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**Decontamination of Glo Germ from  
Military Working Dog Fur**

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#### 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.

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<b>14. ABSTRACT (LESS THAN 200 WORDS)</b> Current guidance relating to the decontamination of radiological or particulate contamination on military working dogs (MWDs) is vague and derived from human decontamination protocols. Animal-specific decontamination guidance and training for Service Members is directed toward individual MWD handlers and veterinary personnel and is best suited to limited decontamination in an ideal environment. Furthermore, this guidance has never been vetted in an operational environment. The current study will begin to address the behavior of radiological threats and proper decontamination methods and solutions based on excised canine skin. German shepherd dog, Belgian malinois, and Labrador retriever canine cadaver skins ( <i>Canis familiaris</i> ) contaminated with Glo Germ powder (Glo Germ Company; Moab, UT) were decontaminated using a microfiber towel, a water rinse, or techniques involving shampoos. Overall, the treatments involving a shampoo removed the most Glo Germ from all breed substrates evaluated.					
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## **PREFACE**

The work described in this report was authorized under project no. A7400A97E12191 with the Defense Centers for Public Health – Aberdeen (Aberdeen Proving Ground, MD). The work was started in June 2021 and completed in December 2022.

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This report has been approved for public release.

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# DECONTAMINATION OF GLO GERM FROM MILITARY WORKING DOG FUR

## 1. INTRODUCTION

U.S. Army Veterinary Services is the sole Department of Defense entity responsible for providing recommendations, monitoring, and treatment of military working dog (MWD) chemical, biological, radiological, and nuclear (CBRN) casualties through detection and decontamination guidance to the Joint Force for CBRN-contaminated working dogs. A variety of capability gaps in both doctrine and equipment have been identified by the MWD community via several capabilities-based assessments. A more extensive list is still to be determined. Currently, CBRN field decontamination recommendations and doctrine for MWDs are vague and derived from human decontamination protocols (Appendix H; HQDA, 2016). Animal-specific decontamination guidance and training for Service Members is directed towards individual MWD handlers and veterinary personnel and is best suited to limited decontamination in an ideal environment. Furthermore, this antiquated guidance was last updated in the mid-1990s and has never been vetted in an operational environment. Knowledge gaps include tactical practicability and efficiency of published MWD decontamination; adequacy of existing inventory to support MWD decontamination; resource requirements such as time, water, decontaminating solutions, personal protective equipment for handlers and veterinary personnel; and efficacy of decontamination. There is no guidance or consideration for decontamination of exposed MWDs provided (HQDA, 2014). The current study will begin to address the behavior of radiological threats and proper decontamination methods and solutions based on excised canine skin.

## 2. EXPERIMENTAL APPROACH

The following basic and applied research goals were addressed through ex vivo laboratory studies: (1) how a radiological “dusty” contaminant acts on MWD casualties with different hair coats, and (2) if a microfiber towel removal demonstrates efficacy in canine hair coat/skin samples compared to the recommended soap/water for human nuclear and radiological casualties. These goals were achieved by applying a known amount of radiological simulant to excised MWD fur, applying various decontamination treatments, and measuring the simulant remaining on the MWD substrate.

### 2.1 Simulant Selection

The primary hazard of contamination following a radioactive event is radioactive material adsorbed by or aggregating on environmental particulate. As such, initial radiological simulant recommendations were made based on the key physical properties of environmental particulate contaminated with radioactive chemical components following a radiological event (i.e., size distributions, conductivity, hydration properties, and typical challenge levels). *Chemical, Biological, and Radiological (CBR) Contamination Survivability, Large Item Interiors* (WDTC, 2016) and *Simulant of radiological contamination* (Martin and Golden, 2009) provided a basis for simulant selection criteria. Based on these documents and discussions with

radiological subject matter experts Dr. Gerald Falo and MAJ Thomas Costeira at the Defense Centers for Public Health, the simulant selected for this effort was Glo Germ powder (item GGP10; Glo Germ Company; Moab, UT), which was applied to the MWD substrates as a radiological simulant. The product was unique for this effort, as the gross contamination can be visualized both before and after decontamination upon exposure to UV light. Glo Germ powder is also readily extracted from the fur after decontamination to provide a quantitative measurement of contaminant removal by the chosen decontamination method.

## **2.2 Test Materials**

German shepherd dog (GSD), Belgian malinois (BM), and Labrador retriever (LR) canine cadaver skins (*Canis familiaris*) were used in these evaluations. Fresh GSD, BM, and LR subject substrates were obtained immediately following euthanasia during standard MWD post-mortem procedures for reasons defined in *DOD Military Working Dog (WMD) Program* (DOD, 2019). No animals were euthanized for the purpose of this study, and researchers obtained exempt status from the governing Institutional Animal Care and Use Committee (College Park, MD).

