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Evaluation of Veriox® as a Skin Decontamination Product after Dermal Exposure to the Nerve Agent VX

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14. ABSTRACT
Veriox® is a topical antimicrobial anti-infective/disinfectant, which is under development for various uses to include medical device sterilization, advanced wound care, surface disinfection, a coating on medical products, hand sanitization, and veterinary wound care. The purpose of these experiments was to determine whether Veriox® had efficacy as a decontamination product (DC) after skin exposure to the chemical warfare agent VX. This study compared the effectiveness of Veriox® to Reactive Skin Decontamination Lotion (RSDL) when each was used as a DC product 2 min after dermal exposure to VX in hair-clipped, unanesthetized guinea pigs. Efficacy was established by generating VX dose-lethality curves for each DC product based on 24 survival/lethality responses and calculating the VX dose at which 50% (LD50) of animals died. The dermal LD50 of VX in Veriox®-treated animals was 5959 ug/kg, which was 1.8-fold higher than the VX LD50 of 3380 µg/kg in RSDL-treated animals. Veriox® was significantly ($p<0.05$) more effective than RSDL. Further studies with Veriox® are needed to determine its ultimate usefulness as a skin DC product for military use.

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Executive Summary:

The skin is a primary route of exposure to chemical agents used as weapons of mass destruction. Because of this threat, the U.S. military has invested considerable resources in developing detectors, protective garments, and products to remove and/or decontaminate chemical agent exposure on the skin. The currently fielded personal decontamination (DC) product is a lotion known as Reactive Skin Decontamination Lotion (RSDL). RSDL is FDA approved for use on the skin, near eyes, around wounds and equipment against all organophosphorus (OP) chemical warfare agents, sulfur mustard, T-2 toxin, and OP pesticides. Veriox® is a topical antimicrobial anti-infective/disinfectant, which is under development for various uses to include medical device sterilization, advanced wound care, surface disinfection, a coating on medical products, hand sanitization, and veterinary wound care. The purpose of these experiments was to determine whether Veriox® had efficacy as a DC product after skin exposure to the chemical warfare agent VX. This study compared the effectiveness of Veriox® to RSDL when each was used as a DC product 2 min after dermal exposure to VX in hair-clipped, unanesthetized guinea pigs. Efficacy was established by generating VX dose-lethality curves for each DC product based on 24 survival/lethality responses and calculating the VX dose at which 50% (LD_{50}) of animals died. The dermal LD_{50} of VX in Veriox®-treated animals was 5959 ug/kg, which was 1.8-fold higher than the VX LD_{50} of 3380 µg/kg in RSDL-treated animals. Veriox® was significantly ($p<0.05$) more effective than RSDL. Further studies with Veriox® are needed to determine its ultimate usefulness as a skin DC product for military use.

Introduction:

The skin is a primary route of exposure to chemical agents used as weapons of mass destruction. Because of this threat, the U.S. military has invested considerable resources in developing detectors, protective garments, and products to remove and/or decontaminate chemical agent exposure to the skin. The currently fielded skin decontamination (DC) product is a lotion known as Reactive Skin Decontamination Lotion (RSDL), which is a mixture of potassium 2,3-butanedione monoximate (KBDO) and diacetylmonoxime (DAM) in a solvent of polyethylene glycol monomethyl ether (MPEG) and water. RSDL is FDA approved for use on the skin, near eyes, around wounds and equipment against all OP chemical agents, sulfur mustard and T-2 toxin.[1] Military personnel are issued three pouches of RSDL; each pouch contains three packets with a sponge pad saturated with RSDL. After a suspected exposure to a chemical agent, RSDL is applied by scrubbing the exposed area(s) vigorously with the sponge and allowing it to remain on the skin for at least 2 minutes before removing.[2,3] RSDL can be reapplied and left on the skin for up to twenty-four hours.

While RSDL is an effective broad spectrum DC product, the user community has complained about its expense and some of the physical characteristics of the product. This has renewed interest in identifying a more acceptable broad spectrum personal DC product. We were recently asked by DTRA/JSTO to evaluate a product called Veriox®, which is a topical antimicrobial anti-infective/disinfectant comprised of proprietary peracids. Veriox® is under development for various uses to include medical device sterilization, advanced wound care, surface disinfection, as a coating for medical products, hand sanitization, and veterinary wound care.

