

EVALUATION OF DECONTAMINATION SYSTEMS CHALLENGED WITH NERVE AGENTS

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Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the US Army.

This research complied with the Animal Welfare Act and implementing Animal Welfare Regulations and adhered to the principles noted in *The Guide for the Care and Use of Laboratory Animals*.

ABSTRACT

Current doctrine describes the use of the M291 Skin Decontamination Kit (SDK), 0.5% hypochlorite solution (household bleach diluted 1 to 10) and 1% soapy water solution to decontaminate skin exposed to chemical warfare agents. Reactive Skin Decontamination Lotion (RSDL) is a new product being considered and was recently approved by the FDA. This study directly compares the efficacy of these four decontamination products, in the clipped haired guinea pig model, following challenges by soman (GD), VX, and Russian VX (VR). The protective ratio (PR) was calculated from the derived median lethal dose-response curves established for each agent and non-decontaminated control animals. RSDL was found to be the most effective decontamination system, whereas the M291 SDK was the least effective.

1. INTRODUCTION

The U.S. military and civilians face a growing threat from chemical warfare agents (CWAs), particularly nerve agents. This creates an immediate need for the most effective protection and decontamination systems to limit the harmful and sometimes fatal symptoms of exposure. The military currently provides its members with protective suits, barrier skin creams and decontaminating systems, which have been found to be effective. However, research continues in the hopes of finding improved products that will be highly effective against most nerve agents.

Nerve agents are organophosphorous compounds that act by inhibiting acetylcholinesterase, an enzyme that aids in the breakdown of the neurotransmitter acetylcholine. This neurotransmitter then accumulates at synaptic sites and causes bodily systems to function hyperactively. Symptoms of nerve

agent poisoning include miosis (pinpoint pupils), rhinorrhea (runny nose), lacrimation (watery eyes), vomiting, bronchial constriction, muscle fasciculations, seizures, and ultimately death.

Modern-day nerve agents were first developed by Germany in the 1930s. While German researchers were developing new insecticides, the nerve agent tabun (GA) was accidentally formed. Later, sarin (GB) and soman (GD) were developed. Cyclosarin (GF) was later discovered, as well as VX, which was developed by the British in the 1950s; VR, an isomer of VX, was also developed in the 1950s by the Russians.

Nerve agents were used during the Iran-Iraq war (UN Security Council, 1984) and the Iraqis exposed the Kurdish village of Halabja to nerve agents (Spiers, 1994). Nerve agents were reportedly not used during the Persian Gulf war, but when a chemical depot was destroyed, U.S. soldiers became exposed to nerve agents. It has been suggested that this exposure may have contributed to the Gulf War Syndrome (Winkenwerder, 2000). The most notable use of nerve agents occurred in 1995 when a Japanese terrorist group released sarin throughout the Tokyo subway. This incident resulted in the deaths of 12 people and injured over a thousand more (Woodall, 1997).

The importance of nerve agent decontamination cannot be overstated. If a nerve agent exposure occurs, quick and thorough cleansing of the site is essential. The warfighter is currently issued the M291 kit for nerve agent removal. It is packaged in two flexible pockets containing three decontamination packets each. The Soldier is to scrub the contaminated skin until there is an even layer of resin covering the site. An adsorbent, polystyrene polymeric and ion exchange resins are the main ingredients that create this black resin product. They act to physically remove and adsorb the agent while reactively destroying the agent (O'Hern et al., 1997). Another product that can be used is a chlorine releaser, such as house-hold bleach (5% sodium hypochlorite), which will neutralize the agent. However, because bleach may cause skin damage, a 0.5% solution is recommended for skin decontamination (Sidell, 1997). If these items are not available, a solution of soap and water may be used to physically remove and neutralize the agent.

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Reactive Skin Decontamination Lotion (RSDL) is a new product that was approved by the FDA in 2003 for the removal and neutralization of vesicants and nerve agents. However, the product has not yet been approved for military use. A sponge is saturated with RSDL and sealed in an aluminum coated packet ready for the Soldier to apply to the contaminated skin. The ingredients of RSDL are 2, 3 butanedione monoxime as a free oxime and potassium salt in a solvent of polyethylene glycol ethyl ether (MPEG) and water. This product acts to remove and neutralize the agent on the exposed skin.

The following study examines the effectiveness of four decontamination products: the M291 kit, 0.5% bleach, 1% soapy water, and RSDL. These products were tested against soman (GD), VX and Russian VX (VR) in the clipped haired guinea pig model.

