



U.S. Army Medical Research
Institute of Chemical Defense

USAMRICD-TR-09-01

Evaluation of RSDL, M291 SDK, 0.5% Bleach, 1% Soapy Water and SERPACWA

Part 1: Challenge with VX

Ernest H. Braue, Jr.
Kelly A. Hanssen
Bryce F. Doxzon
Horace L. Lumpkin
Edward D. Clarkson

June 2009

Approved for public release; distribution unlimited

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

DISPOSITION INSTRUCTIONS:

Destroy this report when no longer needed. Do not return to the originator.

DISCLAIMERS:

The opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army or the Department of Defense.

The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, Publication No. 85-23, 1996), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

The use of trade names does not constitute an official endorsement or approval of the use of such commercial hardware or software. This document may not be cited for purposes of advertisement.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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 Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE (DD-MM-YYYY) June 2009		2. REPORT TYPE Technical Report		3. DATES COVERED (From - To) 3 November 2004 to 4 December 2007	
4. TITLE AND SUBTITLE Evaluation of RSDL, M291 SDK, 0.5% Bleach, 1% Soapy Water and SERPACWA. Part 1: Challenge with VX				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Braue, EH Jr, Hanssen, KA, Doxzon, BK, Lumpkin, HL, Clarkson, ED				5d. PROJECT NUMBER	
				5e. TASK NUMBER TC2	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) US Army Medical Research Institute of Chemical Defense ATTN: MCMR-CDT-T 3100 Ricketts Point Road Aberdeen Proving Ground, MD 21010-5400				8. PERFORMING ORGANIZATION REPORT NUMBER USAMRICD-TR-09-01	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) US Army Medical Research Institute of Chemical Defense ATTN: MCMR-CDZ-I 3100 Ricketts Point Road Aberdeen Proving Ground, MD 21010-5400				10. SPONSOR/MONITOR'S ACRONYM(S)	
				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 Service and Technology Office, Medical Science and Technology Division.					
14. ABSTRACT There is a need for Joint forces to effectively operate across the continuum of global contingency operations. The requirement exists for a pre-exposure barrier skin cream to increase the efficacy of the protective suit and for the ability to decontaminate the skin, individual equipment, and casualties, including those with wounds that have been exposed to chemical, biological, radiological, and nuclear (CBRN) warfare agents. Current doctrine describes the use of Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA) as a barrier skin cream and the M291 Skin Decontamination Kit (SDK), 0.5% hypochlorite solution (household bleach diluted 1 to 10) and 1% soapy water solution to decontaminate intact skin exposed to chemical warfare agents. Reactive Skin Decontamination Lotion (RSDL) is a new product approved by the FDA and selected in March 2007 by the Joint Program Executive Office for Chemical and Biological Defense to eventually replace the M291 SDK. This report, the first in a series, directly compares the efficacy of SERPACWA and the four listed decontamination products in the haired guinea pig model following exposure to VX.					
15. SUBJECT TERMS decontamination, delayed decontamination, Reactive Skin Decontamination Lotion (RSDL), bleach, soapy water, M291 Skin Decontamination Kit, VX, nerve agents, Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA), skin barrier creams					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UNLIMITED	18. NUMBER OF PAGES 81	19a. NAME OF RESPONSIBLE PERSON Ernest H. Braue, Jr.
a. REPORT UNCLASSIFIED	b. ABSTRACT UNCLASSIFIED	c. THIS PAGE UNCLASSIFIED			19b. TELEPHONE NUMBER (include area code) 410-436-2848

ACKNOWLEDGMENTS

The authors wish to give a special thanks to our statistician, Ms. Robyn Lee. She provided expert guidance in selecting appropriate challenge doses and determining the required number of animals to establish rigorous dose-response curves. She also trained the authors to perform a probit analysis of the data using the SAS program and provided a critical review of the draft manuscript. Her dedication and assistance is greatly appreciated.

EXECUTIVE SUMMARY

There is a need for Joint forces to effectively operate across the continuum of global contingency operations. The requirement exists for a pre-exposure barrier skin cream to increase the efficacy of the protective suit and for the ability to decontaminate the skin, individual equipment, and casualties, including those with wounds that have been exposed to chemical, biological, radiological, and nuclear (CBRN) warfare agents. Current doctrine describes the use of Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA) as a barrier skin cream and the M291 Skin Decontamination Kit (SDK), 0.5% hypochlorite solution (household bleach diluted 1 to 10) and 1% soapy water solution to decontaminate intact skin exposed to chemical warfare agents. Reactive Skin Decontamination Lotion (RSDL) is a new product approved by the FDA and selected in March 2007 by the Joint Program Executive Office for Chemical and Biological Defense to eventually replace the M291 SDK. This report, the first in a series, directly compares the efficacy of SERPACWA and the four listed decontamination products in the haired guinea pig model following exposure to VX.

In all experiments, guinea pigs were close-clipped and given anesthesia. SERPACWA was applied as a thin coating (0.1 mm thick), allowed to dry for 15 minutes and challenged with VX. After a 2-hour challenge any remaining VX was blotted off the animal but no additional decontamination was done. In decontamination experiments, the animals were challenged with VX and decontaminated after a 2-minute delay for the standard procedure or at longer times for the delayed decontamination experiments. Positive control animals were challenged with VX in the same way as the treated animals except that they received no treatment. In addition, positive control animals always were challenged with 5% VX in isopropyl alcohol (IPA) solution, whereas the treatment animals received either neat VX or 5% VX in IPA solution. All animals were observed during the first 4 hours and again at 24 hours postexposure for signs of toxicity and death. The protective ratio (PR, defined as LD_{50} of the treatment group divided by the LD_{50} of the untreated positive control animals) was calculated from the derived median lethal dose-response curves established for each treatment group and non-treated control animals. Significance in this report is defined as $p < 0.05$ unless otherwise stated.

The results showed that SERPACWA provided significant, but modest, protection against neat VX with a PR of 2.1. In the standard 2-minute neat VX decontamination experiments, the calculated PRs for RSDL, 0.5% bleach, 1% soapy water, and M291 SDK were 66, 17, 16, and 1.1, respectively. RSDL was by far the most effective decontamination product tested and significantly better than any of the other products. Bleach and soapy water provided equivalent and good protection. They were both significantly better than the M291 SDK. The M291 SDK did not provide significant protection compared to positive controls. In the neat VX delayed decontamination experiments, the calculated LT_{50} values (the delayed decontamination time where 50% of the animals died in the test population following a 5 LD_{50} challenge) for RSDL, 0.5% bleach and 1% soapy water were 31, 48, and 26 minutes, respectively. These results suggest that a much wider window of opportunity may exist for effective

decontamination of VX than previously believed. The LT_{50} value for 0.5% bleach was significantly longer than for RSDL or 1% soapy water. There was no significant difference between the LT_{50} values for RSDL and 1% soapy water.

Battelle Memorial Institute conducted a few similar, but not identical, efficacy evaluation experiments in a rabbit model. In these decontamination experiments the PR values for RSDL, 0.5% bleach, 1% soapy water, and M291 SDK were observed to be 66, 15, 9.3, and 9.6, respectively. There was excellent correlation between the two animal models except for the M291 SDK. The M291 SDK showed no significant efficacy in the guinea pig model but good and significant efficacy in the rabbit model. In another rabbit study the Battelle group evaluated the efficacy of SERPACWA. The observed PR was 52. This result was radically different from the observed PR of 2.1 in our guinea pig experiments. The large difference is most likely explained by the different experimental procedures used in the two studies. In the Battelle rabbit studies the experimental sites were thoroughly wiped and decontaminated with 10% bleach and water following the 4-hour exposure period. In our guinea pigs studies, however, the experimental sites were only gently blotted to remove visible VX but received no decontamination. It is likely that VX penetrated the SERPACWA layer during the exposure period. The vigorous decontamination used in the rabbit studies removed this trapped VX and prevented it from reaching the systemic circulation. In the guinea pig experiments, however, the gentle blotting process did not remove the trapped VX, which eventually caused the observed toxicity.

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, a barrier skin cream known as Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA, [McCreery, 1997](#)) and decontaminating systems, which have been found to be effective ([Hurst, 1997](#)). Research continues, however, in the hopes of finding improved products that will be even more effective against a wide range of 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 consist of 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, ethyl N, N-dimethyl-phosphoramidocyanidate) was accidentally formed. Later, sarin (GB, isopropyl-methylphosphonofluoridate) and soman (GD, 1,2,2-trimethylpropyl methylphosphonofluoridate) were developed. Cyclosarin (GF, cyclohexyl methylphosphonofluoridate) was later discovered, as was VX (o-ethyl S-[2-(diisopropylamino)ethyl]methylphosphonothiolate), which was developed by the British in the 1950s; VR (O-isobutyl S-(2-diethylaminoethyl)methyl thiophosphonate O-isobutyl S-(N,N-diethylaminoethyl)methylphosphonothioate), 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](#)) where the Iraqis exposed people from 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, 2002](#)). The most notable use of nerve agents occurred in 1995 when a Japanese terrorist group released sarin in the Tokyo subway system. This incident resulted in the deaths of 12 people and injury of over a thousand more ([Woodall, 1997](#)).

The importance of preventing a nerve agent from being systemically absorbed through the skin 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 Skin Decontamination Kit (SDK) for nerve agent removal. The user is to scrub the contaminated skin until there is an even layer of resin covering the site. The resin acts 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 household bleach (5% sodium hypochlorite), which will neutralize the agent. However, because household 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 the agent.

The M291 SDK was first issued to U.S. forces in 1989 and remains the primary kit that soldiers use to remove CWA from skin. The kit consists of a wallet-like carrying pouch containing six separate decontaminating pads, enough to perform three decontamination procedures. Each pad has a loop that fits over the fingers so that the user can easily wipe it over contaminated skin. The pads are designed to absorb and slowly neutralize liquid CWAs and are particularly useful when water is limited and at cold temperatures when water would be frozen or casualties might suffer from hypothermia. The M291 pads are non-woven, fiber-fill, laminated and impregnated with the decontaminating compound Ambergard XE-555 resin (Rohm and Haas, Philadelphia, PA), which is a black, free-flowing powder. This powder is a combination of a carbonaceous adsorbent that can remove agent from the skin and two ion exchange resins that neutralize the agent. Each pad provides the Soldier with a single-step, non-toxic, non-irritating decontamination application, which is safe to use on intact skin. However, the pads should not be used in wounds, in eyes, or on abraded skin (Hurst, 1997).

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. The Joint Services of the United States (U.S.) established an operational requirement in 2004 (Joint Requirements Office, 2004) for a new skin decontaminant that could be used effectively on the skin, near eyes, around wounds, and on equipment against all chemical, biological, radiological, and nuclear (CBRN) agents as well as against other toxic industrial materials. RSDL was selected as the Joint Service Personnel Decontamination System (JSPDS) by the Joint Program Executive Office for Chemical and Biological Defense at the Milestone C review in March 2007. A manufacturing contract was established and RSDL began to replace the M291 SDK. In this kit, a sponge is saturated with RSDL and sealed in an aluminum-coated packet ready for the service members or civilians to apply to the contaminated skin. RSDL is a mixture of potassium 2,3-butanedione monoximate (KBDO), potassium 2,3 butanedione monoxime (also called diacetylmonoxime, DAM) in a solvent of polyethylene glycol monomethyl ether (MPEG) and water. The RSDL formulation is 1.25 molal KBDO in 9:1 MPEG:water with about 5 per cent DAM added to the solution (Bide, 1996 and 2002). The nominal molecular weight of MPEG is 550 daltons. This product acts to remove and neutralize the agent on the exposed skin. However, it is not to be used in wounds or in the eyes.

Liquids are best for decontaminating large or irregular surface areas. Current U.S. doctrine (Hurst 1997) describes the use of soapy water and 0.5% bleach (sodium or calcium hypochlorite solution) for skin decontamination. Soapy water solutions are well suited for mass casualty situations with adequate water supplies. Calcium hypochlorite

powder and Dakin's solution (a mixture of 0.5% sodium hypochlorite and 4% boric acid) have been used for chemical agent decontamination since World War I (Smart, 1997).

The use of a topical skin protectant was proposed as a protective measure against percutaneous exposure shortly after the first use of sulfur mustard (HD) by Germany at Ypres, Belgium, in 1917 (Papirmeister, 1991). The U.S. Army Medical Research Institute of Chemical Defense (USAMRICD), Aberdeen Proving Ground, MD, developed an effective topical skin protectant in the early 1990s. It was approved by the Food and Drug Administration (FDA) in 2000. This new product, known as SERPACWA, is now a standard issue item to U.S. forces when there is a threat of CWA use. Operationally, SERPACWA is designed to be used on the skin at the battledress overgarment (BDO) closures and on other vulnerable skin areas to enhance protection (Braue, 2006).

OBJECTIVE

The first objective of this study was to determine the efficacy of four decontamination products: the M291 SDK, 0.5% bleach, 1% soapy water, and RSDL challenged with VX. The second objective was to determine how the efficacy was affected by delaying application of these decontamination products following challenge with VX. The third objective was to determine the efficacy of SERPACWA challenged with VX.

MATERIALS AND METHODS

Experimental Design

Efficacy was based on the protective ratio (PR) defined as the median lethal dose (MLD, LD₅₀) of the treatment group divided by the MLD of the untreated control animals. The MLD value was determined by establishing 24-hour dose-response curves in sets of animals receiving various doses of agent spanning the non-lethal to lethal dose range. The dose-response curves were generated using the sequential stage-wise methods described by Feder et al. (Feder, 1991A and 1991B) allocating animals to several agent challenge levels per treatment group per stage. In the first stage, a range of agent doses for each treatment group was selected to span the predicted range of lethality from 0-100%. When possible, previously determined experimental data were used to select the range of doses for the first stage. The animals were randomly assigned to the challenge levels in each treatment group, and mortality was assessed 24 hours after agent exposure. Doses for subsequent test days (stages) were based on interim probit analyses after each test day and were selected to minimize the variance around the respective MLDs. The MLD estimation was considered complete when the ratio of the upper 95% minus the lower 95% confidence limit divided by two times the MLD was approximately 0.40 or less.

On a given day, either the decontamination products or SERPACWA was evaluated. Non-treated positive control animals were run during both decontamination and SERPACWA experiments. The data for the decontamination groups and the SERPACWA group were pooled to determine the lethality dose-response curve for

these treatment groups. If the decontamination experiments were performed around the same general time frame as the SERPACWA experiments (within 1-2 months) the positive control animals were pooled to determine the lethality dose-response curve for this group. Occasionally, negative control animals (animals anesthetized and treated but receiving no agent) were included to evaluate the effect of anesthesia.

Animals

The animals used in this study were haired guinea pigs [Hartley, Cri(HA)BR] obtained from Charles Rivers Labs (Montréal, Québec, Canada). The animals were all males in the weight range of 275-400 g. The animals were maintained under an AAALAC accredited animal care and use program. The animals were quarantined and observed for evidence of disease prior to protocol use. They were housed and maintained under USAMRICD SOP-VMSB-203, titled "Guinea Pig Husbandry." During quarantine the animals were housed 2 per polycarbonate cage on corncob bedding changed twice weekly. The animals were provided commercial guinea pig ration (Harlan Teklad guinea pig Diet, W, #7006) as appropriate and tap water *ad libitum*. Animal holding rooms were maintained at $21^{\circ} \pm 2^{\circ}\text{C}$ with $50\% \pm 10\%$ relative humidity using at least 10 complete air changes per hour of 100% conditioned fresh air. All animals were on a 12-hour light/dark, full-spectrum lighting cycle with no twilight. During the experimentation the animals were housed singly in polycarbonate containers containing contact bedding and kept in the exposure hoods for the postexposure holding period. Animals were observed periodically throughout the normal work day (0800 to 1700) until euthanasia.

All guinea pigs were fully sedated before agent exposure. The standard 2-minute decontamination experimental animals were given an intramuscular (i.m.) injection (in a rear leg) using the combination of ketamine (32 mg/kg) and xylazine (4 mg/kg) 5 minutes prior to agent exposure. The delayed decontamination experimental animals were given an initial i.m. injection of the combination of ketamine (87 mg/kg) and xylazine (13 mg/kg) 5 minutes prior to agent exposure and a second half dose i.m. injection of ketamine (44 mg/kg) and xylazine (7 mg/kg) if the animal showed signs of waking up before the scheduled decontamination time. The SERPACWA experimental animals were given an initial i.m. injection of the combination of ketamine (87 mg/kg) and xylazine (13 mg/kg) 5 minutes prior to agent exposure and a second half dose i.m. injection of ketamine (44 mg/kg) and xylazine (7 mg/kg) at 60 minutes postexposure. All injections were made using 1 ml syringes with 25 gauge, 5/8" needles.

The endpoint of these experiments was lethality measured 24 hours after exposure to VX. Lethality was selected because it has been historically the most quantitative and objective endpoint with which to determine the toxicity of nerve agents and to evaluate the effect of pretreatments, therapeutic countermeasures and decontamination. The 24-hour time was selected because it has been the standard time point used in these types of studies, and there was a large database on 24-hour lethality.

All animals were euthanized 24 hours postexposure in a halothane or isoflurane-filled chamber IAW USAMRICD SOP-VMSB-301, titled "Animal Euthanasia." After euthanasia, the area of skin receiving VX was excised down to the fat layer and placed in 5% bleach. The carcasses were disposed of IAW USAMRICD SOP-VMSB-301.

Standard 2-Minute Decontamination Procedure

On the morning of the experiment, animals (typically 16 per day) were assigned animal numbers (ear marked with permanent marker) and randomly assigned to treatment groups and transported from the animal holding room to the exposure laboratory. All animals were weighed, and a large area along the left ribcage was close-clipped with The Dander Free Clipper System (Hazard Technology, Millersville, MD 21108) using Oster Brand clippers (model: Golden A5) with a number 40 CryogenX blade. Animals were anesthetized and exposed using a 5-minute cycle. The cycle started by injecting the first animal with anesthesia solution. Once under anesthesia a small amount of Puralube Vet Ointment (Pharmaderm, Melville, NY 11747) was placed in each eye to prevent the eyes from drying out while under anesthesia. A rectangle about 2.5 by 4.0 cm was marked with a permanent marker within the clipped area on the side of the animal. Each animal was moved into the fume hood about 4 minutes after anesthesia administration. After the first animal received anesthesia, each additional animal was injected every 5 minutes.

At 5 minutes after anesthesia, agent was applied to the application site (Figure 1). Two minutes after agent application, the dosing site was either left untreated or decontaminated with one of the four decontamination products following current doctrine. The M291 SDK and RSDL were applied to the skin of test animals in a similar way using a decontamination applicator. The M291 SDK decontamination applicator was made by opening the M291 SDK packet, bisecting the enclosed mitt and trimming it to form two pads, approximately 2.5 x 6.0 cm, and attaching each pad to a wooden tongue depressor with ½-inch staples. The RSDL decontamination applicator was made by opening the RSDL packet and trimming the enclosed pad to form four pads, approximately 2.5 x 6.0 cm, and attaching the pads to a wooden tongue depressor with ½-inch staples. The applicators were prepared the morning of the experiment. The decontamination applicator was held by the end of the tongue depressor opposite the attached pad to position the pad over the exposure site. The decontamination process (Figure 2) involved ten strokes across the test site in a head-to-tail direction. The decontamination applicator for 0.5% bleach and 1% soapy water was made using a 10 x 10 cm gauze. The gauze was folded in half, wrapped around the tongue depressor, and fastened with two ½-inch staples. This created a wiping surface about 2.5 x 5.0 cm with four layers of gauze. Just before use, 5.0 ml of the either the 0.5% bleach or the 1% soapy water was applied to the gauze. The decontamination process involved ten strokes across the test site in a head-to-tail direction followed by another 10 strokes from a similar applicator wetted with 5.0 ml of distilled water. The 0.5% bleach and the 1% soapy water were prepared fresh the morning of the experiment. Exposed guinea pigs remained in the fume hood throughout the 24-hour observation period. They were provided with food and water *ad libitum*. The animals were observed for signs of toxicity

for the first 4 hours after exposure 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. These experiments were conducted between 3 Nov 04 and 28 Mar 05, except that 5 bleach animals and 3 soap animals were run on 25 Oct 05.

Figure 3 the decontamination applicators. The 0.5% bleach solution was prepared from certified lots of 5% household bleach diluted 1 to 10. The 1% soapy water solution was prepared using the Original Ultra Palmolive liquid dish detergent (Colgate-Palmolive, New York, NY 10022). RSDL packets (Figure 4) were procured from E-Z-EM, Inc. (Lake Success, NY 11042). M291 SDK packets (Figure 5) are manufactured at the Pine Bluff Arsenal in Pine Bluff, Arkansas, and were obtained from the U.S. Army Tank-Automotive and Armaments Command (TACOM, Rock Island, IL).

The evaluation of the M291 SDK was repeated about 8 months after the initial experiments were completed using only neat VX to challenge the M291 SDK animals. During these experiments a new positive control group of animals was included. The experiments were conducted between 25 Oct 2005 and 8 Dec 2005.

Delayed Decontamination Procedure

If a decontamination product provided good protection (protective ratios > 5) follow-up decontamination experiments were conducted that delayed the decontamination process to times greater than the standard 2 minutes postexposure. For these animals an LT_{50} was determined. The LT_{50} is the delayed decontamination time at which 50% of the animals die following a 0.625 mg/kg (5 LD_{50}) challenge. These experiments were conducted in a manner similar to the standard decontamination experiments, except for the delay in starting the decontamination process and the additional anesthesia given to these animals. These experiments were conducted between 25 Oct 2005 and 8 Dec 2005.

Standard SERPACWA Procedure

SERPACWA-treated animals were handled and exposed in much the same way as the decontaminated animals. The differences are noted in this section. SERPACWA (Figure 6) was applied according to current doctrine. SERPACWA animals were secured to a hold down board in sternal recumbency after animals were under anesthesia. A test site received a calculated rate of application of approximately 0.01 mL/cm² of SERPACWA (equivalent to an average depth of approximately 0.1 mm). A 1-mL disposable syringe (no needle) was used to deliver approximately 0.07 ml of SERPACWA to each circular test site, 3.0 cm in diameter. After application, the SERPACWA was uniformly spread over the marked site with a small spatula. Special care was taken to work the SERPACWA under the short hair stubble to obtain a uniform coating. This process was completed within the first 5 minutes after anesthesia injection. The SERPACWA was allowed to dry for an additional 15 minutes. VX challenge was administered to the SERPACWA protected skin site 20 minutes after the

initial anesthesia injection. Agent remained on the animal for a period of 2 hours. After the 2-hour exposure period, any remaining agent was removed with a dry wipe, and the animal was transferred to the holding cage in the fume hood. Special care was taken to position these animals in the hood to be sure that the exposure site was level prior to VX application. The maximum volume of VX that could be applied to SERPACWA-protected skin without the agent running off the site was about 70 μl . If VX was observed to run off the SERPACWA protected site during the exposure period, the animal was excluded from the study results. These experiments were conducted between 3 May 2005 and 24 May 2005.

The SERPACWA evaluation was repeated in a similar way except the agent challenge on SERPACWA was neat VX. These experiments were conducted between 26 Oct 2005 and 8 Dec 2005.

