EDGEWOOD CHEMICAL BIOLOGICAL CENTER
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ECBC-TR-1430

PYROTECHNICALLY GENERATED AND DISSEMINATED AEROSOL FOR BIOAGENT DEFEAT

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December 2016

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
XX-12-2016	Final	Oct 2014 – Jan 2016

4. TITLE AND SUBTITLE
Pyrotechnically Generated and Disseminated Aerosol for Bioagent Defeat

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Polk, Amee L.; Kauzlarich, Michael F.; Bonavito, Nino L.; Smith, Lisa S.; and Rastogi, Vipin K.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, ECBC, ATTN: RDCB-DRT-S, APG, MD 21010-5424

8. PERFORMING ORGANIZATION REPORT
ECBC-TR-1430

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Defense Threat Reduction Agency
8725 John J. Kingman Road, MSC 6201
Fort Belvoir, VA 22060-6201

10. SPONSOR/MONITOR'S ACRONYM(S)
DTRA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT:

Iodine-containing compounds are currently the most commonly used antimicrobial agents. Low concentrations of iodine and heavy metals have been shown to cause the precipitation of cell proteins, thus resulting in cell death. Oligo-dynamic metals (such as copper) cause cell membrane destruction as well as coagulation of cell materials. To date, there exist no military pyrotechnic systems designed to generate iodine vapor from metal fuels and iodine oxides. These metal/iodine oxide compounds readily and thoroughly react when provided with an ignition source to produce iodine vapor and metal oxides. The use of certain metals, such as brass (a copper/zinc blend), aluminum, copper, iron, and ferrotitanium (an iron/titanium blend) provides an additional layer of antimicrobial effect because each of these metals has biocidal properties and their metal oxides are biocidal. In this report, we discuss the theoretical modeling and results of experimentation with the metal fuel/iodine pentoxide reactions. These results show a strong biocidal action against the tested biological targets, suggesting use for these reactions in bioagent defeat applications.

15. SUBJECT TERMS
Iodine Bioagent Pyrotechnic Antimicrobial Iodine pentoxide

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Renu B. Rastogi
U	U	U	UU	22	**19b. TELEPHONE NUMBER** *(include area code)* (410) 436-7545

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PREFACE

The work described in this report was authorized under the U.S. Army Research, Development and Engineering Command Free Trade Agreements program. The work was started in October 2014 and completed in January 2016.

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This report has been approved for public release.

Acknowledgments

The authors acknowledge the following individuals for their hard work and assistance with the execution of this technical program:

- Giancarlo Diviacchi (Research and Technology Directorate, U.S. Army Edgewood Chemical Biological Center [ECBC]; Aberdeen Proving Ground, MD);
- Mark Hull (Engineering Directorate, ECBC); and
- Christine Franklin (Leidos, Inc.; Gunpowder, MD) for administrative support in preparing this document.

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CONTENTS

1. INTRODUCTION 1

2. CONCEPT 1

3. EXPERIMENTAL PROCEDURES 2

3.1 Modeling 2
3.2 Dräger Tube Sampling 2
3.3 Live Microbe and Spore Testing 3
3.3.1 Pyrotechnic Preparation 3
3.3.2 Coupon Preparation 3
3.3.3 Sporicidal and Biocidal Aerosol Exposure 4
3.3.4 Spore Extraction from Coupons 6

4. RESULTS 6

5. CONCLUSIONS 7

6. RECOMMENDATIONS 8

LITERATURE CITED 9

ACRONYMS AND ABBREVIATIONS 11

FIGURES

1. Dräger tubes after sampling 3
2. Test box setup before test pellet initiation 4
3. Test box during sample burning 5
4. Test coupons after aerosol exposure 5
5. Copper/iodine pentoxide data summary 6
6. Brass/iodine pentoxide data summary 7

PYROTECHNICALLY GENERATED AND DISSEMINATED AEROSOL FOR BIOAGENT DEFEAT

1. INTRODUCTION

Iodine has been used as a disinfectant for more than 150 years. Iodine-containing compounds are also the most commonly used antimicrobial agents currently in use (Gottardi, 1985, 1991). Due to its small molecular size, iodine can penetrate microorganisms' cell walls quickly; it then proceeds to cause cell death through the dislocation of protein synthesis and the disruption of respiratory chain enzymes, lipid membrane function, and nucleic acid function (Selvaggi et al., 2003). These direct, toxic effects to the cell make microbial resistance to iodine extremely rare (Sibbald et al., 2011).