Full-thickness skin tissue with the attached hair coat was surgically excised post-mortem from the left and right shoulders, ventral abdomens, and lateral flanks of six working dogs. The fresh substrate was quickly packaged in foil wrapping, labeled, and frozen before shipping to a U.S. military processing laboratory for frozen storage at  $-20\text{ }^{\circ}\text{C}$  or colder until use. All tissue was collected and frozen within 3 h post mortem.

## **2.3 Sample Handling**

Before starting an evaluation, MWD substrates were removed from cold storage and thawed for approximately 30 min. The substrates were cut to sizes that could be safely handled by test operators, had a surface area larger than that of the applied simulant, and resulted in the most samples possible from the available tissue. The substrates were stapled to corkboard for ease of operator handling. The corkboard was placed in a flat horizontal orientation to replicate conditions for maximum exposure to simulant for testing and evaluation.

## **2.4 Contamination**

Individual doses of Glo Germ powder were prepared just before the start of an evaluation. The mass of an empty weigh boat was measured and recorded. A target dose of 50 mg of Glo Germ powder was added to the weigh boat. The mass of the Glo Germ-filled weigh boat (GWB) was recorded. The Glo Germ powder was gently applied to a MWD substrate to ensure that the total dose was fully applied onto the fur. The distribution of the Glo Germ powder on the MWD substrate is illustrated in Figure 1, Figure 2, and Figure 3. The formerly Glo Germ-filled weigh boat (FWB) was measured.

Using the mass measurements, the starting challenge of Glo Germ powder applied to the panel was calculated as

$$\text{GWB} - \text{FWB} = \text{Glo Germ starting challenge}$$

The overall starting challenge throughout the evaluation was  $50.63 \pm 10.45$  g. Contaminated surface area measurements were completed using ImageJ (open source), with the resulting area average of  $138.21 \pm 40.42$  mm<sup>2</sup> per sample.

## 2.5 Decontamination Treatments

Four different decontamination treatments were applied to contaminated MWD substrates: (1) microfiber towel only (MFTO), (2) water only (WO), (3) high water method with a canine shampoo (HWM-C), and (4) high water method with a shampoo containing ethylenediaminetetraacetic acid (EDTA; HWM-E). Table 1 provides the details of each treatment.

Table 1. Decontamination Treatment Descriptions

Treatment	Description
MFTO	This treatment consisted of a rolled piece of 15% nylon and 85% polyester microfiber towel (Part # HC-001-3; SimpleHouseware Microfiber Cleaning Cloth; Amazon; Seattle, WA) held by a binder clip and applied to the MWD substrate. Forceps were used to rub the towel back and forth (with and against the grain of the hair) along the substrate five times for a total of 10 passes. This treatment provides a measure of how much Glo Germ powder is physically removed by the microfiber towel alone without the use of any additional decontaminant agents or measures.
WO	This treatment consisted of 60 mL of deionized (DI) water applied to the contaminated MWD substrate. The WO treatment when compared with the HWM-C or HWM-E treatment could illustrate the impact of the use of shampoo.
HWM-C	This method is based off standard guidance provided in Appendix H of the ATP 4-02.85 detailing a thorough washing of the hair coat and skin with soap or available non-medicated veterinary shampoo and water (HQDA, 2016). Modifications to the guidance were necessary for the current laboratory-scale study as pieces of excised dog hair coat and skin and not an entire canine were used. DI water in the amount of 60 mL was applied to the contaminated MWD substrate followed by 200 µL of canine shampoo (CocoDerm Conditioning Shampoo; Butler Schein Animal Health; Dublin, OH). A small toothbrush (30 Tuft, 4 in. Short Orange Handle; soft bristles with rounded polish tips, Item Number TB20; Dukal Corporation; Ronkonkoma, NY) was used to work the soap into the hair coat by brushing with and against the grain of the hair (back and forth) five times for a total of 10 motions. The shampoo and lather were rinsed from the substrate with 60 mL of DI water. The water and shampoo volumes were selected by the test operators based on visually inspecting the MWD substrate to ensure the hair coat was saturated with water and the shampoo was present on the hair coat in excess.
HWM-E	This treatment is identical to the HWM-C method, but it additionally includes an EDTA-containing shampoo (PerCara Baby Shampoo; PerCara Enterprises, Inc.; Burlington, ONT).

## 2.6 Sample Analysis

Each MWD substrate was extracted after the decontamination treatment. To determine the mass of Glo Germ powder on the substrate after the decontamination treatment, the substrate was placed face up in a 1 oz glass jar with a Teflon-lined polypropylene lid (product number 170801; Scientific Specialties Service, Inc.; Hanover, MD). Twenty milliliters of chloroform (item number 650498; MilliporeSigma; Burlington, MA) was added to the vial using a bottle-top organic solvent dispenser (part number 4701351; BrandTech; Essex, CT) to fully cover the entire substrate. The panel was stirred at 1000 rpm in the extraction solvent for 60 min. At the end of the extraction period, the MWD substrate was removed from the jar and the extractant measured for the Glo Germ concentration using fluorescence spectroscopy on a Jasco FP-8300 spectrofluorometer (Jasco; Easton, MD). The samples were excited at  $\lambda_{\text{ex}} = 375$  nm and measured at  $\lambda_{\text{em}} = 380$ -700 nm. Fluorescence intensity at  $\lambda = 436$  nm was measured corresponding to the  $\lambda_{\text{max}}$  of the Glo Germ powder under the experimental conditions.