Agent Application

VX (O-ethyl S-[2-(diisopropylamino)ethyl]methylphosphonothiolate) was obtained from the US Army Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground, MD. The lot number was VX-U-4076 and had a purity of 94% as determined by NMR spectroscopy. VX was applied to the marked area on the animal (side for decontamination and back for SERPACWA) using one of various pipetting devices, depending on the volume needed. Volumes smaller than 2.0 microliters (μl) used either a Rainin micropipette (P-2, Rainin Instrument, LLC, Oakland, CA 94621) or a Hamilton microsyringe (0.5, 1, 5 μl , The Hamilton Co., Reno, NV 89502). Experiments that required volumes greater than 2.0 μl used a Rainin micropipette (P-10, P-20, P-100, P-200, P-1000) or a Drummond positive displacement microdispenser (10, 25, 50, 100, 1000 μl , The Drummond Scientific Co., Broomall, PA 19008). Either neat VX or a 5% VX isopropyl alcohol (IPA) solution was applied. The initial experiments used neat VX whenever the challenge dose required a volume greater than or equal to 0.5 μl and a 5% VX IPA solution when the volume was less than 0.5 μl . This decision was made to minimize the pipetting uncertainties in precision and accuracy inherent in trying to deliver sub-microliter volumes with a pipette. This pipetting decision resulted in all positive control animals (animals receiving VX but no treatment), animals decontaminated with the M291 SDK, and animals protected with SERPACWA being challenged with 5% VX IPA solution. All of the animals decontaminated with RSDL, 0.5% bleach, and 1% soapy water were challenged with neat VX. M291 SDK evaluation and SERPACWA evaluation were later repeated with neat VX challenge.

Data Analysis

Statistical analysis of the data from these experiments was performed using SAS computer program, version 6.12 (SAS Institute, Inc., Cary, NC 27513). Data sets were analyzed using specialized probit analysis programs for sequential stage-wise designs written using SAS NLIN to estimate the MLD and 95% confidence intervals (CI). The PROBSEP Program (see Appendix B) produced a great deal of statistical information, but only a small portion will be given in this report, including LD₁₀, LD₅₀, and LD₉₀ values with lower and upper 95% confidence intervals based on Fieller's method (Finney, 1971), and the probit slope. An additional specialized program using SAS, called PRORATIO (see Appendix C), used the output from the PROBSEP SAS program to calculate the PR of each of the treatments compared to the positive controls and to each of the other treatment groups. The PRORATIO program also estimated a confidence interval for the PR (using the Delta Method, Nelson, 1982), which was used to determine whether the PR was significant and therefore whether the MLDs of the paired treatment groups were significantly different.

RESULTS

In Appendix A, Tables A1-A5 provide the raw data for the standard decontamination experiments (decontamination 2 min postexposure). These tables provide the 24-hour survival data for positive control animals and animals decontaminated with 0.5% bleach, M291 SDK, RSDL, and 1% soapy water.

In Appendix A, Tables A6 and A7 provide the survival raw data for the repeat experiments with M291 SDK challenged with neat VX instead of a 5% VX IPA solution.

In Appendix A, Tables A8 and A9 provide the survival raw data for the initial SERPACWA experiments. In these experiments the control and SERPACWA animals were challenged with a 5% VX IPA solution. Negative control animals (animals treated with SERPACWA but not exposed to VX) were included as quality control animals because of the high dose of anesthesia administered to these animals to keep them sedated for at least 2 hours.

In Appendix A, Tables A10 and A11 provide the survival raw data for the repeat experiments where SERPACWA was challenged with neat VX instead of a 5% VX IPA solution.

In Appendix A, Tables A12, A13, and A14 provide the survival raw data for the delayed decontamination experiments. In these experiments, the decontamination process was delayed from 2 to 90 minutes postexposure. Some animals were scheduled for longer decontamination times, but were not decontaminated because they did not survive to their scheduled time. All these animals were challenged with 0.625 mg/kg neat VX representing a 5 LD₅₀ dosage.

Figure 8 is a graph of LD₅₀ values for the four decontamination products and positive control animals in the guinea pig model. The error bars represent the 95% CI. The number of animals used per treatment group was between 21 and 34. Positive control and M291 SDK animals were challenged with a 5% VX IPA solution. The 0.5% bleach, RSDL, and 1% soapy water animals were challenged with neat VX. The LD₅₀ values for control, 0.5% bleach, M291 SDK, RSDL, and 1% soapy water were 0.21, 3.7, 0.39, 14, and 3.4 mg/kg, respectively. Figure 9 is a graph of PR values calculated from the data in Figure 8. PR values with the same letter were not statistically different at the 0.05 decision level. The PR values for 0.5% bleach, M291 SDK, RSDL, and 1% soapy water were 17, 1.8, 66, and 16, respectively.

Figure 10 is a graph of LD₅₀ values for the M291 SDK repeat experiments in the guinea pig model. The error bars represent the 95% CI. The number of animals used per treatment group was between 27 and 48. Positive control animals were challenged with a 5% VX IPA solution. M291 SDK animals were challenged with neat VX. The LD₅₀ values for control and the M291 SDK were 0.13 and 0.14 mg/kg respectively. Figure 11 is a graph of protective ratio (PR) values calculated from the data in Figure 10. PR values with the same letter were not statistically different at the 0.05 decision level. The PR value for the M291 SDK with neat VX challenge was 1.1.

Figure 12 is a graph of LD₅₀ values for SERPACWA and positive control animals in the guinea pig model. The error bars represent the 95% CI. The number of animals used for positive controls and SERPACWA was 26 and 37, respectively. Positive control and SERPACWA animals were challenged with 5% VX IPA solution. The LD₅₀ values for control and the SERPACWA were 0.18 and 0.57 mg/kg respectively. Figure 13 is a graph of protective ratio (PR) values calculated from the data in Figure 12. PR values with the same letter were not statistically different at the 0.05 decision level. The PR value for SERPACWA with 5% VX IPA solution challenge was 3.2.

Figure 14 is a graph of LD₅₀ values for positive controls and SERPACWA in the guinea pig model. Error bars represent the 95% CI. The number of animals used for positive controls and SERPACWA was 48 each. SERPACWA animals were challenged with neat VX. Positive control animals were challenged with 5% VX IPA solution. The LD₅₀ values for control and the SERPACWA were 0.13 and 0.26 mg/kg respectively. Figure 15 is a graph of protective ratio (PR) values calculated from the data in Figure 14. PR values with the same letter were not statistically different. The PR value for SERPACWA with neat VX challenge was 2.1.

Figure 16 is a graph of percent lethality when RSDL decontamination is delayed following challenge by 0.625 mg/kg (5 LD₅₀s) of VX. The LT₅₀ (50% lethality time) was 31 minutes with a 95% CI of 30 to 32 minutes. The probit slope was 58 using a total of 63 animals. There was one observed animal death at the 2-minute delay time, but this data point was excluded from the analysis. Evaluation of the large data set available from the standard 2-minute decontamination with RSDL combined with the data set from the delayed decontamination provided overwhelming evidence that this data point was an outlier.

Figure 17 is a graph of percent lethality when 0.5% bleach decontamination is delayed following challenge by 0.625 mg/kg (5 LD₅₀s) of VX. The LT₅₀ was 48 minutes with a 95% CI of 32 - 53 minutes. The probit slope was 3.3 using a total of 41 animals.

Figure 18 is a graph of percent lethality when 1% soapy water decontamination is delayed following challenge by 0.625 mg/kg (5 LD₅₀s) of VX. The LT₅₀ was 25 minutes with a 95% CI of 23 to 43 min. The probit slope was 3.0 using a total of 37 animals. There was one observed animal death at the 5-minute delay time. We believe this death was associated with the large dose of anesthesia given to these animals, but there was insufficient data to justify rejecting this data point as an outlier.

Tables 1-3 provide a summary of the SAS probit analysis for all the experiments. It consists of, for each treatment, the number of animals, the LD₁₀, LD₅₀ and LD₉₀ (LT₁₀, LT₅₀ and LT₉₀ for delayed decontamination experiments), the lower and upper 95% CI, the dose-response curve slope and y-intercept, and the PR. Within a given experimental group, the SAS analysis using the Delta method determined which PRs were significantly different at both the 95 and 99.5% level. PRs with the same letter were not statistically different.

AGENT	No. of G.P.	Treatment	LD ₅₀ mg/kg		Slope	PR	PR 95% CI	95% Sig	99.5% Sig	LD ₁₀ mg/kg			LD ₉₀ mg/kg			
			LCL	UCL						LCL	UCL	LCL	UCL			
VX Sol'n	21	Control	0.215	0.141	0.256	10.73	1.00	1	a	a	0.163	0.035	0.198	0.283	0.242	0.773
VX Neat	30	Bleach	3.74	2.37	5.31	4.18	17.4	12.1-25.0	b, e	b, e	1.85	0.528	2.75	7.58	5.34	20.4
VX Sol'n	27	M291	0.387	0.253	0.469	7.62	1.80	1.40-2.31	c	c	0.263	0.067	0.338	0.570	0.470	1.32
VX Neat	34	RSDL	14.3	11.2	19.2	5.92	66.4	50.8-86.9	d	d	8.68	4.34	11.1	23.5	17.9	53.7
VX Neat	30	Soap	3.44	2.16	4.74	4.45	16.0	11.5-22.3	e, b	e, b	1.77	0.383	2.59	6.68	4.82	21.8
VX Sol'n	48	Control Repeat	0.125	0.116	0.140	18.6	1.00	1	a	a	0.107	0.087	0.116	0.147	0.134	0.196
VX Neat	32	M291 Repeat	0.141	0.091	0.218	5.09	1.12	0.72-1.75	a	a	0.079	0.071	0.130	0.251	0.190	0.020

Table 1. Data summary of efficacy experiments for decontamination products with animals challenged with VX. Includes decontamination experiments from 3 Nov 04 to 28 Mar 05 and M291 repeat experiments from 25 Oct 05 to 8 Dec 05.

AGENT	No. of G.P.	Treatment	LT ₅₀ min	LCL	UCL	Slope	95% Sig	99.5% Sig	LT ₁₀ min			LT ₉₀ min		
									LCL	UCL		LCL	UCL	LCL
VX Neat	41	Bleach delayed	47.5	31.7	71.6	3.34	a	a, b, c	19.6	4.61	30.0	115	75.0	498
VX Neat	63	RSDL delayed	30.7	29.6	31.7	55.8	b, c	b, a, c	29.1	28.1	30.1	32.3	31.2	33.5
VX Neat	38	Soap delayed	25.5	12.1	39.8	2.72	c, b	c, a, b	8.60	0.980	15.9	75.4	46.7	316

Table 2. Data summary of efficacy experiments for delayed decontamination with animals challenged with VX. Includes delayed decontamination experiments from 6 Dec 06 to 4 Dec 07.

AGENT	No. of GP	Treatment	LD ₅₀	LCL	UCL	Slope	PR	PR	95%	99.5%	LD ₁₀	LCL	UCL	LD ₉₀	LCL	UCL
								95% CI	Sig	Sig						
VX Sol'n	26	Control	0.175	0.155	0.207	13.3	1.00	1	a	a	0.140	0.080	0.158	0.219	0.192	0.426
VX Sol'n	37	SERPACWA	0.569	0.387	0.806	3.64	3.25	2.35-4.48	b	b	0.253	0.080	0.376	1.28	0.879	3.69
VX Sol'n	48	Control repeat	0.125	0.116	0.140	18.6	1.00	1	a	a, b	0.107	0.087	0.116	0.147	0.134	0.196
VX Neat	48	SERPACWA repeat	0.265	0.088	0.426	1.94	2.11	1.21-3.68	b	b, a	0.058	0.001	0.136	1.22	0.679	10.9

Table 3. Data summary of efficacy experiments for SERPACWA with animals challenged with VX. Includes SERPACWA VX solution experiments from 3 May 05 to 24 May 05, and SERPACWA repeat experiments with neat VX from 26 Oct 05 to 8 Dec 05.

Notes for Tables 1-3:

- LD₁₀, LD₅₀, and LD₉₀ = the dosage (mg/kg body weight) required to kill 10, 50, and 90% respectively of the test population.
- LT₁₀, LT₅₀, and LT₉₀ = the delayed decontamination time in minutes at which 10, 50, and 90% of the animals in the test population die following a 0.625 mg/kg (5 LD₅₀) challenge.
- LCL = Lower confidence limit at p < 0.05 (Fieller's Method).
- UCL = Upper confidence limit at p < 0.05 (Fieller's Method).
- PR = Protective ratio (LD₅₀ of treatment/LD₅₀ of control).
- PR 95% CI = Protective ratio 95% confidence interval (Delta method)
- 95% Sig = Protective ratios with same letter were not statistically different at the 0.05 decision level (Delta method).
- 99.5% Sig = Protective ratios with same letter were not statistically different at the 0.005 decision level (Delta method).
- Slope = The probit analysis slope.
- The LCL and UCL for the M291 repeat neat experiments were calculated using the Delta Method.

DISCUSSION

The initial scope of this project included only the efficacy of RSDL, M291 SDK, and SERPACWA challenged with several toxic agents. After the capabilities area program officer (CAPO) initiated discussions with the Joint Program Executive Office (JPEO) and the Chemical Biological Medical Systems (CBMS) staff, the decision was made to expand the scope in two areas. First, 0.5% bleach and 1% soapy water would be included in the evaluation because these decontamination products are currently listed in Army doctrine and used in the field. The second area was to include traditional chemical warfare agents as well as the other toxic agents. There was a limited amount of efficacy data available for decontamination products and SERPACWA in a rabbit model challenged with traditional nerve agents. Expanding this project to include the traditional agents that were included in the rabbit evaluations would serve as a bridging study to compare the old data in the rabbit model with the new data in the haired guinea pig model.

The four decontamination products evaluated can be divided into two categories based on cost and use. The M291 SDK and RSDL are relatively expensive. The cost of a packet of RSDL is about \$14, and the cost of a packet of M291 SDK is about \$0.70. Both decontamination products were designed to be carried by individuals for immediate lifesaving spot decontamination on small intact skin areas following exposure. On the other hand, 0.5% bleach and 1% soapy water are relatively inexpensive and generally used for whole body decontamination of an exposed person prior to moving them from a dirty zone into a clean zone. All four of these products are described in U.S. doctrine for CWA decontamination of intact skin; however, a comprehensive evaluation comparing the efficacy of these products was never accomplished. This report is the first in a series to provide a comprehensive comparison of the efficacy of these decontamination products and SERPACWA against all of the traditional agents and other toxic agents.

There was a great deal of discussion over which was the best animal model to use for these studies. In the end, the haired guinea pig was selected as the most suitable animal model for the reasons outlined below:

- Pharmacological screening of countermeasures (oxime, anticonvulsant, and bioscavenger) was conducted in guinea pigs.
- Traditional agent and other toxic agent toxicokinetic and pharmacological data were from studies conducted with guinea pigs.
- All screening data for decontamination products at USAMRICD were from studies conducted with guinea pigs.
- Most toxicological and pharmacological data from other sources were from guinea pig studies.
- The CAPO at the Defense Threat Reduction Agency (DTRA), John Oprandy, recommended that a single small animal model be used in all toxic agent work.
- The guinea pig model was efficient to use: small and easy to fit in the hood, low cost, and low body weight, which required small amounts of agent.

The real world threat scenario is for exposure to neat agent not agent in solution. The toxicity of VX was so great, however, that the dosage volumes required to cover the full range of the dose-response curve for untreated animals were so small that they could not be pipetted reliably. The decision was made to minimize the pipetting uncertainties in precision and accuracy by using neat VX whenever the challenge dose required a volume greater than or equal to 0.5 μl and a 5% VX IPA solution whenever the challenge dose required a volume less than 0.5 μl . For our initial experiments, this pipetting decision resulted in all positive control animals (animals receiving VX but no treatment), animals decontaminated with the M291 SDK, and animals protected with SERPACWA being challenged with 5% VX IPA solution. All of the animals decontaminated with RSDL, 0.5% bleach, and 1% soapy water were challenged with neat VX.

During the evaluation of toxic agents, we discovered that the efficacy of a decontamination product can be greatly affected by whether the agent is neat or in solution. We observed that the decontamination products were significantly less effective for animals challenged with agent in solution rather than when the agent was neat. We theorized that this observation resulted from the solvent increasing the penetration rate of the agent through the skin, thus making the surface decontamination process less effective. These observations caused us to re-think our decision to use agent in solution when evaluating cutaneous treatments. Following this analysis, the decision was made in November 2005 to use only a neat agent challenge for evaluating skin treatments in all future experiments. We also made the decision to re-evaluate the M291 SDK and SERPACWA using neat VX challenge instead of 5% VX IPA solution. We recognized that trying to pipette very small volumes (0.05 to 0.5 μl) could not be accomplished with a high degree of accuracy or precision. We decided it was better to use neat agent and accept this uncertainty than to generate false data from using agent in solution. The uncertainty in delivering these very small volumes was mitigated to some degree by using more animals for each experiment. For positive control animals, which received no treatments, agent in solution was still used if the required volume was < 0.5 μl . The rationale for this decision was that the agent had 24 hours to reach the systemic circulation; thus a modified penetration rate should not significantly affect the observed 24-hour lethality. Experiments conducted in our laboratory by Edward Clarkson (Clarkson, 2002) showed that the lethality rate did not change for VX exposure whether the solvent was IPA or methylene chloride. The LD₅₀ values observed in guinea pigs challenged with 10% VX in IPA solution and 10% VX in methylene chloride solution were 0.15 and 0.14 mg/kg, respectively. Since GD is less toxic than VX both the neat and solution toxicities were determined by Clarkson. The observed LD₅₀ values for neat GD and 10% GD in methylene chloride solution were 6.8 and 11.3 mg/kg, respectively. The LD₅₀ value was also determined in guinea pigs following challenge with 10% GD in methylene chloride solution occluded for 2 hours following exposure. The LD₅₀ value was observed to be 1.8 mg/kg. Clearly the toxicity of a volatile agent does depend on whether or not the agent is in solution. Since VX is a non-volatile agent, it is unclear how this observation relates to VX challenge in guinea pigs. Even if the observed 24-hour lethality were affected slightly by the use of 5% VX

IPA solution, the relative efficacy of the decontamination products as measured by the relative PR values would be the same.

Army doctrine instructs that skin decontamination should be done “within the first minute or two after exposure” ([Medical Management of Chemical Casualties Handbook, 2000](#)). Using this guidance and since most of the earlier decontamination evaluations were performed 2 minutes after exposure we decided to use a 2-minute delay as the standard decontamination time. Significance in this report is defined as $p < 0.05$ unless otherwise stated. In the initial series of experiments the calculated PRs for the standard 2-minute decontamination experiments with RSDL, 0.5% bleach, 1% soapy water, and M291 SDK (solution) were 66, 17, 16, and 1.8, respectively. RSDL was by far the most effective decontamination product tested and significantly better than any of the other products. Bleach and soapy water provided equivalent and good protection ($PR > 5$). They were both significantly better than the M291 SDK. The M291 SDK provided only modest protection with a PR of 1.8. When the M291 SDK evaluation was repeated using a neat VX challenge, the PR decreased to 1.1 and was not significantly different from that in animals receiving no treatment at all. In the repeat experiments, we could not deliver doses small enough to accurately define the low end of the dose-response curve for the M291 SDK. The less stringent Delta method was used to calculate the 95% CIs because the SAS program could not calculate results using the Fieller's method.

The LD_{50} value is traditionally used to compare the toxicity of chemicals; however, the probit slope from the dose-response curve is also an important parameter to indicate how quickly the percent lethality changes with applied dose. If the probit slope is flat, one observes a significant percentage of deaths or survivors at doses far removed from the median lethal dose. The PROBSEP program used by SAS to analyze this data set not only provided the LD_{50} values but also gave effective doses for the complete range of lethality percentiles including 1, 10, 16, 30, 50, 70, 84, 90, and 99. The slope, of course, is a reflection of the effective doses over this complete range. The effective doses over this entire range are recorded in the lab notebooks but are not provided in this report. We did provide the slope, LD_{10} , LD_{50} , and LD_{90} values along with the 95% CI values to provide the reader with the information necessary to fully understand the toxicity of VX and the effectiveness of the products tested.

In these decontamination experiments, the positive controls had the steepest slope with values of 11 (original) and 19 (repeat experiments). The slopes for the decontamination products were much less but still relatively steep with values of 5.9, 4.2, 4.4, and 5.1 for RSDL, bleach, soapy water, and M291 SDK (neat), respectively. Although the LD_{50} values have been traditionally used as the measure of toxicity, it may be more relevant to know the LD_1 or LD_{10} value. It may be most relevant to know the dose when very few or no deaths are expected. One reason the LD_{50} value is generally used for toxicity assessment is that it is the place on the dose response curve where the most information is collected and, thus, has the smallest CI. If one uses the LD_{10} values to calculate the PRs the resulting values for RSDL, bleach, soapy water, and M291 SDK (neat) are 53, 11, 11, and 0.74, respectively. These values are the same order of

magnitude and relative efficacy as the values calculated with the LD₅₀ values reflecting the similar slopes.

A possible reason for the poor performance of M291 SDK in the guinea pig studies may be understood from Figure 19. Every time the M291 SDK is used to wipe away agent, the agent is observed to streak or smear. The result is that the agent is spread out over a larger skin area. The smearing is observed because the M291 SDK absorbent resin does not instantly nor irreversibly adsorb the agent. Preliminary results from another agent in this study suggest that the efficacy of the M291 SDK can be significantly improved by first blotting the agent followed by wiping with a new or clean part of the M291 SDK pad. Detailed results of this observation will be reported in a future manuscript.

Battelle Memorial Institute (Columbus, OH) conducted three evaluations of decontamination products challenged with VX sponsored by the U.S. Army Medical Research and Materiel Command. These evaluations were conducted using the same general methodology as the experiments described in this report with three exceptions. The animal model was the clipped rabbit (New Zealand White, albino male), the bleach and soapy water decontamination process did not use a second 10 wipes with distilled water, and all animals (including positive controls) were challenged with neat VX. The first study conducted in September 2001 (Snider, 2002, Battelle Task 0008, Module 1) involved a direct comparison of RSDL and the M291 SDK. The results are summarized in Table 4.

Decon System	Total No. of Animals	Probit Dose-Response Slope		VX LD ₅₀ Dose (mg/kg)	Fieller's 95% Confidence Interval (CI)	Delta Method PR	
		Slope Estimate (Std.)	P-value for Signif.			Protective Ratio	95% CI
RSDL	24	7.98 (3.70)	0.0311	2.32	1.43 - 3.65	66	53 – 82
M291 SDK	24	3.31 (1.28)	0.0096	0.335	0.144 - 0.547	9.6	6.2 – 15
None	24	16.4 (7.1)	0.0207	0.035	0.029 - 0.042	1	-

Table 4. Data summary of efficacy evaluation studies for RSDL and M291 SDK conducted at Battelle Memorial Institute in September 2001 (Snider, 2002, Battelle Task 0008, Module 1). Std. = standard deviation.