To date, no pyrotechnic systems exist that are designed to generate iodine vapor from metal fuels and iodine oxides. Other halogenated fumigants, such as chlorine dioxide and methyl bromide, have been shown to have biocidal and sporicidal activities. However, because these fumigants are not pyrotechnically derived, they require more complicated production processes.

2. CONCEPT

This work focused on the development of an innovative method for the pyrotechnic generation of aerosolized iodine via a thermite reaction of iodine pentoxide and a metal fuel. Depending on the metal fuel selected, this reaction generates not only iodine, the most common antimicrobial substance currently in use, but also an antimicrobial metal oxide. This thermite-like reaction is a simple two-reactant/two-product system wherein a metal powder reacts immediately with the oxygen in the iodine pentoxide to produce a metal oxide and iodine.

The iodine pentoxide/metal fuel mixture can be pressed into grenades, smoke pots, or other standard ammunition configurations for use in a manner similar to a fumigant. The device would be placed in a contaminated room, initiated, and then allowed to remain undisturbed for an optimum contact time. These devices would provide a smaller logistical load as compared with liquid decontamination systems such as the commonly used bleach application systems. The iodine pentoxide/metal fuel munitions offer several benefits: they require less material to perform the same amount of decontamination; are lower in toxicity; require no additional equipment to operate; and are small, man-portable devices.

According to the material safety data sheet (MSDS) for iodine, the oral dose that is lethal to 50% of test subjects (LD50) is 14,000 mg/kg (for rats). In comparison, the oral LD50 for standard household bleach (i.e., 5.25% sodium hypochlorite), which is typically used in decontamination, is 8,200 mg/kg (for rats). Therefore, iodine is almost twofold less toxic than the standard decontamination method.

Damage and disruption to the existing building and room infrastructure would be minimal after application of an iodine aerosol using a standard ammunition device. Once an appropriate contact time had elapsed, the previously contaminated space could be reentered. One possible scenario could involve Special Forces or counterintelligence assets conducting a sensitive site exploitation. If there were suspicion of biocontamination, one or several of these devices could be ignited inside a building space. After an appropriate amount of time, personnel could enter the building and remove equipment that would be unaffected by the aerosol, such as computers, tools, and machines. If necessary, equipment could be removed from the room, and a single application of the aerosol-generating device could be used to further decontaminate individual items. This system also has relevant application in the civilian arena; for example, for the decontamination of a postal facility after mail containing anthrax has been discovered, or for a single-stage decontamination of a hospital so that first responders could enter.

3. EXPERIMENTAL PROCEDURES

3.1 Modeling

Before testing began, theoretical modeling was conducted using ICT Thermodynamic Code software (Fraunhofer Institut für Chemische Technologie; Pfinztal, Germany). This modeling showed that metal oxide and iodine are the favored products for all metal and iodine pentoxide combinations computed, as given by

$$I_2O_5 + M \rightarrow I_2 + M_xO_y \quad (1)$$

where M is the metal fuel, and M_xO_y is the metal oxide of the tested metal fuel. Metals were checked for reaction with the iodine pentoxide to verify production of the metal oxide before mixing was performed. Six metal powders were chosen for live testing: aluminum, iron, brass (70:30 copper/zinc mixture), copper, ferrotitanium (50:50 blend), and zinc. An approximately 40% yield of iodine was predicted.

3.2 Dräger Tube Sampling

An 8 ft^3 Plexiglas test chamber with a sampling port was constructed for use in all live testing. A qualitative test was performed using Dräger tubes (Drägerwerk AG; Lübeck, Germany) to detect the presence of iodine in six test metal/iodine pentoxide mixtures. Brass/iodine pentoxide and copper/iodine pentoxide were down-selected for testing against the spores of *Bacillus thuringiensis* var. *kurstaki* (BtK) and vegetative cells of *Staphylococcus aureus* because these mixtures produced the most saturated Dräger tubes (as evidenced by visual inspection). Figure 1 shows the Dräger tubes after testing.

