USAMRICD-TR-17-01

Comparison of Four Skin Decontamination Procedures Using Reactive Skin Decontamination Lotion (RSDL) Following Cutaneous VX Exposure in Guinea Pigs

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January 2016

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an element of the

US Army Medical Research and Materiel Command

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
January 2017	Technical Report	January 2016-July 2016

4. TITLE AND SUBTITLE
Comparison of Four Skin Decontamination Procedures Using Reactive Skin Decontamination Lotion (RSDL) Following Cutaneous VX Exposure in Guinea Pigs

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Koplovitz, I, Schulz, S, Morgan, J, Reed, R., Clarkson, ED, Hurst, CG

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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2900 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

8. PERFORMING ORGANIZATION REPORT NUMBER

USAMRICD-TR-17-01

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Defense Threat Reduction Agency
8725 John J. Kingman Road, STOP 6201
Fort Belvoir, VA 22060-6201

10. SPONSOR/MONITOR'S ACRONYM(S)
DTRA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES
This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division

14. ABSTRACT
The objective of this study was to determine whether the effectiveness of Reactive Skin Decontamination Lotion (RSDL) as an immediate decontamination (DC) product following cutaneous exposure to VX was affected by the DC procedure. Fur-clipped, male, unanesthetized guinea pigs were used as subjects. Neat, liquid VX was applied to the clipped skin of the left flank. Two minutes after VX application, the exposure site was decontaminated with one of four RSDL DC procedures: 1) RSDL was applied and left on the skin; 2) RSDL was applied and removed after two min; 3) RSDL was applied, removed after two minutes, reapplied by placing an RSDL pad directly on the exposure site for ten minutes; and 4) RSDL was applied, removed after two minutes, and reapplied by dabbing the exposure site once with a fresh RSDL pad. Dose-lethality curves for VX were established for the RSDL DC procedures based on 24-hour alive-or-dead responses. LD_{50}s of VX were calculated by probit analysis. RSDL DC procedures 1, 2 and 3 resulted in VX LD_{50}s that were similar, while procedure 4 resulted in a VX LD_{50} that was significantly higher than each of the other three VX LD_{50}s. The results suggest that the effectiveness of RSDL as a skin DC product is dependent on the DC procedure, with physical removal and reapplication being the most effective.

15. SUBJECT TERMS
Decontamination procedures, RSDL, VX, cutaneous exposure, nerve agents

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Irwin Koplovitz
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED	11	**19b. TELEPHONE NUMBER** *(include area code)* 410-436-5342

Abstract

The objective of this study was to determine whether the effectiveness of Reactive Skin Decontamination Lotion (RSDL) as an immediate decontamination (DC) product following cutaneous exposure to VX was affected by the DC procedure. Fur-clipped, male, unanesthetized guinea pigs were used as subjects. Neat, liquid VX was applied to the clipped skin of the left flank. Two minutes after VX application, the exposure site was decontaminated with one of four RSDL DC procedures. Briefly, the procedures were as follows: 1) RSDL was applied and left on the skin; 2) RSDL was applied and removed after two min; 3) RSDL was applied, removed after two minutes, reapplied by placing an RSDL pad directly on the exposure site for ten minutes; and 4) RSDL was applied, removed after two minutes, and reapplied by dabbing the exposure site once with a fresh RSDL pad. Dose-lethality curves for VX were established for the RSDL DC procedures based on 24-hour alive-or-dead responses. LD_{50}s of VX were calculated by probit analysis. RSDL DC procedures 1, 2 and 3 resulted in VX LD_{50}s that were similar, while procedure 4 resulted in a VX LD_{50} that was significantly higher than each of the other three VX LD_{50}s. The results suggest that the effectiveness of RSDL as a skin DC product is dependent on the DC procedure, with physical removal and reapplication being the most effective.

Introduction

Decontamination (DC) is "the process of removing or neutralizing hazardous substances from people, equipment, structures and the environment."[1] DC of hazardous chemicals from the skin is an integral part of the medical management of cutaneous exposures. Experimental studies have shown that rapid and early DC can prevent and/or greatly reduce injury and the severity of intoxication. U.S. military doctrine recommends DC within two to three minutes of exposure for optimal effectiveness.[2,3] While the effectiveness of the immediate DC process depends greatly on how quickly it is performed, the DC procedures and how thoroughly they are conducted are also likely to impact effectiveness. Although military personnel are well-trained to perform personal DC, the procedures and techniques used will vary between individuals, especially during the "fog of war." The purpose of this study was to evaluate whether the efficacy of RSDL as an immediate DC product was affected by the DC procedure, using an unanesthetized guinea pig model exposed dermally to VX and decontaminated two minutes after exposure.