The purpose of these experiments was to conduct an exploratory study to determine whether Veriox® had efficacy as a skin DC product after dermal exposure to a chemical warfare agent. This study compared the effectiveness of Veriox® to RSDL when each was used to decontaminate dermal exposure to VX in unanesthetized, fur-clipped guinea pigs.

Experimental Methods:

Animals: Male guinea pigs [Hartley, Crl(HA)BR] ranging in weight from 340-503 gm at the time of experimentation were obtained from Charles River (Canada). After arrival, the animals were maintained in quarantine for at least 5 days prior to use in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALACI) accredited animal care and use facility. On the morning of an experiment, around 0800 hr, animals were weighed, the fur was carefully removed from 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 hr). Food and water were provided *ad libitum* after exposure and DC.

Materials: 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. Veriox® was obtained from CHD Bioscience, Ft. Collins, CO, as a concentrated solution of proprietary peracids. A 1:6 dilution in deionized water was prepared according to the manufacturer's formula each test day.

VX Exposure: 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. Animals were hand restrained by a trained technician for exposure.

DC procedure: Two minutes after applying VX to the skin, the exposure site was decontaminated with RSDL or Veriox®. Animals were hand restrained by a trained technician during the DC procedure. RSDL was applied with an applicator made by stapling ¼ (25 mm x 50 mm) of a RSDL sponge pad to a wooden tongue depressor. Veriox® applicators were made by stapling a similar size folded gauze pad to wooden tongue depressors. A fresh applicator of each DC product was used on each animal. The RSDL applicators were made just before the start of the experiment and were placed into small plastic bags until use. The Veriox® applicators were wetted with 10 ml of the diluted Veriox® solution just before DC. Ten ml was sufficient to saturate the applicator pad without run-off based on previous experience using dilute bleach or soap and water. RSDL and Veriox® DC were performed by swiping the applicator across the exposure site 10 times from a head to tail direction. Neither DC product was removed after application.

Experimental Design: VX dose-lethality curves were generated for RSDL and Veriox® based on 24 hr responses. After exposure and DC, each animal was monitored continuously until the onset of toxic signs, and then at 2 and 4 hr after DC, and again 24 hr after exposure. A modified stage-wise adaptive dose design was used to generate the VX dose-lethality curves for each DC product. The first stage utilized the classic up-down dose design of Dixon to estimate the LD_{50} of VX for each DC product.[4] Briefly, one animal at a time was challenged with a dose of VX for each DC product during Stage1. After the 24 hr response was determined, the next animal in each DC product group received a higher (if alive @ 24 hr) or lower (if dead @ 24 hr) dose of VX depending on the response of the previous animal. The up-down procedure continued until four response reversals were observed. The 24 hr responses for each DC product 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 3-8 animals per stage and various doses of VX in each stage for each DC product to improve the LD_{50} estimate and 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 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 2 times the LD_{50} estimate was < 0.4[5]. A total of 15 and 26 animals were used to generate the RSDL and Veriox® dose-lethality curves, respectively.

Statistical Analysis: A final probit analysis was conducted on all stages from the 24 hr responses for RSDL and Veriox®. The slopes, 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. Probit estimates were calculated using both target and actual doses of VX and were not statistically different; therefore, the target doses were used for all statistical comparisons and in the graphs and tables. LD_{50} estimates for RSDL and Veriox® were compared using SAS and another specialized probit program, which determined whether the ratio of the LD_{50}s was statistically different at $p<0.05$.[5] A significant ($p<0.05$) difference was achieved when the delta 95% CI of the LD_{50} ratio did not include the value of 1.[5] The slopes of the dose-lethality curves were compared according to Zar.[8] A protective ratio (PR) defined as LD_{50} of VX in animals treated with the DC product divided by the LD_{50} of VX in untreated animals was estimated, using a historic value of 140 µg/kg in fur-clipped unanesthetized guinea pigs (Clarkson, personal communication) for the denominator in the ratio. The PR expresses the magnitude of the increase in the LD_{50} by the DC product. Another ratio called an absolute efficacy ratio (AER) was also calculated. The AER was defined as the LD_{10} of VX in animals treated with a DC product divided by the dermal LD_{90} of VX in untreated animals. A LD_{90} value of 188 µg/kg generated in hair-clipped, unanesthetized guinea pigs (Clarkson, personal communication) was used for the denominator for the AER. The AER expresses the magnitude of the increase in the LD_{10} relative to the untreated LD_{90}, and is a more operationally relevant measure of efficacy than the PR, especially if the slopes of the dose-lethality curves are significantly different.[9] Military requirements documents prescribe 80-90% survival for acceptance of new medical countermeasures against nerve agent intoxication.

