EPA/600/R-21/105 | August 2021
www.epa.gov/emergency-response-research

United States
Environmental Protection
Agency

Remediation Options for Fentanyl Contaminated Indoor Environments

Office of Research and Development
Homeland Security Research Program

Remediation Options for Fentanyl-contaminated Indoor Environments

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Remediation Options for Fentanyl-contaminated Indoor Environments

U.S. Environmental Protection Agency
Office of Research and Development
Center for Environmental Solutions and Emergency Response
Research Triangle Park, NC 27711

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FOREWORD

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The Center for Environmental Solutions and Emergency Response (CESER) within the Office of Research and Development (ORD) conducts applied, stakeholder-driven research and provides responsive technical support to help solve the Nation’s environmental challenges. The Center’s research focuses on innovative approaches to address environmental challenges associated with the built environment. We develop technologies and decision-support tools to help safeguard public water systems and groundwater, guide sustainable materials management, remediate sites from traditional contamination sources and emerging environmental stressors, and address potential threats from terrorism and natural disasters. CESER collaborates with both public and private sector partners to foster technologies that improve the effectiveness and reduce the cost of compliance, while anticipating emerging problems. We provide technical support to EPA regions and programs, states, tribal nations, and federal partners, and serve as the interagency liaison for EPA in homeland security research and technology. The Center is a leader in providing scientific solutions to protect human health and the environment.

This report assesses decontamination options for fentanyl contaminated building materials as well as responder gear and personal protective equipment related materials. This report builds on a previous fentanyl decontamination efficacy study that looked at other decontaminants. The focus in this report is on decontamination products that were previously not tested and variations in decontamination applications and their use to clean responder gear or personal protective equipment (PPE).

Gregory Sales, Director
Center for Environmental Solutions and Emergency Response

ACKNOWLEDGMENTS

This research is part of the U.S. Environmental Protection Agency's (EPA's) Homeland Security Research Program's (HSRP) efforts to evaluate surface-applied liquid-based decontamination methodologies for decontamination of fentanyl on building materials and responder gear or personal protective equipment (PPE). Funding was provided through the regional applied research effort (RARE) program, administrated by the Office of Science, Policy and Engagement (OSAPE) under RARE Project 2082 entitled "Remediation of Fentanyl Contaminated Indoor Environments".

This effort was directed by the principal investigator (PI) from the Office of Research and Development's (ORD's) Homeland Security and Materials Management Division (HSMMD) within the Center for Environmental Solutions and Emergency Response (CESER). The contributions of the following individuals have been a valued asset throughout this effort.

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EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency's (EPA's) Homeland Security Research Program (HSRP) conducts research necessary for identification of methods and technologies that can be used during hazardous materials remediation and cleanup efforts. The recent increase in the number of unintentional fentanyl-related overdose fatalities in multiple states across the U.S. has resulted in scenarios wherein local and state authorities request (technical) support from EPA in the remediation of indoor fentanyl contamination at a home or other facility.

One of the main scientific gaps in the development of an adequate fentanyl contamination remediation response is related to a lack of knowledge of effective decontamination technologies and the conditions for their application for the degradation of fentanyl on a material or surface. Another decontamination-related gap is associated with a lack of information on suitable cleaning approaches for first responder gear or hazardous material responder personal protective equipment (PPE) that may have become contaminated with fentanyl during the response or remediation activities.

This project builds on an earlier fentanyl decontamination study which assessed efficacies of several decontamination technologies given a single set of application conditions for the degradation of fentanyl on the surface of selected commonly encountered indoor materials. The purpose of this project was to evaluate the efficacy of two hydrogen peroxide-based decontamination technologies that were either low cost, easy to acquire commercial off the shelf (COTS) alternatives to specialized decontaminants, or technologies that were included as part of a completed remediation of a fentanyl-contaminated property. For two other previously studied decontaminants, namely, Dahlgren Decon™ and pH 5 adjusted bleach, the current study assessed whether a reapplication of these decontaminants could improve overall efficacy, especially in the presence of a benign additive that was demonstrated to create a demand on the decontamination solution. Lastly, Dahlgren Decon™ and pH 5 adjusted bleach were also assessed on their ability to degrade fentanyl on a short dwell timescale of only a few minutes. Such a short dwell time would occur if these decontaminants were applied as part of a decontamination line procedure in which the PPE or other response gear is cleaned to reduce exposure to support personnel and eliminate spread of contamination when exiting a (fentanyl) contaminated site.

Decontaminants that were considered included: Meth Remover® and ZEP® Professional Stain Remover with Peroxide (both hydrogen peroxide-based), Dahlgren Decon™ (activated peracetic acid as active ingredient), and pH 5 adjusted bleach with surfactant (hypochlorite based) derived from Clorox™ ProResults® Garage and Driveway Cleaner (referred to as pH 5 modified surfactant bleach). Four indoor-related materials were selected for this study: painted drywall, laminate, powder-coated steel, and wood. First responder PPE materials included neoprene, Saranex®, DuraChem® 500 Level B HazMat suit, and bunker gear. Fentanyl hydrochloride (HCl; 1 mg) was applied as a solid powder to the surface of replicate materials with 10-cm^2 surface area.

Decontaminants were applied via a spray at a target application volume of 60 µL/cm^2. Following the specific decontaminant dwell period, coupons and decontaminant runoff were extracted with an organic solvent and extracts were analyzed via gas chromatography/mass spectrometry (GC/MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantify the amount of fentanyl HCl remaining in the extracts.

Results

- Measured efficacies for both hydrogen peroxide-based decontaminants were noticeably modest and ranged from 14% to 46% (ZEP® product) and 23% to 58% for Meth Remover® across the four materials. We can conclude that hydrogen peroxide is not a highly effective degradant for fentanyl.
- The reapplication of Dahlgren Decon™ and pH 5 modified surfactant bleach did not significantly improve efficacy versus the single application investigated as part of the previous fentanyl decontamination study. Here, Dahlgren Decon™ yielded a greater than 99.8% decontamination efficacy across all materials while the pH 5 modified surfactant bleach yielded 80% - 96% efficacy, depending on the material. A direct comparison between the previous and current study was complicated by the differences in materials and recoveries from positive controls.
- Both Dahlgren Decon™ and pH 5 modified surfactant bleach achieved lower efficacies for decontamination of fentanyl mixed with ascorbic acid as a challenging benign additive, namely 97% (99.8% without additive) on wood for Dahlgren Decon™ and 80% (84% without additive) on wood for pH 5 modified surfactant bleach.
- A diluted (1:4) Dahlgren Decon™ and pH 5 modified surfactant bleach solutions were able to degrade fentanyl over a short (5-minute), duration with efficacies ranging from 89% - 98% across materials for diluted Dahlgren Decon™ and 55% - 66% for the pH 5 modified surfactant bleach.

Figure ES-1 summarizes the average percent decontamination efficacies measured for each test condition.

In many decontamination tests, the observed large variation in amounts recovered can be linked to the presence of agglomerated fentanyl on the surface during the application of the decontaminant spray, resulting in a slower mass transfer rate between decontaminant and fentanyl and hence, higher amounts recovered even in the presence of an otherwise effective decontaminant.

The results of this work inform EPA responders, governments, and health departments in their guidance development for decontamination technology recommendations for building materials contaminated with fentanyl.

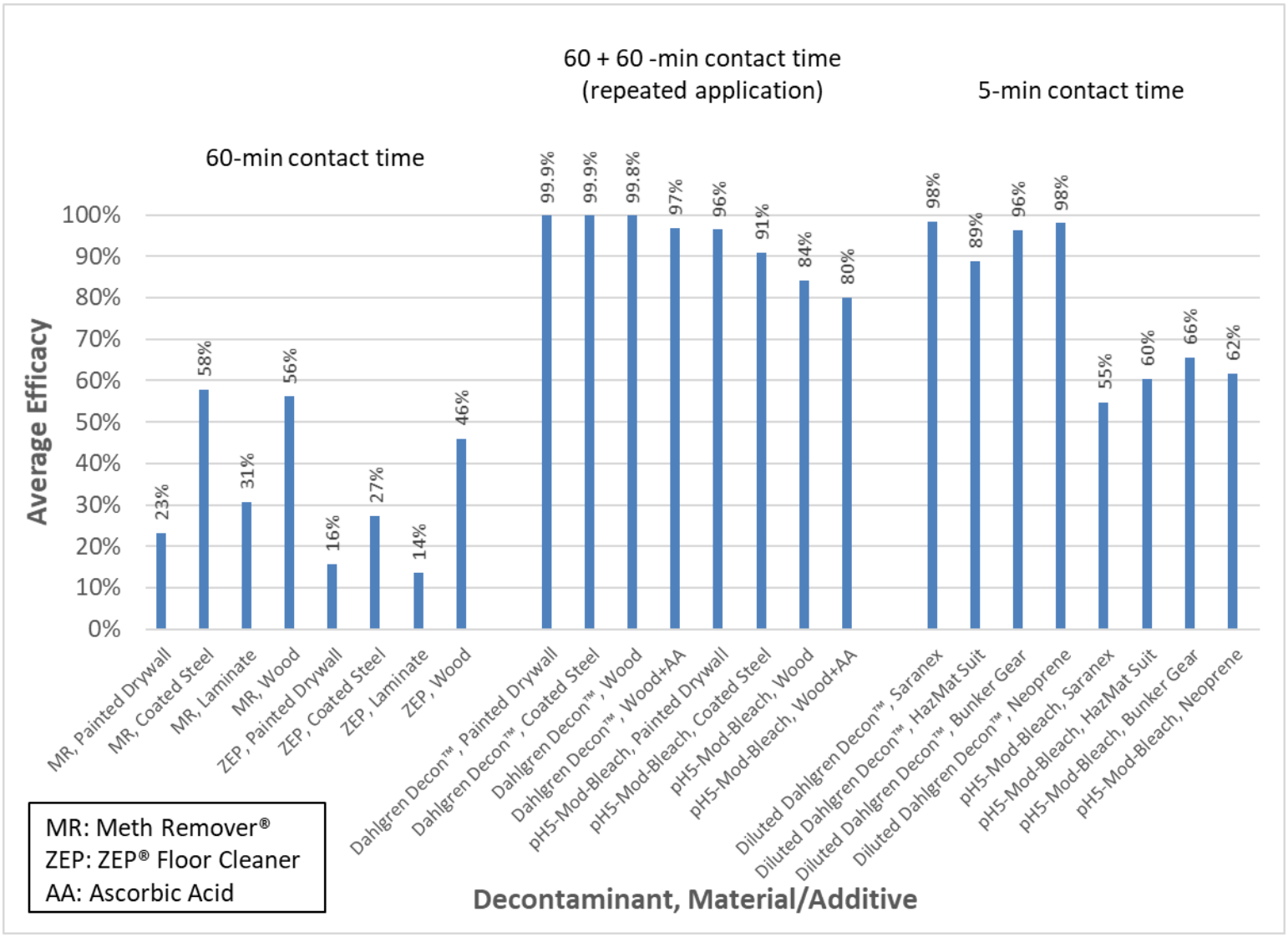

Figure ES-1. Average decontamination efficacies.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	analysis of variance
°C	degrees Celsius
CAS	Chemical Abstracts Service
CBRN	Chemical, Biological, Radiological, Nuclear
CCV	continuing calibration verification
CESER	Center Environmental Solutions and Emergency Response (U.S. EPA)
cm	centimeter(s)
cm^2	square centimeter(s)
CoC	chain of custody
COTS	commercial off the shelf
CoV	coefficient of variation
DEA	U.S. Drug Enforcement Agency
DFTPP	decafluorotriphenylphosphine
EPA	U.S. Environmental Protection Agency
FEP	fluorinated ethylene propylene
g	gram(s)
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
h	hour(s)
HCl	hydrochloride
HMRC	Hazardous Materials Research Center
HPLC	high performance liquid chromatography
HSMMD	Homeland Security and Materials Management Division (U.S. EPA)
HSRP	Homeland Security Research Program (U.S. EPA)
in^2	square inch(es)
IPA	isopropyl alcohol
IS	internal standard
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LLOQ	lower limit of quantitation
LRB	laboratory record book
M	Molar
µg	microgram(s)
µL	microliter(s)
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
mm	millimeter(s)
MRM	multiple reaction monitoring
MSD	mass selective detector
NFPA	National Fire Protection Agency
ng	nanogram(s)
NIST	National Institute of Standards and Technology
ORD	Office of Research and Development (U.S. EPA)
OSAPE	Office of Science, Policy and Engagement (U.S. EPA)
PE	performance evaluation
PFPP	pentafluorophenylpropyl
PI	principal investigator
PP	polypropylene

PPE	personal protective equipment
psi	pound(s) per square inch
PTFE	polytetrafluoroethylene
PVC	polyvinyl chloride
QA	quality assurance
QAPP	quality assurance project plan
QC	quality control
r^2	coefficient of determination
RARE	Regional Applied Research Effort
RH	relative humidity
RSD	relative standard deviation
RT	retention time
RTU	ready to use
SC	spike control
sec	seconds
SIM	selected ion monitoring
STS	sodium thiosulfate ($Na_2S_2O_3$)
TPCS	test parameter control sheet
TSA	technical systems audit
V	Volt

INTRODUCTION

The U.S. Environmental Protection Agency (U.S. EPA) is responsible for preparing for, responding to, and recovering from threats to public health, welfare, or the environment caused by actual or potential hazardous materials incidents. Hazardous materials include chemical, biological, and radiological substances, whether accidentally or intentionally released. The imminent threat of a chemical agent release into the environment is driving EPA's Homeland Security Research Program (HSRP) to systematically evaluate potential decontamination technologies for chemical agents.

Fentanyl is a synthetic, short-acting opioid analgesic that is 50-100 times more potent than morphine. Fentanyl has been approved for managing acute or chronic pain associated with advanced cancer. Although pharmaceutical fentanyl can be diverted for misuse, most cases of fentanyl-related morbidity and mortality have been linked to illicitly imported or manufactured fentanyl that is sold via illicit drug markets. Illicit-use fentanyl is often mixed with heroin, cocaine, or more benign additives. The recent increase in unintentional fentanyl-related overdose fatalities in multiple states across the U.S. has resulted in scenarios where local and state authorities request (technical) support from EPA in the remediation of indoor fentanyl contamination at a residence or other type of facility. One of the main scientific gaps in development of an adequate remediation response is related to a lack of knowledge of effective decontamination technologies and their application conditions for degradation of fentanyl on a material or surface. Current remediation efforts tend to rely on physical removal approaches including careful dry vacuuming followed by "soap and water" cleaning.

A first systematic fentanyl decontamination study has recently been completed [1]. That study assessed efficacy of multiple decontamination solutions including water (reference solution), OxiClean™, bleach, pH adjusted bleach (both at pH 7 and 5), EasyDecon DF200, and Dahlgren Decon™. All tests were conducted with fentanyl applied to stainless steel, laminate, acrylic and painted drywall as a dry powder. Decontaminants were applied via spray for a fixed 1-hour (h) dwell period.

The fentanyl decontamination tests described in this report build on the knowledge gained from this previous study through the addition of decontamination solutions, changes in application procedures as well as an investigation into the ability of two of the better performing decontamination solutions to degrade fentanyl on personal protective equipment (PPE)-related materials over a short (5-minute [min]) dwell time.

1.1 Purpose

The purpose of this project was to evaluate the efficacy of various decontamination technologies to degrade fentanyl on the surface of commonly encountered building- or PPE-related materials.

1.2 Project Objectives

Specific objectives of this study included:

- Assessment of hydrogen peroxide-based commercially available decontamination technologies that are anticipated to be efficacious in degrading fentanyl contamination on hard nonporous material surfaces.
- Determine change in overall efficacy after reapplication of technologies that demonstrated the highest degree of efficacy during the previous decontamination tests and materials commonly found in indoor settings [1].
- Evaluating decontamination efficacy through additional testing with a significantly shorter dwell time of 5 min for responder gear or PPE materials.

1.3 Test Facility Description

All tests were performed at Battelle's Hazardous Materials Research Center (HMRC) located in West Jefferson, Ohio. The HMRC is registered with both the U.S. Drug Enforcement Agency (DEA) and the Ohio Board of Pharmacy to procure, store, synthesize, and use controlled substances up to and including DEA Schedule I material. Wherever applicable and required, the reporting requirements of these registrations were followed.

1.4 Staff and Resources

Surface decontamination efficacy testing and all associated method development testing and sample analyses were completed using staff and resources from Battelle's HMRC in consultation with the EPA Center for Environmental Solutions and Emergency Response (CESER) Homeland Security and Material Management Division (HSMMD).

EXPERIMENTAL METHODS

2.1 Experimental Design

Decontamination efficacy was evaluated through execution of surface decontamination tests. Research was limited to decontamination of fentanyl hydrochloride (HCl) and did not include analogs (e.g., carfentanil). Four (4) commercially available decontaminants were tested (refer to Section 2.1.2) to evaluate the efficacies of the technologies in the degradation of solid fentanyl HCl (CAS 1443-54-5, from here on referred to as fentanyl unless otherwise specified) from the surface of four (4) commonly encountered, indoor-related building materials as well as from the surface of four (4) responder gear or PPE materials. Prior to the surface decontamination efficacy tests, the test methods were experimentally demonstrated, and results were evaluated against predefined criteria to ensure valid data would be generated.

Individual test articles consisted of small coupons of the indoor building or gear-/PPE-related materials. Coupons measured 2.5 centimeters (cm) x 4 cm (10 square centimeters [cm^2] contamination/decontamination surface area). Coupon thickness was dependent on the specific material type. Refer to Section 2.2.1 for more information on the test articles and indoor and PPE-related materials used during the evaluation.

During all decontamination testing, decontaminants were delivered by a sprayer system that produced a low-pressure spray similar to common commercially available, backpack-type sprayers and were applied via spray directly onto the surface of the test articles.

Following decontamination (dwell time varied based on purpose of the test), test articles were extracted with a solvent and the extract as well as decontamination rinsate were analyzed by gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/tandem mass spectrometry (LC-MS/MS) to quantify the total amount of residual fentanyl (freebase and salt). Analysis via GC/MS was anticipated for samples with fentanyl concentrations in the range of 350 micrograms (µg)/milliliter (mL) down to 0.05 µg/mL. LC-MS/MS was used for analysis of samples with fentanyl concentrations that fall within the range of 5 nanograms (ng)/mL down to 0.01 ng/mL.

2.1.1 Test Methods

The test methods used were experimentally verified/demonstrated, and results were evaluated against predefined criteria to ensure valid data would be generated.

2.1.1.1 Fentanyl Delivery (Spiking) Characterization

Solid fentanyl was applied to test articles using a 50-µL Drummond Series 500 Digital microdispenser (3-000-550, Drummond, Broomall, PA) (Figure 1).

Figure 1. Drummond pipettor for delivery of solids.

During use, the capillary was pressed into the solid fentanyl at least three times to ensure adequate packing of material into the capillary underneath the plunger. The solid agent was then dispensed onto the test article surface. Refer to Section 2.2.2.2 for additional information regarding application of fentanyl to coupons. The mass application target was 1 milligram (mg), which required a Drummond setting of 1.9 microliters (µL) based on previous use to deliver fentanyl.

The adequacy of the Drummond to deliver accurate and reproducible amounts of solid fentanyl onto the surfaces of coupons was also continuously evaluated during execution of the method demonstration tests (Sections 2.1.1.4) and decontamination efficacy tests (Section 2.1.2). As described for the approaches for these test phases, fentanyl spike controls were generated during tests by delivering the same mass of fentanyl as the mass applied to test coupons into extraction jars, dissolving the fentanyl in extraction solvent, and analyzing the spike control extracts alongside the test and control samples. A comparison between the spike control sample results and the theoretical target fentanyl delivery mass provided information on the accuracy and reproducibility of the Drummond method to deliver solid fentanyl onto coupons. The target spike control recovery criteria were set at 80% to 120% of the theoretical mass with less than 30% relative standard deviation [RSD], see Table 34 in Section 4.1. The spike control results are provided together with the associated test sample results in Sections 3.1.3 and 3.2.

During the previous study, it was postulated that the Drummond capillary was repeatedly pressed into the solid fentanyl in the working vial leaving void spaces in the powder after the Drummond capillary was withdrawn. These void spaces led to inconsistent Drummond capillary loading. To eliminate the void spaces, regular stirring of the fentanyl powder in the working vial using the Drummond capillary was implemented. Specifically, a stainless steel microspatula was used to stir and mix the powder prior to each fentanyl spiking operation and then again during the operation (after approximately half of the samples of the test had been spiked). Decreased variability (that is, variability within specification, i.e., < 30% RSD, refer to Section 4.1) across spike control replicates included in subsequent tests was then achieved following implementation of this regular stirring and mixing of the fentanyl within the vial to promote uniformity of the powder.

Fentanyl was applied to the 10-cm^2 coupons as a single (target) 1-mg pile placed in the center of the coupon. Following application of fentanyl onto the surface of test and control coupons, the solid was spread evenly across the test article using an antistatic spatula (14-245-99, Fisher Scientific, Pittsburgh, PA). Fentanyl was spread evenly (subjective, visually) across approximately 50% of the 10-cm^2 area of the coupons. As part of the previous study, we had

determined that the amount of fentanyl typically lost to the spatula was minimal (average of 9% or less of the amount spiked), so the spatulas were not extracted during decontamination testing. A different spatula was used for each test/control coupon subsection (i.e., spatulas were not reused for multiple samples).