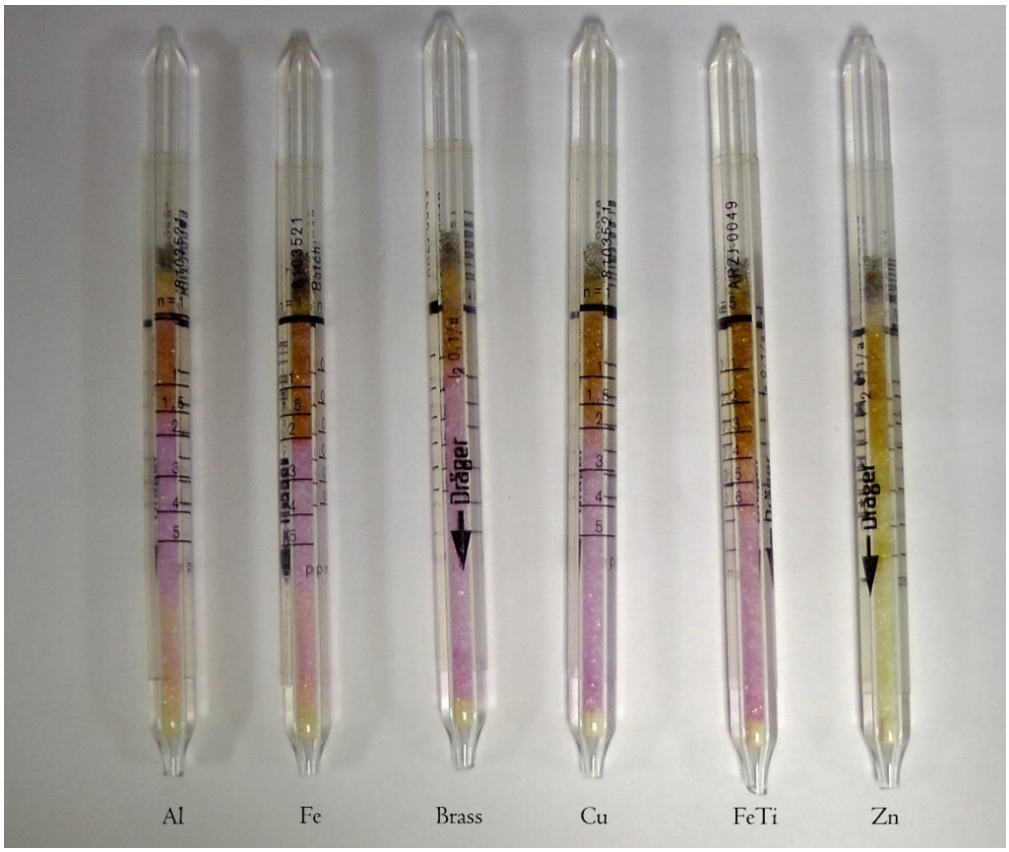

Figure 1. Dräger tubes after sampling.

3.3 Live Microbe and Spore Testing

Small-scale testing was performed against live BtK spores and *S. aureus* cells. The testing was performed in the 8 ft^3 Plexiglas test box. A thermite consisting of aluminum and red iron oxide was used as a control. Temperatures and exposure times were recorded.

3.3.1 Pyrotechnic Preparation

Samples were prepared by mixing equal parts of metal powder and iodine pentoxide and then adding binders. All mixes were allowed to dry thoroughly in a Friction-Aire oven (Thermal Product Solutions; New Columbia, PA) at 71 °C for 1 h. Mixes were pressed into cylindrical test pellets using a Denison press (Abex Corporation; Columbus, OH) with a 1/2 in. mold, set at a 1500 lb dead load with a 3 s dwell time.

3.3.2 Coupon Preparation

Two coupon materials were chosen for sampling based on availability and probability of encounter in the real world. The polycarbonate and steel coupons (3 × 1 in.) were washed with 70% ethanol and then rinsed with distilled water. The coupons were dried and autoclaved at 121 °C for 30 min.

BtK spores were prepared in accordance with established internal operating procedures. *S. aureus* cells were procured from American Type Culture Collection (ATCC; Manassas, VA) and grown overnight before use. A 100 µL aliquot of BtK working spore stock (1E8/mL titer) was added as small droplets across the coupon surface. The aliquot was dried overnight in a biosafety level 2 (BSL-2) cabinet with an open surface. A 100 µL aliquot of *S. aureus* broth (an overnight culture that was approximately 1E8/mL titer) was inoculated across the coupon surface as small droplets. The coupons were left uncovered in a BSL-2 cabinet to dry, which was complete in <90 min. The coupons were used within 1 h of drying.