Results:

Figure 1 graphs the probit dose-lethality curves for VX in Veriox® and RSDL-decontaminated animals, and Table 1 summarizes the results based on LD_{50}s. A total of 15 and 26 animals were needed to generate the dose-lethality curves for RSDL and Veriox®, respectively, using the stopping criteria described in the methodology. The 24 hr dermal LD_{50} of VX was 5959 µg/kg in animals decontaminated with Veriox® and 3380 µg/kg in animals decontaminated with RSDL. Veriox® was 1.8-fold ($p<0.05$) more effective than RSDL. The slope of the Veriox® dose-lethality curve was significantly ($p<0.05$) different from the slope of the RSDL dose-lethality curve. The estimated PR (treated to untreated) was 42.6 for Veriox® and 24.1 for RSDL.

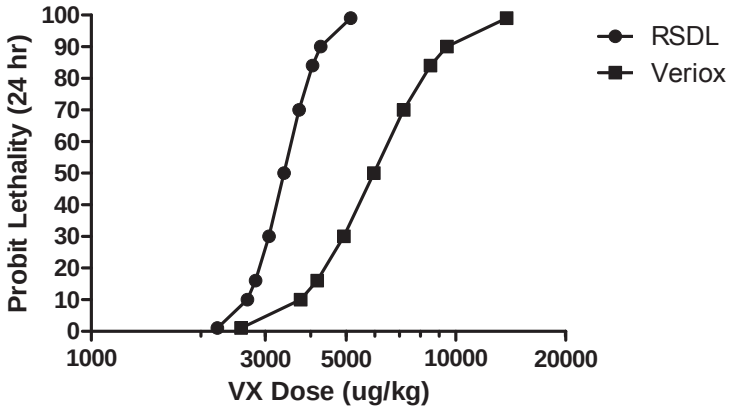

Figure 1. VX dose lethality curves in animals decontaminated with either RSDL or Veriox® 2 min after cutaneous exposure.

Table 1: Twenty-four hour VX LD_{50} estimates in guinea pigs decontaminated with Veriox® or RSDL 2 min after dermal exposure

DC Product	Number of Animals	Slope of the Dose-Lethality Curve	24 hr VX LD_{50}, µg/kg, p.c. (95% CI)	Estimated Protective Ratio[1]
Veriox®	26	6.4	5959 (4858-7309)	42.6
RSDL	15	12.7	3380 (2921-3910)	24.1
Efficacy Ratio 50% Survival		Veriox/RSDL =	1.8 $p<0.05$	

[1]Estimated using a 24-hour dermal VX LD_{50} of 140 µg/kg in fur-clipped unanesthetized guinea pigs (Clarkson, personal communication)

Table 2 summarizes the results based on LD_{10}s. The 24 hr dermal LD_{10} of VX was 3755 µg/kg in animals decontaminated with Veriox® and 2681 µg/kg in animals decontaminated with RSDL. Veriox® was 1.4-fold more effective than RSDL; this difference was not significant. Also, presented in Table 2 is the ratio of the VX LD_{10} in animals receiving DC to the VX LD_{90} in animals that were not treated with a DC product. The LD_{10}/LD_{90} ratio for Veriox® was 20 and the ratio for RSDL was 14.

Table 2: Twenty-four hour VX LD_{10} estimates in guinea pigs decontaminated with Veriox® or RSDL 2 min after dermal exposure

DC Product	Number of Animals	24 hr VX LD_{10}, µg/kg, p.c. (95% CI)	LD_{10}/LD_{90}[1]
Veriox®	26	3755 (2390-5500)	20
RSDL	15	2681 (2096-3429)	14
Efficacy Ratio 90% Survival		Veriox/RSDL =	1.4

[1]A VX LD_{90} of 188 µg/kg was used for the denominator. This value was estimated from the dose-lethality curve generated in fur-clipped, unanesthetized guinea pigs (Clarkson, personal communication)

Discussion:

Decontamination is the process by which hazardous chemicals are removed and or neutralized from people, equipment and the environment.[10] Prompt DC of the skin can mean the difference between life and death. Military doctrine advises DC within 2-3 min after an exposure to a chemical agent for maximum benefit.[2,3] The results show that, under the conditions of this study, Veriox® was more effective than RSDL when used as a DC product 2 min after dermal VX exposure.