2.1.1.2 Solvent Extraction of Fentanyl from Coupons

The methods developed for solvent extraction of fentanyl from similar materials during previous work [1] were not evaluated for effectiveness in recovery of fentanyl from the new materials that were introduced in this study. Fentanyl-HCl as a powder binds loosely to nonporous surfaces and can be extracted without difficulty. Confirmation of the high recoveries can be found in the comparison of fentanyl recoveries from spike controls and associated positive controls for each decontamination test. Based on solvent extraction recovery means and coefficient of variance (CoVs) for each solvent evaluated during the previous fentanyl decontamination study (highest recovery with lowest CoV), isopropyl alcohol (IPA) was selected for use during all phases of testing.

2.1.1.3 Decontaminant Delivery Characterization

Liquid decontaminants were applied to test and control sample coupons via moderately low flow spray using a nozzle typical of a pump pressurization style sprayer (12U469, Grainger, Lake Forest, IL). Section 2.2.3.5 provides information on the sprayer that was used and how the sprayer was interfaced with the test chamber and operated to deliver decontaminants to coupons via uniform spray at the target application volume.

Prior to testing, operation of the sprayer was characterized using each decontamination technology to determine the sprayer settings and use procedures (e.g., sprayer nozzle stand-off distance, sprayer pressurization, spray sweep speed, etc.) necessary to deliver a target decontaminant volume per unit area of 60 $\mu L/cm^2$. Additionally, spray settings were determined such that in addition to delivering 60 $\mu L/cm^2$ of decontaminant to the surface of coupons, spray impact pressure was minimized to reduce, to the greatest extent possible, physical removal of spiked fentanyl (powder) from the surface of coupons (so that quantification of residual fentanyl post decontamination could be attributed to chemical degradation rather than to physical removal).

2.1.1.4 Decontamination Technology Quench and Matrix Effect Evaluation

During decontamination efficacy tests, residual decontaminant on the materials or in the runoffs could be collected in the sample extracts and continue to decontaminate fentanyl. Additionally, chemical compounds extracted from the indoor materials, residual decontaminants, and/or quench agents could create complex sample matrices, which could lead to false-positive or false-negative results and/or analytical interferences. Effective decontaminant quench methods were necessary to allow measured decontamination efficacies to be associated with specific

decontaminant dwell times. Similarly, assessment of matrix effects was also necessary to ensure the matrices would not interfere with analyses.

During the previous fentanyl decontamination effort [1], two approaches were considered to quench the reaction of the decontaminants with fentanyl. Dilution in extraction solvent alone was first evaluated as a method for quenching reaction of the decontaminants with fentanyl. The hypothesis was that dilution in excess extraction solvent would slow the decontamination reaction enough to allow for measurement of efficacy after a defined period. Results of the quench method test suggested that dilution in extraction solvent was ineffective in preventing decontamination of the post-spiked fentanyl by Dahlgren Decon™ and pH 7 bleach. In parallel, an alternative quench method was identified by the addition of 5 mL of a 3-molar (M) solution of sodium thiosulfate ($Na_2S_2O_3$; STS) in water to the IPA used to extract coupons and runoff boxes. This approach was demonstrated to effectively quench the decontamination of fentanyl by Dahlgren Decon™ and pH 7 bleach. In this study, a procedure and single-test matrix was developed to demonstrate the adequacy of 3M STS as a quench agent for halting decontamination of fentanyl by the hydrogen peroxide decontaminants (Meth Remover® and ZEP® Professional Stain Remover with Peroxide; hereafter ZEP®). Meth Remover® was selected based on its use during the 2017 remediation of a fentanyl contaminated house [2]. ZEP® was selected as a low-cost hydrogen peroxide-based decontaminant with a relatively high (approximately 4% v/v) hydrogen peroxide concentration and similar to the EasyDecon DF200 decontamination product that was evaluated during the previous study [1]. Table 1 provides the experimental matrix that was intended to serve two purposes: to evaluate 3M STS as an appropriate quench method for quenching the residual fentanyl decontamination reactions of Meth Remover® and Zep® Professional Stain Remover with hydrogen peroxide, and to evaluate any effects of sample matrices due to residual decontaminant and/or the 3M STS quench agent itself on analysis of fentanyl and the response of the fentanyl-d5 internal standard (IS).

Table 1. Quench Method Scoping Test Matrix

Decontaminant	Fentanyl Target Concentration	Description	Quench	Analysis
Meth Remover®	2 µg/mL	Quench Samples	3M STS	GC/MS
ZEP®	2 µg/mL	Quench Samples	3M STS	GC/MS
None	2 µg/mL	Spike Controls	NA	GC/MS
Meth Remover®	2 ng/mL	Quench Samples	3M STS	LC-MS/MS
ZEP®	2 ng/mL	Quench Samples	3M STS	LC-MS/MS
None	2 ng/mL	Spike Controls	NA	LC-MS/MS

Each decontaminant/quench combination described in Table 1 was tested in triplicate. Spike controls were generated throughout the test (i.e., one spike control prior to delivery of fentanyl to the quench samples, one in the middle of the operation, and one after all quench samples had been spiked).

Sample extract matrices representative of decontaminant runoff test samples (regarding the amount of decontaminant anticipated to be present in the runoff extracts following

decontaminant application via spray onto coupons) were also prepared. During the previous study, the volumes of decontaminant remaining on the surface of coupons and collected in the decontaminant runoff following spray-application were characterized using water. The average volume of water/decontaminant remaining on the surface of coupons following spray-application was measured to be 0.21 mL, and the average volume of water/decontaminant collected in the runoff was 0.99 mL. Thus, representative decontaminant runoff sample extracts were prepared by adding 0.99 mL of test decontaminant to 20 mL of IPA with 5 mL of 3M STS (i.e., no indoor- or PPE-related material coupons or spray application of decontaminants were used). Representative extracts were thoroughly mixed via vortex following preparation. Representative decontaminant runoff extract samples were prepared and used for quench method demonstration testing since the runoff decontaminant volume is greater than the coupon decontaminant volume (0.99 mL versus 0.21 mL) and provides the most technically conservative "worst case" quench test scenario (since the same volume of 3M STS quench [5 mL] was used to quench residual decontaminant in both coupon and runoff extracts).

Following preparation of the representative decontaminant runoff extracts, a dilute fentanyl solution was post-spiked into the IPA layer of the extracts. Extracts intended for GC/MS analysis were spiked with 40 µL of a 1 mg/mL fentanyl solution to target a final concentration of 2 µg/mL in the extracts. Extracts intended for LC-MS/MS analysis were spiked with 40 µL of a 1 µg/mL fentanyl solution to target a final concentration of 2 ng/mL in the extracts. Extracts were mixed thoroughly again via vortex post-spike. Aliquots of the extracts were collected from the IPA (top) layer and stored at -20 ± 10 degrees Celsius (°C) for 72 h. Following the 72-h period, the extract aliquots were equilibrated to room temperature and analyzed via GC/MS or LC-MS/MS (depending on post-spike concentration) to quantify fentanyl, and results were compared to the anticipated concentrations (based on the post-spiked mass and assuming no decontamination occurred) and appropriate control samples containing no decontaminant to determine the effectiveness of the quench methods.

Addition of 5 mL of 3M STS to the IPA used to extract coupons (10 mL) and runoff samples (20 mL) during decontamination efficacy tests were considered sufficient to preserve residual fentanyl in the extracts (i.e., prevent further decontamination of fentanyl past the tested decontaminant dwell period) during storage for up to 72 h at -20 ± 10°C if the amounts of fentanyl recovered from post-spiked extracts containing decontaminants (quench samples; three (3) replicates per decontaminant/fentanyl post-spike concentration/analysis method combination) were each at least 70% of the mean amount of fentanyl recovered from post-spiked extracts that did not contain decontaminants (positive controls; three (3) replicates per fentanyl post-spike/analysis method combination). The impact of extract matrix effects due to residual decontaminants and/or the 3M STS quench on the accuracy of quantitative analyses was considered negligible as well if both of the following criteria were met:

- Recovery of fentanyl ≥ 70% of the theoretical post-spiked amount in representative decontaminant runoff extract matrix samples (quench samples).

- The quality assurance/quality control (QA/QC) criteria for fentanyl-d5 IS response discussed in Sections 4.2.2 and 4.2.3 were satisfied.

If the above criteria were satisfied, we concluded that any matrix effects observed were influencing response of both fentanyl and the fentanyl-d5 IS in an identical manner, and that the IS was adequately and appropriately compensating for any effects and facilitating accurate fentanyl quantitation. As described in Sections 4.2.2 and 4.2.3, IS was added to analytical samples just prior to analysis to lessen the concern for decontamination/degradation of the IS in the samples due to unquenched decontaminant.

2.1.2 Decontamination Efficacy Evaluation

A post-test only control group experimental design was used for the decontamination efficacy evaluation. Decontamination was the experimental variable. Test coupons were contaminated, decontaminated, sampled, and analyzed for fentanyl. Positive control coupons were contaminated but not decontaminated and subsequently sampled and analyzed for fentanyl along with the test coupons. The effect of decontamination (efficacy) was defined as the percentage of fentanyl remaining (total residual active fentanyl, i.e., salt and freebase) on the test coupons compared to the positive control coupons. The higher the efficacy, the greater the effect of decontamination by the specific technology.

Procedurally, a target 1 mg of solid fentanyl was spiked and distributed onto the center portion of each test (3 replicates) and positive control (3 replicates) material coupon as described in Section 2.2.2.2. Material coupons measured 4.0 cm long by 2.5 cm wide (10-cm^2 coupon surface area). The spiked coupons were allowed to remain undisturbed during a set fentanyl contact period of 60 min. Following the fentanyl contact period, the decontamination technology under test was applied as a liquid spray directly to the fentanyl challenge on each test coupon and allowed to remain in contact with the fentanyl for the targeted dwell time.

Decontamination technology application procedures are described in Section 2.2.3.5. Following the decontamination period, the test and positive control coupons were sampled for residual fentanyl via solvent extraction according to Section 2.1.1.4 using 10 mL of IPA with 5 mL of 3M STS quench. Coupon extracts were then analyzed for residual fentanyl via GC/MS or LC-MS/MS according to Section 2.3.1 and 2.3.2.

During application of the decontaminants via moderately low flow spray, we anticipated that a portion of the (target) 600 µL delivered over each coupon would run off the coupon surface. Coupons were placed in individual acrylic boxes on top of polypropylene (PP) mesh disks to allow for collection of the runoff while elevating the coupons out of the decontaminant liquid that was collected (refer to Section 2.2.3.6). Decontaminant runoff from each coupon was collected, and each runoff sample was analyzed via GC/MS or LC-MS/MS to quantify any residual fentanyl. Refer to Sections 2.2.3.5 and 2.2.3.6 for details related to spray application of decontaminants and collection of the associated decontaminant runoff from each coupon. Runoff analysis results provided indication of physical removal of fentanyl from the coupon surface.

The matrix for decontamination efficacy testing for the two hydrogen-peroxide-based decontaminants is provided in Table 2. During each test, environmental conditions (temperature and relative humidity [RH]) were monitored and recorded, but not controlled.

Table 2. Decontamination Efficacy Test Matrix – Hydrogen Peroxide Solutions

Test No.	Sample Type	Material	Fentanyl Challenge	Decontamination Technology	Dwell Time* (min)	Replicates
1	Test Sample	Painted drywall	1 mg	Meth Remover®	60	3
	Positive Control	Painted drywall	1 mg	None	60	3
	Test Sample	Laminate	1 mg	Meth Remover®	60	3
	Positive Control	Laminate	1 mg	None	60	3
	Test Sample	Coated steel	1 mg	Meth Remover®	60	3
	Positive Control	Coated steel	1 mg	None	60	3
	Test Sample	Wood	1 mg	Meth Remover®	60	3
	Positive Control	Wood	1 mg	None	60	3
2	Test Sample	Painted drywall	1 mg	Zep®	60	3
	Positive Control	Painted drywall	1 mg	None	60	3
	Test Sample	Laminate	1 mg	Zep®	60	3
	Positive Control	Laminate	1 mg	None	60	3
	Test Sample	Coated steel	1 mg	Zep®	60	3
	Positive Control	Coated steel	1 mg	None	60	3
	Test Sample	Wood	1 mg	Zep®	60	3
	Positive Control	Wood	1 mg	None	60	3

In the previous fentanyl decontamination study [1], high efficacies (better than 99%) were measured for the peracetic acid-based Dahlgren Decon™ product as well as the hypochlorite-based chemistry in pH 5 modified surfactant bleach. Here, these two decontaminants were applied as a liquid spray directly to the fentanyl challenge on each test coupon and allowed to remain in contact with the fentanyl for 60 min. At that time, coupons were tilted to allow residual decontaminant to run off the surface into the collection box. This process was followed by a second application of the decontaminant as a liquid spray, which was allowed to remain in contact with the fentanyl on the surface for another 60 min. The matrix for decontamination efficacy testing for these two decontaminants is provided in Table 3.

As a continuation and natural progression of the decontamination efficacy evaluations, additional decontamination efficacy tests were conducted that focused on evaluation of the efficacy of selected decontaminants for degradation of a formulation of fentanyl directly on the surface of indoor-related materials. As it pertains to this testing, a formulation of fentanyl was defined as fentanyl mixed with a benign additive as may be encountered in samples collected in the field. In this study, ascorbic acid (Vitamin C; PHR1008-2G, Millipore Sigma, St. Louis, MO) as a benign additive was applied to the surface of selected test and control coupons (according to procedures described in Section 2.2.2.2) along with the fentanyl, and the fentanyl and benign additive were thoroughly mixed on the coupon surface prior to the double 60-min fentanyl dwell period.

Select test coupons (wood only) included in the matrix provided in Table 3 were spiked with a target 1 mg of fentanyl, which accounted for either a target 5% or 100% of the total challenge applied. For samples challenged with 100% fentanyl HCl by weight, coupons were spiked with

only the target 1 mg of fentanyl HCl solid. For samples challenged with 5% fentanyl HCl by weight, the ascorbic acid accounted for the remaining 95% of the total applied solid (i.e., 19 mg of a total 20 mg challenge; refer to Section 2.2.2.2).

Table 3. Decontamination Efficacy Test Matrix

Test No.	Decontaminant	Material Surface Type(s)	Fentanyl HCl Formulation	Dwell Time* (min)	Replicates	Fentanyl-HCl by Weight (%)
1	Dahlgren Decon™	Painted drywall	Fentanyl HCl only	60+60	3	100
		Coated steel	Fentanyl HCl only	60+60	3	100
		Wood	Fentanyl HCl only	60+60	3	100
		Wood	Fentanyl-HCl/ascorbic acid	60+60	3	5
2	Modified Clorox™ ProResults® Garage and Driveway Cleaner	Painted drywall	Fentanyl HCl only	60+60	3	100
		Coated steel	Fentanyl HCl only	60+60	3	100
		Wood	Fentanyl HCl only	60+60	3	100
		Wood	Fentanyl-HCl/ascorbic acid	60+60	3	5
*: 60+60 equates to a 60-min dwell time followed by reapplication and a second 60-min dwell time of the decontaminant.						

The test matrix for short (less than 15 min) dwell times and responder gear- or PPE-related materials is shown in Table 4. The first test was executed for three (3) materials with no replicates to allow for multiple dwell timepoints in one test. The second and third test used a fixed five (5)-min dwell time between the decontaminant and the fentanyl on the surface.

Table 4. Decontamination Efficacy Test Matrix for PPE Materials

Test No.	Decontaminant	Material Surface Type(s)	Fentanyl HCl Formulation	Dwell Time (min)	Replicates
1	Diluted Dahlgren Decon™	Saranex®	Fentanyl HCl only	1, 2, 6, 10, 15	1
		HazMat suit	Fentanyl HCl only	1, 2, 6, 10, 15	1
		Bunker gear	Fentanyl HCl only	1, 2, 6, 10, 15	1
2	Diluted Dahlgren Decon™	Neoprene	Fentanyl HCl only	5	3
		Saranex®	Fentanyl HCl only	5	3
		HazMat suit	Fentanyl HCl only	5	3
		Bunker gear	Fentanyl HCl only	5	3
3	Modified Clorox™ ProResults® Garage and Driveway Cleaner	Neoprene	Fentanyl HCl only	5	3
		Saranex®	Fentanyl HCl only	5	3
		HazMat suit	Fentanyl HCl only	5	3
		Bunker gear	Fentanyl HCl only	5	3

As indicated in Table 3 and Table 4, each decontaminant/material/fentanyl formulation combination was tested in triplicate (except for the efficacy time series, Test 1 in Table 4). In addition to the test coupons identified above, positive, blank and spike control samples were incorporated into each test, including:

- Positive Controls – Indoor material coupons that were spiked with fentanyl (with or without benign additive) using the same equipment and procedures as used to spike the test coupons, but to which no decontaminant was applied. Following the fentanyl contact and decontaminant (for test coupons) dwell periods, positive controls were extracted with solvent, and extracts were analyzed alongside the test coupons.
- Procedural Blanks – Indoor material coupons that were not spiked with fentanyl (with or without benign additive) but that were decontaminated, extracted with solvent, and

analyzed alongside the test coupons using the same procedures and equipment (one replicate per material/decontaminant combination per test).

- Laboratory Blanks – Indoor material coupons that were not spiked with fentanyl (with or without benign additive) or decontaminated but that were extracted with solvent and analyzed alongside the test coupons using the same procedures and equipment (one replicate per material per test).
- Spike Control Samples – A mass of fentanyl consistent with the amount applied to the test coupons and positive controls (target 1 mg) that was dissolved in extraction solvent (three replicates per test; refer to Section 2.2.2.2). Spike control replicates were generated throughout the fentanyl (with or without benign additive) spiking operation (i.e., one spike control prior to application of fentanyl to test/positive control coupons, one in the middle of the operation, and one after all test/positive control coupons had been spiked).

Positive controls, procedural blank samples, and laboratory blank samples consisted of coupons of the same indoor materials of the same dimensions as the test coupons to which they were associated.

2.2 Experimental Methods and Materials

Experimental methods and materials used to conduct the testing described in Sections 2.1.1 and 2.1.2 are described in the subsections below.

2.2.1 Coupon Materials

Method demonstration and decontamination efficacy testing were conducted using the following types of indoor materials: painted drywall, laminate, wood, and coated steel while Saranex® (Tychem®), a Level B HazMat suit, bunker gear, and neoprene were used as responder/PPE materials.

Materials were cut into coupons of uniform length (4.0 cm) and width (2.5 cm). Therefore, the top surface area to which the fentanyl (and benign additive, as required) challenge and decontamination technologies were applied measured 10 cm^2. These dimensions enabled the coupons to fit lying flat at the bottom of the 60-mL glass jars that were used for solvent extraction of coupons.

Coupon thicknesses were dependent upon the material type. All coupons were visually inspected prior to use during testing to confirm the integrity and representativeness of the material. Coupons with irregular edges and/or damaged areas were discarded. Following cutting, coupons were cleaned using dry air to remove dust and debris prior to use in tests. Coated steel coupons were also wiped using IPA-soaked wipes to remove any machining/cutting grease residue.

Painted drywall is a panel made of gypsum typically pressed between two thick sheets of paper with a layer of paint coating one side of the thick sheet of paper. Painted drywall is typically used for walls and ceilings of indoor structures. During this work, white joint tape (Sheetrock®

brand, Lowes, Hilliard, OH) was used to simulate the thick sheet of drywall paper and was painted with latex paint (KILZ® latex primer, Lowes, Hilliard, OH; Behr® Premium Plus interior flat white latex paint, Home Depot, Columbus, OH) simulating the painted surface of a drywall board. The painted joint tape was cut into individual 10 cm^2 coupons.

Laminate, Formica™, Arborite™ or Garolite™ is a sheet created by combining fiber, paper and/or fabric with epoxy or resin and set under heat. Typical uses of laminate include household countertops and flooring. During this work, laminate coupons with a thickness of 3.2 millimeter (mm) (0.125 inch) were used. The 24 by 24-inch Garolite™ G-10 sheets (McMaster-Carr®, Aurora, OH) were obtained and cut into individual 10 cm^2 coupons (2.5 cm by 4 cm).

Wood refers to structural wood used for framing in commercial or residential construction and is also referred to as dimensional lumber. Douglas Fir is commonly sold and used as dimensional lumber due to its strength, hardness, and durability. Coupons for this testing were cut from 4-inch by 4-inch by 8-foot untreated kiln-dried Douglas Fir dimensional lumber (Home Depot, Columbus, OH). A target coupon thickness of approximately 0.375-inches (3/8-inches) was used. Wood surface was cross-grain and the exposed surfaces were not sanded or sealed. After cutting to size any remaining dust was blown off with air.

Coated Steel refers to a powder-coated hard finish of steel that is similar to but generally considered to be more durable than conventional paint. The coating is applied electrostatically as a free-flowing dry powder and then cured under heat. The powder may be a thermoplastic or a thermoset polymer, mainly used for coating metals, such as household appliances, aluminum extrusions, drum hardware, and automobile, motorcycle, and bicycle parts. For this testing, black powder-coated steel landscape edging (Lowe's, Hilliard, OH) was used as a representative powder-coated surface. Coupons needed for this testing were cut from the edging sections.

Saranex® is the Transcendia, Inc., brand name for polyvinylidene chloride, a vinylidene chloride homopolymer. Saranex® offers barrier protection against gases and vapors, so among many other uses, it is often incorporated into textile laminates to produce chemical-protective clothing. For this work, coupons were excised from Tychem® SL hooded disposable coveralls (Grainger, Lake Forest, IL) which are constructed of Saranex® 23P film-laminated Tyvek®.