The probit dose-response slopes and the PR observed for RSDL in the Battelle study (slope = 7.98, PR = 66) using rabbits correlate very well with the values from our study using guinea pigs (slope = 5.9, PR = 66). The PR observed for the M291 SDK in the Battelle study (9.6), however, is significantly different from the PR value observed in our study (1.1). In the rabbit model the M291 SDK provided good protection, but in the

guinea pig model the M291 SDK provided no protection. We do not understand why this difference was observed.

The second study conducted in February 2002 evaluated RSDL (Snider, 2002, Battelle Task 0008, Module 2). In this repeat evaluation for RSDL using 26 rabbits the LD₅₀, slope, and PR values for RSDL were 7.25 (4.70-11.5 Fieller's 95% CI), 3.0, and 207 (137-314 Delta 95% CI), respectively. The PR observed in this study was significantly different from the value in our study and the value from Battelle's previous study. In this second study, Battelle calculated the PRs using the LD₅₀ values for control animals determined in Task 8, Module 1. Possible explanations for the significant increase in the observed PR in this study include degradation of VX and/or change in season from fall to winter affecting the response of animals due to lighting cycle, temperature, and/or humidity. Even with the conflicting results, RSDL provided excellent protection from VX. In our studies, we determined the LD₅₀ of untreated control animals three different times with values of 0.215 (Jan 2005), 0.175 (May 2005), and 0.125 (Dec 2005). The LD₅₀ values vary significantly with time; therefore, it is always best to run the untreated control animals concurrent with treated animals for the most reliable results.

The third study conducted in January 2003 (Snider, 2005, Battelle Task 0015, Part 1) evaluated 0.5% bleach and 1% soapy water (Tincture Green Soap, USP). The results are summarized in Table 5.

Decon System	Total No. of Animals	Probit Dose-Response Slope		VX LD ₅₀ Dose (mg/kg)	Fieller's 95% Confidence Interval (CI)	Delta Method PR	
		Slope Estimate	P-value for Signif.			Protective Ratio	95% CI
0.5% bleach	18	5.97	< 0.0001	0.72	0.54 - 0.98	14.8	9.91 - 22.1
1% soapy water	18	5.97	< 0.0001	0.45	0.33 - 0.62	9.29	6.15 - 14.0
None	18	5.97	< 0.0001	0.048	0.035 - 0.066	1	–

Table 5. Data summary of efficacy evaluation studies for 0.5% bleach and 1% soapy water conducted at Battelle Memorial Institute in January 2003 (Snider, 2005, Battelle Task 0015, Part 1).

In this Battelle study the slopes are all the same because the best fit of the data used a common slope probit model. Again there is generally good correlation of the slopes and PRs between the rabbit and guinea pig models. The PRs for 0.5% bleach (rabbit = 14.8 [9.91-22.1] and guinea pig = 17.4 [12.1 – 25.0]) have overlapping CI, and the PRs for 1% soapy water (rabbit = 9.29 [6.15 – 14.0] and guinea pig = 16.0 [11.5 – 22.3]) are just outside the CI. Perhaps the PR for soapy water was a little less for the

Battelle study because of the extra 10 wipes with distilled water done in the guinea pig studies in our lab.

SERPACWA was observed to provide only modest protection against VX in our guinea pig model with PRs of 3.2 (5% VX IPA solution) and 2.1 (VX neat). There was not a significant difference in efficacy whether the agent was applied neat or in IPA solution. The observed slopes for the VX solution and repeat VX neat experiments were 3.6 and 1.9, respectively. These values represent relatively flat dose-response curves and are a reflection of the variability in the experimental data. There is a fair degree of uncertainty in getting uniform coverage of SERPACWA on the experimental test site. The hair is so thick that even after close clipping dense hair stubble remains. The SERPACWA is applied by spreading it out over the test site and working it into and under the hair shafts; but even with extreme care it is difficult to get uniform coverage of the experimental area. There is also a high degree of variability in the applied dose to SERPACWA-treated animals. When VX was applied neat, it was difficult to accurately apply the very small doses (0.05 - 0.5 μ l) required for the low end of the dose-response curve. The PR calculated using the LD₁₀ values instead of the LD₅₀ values was only 0.54. Again this is a reflection of the relatively flat dose-response curve.

Battelle conducted a study to evaluate SERPACWA (Snider, 2005, Battelle Task 0015, Part 1) in February 2003. The testing procedure was similar to the method described in this report except a clipped rabbit model was used, the dry time was 60 minutes instead of 15 minutes, the challenge time was 4 hours instead of 2 hours, and after the 4-hour exposure period the sites were wiped with a dry gauze to remove the agent and SERPACWA, followed by two skin decontaminations with 10% Ca(OCl)₂ solution and two more decontaminations with water. The results from the Battelle study are summarized in Table 6.

Pretreatment	Total No. of Animals	Probit Dose-Response Slope		VX LD ₅₀ Dose (mg/kg)	Fieller's 95% Confidence Interval	Delta Method PR	
		Slope Estimate	P-value for Signif.			Protective Ratio	P-value for Signif.
None	18	5.97	< 0.0001	0.048	0.035 - 0.066	1	-
SERPACWA	17	5.97	< 0.0001	2.5	1.85 - 3.32	52	< 0.0001

Table 6. Data summary of efficacy evaluation study for SERPACWA conducted at Battelle Memorial Institute in February 2003 (Snider, 2005, Battelle Task 0015, Part 1).

The probit slope was the same for controls and SERPACWA animals because a common slope model was fitted to all the treatment groups evaluated. Throughout the development of the SERPACWA final formulation, a 4-hour challenge was considered the standard exposure time to use for efficacy evaluations. Rabbits in the weight range

of 2 to 4 kg were used for most of the *in vivo* studies, and this model allowed the animals to be kept under anesthesia for 4 hours. Guinea pigs with their lower body weight of 250 – 400 g, however, could not be kept under anesthesia for 4 hours without a high mortality rate. Preliminary experiments determined that 2 hours was about as long as these animals could be safely kept under anesthesia with this particular anesthesia regimen, so this time was used for the agent exposure.

The efficacy of SERPACWA observed with rabbits in the Battelle study (PR = 52) is strikingly different from the efficacy observed with guinea pigs in our study (PR = 2.1). A very likely explanation for the large difference in observed efficacy is the difference in the procedure. In our study with guinea pigs, the experimental sites were not decontaminated at the end of the exposure period. In the Battelle study, however, the sites were blotted with decontamination solution 4 times, twice with concentrated bleach (10%) and twice with water. While SERPACWA acts as a good physical barrier, it is likely that during the long exposure periods of either 2 or 4 hours, some agent penetrates into the SERPACWA layer. The simple blotting procedure used in the guinea pig studies would not remove the agent that had penetrated below the surface of SERPACWA. This trapped agent would eventually migrate to the skin surface and be absorbed into the systemic circulation causing toxicity. The rabbit experiments, however, used a vigorous decontamination procedure that would remove most if not all of the agent from the SERPACWA barrier. Our hypothesis is that this decontamination step in the rabbit experiments is the explanation for the much higher observed PR in the rabbit experiments. The results from the delayed decontamination experiments with VX that demonstrated that delayed decontamination with 0.5% bleach is significantly effective even when delayed for 30 minutes postexposure support this hypothesis. We plan to test this hypothesis in future experiments.

In a real-life scenario, warfighters or civilians may not realize that they have been contaminated with a toxic agent. Thus, they may not start the decontamination process until well after the recommended time of 1 or 2 minutes postexposure. The conventional wisdom for many years was that decontamination would only be effective if performed in the first few minutes after exposure. When this study started in fiscal year (FY) 2005, there were literally no comprehensive evaluations available on the effectiveness of decontamination products beyond the standard 2-minute delay time. A limited study (Hamilton, 2004) using only 3 animals per treatment group evaluated VX decontamination with RSDL in swine (Yorkshire-Landrace cross, 20 kg). In this study, RSDL was found to be significantly effective 15 minutes postexposure for neat VX challenge to the ear but not significantly effective 30 or 60 minutes postexposure for neat VX challenged to the epigastrium (belly). Recognizing the need for a comprehensive study, the scope our current study was expanded to include delayed decontamination studies.

A fixed challenge dose of 0.625 mg/kg (5 LD₅₀) was used for all delayed decontamination studies. This dose was selected because historically a 5 LD₅₀ dose was the suggested minimum target for therapeutics selected for fielding. The lethality delay time-response curves were generated using the sequential stage-wise method

similar to the LD₅₀ dose-response curves using the delay time in place of the mg/kg dose. The standard probit analysis program was used to find the lethality percentiles associated with a given decontamination delay time. The LT₁₀, LT₅₀, and LT₉₀ values were defined as the delayed decontamination times at which 10, 50, and 90% of the animals in the test population die following a 0.625 mg/kg (5 LD₅₀) challenge. A PR of 5, which is directly related to protection from a 5 LD₅₀ challenge, was the decision criteria for choosing which decontamination products were selected for delayed decontamination experiments. Any decontamination products with a PR > 5 would be evaluated for delayed decontamination. For the VX experiments RSDL, 0.5% bleach and 1% soapy water were all selected for delayed decontamination evaluation. The M291 SDK with a PR of only 1.1 was not significantly efficacious and omitted from the delayed decontamination experiments.

RSDL was the first decontamination product evaluated for delayed decontamination and the results were unexpected. No animal deaths were observed for delay times through 25 minutes. The LT₁₀, LT₅₀, and LT₉₀ values were 29, 31, and 32 min respectively. These values reflect the very steep probit slope of 56. The results for bleach and soapy water also were unexpected with LT₅₀ values of 48 and 26, respectively. Neither of these was statistically ($p < 0.05$) different from the RSDL value. All three of these decontamination products provided significant protection when decontamination was delayed. These results suggest a much wider window of opportunity for effective decontamination than previously believed. It is important to note, however, that similar results may not be observed for other agents. The best policy is still to start the decontamination process as soon as practically possible after suspected exposure to toxic agents.

A goal of this project was to act as a bridging study between the early results observed in a clipped rabbit model with the current results observed in the clipped guinea pig model. The correlation between the two models is mixed. There is good correlation between the decontamination data (PRs and slope) for RSDL, 0.5% bleach, and 1% soapy water. There is poor correlation between the decontamination data for the M291 SDK and SERPACWA. There is a reasonable explanation for the discrepancy in the SERPACWA data (rabbit PR = 52 and guinea pig PR = 2.1). In the rabbit SERPACWA experiments, the skin was thoroughly decontaminated of agent, but the agent was only dry blotted in the guinea pig experiments; this is a very plausible explanation for the observed differences. In the rabbit model, the M291 SDK provided significant protection (PR = 9.6), but in the guinea pig model it provided no protection (PR = 1.1). The difference in PRs for the M291 SDK data is not large but statistically significant. There is no readily apparent explanation for this observed difference. There is also a statistically significant difference in the PRs (66 vs. 207) observed for RSDL in two different studies conducted by Battelle in the rabbit model. A possible explanation is the fact that the second study, conducted 4 months after the first study, used the LD₅₀ calculated for the control animals in the first study instead of determining a new value. Agent degradation or seasonal differences in temperature, humidity, and light cycle may have contributed to the observed difference in the PR values. Considering all the data,

there was reasonable correlation between the two models. A better understanding of the correlation may be achieved when data from other agents are available.

CONCLUSIONS

- RSDL provided superior protection against VX compared to the other products tested.
- 0.5% bleach and 1% soapy water were less effective than RSDL, but still provided good protection against VX.
- The M291 SDK was the least effective decontamination product and did not provide significant protection against VX.
- Agent was observed to streak when using the M291 SDK and efficacy was improved when agent was first blotted followed by wiping.
- RSDL, 0.5% bleach, and 1% soapy water provided significant protection against a 5 LD₅₀ challenge of VX even when decontamination was delayed for up to about 30 minutes.
- SERPACWA provided significant, but modest protection against VX.
- There was reasonable correlation between using the clipped rabbit model and the clipped guinea pig model for decontamination efficacy evaluations.

FIGURES



Figure 1. Agent challenge to clipped haired guinea pig in decontamination experiments.



Figure 2. Guinea pig decontamination using decontaminant material (M291 SDK) fixed to a tongue depressor.

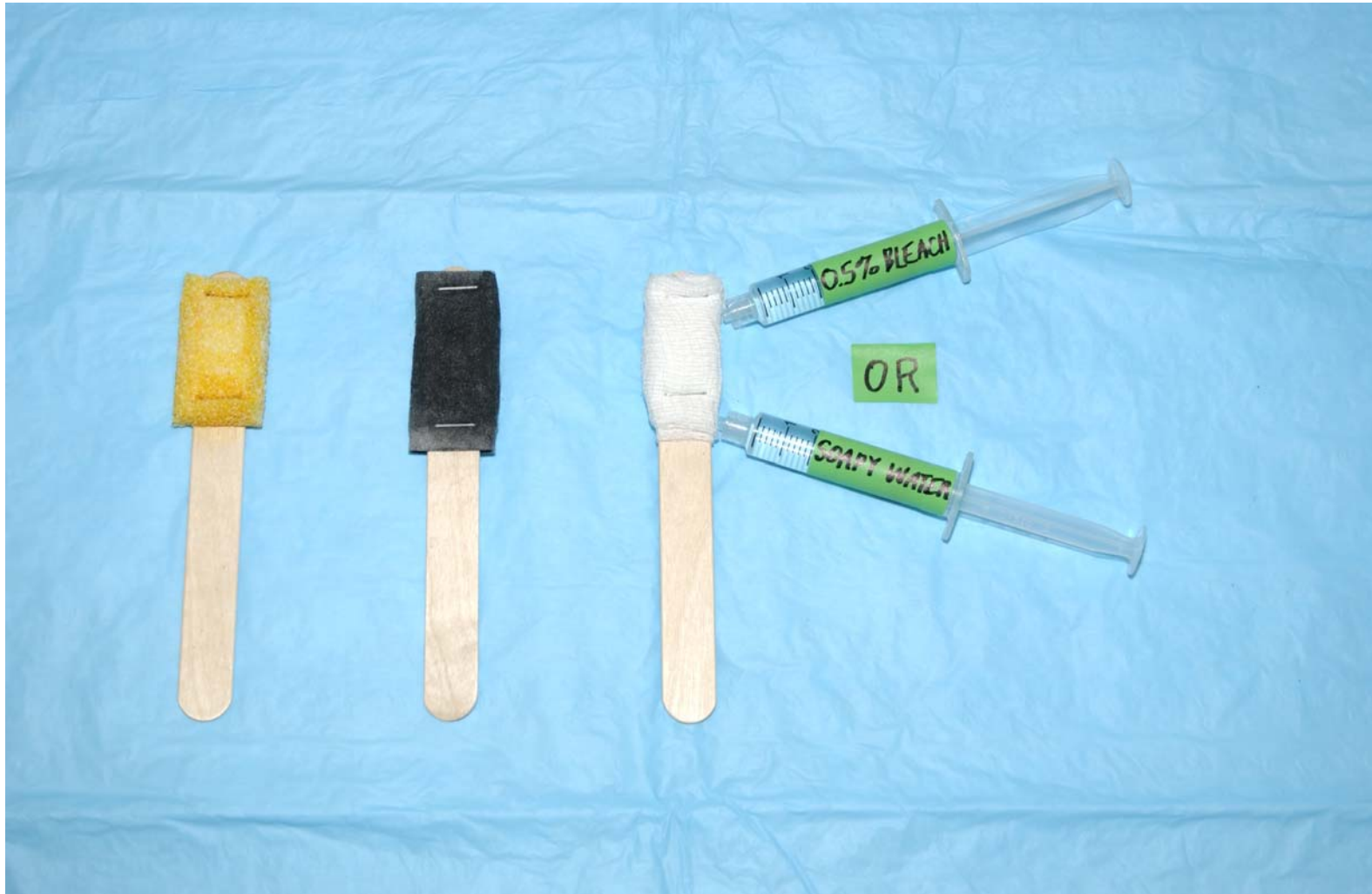


Figure 3. Decontaminant applicators (left to right) for RSDL, M291, and 0.5% bleach or 1% soapy water. Gauze applicator is wetted with 5 ml bleach or soapy water from syringe.



Figure 4. Reactive Skin Decontamination Lotion (RSDL) Kit, front and back of packet with decontaminant sponge (left to right).



Figure 5. M291 Skin Decontamination Kit, front and back of packet with decontaminant sponge (left to right).



Figure 6. Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA), front and back of packet.



Figure 7. SERPACWA application and agent challenge.

VX Liquid Challenge

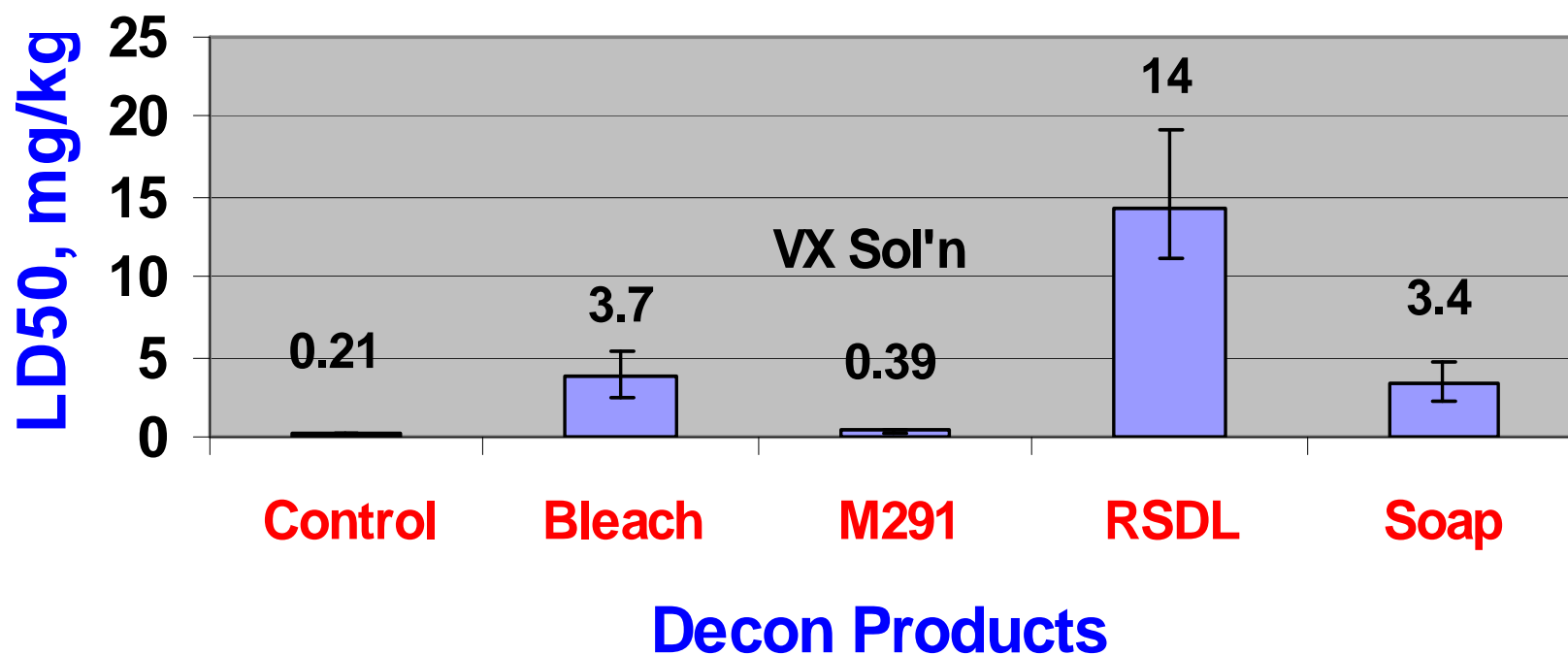


Figure 8. Graph of LD₅₀ values for decontamination products in guinea pig model. Error bars = 95% CI. The number of animals used per treatment group was 21-34. 5% VX IPA solution was used for control and M291 SDK animals. Neat VX was used for 0.5% bleach, RSDL, and 1% soapy water animals.

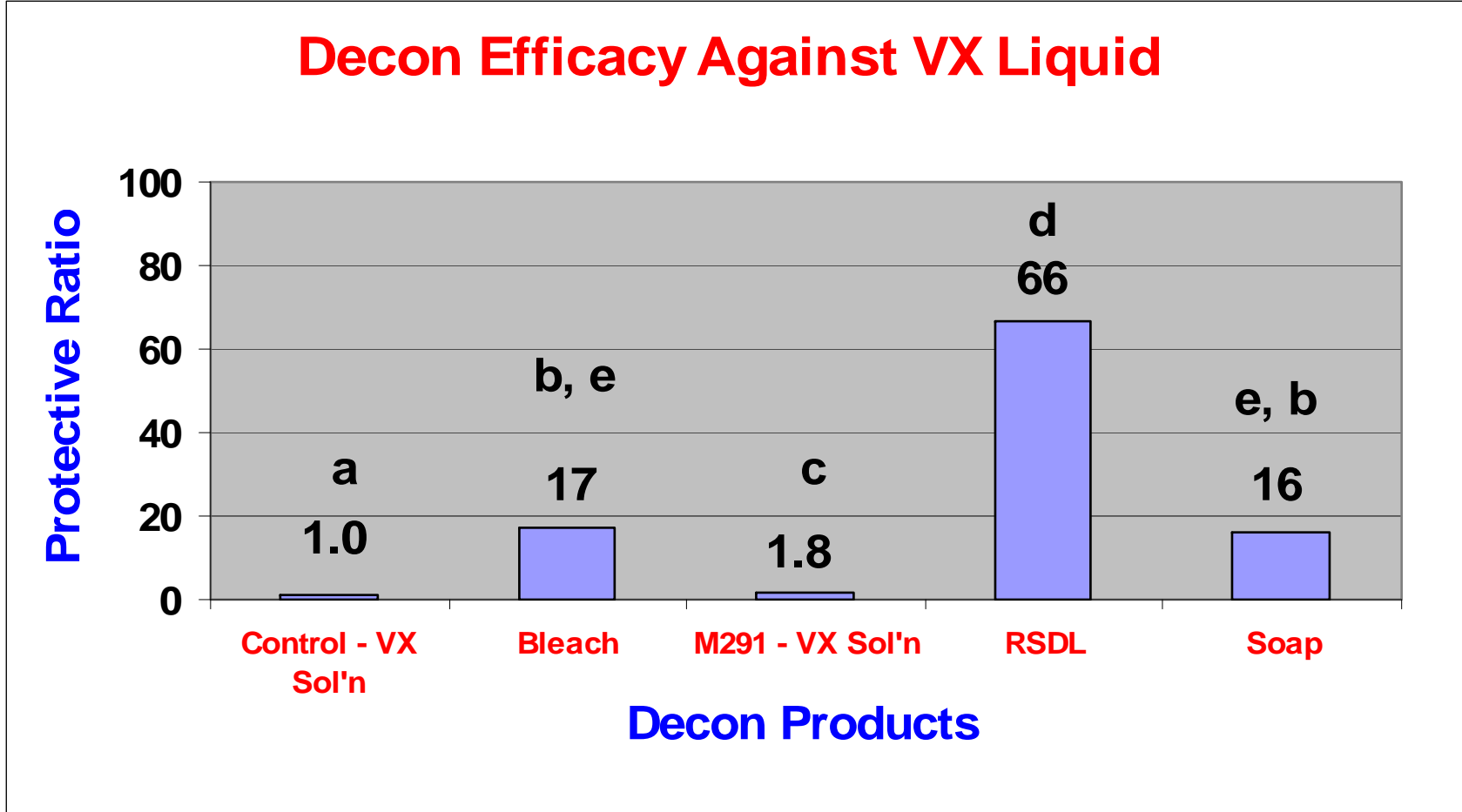


Figure 9. Graph of protective ratio (PR) values for decontamination products in guinea pig model. The 5% VX IPA solution was used for control and M291 SDK animals. Neat VX was used for 0.5% bleach, RSDL, and 1% soapy water animals. PRs with same letter were not statistically different at the 0.05 decision level.

