Matthews¹, JS Graham², BF Doxzon², HL Lumpkin²,
RS Stevenson², KA Hanssen², RR Deckert² and EH Braue Jr.²

¹Battelle Memorial Institute, Medical Research and Evaluation Facility,
Columbus, OH 43201-2693

²U.S. Army Medical Research Institute of Chemical Defense,
Aberdeen Proving Ground, MD 21010-5400

ABSTRACT

Previous work with rabbits has demonstrated the protective efficacy of Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA) against sulfur mustard (SM), soman (GD), or VX. SERPACWA has been licensed by the FDA and fielded to U.S. troops. Over a 4-year program, the U.S. Army Medical Research and Materiel Command has investigated the prophylactic efficacies of more than 270 candidate active topical skin protectants (aTSPs). Each aTSP was formulated with one or more reactive moieties intended to actively bind, decontaminate, and/or render inactive a challenge agent before it can penetrate the skin. A series of three *in vitro* and six *in vivo* evaluation modules involving fixed-dose challenges was designed and implemented to identify lead candidates. Rabbits with hair clipped from the dorsum were used to assess candidates using an eight-block grid on each back, and challenging with 1- μ L volumes of SM covered with Teflon[®] disks. The area of each lesion 24 hr later was estimated and normalized by dividing by the lesion area at a control site on each rabbit. Weanling swine with hair clipped from the dorsum were used in a model involving a saturated SM vapor within a 12-mm diameter plastic cap. Candidate aTSPs were screened at a 30-min SM vapor exposure, from which passing candidates were reassessed over a range of 30- to 90-min exposures. Change in skin erythema (24 hr minus pre-dose baseline) at each test site was measured with a colorimeter. In one of the nerve agent modules, VX was placed onto a candidate test site on each rabbit and covered with a Teflon[®] disk. Survivability in 24 rabbits was evaluated at 24 hr and compared with that for SERPACWA to determine a pass/fail result. A similar model was used to evaluate GD challenges on clipped backs of guinea pigs. Candidates also were evaluated in the rabbit model against a saturated GD vapor generated within a plastic cap placed over each test site. Protective ratios for candidates against liquid GD or VX over a full range of 4-hr challenge levels were determined for three lead candidates and compared with that for SERPACWA.

This work was conducted under the U.S. Army Medical Research and Materiel Command Contract DAMD17-99-D-0010, Task Order 0003.

Assessment of Personal Skin Decontaminants Against Topical Challenges with Sulfur Mustard

TH Snider¹, RJ Jarvis¹, MC Matthews¹, and EH Braue Jr.²

¹Battelle Memorial Institute, Medical Research and Evaluation Facility,
Columbus, OH 43201-2693

²U.S. Army Medical Research Institute of Chemical Defense,
Aberdeen Proving Ground, MD 21010-5400

ABSTRACT

Several Personal Skin Decontaminants (PSD) were evaluated for efficacy against topical exposure to 2,2'-dichlorodiethyl sulfide (sulfur mustard; SM). Rabbits were used to assess, relative to no decontamination of 1 μL of neat SM, the efficacies of five treatments: D-TAM[®], PERC Formulation[®] (PERC), Swiss Decon Powder[®] (Swiss DP), Modec Decon Formulation 200[®] (MDF-200), and Head & Shoulders[®] shampoo (H&SS). Results with this model were previously obtained for distilled water, 0.5% bleach, the M291 SDK, Diphoterine[®], Reactive Skin Decontamination Lotion (RSDL[®]), MDF-100[®], and an enzyme-linked polyurethane sponge containing a solution of pralidoxime chloride in 2,5,8,11,14-pentaoxapentadecane (Gordon sponge). Decontamination applicators were made by attaching a wooden tongue depressor to a section of either cotton gauze or the specific pad material designated for RSDL, M291 SDK, and Gordon sponge. Swiss DP was applied according to the manufacturer's instructions. Rabbits were anesthetized, and dorsal hair was clipped. Baseline chromaticity (the red parameter, a^*) assessments were made at each of eight test sites drawn in a grid on each rabbit's back. A 1- μL volume of SM was applied at each test site. At 2 min after SM application, each test site was either treated with one of the candidates or left undisturbed. At 24 hr after dosing, test sites were rinsed, chromaticity assessments were made, and lesion areas were estimated and normalized by dividing by the lesion area at the no-treatment site to determine lesion area ratios (LARs). Change in a^* ($\Delta a^* = a^*_{24\text{-hr}} - a^*_{\text{baseline}}$) was calculated and normalized by dividing by Δa^* at the no-treatment site to determine relative erythema. Statistical comparisons of mean LARs and relative erythema from each PSD-treated group were made to determine efficacy. Relative erythema was not a useful endpoint for distinguishing among PSD efficacies. In order of decreasing efficacy, according to the LAR endpoint, the significantly effective PSDs were: MDF-200, Swiss DP, RSDL, MDF-100, D-TAM, and M291 SDK. Among these, MDF-200, Swiss DP, and RSDL were significantly better than the currently fielded M291 SDK against SM.