"HazMat Suit" refers to the DuraChem® 500, a National Fire Protection Agency (NFPA) 1994-certified (2018 edition; Class 1 and Class 2) chemical, biological, radiological, nuclear (CBRN) protective "multiuse, single-exposure" garment manufactured by Kappler, Inc. (Guntersville, AL). Coupons cut from larger swatches of DuraChem® 500 suit material received directly from Kappler, Inc., were included during testing.

"Bunker gear" (i.e., turnout gear) refers to the PPE worn by firefighters, most often during structural fire operations. A typical set of turnout gear includes coat and trousers that, according to NFPA Standard 1971, must incorporate: (1) an outer shell (typically of Nomex®/Kevlar® construction) for heat, fire, abrasion, and chemical resistance, (2) a waterproof moisture barrier, and (3) an inner thermal barrier. Turnout coupons used during this testing were harvested from

the outermost layer (outer shell) of a Chieftain® 32XTM turnout coat (Grainger, Lake Forest, IL).

Neoprene, a synthetic rubber produced by polymerization of chloroprene, is used in a wide variety of products and applications, including gasketing and sealing of electrical enclosures, sports, and medical equipment (e.g., joint braces and supports), wetsuits, and safety gloves. Individual coupons for this testing were cut from a larger 12-inch by 24-inch sheet of multipurpose neoprene rubber (0.016-inch thickness; McMaster-Carr®, Aurora, OH).

Table 5 provides a summary of test coupon information for this work.

Table 5. Coupon Materials

Material	Description	Supplier	Coupon Thickness (mm)	Preparation
Painted Drywall	White joint tape, Sheetrock® brand, item number 15335, model number 380041; KILZ® latex primer, item number 45548, model number 20902	Lowes Hilliard, OH	~0.5	1. Apply one coat of latex primer; 2. Allow to dry; 3. Apply one coat of paint; 4. Allow to dry. 5. Clean using dry air to remove debris
	Behr® Premium Plus Interior Flat White Latex Paint, item number 923827	Home Depot Columbus, OH		
Laminate	Garolite™ G-10 sheet, 24" x 24", epoxy resin with fiberglass fabric reinforcement, item number 9910T2	McMaster-Carr Aurora, OH	3.2	• Coupons cut from sheet • Clean using dry air to remove debris.
Wood	4" x 4" x 8' Untreated Kiln-Dried Douglas Fir Dimensional Lumber, item number 137195	Home Depot Columbus, OH	9.5	• Cut coupons to size • Clean using dry air to remove debris
Coated Steel	Black powder-coated steel landscape edging section, item number 959658	Lowes Hilliard, OH	3.2	• Cut coupons to size • Clean using dry air to remove debris
Saranex®	Tychem® SL coveralls (item number 34CL41) with elastic material (Saranex® 23P film laminated Tyvek construction; white)	Grainger Lake Forest, IL	0.3	• Cut coupons to size • Clean using dry air to remove debris
HazMat Suit	DuraChem® 500 HazMat and CBRN Protective Suit material	Kappler Guntersville, AL	0.4	• Cut coupons to size • Clean using dry air to remove debris
Bunker gear	Chieftain® 32XTM khaki turnout coat, item number 1370N51; Nomex® construction and polymer-coated Kevlar® cuff reinforcements	Grainger Lake Forest, IL	0.4	• Cut coupons to size • Clean using dry air to remove debris
Neoprene	Multipurpose neoprene rubber sheet, item number 1370N51	McMaster-Carr	0.4	• Cut coupons to size • Clean using dry air to remove debris

2.2.2 Fentanyl

2.2.2.1 Fentanyl Source

Fentanyl HCl (3.5 grams [g]) was purchased from Cayman Chemical Company (14719, Cayman Chemical Company, Ann Arbor, MI). All fentanyl originated from the same synthesis/production lot. Upon receipt, fentanyl was stored at ambient laboratory temperature in accordance with facility and DEA security and storage policies until needed for testing. Fentanyl was stored in a single capped vial from which working quantities were drawn for use when needed.

The purity of the fentanyl received from Cayman Chemical Company was 99.59% ± 0.18%, as provided on the certificate of analysis received with the compound. The certificate of analysis for the fentanyl received and used for all testing that was performed is provided as Attachment A.

2.2.2.2 Fentanyl Application

Test and positive control coupons were inspected visually prior to contamination with fentanyl and any coupons with surface anomalies were not used. Fentanyl was applied to the center of each designated test and positive control coupon as a single (target) 1-mg pile using a 50-µL Drummond Series 500 Digital Microdispenser (part no. 3-000-550, Drummond, Broomall, PA) utilizing a borosilicate capillary tube and Teflon® plunger. The fentanyl was spread over approximately 50% of the coupon surface (as determined visually) using an antistatic spatula (14-245-99, Fisher Scientific, Pittsburgh, PA). This spread equates to a coupon contamination level of approximately 200 µg/cm^2 (based on the 10-cm^2 coupon contamination/decontamination surface area). Spike control samples were generated by delivering the same quantity of fentanyl as that applied to the surface of coupons (target 1 mg) into an empty 60-mL glass extraction jar with subsequent addition of 10 mL of IPA to dissolve the fentanyl. Following preparation, spike controls were processed in a manner like the coupon extracts (that is, spike controls were sonicated and aliquoted for analysis as described for coupon extracts in Section 2.2.4).

During the fentanyl/benign additive decontamination efficacy evaluation, the benign additive ascorbic acid was applied to the surface of the 10-cm^2 coupons along with fentanyl. A target 19 mg of the ascorbic acid was applied to the center of each designated test or positive control coupon using a 100-µL Drummond Series 500 Digital Microdispenser (3-000-575, Drummond, Broomall, PA). A setting of 19 µL on the 100-µL Drummond was used to deposit (target) 19-mg piles of ascorbic acid. A single (target) 19-mg pile was thus applied onto the surface of the coupon in the center. Following application of the (target) 19 mg of the ascorbic acid, the (target) 1 mg of fentanyl was applied using a 50-µL Drummond Series 500 microdispenser (as discussed earlier). The fentanyl/ ascorbic acid applied to each coupon was then spread over approximately 50% of the coupon surface using an antistatic spatula as described above, and the two compounds (fentanyl and ascorbic acid) were mixed concurrently with the spreading step.

2.2.2.3 Fentanyl Contact Period

Following application of fentanyl, the contaminated coupons were allowed to remain undisturbed for a 60-min fentanyl contact period. During this contact period, the coupons were subjected to the ambient atmosphere within the test chamber. Coupons remained uncovered during the 60-min fentanyl contact period. While temperature and RH inside the test chamber were not controlled to a specific target, extreme conditions were avoided. Generally, test chamber temperature ranged from 18°C to 28°C, and RH from 30% to 70%. Temperature and RH for each test were monitored and recorded via a HOBO UX100-003 Temperature/RH datalogger (part no. UX100-003, Onset®, Bourne, MA). Environmental data from each test are provided in Attachment B.

2.2.3 Application of Decontamination Technologies

2.2.3.1 Meth Remover®

Meth Remover® is a formulated aqueous alkaline decontamination solution from Apple Environmental. It is a hydrogen peroxide-based decontaminant that is intended to be "environmentally-friendly", non-corrosive, and used for cleanup and remediation of methamphetamine contamination. Meth Remover® is a two-component system that includes disodium carbonate, ethanol, and a water-based buffer (Part 1), and stabilized hydrogen peroxide (< 8%; Part 2). The decontaminant is prepared by mixing Parts 1 and 2 in equal amounts. Meth Remover® was prepared in accordance with manufacturer instruction prior to each test during this work.

Prior to use during decontamination efficacy tests, hydrogen peroxide concentration and pH of the prepared Meth Remover® decontaminant were measured. Hydrogen peroxide concentration was measured using a Hach® hydrogen peroxide test kit (HYP-1, Hach Company, Loveland, CO), and pH was measured using a pH meter (Orion Star™ A221 pH portable meter, STARA2210, Thermo Fisher Scientific, Waltham, MA).

2.2.3.2 Zep® Professional Stain Remover with Peroxide

Zep® Professional Stain Remover with Peroxide (ZEP®) is a hydrogen peroxide-based (≥5% to < 10%) cleaner intended for use on natural and synthetic textiles including carpet and upholstery. In addition to the hydrogen peroxide active ingredient, the cleaner includes water, sodium acrylate copolymer (film-forming agent), and ethoxylated alcohols.

Prior to use during decontamination efficacy tests, hydrogen peroxide concentration and pH of the cleaner were measured. Hydrogen peroxide concentration was measured using a Hach® hydrogen peroxide test kit (HYP-1, Hach Company) and pH was measured using a pH meter (Orion Star™ A221 pH portable meter, STARA2210, Thermo Fisher Scientific, Waltham, MA).

2.2.3.3 Modified Clorox™ ProResults® Garage and Driveway Cleaner

Clorox™ ProResults® Garage and Driveway Cleaner (564084310, Walmart) is a hypochlorite-based cleaner that includes a surfactant (myristamine oxide, CAS 3332-27-2). The cleaner was modified in pH for use as a test decontaminant. Prior to use during testing, the necessary ratio of cleaner to vinegar (Heinz Distilled White Vinegar, 5% acidity; 700667856063, Amazon) to water (Crystal Springs Water) required to adjust both the pH of the cleaner to 5 and the hypochlorite concentration to 0.5% were determined. Such adjustments were intended to produce a decontaminant like the pH 5 bleach tested previously [1], but that also included a surfactant to promote spreading of the decontaminant across a material surface. The pH was adjusted using vinegar (as measured using a pH meter) and then diluted as necessary using water to target a hypochlorite concentration of 0.5% (measured using a Hach® hypochlorite test kit). A ratio of 1 part Clorox™ ProResults® Garage and Driveway Cleaner to 0.66-parts vinegar to 1.5 parts water was determined to produce an adjusted cleaner at the pH and hypochlorite concentration targets.

Prior to initial application of decontaminant and prior to reapplication during indoor-related material decontamination efficacy tests (at 60 min into the total 120-min decontaminant dwell period), hypochlorite concentration and pH of the prepared pH-adjusted surfactant bleach were measured. Hypochlorite concentration was measured using a Hach® hypochlorite test kit (CN-HRDT, 2687100, Hach Company) and pH was measured using a pH meter (Orion Star™ A221 pH portable meter, STARA2210, Thermo Fisher Scientific).

2.2.3.4 Dahlgren Decon™

Dahlgren Decon™ (DD-006-RTU, First Line Technology, Chantilly, VA) is a three-component decontaminant system including water and a surfactant package (Part A), sodium hydroxide (Part B1), and peracetyl borate (active ingredient; Part B2; releases peracetic acid upon dissolution in water). Normally, Part A comes as a solid and must be dissolved in water before mixing with Parts B1 and B2, but for this testing a “ready-to-use” (RTU) version was used that provides Part A already dissolved in water from the manufacturer.

Approximately 1 liter of Dahlgren Decon™ was prepared for use prior to each test by mixing the three parts in accordance with directions provided by the manufacturer. Per manufacturer direction, the prepared decontaminant must be used (i.e., applied via spray to designated coupons, as it pertains to this testing) within 6 h of preparation. Preparation and use of Dahlgren Decon™ adhered to this requirement during this testing. Prior to initial application of decontaminant and prior to reapplication during indoor-related material decontamination efficacy tests (at 60 min into the total 120-min decontaminant dwell period), peracetic acid concentration and pH of the prepared decontaminant were measured. Peracetic acid concentration was measured using a LaMotte test kit (7191-02, LaMotte Company, Chestertown, MD), and the pH was measured using a pH meter (Orion Star™ A221 pH portable meter, STARA2210, Thermo Fisher Scientific).

During PPE-related material decontamination efficacy testing, Dahlgren Decon™ was diluted by a factor of 5 in distilled water (Crystal Springs Water) prior to use. Full-strength Dahlgren Decon™ was first prepared as described above, then 1 part (undiluted) Dahlgren Decon™ was mixed with 4 parts water. Peracetic acid concentration and pH of the 5-fold diluted Dahlgren Decon™ were measured as described above for full strength Dahlgren Decon™.

2.2.3.5 Decontaminant Application

Figure 2. Handheld pump pressurization-style sprayer.

Liquid decontaminants were applied to test and control sample coupons via moderately low flow spray using a handheld 1-gallon pump-pressurization-style sprayer (12U469, Grainger, Lake Forest, IL; Figure 2) equipped with a polyethylene nozzle (2ZV94, Grainger).

The sprayer was integrated into the test chamber (nozzle inside; tank maintained outside) to allow for moderately low flow spray application of the decontaminants onto coupons while still enabling operators to work with solid fentanyl within the safety of the test chamber. The handheld pump sprayer was selected as it is readily commercially available, and the pump pressurization mechanism allows for better control of spray impact pressure.

Integration of the sprayer into the test chamber involved replacement of the sprayer extension wand with flexible tubing that was run into the test chamber through a port on the side wall. Specifically, the spray shut-off assembly and complete nozzle assembly were removed from the extension wand and attached to each end of a length of chemical-resistant Versilon™ polyvinyl chloride (PVC) tubing lined with fluorinated ethylene propylene (FEP, 6519T14, McMaster-Carr, Aurora, OH; coiled red tubing in Figure 3 below). The nozzle assembly was then mounted to a rail installed at the top of the test chamber that allowed the spray delivered from the nozzle to be swept from side to side. The nozzle standoff distance (i.e., distance from the nozzle outlet to the top surface of the coupons placed underneath) was approximately 10.25-inches. A variable speed motorized pulley system was used to move the nozzle across the rail at a uniform and constant rate. The sprayer was cleaned after use and in between changes in decontamination solution. At least one liter of distilled water was passed through the sprayer, with any residual water discharged under air pressure.

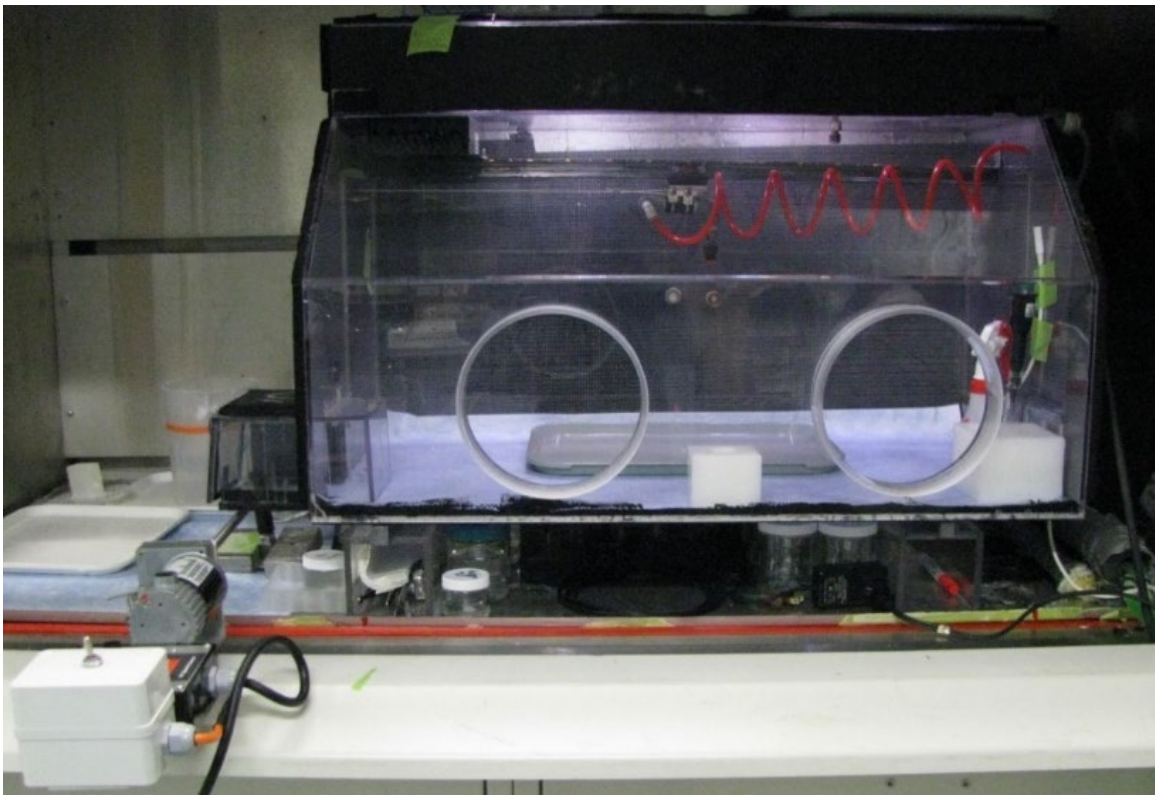


Figure 3. Sprayer and test chamber setup.

During tests with the 10-cm^2 coupons, test and procedural blank coupons were placed into separate acrylic boxes (1.75-inch square by 1-inch height; part no. 3790-CL, G&G Distributors, Saddle Brook, NJ) on top of small PP mesh disks (1.375-inch diameter, 0.05-inch thickness, cut from larger sheet of PP mesh (part no. 9265T47, McMaster-Carr). The acrylic boxes holding individual coupons were placed onto a tray that was positioned underneath the sprayer nozzle. The plastic boxes containing the coupons were arranged in two rows of eight boxes as shown in Figure 4. The sprayer nozzle stand-off distance was set such that the spray fan/cone delivered from the nozzle extended past the outer edges of the plastic boxes placed on the tray below within the characterized area of the spray (Figure 5).

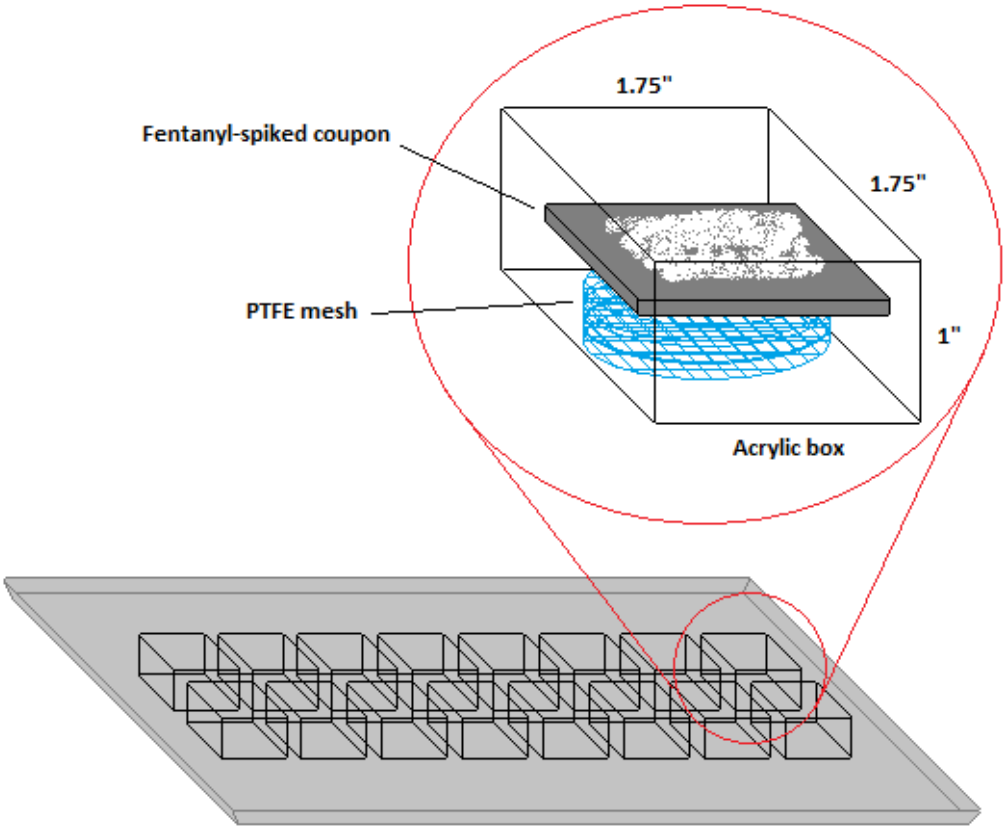


Figure 4. Decontaminant spray tray setup.

When applying decontaminant to the coupons, the sprayer was pressurized to 20 pounds per square inch (psi), the variable speed motor was set to the necessary rate (dependent upon the decontaminant), and the sprayer shut-off assembly (outside the chamber/hood) was actuated to begin spray delivery. The motorized pulley system was then activated, and the nozzle (inside the chamber) was swept from one side to the other at a rate/speed required to deliver the target volume of decontaminant per unit area (60 µL/cm^2) to each coupon as illustrated in Figure 5. Following application of the decontaminant, the spray was stopped, and the nozzle was returned to the starting position on the rail. Any decontaminant dripping from the nozzle after spray had been stopped was collected/captured so excess decontaminant did not fall on top of the coupons below.