The effectiveness of Veriox® and RSDL was determined by establishing dose-lethality curves based on 24 hr survival/lethality responses after dermal VX exposure, from which LD_{50}s were estimated. A combination of an up-and-down design and a stage-wise adaptive dose design was used to generate the dose-lethality curves. The up-and-down design provided initial LD_{50} estimates, while the stage-wise adaptive design refined the LD_{50} estimates, generated confidence limits for the LD_{50} and provided slopes for the dose-lethality curves. This combined design, with the aid of specialized probit programs, minimized animal use and did not reduce our ability to generate the data needed to evaluate the effectiveness of Veriox®. A comparison of the LD_{50} estimates showed that Veriox® was significantly more effective than RSDL. In addition, the slope of the Veriox® dose-lethality curve was more shallow than the slope of the RSDL curve. It is not unusual for the slope of the dose-lethality curve to become more shallow as the effectiveness of medical countermeasures against organophosphate intoxication increases.[9,11] However, Braue et al.[1] observed no difference in the slopes of the dose-lethality curves for RSDL, 1% soapy water, and 0.5% bleach, even though RSDL was greater than 3-fold more effective than the other two DC products; all three slopes were similar to the slope for Veriox® in the our study.

When the slopes are different, comparison of LD_{50}s may not be as valuable, because the lower doses of agent in the curve with the shallower slope may still show lethality.[9] Since the slope of the Veriox® dose-lethality curve was shallower than the slope of the RSDL curve, we compared the ratio of the LD_{10} doses of VX. This might reveal whether the shallower slope of the Veriox® dose-lethality curve resulted in higher lethality at lower doses of VX compared to RSDL. The LD_{10} was selected because military requirements documents prescribe 80-90% survival rates as criteria for accepting new medical countermeasures for use by warfighters. Veriox® was still more effective than RSDL, but the ratio of the LD_{10}s was not significantly different. This was probably due to the wider confidence intervals around the LD_{10} than the LD_{50} estimate. The ratio of the LD_{10} in the animals receiving DC to LD_{90} in animals not receiving DC provides another way of comparing efficacy which is independent of the slope. This ratio value represents the number of LD_{90}s of exposure that can be tolerated without sustaining more than 10% lethality. This value was 20 for Veriox® and 14 for RSDL.

The methodology for evaluating Veriox® was based on previous work by Braue et al.[1] In this seminal paper, the authors evaluated and compared the efficacy of RSDL, M291, 0.5% bleach, and 1% soapy water as DC products in clipped haired, anesthetized guinea pigs following dermal exposure to VX. RSDL was by far the most effective DC product when used 2 min after exposure, increasing the LD_{50} of VX 66-fold compared to 2, 17 and 16 for the other DC products, respectively. Exposure, DC procedures and efficacy endpoints in the current study were very similar to those used in the above referenced study. In the current study, RSDL was much less effective, increasing the LD_{50} of VX only about 24-fold. The reason for the difference is unclear, but the use of anesthesia by Braue et al. likely contributed to the difference in the efficacy of RSDL between the two studies, although other factors may be involved. Dermal nerve agent exposure studies are fraught with variability because of the nature of the exposure route, the small volumes of agents applied to the skin, day-to-day differences in environmental conditions, and the inability to quantitate the actual exposure dose to the animal. Any or all of these factors may have also contributed to the difference in the efficacy of RSDL between the two studies.

The RSDL-decontaminated animals included in the current study were also part of another study, which was investigating 3 alternate RSDL DC procedures at 2 min after dermal VX exposure to the one described the Methods section. Two of the 3 RSDL DC procedures resulted in increases in the VX LD_{50}, which were similar to the RSDL LD_{50} reported herein. However, one of the alternate RSDL procedures increased the LD_{50} of VX 40-fold, which is very similar to the 42-fold increase we observed with Veriox®. We are going to repeat the experiment with this one RSDL DC procedure to corroborate the finding. If the results can be corroborated, it would be interesting to speculate whether the efficacy of Veriox® would also be increased using this alternate DC procedure.

In summary, the results suggest that Veriox® merits further investigation as a DC product against chemical agent exposure. Further studies should include *in vitro* studies and additional *in vivo* studies to assess the broad spectrum potential of Veriox® as a skin DC product against other OP chemical agents and sulfur mustard. Additional *in*

vivo studies should also evaluate the DC potential of Veriox® when used at delayed time points after chemical agent exposure on the skin.

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