U.S. ARMY COMBAT CAPABILITIES DEVELOPMENT COMMAND
CHEMICAL BIOLOGICAL CENTER
ABERDEEN PROVING GROUND, MD 21010-5424

DEVCOM CBC-TR-1818

# Rapid and Effective Bio-decontamination of Military Working Dog Skin

**Vipin K. Rastogi**
**Sarah Katoski**
**RESEARCH AND OPERATIONS DIRECTORATE**

**Orshuntis Cross**
**OAK RIDGE INSTITUTE FOR SCIENCE AND EDUCATION**
**Belcamp, MD 21017-1543**

**Brianna M. Leija**
**EXCET, INC.**
**Springfield, VA 22150-2519**

**April 2023**

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

# REPORT DOCUMENTATION PAGE

| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED | |
|---|---|---|---|
| | | START DATE | END DATE |
| XX-04-2023 | Final | Jan 2022 | Jun 2022 |

**4. TITLE AND SUBTITLE**
Rapid and Effective Bio-decontamination of Military Working Dog Skin

| 5a. CONTRACT NUMBER | 5b. GRANT NUMBER | 5c. PROGRAM ELEMENT NUMBER |
|---|---|---|
| | | |

| 5d. PROJECT NUMBER | 5e. TASK NUMBER | 5f. WORK UNIT NUMBER |
|---|---|---|
| A7400A97EI2191 | | |

**6. AUTHOR(S)**
Rastogi, Vipin K.; Katoski, Sarah (DEVCOM CBC); Cross, Orshuntis (ORISE); Leija, Brianna M. (Excet)

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Director, DEVCOM CBC, ATTN: FCDD-CBR-BD, APG, MD 21010-5424
Excet Inc.; 6225 Brandon Ave, Suite 360, Springfield VA 22150-2519
Oak Ridge Institute for Science and Education; 4692 Millennium Drive, Suite 101, Belcamp, MD, 21017-1543

**8. PERFORMING ORGANIZATION REPORT NUMBER**
DEVCOM CBC-TR-1818

| 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) | 10. SPONSOR/MONITOR'S ACRONYM(S) | 11. SPONSOR/MONITOR'S REPORT NUMBER(S) |
|---|---|---|
| Office of the Surgeon General/U.S. Army Medical Command, Chemical, Biological, Radiological, and Nuclear through U.S. Army Public Health Center, 8252 Blackhawk Road, APG, MD 21010-5403 | OSG/MEDCOM CBRN | |

**12. DISTRIBUTION/AVAILABILITY STATEMENT**
Distribution Statement A. Approved for public release: distribution unlimited.

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT (LESS THAN 200 WORDS)**
More than 1600 military working dogs (MWDs) are deployed in conflict regions by the U.S. armed forces. Dogs are trained for specific jobs, including tracking, explosive detection, patrol, search and rescue, and attack. One of their key functions is detection of chemical, biological, radiological, nuclear, and energetics threat materials. MWDs operate under a high risk of exposure to chemical and biological warfare agents. If MWDs are exposed to a biological warfare agent, such as spores of *Bacillus anthracis*, current protocols used by handlers include rinse–wash–rinse and a chlorhexidine sponge wipe. Both procedures fail to inactivate spores. As a result, viable spores remain in high numbers on canine skin. As an alternative, we have previously shown partial inactivation of spores by use of wipes soaked in 5% Bioxy (Atomes, Inc.; Quebec, Canada). Here we report highly efficient, rapid, and complete inactivation of spores by use of wipes soaked in Veriox decontaminant (Armis Biopharma; Fort Collins, CO). A 6% Veriox solution was highly effective after a 15 min contact period. In conclusion, Veriox decontaminant is a superior alternative for dog handlers in the field to use in bio-decontamination of dog skin surfaces.

**15. SUBJECT TERMS**
Spores *Bacillus anthracis* Military working dog Bio-decontamination
Veriox

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES |
|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | C. THIS PAGE | | |
| U | U | U | UU | 22 |

| 19a. NAME OF RESPONSIBLE PERSON | 19b. PHONE NUMBER *(Include area code)* |
|---|---|
| Renu B. Rastogi | (410) 436-7545 |

**STANDARD FORM 298 (REV. 5/2020)**
*Prescribed by ANSI Std. Z39.18*

Blank

## PREFACE

The work described in this report was authorized under agreement number A7400A97E12191 with the U.S. Army Public Health Center (APHC; Aberdeen Proving Ground, MD). The work was started in January 2022 and completed in June 2022.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release.