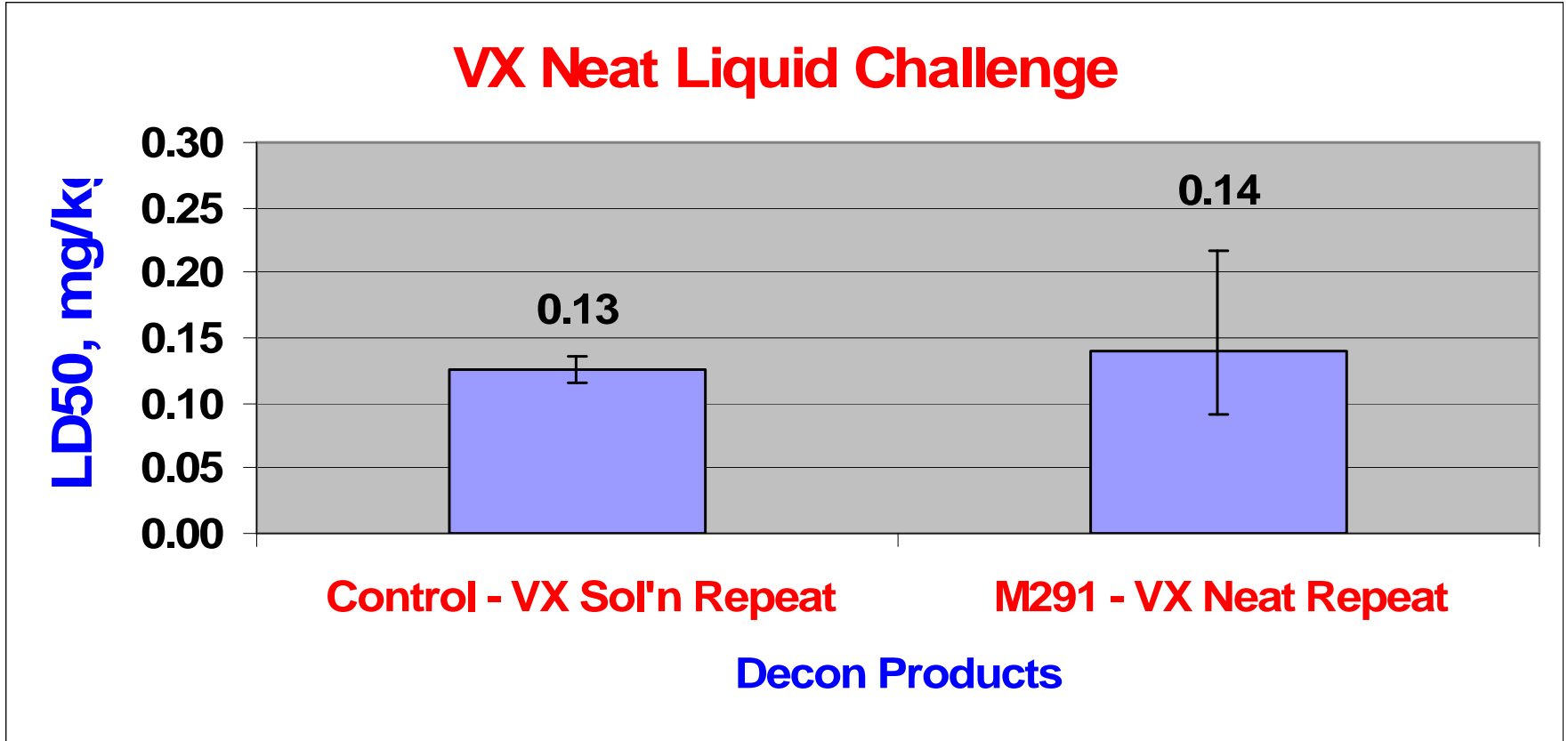


Figure 10. Graph of LD₅₀ values for the M291 SDK repeat experiments in guinea pig model. Error bars = 95% CI. The number of animals used per treatment group was 27-48. The 5% VX IPA solution was used for control animals. Neat VX was used for M291 SDK animals.

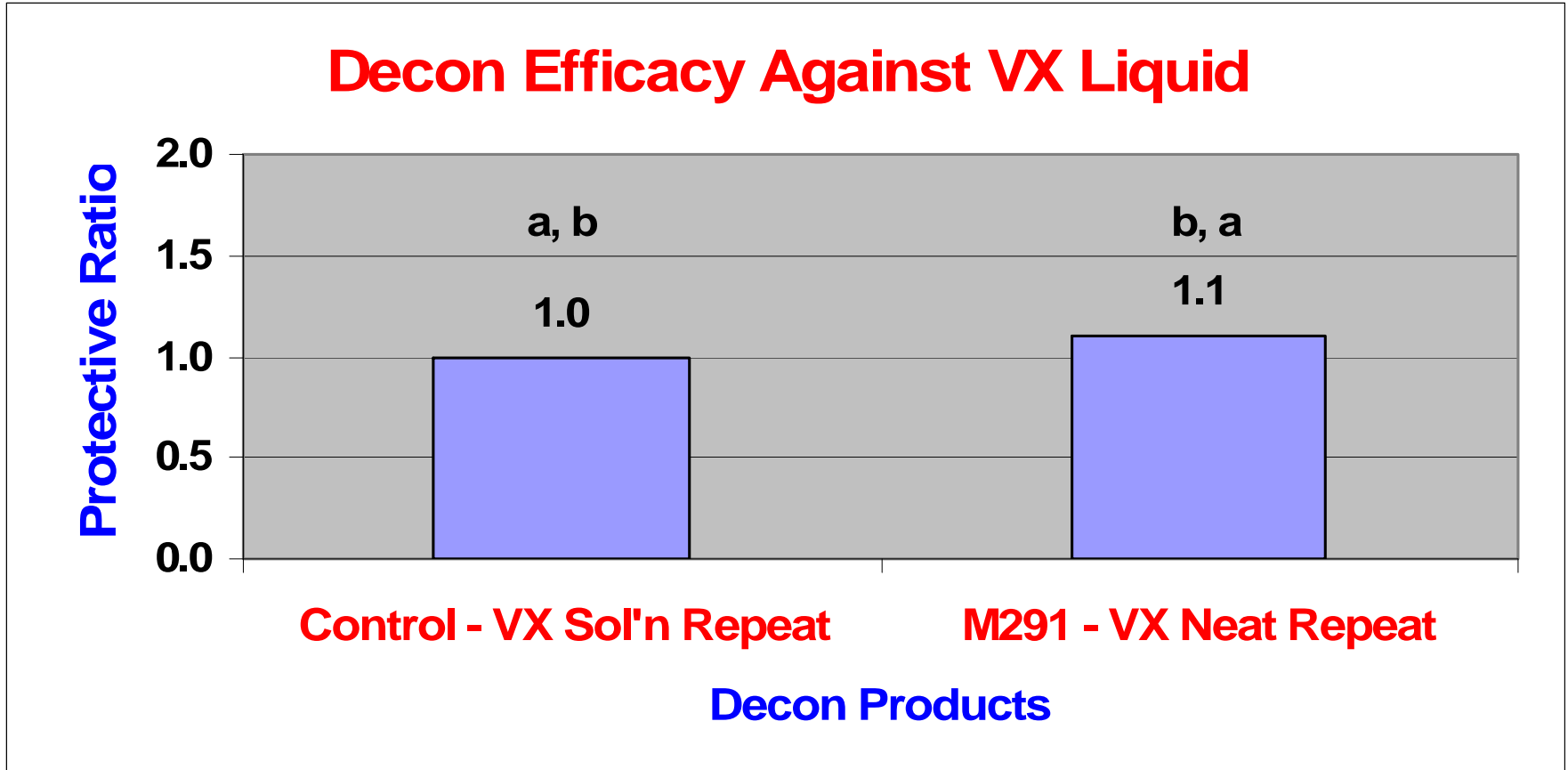


Figure 11. Graph of protective ratio (PR) values for the M291 SDK repeat experiments in guinea pig model. The 5% VX IPA solution was used for control animals. Neat VX was used for M291 SDK animals. PRs with same letter were not statistically different at the 0.05 decision level.

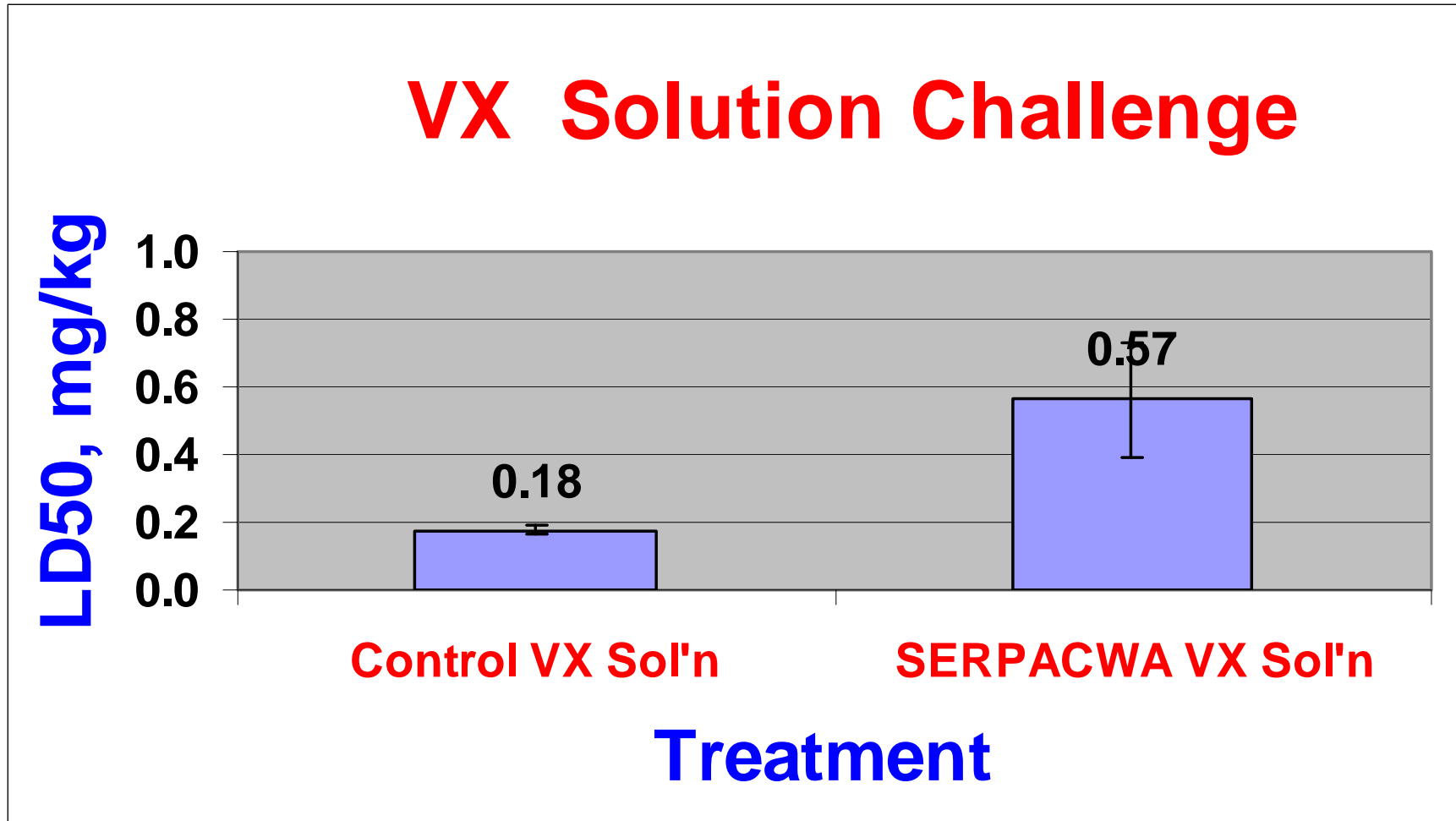


Figure 12. Graph of LD₅₀ values for positive controls and SERPACWA in the guinea pig model. Error bars = 95% CI. The number of animals used for positive controls and SERPACWA was 26 and 37, respectively. Positive control and SERPACWA animals were challenged with 5% VX IPA solution.

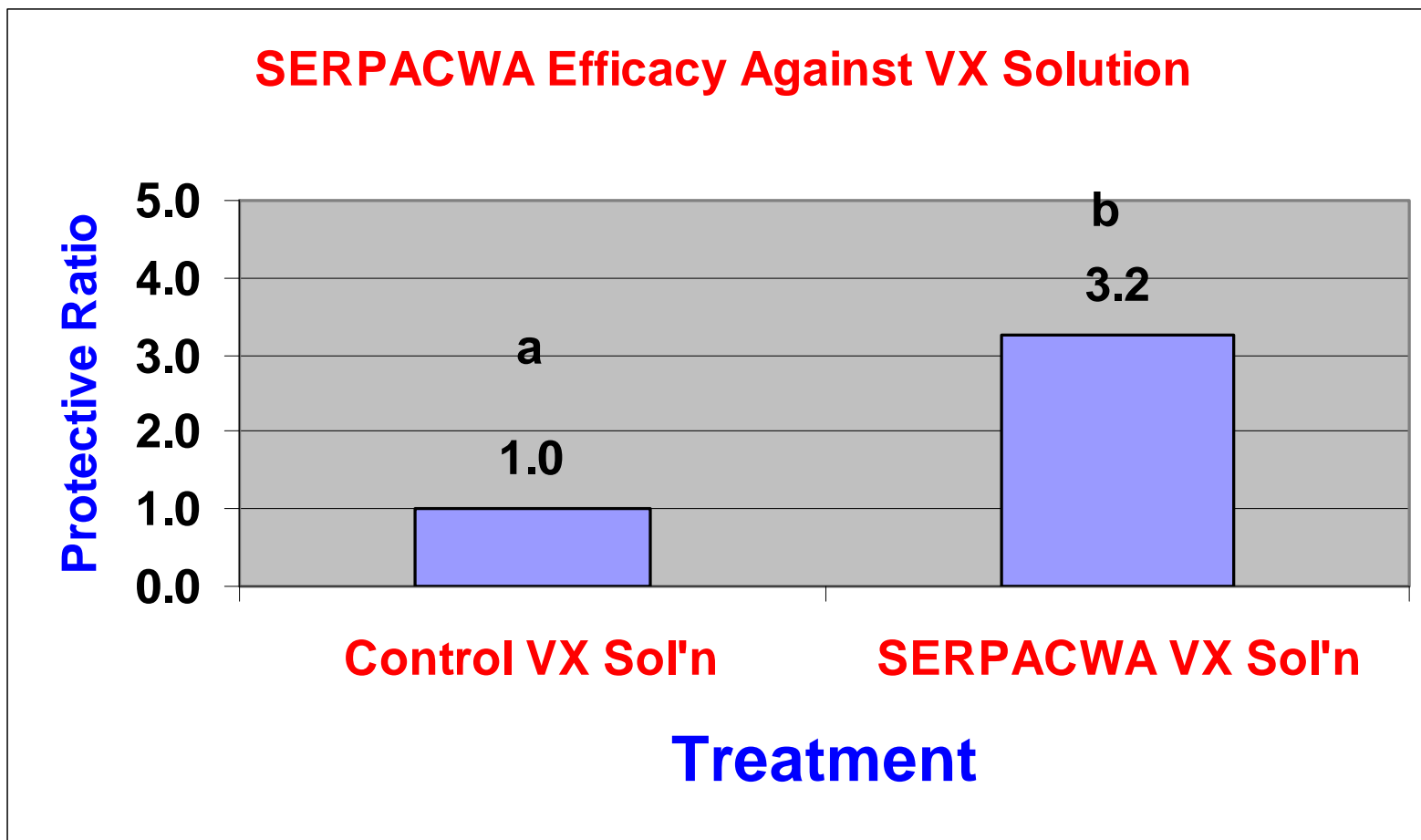


Figure 13. Graph of PR values for positive controls and SERPACWA in the guinea pig model. The number of animals used for positive controls and SERPACWA was 26 and 37, respectively. Positive control and SERPACWA animals were challenged with 5% VX IPA solution. PRs with same letter were not statistically different at the 0.05 decision level.

VX Liquid Challenge

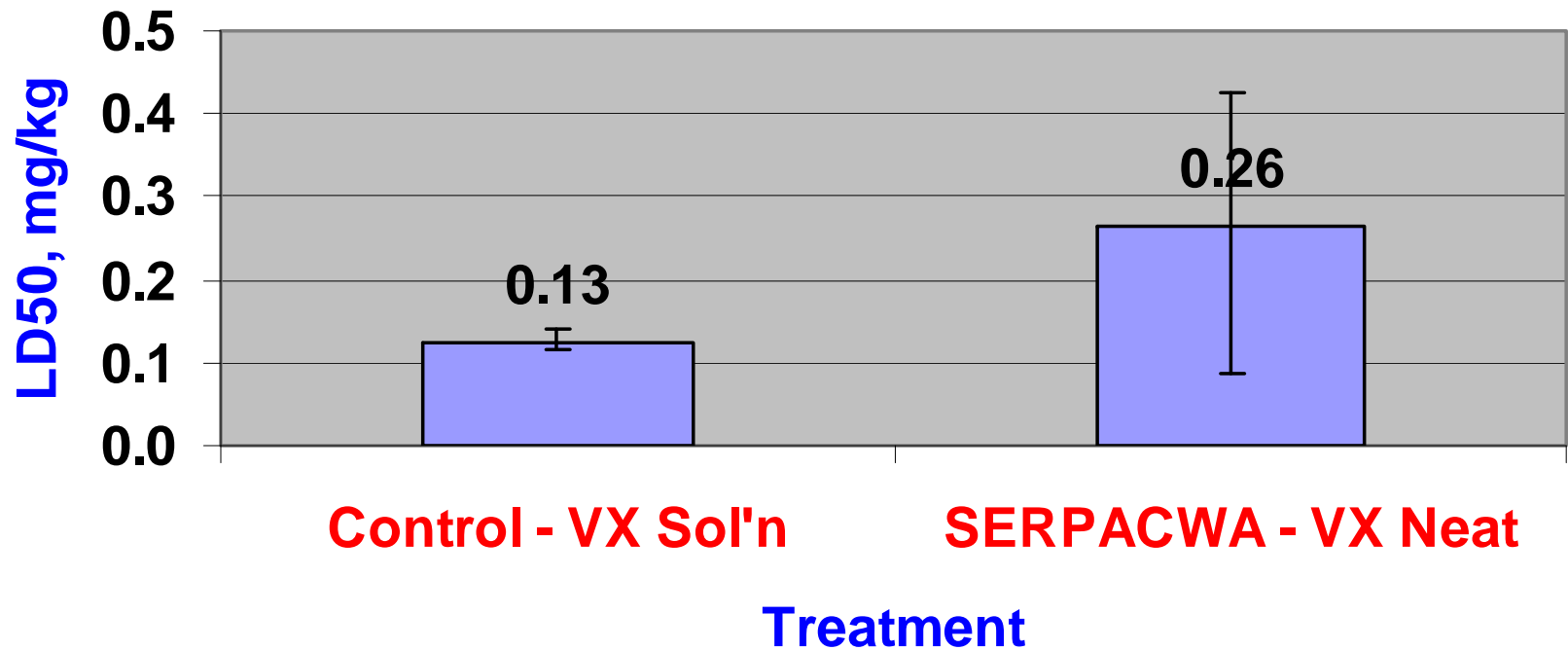


Figure 14. Graph of LD₅₀ values for positive controls and SERPACWA in the guinea pig model. Error bars = 95% CI. The number of animals used for positive controls and SERPACWA was 48 each. SERPACWA animals were challenged with neat VX. Positive control animals were challenged with 5% VX IPA solution.

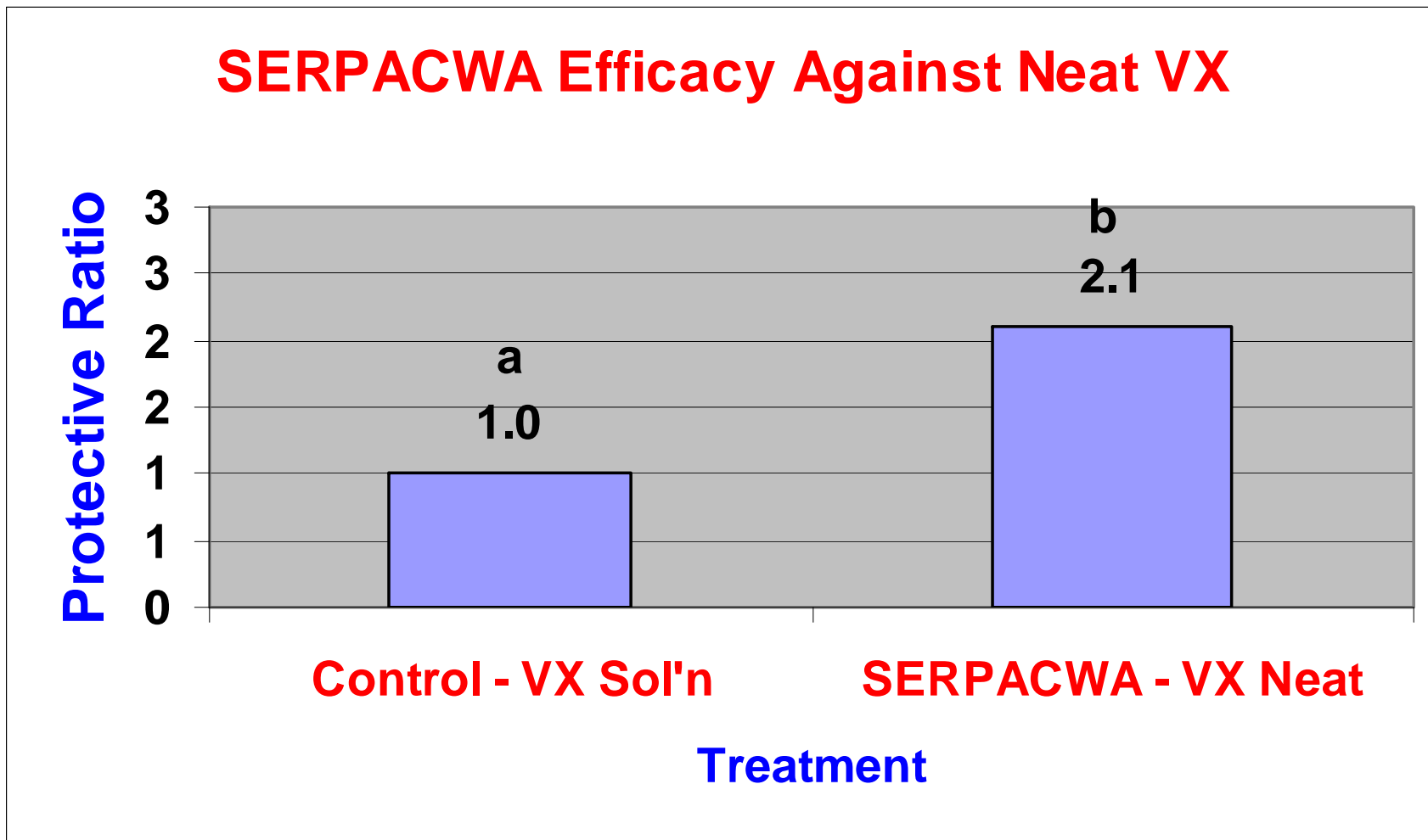


Figure 15. Graph of PR values for positive controls and SERPACWA in the guinea pig model. The number of animals used for positive controls and SERPACWA was 48 each. SERPACWA animals were challenged with neat VX. Positive control animals were challenged with 5% VX IPA solution. PRs with same letter were not statistically different at the 0.05 decision level.