Materials and Methods

Animals: Male guinea pigs [Hartley, Crl(HA)BR] were obtained from Charles River (Canada) and ranged in weight from 300 to 450 grams at the time of experimentation. After arrival, the animals were maintained in quarantine for at least five days prior to use in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited animal care and use facility. On the morning of an experiment, around 0800 hrs, animals were weighed, the fur was carefully removed on the left side with electric clippers, and excess loose fur was removed with a vacuum. An exposure site was outlined with an indelible marker at approximately the same location on the left side of each animal midway between the spine and the ventral midline. The animals remained unanesthetized during the entire experiment. After VX exposure and DC, animals were housed in individual cages without bedding in a fume hood for the duration of the experiment (24 hours). Food and water were provided *ad libitum* after VX exposure and DC. Animals were hand restrained by trained technicians for all procedures.

VX Exposure: Each exposure day a 50 µl aliquot of neat VX was obtained from the Chemical Exclusion Area, USAMRICD. RSDL was purchased in sealed packages from First Line Technology, Chantilly, VA. Neat VX was applied in a fume hood to the marked exposure site of each animal using either a 5 µl Hamilton syringe for volumes greater than 1 µl, or a 0.5 µl or 1.0 µl Hamilton digital syringe for volumes less than 1 µl. Both syringes had blunt tips. The syringe was lowered into the primary agent vial, excess agent was drawn up, the syringe was removed from the vial, and while making sure the syringe was not in contact with any surface, the volume was adjusted to the target volume. Excess agent was removed from the outside of syringe by wiping the syringe with a poly-urethane swab. The guinea pig was restrained with the previously marked exposure site pointing up in the fume hood by the assistant. The syringe was held perpendicular to the exposure site, the agent was expelled from the syringe, and contact with the exposure site was briefly made with the tip of the needle. Care was taken to ensure that the exposure site did not show any signs of irritation (nicks, scratches or abrasions).

DC procedure: Two minutes after applying VX to the skin, the exposure site was decontaminated with RSDL. RSDL was applied with an applicator made by stapling one fourth of an RSDL sponge pad (25 mm x 50 mm) to a wooden tongue depressor. The RSDL applicators were made just before the start of the experiment, and until use were placed in the opened original packaging. RSDL was applied by swiping the applicator across the exposure site ten times in a head-to-tail direction with steady pressure on the exposure site during each swipe. A fresh applicator was used for each animal. Four DC procedures were evaluated. In the first procedure, after application, RSDL remained on the skin for the duration of the experiment (hereafter referred to as ON). In the second, two minutes after its application, RSDL was removed by swiping the exposure site ten times in a head-to-tail direction with a similar size applicator made from a folded gauze pad dampened with 5 ml of water (hereafter referred to as OFF). In the third, two minutes after its application, RSDL was removed as described in procedure 2 and immediately reapplied by placing half of a fresh RSDL sponge pad (50 mm x 100 mm) on the site and securing it in place by wrapping the animal with Vetrap®. The pad was allowed to remain on the animal for ten minutes and was then removed, leaving any RSDL residue on the skin for the remainder of the experiment (hereafter referred to as PAD). In fourth, two minutes after its application, RSDL was removed as in procedure 2 and then immediately reapplied by dabbing the exposure site once with a fresh RSDL applicator, and leaving any residue on the skin for the remainder of the experiment (hereafter referred to as DAB).