## Acknowledgments

The authors acknowledge LTC Emilee Venn and her team at APHC for providing overall project oversight. Special thanks are extended to Dr. Linnzi Wright and Dr. Kelly Evans (U.S. Army Combat Capabilities Development Command Chemical Biological Center; Aberdeen Proving Ground, MD) for coordinating receipt, handling, and distribution of skin substrates.

Blank

## CONTENTS

## FIGURES



## TABLE

# RAPID AND EFFECTIVE BIO-DECONTAMINATION OF MILITARY WORKING DOG SKIN

## 1. INTRODUCTION

In an address given on 8 February 2008, Gen. David H. Petraeus, the commanding general of Multi-National Force, Iraq, said, "The capability that military working dogs bring to the fight cannot be replicated by man or machine. By all measures of performance, their yield outperforms any asset we have in our inventory. Our Army would be remiss if we failed to invest more in this incredibly valuable resource" (Ponga, 2017). Since World War II, dogs have been trained for specific jobs in the U.S. armed forces. In this war, the Coast Guard, Marines, and the Army deployed approximately 20,000 dogs for various functionalities (Gordon, 2009; Lavallée et al., 2020; Perry et al., 2021; Rastogi et al, 2022). Currently, military working dogs (MWDs) actively serve as an integral asset to Soldiers in the field. They fill roles ranging from security patrol work to scouting and threat detection. These roles are highly focused on the canine's ability to detect targets by smell. Members of the 637th Chemical Company, the 719th Medical Detachment Veterinary Service Support, and the 386th Expeditionary Security Forces Squadron came together to conduct a live exercise to train MWDs in the event they were exposed to a chemical, biological, radiological, and nuclear substance. Since dogs remain close to ground level, they are at high risk of being exposed to chemical and biological warfare threats. The daunting challenge of rapid cleanup and decontamination of dog skin by handlers or decontamination experts is an absolute requirement for continued field operations (Melekwe et al., 2018; Claudio, 2019; MCTP 10-10F, 2020)

Even though the MWD fur can serve as a barrier to dermal pathogens, long fur can offer a reservoir for pathogens including common biological warfare (BW) agents, such as spores of *Bacillus anthracis*, the causative pathogen for anthrax. Therefore, contaminated MWDs pose a risk to their handlers by serving as carriers or sources of transmission for biothreat agents. The current decontamination protocol is focused on full mechanical decontamination; however, it is water-intensive and generates potentially infectious waste (Gordon, 2009; Perry et al., 2021; Rastogi et al., 2022). It involves a multi-cycle rinse-wash-rinse method that uses a high volume of low-pressure water with soap to remove the contaminant; contaminated runoff is drained to mitigate the risk of infection (MCTP 10-10F, 2020). Washing can push contaminants deeper through fur onto the skin surface. At the same time, over-washing can compromise the integrity of the skin barrier through removal of natural skin oils and over-drying (Lavallée et al., 2020; Perry et al., 2021).

Anthrax, the pathogenic Ames strain of *B. anthracis*, is a notoriously efficient spore-forming gram-positive bacterium. The spores are easily aerosolized, persistent, and highly infectious via inhalation, cutaneous, and gastrointestinal routes of exposure, making it an ideal BW agent. After the 2001 'Amerithrax' attack, the Centers for Disease Control and Prevention and U.S. Department of Defense elevated it to a Tier One Select Agent and, to date, this remains a significant target for biological decontamination efforts. In this study, *B. anthracis* Sterne (BaS) was used as a surrogate for the Ames strain to evaluate the efficacy of current decontamination protocols at neutralizing the risk posed by BW agent (spores) to MWD. Wipes

soaked in Veriox decontaminant (Armis Biopharma; Fort Collins, CO) were included as test wipes for decontamination of dog skin. The Veriox concentration ranged from 2 to 6%, and contact time varied from 15 to 60 min.