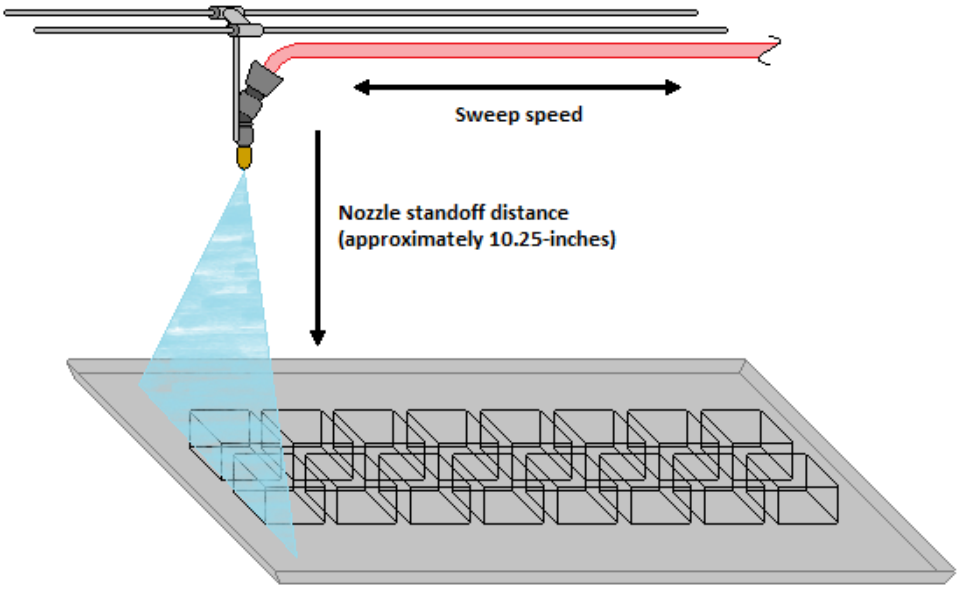


Figure 5. Spray application.

As discussed in Section 2.1.1.3, the sprayer pressure and sweep rate/speed necessary to deliver 60 µL/cm^2 of decontaminant to the top surface of coupons was determined prior to testing. Determination of the necessary sprayer pressure and sweep speed was made with the 1.75 square inch (in^2) acrylic boxes present in the tray so that uniformity of the spray delivery could be assessed by measuring the weight of liquid added to each acrylic box. Each plastic box had an internal area (bottom internal surface) of 18.87 cm^2. The target decontaminant volume delivery of 60 µL/cm^2 would thus equate to approximately 1.13 mL of decontaminant delivered into each box. Various combinations of sweep speeds and sprayer pressures were evaluated, and the liquid delivered into each box was weighed to determine successful delivery of the target 1.13 mL volume of decontaminant.

Following application, the decontaminants were allowed to remain undisturbed on the coupons (to react with the fentanyl challenge, in the case of test coupons) for a predetermined dwell period. Visual observation of the wetness of each coupon was recorded. The 10-cm^2 coupons in acrylic boxes were left uncovered during the decontamination dwell period. Following the decontaminant dwell period, 10-cm^2 coupons were extracted in solvent according to procedures described in Section 2.2.4.

2.2.3.6 Decontaminant Runoff

Decontaminant that ran off the test and procedural blank coupons following spray delivery was collected for analysis for fentanyl by GC/MS or LC-MS/MS.

During decontamination efficacy tests described in Section 2.1.2, each coupon was placed into a separate acrylic box during application of decontaminant via spray (see Figure 5), so that the decontaminant runoff from each coupon was segregated for collection. A PP mesh placed underneath the coupons in the acrylic boxes provided stable elevation of the coupons off the

bottom of the acrylic boxes to prevent the coupons from contacting any decontaminant runoff (that may potentially contain fentanyl physically removed from the coupon by the spray-application of decontaminant). Following removal of coupons from the acrylic boxes for extraction with solvent (Section 2.2.4), the acrylic boxes (containing decontaminant runoff and PP mesh) were placed into individual 250-mL glass jars (05-719-61, Fisher Scientific), and the acrylic boxes and runoff contents were extracted with 20 mL of IPA and 5 mL 3M STS. Following extraction of the acrylic box and runoff contents, aliquots of the IPA layers of the extracts were transferred into individual gas chromatograph (GC) vials (21140 (vial), 24670 (cap), Fisher Scientific (Restek Corp.), and extracts were analyzed via GC/MS (Section 2.3.1) or LC-MS/MS (Section 2.3.2).

2.2.4 Extraction of Fentanyl from Coupons

All coupons were extracted by placing each into a separate 60-mL glass jar (05-719-257, Fisher Scientific) containing 10 mL of IPA and 5 mL of 3M STS quench. IPA was selected based on the results of previous solvent extraction method testing [1]. Using the dimensions provided in Section 2.2.1 and Table 5, coupons of the indoor materials fit lying flat within the inside diameter of the extraction jar identified above. The 10 mL of IPA reached a height within the jar of approximately 1 cm. This jar and volume of solvent were sufficient to submerge all coupon types fully. Wood coupons were placed face down as they floated to the top surface of the solvent due to buoyancy.

Following the addition of coupons to the extraction solvent within each jar, the jars were swirled by hand for approximately 5-10 seconds and placed into a sonicator (Branson Model 5510R-DTH). Extraction jars were sonicated at 40 to 60 kilohertz for 10 min. Within 30 min of completing this process, aliquots of approximately 0.5 mL from each extraction jar were transferred to individual GC vials and sealed (21140 (GC vial), 24670 (GC vial cap), Fisher Scientific (Restek Corp.)). Samples that were not analyzed the same day were stored at -20 ± 10°C.

2.3 Analytical Methods

As described in Section 2.1.1.4, the strategy for quantification of residual fentanyl in coupon and decontaminant runoff extracts included both GC/MS and LC-MS/MS analyses. GC/MS was used for quantitation of fentanyl in control samples of known concentration as well as initial analyses of samples of unknown concentration (i.e., decontamination test samples). Samples below the quantitation range of GC/MS were then analyzed via LC-MS/MS.

The GC/MS and LC-MS/MS analyses did not include a qualitative assessment of fentanyl degradation byproducts and neither were other analytical methods considered. The previous fentanyl decontamination effort [1] included some qualitative interpretation of byproduct formation.

2.3.1 Quantitative Fentanyl Analysis – GC/MS

Samples were analyzed in selected ion monitoring (SIM) mode on an Agilent 6890 using an Agilent 5973A mass selective detector (MSD; Agilent Technologies, Santa Clara, CA). Modern instrumental SIM analysis allows for multiple ion selections while still providing increased sensitivity. Fentanyl was detected using ions *m/z* 245 (quantifier ion), 146, 189, 105, and 202. A decafluorotriphenylphosphine (DFTPP) tune check was performed on the MSD to ensure proper operation prior to sample analysis. Prior to GC/MS analysis, samples were spiked with a known amount of fentanyl-d5 (F-001-1ML, Sigma-Aldrich) to use as an IS (*m/z* 250 as quantifier ion). The concentration of analyte in samples was interpolated using the analyte area/IS area ratio and the regression equation generated from calibration standards. See Section 4.2.3 for GC/MS calibration details.

Table 6 provides the GC/MS conditions that were used during fentanyl analyses. Refer to Section 4.2.3 for QA/QC provisions that were included during analyses to ensure adequate performance of the GC/MS across the calibration range.

Table 6. GC/MS Conditions for Quantitative Fentanyl Analysis

Parameter	Description
Instrument	Agilent Model 6890 Gas Chromatograph equipped with HP 5973A Mass Selective Detector and Model 7683 Automatic Sampler
Data System	MSD ChemStation
Column	Rxi-5Sil MS, 30.0 meters × 0.25 mm, 0.25 µm film thickness
Liner Type	4 mm split/splitless
Carrier Gas Flow rate	1.2 mL/min
Column Temperature	50 °C initial temperature, hold 0.5 min, 30 °C/min to 280 °C, hold 1.0 min
Injection Volume	3.0 µL
Injection Temperature	250 °C
MS Quad Temperature	150 °C
MS Source Temperature	230 °C
Solvent Delay	3.1 min

2.3.2 Quantitative Fentanyl Analysis – LC-MS/MS

Coupon extracts and aliquots of decontaminant runoff were analyzed using LC-MS/MS to quantify the amount of residual fentanyl present. An AB Sciex 5500 triple quadrupole MS (SCIEX, Framingham, MA) coupled to a Shimadzu 20 XR series LC (Shimadzu, Columbia, MD) was used for sample analyses. Fentanyl was quantitated in sample extracts using a reversed-phase high performance liquid chromatography (HPLC) method and multiple reaction monitoring (MRM). MRM provides high specificity and sensitivity and is typically used in quantitative applications. The MRM transition with the best signal-to-noise ratio is usually selected for quantitation. Fentanyl-d_5 (F-001-1ML, Sigma-Aldrich) was used as the IS for quantitation of fentanyl and was added to calibration standards, controls, and test samples just prior to LC-MS/MS analysis (nominal concentration in samples after addition of 0.45 ng/mL). Table 7 provides the ion transitions that were used for detection and quantitation of fentanyl.

Table 7. LC-MS/MS Analyte Ion Transitions

Analyte	Precursor Ion (*m/z*)	Product Ion Quantifier (*m/z*)
Fentanyl	337	188
Fentanyl-d_5	342	188

The lower limit of quantitation for fentanyl free base was 0.010 ng/mL, which was equal to the concentration of the lowest standard used to generate the calibration curve.

The concentration of analyte in samples was interpolated using the analyte area/IS area ratio and the regression equation generated from calibration standards. Samples that quantitated below the lowest calibration standard concentration or displayed area counts below the lowest concentration on the calibration curve were reported as less than the Lower Limit of Quantitation (LLOQ; e.g., <0.01 ng/mL). The less-than-the-LLOQ value was corrected to account for the sample dilution factor. Samples that quantitated above the highest calibration standard were re-diluted and reanalyzed. See Section 4.2.2 for LC-MS/MS calibration details. All data were reported to two significant figures.

LC-MS/MS parameters that were used are provided in Table 8.

Table 8. LC-MS/MS Conditions for Quantitative Fentanyl Analysis

Parameter	Description		
Ionization Mode and Polarity	Electrospray Ionization, Positive Mode		
HPLC Column	Restek Allure PFPP [A], 2.1 x 50 mm, 5 µm (part no. 9169552)		
Column Temperature	35 °C		
Curtain Gas	Nitrogen (20 psi pressure)		
IonSpray Voltage	2500 V		
Ion Source Temperature	500 °C		
Entrance Potential	10 V		
Cell Exit Potential	15 V		
Mobile Phase	A: 2 mM Formic Acid/2 mM Ammonium Formate in Water		
	B: 2 mM Formic Acid/2 mM Ammonium Formate in Methanol		
Mobile Phase Gradient	**Time (min)**	**%B**	Flow Rate (mL/min)
	0.0	20	0.5
	1.0	20	0.5
	2.0	100	0.7
	4.0	100	0.7
	4.1	20	0.5
	5.0	20	0.5
Injection Volume	4 µL		
Run Time	5 min		

[A] Pentafluorophenylpropyl phase.

Samples in IPA were diluted at least 10-fold prior to LC-MS/MS analysis. Samples (e.g., aliquots of decontaminant runoff) were matrix-matched to the calibration standards by addition of IPA to a final concentration of approximately 10%. Alternative dilution factors were used for

samples of high analyte concentration or to reduce sample matrix concentration (such as residual Dahlgren Decon™). Sample dilutions were performed using calibrated positive displacement pipettes and were documented on the sample chain of custody (CoC; refer to Section 4.3) and laboratory record book (LRB).

2.4 Calculations

For each fentanyl formulation/indoor material/decontaminant combination, means of the coupon mass recoveries, coupon residual contamination, and decontamination efficacy values were calculated and reported, along with percent RSD.

2.4.1 Decontamination Efficacy Evaluation

Test, control, and blank coupon and runoff extract concentrations were provided in units of µg of fentanyl per mL of extract by the GC/MS ChemStation software (ver. E.02.02 SP1) or in units of ng of fentanyl HCl per mL of extract by the LC-MS/MS Analyst® software (ver. 1.6.2) through comparison of the analyte and IS peak areas to the GC/MS or LC-MS/MS calibration curves. GC/MS calibration data were fitted to a quadratic regression while the LC-MS/MS calibration data fit a linear regression ($1/x^2$ weighting). Based on the regression, concentrations of fentanyl in the coupon and decontaminant runoff extracts were determined (calculated by the software) according to either Equation 1 (quadratic regression) or Equation 2 (linear regression):

Quadratic regression:

$$\frac{A_A}{A_{IS}} = a\left(\frac{C_A}{C_{IS}}\right)^2 + b\left(\frac{C_A}{C_{IS}}\right) + c \qquad (1)$$

where: A_A = Analyte peak area

A_{IS} = Internal standard peak area

C_A = Actual analyte concentration (µg/mL)

C_{IS} = Internal standard concentration (µg/mL)

a, b, c = quadratic regression coefficients.

Linear regression:

$$\frac{A_A}{A_{IS}} = m\frac{(C_A/C_{IS})}{DF} + b \qquad (2)$$

where: A_A = Analyte peak area

A_{IS} = Internal standard peak area

b = y-intercept of regression curve

C_A = Actual analyte concentration (ng/mL)

C_{IS} = Internal standard concentration (ng/mL)

DF = Dilution factor (set to 1 in the software; actual dilution factor is applied in the raw analytical data spreadsheet)

m = slope of regression curve.

In Equations 1 and 2, C_A (actual fentanyl concentration in µg/mL (GC/MS) or ng/mL (LCMS/MS)) is determined as fentanyl free base equivalents (since the calibrations standards are prepared from free base fentanyl). Equation 3 was applied to the results to convert to the equivalent fentanyl HCl concentration:

$$Conc_{Ext} = C_A \times \left(\frac{MW_{HCl}}{MW_{Free}}\right) \quad (3)$$

where: $Conc_{Ext}$ = Coupon/runoff extract concentration in terms of fentanyl HCl (ng/mL (LC-MS/MS) or µg/mL (GC/MS))

C_A = Coupon/runoff extract concentration (fentanyl free base equivalents) provided by the LC-MS/MS software (ng/mL) or GC/MS software (µg/mL)

MW_{HCl} = Fentanyl HCl molecular weight (372.94 g/mol)

MW_{Free} = Free base fentanyl molecular weight (336.47 g/mol).

Mass recovered from the coupons or runoff samples via extraction was determined according to Equation 4:

$$Mass_{Rec} = \frac{Conc_{Ext} \times Vol_{Ext}}{Conv} \quad (4)$$

where: $Mass_{Rec}$ = Fentanyl mass recovered from the coupon/runoff (µg)

$Conc_{Ext}$ = Coupon/runoff extract concentration in terms of fentanyl HCl (ng/mL (LC-MS/MS) or µg/mL (GC/MS))

Vol_{Ext} = Volume of coupon/runoff extraction solvent (mL)

Conv = Conversion factor (1000 for LC-MS/MS analyses; 1 for GC/MS analyses)

Residual fentanyl contamination for each coupon was determined using the calculated mass recovered from the coupon and the coupon contamination/decontamination surface area, according to Equation 5:

$$Cont_{Res} = \frac{Mass_{Rec}}{A_{Coupon}} \quad (5)$$

where: $Cont_{Res}$ = Residual coupon contamination (µg/cm^2)

$Mass_{Rec}$ = Fentanyl mass recovered (µg)

A_{Coupon} = Contamination/decontamination surface area of the coupon (cm^2).

Total sample mass was determined using the masses recovered from extraction of the coupon and extraction of the associated runoff sample, according to Equation 6:

$$Mass_{Tot} = Mass_{Rec\,(coupon)} + Mass_{Rec\,(runoff)} \quad (6)$$

where: $Mass_{Tot}$ = Total fentanyl mass recovered (µg)

$Mass_{Rec\,(coupon)}$ = Fentanyl mass recovered from the coupon (µg)

$Mass_{Rec\,(runoff)}$ = Fentanyl mass recovered from the runoff (µg)

Percent efficacy of decontamination from each individual test coupon or percent total efficacy for each coupon/runoff combination was calculated according to Equation 7:

$$Efficacy = \left(\frac{Mass_{Rec\,(pos)} - Mass_{(x)}}{Mass_{Rec\,(pos)}}\right) \times 100\% \quad (7)$$

where: $Mass_{(x)}$ = Either $Mass_{Rec\,(coupon)}$ or $Mass_{Tot}$ from Equation 4 (µg)

$Mass_{Rec\,(pos)}$ = Fentanyl mass recovered from the associated positive control (µg).

Calculation of efficacy using $Mass_{Rec\,(coupon)}$ provided a measurement of the ability of the decontaminant to remove fentanyl contamination from the surface of the material coupons, either by chemical decontamination of fentanyl or by physical removal. Calculation of efficacy using $Mass_{Tot}$ intended to decouple physical removal from the efficacy calculation and provide an indication of the ability of the decontaminant to chemically degrade fentanyl contamination.

2.4.2 Decontamination Efficacy Evaluation (Benign Additive Ascorbic Acid)

Fentanyl mass recoveries, residual contamination, total sample masses, and percent efficacies were calculated according to Equations 1 through 7 used during the initial fentanyl decontamination efficacy evaluations (10-cm^2 coupons, fentanyl HCl only without benign additives present; Section 2.1.2).

2.5 Statistical Analyses

For each test condition as defined by the decontamination technology/material type/decontamination period/challenge additive combinations, mean and percent RSD of the fentanyl recovery from test coupon and positive control sample sets were calculated, and test coupon fentanyl recovery means were compared to associated positive control means to determine if statistically significant decontamination of fentanyl occurred. Geometric means were compared for trial datasets as shown in Tables 3 and 4; and arithmetic means were compared for datasets as shown in Table 2 to be consistent with the transformations used in the comparisons between test conditions described below. These decontamination comparisons were conducted both between mean positive control coupon mass versus mean test coupon extracted mass, and between mean positive control coupon mass and the sum of test coupon extracted mass and the decontaminant runoff mass.

F-tests were used to determine if the variances of the set of three test coupon mass (µg) results were equal to the set of three positive control coupon mass (µg) results. The null hypothesis that the variances of the two sets were equal was rejected if the F-test p-value was ≤ 0.05. One-tailed,

two-sample Student's *t*-tests (homoscedastic or heteroscedastic based on the F-test result) were then used to determine if the means of the test results were significantly less than the positive controls or not [3]. The null hypothesis that the test coupon and positive control coupon means were equal was rejected if the *t*-test p-value was ≤ 0.05. If multiple pairwise comparisons are performed at a 0.05 significance level, the probability of falsely rejecting a true null hypothesis at least once over all tests is greater than 0.05. Bonferroni corrections for multiple comparisons were therefore applied within each trial dataset to maintain a familywise error rate of 0.05 over all tests within a given dataset and test coupon outcome measurement [4]. Rejecting the null hypothesis represents evidence that fentanyl mass was reduced after the application of the decontaminant.

Additionally, four separate groups of analyses were conducted to test whether there were significant differences in fentanyl recovery between the test conditions of interest. Tukey's multiple comparisons procedure was performed following each of the analyses where more than two conditions were compared [5]. Like the Bonferroni procedure, Tukey's procedure adjusts the p-values of the pairwise comparisons to maintain a familywise error rate of 0.05 per each one-way analysis of variance (ANOVA) model over the multiple comparisons being performed. Tukey's procedure was selected to account for multiple comparisons instead of the Bonferroni corrections because Tukey's procedure typically has a higher power to detect differences between conditions but applies only when all pairwise comparisons are made within a model. The Tukey-adjusted p-values are presented only if significant differences were identified.

Within each condition, the characteristics of fentanyl application to the positive controls are assumed to be the same as the characteristics of application to the test coupons with regard to variability from coupon to coupon and in the average amount of fentanyl applied. Acceptance criteria for the spike control results (average within 80% to 120% of theoretical, <30% RSD) are intended to support this assumption. For accurate comparison of the performance of the decontaminants within each material as described above, the amount of fentanyl applied to the test coupons must be consistent across all conditions being compared within a given analysis. To evaluate consistency of fentanyl application across the three samples per material of each decontaminant, the 58 comparisons described above for the test coupons were repeated using the total mass recoveries from the positive control sets of three samples associated with each of the 31 test conditions.

2.5.1 Group 1: Comparisons of Decontaminant Performance within Material Type

For the first group of ANOVA model with an effect for material (painted drywall paper, powder-coated steel, and wood for 60 + 60-min decontamination periods; bunker gear, HazMat suit, neoprene and Saranex® for 5-min decontamination periods) was fitted separately to each of four decontaminant and decontamination period combinations (Dahlgren Decon™ at 60 + 60 min, Diluted Dahlgren Decon™ at 5 min, pH 5 modified surfactant bleach at 60 + 60 min, pH 5 modified surfactant bleach at 5 min) to determine if there were significant performance differences among the different materials. Materials were challenged with a targeted 1 mg of

fentanyl. Tukey's multiple comparisons procedure was performed for the three (for the 60 + 60-min decontamination periods) or six (for 5-min decontamination periods) possible pairwise comparisons between the three or four materials within each decontaminant/decontamination period group to determine which pairs of materials had mean total mass recoveries that were significantly different from each other.

2.5.2 Group 2: Decontamination Period Effect Analysis Plan

For the second group of analyses, a one-way ANOVA model with an effect for decontamination period (60 min, 60 + 60 min) was fitted separately to each of two decontaminants (Dahlgren Decon™, pH 5 modified surfactant bleach) to determine if there were significant performance differences depending on decontamination time. All data for the 60-min decontamination period were taken from the previous study [1], while all data for the 60 + 60-min decontamination period were taken from the current study. Materials were challenged with a targeted 1 mg of fentanyl. The effect of decontamination period was calculated while collapsing across material condition based on the assumption that there is no material effect. The results of Analysis 1 and from the previous study suggested that there was not a statistically significant effect of material on fentanyl recovery within the group of materials tested for each study. However, it should be noted the materials used in the current study (with a decontamination period of 60 + 60 min) were different from the materials used in the previous study (with a decontamination period of 60 min). It was therefore impossible to isolate the material effect from the decontamination period effect for these materials, and impossible to confirm the assumption of no material effect when comparing acrylic, laminate, painted drywall, and stainless steel (previous study materials [1]) to drywall paper, powder-coated steel, and wood (current study materials).