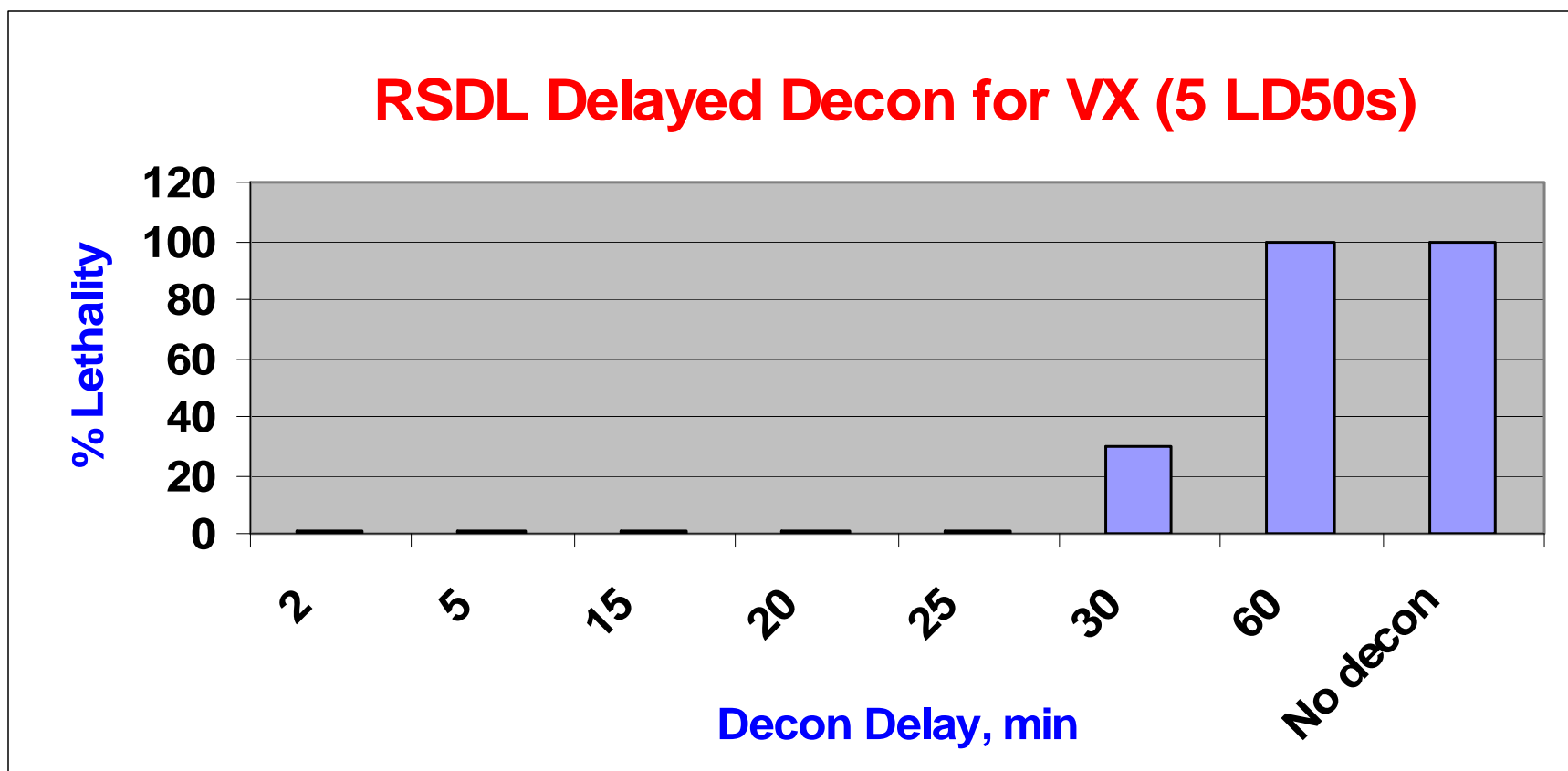


Figure 16. Graph of percent lethality when RSDL decontamination was delayed following challenge by 0.625 mg/kg (5 LD₅₀S) of VX. LT₅₀ (50% lethality time) = 31 minutes (95% CI = 30-32 min). Probit slope = 58. 63 animals total. Note: one animal death at 2 min was omitted as an outlier.

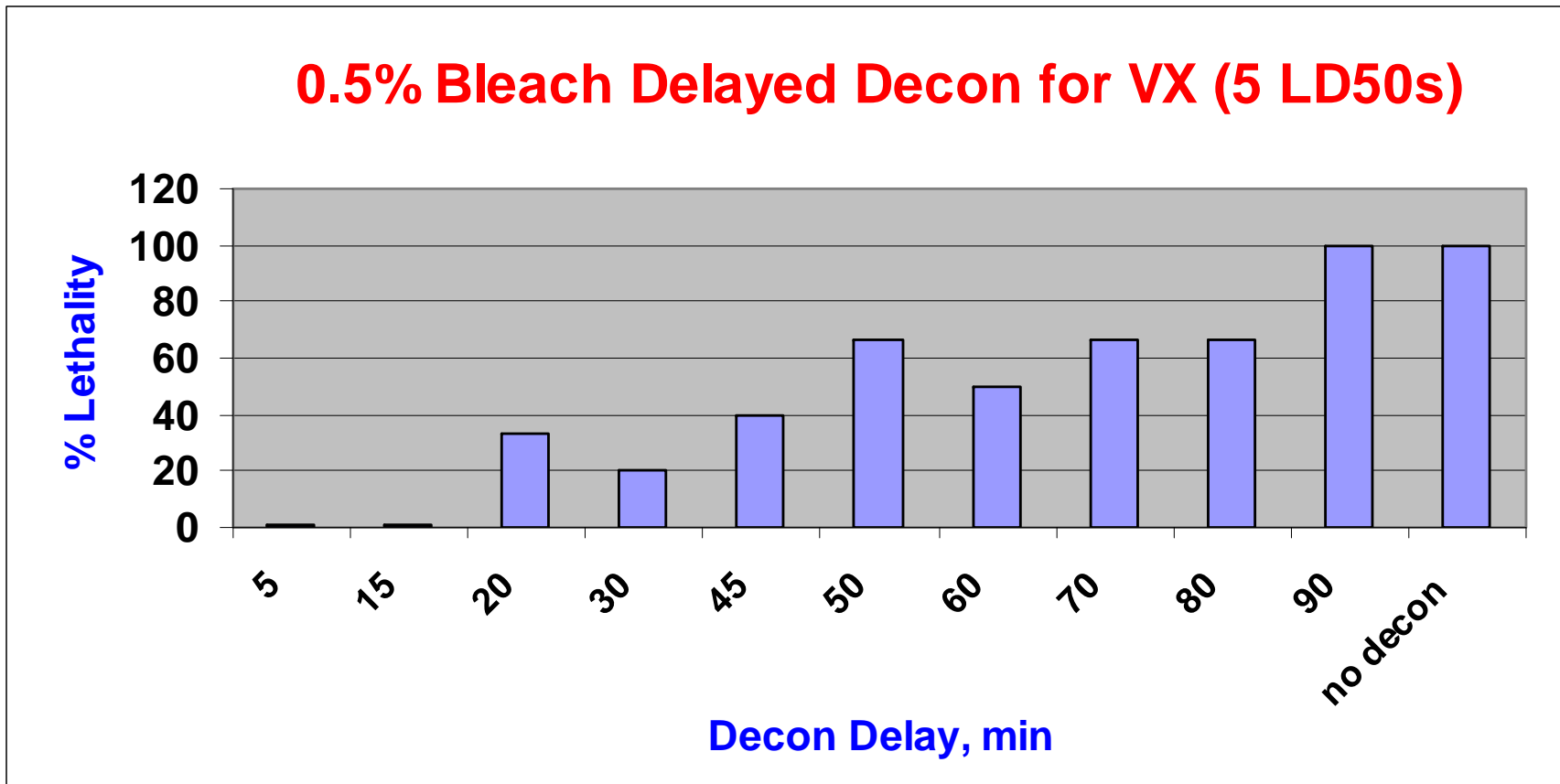


Figure 17. Graph of percent lethality when 0.5% bleach decontamination was delayed following challenge by 0.625 mg/kg (5 LD₅₀s) of VX. LT₅₀ (50% lethality time) = 48 minutes (95% CI = 32-72 min). Probit slope = 3.3. 41 animals total.

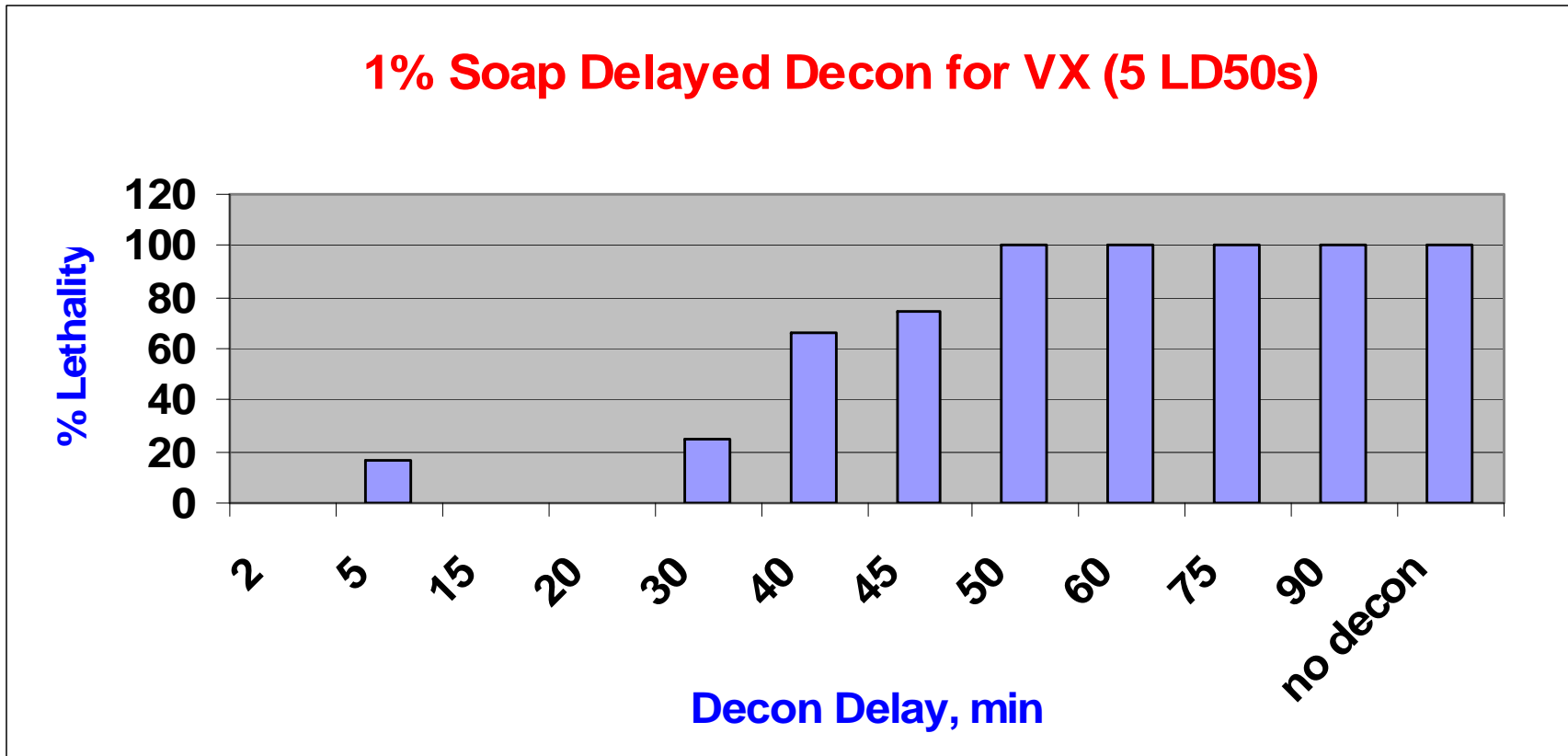


Figure 18. Graph of percent lethality when 1% soapy water decontamination is delayed following challenge by 0.625 mg/kg (5 LD₅₀s) of VX. LT₅₀ (50% lethality time) = 25 minutes (95% CI = 23-43 min). Probit slope = 3.0. 37 animals total. Note: the one death observed at 5 minutes most likely resulted from anesthesia issues, but the data is included in the analysis.

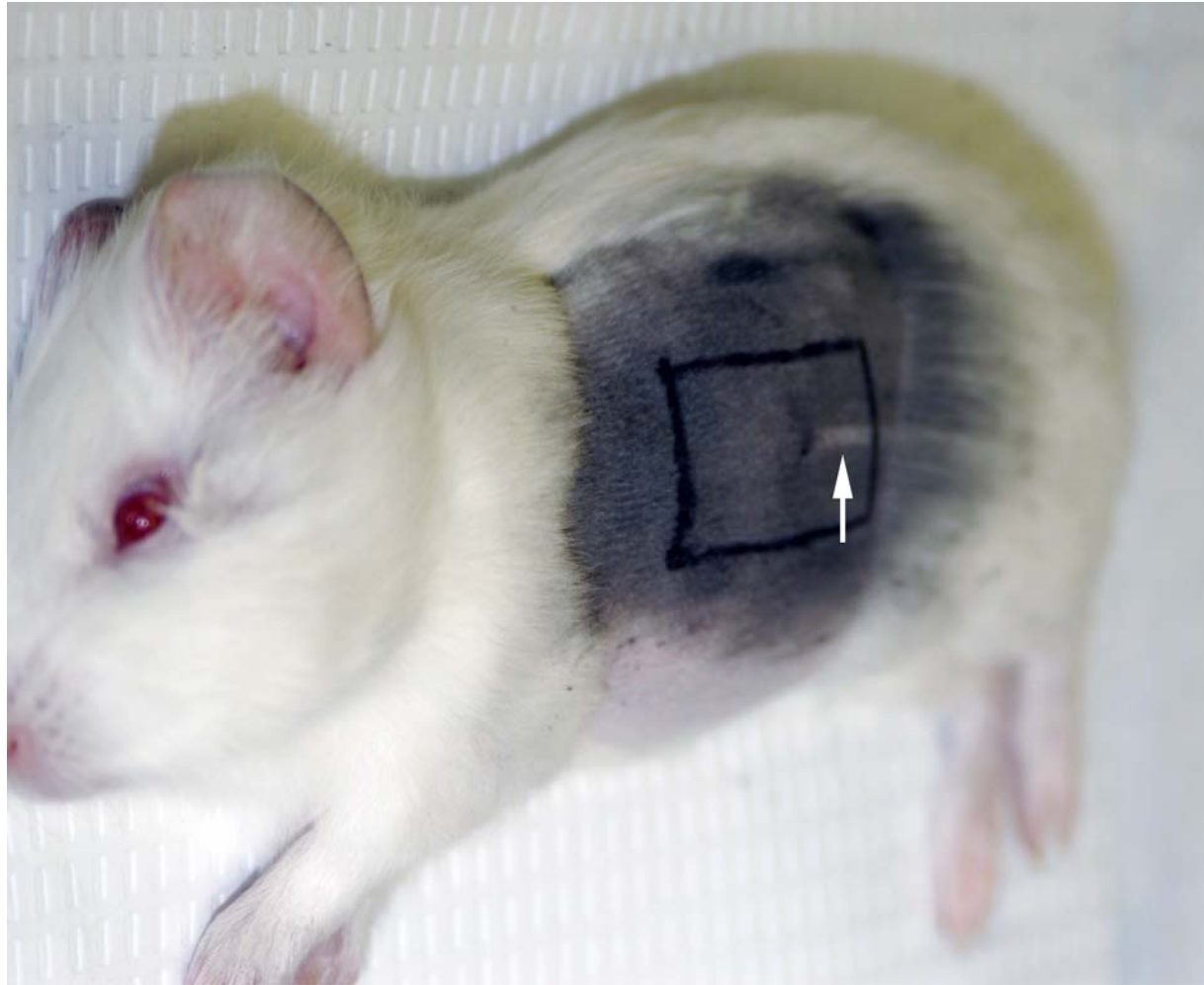


Figure 19. Agent observed to streak following use of the M291 SDK. Blotting first significantly increased the observed protection for another similar toxic agent.

REFERENCES

Bide, RW, Burczyk, AF, and Risk, DJ, Comparison of skin decontaminants for HD: Canadian Reactive Skin Decontaminant Lotion, Canadian Decontaminating Mitt and US Skin Decontaminating Kit, *Proceedings of the 1996 Medical Defense Bioscience Review*, U.S. Army Medical Research Institute of Chemical Defense. Vol No. III, pp 1218-1227, 1996. AD A321842.

Bide, RW and Risk, DJ, Decontamination of GF in vivo by the Reactive Skin Decontamination Lotion (RSDL), Technical Memorandum, Defence R&D Canada (DRDC), Suffield, 2002, TM 2002-046.

Braue, EH, Jr, Doxzon, BF, Lumpkin, HL, Hanssen, KA and Graham, JS, Military Perspectives in chemical penetration retardation. In E.W. Smith and H.I. Maibach, eds., *Percutaneous Penetration Enhancers*-Volume 2, CRC Press, 2006, pp. 385-397.

Clarkson, ED, Logan, TP, Lukey, BJ, Maxwell, D, Newkirk, KT, and Skvorak, JB, Characterization of solvent effects on cutaneous exposure to chemical warfare agents GD and VX. Society for Neuroscience 2002 Meeting Abstracts, Abstract #205.17 (available on-line at http://www.sfn.org/index.cfm?pagename=abstracts_ampublications.)

Feder, PI, Hobson, DW, Olson, CT, Joiner, RL, and Matthews, MC, **A**. Stagewise, adaptive dose allocation for quantal response dose-response studies. *Neurosci. Behavioral Rev*, 1991, **15**, 109-114.

Feder, PI, Olson, CT, Hobson, DW, Matthews, MC, and Joiner, RL, **B**. Stagewise, group sequential experimental designs for quantal responses. One-sample and two-sample comparisons. *Neurosci. Behavioral Rev*, 1991, **15**, 129-133.

Finney, DJ, *Probit Analysis*, 3rd ed. Cambridge: Cambridge UP, 1971.

Hamilton, MG, Hill, I, Conley, J, Sawyer, TW, Caneva, DC, and Lundy, PM, Clinical aspects of percutaneous poisoning by the chemical warfare agent VX: Effects of application site and decontamination, *Military Medicine*, 2004, **169**, 856-862.

Hurst, CG. Decontamination. In: Sidell FR, Takafuji ET, Franz DR, eds. *Medical Aspects of Chemical and Biological Warfare*. In: Zajtchuk R, Bellamy RF, eds. *Textbook of Military Medicine*. Washington, DC: Department of the Army, Office of The Surgeon General, Borden Institute; 1997, Chap 15.

Joint Requirements Office for Chemical, Biological, Radiological and Nuclear Defense. *Joint Service Personnel / Skin Decontamination System (JSPDS)*. Washington, DC: Joint Requirements Office, Operational Requirements Document, 2004.

McCreery, MJ, U.S. Patent 5,607,979, 4 March 1997.

Medical Management of Chemical Casualties Handbook, U.S. Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400, 3rd Edition, 2000, p. 175.

Nelson, WB. *Applied Life Data Analysis*, John Wiley and Sons, New York, NY, 1982, p. 374.

O'Hern, MR, Dashiell, TR, and Tracy, MF, Chemical defense equipment. *Textbook of Military Medicine*, R. Zajtchuck and R. F. Bellamy, Eds., Office of the Surgeon General, 1997, pp. 361-396.

Papirmeister, B, Feister, AJ, Robinson, SI, and Ford, RD, *Medical Defense Against Mustard Gas: Toxic Mechanisms and Pharmacological Implications*, CRC Press, Boston, 1991, p. 2.

Sidell, FR, Nerve agents. *Textbook of Military Medicine*, R. Zajtchuck and R. F. Bellamy, Eds., Office of the Surgeon General, 1997, pp.129-179.

Smart, JK, History of chemical and biological warfare: An American perspective. In Sidell FR, Takafuji ET, Franz DR, eds. *Textbook of Military Medicine*, Part I, Warfare, Weaponry, and the Casualty. Medical Aspects of Chemical and Biological Warfare. Washington D.C.: Office of the Surgeon General, Borden Institute; 1997.

Snider, TH, Hayes, TL, Jarvis, RJ, Matthews, MC, and Estep, JE, Assessment of RSDL as a Decontaminant Against VX and HD: Efficacy, Equivalence to M291 Skin Decontamination Kit, and Compatibility with Fielded Materials, Battelle Memorial Institute Contract No. DAMD17-99-D 0010, Final report, Task 0008, **Module 1**, U.S. Army Medical Research and Materiel Command, Ft. Detrick, MD, November, 2002.

Snider, TH, Hayes, TL, Jarvis, RJ, Matthews, MC, and Estep, JE, Assessment of RSDL as a Decontaminant Against VX and HD: Efficacy, Equivalence to M291 Skin Decontamination Kit, and Compatibility with Fielded Materials, Battelle Memorial Institute Contract No. DAMD17-99-D 0010, Final report, Task 0008, **Module 2**, U.S. Army Medical Research and Materiel Command, Ft. Detrick, MD, November, 2002.

Snider, TH and LeClaire, RD, Evaluation of SERPACWA, Dilute bleach, Water, Soapy Water, and DF-200 Against VX and Other Agents (U), Battelle Memorial Institute Contract No. DAMD17-99-D 0010, Final report, Task 0015, Part 1, U.S. Army Medical Research and Materiel Command, Ft. Detrick, MD, May 2005 (report classified SECRET).

Spiers, EM, *Chemical and Biological Weapons: A Study in Proliferation*. St. Martin's Press, 1994, p. 18.

UN Security Council, Report of the specialists appointed by the Secretary-General to investigate allegations by the Islamic Republic of Iran concerning the use of chemical weapons. 1984, S/16433, p. 2.

Winkenwerder W, US demolition operations at Khamisiyah. Final report. Washington, DC: Department of Defense, 2002: http://www.gulflink.osd.mil/khamisiyah_iii/.