## 2. METHOD

### 2.1 *Bacillus* Spore Preparation

BaS spores were prepared as described previously (Rastogi et al., 2009). Briefly, spores were prepared on Lab-Lemko agar plates. Preparations were verified to be >95% spores and were treated at 65 °C for 30 min to rid the samples of vegetative cells. The stock culture was enumerated and stored at 4 °C for up to 12 weeks.

Based on the colony-forming units (CFU) on tryptic soy agar (TSA) plates, the stock titer averaged ~$3.6 \times 10^9$ CFU/mL. A working stock was prepared by adding 10 ml of ~$4.0 \times 10^9$ CFU/mL parent stock to 90 mL of 0.01% Tween 80 (Croda; Princeton, NJ); the working stock was then divided between two tubes so that each had 5 mL of parent stock and 45 mL Tween 80. The working stocks were enumerated separately and were found to have a titer of ~$4.0 \times 10^8$ CFU/mL.

### 2.2 Dog Skin Pieces

Full-thickness cadaver skin tissue substrates (swatches) were collected during standard postmortem procedures of MWDs following euthanasia for reasons defined in Air Force Instruction 31-126/Army Regulation 700-81/OPNAVINST 5585.3B/MCO 5585.6. No animals were euthanized for the purpose of this study, and researchers obtained exempt status from the governing Institutional Animal Care and Use Committee (IACUC). Skin samples were collected from six dogs representing the most common MWD breeds. Tissue was collected within 3 h of euthanasia from the left and right shoulders, lateral flank, and ventral abdomen. The skin from each region was cut into 1.5 in. strips or 1.5 by 1.5 in. coupons before being wrapped in foil and stored long-term at –80 °C.

Table 1. Dog Skin Characteristics

| Identification | Breed | Sex | Age (years) | Hair Coat | Coat Color |
|---|---|---|---|---|---|
| 1 | German Shepherd | Male | 6 | Rough, long | Black |
| 2 | German Shepherd | Male | 7 | Rough, medium-long | Sable |
| 3 | German Shepherd | Female | 7 | Rough, short | Black |
| 4 | Belgian Malinois | Male | 2 | Rough | Tan |
| 5 | Belgian Malinois | Male | 2 | Rough | Tan |
| 6 | Labrador Retriever | Male | 9 | Rough | Black |

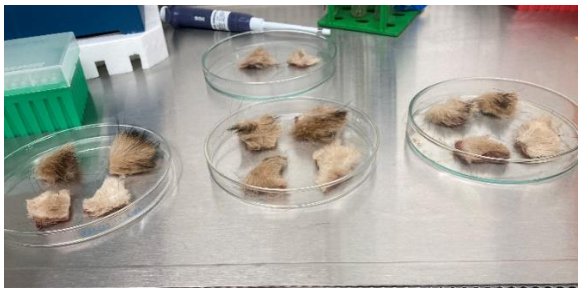
For testing, samples were thawed overnight in a 4 °C refrigerator. Thawed samples were sprayed with 70% isopropanol to saturation (~5 mL), then left to dry overnight in the biosafety cabinet. Larger skin samples were then cut down to roughly 1 by 0.5 in. in size using surgical scissors. After sizing, the length of fur was trimmed down as per experimental design.

Clean disinfected skin samples were inoculated with a 50 µL aliquot of BaS spores containing ~1–2 × $10^7$ CFU/mL titer. The aliquot was left to dry in a biosafety level 2 cabinet for 2 h under sterile laminar flow (Figure 1).

## 2.3 Decontamination Using Veriox Decontaminant

In phase 2, one of the key tasks was to evaluate a novel technology, Veriox, from Armis Biopharma. A full set of three Veriox solutions (2, 4, and 6%) were evaluated. Control wipes were soaked with a 0.01% Tween-80 solution. A 50 µL (containing ~7 logs) aliquot of Sterne strain spore suspension was deposited as microdroplet on each skin piece. Typically, three replicate skin samples were used for each set. As per standard protocol, sterile wipes were scrubbed over skin pieces. Finally, polyester wipes soaked in Veriox solution were used as a novel decontamination wipe. Spores removed mechanically were discarded and not enumerated. Skin pieces with residual spores were placed in 20 mL of Dey–Engley (D/E) neutralizing broth. The tubes were vortexed for 2 min and sonicated for 10 min so spores could be recovered from the skin pieces. After sonication, tubes were vortexed for an additional 2 min, and skin pieces were removed. The control and test sets were diluted ten-fold in a final volume of 1 mL. A 100 µL aliquot was plated on two TSA plates from appropriate control and test set dilutions. The plates were incubated for 24 ± 2 h at 37 °C. CFU were counted from all plates and recorded.