## 2.7 Data Analysis

Data regarding the efficacy of multiple decontamination treatments were collected in this study. Efficacy was measured as the percent of Glo Germ powder removed from the MWD substrate after decontamination treatment. The percent removed was calculated as

$$\text{Percent removed} = 100 \times [1 - (\text{Remaining Glo Germ} \div \text{Glo Germ starting challenge})]$$

The Tukey–Kramer honestly significant difference (HSD) test was used to assess differences in performance. The Tukey–Kramer test controls the type I error, which is the probability of rejecting the null hypothesis when it is true. This test is a single-step, multiple-comparison procedure, which simultaneously considers all pair-wise comparisons. The goal is to compare the average effects of three or more data sets to decide which are different from each other and by how much. The Tukey–Kramer test calculates a critical value that is used to evaluate whether differences between any two pairs of means are significant. The critical value is determined using a studentized range statistic, the mean square error from the overall F-test, and the sample size for each group. The Tukey–Kramer test was used throughout this report to establish if different decontamination methods resulted in statistically significant differences in remaining agent. The software used to conduct the Tukey–Kramer analyses test was JMP version 16.2.0 from SAS Institute, Inc. (Cary, NC).

## 3. RESULTS

### 3.1 Glo Germ Presentation

Images were taken of the fur before and after decontamination for each sample. A cardboard box was lined with flexible Onforu UV black light strips (model ON-DT46-UV-US-NF; Shenzhen Mengzhituo Technology Co., Ltd.; Shenzhen, Guangdong) to create an imaging chamber. The native camera application on an iPhone 13 Pro (Apple Inc.; Cupertino, CA) was used to photograph the fur. Each image was captured using the maximum

exposure presented by the camera application without a flash. Focal plane, ISO setting, aperture, and shutter speed varied across the images. Figure 1 illustrates the MWD fur before and after the decontamination treatments.

Images of Glo Germ powder on the LR, BM, and GSD fur samples are presented in Figures 1, 2, and 3, respectively. The images present a visualization of the relative change in contamination distribution as a result of the decontamination treatment. In each image, the white areas represent the Glo Germ powder fluorescing in the UV imaging chamber. It is visually apparent that a significant amount of Glo Germ powder remained and covered a larger surface area after the MFTO treatment. Notably, the images of the HWM-C and HWM-E treatments show a reduction of Glo Germ powder and reveal a collection of the remaining particulate near the fur/skin boundary.