Woodall, J, Tokyo subway gas attack, Lancet, 1997, **350**, 296.

APPENDIX A
EXPERIMENTAL RAW DATA

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead
VX IPA Solution	03-Nov-04	Control	0.135	-0.8703	1	0
VX IPA Solution	03-Mar-05	Control	0.135	-0.8697	1	0
VX IPA Solution	16-Nov-04	Control	0.156	-0.8069	1	0
VX IPA Solution	03-Mar-05	Control	0.156	-0.8069	1	0
VX IPA Solution	16-Nov-04	Control	0.208	-0.6819	1	0
VX IPA Solution	14-Nov-04	Control	0.208	-0.6819	1	0
VX IPA Solution	10-Mar-05	Control	0.208	-0.6819	1	1
VX IPA Solution	10-Mar-05	Control	0.220	-0.6576	2	2
VX IPA Solution	03-Nov-04	Control	0.241	-0.6184	1	1
VX IPA Solution	14-Nov-04	Control	0.241	-0.6184	1	0
VX IPA Solution	06-Mar-05	Control	0.241	-0.6180	2	2
VX IPA Solution	22-Nov-04	Control	0.279	-0.5544	1	0
VX IPA Solution	09-Dec-04	Control	0.279	-0.5544	2	2
VX IPA Solution	06-Mar-05	Control	0.279	-0.5544	1	1
VX IPA Solution	22-Nov-04	Control	0.322	-0.4921	1	1
VX IPA Solution	01-Mar-05	Control	0.322	-0.4921	1	1
VX IPA Solution	01-Mar-05	Control	0.400	-0.3979	2	2

Table A1. Raw data for positive control animals challenged with VX in the decontamination product experiments.

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead
VX Neat	25-Oct-05	Bleach	1.00	0.0000	1	0
VX Neat	25-Oct-05	Bleach	1.20	0.0792	2	0
VX Neat	25-Oct-05	Bleach	1.40	0.1461	2	0
VX Neat	01-Mar-05	Bleach	1.84	0.2648	1	1
VX Neat	03-Nov-04	Bleach	1.84	0.2658	1	0
VX Neat	24-Mar-05	Bleach	2.00	0.3010	2	0
VX Neat	17-Mar-05	Bleach	2.70	0.4314	2	0
VX Neat	03-Nov-04	Bleach	3.96	0.5975	1	0
VX Neat	01-Mar-05	Bleach	3.96	0.5977	1	1
VX Neat	06-Mar-05	Bleach	3.96	0.5977	2	1
VX Neat	24-Mar-05	Bleach	4.80	0.6812	2	2
VX Neat	16-Nov-04	Bleach	5.82	0.7649	1	0
VX Neat	01-Mar-05	Bleach	5.82	0.7649	1	1
VX Neat	22-Nov-04	Bleach	6.40	0.8062	2	1
VX Neat	10-Mar-05	Bleach	6.40	0.8062	2	2
VX Neat	14-Nov-04	Bleach	7.05	0.8482	2	2
VX Neat	01-Mar-05	Bleach	8.50	0.9294	1	1
VX Neat	16-Nov-04	Bleach	8.54	0.9315	1	1
VX Neat	10-Mar-05	Bleach	11.00	1.0414	1	1
VX Neat	29-Mar-05	Bleach	11.00	1.0414	2	2

Table A2. Raw data for 0.5% bleach animals challenged with VX.

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead
VX IPA Solution	03-Mar-05	M291 SDK	0.100	-1.0000	1	0
VX IPA Solution	06-Mar-05	M291 SDK	0.100	-1.0000	1	0
VX IPA Solution	03-Mar-05	M291 SDK	0.200	-0.6990	2	0
VX IPA Solution	24-Mar-05	M291 SDK	0.245	-0.6108	2	0
VX IPA Solution	16-Nov-04	M291 SDK	0.300	-0.5229	1	0
VX IPA Solution	06-Mar-05	M291 SDK	0.300	-0.5229	1	0
VX IPA Solution	24-Mar-05	M291 SDK	0.358	-0.4461	2	2
VX IPA Solution	22-Nov-04	M291 SDK	0.421	-0.3757	1	1
VX IPA Solution	06-Mar-05	M291 SDK	0.421	-0.3757	1	1
VX IPA Solution	09-Dec-04	M291 SDK	0.459	-0.3382	2	1
VX IPA Solution	17-Mar-05	M291 SDK	0.460	-0.3372	2	1
VX IPA Solution	14-Nov-04	M291 SDK	0.500	-0.3010	1	0
VX IPA Solution	10-Mar-05	M291 SDK	0.500	-0.3010	2	2
VX IPA Solution	22-Nov-04	M291 SDK	0.501	-0.3002	1	0
VX IPA Solution	10-Mar-05	M291 SDK	0.530	-0.2757	1	1
VX IPA Solution	17-Mar-05	M291 SDK	0.530	-0.2757	2	2
VX IPA Solution	14-Nov-04	M291 SDK	0.533	-0.2733	1	1
VX IPA Solution	16-Nov-04	M291 SDK	0.600	-0.2218	1	1
VX IPA Solution	01-Mar-05	M291 SDK	0.600	-0.2218	1	1
VX IPA Solution	01-Mar-05	M291 SDK	0.850	-0.0706	1	1

Table A3. Raw data for M291 SDK animals challenged with VX.

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead
VX Neat	24-Mar-05	RSDL	3.14	0.4969	2	0
VX Neat	06-Mar-05	RSDL	5.00	0.6990	1	0
VX Neat	06-Mar-05	RSDL	6.30	0.7993	2	0
VX Neat	22-Nov-04	RSDL	7.69	0.8859	1	0
VX Neat	03-Mar-05	RSDL	7.69	0.8859	1	1
VX Neat	10-Mar-05	RSDL	7.69	0.8859	2	0
VX Neat	24-Mar-05	RSDL	8.88	0.9484	2	0
VX Neat	22-Nov-04	RSDL	10.26	1.0111	1	0
VX Neat	09-Dec-04	RSDL	10.64	1.0269	2	0
VX Neat	09-Dec-04	RSDL	11.03	1.0426	2	0
VX Neat	14-Nov-04	RSDL	11.85	1.0737	1	1
VX Neat	10-Mar-05	RSDL	11.85	1.0737	1	1
VX Neat	17-Mar-05	RSDL	11.85	1.0737	1	0
VX Neat	17-Mar-05	RSDL	13.60	1.1335	2	0
VX Neat	03-Nov-04	RSDL	15.80	1.1987	1	0
VX Neat	14-Nov-04	RSDL	15.80	1.1987	1	1
VX Neat	01-Mar-05	RSDL	15.80	1.1987	1	1
VX Neat	16-Nov-04	RSDL	18.25	1.2613	1	1
VX Neat	01-Mar-05	RSDL	18.25	1.2613	1	0
VX Neat	16-Nov-04	RSDL	24.33	1.3861	1	1
VX Neat	01-Mar-05	RSDL	24.33	1.3861	1	1
VX Neat	29-Mar-05	RSDL	24.43	1.3879	3	3
VX Neat	03-Nov-04	RSDL	28.06	1.4481	1	1
VX Neat	03-Mar-05	RSDL	31.50	1.4983	2	2

Table A4. Raw data for RSDL animals challenged with VX.

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead
VX Neat	25-Oct-05	Soap	1.00	0.0000	3	0
VX Neat	03-Nov-04	Soap	1.16	0.0645	1	0
VX Neat	03-Mar-05	Soap	2.00	0.3010	2	1
VX Neat	06-Mar-05	Soap	2.00	0.3010	2	0
VX Neat	03-Nov-04	Soap	2.49	0.3962	1	0
VX Neat	01-Mar-05	Soap	2.49	0.3962	1	0
VX Neat	10-Mar-05	Soap	3.50	0.5441	2	1
VX Neat	24-Mar-05	Soap	3.50	0.5441	2	1
VX Neat	16-Nov-04	Soap	3.66	0.5635	1	1
VX Neat	17-Mar-05	Soap	4.30	0.6335	4	3
VX Neat	16-Nov-04	Soap	5.38	0.7308	1	0
VX Neat	01-Mar-05	Soap	5.38	0.7308	1	0
VX Neat	10-Mar-05	Soap	5.38	0.7308	1	1
VX Neat	24-Mar-05	Soap	5.38	0.7308	1	1
VX Neat	09-Dec-04	Soap	5.92	0.7723	2	2
VX Neat	22-Nov-04	Soap	6.51	0.8136	2	2
VX Neat	14-Nov-04	Soap	7.89	0.8971	1	1
VX Neat	01-Mar-05	Soap	7.89	0.8971	1	1
VX Neat	14-Nov-04	Soap	11.58	1.0637	1	1

Table A5. Raw data for 1% soapy water animals challenged with VX.

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead
VX IPA Solution	29-Nov-05	Control	0.010	-2.00	2	0
VX IPA Solution	29-Nov-05	Control	0.020	-1.70	2	0
VX IPA Solution	29-Nov-05	Control	0.040	-1.40	2	0
VX IPA Solution	29-Nov-05	Control	0.080	-1.10	2	0
VX IPA Solution	1-Dec-05	Control	0.089	-1.05	3	0
VX IPA Solution	1-Dec-05	Control	0.100	-1.00	3	0
VX IPA Solution	8-Dec-05	Control	0.100	-1.00	2	0
VX IPA Solution	1-Dec-05	Control	0.110	-0.959	3	0
VX IPA Solution	8-Dec-05	Control	0.110	-0.959	3	1
VX IPA Solution	8-Dec-05	Control	0.115	-0.939	3	1
VX IPA Solution	22-Nov-05	Control	0.120	-0.921	2	2
VX IPA Solution	8-Dec-05	Control	0.120	-0.921	2	0
VX IPA Solution	8-Dec-05	Control	0.135	-0.870	3	1
VX IPA Solution	22-Nov-05	Control	0.150	-0.824	3	3
VX IPA Solution	8-Dec-05	Control	0.150	-0.824	2	2
VX IPA Solution	22-Nov-05	Control	0.170	-0.770	3	3
VX IPA Solution	22-Nov-05	Control	0.190	-0.721	3	3
VX IPA Solution	22-Nov-05	Control	0.210	-0.678	3	3
VX IPA Solution	22-Nov-05	Control	0.230	-0.638	2	2

Table A6. Raw data for positive control animals challenged with VX (M291 repeat experiments).

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead
VX Neat	01-Nov-05	M291	0.150	-0.824	4	1
VX Neat	09-Nov-05	M291	0.165	-0.783	3	3
VX Neat	09-Nov-05	M291	0.182	-0.740	3	3
VX Neat	01-Nov-05	M291	0.200	-0.699	4	3
VX Neat	09-Nov-05	M291	0.250	-0.602	2	1
VX Neat	25-Oct-05	M291	0.300	-0.523	2	2
VX Neat	01-Nov-05	M291	0.300	-0.523	2	2
VX Neat	01-Nov-05	M291	0.400	-0.398	3	3
VX Neat	25-Oct-05	M291	0.500	-0.301	2	2
VX Neat	01-Nov-05	M291	0.500	-0.301	2	2
VX Neat	25-Oct-05	M291	0.750	-0.125	2	2
VX Neat	01-Nov-05	M291	0.750	-0.125	1	1
VX Neat	25-Oct-05	M291	1.00	0.000	2	2

Table A7. Raw data for M291 SDK animals challenged with neat VX (M291 repeat experiments).

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead
VX IPA Solution	19-May-05	Control	0.120	-0.921	2	0
VX IPA Solution	03-May-05	Control	0.140	-0.854	2	0
VX IPA Solution	19-May-05	Control	0.150	-0.824	4	1
VX IPA Solution	24-May-05	Control	0.160	-0.796	4	1
VX IPA Solution	17-May-05	Control	0.170	-0.770	4	3
VX IPA Solution	24-May-05	Control	0.190	-0.721	4	1
VX IPA Solution	05-May-05	Control	0.210	-0.678	4	4
VX IPA Solution	03-May-05	Control	0.260	-0.585	2	2
No VX	03-May-05	Neg Control	0.000	No VX	2	0
No VX	05-May-05	Neg Control	0.000	No VX	2	0
No VX	17-May-05	Neg Control	0.000	No VX	2	0
No VX	19-May-05	Neg Control	0.000	No VX	2	0
No VX	24-May-05	Neg Control	0.000	No VX	1	0

Table A8. Raw data for positive and negative control animals challenged with VX in the SERPACWA experiments. Neg (negative) control animals were handled like positive control animals, except they received no VX.

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead
VX IPA Solution	03-May-05	SERPACWA	0.140	-0.854	1	0
VX IPA Solution	05-May-05	SERPACWA	0.140	-0.854	1	0
VX IPA Solution	03-May-05	SERPACWA	0.220	-0.658	1	0
VX IPA Solution	05-May-05	SERPACWA	0.220	-0.658	1	0
VX IPA Solution	17-May-05	SERPACWA	0.300	-0.523	4	0
VX IPA Solution	24-May-05	SERPACWA	0.350	-0.456	4	1
VX IPA Solution	05-May-05	SERPACWA	0.400	-0.398	2	2
VX IPA Solution	17-May-05	SERPACWA	0.400	-0.398	2	1
VX IPA Solution	05-May-05	SERPACWA	0.600	-0.222	2	1
VX IPA Solution	19-May-05	SERPACWA	0.600	-0.222	2	1
VX IPA Solution	05-May-05	SERPACWA	0.800	-0.097	2	1
VX IPA Solution	17-May-05	SERPACWA	0.800	-0.097	2	0
VX IPA Solution	17-May-05	SERPACWA	1.000	0.000	2	1
VX IPA Solution	19-May-05	SERPACWA	1.000	0.000	2	2
VX IPA Solution	03-May-05	SERPACWA	1.100	0.041	1	1
VX IPA Solution	24-May-05	SERPACWA	1.100	0.041	3	3
VX IPA Solution	19-May-05	SERPACWA	1.200	0.079	2	2
VX IPA Solution	19-May-05	SERPACWA	1.400	0.146	2	2
VX IPA Solution	03-May-05	SERPACWA	2.200	0.342	1	1

Table A9. Raw data for SERPACWA animals challenged with VX in the initial SERPACWA experiments.

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead
VX IPA Solution	29-Nov-05	Control	0.010	-2.000	2	0
VX IPA Solution	29-Nov-05	Control	0.020	-1.699	2	0
VX IPA Solution	29-Nov-05	Control	0.040	-1.398	2	0
VX IPA Solution	29-Nov-05	Control	0.080	-1.097	2	0
VX IPA Solution	1-Dec-05	Control	0.089	-1.051	3	0
VX IPA Solution	1-Dec-05	Control	0.100	-1.000	3	0
VX IPA Solution	8-Dec-05	Control	0.100	-1.000	2	0
VX IPA Solution	1-Dec-05	Control	0.110	-0.959	3	0
VX IPA Solution	8-Dec-05	Control	0.110	-0.959	3	1
VX IPA Solution	8-Dec-05	Control	0.115	-0.939	3	1
VX IPA Solution	22-Nov-05	Control	0.120	-0.921	2	2
VX IPA Solution	8-Dec-05	Control	0.120	-0.921	2	0
VX IPA Solution	8-Dec-05	Control	0.135	-0.870	3	1
VX IPA Solution	22-Nov-05	Control	0.150	-0.824	3	3
VX IPA Solution	8-Dec-05	Control	0.150	-0.824	2	2
VX IPA Solution	22-Nov-05	Control	0.170	-0.770	3	3
VX IPA Solution	22-Nov-05	Control	0.190	-0.721	3	3
VX IPA Solution	22-Nov-05	Control	0.210	-0.678	3	3
VX IPA Solution	22-Nov-05	Control	0.230	-0.638	2	2

Table A10. Raw data for positive control animals challenged with VX in the SERPACWA repeat experiments.

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead
VX Neat	02-Nov-05	SERPACWA	0.150	-0.824	4	1
VX Neat	08-Nov-05	SERPACWA	0.150	-0.824	4	1
VX Neat	02-Nov-05	SERPACWA	0.200	-0.699	4	2
VX Neat	26-Oct-05	SERPACWA	0.300	-0.523	4	2
VX Neat	02-Nov-05	SERPACWA	0.400	-0.398	3	3
VX Neat	08-Nov-05	SERPACWA	0.450	-0.347	3	1
VX Neat	26-Oct-05	SERPACWA	0.500	-0.301	4	4
VX Neat	02-Nov-05	SERPACWA	0.600	-0.222	3	2
VX Neat	08-Nov-05	SERPACWA	0.650	-0.187	3	2
VX Neat	26-Oct-05	SERPACWA	0.750	-0.125	4	3
VX Neat	26-Oct-05	SERPACWA	1.00	0.000	4	4
VX Neat	02-Nov-05	SERPACWA	1.25	0.097	2	1
VX Neat	08-Nov-05	SERPACWA	2.00	0.301	3	3
VX Neat	08-Nov-05	SERPACWA	2.50	0.398	3	3

Table A11. Raw data for SERPACWA animals challenged with VX in the SERPACWA repeat experiments.

Agent	Date	Treatment	Time Delay	Number Animals	Number Dead
VX Neat	29-Mar-07	Bleach delayed	5	1	0
VX Neat	20-Nov-07	Bleach delayed	5	3	0
VX Neat	29-Mar-07	Bleach delayed	15	2	0
VX Neat	20-Nov-07	Bleach delayed	15	3	0
VX Neat	27-Nov-07	Bleach delayed	20	3	1
VX Neat	29-Mar-07	Bleach delayed	30	2	1
VX Neat	20-Nov-07	Bleach delayed	30	3	0
VX Neat	29-Mar-07	Bleach delayed	45	2	1
VX Neat	20-Nov-07	Bleach delayed	45	3	1
VX Neat	27-Nov-07	Bleach delayed	50	3	2
VX Neat	29-Mar-07	Bleach delayed	60	1	0
VX Neat	20-Nov-07	Bleach delayed	60	3	2
VX Neat	27-Nov-07	Bleach delayed	70	3	2
VX Neat	27-Nov-07	Bleach delayed	80	3	2
VX Neat	27-Nov-07	Bleach delayed	90	3	3
VX Neat	29-Mar-07	No decon	120	1	1
VX Neat	20-Nov-07	No decon	240	1	1
VX Neat	27-Nov-07	No decon	240	1	1

Table A12. Raw data for delayed decontamination for 0.5% bleach animals challenged with 0.625 mg/kg VX (5 LD₅₀s). Animals at 120 and 240 min were dead at their decon times.

Agent	Date	Treatment	Time Delay, min	Number Animals	Number Dead
VX Neat	06-Dec-06	RSDL delayed	2	3	1
VX Neat	07-Dec-06	RSDL delayed	2	3	0
VX Neat	13-Dec-06	RSDL delayed	2	3	0
VX Neat	06-Dec-06	RSDL delayed	5	3	0
VX Neat	07-Dec-06	RSDL delayed	5	3	0
VX Neat	13-Dec-06	RSDL delayed	5	3	0
VX Neat	06-Dec-06	RSDL delayed	15	3	0
VX Neat	07-Dec-06	RSDL delayed	15	3	0
VX Neat	13-Dec-06	RSDL delayed	15	3	0
VX Neat	14-Dec-06	RSDL delayed	20	8	0
VX Neat	14-Dec-06	RSDL delayed	25	8	0
VX Neat	06-Dec-06	RSDL delayed	30	3	1
VX Neat	07-Dec-06	RSDL delayed	30	3	2
VX Neat	13-Dec-06	RSDL delayed	30	4	0
VX Neat	06-Dec-06	RSDL delayed	60	3	3
VX Neat	07-Dec-06	RSDL delayed	60	3	3
VX Neat	13-Dec-06	RSDL delayed	60	3	3
VX Neat	06-Dec-06	No decon	120	1	1
VX Neat	07-Dec-06	No decon	180	1	1

Table A13. Raw data for delayed decontamination for RSDL animals challenged with 0.625 mg/kg VX (5 LD₅₀s). The dead animal at 2 min is an outlier and will be omitted for statistical analysis. Animals at 120 and 180 min were dead at their decon times.

Agent	Date	Treatment	Time Delay, min	Number Animals	Number Dead
VX Neat	4-Dec-07	Soap delayed	2	4	0
VX Neat	29-Mar-07	Soap delayed	5	1	0
VX Neat	29-Nov-07	Soap delayed	5	2	1
VX Neat	4-Dec-07	Soap delayed	5	3	0
VX Neat	29-Mar-07	Soap delayed	15	1	0
VX Neat	29-Nov-07	Soap delayed	15	2	0
VX Neat	4-Dec-07	Soap delayed	20	2	0
VX Neat	29-Mar-07	Soap delayed	30	2	0
VX Neat	29-Nov-07	Soap delayed	30	2	1
VX Neat	4-Dec-07	Soap delayed	40	3	2
VX Neat	29-Mar-07	Soap delayed	45	2	1
VX Neat	29-Nov-07	Soap delayed	45	2	2
VX Neat	4-Dec-07	Soap delayed	50	3	3
VX Neat	29-Mar-07	Soap delayed	60	1	1
VX Neat	29-Nov-07	Soap delayed	60	2	2
VX Neat	29-Nov-07	Soap delayed	75	3	3
VX Neat	29-Nov-07	Soap delayed	90	2	2
VX Neat	29-Nov-07	No decon	120	1	1

Table A14. Raw data for delayed decontamination for 1% soapy water animals challenged with 0.625 mg/kg VX (5 LD₅₀s). Animal at 120 minutes was dead at its decontamination time.