2. MATERIALS AND METHODS

2.1 Subjects

Guinea pigs from Charles River Laboratories (Hartley strain, 250 – 400 grams) were sheared with Oster brand animal clippers, blade #40, and anesthetized with a combination of 32 mg/ml ketamine and 4 mg/ml xylazine in saline. Eighteen to fifty animals were used for each set of agent-decontaminant experiments in this study.

2.2 Procedures

Agent was applied with a Rainin micropipettor or a Hamilton microsyringe to the sheared area along the animal's left ribcage, within a 1 by 1.5-inch box drawn on the animal's side. Two minutes after agent application, the dosing site was swabbed ten times, using moderate pressure, with a swab comprising the decontaminant material (Figure 1). For dilute bleach (0.5%) or soapy water (1% Palmolive detergent) decontamination, a swab made of a tongue depressor wrapped and stapled with a single folded sheet of 4-inch gauze was wetted with 5 mL and used for wiping off agent (ten strokes). This was followed by another swabbing (ten strokes) using swabs wetted with distilled water. M291 SDK material was folded around a tongue depressor and stapled to make the decontaminant swab. A Reactive Skin Decontaminant Lotion (RSDL) sponge was cut into four sections; each section was stapled to a tongue depressor to make decontaminant swabs. Decontamination by M291 or RSDL used ten strokes, but was not followed by swabbing with distilled water. Guinea pigs were maintained under an AAALAC-accredited animal care and use program. They were housed, fed, and watered in a fume hood with an air flow

of 100 CFM +/- 20 CFM and a temperature of 70°F-80°F for 24 hours. The animals were observed for 4 hours and at 24 hours. Surviving guinea pigs were euthanized with halothane vapor, and skin areas exposed to agent were excised and decontaminated at the conclusion of each experiment.



Fig. 1. Decontaminant applicators (left to right) for RSDL, M291, and 0.5% bleach or 1% soapy water. Gauze applicator is wetted with 5 mL bleach or soapy water from syringe.

2.3 Measurements

The median lethal dose (LD_{50}) of agent was calculated for the M291 SDK, soapy water (1% Palmolive detergent), dilute bleach (0.5%), RSDL and controls (no decontamination). Protective ratios (PR) were calculated from the derived LD_{50} curves determined for each agent, each decontaminant and the control animals.

2.4 Agent Application

Rainin micropipettors were used to apply agent for volumes down to 0.5 μ l. A Hamilton positive displacement microliter syringe (model 7001) was used for volumes below 0.5 μ l.

For all GD experiments, only neat GD was used on control and treated animals. For VX and VR experiments, agent application used both neat and agent diluted with isopropyl alcohol (IPA). VX and VR control animals were challenged with agent in IPA solution. Animals decontaminated by RSDL, 1% soapy water, and 0.5% bleach used only neat agent. Animals decontaminated by M291 SDK used agent in IPA solution.

Several months after the initial experiments with VX and VR were completed, the MLDs for non-treated control animals and decontamination by the M291 SDK were repeated. In these repeat experiments, the control animals still used agent in IPA solution, but

the animals decontaminated with the M291 SDKs were challenged with neat agent.

3. RESULTS AND DISCUSSION

3.1 GD Experiments

RSDL showed the highest protection against GD with an LD₅₀ of 154 mg/kg and a protective ratio of 14. Soapy water, 0.5 % bleach and M291 SDK had near-equivalent LD₅₀s and protective ratios but were well below that of RSDL (Figures 2 and 3).

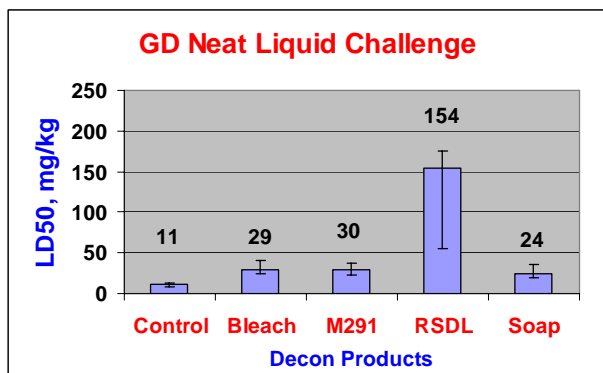


Fig. 2. GD Neat Liquid Challenge. Error bars = 95% C.I.