Experimental Design: VX dose-lethality curves were generated in parallel for each of the RSDL DC procedures by exposing each group of animals to various doses of VX and evaluating the survival or lethality at 24 hours. A modified adaptive, stage-wise, dose design was used to generate the VX dose-lethality curves for each RSDL DC procedure. The first stage utilized the classic up-down dose design of Dixon to estimate a LD_{50} of VX for each RSDL procedure.[4] Briefly, one animal at a time in each RSDL group was challenged with a dose of VX, and the response was assessed at 24 hours. After the 24-hour response was determined, the next animal in each RSDL group received either a higher (if the previous animal was alive at 24 hours) or lower (if the previous animal was dead at 24 hours) dose of VX, depending on the response of the previous animal. The up-down procedure continued until four response reversals were observed. The 24-hour responses in each RSDL group from Stage 1 were analyzed by probit analysis using SAS NLIN and special purpose probit programs developed by Battelle (Columbus, Ohio) to generate an interim LD_{50} estimate.[5] The next stages of the experiment used three to eight animals per stage and various doses of VX in each stage for each RSDL group to improve the LD_{50} estimate and to generate 95% confidence intervals (CI) by both the Fieller's and the delta methods.[6,7] The VX doses in each stage were selected to improve the LD_{50} estimate and 95% CI based on responses from all stages. Interim probit analyses were run after each stage, and the experiment was stopped when the ratio of the upper delta 95% CI minus the lower delta 95% CI divided by two times the LD_{50} estimate was < 0.4.[5] After exposure and DC, each animal was monitored continuously until the onset of toxic signs, again at two and four hours after DC, and then at 24 hours after exposure.

Statistical Analysis: A final probit analysis was conducted on all stages for each RSDL group. The slopes and LD_{50}s, as well as the LD_1, LD_{10}, LD_{16}, LD_{30}, LD_{70}, LD_{84}, LD_{90}, and LD_{99} with their respective 95% CI were calculated by both Fieller's and delta methods. LD_{50} estimates for the four RSDL groups were compared using another SAS program, which compared the ratio of

all pairs of the LD_{50} estimates for the 4 RSDL groups. The slopes of the dose-lethality curves were compared according to Zar.[8]

Results

Table 1 lists the individual VX challenge doses and response fractions for the four RSDL groups. The resulting 24-hour probit dose-lethality curves are shown in Fig 1. Table 2 presents the 24-hour LD_{50} estimates and delta 95% CI along with the slope of each curve. Fifteen to nineteen animals were needed to generate VX LD_{50} estimates with delta confidence intervals meeting the stopping criteria. The VX LD_{50} for the RSDL DC procedures ON (3380 µg/kg), OFF (2956 µg/kg) and PAD (3357 µg/kg) were similar and not statistically different from each other. The VX LD_{50} for the RSDL DC procedure DAB (5639 µg/kg) was significantly ($p<0.05$) greater than each of the other three RSDL DC procedures. Because of the unexpected result with the RSDL DAB procedure, the VX dose-lethality curve for this DC procedure was repeated. In regenerating the VX dose-lethality curve for the DAB procedure, stage 1 of the methodology was not run, since we already had an estimate of the VX LD_{50}.

Table 1: Dermal VX challenge doses and response fractions 24 hours after exposure for four RSDL DC procedures

ON		OFF		PAD		DAB	
µg/kg	No.Dead /N	µg/kg	No.Dead /N	µg/kg	No.Dead /N	µg/kg	No.Dead /N
2310	0/3	1155	0/1	1155	0/1	2310	0/2
2900	1/3	1638	0/2	1638	0/2	3277	0/2
3277	1/3	2310	1/3	1950	0/1	4620	2/3
3800	2/3	3276	1/3	2310	1/4	5490	0/1
4620	3/3	3890	2/2	2600	0/1	6514	0/2
		4620	2/2	2900	0/3	7750	2/2
		6514	1/1	3100	0/3	9240	4/4
				3277	2/2		
				4620	2/2		

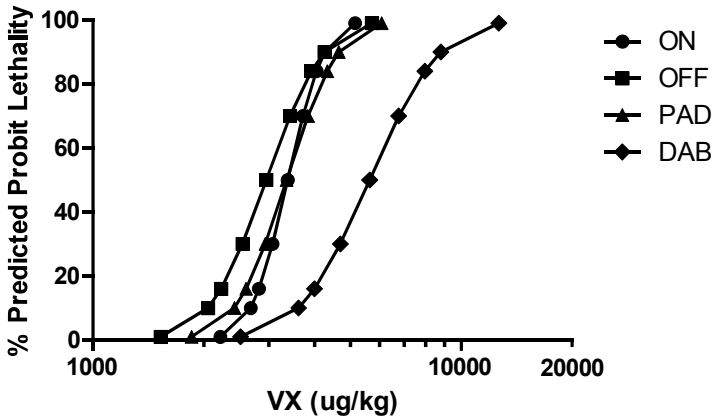

Figure 1: Twenty-four-hour probit dose lethality curves for four RSDL DC procedures. ON = RSDL applied and not removed; OFF = RSDL applied and removed after 2 min; PAD = RSDL applied, removed after 2 min, and immediately reapplied by placing an RSDL pad on exposure site for 10 min; DAB = RSDL applied, removed after 2 min, and immediately reapplied with a single dab with a new RSDL applicator.