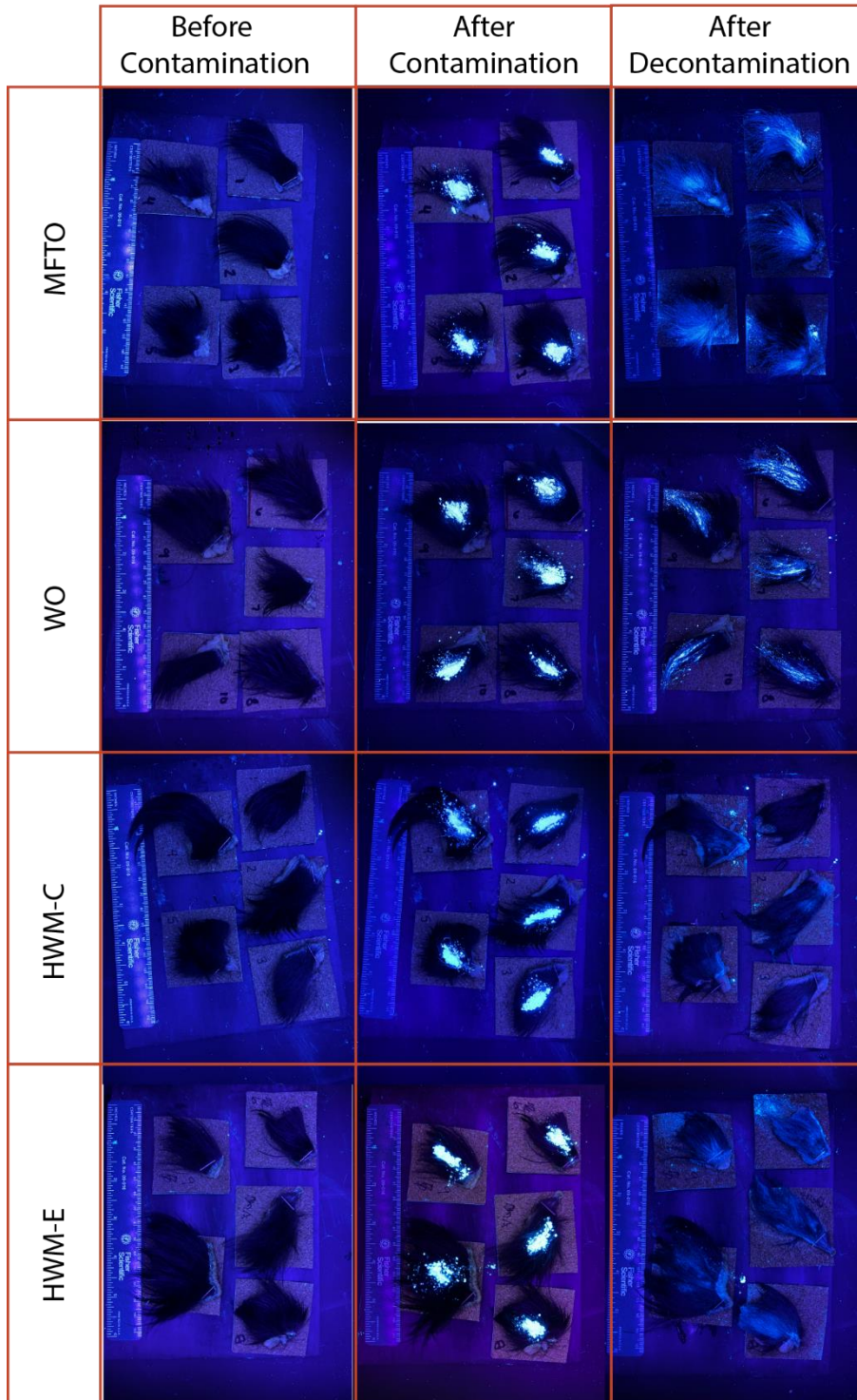


Figure 1. LR MWD fur before and after the decontamination treatments.