3.3.3 Sporicidal and Biocidal Aerosol Exposure

Inoculated coupons were placed in glass Petri dishes and positioned on a steel plate at the base of the Plexiglas box. The test pellet was placed at the center of the Plexiglas test box on the steel plate, and biological samples were placed on either side of the pellet. A thermometer was inserted through a port on the side of the box to record temperature. Approximately 0.5 g of a nitrate-based starter mixture was placed on top of the test pellet, with a sufficient length of quickmatch (cotton string soaked in black powder and covered by a thin paper wrap) taped over the top to allow for remote lighting of the sample. Test mixtures were initiated by lighting the quickmatch. The generated smoke was allowed to contact the biological samples undisturbed for the allotted test time. Figures 2 and 3 show the test box before the test pellet initiation and during the sample burning, respectively. Figure 4 shows the coupons after the aerosol exposure.

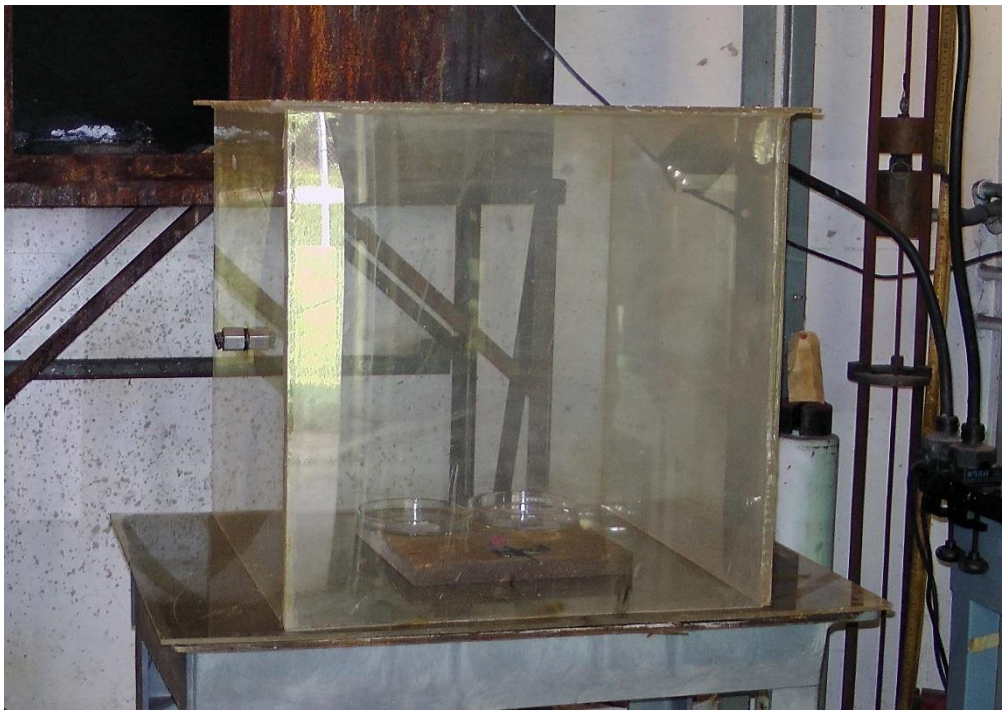

Figure 2. Test box setup before test pellet initiation.