Table 2: Data summary for four RSDL DC procedures

RSDL Group	N	Slope	VX LD_{50}, µg/kg, p.c. (95% CI)
ON	15	12.7	3380* (2921 – 3910)
OFF	14	8.1	2956* (2295 -3807)
PAD	19	9.0	3357* (2704 – 4166)
DAB	16	6.6	5639 (4276 – 7437)

* Significantly different from the DAB RSDL group, $p<.05$.

Table 3 lists the individual VX challenge doses and response fractions for the repeat RSDL DAB (DAB 2) procedure. DAB designates the first result and DAB 2 designates the repeat result. The dose-lethality curve for the DAB 2 responses is shown in Figure 2 along with the curve for the original DAB responses, and the 24-hour LD_{50} estimates and slopes are summarized in Table 4. The VX LD_{50} estimate for DAB 2 (6207 µg/kg) was higher but not significantly different from the DAB (5639 µg/kg) estimate, thus corroborating the initial finding. However, the slope of the DAB 2 (16.2) VX dose-lethality curve was significantly steeper than the slope of the DAB (6.6) VX dose-lethality curve.

Table 3: Dermal VX challenge doses and response fractions 24 hours after exposure for the repeat RSDL DAB dose-lethality curve

DAB 2	
µg/kg	No.Dead /N
2321	0/2
3279	0/2
4631	0/3
5508	1/3
6542	2/4
7773	3/3
9240	3/3

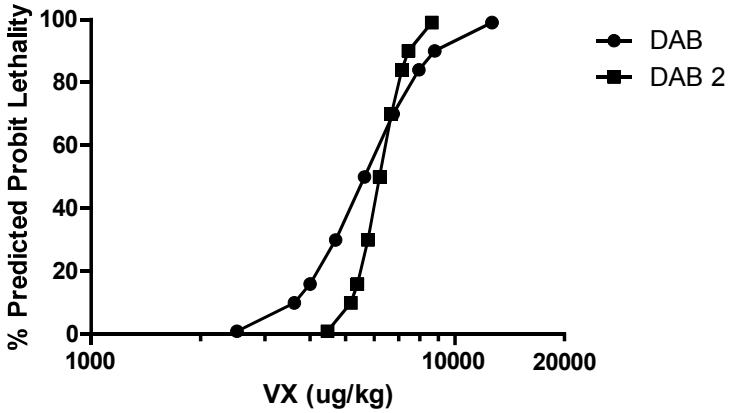

Figure 2: Twenty-four-hour probit dose lethality curves for the initial (DAB) and repeat (DAB 2) RSDL DAB DC procedure experiments. The same DAB procedure was used for generating both curves (see Fig 1 and Material and Methods for procedure description).

Table 4: Comparison of the two RSDL DAB DC procedure LD_{50}s and slopes.

RSDL	N	Slope	VX LD_{50}, µg/kg, p.c.
DAB	16	6.6	5639 (4276 – 7437)
DAB2	20	16.2	6207 (5483 -7027)

Discussion

The results of this study demonstrate that the efficacy of RSDL as an immediate DC product following dermal VX exposure in this guinea pig model depends on the DC procedures. Specifically, the DAB procedure was significantly more effective than any of the other three RSDL DC procedures by about two-fold. Interestingly, the efficacy of RSDL seemed to depend only on how it was reapplied, since the procedure for applying RSDL was the same for all four procedures, and removal after two minutes (OFF) was just as effective as leaving it on the skin (ON).

The reasons for selecting these four RSDL DC procedures were as follows. The ON procedure was selected because it was the standard procedure used originally to characterize the efficacy of RSDL against organophosphorus nerve agents.[9] The OFF procedure was selected because it followed the labeling guidance on the RSDL packet, which says "Allow RSDL to remain on the skin for at least two minutes." The reasons for the two-minute guidance on the package labeling are not clear. Perhaps this time was added to the label to limit systemic absorption of RSDL. The PAD and DAB procedures were suggested by one of the authors (CGH) as possible reapplication procedures that a user might employ in the field following initial removal.