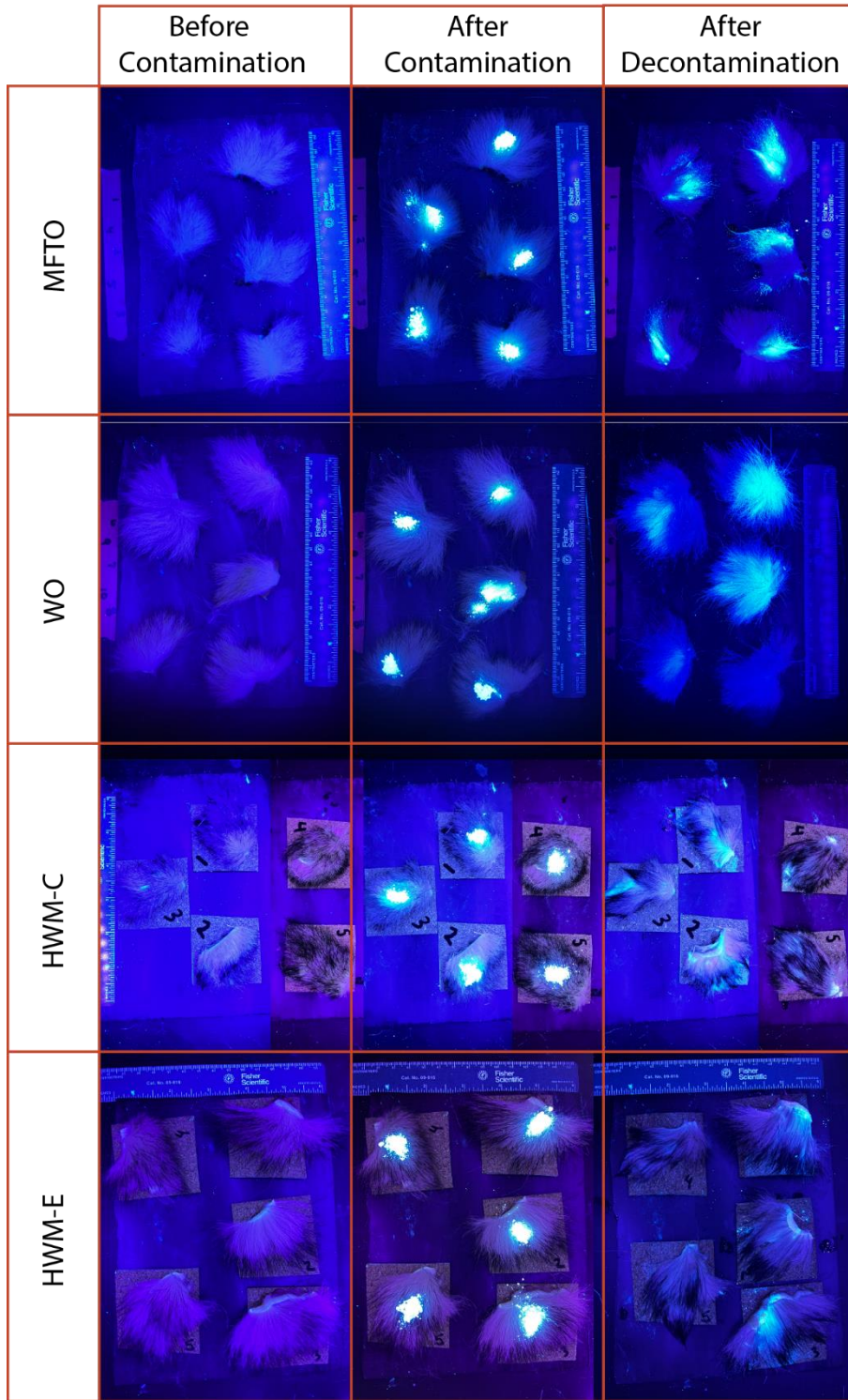


Figure 2. BM MWD fur before and after the decontamination treatments.

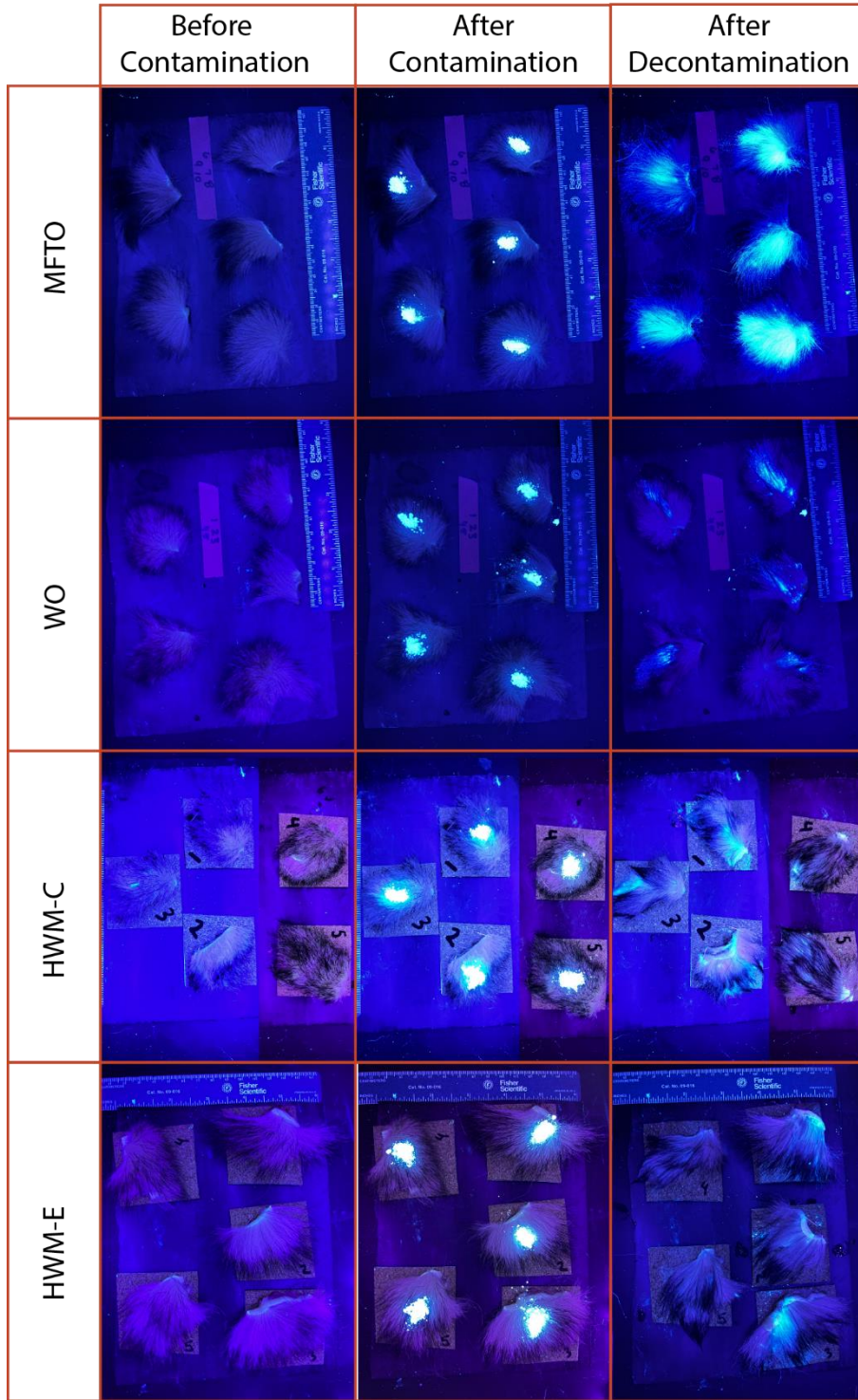


Figure 3. GSD MWD fur before and after the decontamination treatments.