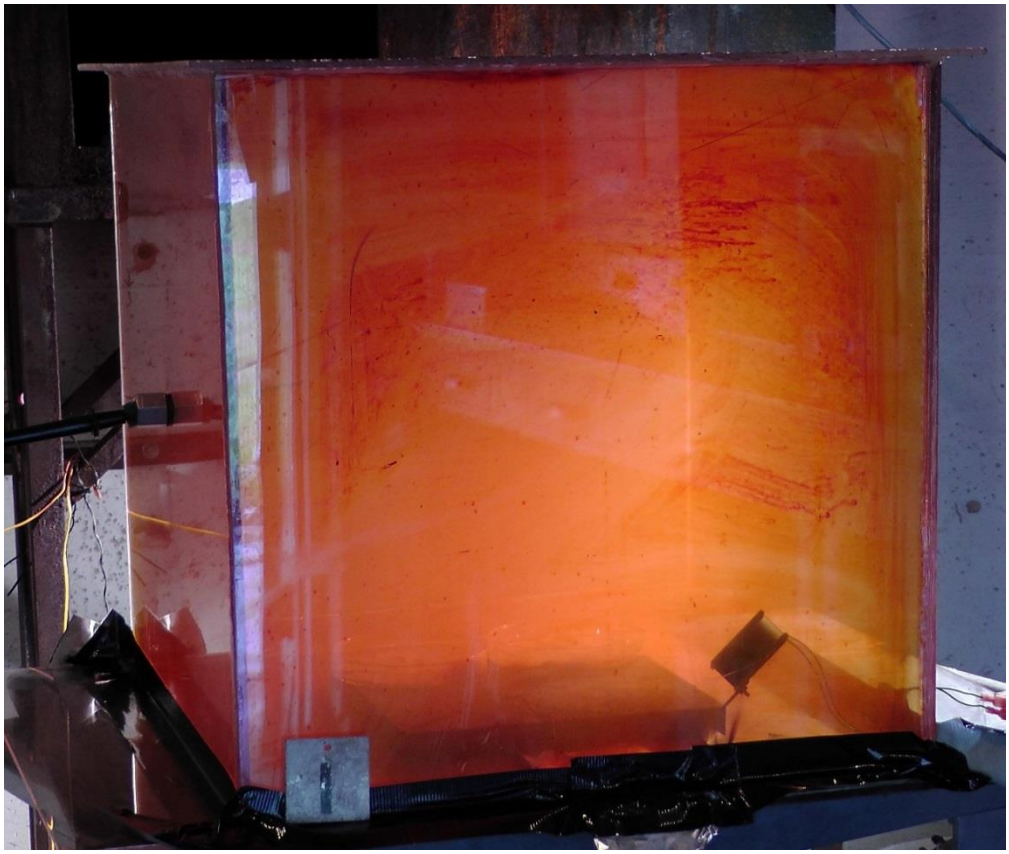

Figure 3. Test box during sample burning.

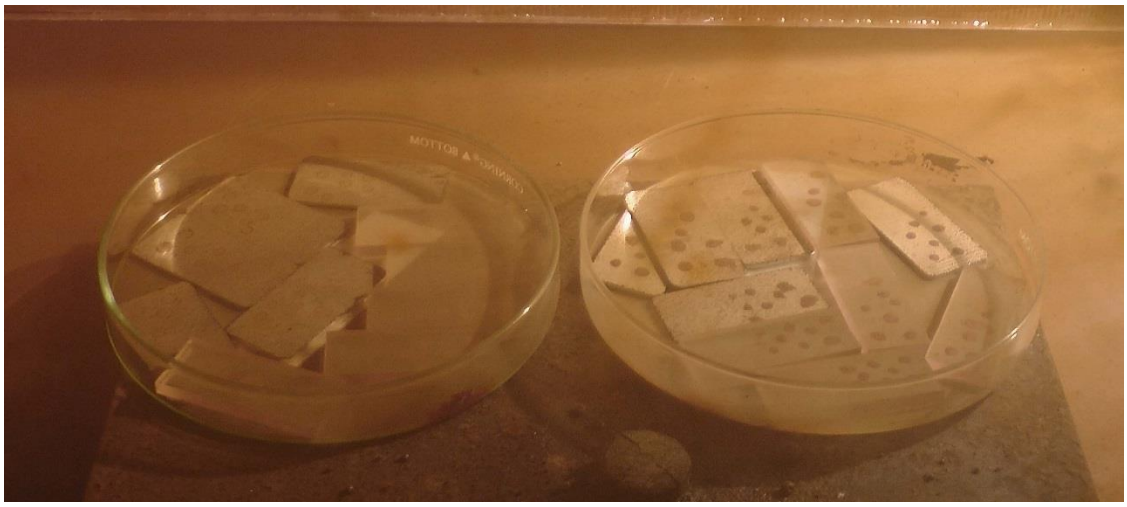

Figure 4. Test coupons after aerosol exposure.

3.3.4 Spore Extraction from Coupons

The coupons were dropped into 20 mL of Tween 80 detergent in a 50 mL sterile tube and were sonicated for 10 min and vortexed for 2 min. The samples were serially diluted (10-fold) and then plated. Aliquots of diluted sample (100 µL each) were spread on duplicate tryptic soy agar (TSA) plates, and the plates were placed in an incubator at 37 °C overnight. The colony-forming units (cfu) on each plate were counted the next day. The average numbers of colony-forming units were multiplied with a volume factor (10) and a dilution factor (1/dilution plated) to estimate the spore number for each coupon. Spore numbers were also calculated for control samples (which were treated in the same fashion, except there was no reactive component in the aerosol) and test samples, and the log density for each sample type was calculated.