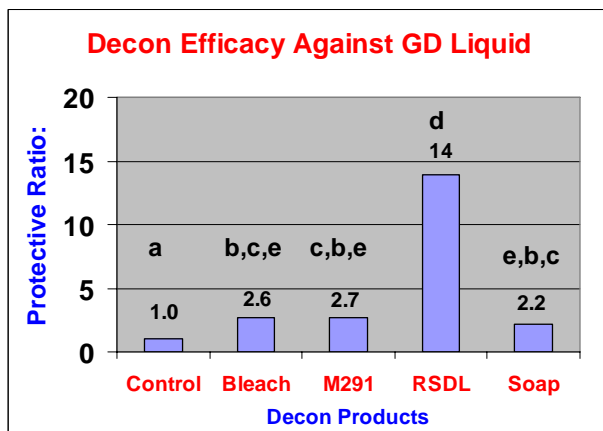


Fig. 3. Decon Efficacy Against GD Liquid. PRs with same letter were not statistically different at the 0.05 decision level.

3.2 VX Experiments

Against VX, RSDL provided the most protection, as evidenced by its LD₅₀ of 14.3 mg/kg and PR of 66. Dilute bleach and soapy water showed smaller levels of protection with PRs of 17 and 16, respectively. M291 SDK showed hardly any protection against VX (in IPA), with LD₅₀s and protective ratios at or near that of controls (Figures 4 and 5).

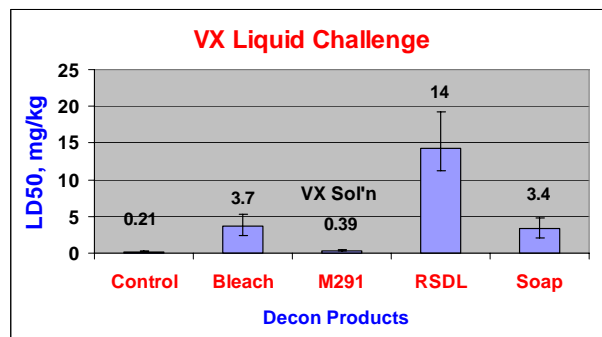


Fig. 4. VX Liquid Challenge. Error bars = 95% C.I.

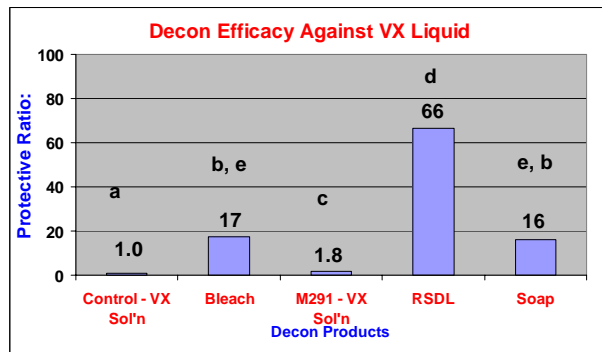


Fig. 5. Decon Efficacy Against VX Liquid. PRs with same letter were not statistically different at the 0.05 decision level.

3.3 VR Experiments

RSDL was also the best decontaminant against VR, with an LD₅₀ of 18.7 mg/kg and a sizable PR of 140. Dilute bleach, with a PR of 60, provided good protection against VR, but soapy water, with its PR of 27, was half as effective against this agent. With an LD₅₀ and PR near that of controls, M291 SDK showed little protection against VR (in IPA) (Figures 6 and 7).

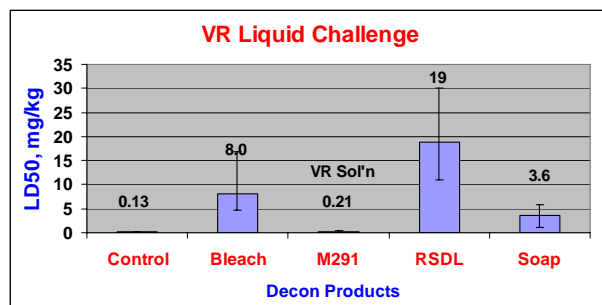


Fig. 6. VR Liquid Challenge. Error bars = 95% C.I.

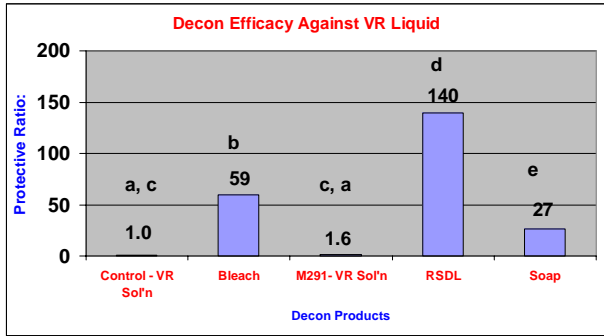


Fig.7. Decon Efficacy Against VR Liquid. PRs with same letter were not statistically different at the 0.05 decision level.