Braue et al.[9] used the ON DC procedure to characterize the efficacy of RSDL. In those experiments, fur-clipped anesthetized guinea pigs were also utilized. When RSDL DC was performed two minutes after dermal VX exposure, a 24-hour MLD of 14300 µg/kg was achieved or a 66-fold increase over the control MLD of VX (215 µg/kg). The RSDL ON DC procedure in the current study resulted in a 24-hour VX MLD of 3380 µg/kg; this represents a 24-fold increase over the historic VX MLD (140 µg/kg). The large difference between the two RSDL ON procedures in the two studies may be due in part to presence of anesthesia in the former study, but other factors may also be responsible. It is interesting to note that the DAB procedure in our experiments increased the MLD of VX by 40- to 44-fold, results that are closer to those from the study by Braue et al.[9]

It is unclear why the DAB procedure was significantly more effective than any of the other three DC procedures, especially the PAD procedure. Both reapplication procedures were performed immediately after removing the initial application of RSDL after two minutes, using the same removal procedure. The reapplication by DAB was made by pressing a fresh RSDL applicator on the exposure site for about one second and leaving any residue on the skin. The PAD procedure placed half of a fresh RSDL sponge over the exposure site, which was secured in place by wrapping an elastic bandage (Vetwrap®) around the animal. The elastic bandage was secured tight enough to cause excess RSDL to ooze from the sides of the sponge. The bandage and the RSDL sponge were removed after ten minutes, leaving any residue on the skin. Perhaps the PAD procedure introduces occlusion which paradoxically 1) doesn't supply as much RSDL as the DAB; 2) drives VX further into the skin; or 3) at least inhibits further uptake of agent.
In summary, the effectiveness of RSDL as an immediate DC product following cutaneous exposure to the nerve agent VX, in this animal model and under the conditions of these experiments, was affected by the RSDL reapplication procedure. Additional experimentation is needed to further explore RSDL DC methods in this model, and in an animal model with skin morphology and thickness closer to human skin than fur-clipped guinea pig skin. The data suggest that physical removal of the first RSDL application followed by reapplication appears to be most effective procedure for using RSDL as an immediate DC product.

References

1. In: *Textbooks of Military Medicine. Medical Aspects of Chemical Warfare*. Shirley D. Tuorinsky, Sr.ed., 2nd edition, pp 528, Office of the Surgeon General, United States Army, Falls Church, VA, and the Borden Institute, Walter Reed Army Medical Center, Washington, DC, 2008.

2. Joint Service Training Manual (ARMY TM 3-6505-001-10, AIR FORCE TO 14S4-2-1, MARINE CORPS TM 11075A, NAVY SS010-AB-MMO-010). Operators Manual for Reactive Skin Decontamination Lotion. Headquarters Departments of Army, Air Force, Marine Corps and Navy. 17 April 2007.

3. Joint Service Training Manual (ATP 4-02.85, NTRP 4-02.22, AFTTP(I) 3-2.69). Multi-Service Tactics, Techniques, and Procedures for Treatment of Chemical Warfare Agent Casualties and Conventional Military Chemical Injuries. Department of Army, August 2015.

4. Dixon WJ. The up-and-down method for small samples. Journal of the American Statistical Association 60: (312), pp. 967-978, Dec., 1965.

5. Feder PI, Olson CT, Hobson DW, Matthews MC, Joiner RL. Statistical analysis of dose-response experiments by maximum likelihood analysis and iteratively reweighed nonlinear least squares regression techniques. Drug Information J. 25: 323-334, 1991b.

6. Finney, DJ. *Probit Analysis*, 3rd ed. Cambridge University Press, Cambridge, England, 1971.

7. Casella G and Berger RL. *Statistical Inference*, 2nd ed. 2002.

8. Zar JH. *Biostatistical Analysis* Third Edition, Comparing 2 slopes. pp 353-357, 1996, Prentice Hall, Upper Saddle River, NJ.

9. Braue EH, Jr, Smith KH, Doxzon BF, Lumpkin HI, Clarkson ED. Efficacy studies of Reactive Skin Decontamination Lotion, M291 Skin Decontamination Kit, 0.5% bleach, 1% soapy water, and Skin Exposure Reduction Paste against Chemical Warfare Agents, Part 1: Guinea pigs challenged with VX. Cut and Occul Toxicol 30(1): 15-28, 2011.