This work was conducted under the U.S. Army Medical Research and Materiel Command Contract DAMD17-99-D-0010, Task Order 0010.

Efficacy of SERPACWA and Personal Skin Decontaminants Against Topical VX Challenges

CM Wilhelm¹, TH Snider¹, MC Matthews¹, and DM Maxwell²

¹Battelle Memorial Institute, Medical Research and Evaluation Facility,
Columbus, OH 43201-2693

²U.S. Army Medical Research Institute of Chemical Defense,
Aberdeen Proving Ground, MD 21010-5400

ABSTRACT

Several Personal Skin Decontaminants (PSD) and SERPACWA were evaluated for efficacy against topical exposure to O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX). Rabbits with hair clipped from the dorsum were used to assess, relative to no decontamination, the efficacies of four PSDs: distilled water, soapy water (tincture of Green Soap USP, i.e., vegetable soap, lavender oil, glycerin, and ethanol, diluted 1:100 in distilled water), 0.5 percent aqueous sodium hypochlorite (bleach), and Modec Decon Formulation 200[®] (MDF-200). A probit analysis based on 24-h lethality data for each treatment was used to estimate a protective ratio (PR) for each of the PSDs. The evaluation model also was used to estimate a PR for test site pretreatment with a 0.1-mm thick layer of Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA), which has been fielded to U.S. troops. The 24-hr median lethal dose (LD₅₀) for VX alone was 0.048 mg/kg. PRs for soapy water, MDF-200, 0.5 percent bleach, and water were statistically ($\alpha = 0.05$) indistinguishable from each other at 9.3, 15, 15, and 17, respectively. Distilled water appeared to be as effective a decontaminant against topical VX as any of the four PSDs. The PR for SERPACWA was 52, indicating a high level of protection in the rabbit. Each of the five PRs was significantly ($P < 0.0001$) greater than unity, indicating significant protection.

This work was conducted under the U.S. Army Medical Research and Materiel Command Contract DAMD17-99-D-0010, Task Order 0015.

Development of a Weanling Swine Model for Sulfur Mustard-Induced Superficial Dermal Injury

FM Reid¹, JS Graham², NA Niemuth¹, C Matthews¹, T Hoffman¹, and D Vasconcelos¹

¹Medical Research and Evaluation Facility, Battelle
505 King Avenue, Columbus, OH, USA 43201

²Comparative Pathology Branch, Comparative Medicine Division, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, USA 21010

ABSTRACT

Sulfur mustard (SM) is a severe vesicating agent. Recommended SM lesion treatment is symptomatic. Discussions with the medical community and a literature review indicated a need for an animal model with superficial (epidermis only) and superficial dermal (upper third of the dermis) injuries. Eight female Yorkshire-cross pigs weighing ~10 kg were used in a dose ranging study. Undiluted liquid SM (400 μ L) was applied at each of six ventral abdominal sites with exposure times ranging from 15-30 s for superficial injuries and 1-16 min for superficial dermal injuries. Exposure times of 7 and 8 min were selected and evaluated in an additional 6 animals. Lesions were photographed, measured, and assessed for edema presence on Day 1 and Day 2 after SM exposure, after which animals were euthanatized and lesions excised for histopathology. In the dose ranging study, one-half of each lesion was processed to prepare hematoxylin and eosin (H&E) stained slides and the other half was frozen in liquid Freon for staining with neutral blue tetrazolium chloride (NBTC). Superficial lesions were characterized by a depth of injury of less than 0.1 mm (NBTC stain) and affected only the epidermis. Morphological findings included basal cell necrosis, endothelial cell damage, presence of intraepidermal pustules and neutrophil infiltrate, minimal to mild dermal congestion and hemorrhage, and occasional necrosis of follicles to the isthmus. Exposures less than 1 min tended to produce lesions with a patchy distribution and focal extensions through the epidermis. Dermal injury (minimal to mild dermal congestion, minimal dermal coagulation, and/or minimal dermal hemorrhage) was observed subjacent to basal cell necrosis. Superficial dermal injury (upper 1/3 of the dermis) occurred at sites exposed for 1 to 8 min, and were characterized by a depth of injury of 0.15 to 0.4 mm (NBTC). Superficial dermal morphology included lesions described as superficial injuries and more frequent dermal elastosis, coagulation, and greater congestion (all scored as mild). Basal cell necrosis affected ≥ 40 percent of the exposed area on average for exposure times of 6 to 8 min, and ≥ 80 percent for exposure times greater than 10 min. Follicular necrosis extended to the isthmus, but not the inferior segment. Dermal edema was not observed. Exposure times of 10 to 16 min produced a depth of injury >0.4 to 0.6 mm and necrosis involving follicles at the level of the infundibulum. Coagulation into the adipose tissue was present in sites exposed for greater than 12 min, and involved the panniculus muscle for exposures of 16 min. The dose-ranging study determined that use of the techniques established to produce SM-induced full-skin-thickness lesions did not produce uniform superficial (epidermis only) lesions. An exposure time of 8 min generated superficial dermal injuries on the ventral abdomen of weanling swine.

Supported by the US Army Medical Research and Materiel Command under Contract No. DAMD17-89-C-9050.