### 3.2 Decontamination Results by Breed

Table 2 and Figure 4 present the percentage of Glo Germ powder removed for each breed–decontamination combination evaluated. Table 2 also provides the Tukey–Kramer HSD results for each decontamination treatment limited by breed. The top performing decontamination method for the LR fur was HWM-C, and the percent removal results were also similar to those of the HWM-E method, as shown by the same letters in the Tukey–Kramer letters report. Analogous performance similarities were observed for the BM and GS fur samples as well, although the performance of the WO method was statistically similar to the HWMs. The MFTO method provided the least amount of Glo Germ powder removal for each breed evaluated.

Table 2. Percent Removed Results for Each Breed–Decontamination Combination

Breed	Decontamination Method	<i>N</i>	Amount Removed (%)	Tukey–Kramer Letters Report
LR	HWM-C	5	93.94 ± 4.09	A
	HWM-E	5	89.72 ± 2.05	AB
	WO	5	76.02±9.75	BC
	MFTO	5	67.81±15.67	C
BM	HWM-E	5	97.24±2.05	A
	HWM-C	5	97.23±1.99	A
	WO	5	89.22 ± 8.56	A
	MFTO	5	54.85 ± 13.54	B
GSD	HWM-E	5	97.90 ± 1.88	A
	HWM-C	5	94.84 ± 2.30	A
	WO	5	87.61 ± 7.96	A
	MFTO	5	49.02 ± 12.51	B

*N*, number of replicates.

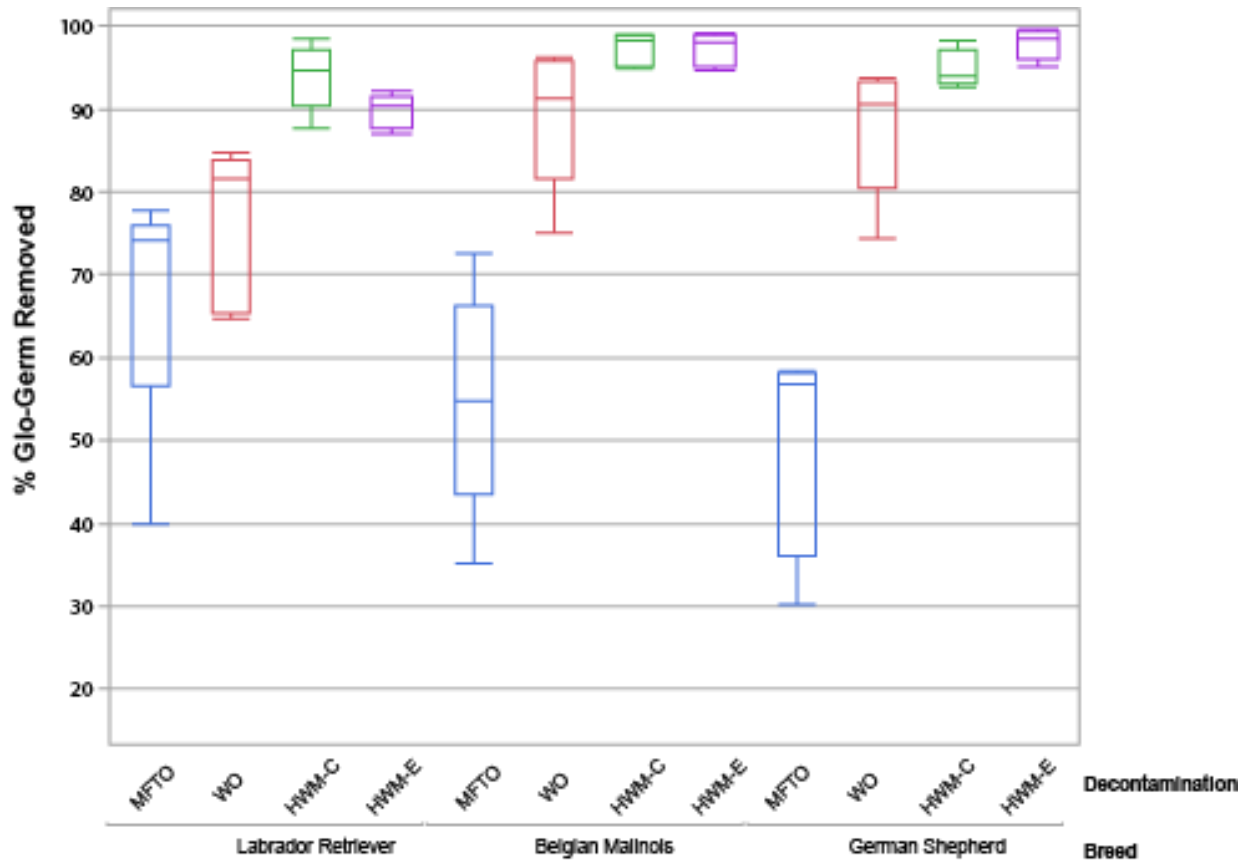


Figure 4. Decontamination results by breed.