Appendix B: PROBSEP Program Code

```

FILENAME DATAIN 'p:\SPAT\744\SAS\VX decon 9 Mar 05.csv';
FILENAME NEWOUT 'p:\SPAT\744\SAS\test.ONL';
LIBNAME SASDB 'p:\SPAT\744\SAS';
OPTIONS LS=78 PS=60;

* PROGRAM NAME IS PROBSEP.SAS; *Does both Fieller's and delta confidence
intervals;
* Use macros to define separate starting values for each treatment group;

DATA D; INFILE DATAIN MISSOVER delimiter=';';
  length trtgrp $20;
  INPUT AGENT$ STAGE TRTGRP$ DOSE N NDEAD;
  * IF NDEAD = . THEN DELETE;
  *   if stage<=4;
  TITLE1 'LD50 VX';
  TITLE3 'SEPARATE-SLOPES DOSE-RESPONSE FITS';
  ANIMAL = 'XXX';
  DATE = '02JUL01'D;
  FORMAT DATE MMDDYY8.;

PROC SORT DATA=D;
  BY agent TRTGRP DOSE;

/*
DATA DCHK; SET D; BY TRTGRP;
*IF FIRST.TRTGRP;
PROC PRINT; by trtgrp;
TITLES'DIAGNOSTIC CHECK ON NAMES';
*/

*****;
* Make sure the variable TRTNO (treatment group number)   *;
* gets a new value for each new value of TRTGRP           *;
*****;

DATA D; SET D; BY AGENT TRTGRP;
RETAIN TRTNO;
IF FIRST.AGENT THEN TRTNO = 1;
ELSE IF (FIRST.TRTGRP) THEN TRTNO = TRTNO + 1;

LOGDOSE = LOG10(DOSE);
PRPDEAD =NDEAD/N;
PRPNDEAD=NDEAD/N;
IF (PRPNDEAD LE .05) THEN PRPNDEAD=.05;
IF (PRPNDEAD GE .95) THEN PRPNDEAD=.95;
PROBT = PROBIT(PRPNDEAD);

*****;
* Use regular least-squares regression followed by Proc Means *;
* to get average slope and intercept to use as starting values *;
* for Proc NLin                                             *;

```

*****;

PROC SORT DATA=D; BY TRTGRP DOSE;

PROC REG NOPRINT DATA=D OUTEST=OUTREG;
BY AGENT TRTNO TRTGRP;
MODEL PROBT = LOGDOSE;

* Figure out total no. of treatments (ntrts) and store in macro variable;

DATA OUTREGC;
SET OUTREG;
BY AGENT;
SLOPE=LOGDOSE;
INTERCEP = INTERCEP + 5;
KEEP AGENT TRTNO TRTGRP SLOPE INTERCEP;
CALL SYMPUT('NTRTS', PUT(TRTNO,2.0));

DATA _NULL_; SET OUTREGC;
PUT 'NO. TREATMENTS=' "&NTRTS";
RUN;

*****;

* Define macro to Retrieve starting values for each treatment group. *;
* Run Proc NLin to get maximum likelihood estimates for the probit *;
* (normal) distibution *;

*****;

%MACRO DONLIN(TR);
DATA D1;
MERGE D OUTREGC;
BY AGENT TRTNO;
IF TRTNO = &TR;
TITLE4 'PROBIT FITS TO DATA USING ALL EXPERIMENTAL STAGES, LOG10(DOSE)';

CALL SYMPUT('INITB1', SLOPE);
CALL SYMPUT('INITB01',INTERCEP);

PROC NLIN DATA=D1
BEST=10 METHOD=GAUSS SIGSQ=1 NOHALVE MAXITER=30 OUTEST=OUTNL&TR;
BY AGENT TRTGRP;

PARAMETERS B1 = &INITB1 B01 = &INITB01 ;

* ORDER OF PARAMETERS IS SLOPE (B1), INTERCEPT (B01) ;
* BOUNDS 0<B1;

ARG1=B01-5+B1*LOGDOSE;
ARG=ARG1;
PROB=PROBNORM(ARG);
SMLPHI1=0.3989*EXP(-0.5*ARG1**2);
MODEL NDEAD=N*PROB;
DER.B01=0; DER.B1=0;

DER.B01=N*SMLPHI1;

```

DER.B1 =N*LOGDOSE*SMLPHI1;

_WEIGHT_ = 1.0/(N*PROB*(1-PROB) + 0.00001);
_LOSS_ =
-2*(NDEAD*LOG(PROB + 1.E-30) + (N-NDEAD)*LOG(1-PROB + 1.E-30))/_WEIGHT_;
ID _WEIGHT_;
OUTPUT OUT=OUTD&TR P=NDDHAT l95m=LCL u95m=UCL STUDENT=STUDRES SSE=SSE R=RESID;
label trtgrp='Treatment Group';
RUN;
%MEND;

```

```

* Define and execute macro to go through do-loop and run above macro to do
proc nlin "ntrts" number of times;

```

```

OPTIONS PAGENO=1;

```

```

%MACRO RUNALL;
%LOCAL TR;
%DO TR = 1 %TO &NTRTS;
%DONLIN (&TR);
%END;
%MEND;

```

```

%RUNALL;

```

```

* Concatenation output data sets for both predicted values and nlin estimates;

```

```

%MACRO OUTLIST(DNAME);
%LOCAL TR;
%DO TR = 1 %TO &NTRTS;
&DNAME&TR
%END;
%MEND;

```

```

DATA D2; SET %OUTLIST(OUTD);

```

```

DATA OUTNL; SET %OUTLIST(OUTNL);

```

```

*****;
* Analyze residuals from Proc NLin for stage effects *;
*****;

```

```

DATA D2; SET D2;
TITLE4
'OUTPUT DATA FROM PROC NLIN -- ANALYSES OF RESIDUALS AND STAGE EFFECTS';

```

```

PREDVAL=NDDHAT/N;
PDD2=PREDVAL;
IF PDD2 LT .05 THEN PDD2=.05;
IF PDD2 GT .95 THEN PDD2=.95;
IF PREDVAL = . THEN PDD2=.;
PREDPROB=PROBIT(PDD2);
RESIDSSQ = _WEIGHT_ * RESID * RESID;
DROP PDD2 PRPNDEAD; * AVGSLOPE AVGINTEr;
ABSRES = ABS(STUDRES);

```

```

label agent='Agent';
label n=' No.*Animals';
label ndead='Observed*N Dead';
label trtgrp='Treatment Group';

label dose='Agent* Dose* (ug/kg)';
label prpdead = 'Prop.*Dead';
label probt='Probit of*Percentile';
label PREDPROB='Probit Pred.*Pct Dead';
label logdose=' Log10* Dose*(ug/kg)';
label nddhat= 'Predicted*No. Dead';
label PREDVAL='Predicted*Prop. Dead';
label studres='Studentized* Residuals';
label date = 'Expmt*Date';

PROC SORT DATA=D2;
  BY AGENT TRTGRP;

PROC PRINT DATA=D2 SPLIT='*';
  BY AGENT TRTGRP;
  VAR stage DOSE LOGDOSE N NDEAD prpdead probt LCL UCL nddhat STUDRES predval predprob;
  FORMAT LOGDOSE PRPDEAD 5.3 DATE MMDDYY8.;
  TITLE4 'OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES AND
  RESIDUALS';

PROC PLOT NOLEGEND DATA=D2;
  PLOT STUDRES*LOGDOSE=TRTgrp / VREF=0;
  TITLE5 'RESIDUALS VS. LOGDOSE - PLOTTING SYMBOL IS TREATMENT GROUP';
  label logdose='Log Base 10 Agent Dose';
  label studres='Studentized Residuals';

PROC PLOT NOLEGEND DATA=D2;
  PLOT STUDRES*LOGDOSE=STAGE / VREF=0;
  TITLE5 'RESIDUALS VS. LOGDOSE - PLOTTING SYMBOL IS EXPERIMENTAL STAGE';
  label logdose='Log Base 10 Agent Dose';
  label studres='Studentized Residuals';

PROC SORT;
  BY AGENT STAGE;
PROC MEANS MEAN N STD STDERR MIN MAX DATA=D2;
  BY AGENT STAGE;
  VAR STUDRES;
  TITLE5 'DESCRIPTIVE STATISTICS OF RESIDUALS BY STAGE';
  label studres='Studentized Residuals';
  label stage='Experimental Stage';

PROC GLM DATA=D2;
  BY AGENT;
  CLASS STAGE;
  MODEL STUDRES=STAGE LOGDOSE;
  label logdose='Log Base 10 Agent Dose';
  label studres='Studentized Residuals';
  label stage='Experimental Stage';
  TITLE5 'ANOVA OF RESIDUALS FOR STAGE EFFECTS';

```

```

PROC PLOT NOLEGEND DATA=D2;
  BY AGENT;
  PLOT PREDVAL * LOGDOSE = TRTGRP/
  VAXIS=0 TO 1 BY .1  VREF= .16 .50 .84;
  TITLE4
'PROBABILITY PLOT OF PREDICTED PERCENT DEAD WITH 16%, 50%, 84% REFERENCE LINES';
label PREDVAL='Predicted Prop. Dead';
label logdose='Log Base 10 Agent Dose (ug/kg)';

/*
PROC PLOT NOLEGEND DATA=D2;
  BY AGENT;
  PLOT PREDVAL * DOSE = TRTGRP/
  VAXIS=0 TO 1 BY .1  VREF= .16 .50 .84; * HAXIS=1 10 100 1000;
  TITLE4
'PROBABILITY PLOT OF PREDICTED PERCENT DEAD WITH 16%, 50%, 84% REFERENCE LINES';
TITLE5 'USING TREATMENT GROUP AS A PLOTTING SYMBOL';
label PREDVAL='Predicted Proportion Dead';
label dose='Agent Dose (ug/kg)';

proc sort; by agent trtgrp;
PROC PLOT NOLEGEND DATA=D2;
  BY AGENT trtgrp;
  PLOT PREDVAL * DOSE = NDEAD/
  VAXIS=0 TO 1 BY .1  VREF= .16 .50 .84; * HAXIS=1 10 100 1000;
  TITLE4
'PROBABILITY PLOT OF PREDICTED PERCENT DEAD WITH 16%, 50%, 84% REFERENCE
LINES';
TITLE5 'USING NUMBERS DEAD AS A PLOTTING SYMBOL';
label PREDVAL='Predicted Proportion Dead';
label dose='Agent Dose (ug/kg)';

PROC PLOT NOLEGEND DATA=D2;
  by agent;
  PLOT STUDRES*LOGDOSE=TRTGRP / VREF=0; * HAXIS=1 10 100 1000;
  TITLE4 'PLOT OF STUDENTIZED RESIDUALS WITH ZERO REFERENCE LINE';
  TITLE5 'USING TREATMENT GROUP AS A PLOTTING SYMBOL';
  label studres='Studentized Residuals';
  label logdose='Log Base 10 Agent Dose (ug/kg)';
  label dose='Agent Dose (ug/kg)';
  */
*****;
* Get file of estimated coefficients and covariance matrix written *;
* by Proc NLin, pull off appropriate values and collapse down to a *;
* single record for each group (response) *;
*****;

DATA OUTNL;
  SET OUTNL;
  IF (_TYPE_ NE 'ITER');

IF (_TYPE_='FINAL') THEN DO;
  SLP=B1;

```

```

INT1=B01;
SSE=_SSE_;
END;

ELSE IF (_TYPE_='COVB' AND _NAME_='B1' ) THEN DO;
  VARB1=B1;
  COVB0B1=B01;
END;

ELSE IF (_TYPE_='COVB' AND _NAME_='B01') THEN DO;
  VARB0=B01;
END;

proc sort; by agent trtgrp;
PROC MEANS MEAN NOPRINT DATA=OUTNL;
  BY agent TRTGRP;
  VAR SLP INT1 SSE VARB0 COVB0B1 VARB1;
  OUTPUT OUT=OUTNLM
    MEAN=SLP INT1 SSE VARB0 COVB0B1 VARB1;

proc sort data=d2; by agent trtgrp;
PROC MEANS NOPRINT DATA=D2; BY AGENT TRTGRP;
VAR RESIDSSQ N;
OUTPUT OUT=SSOUT SUM=RESIDSSQ NTOT
  N=NPTS;

DATA SASDB.NSEP1;
MERGE OUTNLM SSOUT; BY TRTGRP;
DROP _TYPE_ _FREQ_;

PROC PRINT NOOBS DATA=SASDB.NSEP1;
  VAR TRTGRP SSE RESIDSSQ NTOT NPTS SLP INT1 VARB1 COVB0B1 VARB0;
TITLE4 'OUTPUT COEFFICIENTS AND COVARIANCE MATRIX FROM PROBIT REGRESSION';

*****
* Generate percentiles to be estimated from the probit equation *;
*****

DATA OUTPCT;
  SET SASDB.NSEP1;
  DO PCTILE= 1, 10, 16, 30, 50, 70, 84, 90, 99;
    OUTPUT;
  END;

DATA OUTPCT2;
  SET OUTPCT;
  T = 1.96;
  PRP = PCTILE/100;
  PROBT=PROBIT(PRP);

IF (VARB0 GT 0 AND VARB1 GT 0) THEN DO;
  SEB0 = SQRT(VARB0);
  SEB1 = SQRT(VARB1);

```



```

CORRB0B1 = COVB0B1/SQRT(VARB0*VARB1);
END;

IF (SLP NE 0 AND SLP NE .) THEN DO;
  LOGLDPCT = -(INT1 - 5 - PROBT)/SLP;
  VARX = (1/SLP**2)*(SEB0**2) + ((INT1-5-PROBT)**2/SLP**4)*(SEB1**2)
    - 2*((INT1-5-PROBT)/SLP**3)*SEB0*SEB1*CORRB0B1;
  SDX = SQRT(VARX);
END;

A0 = INT1 - 5 - PROBT;
AA = SLP*SLP - T*T*VARB1;
BB = A0 *SLP - T*T*COVB0B1;
CC = A0 *A0 - T*T*VARB0;
DELTA = .000001;
QUAD = BB*BB - AA*CC;

LENGTH COMMENT $9;

IF (QUAD LE DELTA AND QUAD NE .) THEN DO;
  LOGLCB=-100;
  LOGUCB=100;
END;

ELSE IF (QUAD GT DELTA) THEN DO;
  IF (AA GT DELTA) THEN DO;
    LOGLCB = (-BB - SQRT(QUAD))/AA;
    LOGUCB = (-BB + SQRT(QUAD))/AA;
  END;

  ELSE IF (AA LT -1*DELTA AND AA NE .) THEN DO;
    LOGLCB = (-BB - SQRT(QUAD))/AA;
    LOGUCB = (-BB + SQRT(QUAD))/AA;
    COMMENT='(Outside)';
  END;

  ELSE IF (ABS(AA) LE DELTA AND AA NE .) THEN DO;
    THETAS = -CC/(2*BB);
    IF (LOGLDPCT LT THETAS) THEN DO;
      LOGLCB = -100;
      LOGUCB = THETAS;
    END;
    ELSE IF (LOGLDPCT GE THETAS) THEN DO;
      LOGLCB = THETAS;
      LOGUCB = 100;
    END;
  END;
END;

IF (LOGLDPCT NE . AND LOGLCB NE . AND LOGUCB NE .) THEN DO;
IF (ABS(LOGLDPCT) LT 30) THEN LDPCT = (10**(LOGLDPCT));
ELSE IF (LOGLDPCT LT -30) THEN LDPCT = -999999;
ELSE IF (LOGLDPCT GT 30) THEN LDPCT = 999999;
IF (ABS(LOGLCB) LT 30) THEN LCB = (10**(LOGLCB));
ELSE IF (LOGLCB LT -30) THEN LCB = -999999;

```

```

ELSE IF (LOGLCB GT 30) THEN LCB = 999999;
IF (ABS(LOGUCB) LT 30) THEN UCB = (10** (LOGUCB));
ELSE IF (LOGUCB LT -30) THEN UCB = -999999;
ELSE IF (LOGUCB GT 30) THEN UCB = 999999;
END;

FIELLCB = (' || PUT(LCB,7.3) || ',' || PUT(UCB,7.3) || ');

LOGLCBD = LOGLDPCT - 1.96*SDX;
LOGUCBD = LOGLDPCT + 1.96*SDX;
LCBD = (10** (LOGLCBD));
UCBD = (10** (LOGUCBD));
DELTACB = (' || PUT(LCBD,6.3) || ',' || PUT(UCBD,7.3) || ');

label trtgrp='Treatment Group';
label agent='Agent';
label trtgrp='Treatment Group';
label pctile=' Perc-*entile';
label probt='Probit of*Percentile';
label logldpct='Log(Eff. Dose)*for Percentile';
label ldpct='Effective Dose*for Percentile';
label sdX='Std. Error of*Log(Eff. Dose)';
label lcb='Lower Confid-* ence Bound';
label ucb='Upper Confid-* ence Bound';
label fiellcb = 'Fieller's*Conf.*Bounds';
label deltacb = 'Delta*Conf.Bounds';
LABEL COMMENT=* *';

PROC PRINT NOOBS SPLIT=*' DATA=OUTPCT2;
  BY AGENT TRTGRP;
  VAR AGENT PCTILE PROBT LOGLDPCT SDX LDPCT LCB UCB;
  TITLE4 "PERCENTILES WITH CONFIDENCE INTERVALS BASED ON FIELLER'S METHOD";

RUN;

DATA SASDB.PSEP1;
  SET OUTPCT2 (KEEP = TRTGRP PCTILE SDX LDPCT LCB UCB COMMENT);
RUN;

DATA _NULL_; SET OUTNLM;
*TRTGRP = COMPRESS(TRTGRP);

FILE NEWOUT;
PUT
  'S ' AGENT $CHAR4. ' 1 ' TRTGRP $CHAR20.
  / @1 (SLP INT1) (+1 13.9)
  / @1 (VARB1) (+1 13.9)
  / @1 (COVB0B1 VARB0)(+1 13.9)
  / '...'
  / '....'
  / '.....'
  / '.....'
  / '.....';
RUN;

```

APPENDIX C PRORATIO Program Code

```

FILENAME DATAIN 'p:\SPAT\744\SAS\test.ONL';
LIBNAME SASDB 'p:\SPAT\744\SAS';
OPTIONS LS=160 PS=60;
* PROGRAM NAME IS PRORATIO.SAS - protective ratios among treatments;
* Run on SAS Version 6;
* Note: treatment group names on input file (*.ONL) cannot contain
    blanks - edit them out if necessary;

DATA D1; INFILE DATAIN MISSEVER;
    LENGTH TRNAME1 TRNAME2 TRNAME3 TRNAME4 TRNAME5 TRNAME6 $40;

* Read in treatment group names, regression coefficients, and covariance
    matrix as one big record;
INPUT
#1 REGTYPE$ AGENT$ NTRT
    TRNAME1$ TRNAME2$ TRNAME3$ TRNAME4$ TRNAME5$ TRNAME6$
#2 SLP B01 B02 B03 B04 B05 B06
#3 VARS
#4 COVS01 VARB01
#5 COVS02 COV0102 VARB02
#6 COVS03 COV0103 COV0203 VARB03
#7 COVS04 COV0104 COV0204 COV0304 VARB04
#8 COVS05 COV0105 COV0205 COV0305 COV0405 VARB05
#9 COVS06 COV0106 COV0206 COV0306 COV0406 COV0506 VARB06;

IF REGTYPE = 'P' THEN RT = 'PARALLEL';
ELSE IF REGTYPE = 'S' THEN RT = 'SEPARATE';
CALL SYMPUT('RT', RT);

DATA D1; SET D1;
    TITLE1 '';
    TITLE3 "ICD CANDIDATE TREATMENTS FOR VX COMPARISONS AMONG LD50s";
    TITLE4 "ESTIMATED BY SRT SLOPES PROBIT ANALYSES MODEL 95% CI";

* Set covariance matrix elements to missing if the PROC NLIN that they
    resulted from never converged, or the slope was infinite;

ARRAY COVAR {28} VARS-- VARB06;
DO I=1 TO 28;
IF COVAR{I} GT 1.0E+15 OR COVAR{I} LT -1.0E+15 THEN COVAR{I} = .;
END;
DROP I;

*PROC PRINT NOOBS;
*TITLE5 'DIAGNOSTIC INFORMATION - INPUT DATASET D1';

DATA D1; SET D1;
* Calculate std errors of slope and each intercept;
SES = SQRT(VARS);
SE1 = SQRT(VARB01);

```

```

IF (REGTYPE='P') THEN DO;
  SE2 = SQRT(VARB02);
  SE3 = SQRT(VARB03);
  SE4 = SQRT(VARB04);
  SE5 = SQRT(VARB05);
  SE6 = SQRT(VARB06);
END;

```

* Calculate correlations between slope and each intercept;

```

CORRS1 = COVS01/SQRT(VARS*VARB01);

```

```

IF (REGTYPE='P') THEN DO;
  CORRS2 = COVS02/SQRT(VARS*VARB02);
  CORRS3 = COVS03/SQRT(VARS*VARB03);
  CORRS4 = COVS04/SQRT(VARS*VARB04);
  CORRS5 = COVS05/SQRT(VARS*VARB05);
  CORRS6 = COVS06/SQRT(VARS*VARB06);
END;

```

* If parallel-slopes model was used, calculate variances of differences between all pairs of treatments, using covariances between treatment estimates;

```

IF (REGTYPE='P') THEN DO;

```

```

  IF (NTRT GE 2) THEN DO;
    VARDEL12 = (VARB01 + VARB02 - 2* COV0102) /(SLP**2)
      + ((B02-B01)**2)*(VARS) /(SLP**4)
      + 2*(B02-B01)* COVS01 /(SLP**3)
      - 2*(B02-B01)* COVS02 /(SLP**3) ;
  END;

```

```

  IF (NTRT GE 3) THEN DO;
    VARDEL13 = (VARB01 + VARB03 - 2* COV0103) /(SLP**2)
      + ((B03-B01)**2)*(VARS) /(SLP**4)
      + 2*(B03-B01)* COVS01 /(SLP**3)
      - 2*(B03-B01)* COVS03 /(SLP**3) ;

```

```

    VARDEL23 = (VARB02 + VARB03 - 2* COV0203) /(SLP**2)
      + ((B03-B02)**2)*(VARS) /(SLP**4)
      + 2*(B03-B02)* COVS02 /(SLP**3)
      - 2*(B03-B02)* COVS03 /(SLP**3) ;
  END;

```

```

  IF (NTRT GE 4) THEN DO;
    VARDEL14 = (VARB01 + VARB04 - 2* COV0104) /(SLP**2)
      + ((B04-B01)**2)*(VARS) /(SLP**4)
      + 2*(B04-B01)* COVS01 /(SLP**3)
      - 2*(B04-B01)* COVS04 /(SLP**3) ;

```

```

    VARDEL24 = (VARB02 + VARB04 - 2* COV0204) /(SLP**2)
      + ((B04-B02)**2)*(VARS) /(SLP**4)
      + 2*(B04-B02)* COVS02 /(SLP**3)
      - 2*(B04-B02)* COVS04 /(SLP**3) ;

```

```

    VARDEL34 = (VARB03 + VARB04 - 2* COV0304) /(SLP**2)

```

```

+ ((B04-B03)**2)*(VARS)/(SLP**4)
+ 2*(B04-B03)* COVS03/(SLP**3)
- 2*(B04-B03)* COVS04/(SLP**3);
END;

IF (NTRT GE 5) THEN DO;
VARDEL15 = (VARB01 + VARB05 - 2* COV0105)/(SLP**2)
+ ((B05-B01)**2)*(VARS)/(SLP**4)
+ 2*(B05-B01)* COVS01/(SLP**3)
- 2*(B05-B01)* COVS05/(SLP**3);

VARDEL25 = (VARB02 + VARB05 - 2* COV0205)/(SLP**2)
+ ((B05-B02)**2)*(VARS)/(SLP**4)
+ 2*(B05-B02)* COVS02/(SLP**3)
- 2*(B05-B02)* COVS05/(SLP**3);

VARDEL35 = (VARB03 + VARB05 - 2* COV0305)/(SLP**2)
+ ((B05-B03)**2)*(VARS)/(SLP**4)
+ 2*(B05-B03)* COVS03/(SLP**3)
- 2*(B05-B03)* COVS05/(SLP**3);

VARDEL45 = (VARB04 + VARB05 - 2* COV0405)/(SLP**2)
+ ((B05-B04)**2)*(VARS)/(SLP**4)
+ 2*(B05-B04)* COVS04/(SLP**3)
- 2*(B05-B04)* COVS05/(SLP**3);
END;

IF (NTRT GE 6) THEN DO;
VARDEL16 = (VARB01 + VARB06 - 2* COV0106)/(SLP**2)
+ ((B06-B01)**2)*(VARS)/(SLP**4)
+ 2*(B06-B01)* COVS01/(SLP**3)
- 2*(B06-B01)* COVS06/(SLP**3);

VARDEL26 = (VARB02 + VARB06 - 2* COV0206)/(SLP**2)
+ ((B06-B02)**2)*(VARS)/(SLP**4)
+ 2*(B06-B02)* COVS02/(SLP**3)
- 2*(B06-B02)* COVS06/(SLP**3);

VARDEL36 = (VARB03 + VARB06 - 2* COV0306)/(SLP**2)
+ ((B06-B03)**2)*(VARS)/(SLP**4)
+ 2*(B06-B03)* COVS03/(SLP**3)
- 2*(B06-B03)* COVS06/(SLP**3);

VARDEL46 = (VARB04 + VARB06 - 2* COV0406)/(SLP**2)
+ ((B06-B04)**2)*(VARS)/(SLP**4)
+ 2*(B06-B04)* COVS04/(SLP**3)
- 2*(B06-B04)* COVS06/(SLP**3);

VARDEL56 = (VARB05 + VARB06 - 2* COV0506)/(SLP**2)
+ ((B06-B05)**2)*(VARS)/(SLP**4)
+ 2*(B06-B05)* COVS05/(SLP**3)
- 2*(B06-B05)* COVS06/(SLP**3);
END;

STDEL12 = SQRT(VARDEL12);
STDEL13 = SQRT(VARDEL13);