Histopathologic Characterization of a Superficial Dermal Sulfur Mustard-Induced Lesion in the Weanling Swine Model

FM Reid¹, JS Graham², NA Niemuth¹, MC Matthews¹,
TL Hoffman¹, and D Vasconcelos¹

¹Medical Research and Evaluation Facility, Battelle
505 King Avenue, Columbus, OH, USA 43201

²Comparative Pathology Branch, Comparative Medicine Division
U.S. Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD, USA 21010

ABSTRACT

Sulfur mustard (SM) is a severe vesicating agent. Although a SM-induced, full-skin thickness, dermal injury model has been developed in swine, a superficial dermal model, with lesions restricted to the upper third of the dermis, is needed. A SM dose ranging study with exposure times ranging from 5 s to 16 min demonstrated exposure times of 7 and 8 min would produce superficial dermal lesions. Six female, Yorkshire-cross pigs weighing 10 ± 2 kg were exposed to 400 μ L of undiluted SM at each of six ventral abdominal sites for 7 or 8 min. Lesions were photographed, measured, and assessed for edema on Day 1 and Day 2 after SM exposure, after which the animals were euthanized and lesions excised for histopathology. Three sections were taken from medial to lateral of each lesion and processed to provide H&E stained slides. Image analysis indicated an increase in lesion size from Day 1 to Day 2 for both 7 min (5.8 to 6.6 cm^2) and 8 min (6.1 to 7.0 cm^2) exposure times. Lesion area was significantly greater for 8 min than for 7 min exposures ($p = 0.02$) on Day 2. Anterior and middle lesions were greater in size than posterior lesions by Day 2. The depth of lesion (targeted injury depth of 18 to 36 percent) for both exposure times was the upper one third of the dermis, with dermal coagulation reported as 20.8 percent (SE = 1.2) for the 7 min exposure and 21.5 percent (SE = 1.1) for the 8 min exposure. Percent of lesion area affected by basal cell necrosis was not significantly different between the two exposure times, with an average of 40.1 percent for 7 min and 43.1 percent for 8 min exposures. Likewise, no significant differences between the two exposure times were observed for basal cell necrosis, dermal depth, dermal coagulation, intraepidermal blisters/pustules, endothelial cell damage, or follicular necrosis. Basal cell necrosis, bullae (dermoepidermal junction), and dermal hemorrhage were slightly greater on the right side than the left. The medial section had a slightly greater degree of basal cell necrosis than the middle or lateral sections. In general, the magnitude of the differences was not considered biologically significant, but was sufficient to select an 8 min exposure time.

Supported by the US Army Medical Research and Materiel Command under Contract No. DAMD17-89-C-9050.

**Protection against Soman Poisoning by
Human Butyrylcholinesterase in Cynomolgus Monkeys**

DE Lenz, CR Clark, BR Capacio, JM Federko**, C Luo**, A Saxena**,
BP Doctor** and CT Olson#

Pharmacology Division, 3100 Ricketts Pt. Rd. USAMRICD, APG, MD 21010

**Division of Biochemistry, 503 Robert Grant Road

WRAIR, Silver Spring, MD 20910

#Battelle, 505 King Ave., Columbus, Ohio 43201

ABSTRACT

Human butyrylcholinesterase (HuBuChE) purified from outdated human plasma was evaluated for efficacy in preventing nerve agent intoxication in cynomolgus monkeys. Previous studies in rodents and rhesus monkeys demonstrated that pretreatment of animals with enzymes that scavenge nerve agents provides significant protection from behavioral and lethal effects of these agents. To evaluate efficacy of HuBuChE prior to initiating an investigational new drug (IND) application, the pharmacokinetics and efficacy of HuBuChE were evaluated in cynomolgus monkeys. HuBuChE was injected intramuscularly (i.m.) at doses of 5.25 mg/kg or 8.75 mg/kg and periodic blood samples were taken to follow the concentration of HuBuChE in blood for 168 hr. The two doses of HuBuChE produced similar times of maximal blood concentration (T_{max} of 9.3 and 10.3 hr, respectively) and similar elimination half-times ($t_{1/2}$ of 79.3 and 73.5 hr, respectively). Enzyme levels were still 10 fold over baseline at 72 hr. Based on these data, cynomolgus monkeys were injected i.m. with 24.1 mg/kg of HuBuChE and challenged at T_{max} with soman (GD). Soman doses were approximately 1.5, 2.0 and 2.0 x LD_{50} and were administered sequentially i.m. 90-120 min apart. None of the animals displayed signs of organophosphorus (OP) anticholinesterase intoxication after the first two GD doses (a cumulative challenge of 3.5 x LD_{50}). After the third challenge dose, one animal died within one hr. The remaining animals survived for 48 hr. One monkey was sacrificed in moribund condition at 48 hr, and the other four animals showed no signs of intoxication and remain in apparent excellent health after four months. Survival was dependent upon the circulatory level of HuBuChE prior to challenge. These data, coupled with prior results with guinea pigs in which survival of all (n=10) animals occurred using a similar experimental design, provide strong support for the continued development of HuBuChE for protection from nerve agents.