3.4 VX and VR Repeat Experiments

For the initial VX and VR experiments, all the non-treated control animals were challenged with agent diluted with isopropyl alcohol (IPA). Diluted agent was used on these animals because the toxicity on VX and VR required neat volumes less than 0.5 μ l. It is very difficult to deliver such small volumes with high precision and accuracy. Decontamination by RSDL, 1% soapy water, and 0.5 % bleach, however, were sufficiently efficacious to required dosing volumes greater than 0.5 μ l of neat agent.

Observations from other decontamination experiments conducted in our lab suggested that agent in IPA solution may affect the observed efficacy of decontamination products compared to neat agent challenge. It is possible that agent in IPA solution may artificially enhance the absorption of these agents through the skin resulting in less effective decontamination two minutes after exposure.

In order to test this hypothesis, the M291 SDK was tested again against challenge with neat VX and VR. Since there was a time lapse of several months, the LD₅₀s of non-treated control animals were also evaluated along with the decontaminated animals. The volumes of neat agent needed to determine the full dose-response curves were as low as 0.05 μ l. It is very difficult, if not impossible, to deliver these small volumes with high accuracy and precision so larger numbers on animals were used to obtain the power needed for statistical significance. Against both of these agents in neat form, the LD₅₀s of M291 (0.14 mg/kg for VX, 0.22 mg/kg for VR) were not significantly different from those of non-treated controls (Figures 8 and 9).

It should be noted that the marginal efficacy of the M291 SDK against VX observed in these experiments using the clipped guinea pig model are contrary to the results obtained in the clipped rabbit model reported in a study conducted by Battelle

Memorial Institute (Study No. 204-G472508M1). In the Battelle study, a similar decontamination procedure was used except both non-treated control and decontaminated animals were challenged with neat agent. The LD₅₀s of control animals and PR were observed to be 0.035 mg/kg and 9.6 respectively.

While the efficacy reported in the Battelle study using a rabbit model was much different for the M291 SDK, it was identical to the current study using guinea pigs for RSDL with a value of 66. There are no studies available that have evaluated the efficacy of the M291 SDK challenged with VR in the rabbit model for comparison.

While the current study and the Battelle study report different efficacy for the M291 SDK depending on the animal model, both studies clearly demonstrate the superior performance of RSDL.

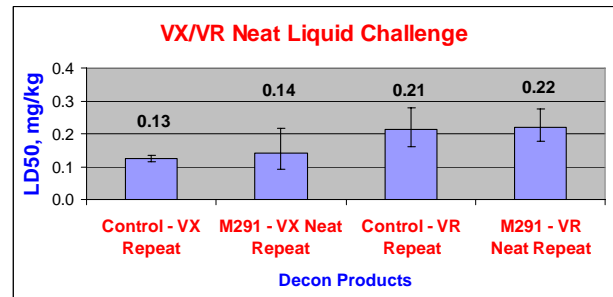


Fig. 8. VX/VR Neat Liquid Challenge. Error bars = 95% C.I.

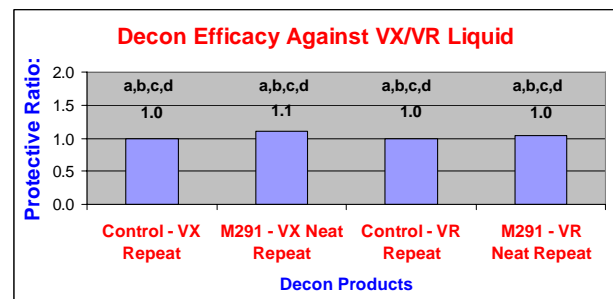


Fig. 9. Decon Efficacy Against VX and VR Liquid. PRs with same letter were not statistically different at the 0.05 decision level.

4. CONCLUSIONS

In tests of potential decontaminants against cutaneous agent exposure in guinea pigs, RSDL provided superior protection against GD, VX, and VR in comparison to all of the other materials. Soapy water and 0.5% bleach, though not as protective as RSDL, provided equivalent levels of protection against GD and VX. The 0.5% bleach solution was twice as effective as soapy water against VR exposure. M291 SDK was the least effective decontaminant overall, providing modest

protection against GD and hardly any protection against VX and VR, whether administered as neat agent or in IPA solution.

These results indicate that even though the M291 SDK is the currently prescribed decontamination system for nerve agent exposure it may not be the most effective product. The study does show that RSDL is the most effective decontaminant out of those products tested and is therefore a viable alternative to the currently available decontamination systems. The growing threat of chemical weapons facing both the military and civilians makes the final approval of RSDL as a fielded decontamination system a high priority.

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