### 3.3 Breed Results Per Decontamination Treatment

Table 3 and Figure 5 present the performance of each decontamination method with respect to breed variations. There were no significant differences in the percentage of Glo Germ powder removal for the HWM-E, HWM-C, and WO methods across the breeds evaluated. While the MFTO method results were similar for the BM and GS breeds, there was a significantly higher percent removal of Glo Germ powder from the LR fur.

Table 3. Percent Removed Results for Each Decontamination–Breed Combination

Decontamination Method	Breed	N	Amount Removed (%)	Tukey–Kramer Letters Report
HWM-E	LR	5	89.72 ± 2.05	A
	BM	5	97.24 ± 2.05	A
	GSD	5	97.90 ± 1.88	A
HWM-C	LR	5	93.94 ± 4.09	A
	GSD	5	94.84 ± 2.3	A
	BM	5	97.23 ± 1.99	A
WO	LR	5	76.02 ± 9.75	A
	GSD	5	87.61 ± 7.96	A
	BM	5	89.22 ± 8.56	A
MFTO	GSD	5	49.02 ± 12.51	A
	BM	5	54.85 ± 13.54	A
	LR	5	67.81 ± 15.67	B

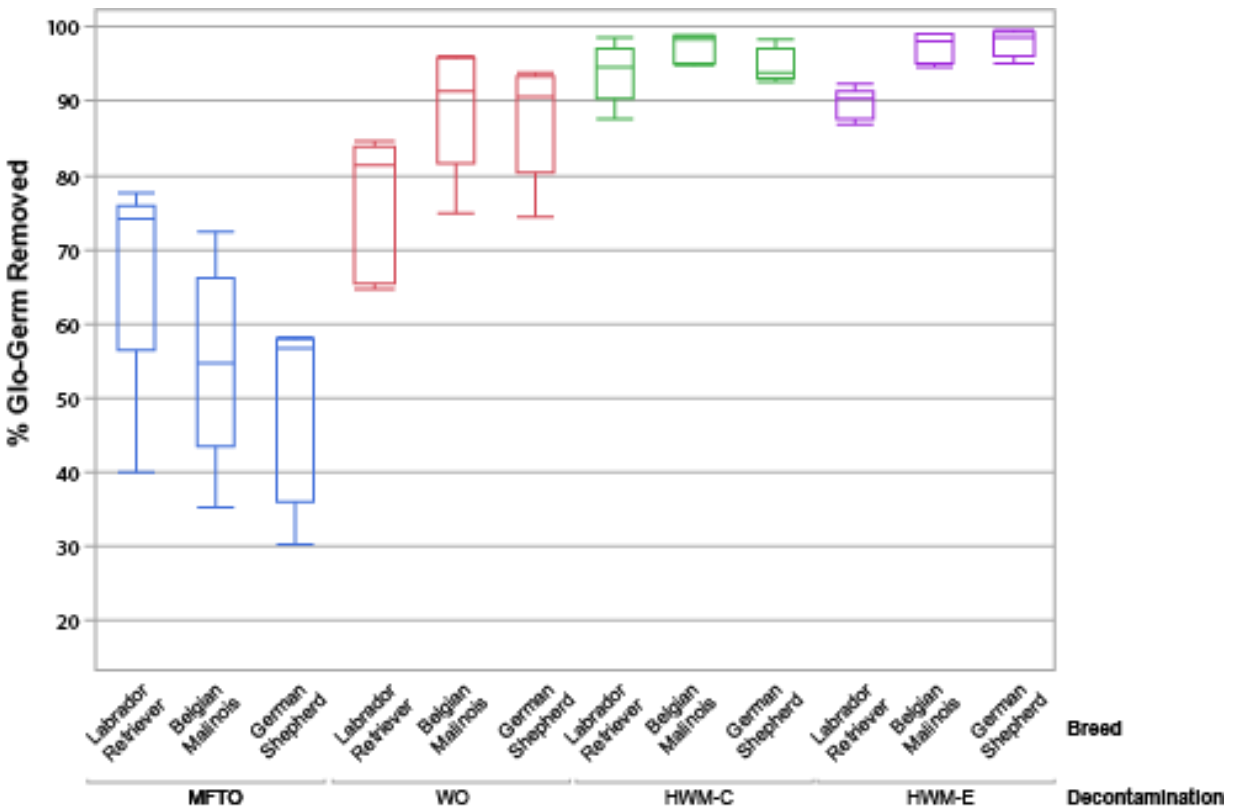


Figure 5. Breed results by decontamination method.