4. RESULTS

Tests involved placing a 20 g sample of the test mixture in an 8 ft^3 test box along with samples of BtK spores and *S. aureus* vegetative cells inoculated onto coupons of steel and polycarbonate. Temperature was recorded for each test; however, no temperatures >35.6 °C were observed. An aluminum/red iron oxide thermite sample was used as a control for each test. No log reductions in spore or cell numbers were observed for any of the thermite control tests. Figures 5 and 6 summarize the log reductions in spore viability data from the copper/iodine pentoxide and the brass/iodine pentoxide tests, respectively.

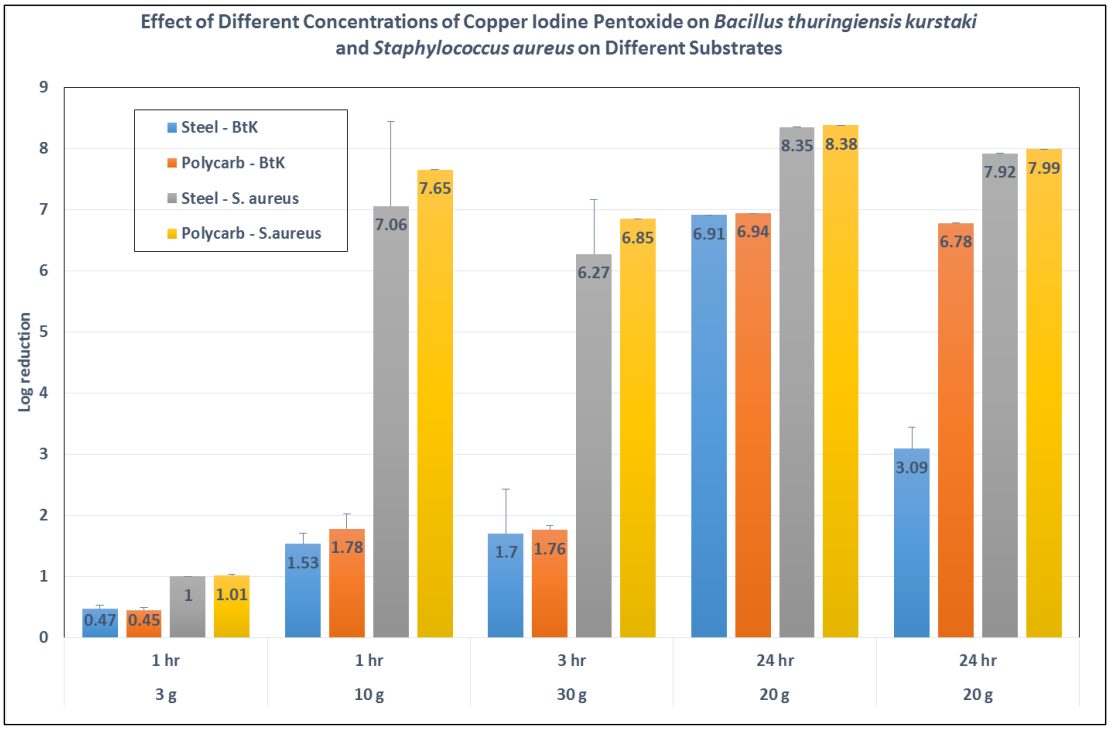

Figure 5. Copper/iodine pentoxide data summary.