A



B

C

Figure 1. Dog skin pieces (A and B), and a typical workstation for experimental run (C).

## 2.4 Data Handling and Reduction

CFU numbers were multiplied with volume factor (20 mL) and dilution factor to estimate spore number per sample. Log values were computed from the spore numbers. Average values and standard deviation were computed. Log reduction was estimated by subtracting the log spore of the test sample from the control sample.

# 3. RESULTS

## 3.1 Log Recovery of *Bacillus* Spores in Control Sets

Across all tests, the dog skin pieces were inoculated with ~7 log of spores. Average spore recovery after the skin was wiped down was consistent in all runs, as shown in Figure 2. A control sample (extracted before the skin was wiped down) was also included. The titer sample showed ~6.8 log of spores. In extracted post-wiping and control samples, ~6.1 and 6.5 log of spores were recorded, respectively (Figure 2).

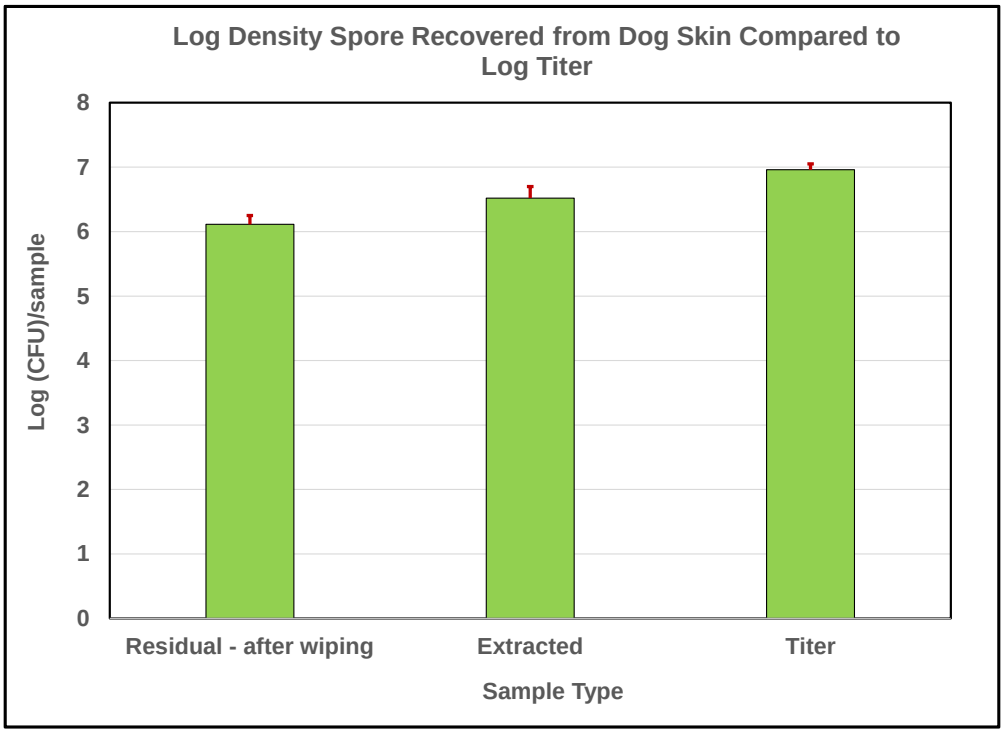



Figure 2. Average spore recovery from skin after wiping down relative to spore titer and spores extracted from dog skin before wiping down.