2.5.3 Group 3: Challenge Additive Effect Analysis Plan

For the third group of analyses, a one-way ANOVA model with an effect for challenge compound (1 mg fentanyl, 1 mg fentanyl + 19 mg ascorbic acid) was fitted separately to each of two decontaminants (Dahlgren Decon™, pH 5 modified surfactant bleach) to determine if there were significant performance differences when the challenge did contain ascorbic acid versus did not contain ascorbic acid. Only the wood material was challenged with both fentanyl and fentanyl + ascorbic acid. Therefore, the model was only fitted to data from the wood material. The decontamination period for all conditions was 60 min.

2.5.4 Group 4: Decontaminant Effect Analysis Plan

For the fourth group of analyses, a one-way ANOVA model with an effect for decontaminant across the two fentanyl decontamination studies (Dahlgren Decon™, EasyDecon DF200, OxiClean™, pH 7 bleach, pH 5 bleach, pH 5 modified surfactant bleach, and water from the previous study [1]; Meth Remover® and ZEP® from the current study) was fitted separately for each positive control and test coupon sample set from the laminate material condition to determine if there were significant performance differences among the decontaminants tested.

Only the laminate material was selected because this was the only material overlapping between the previous and current decontamination study. Materials were challenged with a targeted 1 mg of fentanyl and underwent a 60-min decontamination period.

For all four analysis groups (Sections 2.5.1 – 2.5.4), positive control coupon fentanyl mass (µg) and test coupon total sample fentanyl mass (µg) were evaluated to determine if the total mass recovery data were reasonably normally distributed or if a natural logarithmic transformation would improve adherence to the statistical assumptions of normality and equal variances. The total sample fentanyl mass was calculated as the sum of the coupon extract mass and the decontaminant runoff mass. For the positive control data, a logarithmic transformation improved conformity to the assumptions of normality and equal variance in all but one case. Therefore, fentanyl recovery masses for all positive control samples were log-transformed. For the test sample data, a natural logarithmic transformation also improved conformity to the assumption of normality and equal variances for almost all datasets in analysis groups 1-3. Therefore, the test sample data were log-transformed for the analyses in groups 1-3. However, the data better conformed to the assumptions of normality and equal variances when untransformed for the fourth analysis group, which examined the effect of decontaminant for the laminate material. For this reason and to maintain consistency with the analysis in the previous study, data were left untransformed for the fourth analysis group.

RESULTS

3.1 Methods Demonstration

3.1.1 Fentanyl Delivery (Spiking) Characterization

Under the previous fentanyl decontamination effort [1], the 50-µL Drummond Series 500 Digital Microdispenser with a setting of 1.9 µL on the 50-µL Drummond produced generally accurate and repeatable target 1 mg masses of fentanyl, producing an average percent recovery of 95% with ±14% RSD. This setting was utilized without further verification prior to the method development and decontamination testing. During the method development and decontamination testing, spike controls exceeded the 80% to 120% of the target application. The Drummond setting was gradually adjusted downwards between decontamination tests to get the delivered fentanyl mass within the desired range.

3.1.2 Decontaminant Spray Delivery Characterization

As discussed in Section 2.1.1.3, the sprayer system used to apply the test decontaminants was characterized using each specific decontamination technology to determine system settings necessary to deliver the decontaminants at the target application volume of 60 µL/cm^2.

Empty acrylic runoff boxes (refer to Section 2.2.3.5) were arranged underneath the sprayer as depicted in Figure 6. As described in Section 2.2.3.5, the acrylic boxes were weighed before and after spray delivery of the decontaminants to determine the mass, and thus volume per unit area, of decontaminant delivered.

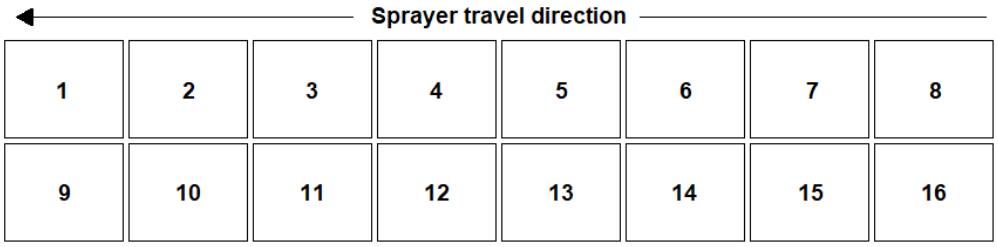


Figure 6. Test sample arrangement under sprayer.

Spray system settings necessary for delivery of each decontaminant at the target volume of 60 µL/cm^2 are summarized in Table 9.

Table 9. Decontaminant Spray Application Settings

Decontaminant	No. of Sprayer Passes	Sprayer Motor Setting	Pass Speed (cm/s)	Sprayer Pressure (psi)	Plastic Box Area (cm^2)	Target Decontaminant Delivery ($\mu L/cm^2$)	Target Decontaminant Weight (g)
Meth Remover®	1	76	7.9	20	18.87	60	1.16
ZEP®		70	6.9				1.16
pH 5 Modified Surfactant Bleach		56	4.6				1.13
Dahlgren Decon™		84	9.1				1.28
Diluted Dahlgren Decon™		75	7.9				1.16

Replicate decontaminant weights measured during characterization, average weights, variabilities (% relative standard deviation [RSD]), and delivery accuracy (percent of target) for each decontaminant are provided in Tables C1 through C5 in Attachment C. A summary of the delivery accuracies for each decontaminant is provided in Table 10.

Table 10. Decontaminant Spray Delivery Summary

Decontaminant	Average Percent of Target (% ± SD)	Lowest Percent of Target (Position) (%)	Highest Percent of Target (Position) (%)
Meth Remover®	111 ± 4	104 (#11)	117 (#13)
ZEP®	95 ± 10	83 (#11)	112 (#6)
pH 5 Modified Surfactant Bleach	99 ± 3	93 (#9)	104 (#14)
Dahlgren Decon™	103 ± 6	92 (#3)	115 (#10)
Diluted Dahlgren Decon™	98 ± 6	86 (#12)	107 (#1)

Results showed that the delivery of the decontaminant was accurate and precise with no evidence for a nonuniform spatial distribution across the 16 coupon locations.

3.1.3 Quench Method Demonstration

The quench method test matrix was intended to demonstrate the adequacy of 3M STS as a quench agent for halting decontamination of fentanyl by the hydrogen peroxide-based decontaminants (Meth Remover® and ZEP®).

As described in Section 2.1.1.4, representative decontaminant runoff sample extracts were prepared by adding 0.99 mL of test decontaminant to 20 mL of IPA with 5 mL of 3M STS solution. Samples were produced in triplicate, and the extracts were post-spiked with a dilute solution of fentanyl. Recovery of fentanyl ≥ 70% of the theoretical post-spiked amount in representative test extract matrix samples (quench samples) during the 72-h delayed analyses of the samples and conformance to the QA/QC criteria for fentanyl-d_5 IS response discussed in Section 4.2.2 and provided in Table 36 would demonstrate the adequacy of the quench method and that no interferences due to the sample matrices were occurring. Results of the quench method scoping test are summarized in Table 11 and Figures 7 and 8.

Table 11. Quench Method Demonstration Test, Average Mass Recovery

Sample Description		Analyses by GC/MS			Analyses by LC-MS/MS			% Recovery vs
		Mass (µg)	Recovery	IS Recovery	Mass (ng)	Recovery	IS Recovery	
Spike Controls	Avg	40	99%	101%	37	93%	105%	Theoretical
	%RSD	3.8%	-	-	3.7%	-	-	
Positive Control	Avg	36	91%	94%	35	93%	102%	SCs
	%RSD	4.3%	-	-	1.1%	-	-	
Meth Remover®	Avg	33	91%	132%	35	100%	100%	PCs
	%RSD	0.7%	-	-	1.9%	-	-	
ZEP®	Avg	33	92%	103%	36	105%	93%	PCs
	%RSD	2.2%	-	-	4.3%			

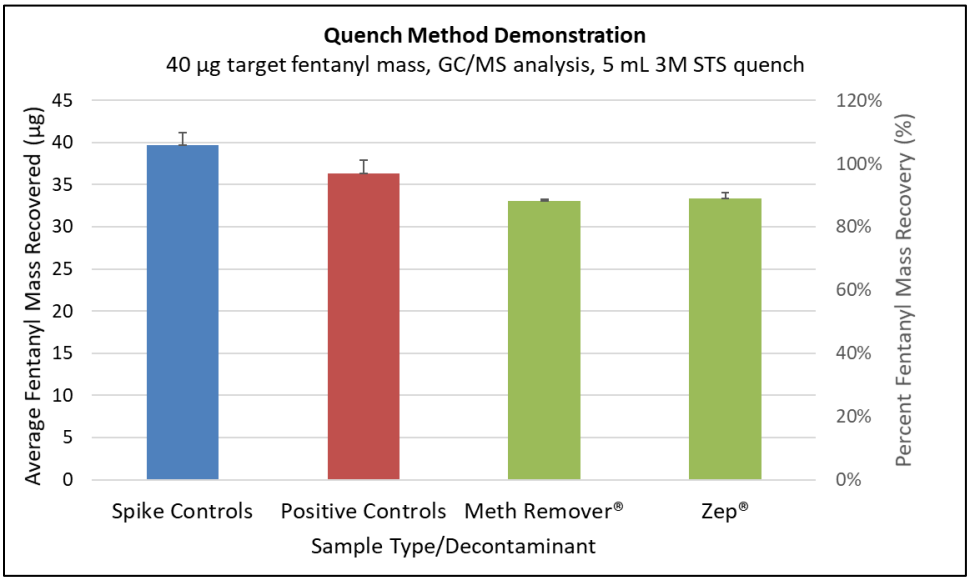


Figure 7. Quench method demonstration test, average percent recovery by GC/MS.

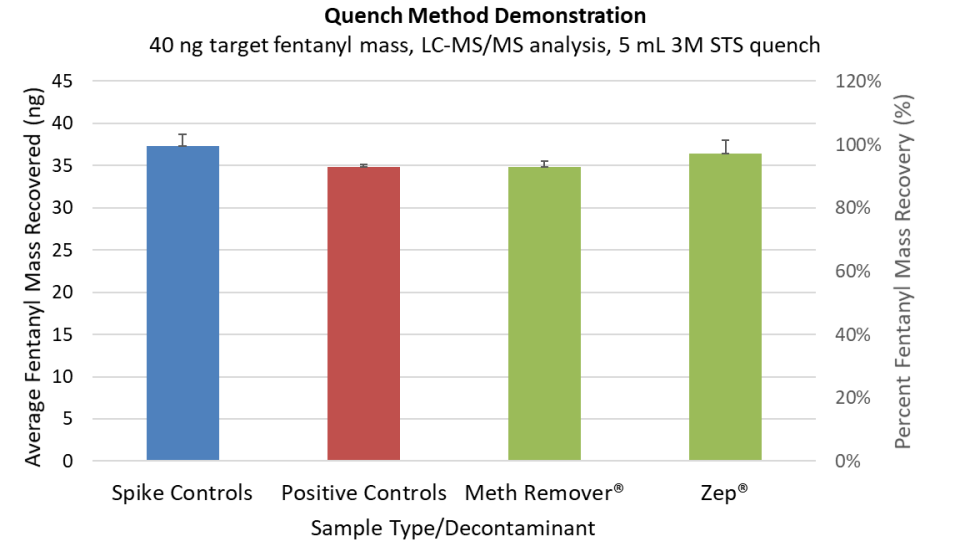


Figure 8. Quench method demonstration test, average percent recovery by LC-MS/MS.

The results suggest that addition of 5 mL of 3M STS with the extraction solvent may be an effective quench for both hydrogen peroxide-containing decontaminants. Recoveries of post-spiked fentanyl in samples containing Meth Remover® and ZEP® ranged from 91% to 92% for the GC/MS results and ranged from 100% to 105% for the LC-MS/MS results, respectively, which satisfy the minimum criterion of 70%. Thus, the results suggested that the defined quench and sample storage procedures (addition of 5 mL of 3M STS to the IPA used to extract coupon and runoff samples and storage of samples at -20°C for up to three days prior to GC/MS or LC-MS/MS analysis) were adequate for preservation of the mass of fentanyl that was post-spiked into the representative coupon and runoff samples extracts.

3.2 Decontamination Efficacy Evaluation – Building Materials

3.2.1 Hydrogen Peroxide-Based Decontaminants

The tests described in Table 2 in Section 2.1.2 evaluated the efficacy of two (2) decontaminants (Meth Remover® and ZEP®) to decontaminate fentanyl on the surface of 10-cm^2 coupons of four (4) materials (painted drywall paper, powder-coated steel, laminate, and wood). Each test included the necessary replicate test and control samples to evaluate efficacy of a single decontaminant on all four material types.

Average spike control recoveries for each test are provided in Table 12. The average amounts recovered exceeded the 80% to 120% of the target application, which may be due to a tighter packing of the fentanyl in the vial leading to higher amounts spiked at the same setting on the Drummond Pipettor from the previous research effort.

Table 12. Decontamination Efficacy Testing, Spike Control Average Recovery

Test Number	Test	Avg Mass (µg)	% RSD	Avg % Recovery (vs theoretical)
1	Meth Remover®	1234	63	122%
2	ZEP®	1398	206	138%

No fentanyl was detected in any laboratory blank samples. Fentanyl was detected in all procedural blank coupon extracts and in runoff samples for all decontaminants, but all were below the criteria provided in Table 34 in Section 4.1. Detections in procedural blank coupon extract and runoff samples were always less than 1% of the associated positive controls for all materials and both decontaminants.

Mass recovery results are summarized in Figure 9. Average fentanyl mass recoveries, standard deviations, and variabilities (%RSD) for replicate test and positive control coupons of all four material types for each decontaminant are provided in Tables D1 and D2 of Attachment D. Average total test sample mass recoveries (mass recovered from extraction of the coupon sample plus mass recovered from extraction of the associated runoff) and percent of the total test sample mass recovery versus the associated positive controls are provided as well. In some instances, solid material (which could potentially be undissolved fentanyl) was observed on the surface of

replicate coupons following the 60-min decontaminant dwell period and prior to solvent extraction of the coupons.

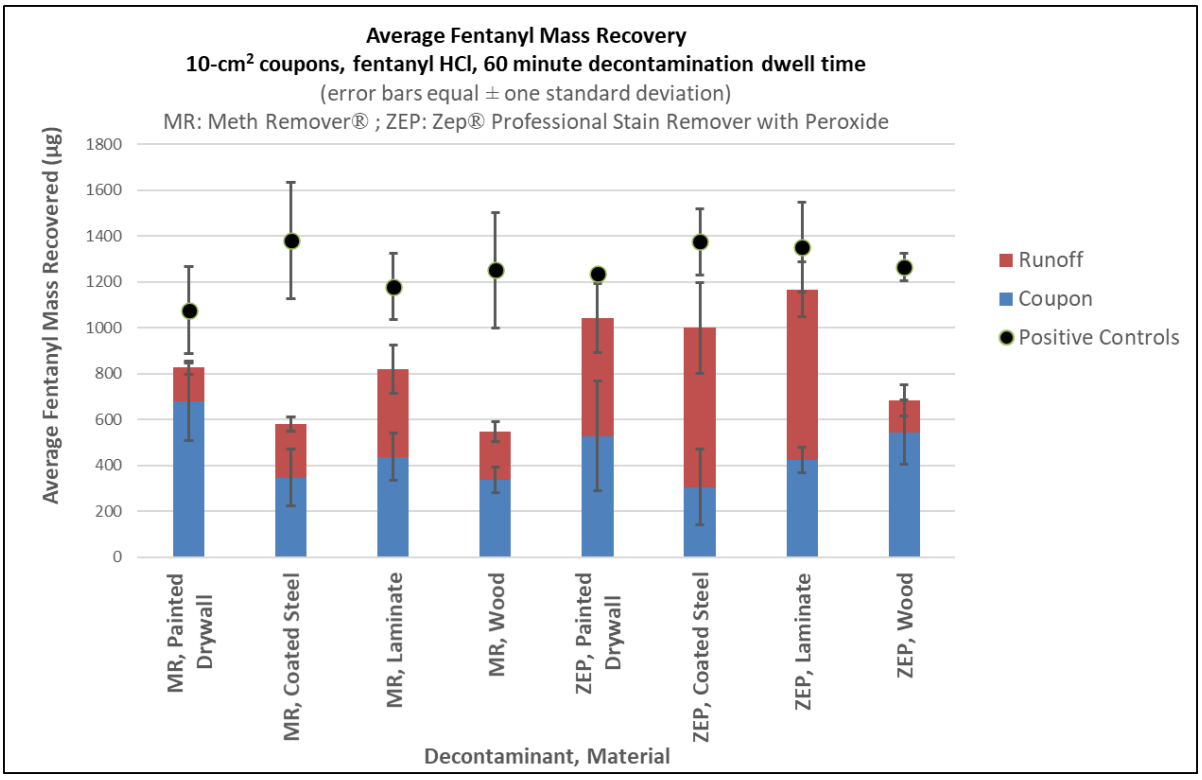


Figure 9. Decontamination efficacy testing, average mass recovery.

Efficacy for each of the decontaminants was calculated by comparing the residual fentanyl mass on the test coupons against the fentanyl mass recovered from the associated positive control. Two efficacy values were calculated – one using only the masses recovered from extraction of the test and positive control coupons (efficacy thus does not differentiate between physical removal and chemical decontamination), and another value wherein the fentanyl mass measured in the runoff extract was added to the test sample coupon mass before comparison to the positive control (to attempt to decouple physical removal from chemical decontamination).

Average percent efficacies (both excluding and including average runoff mass) for each material/decontaminant combination are summarized in Figure 10. Efficacy values are also tabulated in Table D3, Attachment D.

Efficacies ranged from 23% to 58% and from 14% to 46% for the Meth Remover® and ZEP® products, respectively, when considering only the chemical degradation by the decontaminant.

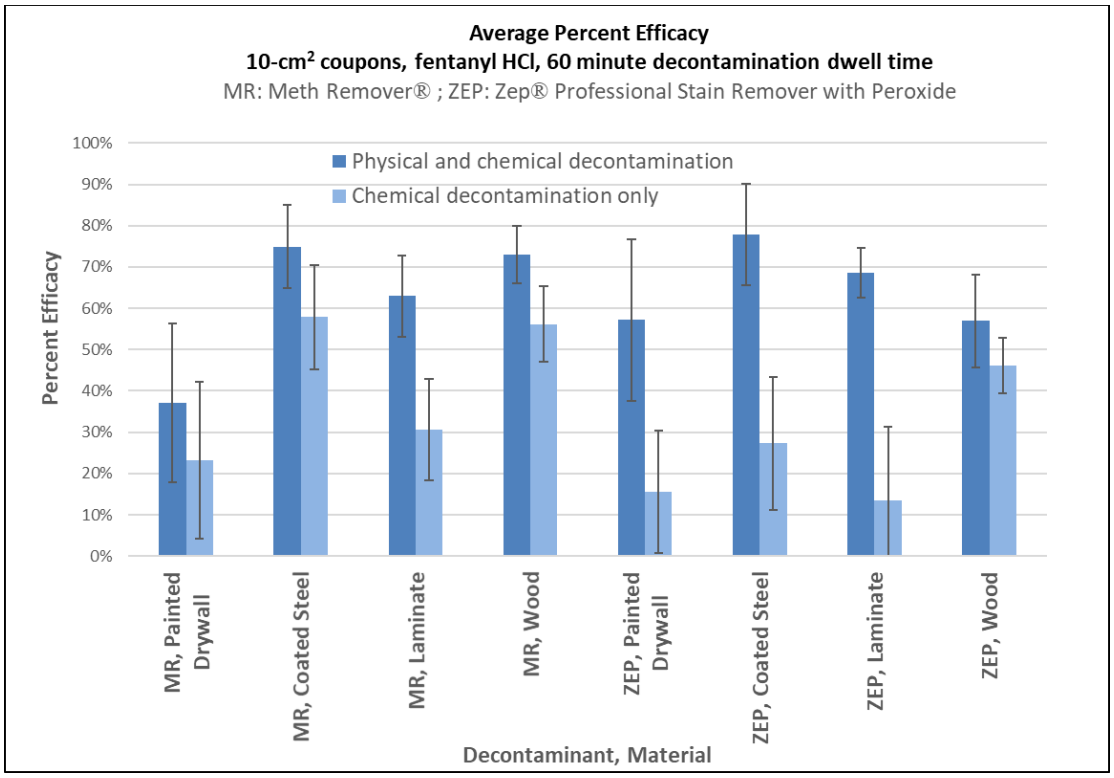


Figure 10. Decontamination efficacy testing, average percent efficacy.