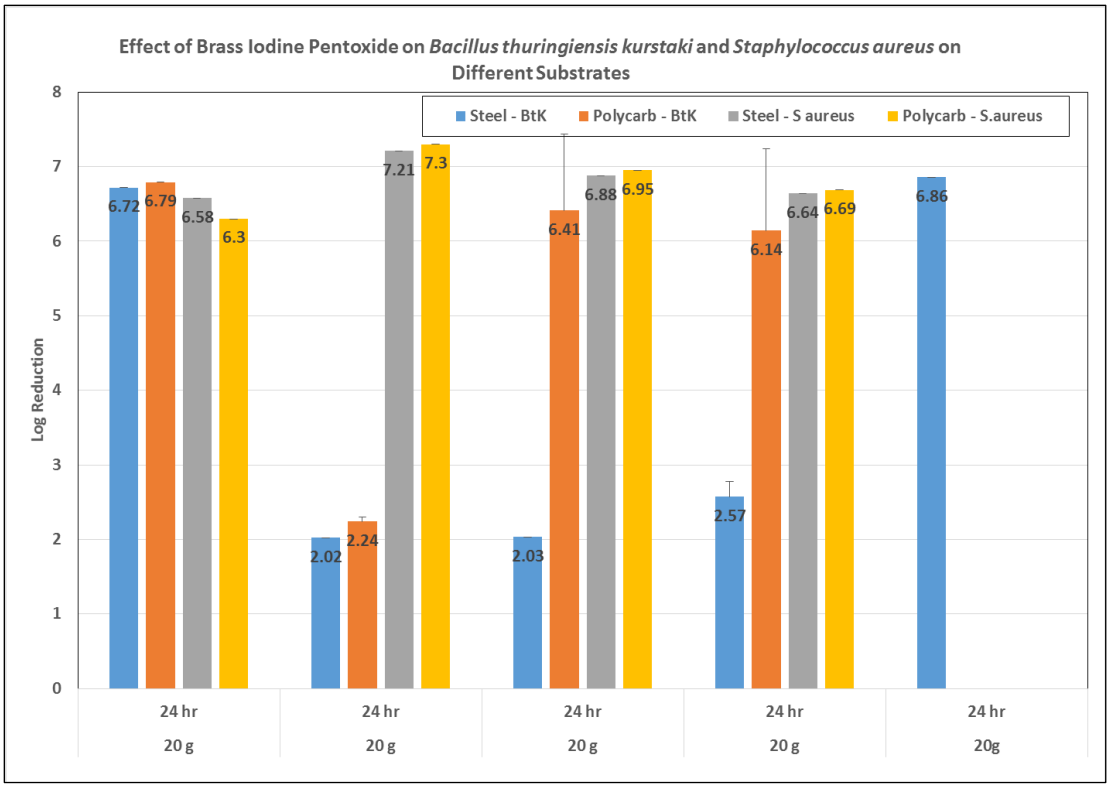

Figure 6. Brass/iodine pentoxide data summary.

Particle size parameters of the initial cloud were determined using an ELPI electrical low-pressure impactor (Dekati, Ltd.; Kangasala, Finland) and an Aerodynamic Particle Sizer system (TSI, Inc.; Shoreview, MN). Within a minute of dissemination, the mass median aerodynamic diameter was determined to be 3.12 µm, with a geometric standard deviation of 1.84. The number median aerodynamic diameter was determined to be 0.66 µm, with a geometric standard deviation of 2.37.

5. CONCLUSIONS

The copper/iodine pentoxide and brass/iodine pentoxide demonstrated complete (7 log reduction) killing of Btk spores and *S. aureus* cells on both surfaces from the aerosol generated by a 20 g sample with a 24 h contact time. Repeatable results were obtained; however, inconsistencies were apparent in the kill rates on certain surfaces. The test sample temperatures were not high enough to affect the organisms; therefore, temperature can be ruled out as supportive of killing the spores and cells. Based on this information, it can be concluded that the aerosolized iodine and metal oxide generated from the reaction were the active constituents in the destruction of the biological agent simulants.

6. RECOMMENDATIONS

To create the most efficient aerosol, optimization of the pyrotechnic mixture is necessary, which should include identification of the optimum particle size for effectiveness. Integration of the pyrotechnics into standard ammunition, such as grenades or smoke pots, is an integral aspect of this system that remains to be developed. Further testing of the mix for safety and pyrotechnic feasibility must be accomplished, and testing with additional complex surfaces is required to more fully investigate the applicability of this technology. Conducting samples testing with only the metal or metal oxide will allow for a better understanding of this reaction process, and testing against other biological materials also should be performed. A full-scale challenge incorporating actual ammunition devices in a large chamber with various contaminated equipment and surfaces will be required to demonstrate the real-world efficacy of this novel approach.

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ACRONYMS AND ABBREVIATIONS

ATCC	American Type Culture Collection
BSL-2	biosafety level 2
BtK	*Bacillus thuringiensis* var. *kurstaki*
cfu	colony-forming units
ELPI	electrical low pressure impactor
LD50	dose that is lethal to 50% of test subjects
MSDS	material safety data sheet
TSA	tryptic soy agar

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