#### 4. DISCUSSION

This study presents results of multiple *ex vivo* decontamination treatments against particulate radiological contamination on cadaver tissue representing typical MWD breeds. Notably, the performance of each method was not breed dependent; that is, the top performing methods were the same in this study regardless of the breeds evaluated. These results suggest a decrease in decontamination burden based on breed, as handlers would not need breed-specific decontamination supplies for their particular contaminated MWDs.

Despite minimal statistical differences across breeds for a given decontamination method, the LR results were notably different from those derived from the GS or BM furs. It should be noted that samples from only one LR were available for this study and these results cannot be used to make generalized conclusions for the breed. More sampling from additional LR subjects to achieve increased statistical power are necessary for further analysis of the breed. The results, however, do provide insight that subtle differences in hair coat between working dog breeds may need to be considered when evaluating priority of decontamination and the type of decontamination methods used after radiological particulate exposure. The two shampoo methods were still the top performers on the LR; however, on average, removal of the powder was less than on the GS or BM fur samples. In addition, the MFTO performance was highest on the LR achieving 67% Glo Germ powder removal, compared to 54 and 49% on the BM and GS, respectively. This may be attributed to the short double-coated LR hair coat that excels at repelling moisture and preventing liquid contaminants from penetrating the hair coat layers as easily as longer hair coats (Whitaker and Ostrander, 2019). Further evaluation of more samples from each breed examined in this study is needed to better define differences relevant to tactical application of canine radiological particulate decontamination.

The results indicate that decontamination methods, including the shampoo method, remove the most amount of Glo Germ powder from the examined canine hair coats followed by the WO and MFTO methods, respectively. In all cases evaluated, the performance of each shampoo method was statistically similar. The two shampoos used were a non-medicated veterinary shampoo and a baby shampoo containing EDTA, a chelating agent. Chelating agents are typically used to bind radioactive materials to enhance their removal. Glo Germ powder is not a radioactive material and does not have properties that render it able to be chelated, so it was not unexpected that the shampoos performed in a similar manner. Further research is necessary to determine if the shampoo methods provide similar performance in canines when the contaminant is more susceptible to being chelated by EDTA. Also, additional work is needed to determine if combining an MFTO method before a follow-on HWM would further increase contaminant removal, especially in LR breeds, or make the HWM performance less efficient than that demonstrated in this study. This research only examined canine hair coat samples representative of MWD breeds. Additional research is needed prior to any correlation of decontamination efficiency in other military working animal species such as horses or donkeys.

As with many decontamination efficacy studies, the results presented herein represent the performance of a given treatment in a highly controlled environment with specific parameters. Continued research is necessary to determine whether the method of performance with respect to a given treatment would impact efficacy, such as varying the amount of water

used, the scrubbing motion used, or the material used to scrub the fur. Furthermore, continued research would be necessary to determine if the decontamination performance is comparable when the radiological particulate challenge is altered, for example, different particulate size or particulate composition. None of the decontamination methods examined in this study are designed to neutralize radiological contaminants, so any tools or resources utilized to remove particulates from a canine would need to be considered contaminated, including any generated wastewater. Incident Commanders, veterinary, medical, CBRN response, and canine handler personnel should be aware of these considerations in determining the most appropriate response procedure.

Whereas the results of this study are supportive of the decontamination potential for MWDs exposed to radiological particulate contamination, it must be stated that training is critically important for the effective application of these procedures. Quality training for working dog handlers improves the outcome of decontamination, and trained handlers are more effective than untrained handlers (Powell et al., 2019). The consequences of failed decontamination could be catastrophic for the MWDs, handlers, and others exposed to MWDs through cross contamination (Perry et al., 2021). Untrained handlers are more likely to not remove collars, leashes, muzzles, or other gear resulting in residual contaminants on the MWD's paws or ventral surfaces. Remaining contaminant from incomplete decontamination procedures can cause further exposure to the canine and lead to cross contamination to the handler, equipment, or environment where others are working (Powell et al., 2019; Perry et al., 2021). The possible repeat contamination could necessitate multiple trips through the decontamination line and associated increased utilization of scarce resources.

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## ACRONYMS AND ABBREVIATIONS

ATP	Army Techniques and Procedures
BM	Belgian malinois
CBRN	chemical, biological, radiological, and nuclear
DI	deionized
EDTA	ethylenediaminetetraacetic acid
FWB	formerly Glo Germ-filled weigh boat
GSD	German shepherd dog
GWB	Glo Germ-filled weigh boat
HSD	honestly significant difference
HWM-C	high water method with a canine shampoo
HWM-E	high water method with a shampoo containing ethylenediaminetetraacetic acid
LR	Labrador retriever
MFTO	microfiber towel only
MWD	military working dog
WO	water only



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