### 3.2 Log Reduction of BaS Spores following Veriox Treatment

After preliminary runs, the effectiveness of wipes soaked in 2, 4, and 6% Veriox solution for inactivating the spores on dog skin was evaluated. Wipes were scrubbed on skin, and after a 60 min contact period, spores were extracted from treated dog skin pieces. Log reduction data are summarized in Figure 3. Results show that all three concentrations were highly effective in inactivating the spores on dog skin. Log reduction values ranged between 4.8 and 5.2, suggesting very effective spore kill. To determine how rapidly the 6% Veriox solution was effective in spore inactivation, spores were extracted from dog skin after 15, 30, and 60 min exposure periods. The log reduction data are summarized in Figure 4. The data show that 6% Veriox solution was highly effective within 15 min exposure period. Nearly complete spore inactivation was observed after the 30 and 60 min exposure periods. Veriox decontaminant, therefore, appears to be highly effective in spore inactivation on dog skin.

A paired t test was done between data sets for control skin samples, which were wiped down, and those treated with Veriox decontaminant. The p value (0.00005) was much less than the standard significance value of 0.05; therefore, the difference between treated samples and controls was statistically significant.

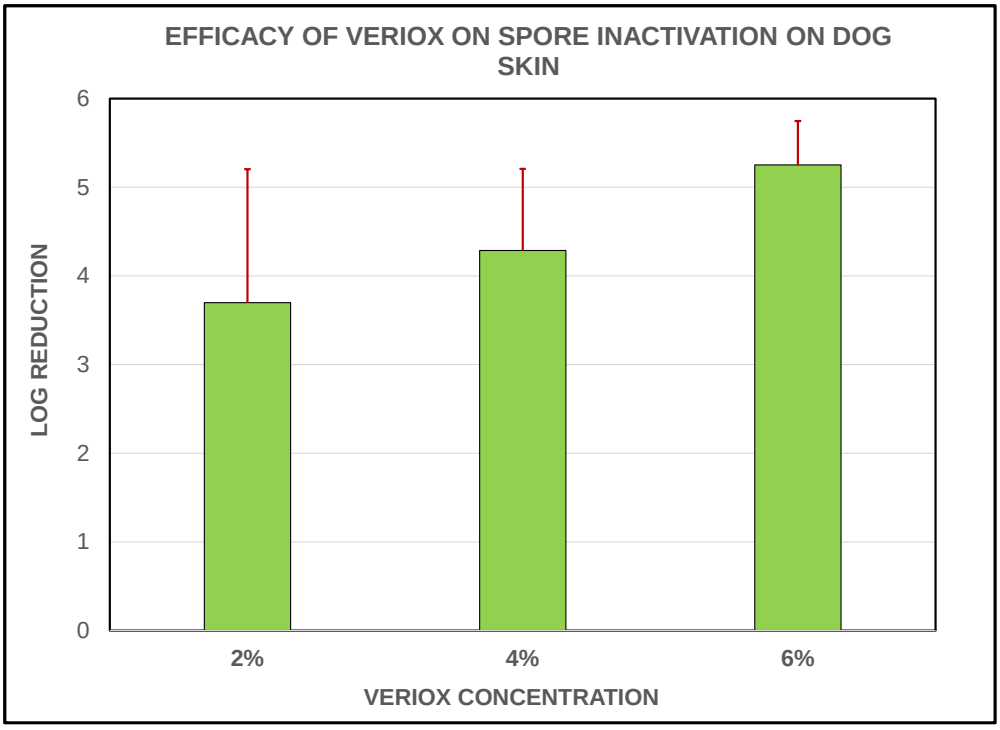



Figure 3. Average log reduction following 60 min contact with three concentrations of Veriox-soaked wipes. The t-test analysis between any two groups (2 vs. 4, 2 vs. 6, or 4 vs. 6), indicated that the data were significantly different, since the p-value was <0.05.