3.2.2 Decontamination Efficacy Evaluation – Reapplication of Decontaminants

The two tests described in Table 3 in Section 2.1.2 evaluated the efficacy of two (2) decontaminants (Dahlgren Decon™ and pH 5 modified surfactant bleach) to decontaminate fentanyl directly on the surface of 10-cm^2 coupons. For these two tests, the recovered fentanyl amounts are for the test coupons to which the decontaminant was applied twice, each with a 60-min dwell time. Each test included three (3) materials (painted drywall paper, powder-coated steel, and wood) with a fourth coupon consisting of the same wood but in the presence of ascorbic acid as a benign additive to the fentanyl on the surface. Each test included the necessary replicate test and control samples to evaluate efficacy of a single decontaminant on all material types.

Average spike control recoveries for each test are provided in Table 13. The average amounts recovered exceeded the 80% to 120% of the target application, which may be due to a tighter packing of the fentanyl in the vial leading to higher amounts spiked at the same setting on the Drummond Pipettor from the previous research effort.

Table 13. Decontamination Efficacy Testing, Spike Control Average Recovery

Test Number	Test	Spike Type	Avg Mass (µg)	% RSD	Avg % Recovery (vs theoretical)
1	Dahlgren Decon™	No AA	1549	10%	153%
		With AA	1703	5.6%	168%
2	pH 5 modified surfactant bleach	No AA	1509	10%	149%
		With AA	1377	6.6%	136%

AA: Ascorbic Acid

No fentanyl was detected in any laboratory blank samples. Fentanyl was also not detected in the procedural blank coupon extracts and in runoff samples associated with the Dahlgren Decon™ applications. For the pH 5 modified surfactant bleach decontamination test, fentanyl was detected in the procedural blanks, but all were below the criteria provided in Table 34 in Section 4.1. Detections in procedural blank coupon extracts and runoff samples were always less than 1% of the associated positive controls for all materials and pH 5 modified surfactant bleach.

Mass recovery results are summarized in Figure 11. Average fentanyl mass recoveries, standard deviations, and variabilities (%RSD) for replicate test and positive control coupons of all three material types and the fourth material with ascorbic acid for each decontaminant are provided in Tables D4 and D5, Attachment D. Average total test sample mass recoveries (mass recovered from extraction of the coupon sample plus mass recovered from extraction of the associated runoff) and percent of the total test sample mass recovery versus the associated positive controls are provided as well. In some instances, solid material (which could potentially be undissolved fentanyl) was observed on the surface of replicate coupons following the first 60-min decontaminant dwell time or after the double 60-min decontaminant dwell periods and prior to solvent extraction of the coupons.

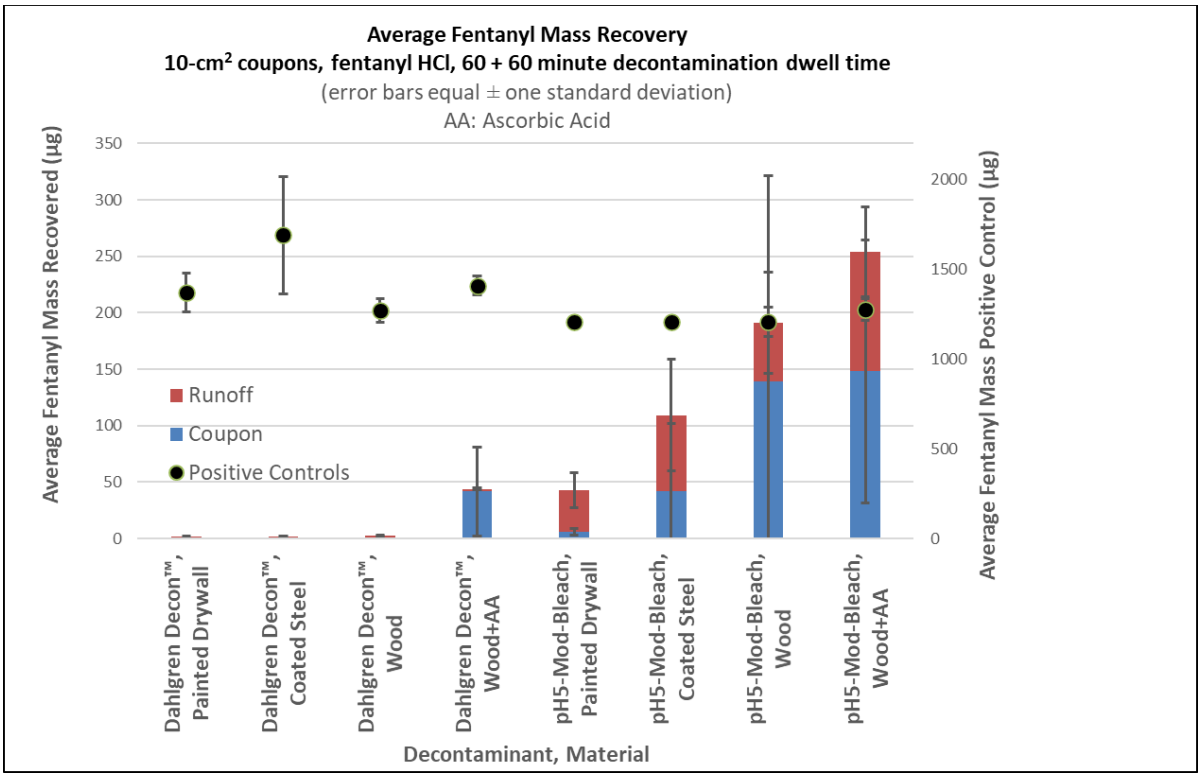


Figure 11. Decontamination efficacy testing, average mass recovery.

Efficacy for each of the decontaminants was calculated by comparing the residual fentanyl mass on the test coupons against the fentanyl mass recovered from the associated positive control. Once again, two efficacy values were calculated – one using only the masses recovered from extraction of the test and positive control coupons (efficacy thus does not differentiate between physical removal and chemical decontamination), and another value wherein the fentanyl mass measured in the runoff extract was added to the test sample coupon mass before comparison to the positive control (to attempt to decouple physical removal from chemical decontamination).

Average percent efficacies (both excluding and including average runoff mass) for each material/decontaminant combination are summarized in Figure 12. Efficacy values are also tabulated in Table D6, Attachment D.

Efficacies ranged from 88% to more than 99.9% and from 80% more than 99.9% for the Dahlgren Decon™ and modified pH 5 modified surfactant bleach products, respectively, when considering only the chemical degradation by the decontaminant. Recovered fentanyl mass was higher in the presence of ascorbic acid.

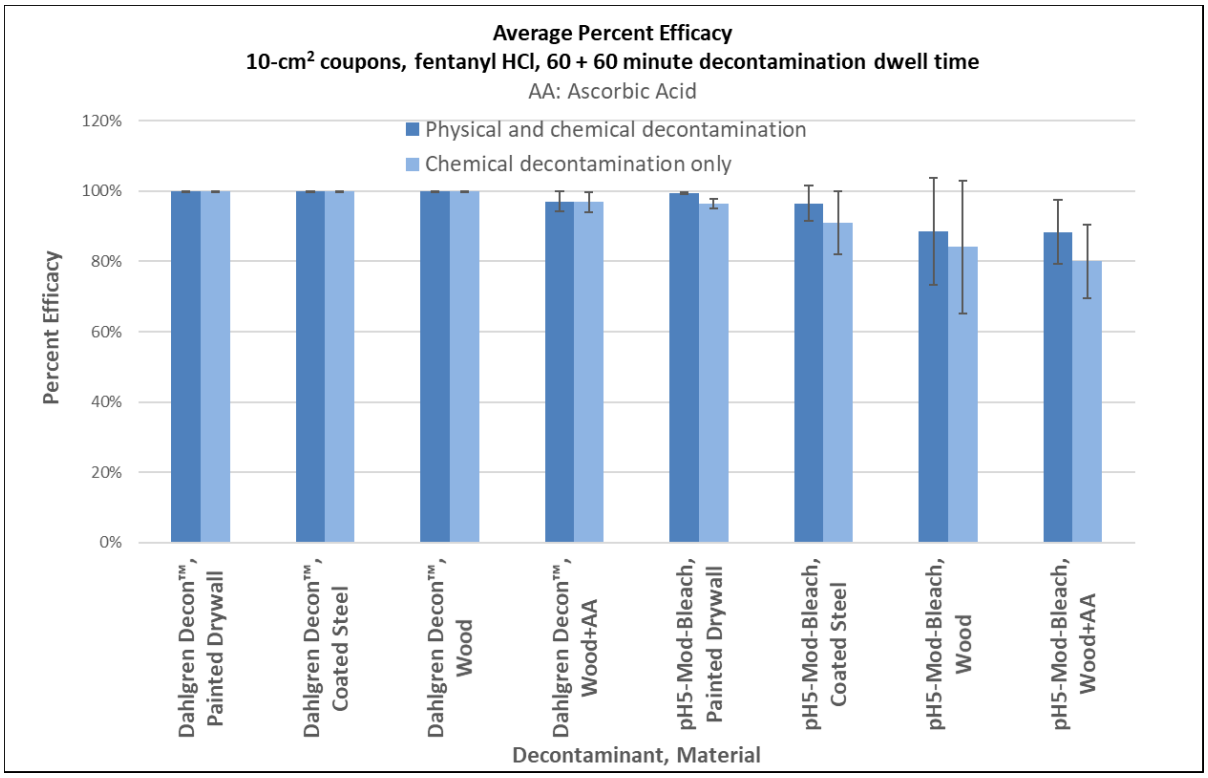


Figure 12. Decontamination efficacy testing, average percent efficacy.

3.2.3 Decontamination Efficacy Evaluation – PPE/Gear Materials

The first test described in Table 4 in Section 2.1.2 evaluated the efficacy of dilute Dahlgren Decon™ as a function of time (1-15 min) for three materials (Saranex®, HazMat suit, and bunker gear). Average mass recovered for the spike controls (n=3) was 1366 µg with a 14% RSD. As mentioned before, the amount spiked was higher (135% of theoretical mass spiked) than expected.

No fentanyl was detected in any laboratory or procedural blank samples taken at the longest 15-min dwell time.

Recovered fentanyl mass (no replicates) for the five timepoints (coupon, runoff, and sum) are tabulated in Table 14. Positive controls are reported as recovered mass at 0 min (start fentanyl mass on material).

Table 14. Average Mass Recovery, Diluted Dahlgren Decon™

Time (min)	Saranex®			HazMat suit			Bunker gear		
	Coupon Mass (µg)	Runoff Mass (µg)	Sum (µg)	Coupon Mass (µg)	Runoff Mass (µg)	Sum (µg)	Coupon Mass (µg)	Runoff Mass (µg)	Sum (µg)
0	1205	-	1205	1360	-	1360	1127	-	1127
1	36	4.1	40	11	2.2	13	15	4.6	19
3	0.63	2.0	2.7	14	0.67	15	316 [A]	2.0	318
6	0.58	7.2	7.8	47 [A]	1.4	48	25	5.5	31
10	4.6	2.8	7.5	11	1.5	12	41 [A]	2.3	43
15	20	1.9	22	0.31	0.53	0.84	61 [A]	0.87	62

[A] Solid material observed on coupon surface following application.

Recovered fentanyl mass from coupon and runoff combined was in general less than 50 µg except for one outlier of 318 µg which was accompanied by observed solid material remaining on the coupon surface. A recovered total mass of 50 µg equates to approximately a 96% efficacy, which can be reached within minutes following interaction with the diluted Dahlgren Decon™ decontaminant on these gear/PPE materials.

The second and third tests described in Table 4 in Section 2.1.2 evaluated the efficacy of two (2) decontaminants (Dahlgren Decon™ and pH 5 modified surfactant bleach) with a 5-min dwell time of the solution to decontaminate fentanyl directly on the surface of 10-cm^2 gear/PPE material coupons. Each test included the necessary replicate test and control samples to evaluate efficacy of a single decontaminant on all material types.

Average spike control recoveries for each test are provided in Table 15. The average amounts recovered slightly exceeded the 80% to 120% of the target application, which may be due to a tighter packing of the fentanyl in the vial leading to higher amounts spiked. However, this exceedance of the target application does not impact the decontamination process itself as the amount of decontaminant significantly exceeds the amount of fentanyl on the material.

Table 15. Decontamination Efficacy Testing, Spike Control Average Recovery

Test Number	Test	Avg Mass (µg)	% RSD	Avg % Recovery (vs theoretical)
1	pH 5 modified surfactant bleach	1280	15%	130%
2	Diluted Dahlgren Decon™	1261	25%	128%

No fentanyl was detected in any laboratory blank samples. Fentanyl was also not detected in the procedural blank coupon extracts and in runoff samples associated with the Dahlgren Decon™ applications. For the pH 5 modified surfactant bleach decontamination test, fentanyl was detected in the procedural blanks, but all were well below the criteria provided in Table 34 in Section 4.1. Detections in procedural blank coupon extract and runoff samples were always less than 0.1% of the associated positive controls for all materials and pH 5 modified surfactant bleach.

Mass recovery results are summarized in Figure 13. Average fentanyl mass recoveries, standard deviations, and variabilities (%RSD) for replicate test and positive control coupons of all four

PPE/gear material types are provided in Tables D7 and D8 in Attachment D. Average total test sample mass recoveries (mass recovered from extraction of the coupon sample plus mass recovered from extraction of the associated runoff) and percent of the total test sample mass recovery versus the associated positive controls are provided as well. As expected, based on previous research results, in some instances, solid material (which could potentially be undissolved fentanyl) was observed on the surface of replicate coupons following the 5-min decontaminant dwell time and prior to solvent extraction of the coupons

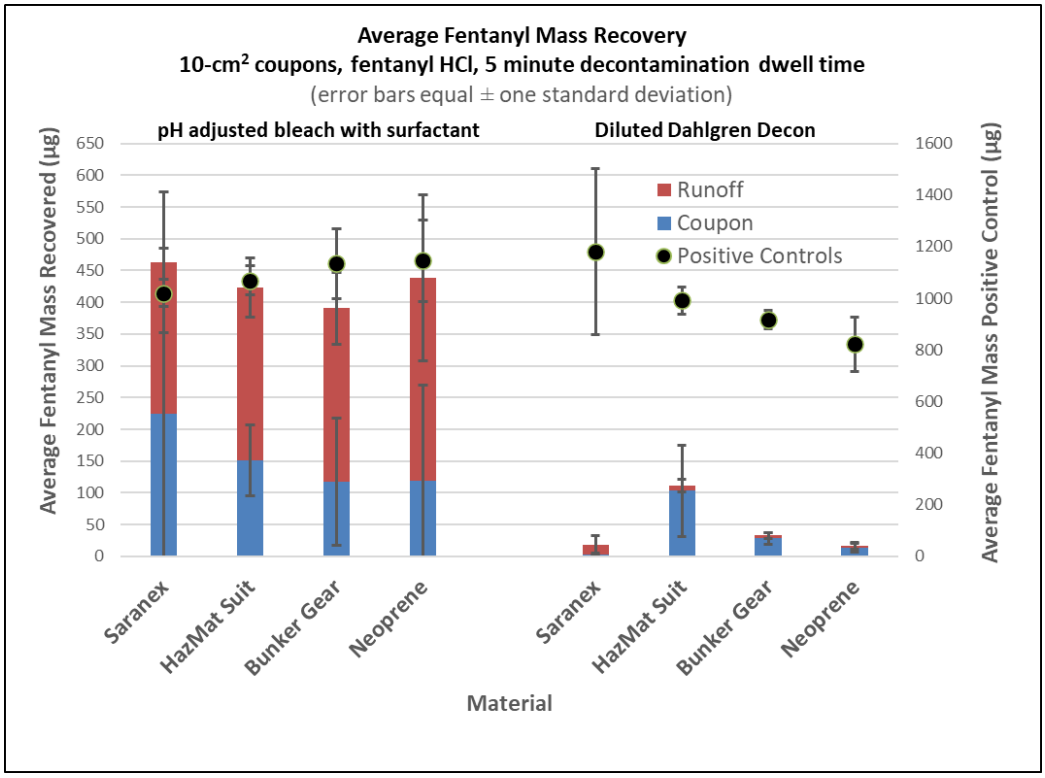


Figure 13. Decontamination efficacy testing of PPE materials, average mass recovery.

Efficacy for each decontaminant was calculated by comparing the residual fentanyl mass on the test coupons against the fentanyl mass recovered from the associated positive control. Two efficacy values were calculated – one using only the masses recovered from extraction of the test and positive control coupons (efficacy thus does not differentiate between physical removal and chemical decontamination), and another value wherein the fentanyl mass measured in the runoff extract was added to the test sample coupon mass before comparison to the positive control (to attempt to decouple physical removal from chemical decontamination).

Average percent efficacies (both excluding and including average runoff mass) for each material/decontaminant combination are summarized in Figure 14. Efficacy values are also tabulated in Table D9, Attachment D.

Efficacies ranged from 55% to 66% and from 89% to 98% for modified pH 5 modified surfactant bleach and Dahlgren Decon™ products, respectively, when considering only the chemical degradation by the decontaminant.

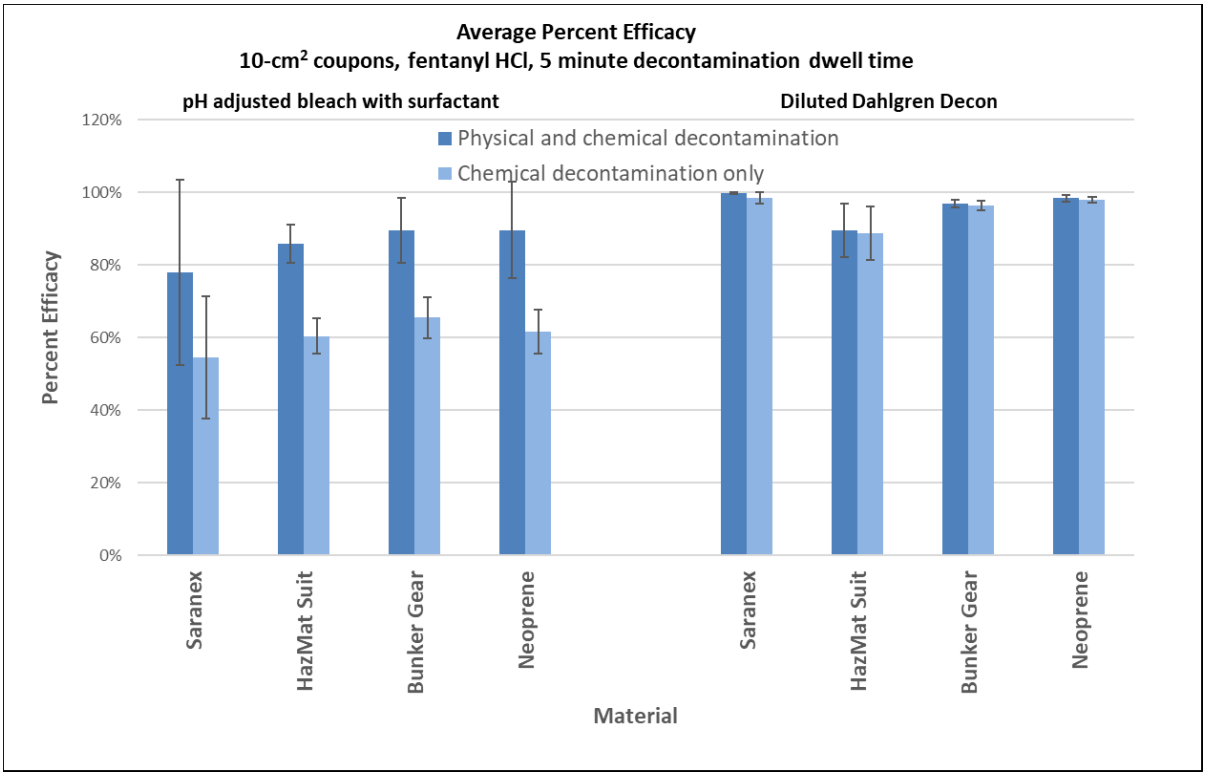


Figure 14. Decontamination efficacy testing, average percent efficacy.

3.3 ANOVA Results

The mean mass for each material from the ANOVA model is presented for each decontaminant group in Tables 16-33. Estimated geometric mean masses are presented for the analyses with log-transformed data, and estimated arithmetic mean masses are presented for each analysis with untransformed data.

3.3.1 Positive Control Comparison Results

Tables 16 through 24 present the estimated geometric mean mass recoveries of the positive controls corresponding to each test condition, sorted from lowest to highest mean mass recovery. Results from the Tukey-adjusted multiple comparisons of the geometric mean total mass recovery between conditions based on the ANOVA models are also shown. The capital letters in the "Similarity Designation" column indicate the statistical similarity of the mean mass recovery of a condition to the mean mass recovery of the other conditions tested. All rows with the same similarity designation value are not statistically significantly different from each other, while rows that did not share any similarity designation values are significantly different. For example, in Table 24, the decontaminant Dahlgren Decon™ has the similarity designation value A, Water has the similarity designation value AB, and pH 5 modified surfactant bleach has the similarity designation value BCD, implying that the positive control mean for Dahlgren Decon™ was not significantly different from the positive control mean for Water (they share the "A" designation), but that the positive control means were different for Dahlgren Decon™ and pH 5 modified

surfactant bleach with Surfactant (they do not share a similarity designation). However, the positive control means were not significantly different for Water and pH 5 modified surfactant bleach (they share the "B" similarity designation).