```

```
STDEL14 = SQRT(VARDEL14);
STDEL15 = SQRT(VARDEL15);
STDEL16 = SQRT(VARDEL16);
```

```
STDEL23 = SQRT(VARDEL23);
STDEL24 = SQRT(VARDEL24);
STDEL25 = SQRT(VARDEL25);
STDEL26 = SQRT(VARDEL26);
```

```
STDEL34 = SQRT(VARDEL34);
STDEL35 = SQRT(VARDEL35);
STDEL36 = SQRT(VARDEL36);
```

```
STDEL45 = SQRT(VARDEL45);
STDEL46 = SQRT(VARDEL46);
STDEL56 = SQRT(VARDEL56);
```

```
END;
```

```
DROP VARS--VARB06 VARDEL12--VARDEL56;
```

```
DATA STDELS; SET D1;
```

```
* Info from covariance matrix only relevant to parallel-slopes model;
IF REGTYPE = 'P';
ALLKEY=1;
KEEP ALLKEY TRNAME1--TRNAME6 STDEL12--STDEL56;
```

```
DATA D1; SET D1;
```

```
ALLKEY=1;
LENGTH TRTGRP $40;
```

```
* Just need variances for slope and intercepts, and correlations of the
joint slope with individual intercepts, for the confidence intervals
around the percentile estimates ;
```

```
DROP I;
```

```
DO I = 1 TO NTRT;
```

```
IF (I=1) THEN DO; TRTGRP=TRNAME1; INT=B01; CORRSI=CORRS1; SEI=SE1; END;
```

```
IF (I=2) THEN DO; TRTGRP=TRNAME2; INT=B02; CORRSI=CORRS2; SEI=SE2; END;
```

```
IF (I=3) THEN DO; TRTGRP=TRNAME3; INT=B03; CORRSI=CORRS3; SEI=SE3; END;
```

```
IF (I=4) THEN DO; TRTGRP=TRNAME4; INT=B04; CORRSI=CORRS4; SEI=SE4; END;
```

```
IF (I=5) THEN DO; TRTGRP=TRNAME5; INT=B05; CORRSI=CORRS5; SEI=SE5; END;
```

```
IF (I=6) THEN DO; TRTGRP=TRNAME6; INT=B06; CORRSI=CORRS6; SEI=SE6; END;
```

```
OUTPUT;
```

```
END;
```

```
*PROC PRINT NOOBS DATA=D1;
```

```
*TITLES 'DIAGNOSTIC INFORMATION - LAST DATA SET D1';
```

```
/*
```

```
FILENAME PCTILE 'PCTILE.DATA';
```

```
DATA PC; INFILE PCTILE MISSOVER;
```

```
INPUT P1 P2 P3 P4 P5 P6 P7 P8 P9 P10;
```

```
ALLKEY=1;
```

*/

* Create one record for each treatment and percentile of interest;

```
DATA PC;
P1=50; P2=.; P3=.; P4=.; P5=.; P6=.; P7=.; P8=.; P9=.; P10=.;
ALLKEY=1;
```

```
DATA DPCT; MERGE D1 PC; BY ALLKEY; DROP ALLKEY;
ARRAY PS {10} P1-P10;
DO I=1 TO 10;
IF (PS{I} NE .) THEN DO;
  PCT = PS{I};
  OUTPUT;
END;
END;
```

```
DATA DPCT; SET DPCT (DROP= I P1--P10);
IF (PCT GT 1) THEN PCT = PCT/100;
```

```
RPCT=PROBIT(PCT); *INPUT DESIRED PERCENTILE (AS A PROPORTION);
T=1.96; *INPUT T FACTOR FOR USE WITH CONF INTVLS;
```

```
IF (SLP NE 0 AND SLP NE .) THEN DO;
LOGLD = -(INT - 5 - RPCT)/SLP;
VARX = (1/SLP**2)*(SEI**2) + ((INT-5-RPCT)**2/SLP**4)*(SES**2)
- 2*((INT-5-RPCT)/SLP**3)*SEI*SES*CORRSI;
SDX = SQRT(VARX);
LOGLCB = LOGLD - T*SDX;
LOGUCB = LOGLD + T*SDX;
```

```
IF (ABS(LOGLD) LT 30) THEN LD = 10**(LOGLD);
ELSE IF (LOGLD LT -30) THEN LD = -999999;
ELSE IF (LOGLD GT 30) THEN LD = 999999;
IF (ABS(LOGLCB) LT 30) THEN LCB = 10**(LOGLCB);
ELSE IF (LOGLCB LT -30) THEN LCB = -999999;
ELSE IF (LOGLCB GT 30) THEN LCB = 999999;
IF (ABS(LOGUCB) LT 30) THEN UCB = 10**(LOGUCB);
ELSE IF (LOGUCB LT -30) THEN UCB = -999999;
ELSE IF (LOGUCB GT 30) THEN UCB = 999999;
END;
```

```
KEEP AGENT PCT REGTYPE TRTGRP LOGLD SDX LOGLCB LOGUCB LD LCB UCB;
```

```
*label time = 'Time (Minutes)';
label agent='Agent';
label pct='Percentile';
label trtgrp='Treatment* Group';
label logld='Log(Leth Dose)*for Percentile';
label sdx='Standard Error*for Log(L.D.)';
label loglcb='Log (Lower*Conf. Bnd)';
label logucb='Log (Upper*Conf. Bnd)';
label ld=' Leth Dose*for Percentile';
label lcb='Lower Confid-* ence Bound';
label ucb='Upper Confid-* ence Bound';
```

```

*PROC SORT;
* BY AGENT PCT; * TRTGRP;

PROC PRINT NOOBS DOUBLE SPLIT='*'; BY AGENT PCT;
VAR TRTGRP LOGLD SDX LOGLCB LOGUCB LD LCB UCB;
TITLE5 'PERCENTILE ESTIMATES OF DOSES PRODUCING SPECIFIED RESPONSE';
TITLE6 'RATES SHOWN WITH DELTA-TYPE CONFIDENCE INTERVALS';

* Compute lag functions to get variables needed to get all 15 pairwise
  comparisons of treatments for six groups;

DATA DPCT; SET DPCT;
LENGTH TRTGRP1 TRTGRP2 TRTGRP3 TRTGRP4 TRTGRP5 TRTGRP6
  TRTGRP7 TRTGRP8 TRTGRP9 TRTGRP10 $40;
*LENGTH TRTGRP11 TRTGRP12 TRTGRP13 TRTGRP14 TRTGRP15 $40;

TRTGRP1 = LAG1(TRTGRP); PCT1 = LAG1(PCT);
TRTGRP2 = LAG2(TRTGRP); PCT2 = LAG2(PCT);
TRTGRP3 = LAG3(TRTGRP); PCT3 = LAG3(PCT);
TRTGRP4 = LAG4(TRTGRP); PCT4 = LAG4(PCT);
TRTGRP5 = LAG5(TRTGRP); PCT5 = LAG5(PCT);
TRTGRP6 = LAG6(TRTGRP); PCT6 = LAG6(PCT);
TRTGRP7 = LAG7(TRTGRP); PCT7 = LAG7(PCT);
TRTGRP8 = LAG8(TRTGRP); PCT8 = LAG8(PCT);
TRTGRP9 = LAG9(TRTGRP); PCT9 = LAG9(PCT);
TRTGRP10=LAG10(TRTGRP); PCT10=LAG10(PCT);
/*
TRTGRP11=LAG11(TRTGRP); PCT11=LAG11(PCT);
TRTGRP12=LAG12(TRTGRP); PCT12=LAG12(PCT);
TRTGRP13=LAG13(TRTGRP); PCT13=LAG13(PCT);
TRTGRP14=LAG14(TRTGRP); PCT14=LAG14(PCT);
TRTGRP15=LAG15(TRTGRP); PCT15=LAG15(PCT);
*/

LOGLD1 = LAG1(LOGLD); LOGSD1 = LAG1(SDX); REGTYP1 = LAG1(REGTYPE);
LOGLD2 = LAG2(LOGLD); LOGSD2 = LAG2(SDX); REGTYP2 = LAG2(REGTYPE);
LOGLD3 = LAG3(LOGLD); LOGSD3 = LAG3(SDX); REGTYP3 = LAG3(REGTYPE);
LOGLD4 = LAG4(LOGLD); LOGSD4 = LAG4(SDX); REGTYP4 = LAG4(REGTYPE);
LOGLD5 = LAG5(LOGLD); LOGSD5 = LAG5(SDX); REGTYP5 = LAG5(REGTYPE);
LOGLD6 = LAG6(LOGLD); LOGSD6 = LAG6(SDX); REGTYP6 = LAG6(REGTYPE);
LOGLD7 = LAG7(LOGLD); LOGSD7 = LAG7(SDX); REGTYP7 = LAG7(REGTYPE);
LOGLD8 = LAG8(LOGLD); LOGSD8 = LAG8(SDX); REGTYP8 = LAG8(REGTYPE);
LOGLD9 = LAG9(LOGLD); LOGSD9 = LAG9(SDX); REGTYP9 = LAG9(REGTYPE);
LOGLD10=LAG10(LOGLD); LOGSD10=LAG10(SDX); REGTYP10=LAG10(REGTYPE);
/*
LOGLD11=LAG11(LOGLD); LOGSD11=LAG11(SDX); REGTYP11=LAG11(REGTYPE);
LOGLD12=LAG12(LOGLD); LOGSD12=LAG12(SDX); REGTYP12=LAG12(REGTYPE);
LOGLD13=LAG13(LOGLD); LOGSD13=LAG13(SDX); REGTYP13=LAG13(REGTYPE);
LOGLD14=LAG14(LOGLD); LOGSD14=LAG14(SDX); REGTYP14=LAG14(REGTYPE);
LOGLD15=LAG15(LOGLD); LOGSD15=LAG15(SDX); REGTYP15=LAG15(REGTYPE);
*/

IF (PCT NE PCT1 )THEN DO; TRTGRP1=' '; LOGLD1=.; LOGSD1=.; REGTYP1=' '; END;
IF (PCT NE PCT2 )THEN DO; TRTGRP2=' '; LOGLD2=.; LOGSD2=.; REGTYP2=' '; END;
IF (PCT NE PCT3 )THEN DO; TRTGRP3=' '; LOGLD3=.; LOGSD3=.; REGTYP3=' '; END;

```



```

IF (PCT NE PCT4 )THEN DO; TRTGRP4=' '; LOGLD4=.; LOGSD4=.; REGTYP4=' '; END;
IF (PCT NE PCT5 )THEN DO; TRTGRP5=' '; LOGLD5=.; LOGSD5=.; REGTYP5=' '; END;
IF (PCT NE PCT6 )THEN DO; TRTGRP6=' '; LOGLD6=.; LOGSD6=.; REGTYP6=' '; END;
IF (PCT NE PCT7 )THEN DO; TRTGRP7=' '; LOGLD7=.; LOGSD7=.; REGTYP7=' '; END;
IF (PCT NE PCT8 )THEN DO; TRTGRP8=' '; LOGLD8=.; LOGSD8=.; REGTYP8=' '; END;
IF (PCT NE PCT9 )THEN DO; TRTGRP9=' '; LOGLD9=.; LOGSD9=.; REGTYP9=' '; END;
IF (PCT NE PCT10)THEN DO; TRTGRP10=' '; LOGLD10=.; LOGSD10=.; REGTYP10=' '; END;
/*
IF (PCT NE PCT11)THEN DO; TRTGRP11=' '; LOGLD11=.; LOGSD11=.; REGTYP11=' '; END;
IF (PCT NE PCT12)THEN DO; TRTGRP12=' '; LOGLD12=.; LOGSD12=.; REGTYP12=' '; END;
IF (PCT NE PCT13)THEN DO; TRTGRP13=' '; LOGLD13=.; LOGSD13=.; REGTYP13=' '; END;
IF (PCT NE PCT14)THEN DO; TRTGRP14=' '; LOGLD14=.; LOGSD14=.; REGTYP14=' '; END;
IF (PCT NE PCT15)THEN DO; TRTGRP15=' '; LOGLD15=.; LOGSD15=.; REGTYP15=' '; END;
*/

DATA P1; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP1;
REGTYTWO=REGTYPE; REGTYONE=REGTYP1;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD1; LOGSDTWO=SDX; LOGSDONE=LOGSD1;
DROP TRTGRP1--REGTYP10;
*PROC PRINT;

DATA P2; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP2;
REGTYTWO=REGTYPE; REGTYONE=REGTYP2;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD2; LOGSDTWO=SDX; LOGSDONE=LOGSD2;
DROP TRTGRP1--REGTYP10;

DATA P3; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP3;
REGTYTWO=REGTYPE; REGTYONE=REGTYP3;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD3; LOGSDTWO=SDX; LOGSDONE=LOGSD3;
DROP TRTGRP1--REGTYP10;

DATA P4; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP4;
REGTYTWO=REGTYPE; REGTYONE=REGTYP4;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD4; LOGSDTWO=SDX; LOGSDONE=LOGSD4;
DROP TRTGRP1--REGTYP10;

DATA P5; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP5;
REGTYTWO=REGTYPE; REGTYONE=REGTYP5;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD5; LOGSDTWO=SDX; LOGSDONE=LOGSD5;
DROP TRTGRP1--REGTYP10;

DATA P6; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP6;
REGTYTWO=REGTYPE; REGTYONE=REGTYP6;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD6; LOGSDTWO=SDX; LOGSDONE=LOGSD6;
DROP TRTGRP1--REGTYP10;

DATA P7; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP7;
REGTYTWO=REGTYPE; REGTYONE=REGTYP7;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD7; LOGSDTWO=SDX; LOGSDONE=LOGSD7;
DROP TRTGRP1--REGTYP10;

DATA P8; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP8;
REGTYTWO=REGTYPE; REGTYONE=REGTYP8;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD8; LOGSDTWO=SDX; LOGSDONE=LOGSD8;
DROP TRTGRP1--REGTYP10;

```

```
DATA P9; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP9;
REGTYTWO=REGTYPE; REGTYONE=REGTYP9;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD9; LOGSDTWO=SDX; LOGSDONE=LOGSD9;
DROP TRTGRP1--REGTYP10;
```

```
DATA P10; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP10;
REGTYTWO=REGTYPE; REGTYONE=REGTYP10;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD10; LOGSDTWO=SDX; LOGSDONE=LOGSD10;
DROP TRTGRP1--REGTYP10;
```

```
/*
```

```
DATA P11; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP11;
REGTYTWO=REGTYPE; REGTYONE=REGTYP11;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD11; LOGSDTWO=SDX; LOGSDONE=LOGSD11;
DROP TRTGRP1--REGTYP15;
```

```
DATA P12; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP12;
REGTYTWO=REGTYPE; REGTYONE=REGTYP12;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD12; LOGSDTWO=SDX; LOGSDONE=LOGSD12;
DROP TRTGRP1--REGTYP15;
```

```
DATA P13; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP13;
REGTYTWO=REGTYPE; REGTYONE=REGTYP13;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD13; LOGSDTWO=SDX; LOGSDONE=LOGSD13;
DROP TRTGRP1--REGTYP15;
```

```
DATA P14; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP14;
REGTYTWO=REGTYPE; REGTYONE=REGTYP14;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD14; LOGSDTWO=SDX; LOGSDONE=LOGSD14;
DROP TRTGRP1--REGTYP15;
```

```
DATA P15; SET DPCT; LENGTH GRPONE GRPTWO $40; GRPTWO=TRTGRP; GRPONE=TRTGRP15;
REGTYTWO=REGTYPE; REGTYONE=REGTYP15;
LOGLDTWO=LOGLD; LOGLDONE=LOGLD15; LOGSDTWO=SDX; LOGSDONE=LOGSD15;
DROP TRTGRP1--REGTYP15;
```

```
*/
```

```
**** Shrink this dataset since it takes up too much workspace;
DATA DPCT; SET DPCT (KEEP=AGENT);
```

```
DATA PALL; SET P1 P2 P3 P4 P5 P6 P7 P8 P9 P10; * P11 P12 P13 P14 P15;
IF (GRPONE NE ' ' AND GRPTWO NE ' ');
ALLKEY=1;
```

```
DATA PALL; MERGE PALL STDELS; BY ALLKEY;
DROP ALLKEY;
T=1.96; *INPUT T FACTOR FOR USE WITH CONF INTVLS;
```

```
LOGDEL = LOGLDTWO - LOGLDONE;
```

```
IF (REGTYONE = 'S' OR REGTYTWO = 'S') THEN
STDEL = SQRT(LOGSDONE**2 + LOGSDTWO**2);
```

```
ELSE IF (REGTYONE = 'P' AND REGTYTWO = 'P') THEN DO;
```

```
IF (GRPONE = TRNAME1 AND GRPTWO = TRNAME2) OR
```

```

        (GRPONE = TRNAME2 AND GRPTWO = TRNAME1) THEN STDEL = STDEL12;
ELSE IF (GRPONE = TRNAME1 AND GRPTWO = TRNAME3) OR
        (GRPONE = TRNAME3 AND GRPTWO = TRNAME1) THEN STDEL = STDEL13;
ELSE IF (GRPONE = TRNAME1 AND GRPTWO = TRNAME4) OR
        (GRPONE = TRNAME4 AND GRPTWO = TRNAME1) THEN STDEL = STDEL14;
ELSE IF (GRPONE = TRNAME1 AND GRPTWO = TRNAME5) OR
        (GRPONE = TRNAME5 AND GRPTWO = TRNAME1) THEN STDEL = STDEL15;
ELSE IF (GRPONE = TRNAME1 AND GRPTWO = TRNAME6) OR
        (GRPONE = TRNAME6 AND GRPTWO = TRNAME1) THEN STDEL = STDEL16;
ELSE IF (GRPONE = TRNAME2 AND GRPTWO = TRNAME3) OR
        (GRPONE = TRNAME3 AND GRPTWO = TRNAME2) THEN STDEL = STDEL23;
ELSE IF (GRPONE = TRNAME2 AND GRPTWO = TRNAME4) OR
        (GRPONE = TRNAME4 AND GRPTWO = TRNAME2) THEN STDEL = STDEL24;
ELSE IF (GRPONE = TRNAME2 AND GRPTWO = TRNAME5) OR
        (GRPONE = TRNAME5 AND GRPTWO = TRNAME2) THEN STDEL = STDEL25;
ELSE IF (GRPONE = TRNAME2 AND GRPTWO = TRNAME6) OR
        (GRPONE = TRNAME6 AND GRPTWO = TRNAME2) THEN STDEL = STDEL26;
ELSE IF (GRPONE = TRNAME3 AND GRPTWO = TRNAME4) OR
        (GRPONE = TRNAME4 AND GRPTWO = TRNAME3) THEN STDEL = STDEL34;
ELSE IF (GRPONE = TRNAME3 AND GRPTWO = TRNAME5) OR
        (GRPONE = TRNAME5 AND GRPTWO = TRNAME3) THEN STDEL = STDEL35;
ELSE IF (GRPONE = TRNAME3 AND GRPTWO = TRNAME6) OR
        (GRPONE = TRNAME6 AND GRPTWO = TRNAME3) THEN STDEL = STDEL36;
ELSE IF (GRPONE = TRNAME4 AND GRPTWO = TRNAME5) OR
        (GRPONE = TRNAME5 AND GRPTWO = TRNAME4) THEN STDEL = STDEL45;
ELSE IF (GRPONE = TRNAME4 AND GRPTWO = TRNAME6) OR
        (GRPONE = TRNAME6 AND GRPTWO = TRNAME4) THEN STDEL = STDEL46;
ELSE IF (GRPONE = TRNAME5 AND GRPTWO = TRNAME6) OR
        (GRPONE = TRNAME6 AND GRPTWO = TRNAME5) THEN STDEL = STDEL56;
END;

LOGLCB = LOGDEL - T*STDEL;
LOGUCB = LOGDEL + T*STDEL;

IF (ABS(LOGDEL) LT 30) THEN PR = 10**(LOGDEL);
ELSE IF (LOGDEL LT -30) THEN PR = -999999;
ELSE IF (LOGDEL GT 30) THEN PR = 999999;
IF (ABS(LOGLCB) LT 30) THEN LCB = 10**(LOGLCB);
ELSE IF (LOGLCB LT -30) THEN LCB = -999999;
ELSE IF (LOGLCB GT 30) THEN LCB = 999999;
IF (ABS(LOGUCB) LT 30) THEN UCB = 10**(LOGUCB);

```

```
ELSE IF (LOGUCB LT -30) THEN UCB = -999999;
ELSE IF (LOGUCB GT 30) THEN UCB = 999999;
```

```
*KEEP AGENT PCT GRPONE GRPTWO REGTYONE REGTYTWO
  LOGLDONE LOGLDTWO LOGDEL STDEL PR LCB UCB;
```

```
label agent='Agent';
*label time = 'Time (Minutes)';
label pct='Percentile';
label grpone='1st Group*(Denominator)';
label grptwo='2nd Group*(Numerator)';
label logldone='Log(L.D.)*1st Group';
label logldtwo='Log(L.D.)*2nd Group';
label logdel='Del(LogLD)*2nd - 1st';
label stdel='Std Err,*Delta';
label loglcb='Log (Lower*Conf. Bnd)';
label logucb='Log (Upper*Conf. Bnd)';
label pr='Protective*Ratio';
label lcb='Lower Confid-*ence Bound';
label ucb='Upper Confid-*ence Bound';
```

```
PROC SORT;
  BY AGENT PCT GRPONE GRPTWO;
```

```
*PROC PRINT;
*TITLE5 'DIAGNOSTIC INFORMATION - LAST DATA SET PALL';
```

```
PROC PRINT NOOBS DOUBLE SPLIT='*' DATA=PALL; BY AGENT PCT;
  VAR GRPONE GRPTWO LOGLDONE LOGLDTWO LOGDEL STDEL PR LCB UCB;
TITLES 'PROTECTIVE RATIOS AND CONFIDENCE BOUNDS FOR SPECIFIED PERCENTILES';
```

```
RUN;
```