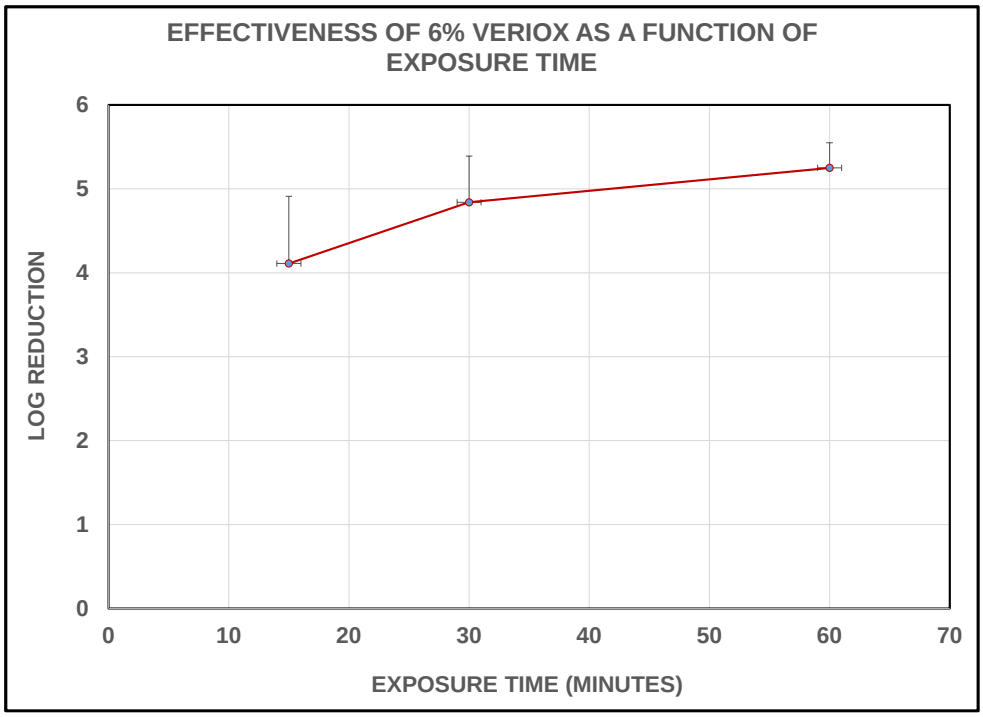


Figure 4. Efficacy of 6% Veriox solution as a function of exposure period.

## 4. DISCUSSION AND CONCLUSIONS

If BW threat material, such as anthrax spores, is encountered, MWD skin is very likely to be contaminated. Rapid and effective decontamination of dog skin is highly desirable not only to contain the spread of hazardous material, but also to minimize the potential for infection. Current field protocols rely solely on mechanical removal of spores from the surface of canine skin. Recently, we reported comparison of current protocols with improved methods in spore decontamination on canine tissues (Rastogi et al., 2022). Even though use of Bioxy solution (Atomes; Quebec, Canada) was found to cause significant spore inactivation, complete spore inactivation was not achieved with a 5% solution strength.

Bioxy powder is a surface sanitizer that generates peracetic acid and peroxide when dissolved in water at neutral pH (Rastogi et al., 2017). The key component of Bioxy powder is sodium carbonate peroxyhydrate. The Veriox solution used in this study contains peracids and α-keto peracids (Neas, 2018). From a chemical standpoint, the Bioxy and Veriox decontaminants are similar in their composition. Our results with Veriox decontaminant, especially the 4 and 6% solutions, indicated that it was highly effective in spore inactivation. The whole study was performed on skin pieces from cadavers. It remains to be seen whether Veriox decontaminant causes skin sensitivity when in contact for a period of up to 60 min. If this technology is to be widely accepted by MWD handlers as the first choice for skin decontamination, skin sensitivity tests must be performed.

This laboratory-scale study required shaving the hair coat to expose the surface of the dog's skin *in vitro*. In practice, shaving a dog for decontamination at this time is not advised

due to secondary risks of morbidity, and future studies should focus on the efficacy of spore removal from unadulterated canine hair coats. In conclusion, Veriox decontaminant appears to be highly effective for inactivating the anthrax surrogate, BaS spores, on dog skin. Even though a 6% Veriox solution with a 60 min contact time was highly effective in complete inactivation of spores, 30 and 15 min contact times and lower concentrations were also very effective. Future studies should focus on evaluating skin sensitivity to Veriox decontaminant and assessing other methods of applying Veriox to dog skin, such as spraying followed by washing with a neutralizer or water.

Blank

## LITERATURE CITED


Army Regulation 700-81, OPNAVINST 5585.3B, MCO 5585.6, Distibution: PCN102 08621200, DOD Military Working DOG (MWD) Program, https://static.e-publishing.af.mil/production/1/af_a4/publication/afi31-126/afi31-126.pdf (accessed April 1 November 2022).

Claudio, A. Military Working Dogs Train for CBRN Exposure in Kuwait. 2019. https://www.army.mil/article/220856/military_working_dogs_train_for_cbrn_exposure_in_Kuwait (accessed 1 November 2022).