3.3.1.1 Group 1 Material Effect Analysis Effects

There were no significant differences in fentanyl recovery mass for the positive controls between any pairs of materials within a given decontaminant trial (Dahlgren Decon™ at 60 + 60 min, Diluted Dahlgren Decon™ at 5 min, or pH 5 bleach with surfactant at 60 + 60 min and 5 min). Refer to Tables 16 through 19.

3.3.1.2 Group 2 Decontamination Period Effect Analysis Results

Collapsing across materials, positive control fentanyl recovery masses for a 60-min decontamination period [1] were found to be statistically significantly different from the fentanyl recovery masses of a 60 + 60-min decontamination period for both the Dahlgren Decon™ and the pH 5 modified surfactant bleach decontaminants. We can therefore not say that fentanyl was applied equally across the two fentanyl decontamination studies materials. Refer to Tables 20 and 21.

3.3.1.3 Group 3 Challenge Additive Effect Analysis Results

Positive control materials challenged with 1 mg fentanyl only were found to have a significantly different fentanyl recovery mass than materials challenged with 1 mg fentanyl + 19 mg ascorbic acid for the Dahlgren Decon™ test condition. We cannot say that fentanyl was applied equally in the 1-mg fentanyl and the 1-mg fentanyl + 19 mg ascorbic acid test conditions with Dahlgren Decon™. Positive control fentanyl recovery mass was not found to be different for the 1 mg fentanyl challenge compound versus the 1 mg fentanyl + 19 mg ascorbic acid compound for the pH 5 modified surfactant bleach decontaminant test condition. Refer to Tables 22 and 23.

3.3.1.4 Group 4 Decontaminant Effect Analysis Results

In the previous fentanyl decontamination study [1], only EasyDecon DF200 and pH 5 modified surfactant bleach resulted in significantly different fentanyl recovery masses for the positive control samples on the laminate material. The positive control geometric mean recovery masses for EasyDecon DF200 and pH 5 modified surfactant bleach, which were identified as significantly different in the previous study, remained significantly different for the current analysis. Additionally, the positive control fentanyl recovery mass for Meth Remover® differs significantly from the positive control fentanyl recovery mass of EasyDecon DF200, and fentanyl recovery mass for ZEP® differs significantly from the positive control fentanyl recovery mass of seven of the nine other decontaminant positive controls. We cannot conclude that fentanyl was applied equally across the laminate in both studies. Refer to Table 24.

Group 1:

Table 16. ANOVA Results for Dahlgren Decon™ at 60 + 60 min (Positive Controls)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	Tukey-Adjusted p-Value *
Dahlgren Decon™	Wood	60 + 60	fentanyl	A	1267	No significant differences.
Dahlgren Decon™	Painted drywall	60 + 60	fentanyl	A	1368	
Dahlgren Decon™	Coated steel	60 + 60	fentanyl	A	1668	

* There were no significant differences between any pairs of materials.

Table 17. ANOVA Results for Diluted Dahlgren Decon™ at 5 min (Positive Controls)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	Tukey-Adjusted p-Value *
Diluted Dahlgren Decon™	Neoprene	5	fentanyl	A	818	No significant differences.
Diluted Dahlgren Decon™	Bunker gear	5	fentanyl	A	916	
Diluted Dahlgren Decon™	HazMat suit	5	fentanyl	A	991	
Diluted Dahlgren Decon™	Saranex®	5	fentanyl	A	1150	

* There were no significant differences between any pairs of materials.

Table 18. ANOVA Results for pH 5 Modified Surfactant Bleach at 60 + 60 min (Positive Controls)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	Tukey-Adjusted p-Value *
pH 5 modified surfactant bleach	Wood	60 + 60	fentanyl	A	1203	No significant differences.
pH 5 modified surfactant bleach	Painted drywall	60 + 60	fentanyl	A	1208	
pH 5 modified surfactant bleach	Coated steel	60 + 60	fentanyl	A	1208	

* There were no significant differences between any pairs of materials.

Table 19. ANOVA Results for pH 5 Modified Surfactant Bleach at 5 min (Positive Controls)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	Tukey-Adjusted p-Value *
pH 5 modified surfactant bleach	Saranex®	5	fentanyl	A	1020	No significant differences.
pH 5 modified surfactant bleach	HazMat suit	5	fentanyl	A	1068	
pH 5 modified surfactant bleach	Bunker gear	5	fentanyl	A	1129	
pH 5 modified surfactant bleach	Neoprene	5	fentanyl	A	1138	

* There were no significant differences between any pairs of materials.

Group 2:

Table 20. ANOVA Results for Dahlgren Decon™, Collapsed over Multiple Materials (Positive Controls)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	p-Value
Dahlgren Decon™ *	Multiple	60	fentanyl	A	859	<0.0001 (60 min < 60 + 60 min)
Dahlgren Decon™	Multiple	60 + 60	fentanyl	B	1425	

* Data from previous study [1].

Table 21. ANOVA Results for pH 5 Modified Surfactant Bleach, Collapsed over Multiple Materials (Positive Controls)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	p-Value
pH 5 modified surfactant bleach *	Multiple	60	fentanyl	A	947	<0.0001 (60 min < 60 + 60 min)
pH 5 modified surfactant bleach	Multiple	60 + 60	fentanyl	B	1206	

* Data from previous study [1].

Group 3:

Table 22. ANOVA Results for Dahlgren Decon™ on Wood at 60 + 60 min (Positive Controls)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	p-Value
Dahlgren Decon™	Wood	60 + 60	fentanyl	A	1267	0.0464 (fentanyl < fentanyl + ascorbic acid)
Dahlgren Decon™	Wood	60 + 60	fentanyl + ascorbic acid	B	1406	

Table 23. ANOVA Results for pH 5 Modified Surfactant Bleach on Wood at 60 + 60 min (Positive Controls)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	p-Value *
pH 5 modified surfactant bleach	Wood	60 + 60	fentanyl	A	1203	No significant differences.
pH 5 modified surfactant bleach	Wood	60 + 60	fentanyl + ascorbic acid	A	1273	

Group 4:

Table 24. ANOVA Results on Laminate at 60 min (Positive Controls)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	Tukey-Adjusted p-Value
EasyDecon DF200*	Laminate	60	fentanyl	A	736	0.0337 (DF200 < Meth Remover®) 0.0215 (DF200 < pH 5 bleach) 0.0039 (DF200 < ZEP®) 0.0140 (OxiClean™ < ZEP®) 0.0247 (Dahlgren Decon™ < ZEP®) 0.0398 (Water < ZEP®) 0.0229 (pH 7 bleach < ZEP®) 0.0429 (pH 5 modified surfactant bleach < ZEP®)
OxiClean™ *	Laminate	60	fentanyl	A	796	
pH 7 bleach*	Laminate	60	fentanyl	A	820	
Dahlgren Decon™ *	Laminate	60	fentanyl	A	824	
Water*	Laminate	60	fentanyl	AB	849	
pH 5 modified surfactant bleach	Laminate	60	fentanyl	BCD	854	
Meth Remover®	Laminate	60	fentanyl	CD	1174	
pH 5 bleach*	Laminate	60	fentanyl	DE	1208	
ZEP®	Laminate	60	fentanyl	E	1341	

* Data from previous study [1].

3.3.2 Comparison of Test Sample Results

Tables 25 through 33 present the mean mass recoveries of the ANOVA models for each of the analysis groups ordered from lowest to highest mean, along with the significant Tukey-adjusted comparisons. The estimated geometric mean is presented in Tables 25 through 32, corresponding to the natural log-transformed data in analyses for Group 1 through Group 3. The estimated arithmetic mean is presented in Table 33, corresponding to the untransformed data for analysis of Group 4. As in Tables 16 through 24, the characters in the "Similarity Designation" column indicate the statistical similarity of the mean total mass recovery of a given condition to the mean total mass recovery of the other conditions tested. All rows with the same similarity designation value are not statistically significantly different from each other.

3.3.2.1 Group 1 Material Effect Results

There were no significant differences in fentanyl recovery mass between any pairs of materials within a given decontaminant (Dahlgren Decon™ at 60 + 60 min, Diluted Dahlgren Decon™ at 5 min, or pH 5 modified surfactant bleach with surfactant at 60 + 60 min and 5 min). Refer to Tables 25 through 28. Hence, there are no significant impacts of the materials on the efficacies within the limitations of this study.

3.3.2.2 Group 2 Decontamination Period Effect Analysis Results

Collapsing across materials, fentanyl recovery mass for a 60-min decontamination period was not found to be statistically significantly different from the fentanyl recovery mass of a 60 + 60 min decontamination period for the Dahlgren Decon™ or pH 5 modified surfactant bleach decontaminants. The reason that no significant differences were observed may be questioned, however, due to significant differences between positive control masses for the 60 min and 60 + 60 min test conditions (see Limitations Section 3.3.3). Refer to Tables 29 and 30.

3.3.2.3 Group 3 Challenge Additive Effect Analysis Results

Materials challenged with 1 mg fentanyl only were found to have a significantly lower fentanyl recovery mass than materials challenged with 1 mg fentanyl + 19 mg ascorbic acid for the Dahlgren Decon™ test condition. The cause of this significant difference may be questioned, however, due to significant differences between positive control masses for the 1 mg fentanyl and 1 mg fentanyl + 19 mg ascorbic acid conditions (see Limitations Section 3.3.3). Fentanyl recovery mass was not found to be different for the 1 mg fentanyl challenge compound versus the 1 mg fentanyl + 19 mg ascorbic acid compound for the pH 5 modified surfactant bleach decontaminant. Refer to Tables 31 and 32.

3.3.2.4 Group 4 Decontaminant Effect Results

In the previous decontamination study [1], Water and OxiClean™ had the highest fentanyl recovery masses of any of the seven decontaminants tested and were the most statistically

different of the decontaminants. Water had been significantly different from four and OxiClean™ had been significantly different from five of the other decontaminants tested on laminate.

For the current analysis, Meth Remover® and ZEP® numerically resulted in the highest fentanyl recovery masses. Meth Remover® was significantly different from five of the other eight decontaminants tested (all decontaminants except for Water, OxiClean™, and ZEP®). ZEP® was different from all other decontaminants tested except for Meth Remover®. The cause of significant differences between Meth Remover® or ZEP® and the decontaminants from the previous study [1] may be questioned, however, due to significant differences between the positive control masses for Meth Remover® and ZEP® compared to the remaining decontaminants. Refer to Table 33.

Group 1:

Table 25. ANOVA Results for Dahlgren Decon™ at 60 + 60 min (Test Samples)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	Tukey-Adjusted p-Value *
Dahlgren Decon™	Painted drywall	60 + 60	fentanyl	A	2	No significant differences.
Dahlgren Decon™	Coated steel	60 + 60	fentanyl	A	2	
Dahlgren Decon™	Wood	60 + 60	fentanyl	A	2	

* There were no significant differences between any pairs of materials.

Table 26. ANOVA Results for Diluted Dahlgren Decon™ at 5 min (Test Samples)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	Tukey-Adjusted p-Value *
Diluted Dahlgren Decon™	Saranex®	5	fentanyl	A	12	No significant differences.
Diluted Dahlgren Decon™	Neoprene	5	fentanyl	A	15	
Diluted Dahlgren Decon™	Bunker gear	5	fentanyl	A	32	
Diluted Dahlgren Decon™	HazMat suit	5	fentanyl	A	86	

* There were no significant differences between any pairs of materials.

Table 27. ANOVA Results for pH 5 Modified Surfactant Bleach at 60 + 60 min (Test Samples)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	Tukey-Adjusted p-Value *
pH 5 modified surfactant bleach	Painted drywall	60 + 60	fentanyl	A	40	No significant differences.
pH 5 modified surfactant bleach	Coated steel	60 + 60	fentanyl	A	77	
pH 5 modified surfactant bleach	Wood	60 + 60	fentanyl	A	118	

* There were no significant differences between any pairs of materials.

Table 28. ANOVA Results for pH 5 Modified Surfactant Bleach at 5 min (Test Samples)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	Tukey-Adjusted p-Value *
pH 5 modified surfactant bleach	Bunker gear	5	fentanyl	A	389	No significant differences.
pH 5 modified surfactant bleach	HazMat suit	5	fentanyl	A	422	
pH 5 modified surfactant bleach	Neoprene	5	fentanyl	A	437	
pH 5 modified surfactant bleach	Saranex®	5	fentanyl	A	444	

* There were no significant differences between any pairs of materials.

Group 2:

Table 29. ANOVA Results for Dahlgren Decon™, Collapsed over Multiple Materials (Test Samples)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	p-Value *
Dahlgren Decon™	Multiple	60 + 60	fentanyl	A	2	No significant differences.
Dahlgren Decon™ **	Multiple	60	fentanyl	A	5	

* There were no significant differences between the decontamination periods.

** Data from previous study [1].

Table 30. ANOVA Results for pH 5 Modified Surfactant Bleach, Collapsed over Multiple Materials (Test Samples)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	p-Value *
pH 5 modified surfactant bleach **	Multiple	60	fentanyl	A	35	No significant differences.
pH 5 modified surfactant bleach	Multiple	60 + 60	fentanyl	A	71	

* There were no significant differences between the decontamination periods.

** Data from previous study [1].

Group 3:

Table 31. ANOVA Results for Dahlgren Decon™ on Wood at 60 + 60 min (Test Samples)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	p-Value
Dahlgren Decon™	Wood	60 + 60	fentanyl	A	2	0.0124 (fentanyl < fentanyl + ascorbic acid)
Dahlgren Decon™	Wood	60 + 60	fentanyl + ascorbic acid	B	33	

Table 32. ANOVA Results for pH 5 Modified Surfactant Bleach on Wood at 60 + 60 min (Test Samples)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Geometric Mean Mass Recovery (µg)	p-Value *
pH 5 modified surfactant bleach	Wood	60 + 60	fentanyl	A	118	No significant differences.
pH 5 modified surfactant bleach	Wood	60 + 60	fentanyl + ascorbic acid	A	233	

* There were no significant differences between the challenge compounds.

Group 4:

Table 33. ANOVA Results on Laminate at 60 min (Test Samples)

Decontaminant	Material	Period (min)	Challenge Compound	Similarity Designation	Arithmetic Mean Mass Recovery (µg)	Tukey-Adjusted p-Value
pH 5 modified surfactant bleach	Laminate	60	fentanyl	A	64	0.0050 (pH 5 modified surfactant bleach < Water) 0.0010 (pH 5 modified surfactant bleach < OxiClean™) 0.0002 (pH 5 modified surfactant bleach < Meth Remover®) <0.0001 (pH 5 modified surfactant bleach < ZEP®) 0.0056 (DF200 < Water) 0.0011 (DF200 < OxiClean™) 0.0002 (DF200 < Meth Remover®) 0.0001 (DF200 < ZEP®) 0.0088 (pH 5 bleach < Water) 0.0017 (pH 5 bleach < OxiClean™) 0.0003 (pH 5 bleach < Meth Remover®) <0.0001 (pH 5 bleach < ZEP®) 0.0106 (Dahlgren Decon™ < Water) 0.0020 (Dahlgren Decon™ < OxiClean™) 0.0004 (Dahlgren Decon™ < Meth Remover®) <0.0001 (Dahlgren Decon™ < ZEP®) 0.0102 (pH 7 bleach < OxiClean™) 0.0024 (pH 7 bleach < Meth Remover®) <0.0001 (pH 7 bleach < ZEP®) 0.0064 (Water < ZEP®) 0.0421 (OxiClean™ < ZEP®)
EasyDecon DF200*	Laminate	60	fentanyl	A	72	
pH 5 bleach*	Laminate	60	fentanyl	A	102	
Dahlgren Decon™ *	Laminate	60	fentanyl	A	114	
pH 7 bleach*	Laminate	60	fentanyl	AB	223	
Water*	Laminate	60	fentanyl	BCD	628	
OxiClean™ *	Laminate	60	fentanyl	CD	739	
Meth Remover®	Laminate	60	fentanyl	DE	818	
ZEP®	Laminate	60	fentanyl	E	1168	

* Data from previous study [1].

3.3.3 Limitations of Statistical Analysis

The significant differences between the positive controls for the various test conditions is an important limitation for the current analysis, especially when comparing the results obtained in this study against the results from previous study [1]. The positive control fentanyl recovery masses differed significantly for 11 of the 58 total comparisons with 8 of the 36 comparisons between the two studies, suggesting that fentanyl application was mostly inconsistent across the two studies. This inconsistency could result in artificial differences being detected if conditions that were otherwise equal received significantly different amounts of fentanyl and could also conceal differences between conditions if conditions that truly have lower fentanyl recovery mass received more fentanyl and conditions that truly had greater fentanyl recovery mass received less.

Further, the assumption of no material effect that was made to justify collapsing the data across materials in the analysis of Group 2 could not be completely assessed. While there is not strong evidence of differences between material groups within the past study [1] and results discussed here, the materials tested at the 60-min decontamination period [1] differed from the materials tested at a 60 + 60-min period. Therefore, the differences between the materials in the 60-min condition [1] and 60 + 60-min condition could not be assessed separately from the differences between the decontamination periods. The lack of significant differences between 60 versus 60 + 60-min decontamination periods could be due to no true differences between decontamination periods or could be due to material differences and decontamination period differences balancing out.

QUALITY ASSURANCE/QUALITY CONTROL

Quality objectives and performance criteria described in the sections below provide the requirements for determining the adequacy of data generated during this project. Methods were considered acceptable and valid data were assumed if the data quality objectives for the test measurements were met, and the Technical Systems Audit (TSA), Performance Evaluation (PE), and data quality audits demonstrated acceptable results, as described in Sections 4.5, 4.6, and 4.7. Accuracy was ensured by the calibration of the instruments. The PE audits further confirmed that valid data were generated (refer to Section 4.6). The consistently higher amounts of spiked fentanyl based on the recovered mass from the spike controls did not impact the results of this study. The only difficulty was that a direct comparison of the collected data (amounts recovered from positive controls and test coupons) against the results from the previous study was more complicated.

4.1 Data Quality Indicators

Data quality indicators and results are provided in Table 34. In general, the data quality indicator results were acceptable per the Quality Assurance Project Plan (QAPP) titled *Quality Assurance Project Plan for Remediation of fentanyl Contaminated Indoor Environments* (version 1.0, 20 February 2020), as amended, including checks of the measurement methods for temperature, RH, time, volume, mass, fentanyl recovery from blank samples and spike controls, and pH. Attainment of these data quality indicator results limited the amount of error introduced into the evaluation results except for the amount of fentanyl recovered in the spike control (SC) extracts from multiple tests.

Table 34. Data Quality Indicators and Results

Parameter	Measurement Method	Data Quality Indicators	Results
Temperature (°C)	National Institute of Standards and Testing (NIST)-traceable thermometer	Compare against calibrated thermometer once before testing; agree ±1 °C through 60 min.	The HOBO UX100 datalogger used in the test chamber remained within 0.1°C of the calibrated reference through 1 h.
Relative Humidity (%)	NIST-traceable hygrometer	Compare against calibrated hygrometer once before testing; agree ±10% through 60 min.	The HOBO UX100 datalogger used in the test chamber during the TO remained within 1.9% of the calibrated reference through one hour.
Time (seconds, sec)	Timer/data logger	Compare to time provided at NIST.time.gov once before testing; agree ±2 sec/h.	No difference was observed between the timer and NIST.time.gov after 1 h.
Volume (mL, µL)	Calibrated pipette (LC-MS/MS sample dilution)	Pipettes were checked for accuracy and repeatability once before use by determining mass of water delivered. The syringe/pipette was acceptable if the range of observed masses for five replicate droplets was ±10% of expected.	Five pipettes used for LC-MS/MS sample dilution were checked. Systematic and random percent error ranges for each are provided below: • Pipette 1 at 1, 5, and 10 µL – 0.18% to 7.7% • Pipette 2 at 3, 10, and 25 µL – 0.34% to 8.0% • Pipette 3 at 20, 35, and 50 µL – 0.00% to 1.2% • Pipette 4 at 50, 100, and 250 µL – 0.12% to 1.2% • Pipette 5 at 100, 500, and 1,000 µL – 0.16% to 1.2%

Parameter	Measurement Method	Data Quality Indicators	Results
Volume (mL, µL)	Pump pressurization-style sprayer (decontaminant delivery)	Sweep speed and sprayer pressure for the sprayer nozzle to achieve the 600 µL/coupon target application volume was determined once prior to testing by weighing the amount of decontaminant delivered. Spray procedures were acceptable if the range of measured volumes for 5 applications is ± 20% of the nominal target volume.	Three (3) replicate spray applications were delivered to acrylic runoff boxes at all sixteen (16) positions across the characterized spray area. Average spray delivery across the 16 positions across the 3 replicates were: • Meth Remover® – 111% of theoretical • ZEP® – 95% of theoretical • pH 5 modified surfactant bleach – 99% of theoretical • – 103% of theoretical • Diluted Dahlgren Decon™ – 98% of theoretical Detailed data are provided in Section 3.1.2.
Fentanyl in Laboratory Blank Coupon Extracts (µg/mL)	Extraction, LC/MS/MS or GC/MS	Laboratory blanks (coupons without applied fentanyl that are not decontaminated) should have less than 50% of the lowest detected amount on the test coupon or 1% of the amount on the positive controls, whichever is lower.	No fentanyl outside the stated criteria was measured in any laboratory blank sample extract throughout testing.
Fentanyl in Procedural Blank Coupon Extracts (µg/mL)	Extraction, LC/MS/MS or GC/MS	Procedural blanks (coupons without applied fentanyl that are decontaminated) should have less than 50% of the lowest detected amount on the test coupon or 1% of the amount on the positive controls, whichever is lower.	No fentanyl outside the stated criteria was measured in any procedural blank sample extracts throughout testing.
Fentanyl in Spike Control Extracts (µg/mL)	LC/MS/MS or GC/MS	The mean of the spike controls included with each day of testing was within 80% to 120% of the target application and had a CoV of <30% between replicates.	Mean and RSD of the following SC sets were outside tolerance: • Test 1 of Table 12, 122% avg SC recovery, 5.1% RSD • Test 2 of Table 12, 138% avg SC recovery, 15% RSD • Test 1 of Table 13 (no ascorbic acid), 153% avg SC recovery, 10% RSD • Test 1 of Table 13 (with ascorbic acid), 168% avg SC recovery, 5.6% RSD • Test 2 of Table 13 (no ascorbic acid), 149% avg SC recovery, 10% RSD • Test 2 of Table 13 (with ascorbic acid), 136% avg SC recovery, 6.6% RSD • Time dependence test (Section 3.2.3), 135% avg SC recovery, 14% RSD • Test 1 of Table 15, 130% avg SC recovery, 15% RSD • Test 2 of Table 15, 128% avg SC recovery, 25% RSD
pH	Calibrated pH meter	Meter was checked for accuracy prior to each use using unexpired buffer solutions at: • pH 4 (SB101-500, Fisher Scientific) • pH 7 (1552-16, Fisher Scientific) • pH 10 (1602-16, Fisher Scientific) • pH 12.5 (1618-16, Fisher Scientific) Check value must be within ± 0.1 pH units of the buffer value.	Meter was checked before each use using the specified unexpired buffer solutions and was within tolerance during all checks.