Gordon, L. Canine Decontamination: Guidelines for Emergency, Gross, and Technical Decontamination of the Urban Search and Rescue Canine. *J. Vet. Behav.* **2009,** *4* (6), 248–249. DOI: 10.1016/j.jveb.2009.05.023

Lavallée, J.M.; Shimon, C.; Beaufrère, H.; Chirino-Trejo, M.; Linn, K. Influence of Clipping on Bacterial Contamination of Canine Arthrocentesis Sites before and after Skin Preparation. *Vet. Surg.* **2020,** *49* (7), 1307–1314. DOI: 10.1111/vsu.13468

Melekwe, G.O.; Uwagie-Ero, E.A.; Zoaka, H.A.; Odigie, E.A. Comparative Clinical Effectiveness of Preoperative Skin Antiseptic Preparations of Chlorhexidine Gluconate and Povidone Iodine for Preventing Surgical Site Infections in Dogs. *Int. J. Vet. Sci. Med.* **2018,** *6* (1), 113–116. DOI: 10.1016/j.ijvsm.2018.03.005

*Military Police Operations*; MCTP 10-10F; United States Marine Corps: Washington, DC, 2020; UNCLASSIFED Regulation; https://www.marines.mil/Portals/1/Publications/MCTP%2010-10F.pdf?ver=2020-03-24-083204-067 (accessed 15 November 2022).

Neas, E.D. Compositions Comprising Peroxyacid and Methods for Producing and Using the Same. U.S. Patent 9,877,483 B2; 30 January 2018; https://patentimages.storage.googleapis.com/07/9d/40/baa355d776ada3/US9877483.pdf.

Perry, E.B.; Discepolo, D.R.; Liang, S.Y.; Jenkins, E.K. Removal of Aerosolized Contaminants from Working Canines via a Field Wipe-Down Procedure. *Animals* **2021**, *11*, 120. DOI: 10.3390/ani11010120

Ponga, A. (2017). Military working dog, human handler bond in Baghdad. https://www.defense.gov/News/News-Stories/Article/Article/1097173/military-working-dog-human-handler-bond-in-baghdad/ (accessed 15 November 2022).

Rastogi, V.K.; Katoski, S.; Cross, O.; Leija, B.M. *Decontamination of Bacillus Anthracis Spores on Military Working Dogs*; DEVCOM-CBC-TR-1785; U.S. Army Combat Capabilities Development Command Chemical Biological Center: Aberdeen Proving Ground, MD, 2022; UNCLASSIFIED Report (AD1175012).

Rastogi, V.K.; Smith, L.S.; Edgington, G.; Dagher, M.; Dagher, D.; Dagher, F. The Sporicidal Potency of Bioxy Formulations in Decontaminating Bio-Warfare Agents. *Clin. Microbiol. Infect. Dis*. **2017,** *2* (2), 1–4. DOI: 10.15761/CMID.100125

Rastogi, V.K.; Wallace, L.; Smith, L.S.; Ryan, S.P.; Martin, B. Quantitative method to determine sporicidal decontamination of building surfaces by gaseous fumigants, and issues related to laboratory-scale studies. *Appl. Environ. Microbiol.* **2009**, *75* (11), 3688–3694.

## ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| BaS | *Bacillus anthracis* Sterne |
| BW | biological warfare |
| CFU | colony-forming units |
| D/E | Dey–Engley |
| MWD | military working dog |
| TSA | tryptic soy agar |

Blank

## DISTRIBUTION LIST

The following individuals and organizations were provided with one electronic version of this report:

U.S. Combat Capabilities Development Command Chemical Biological Center (DEVCOM CBC)
FCDD-CBR-BD
ATTN: Rastogi, V.
Nobles, K.

DEVCOM CBC
Technical Library
FCDD-CBR-L
ATTN: Foppiano, S.
Stein, J.

Defense Technical Information Center
ATTN: DTIC OA

U.S. Army Public Health Center
JBSA-Ft Sam Houston/MED365
ATTN: LTC Venn, E.


U.S. ARMY COMBAT CAPABILITIES DEVELOPMENT COMMAND
CHEMICAL BIOLOGICAL CENTER