4.2 Instrument Calibration

4.2.1 Calibration Schedules

Instrumentation needed for the investigation was maintained and operated according to the quality and safety requirements and documentation of Battelle's HMRC. Except for the GC/MS and LC-MS/MS, all instruments utilized during the project were calibrated as stipulated by the manufacturer or, at a minimum, annually. The GC/MS and LC-MS/MS were calibrated as described in Sections 4.2.2 and 4.2.3. Table 35 provides calibration schedules for instruments that were used during the evaluation.

Table 35. Equipment Calibration Schedule

Equipment	Frequency
Calibrated pipettes	Prior to the investigation and annually thereafter. Calibration/accuracy was also verified as described in Table 34.
Calibrated UX100 HOBO Hygrometer/Thermometer	Prior to the investigation by the manufacturer. After the manufacturer-provided calibration expired, use of the expired unit was discontinued and the unit was discarded. A new manufacturer-calibrated unit was obtained for use.
Timer	Prior to the investigation by the manufacturer. After the manufacturer-provided calibration expired, use of the expired unit was discontinued and the unit was discarded. A new manufacturer-calibrated unit was obtained for use.
LC-MS/MS	Calibrated prior to analysis of each set of test samples (calibration curve) and a calibration verification standard was analyzed after every ten samples (see Section 4.2.2).
GC/MS	Calibrated prior to analysis of each set of test samples (calibration curve) and a calibration verification standard was analyzed after every ten samples (see Section 4.2.3).
pH meter	Prior to the investigation and annually thereafter. Calibration/accuracy was verified prior to each use as described in Table 34.

4.2.2 LC-MS/MS Calibration

Fentanyl (certified analytical reference material; separate source from the fentanyl used to contaminate test coupons; part numbers F-013-1ML and F-002-1ML, Sigma Aldrich) was used to create calibration standards (concentrations corrected for percent purity; see Section 2.2.2.1) encompassing the appropriate analysis range. Calibration standards were kept and used for no longer than two months from the date of creation. After two months of use, calibration standards and continuing calibration verifications (CCVs) were replaced with a new (fresh) set prepared from an unopened stock ampoule. The old and new sets were then analyzed, and the results were compared to ensure consistency, accuracy, and precision (in terms of the criteria provided in Table 36) and to demonstrate that degradation of the old standards during the two-month period of use had not occurred. In all cases, calibration standard and CCV concentrations remained stable (i.e., no degradation occurred) during the two-month use period.

A seven-point calibration curve for fentanyl was used with a lower calibration level of 0.010 ng/mL and an upper limit of 5.0 ng/mL. A linear or quadratic regression (specified in the raw data product) was used to describe the data with $1/x^2$ weighting. The origin was not included for regression. Limits were also placed on the percent bias (Equation 8) observed in the standards.

$$Bias = \left(\frac{E_V - O_V}{E_V}\right) \times 100\% \qquad (8)$$

where: E_V = expected value from calibration curve

O_V = observed value from standard

The percent bias for the low standard had to be less than or equal to 25%, and the percent bias for the remaining standards had to be less than or equal to 15%. The signal-to-noise ratio of the lowest calibration standard had to be approximately 3:1 at minimum. The retention time (RT) for each target compound and IS in each injection had to be within ± 0.1 min of the RT for the same components in the mid-level calibration standard.

Solvent blank and double blank samples were included during analytical runs to confirm that no fentanyl carryover occurred. Solvent blank sample analysis results had to be below the value of the lowest calibration standard.

Independently prepared CCVs were analyzed prior to sample analysis, following every ten (or fewer) test/control samples (not including blanks or matrix samples), and at the end of each set of samples. Two CCV concentrations were used, one of which was equal to the low calibration standard (0.010 ng/mL) and the other within the calibration range (2 ng/mL). CCV response had to be within 25% of the nominal concentration for the low level CCV used and within 15% of the nominal concentration for the mid-range CCV for fentanyl analyses to be considered acceptable.

Calibration standards and CCVs were matched to the samples undergoing analysis as closely as possible. For example, test samples in IPA prepared for analysis by a 10-fold dilution in water were quantitated using standards and CCVs prepared in 10% IPA.

The area of fentanyl-d_5 IS in the test samples was compared to the area of fentanyl-d_5 IS in the nearest passing calibration standard or passing CCV. Fentanyl-d_5 area in the test samples had to fall within 50% to 200% of the area of the IS in the calibration standard or CCV to which it was compared (criteria per EPA Method 8000D [6]). As described in Section 2.1.1.4 (Decontamination Technology Quench and Matrix Effect Evaluation), the validity of the assumption that any test sample matrix would affect analysis of fentanyl and fentanyl-d_5 IS in an identical manner was evaluated prior to decontamination efficacy testing. Based on the data and criteria (refer to Section 3.1.3) the assumption held, so IS response variability within the range of 50% to 200% of that of the nearest passing calibration standard or CCV was considered acceptable and IS was assumed to properly compensate for identical effects on fentanyl response due to sample matrices. In certain cases, IS area was found to be outside this acceptance range, so the test sample dilution factor was increased to reduce the effect of sample matrix.

Table 36 summarizes LC-MS/MS analysis performance parameters and acceptance criteria.

Table 36. LC-MS/MS Analysis Performance Parameters and Acceptance Criteria

Parameter	Criterion
Coefficient of determination (r^2)	≥ 0.990
% Bias for the lowest calibration standard	≤ 25%
% Bias for remaining calibration standards (except lowest standard)	≤ 15%
Solvent blank samples	< lowest calibration standard
% Bias for the low CCV	≤ 25%
% Bias for the high CCV	≤ 15%
Signal-to-noise ratio for the lowest calibration standard	Minimum of 3:1
RT for target compound and IS	± 0.1 min. as same compounds in mid-level calibration standard
Fentanyl-d_5 IS area in samples	50% to 200% area of nearest passing calibration standard or passing CCV

4.2.3 GC/MS Calibration

As with LC-MS/MS calibration, fentanyl (certified analytical reference material; separate source from the fentanyl used to contaminate test and control coupons; part numbers F-013-1ML and F-002-1ML, Sigma Aldrich) were used to create calibration standards encompassing the appropriate analysis range. Use and retention schedules and replacement procedures for calibration standards and CCVs for GC/MS calibration were identical to those described for LC-MS/MS calibration standards and CCVs in Section 4.2.2. Calibration standards and CCVs were stored in a freezer at -20 ± 10°C when not in use. A five-point calibration for fentanyl was used with a lower calibration level of 0.25 µg/mL and an upper limit of 25 µg/mL. As discussed in Section 2.3.1, fentanyl-d5 was used as an IS during GC/MS fentanyl analyses, and the IS was added to samples just prior to GC/MS analyses. Target fentanyl-d5 concentration in samples was 5 µg/mL. Fentanyl-d5 IS area in the test samples was compared to the area of fentanyl-d5 IS in the nearest passing calibration standard or passing CCV and IS acceptance criteria was identical to that described for acceptance of fentanyl-d5 IS response during LC-MS/MS analyses in Section 4.2.2 and indicated in Table 36.

A quadratic regression curve fit was applied to the calibration data. As during LC-MS/MS calibration (as indicated in Table 36), the GC/MS was recalibrated if the r^2 from the regression analysis of the standards was less than 0.990. Limits were also placed on the percent bias (Equation 8) observed in the standards. As required during LC-MS/MS analyses (as described in Section 4.4.2 and indicated in Table 36), the percent bias for the low standard must be less than or equal to 25%, and the percent bias for the remaining standards must be less than or equal to 15%. The GC/MS was tuned initially and as needed following manufacturer's guidelines. A tune check was performed before running each set of samples using DFTPP. A 12-h tune time was not employed.

Following analysis of the calibration standards at the beginning of each analytical run, a solvent blank sample was analyzed to confirm that no fentanyl carryover was occurring. Solvent blank sample analysis results were always below the value of the lowest calibration standard. As with LCMS/MS analysis, independently prepared CCV standards were analyzed prior to sample

analysis, following every five test/control samples and at the end of each set of samples. Use of CCVs and CCV acceptance criteria during GC/MS analyses were identical to those described for LC-MS/MS analyses in Section 4.4.2 and summarized in Table 36.

4.3 Sample Handling and Custody

At all times during the project, protocols required by the U.S. DEA and Battelle's HMRC were followed in the movement and use of fentanyl within the test facility. CoC forms were used to ensure that test samples generated during the work were traceable throughout all phases of testing.

4.4 Test Parameter Control Sheets

Test measurements and information were recorded on test parameter control sheets (TPCSs) or in an LRB. Monitoring of test conditions, parameters, and times was performed by technical staff familiar with the QAPP and testing and was documented on the TPCS.

4.5 Technical Systems Audit

The QA Officer performed a TSA at the HMRC facility in West Jefferson, Ohio, during decontamination efficacy testing on July 13, 2020. The purpose of the TSA was to ensure that testing was performed in accordance with the QAPP. The Battelle QA Officer reviewed the investigation methods, compared test procedures to those specified in the QAPP, and reviewed data acquisition and handling procedures. The Battelle QA Officer did not identify any findings that required corrective action.

4.6 Performance Evaluation Audits

PE audits, provided in Table 37 with results, addressed those reference measurements that factored into the data used in quantitative analysis during the evaluation, including volume, mass, and time measurements and GC/MS or LC-MS/MS calibration and performance. The mass of fentanyl dispensed correlated directly to the mass of fentanyl on the coupons. The measured times that fentanyl and the decontamination technologies were allowed to remain in contact with the coupons directly influenced efficacy of the decontaminants. Calibration of the GC/MS and LC-MS/MS and IS recovery provided confidence that the analysis system was providing accurate data.

Temperature and RH were measured and recorded on each day of testing, but not monitored or controlled. Therefore, no PE audit of these parameters was performed. See Attachment B for a summary table of measured temperature and RH ranges.

During the decontaminant spray delivery characterization (Section 3.1.2), two minor spills and one loss of a sample were reported (Tables C1-C3, Attachment C) leading to a lower average and larger standard deviation in two of the average weights while for the third lost sample, the average was taken over two instead of three measurements. The spray delivery characterization

experiment was not repeated as the impact of these three spills was negligible. Despite the lower measured average weight, the percent of target weight was more than 100%.

Table 37. Performance Evaluation Audit Results

Parameter	Audit Procedure	Required Tolerance	Results
Volume (mL, µL)	Pipettes were checked for accuracy and repeatability one time before use by determining the mass of water delivered. The pipette was acceptable if the range of observed masses for five droplets is ±10% of expected.	±10%	Five pipettes used for LC-MS/MS sample dilution were checked. Systematic and random percent error ranges for each are provided below: • Pipette 1 at 1, 5, and 10 µL – 0.18% to 7.7% • Pipette 2 at 3, 10, and 25 µL – 0.34% to 8.0% • Pipette 3 at 20, 35, and 50 µL – 0.00% to 1.2% • Pipette 4 at 50, 100, and 250 µL – 0.12% to 1.2% • Pipette 5 at 100, 500, and 1,000 µL – 0.16% to 1.2%
Time (sec)	Compare to time provided at NIST.time.gov once before testing; agree ±2 seconds/h.	±2 seconds/h	No difference was observed between the timer and NIST.time.gov after 1 h.
Fentanyl in Spike Control Extracts (µg/mL)	Use LC-MS/MS to determine mass of fentanyl delivered as a 1 mg pile into 10 mL of extraction solvent and compare to target application level.	≥80% of spike target ≤ 120% of spike target ≤ 30% CoV	Mean and RSD of the following SC sets were outside of tolerance: • Test 1 of Table 12, 122% avg SC recovery, 5.1% RSD • Test 2 of Table 12, 138% avg SC recovery, 15% RSD • Test 1 of Table 13 (no ascorbic acid), 153% avg SC recovery, 10% RSD • Test 1 of Table 13 (with ascorbic acid), 168% avg SC recovery, 5.6% RSD • Test 2 of Table 13 (no ascorbic acid), 149% avg SC recovery, 10% RSD • Test 2 of Table 13 (with ascorbic acid), 136% avg SC recovery, 6.6% RSD • Time dependence test (Section 3.2.3), 135% avg SC recovery, 14% RSD • Test 1 of Table 15, 130% avg SC recovery, 15% RSD • Test 2 of Table 15, 128% avg SC recovery, 25% RSD
LC-MS/MS Fentanyl Calibration Standards (%)	Verify all standards and CCVs used to calibrate and confirm calibration of the LC-MS/MS system used for analysis fall within the requirements provided in Section 4.2.2.	Refer to Table 36	All standards and CCVs were within specification for all reported data.
Fentanyl-d_5 IS Recovery	Use LC-MS/MS to measure from a secondary source and compare to the primary source one time.	±10%	IS used during analyses was compared to a secondary source. Five (5) replicate analyses of a 1 ng/mL standard prepared from each source were conducted. 2.2% relative percent difference in mean areas obtained.
pH	Meter was checked for accuracy prior to each use using unexpired buffer solutions at: • pH 4 (SB101-500, Fisher Scientific) • pH 7 (1552-16, Fisher Scientific), • pH 10 (1602-16, Fisher Scientific), • pH 12.5 (1618-16, Fisher Scientific)	± 0.1 pH	Meter was checked before each use using the specified unexpired buffer solutions and was within tolerance during all checks.

4.7 Data Quality Audit

Validation of the data included verification of the completeness of the data, compliance with the acceptance criteria in the QAPP, recalculation checks, and tracing of the data from instrument outputs through the final report. One hundred percent (100%) of the data was reviewed prior to use in calculations or any data manipulation, and review was completed before the data were provided to QA for the data quality audit.

The QA Manager, operating independently of the laboratory testing effort, audited at least 10% of the data generated during testing. Data were traced from initial acquisition through reduction and to final reporting. All calculations were checked.

Through the data quality audit, the TSA, and review of reports, the QA Manager ensured that data generated during testing were valid, meeting the requirements of the QAPP.

4.8 QAPP Amendments

Two (2) amendments to the QAPP were prepared during the project:

- Amendment 1 (dated June 18, 2020) revised the test matrix provided as Table 2 for the indoor-related material decontamination efficacy evaluation described in Section B.1.2 of the QAPP.
- Amendment 2 (dated September 17, 2020) revised the test matrix provided as Table 3 for the first responder PPE material-related decontamination efficacy evaluation described in Section B.1.3 of the QAPP. An additional (third) PPE material-related decontamination efficacy test was added to the matrix.

4.9 QAPP Deviations

Two (2) deviations from the procedures defined in the QAPP were noted during the TO:

- Per Sections B.2.3.5 and B.2.3.6 of the QAPP, the 10-cm^2 test and control coupons are placed into separate acrylic boxes during each test to collect any decontaminant (and fentanyl) that runs off the coupon following spray-application of decontaminant. Plastic mesh disks were placed underneath the coupons in the acrylic boxes to elevate the coupons and prevent contact of the coupons with the decontaminant runoff that is collected. The QAPP indicates that the plastic mesh disks will be made of polytetrafluoroethylene (PTFE), but mesh disks made of polypropylene (PP) were used during all tests. The PP mesh disks were cut from a larger sheet of PP mesh (part number 9265T47, McMaster-Carr) using a 1.5-inch diameter die.
- Table 8 in Section B.5 of the QAPP indicates that five (5) replicate decontaminant spray applications will be performed during determination of necessary spray parameters for each decontaminant to achieve the target application volume of 600 µL/coupon (60 µL/cm^2). During characterization of the sprayer using the test decontaminants, only three (3) replicate decontaminant spray applications were performed for each decontaminant.

DISCUSSION/CONCLUSIONS

Bench scale decontamination efficacy tests were performed in which candidate decontaminants were assessed for efficacy in decontamination of fentanyl on the surface of 10-cm^2 material coupons. Decontaminants included Meth Remover® (hydrogen peroxide active ingredient), Zep® Professional Stain Remover with Peroxide (hydrogen peroxide), Dahlgren Decon™ (activated peracetic acid), and pH 5 modified surfactant bleach (nominal hypochlorite concentration of 5%) derived from Clorox™ ProResults® Garage and Driveway Cleaner. Decontaminants were applied via spray at a target application volume of 60 $\mu L/cm^2$. Decontaminant that ran off the coupon surface during and after spray-application was collected to assess the runoff of fentanyl from the material coupons in addition to chemical decontamination of fentanyl.

Decontamination efficacies were calculated by both including and excluding mass detected in the decontaminant runoff. Efficacy calculated without consideration of mass present in the runoff describes the performance of the decontaminant with regard to both propensity for physical removal of fentanyl by the decontaminant during spray-application as well as by chemical degradation of fentanyl. Conversely, addition of fentanyl runoff mass to the mass recovered from coupons via solvent extraction enables calculation of efficacy that is attributable primarily to degradation of fentanyl.

5.1 Building Material Decontamination

After a 1-h dwell time with the fentanyl-contaminated surfaces, the Meth Remover® and ZEP® product demonstrated similar average efficacies of 62% and 65%, respectively, across the four materials attributable to a combination of physical removal and chemical decontamination. The average percent efficacy dropped to 42% and 26%, respectively, across the four materials when physical removal was decoupled from the efficacy calculation. While the application of these two, hydrogen peroxide-containing, products led to degradation of fentanyl, their efficacies are not as high as seen for some of the other decontamination solutions. It is possible that a longer contact time or repeated application would improve efficacy. However, such a study was not conducted. The Meth Remover® product was included in the completed remediation of a fentanyl-contaminated home [2]. While this suggests that residual fentanyl would have been present after the application (and 1-h dwell time) of this product, it should be noted that the remediation effort of the home included multiple applications of wiping and water rinsing of the surfaces in addition to the Meth Remover® product application. The combination of the physical removal and chemical degradation appears to have led to the successful remediation. Any future cleanups should consider the combination of physical removal and chemical degradation.

After a double application and a total 2-h dwell time, the pH 5 modified surfactant bleach decontaminant demonstrated average efficacies of 95% across the four materials attributable to a combination of physical removal and chemical decontamination. The average percent efficacy dropped to 91% across the three materials when physical removal was decoupled from the

efficacy calculation. The addition of ascorbic acid mixed with the fentanyl lowered the efficacy on wood from 84% to 80% based on chemical degradation only.

Under the same test conditions, the Dahlgren Decon™ decontaminant demonstrated an average efficacy of 99.97% across the four materials attributable to a combination of physical removal and chemical decontamination and the average percent efficacy dropped to 99.86% across the four materials when physical removal was decoupled from the efficacy calculation. The addition of ascorbic acid mixed with the fentanyl lowered the efficacy on wood from 99.8% to 97% based on chemical degradation only.

A direct comparison between the double application data and the single application data [1] is difficult to make as the amount of fentanyl that was applied was significantly different with more fentanyl applied in the current study. Since efficacy values did not improve significantly, it appears that a reapplication of these two products may not be useful unless there is evidence that a double application can overcome any material demand of the decontamination solution. The presence of additives such as ascorbic acid may result in lower efficacy due to a higher demand of the decontaminant.

5.2 PPE/Responder Gear Decontamination

The Dahlgren Decon™ and pH 5 modified surfactant bleach decontaminants were also considered in efficacy studies with a short 5-min dwell time, simulating a short dwell time as part of decontamination line procedures.

After a 5-min dwell time, the pH 5 modified surfactant bleach decontaminant demonstrated efficacies of 86% across the four PPE/responder gear materials attributable to a combination of physical removal and chemical decontamination. The average percent efficacy dropped to 61% across the four materials when physical removal was decoupled from the efficacy calculation.

Under the same test conditions, the 1:4 diluted Dahlgren Decon™ decontaminant demonstrated efficacies of 96% across the four materials attributable to a combination of physical removal and chemical decontamination and the average percent efficacy dropped to 95% across the four materials when physical removal was decoupled from the efficacy calculation.

Generally, the 1:4 diluted Dahlgren Decon™ demonstrated noticeable higher average decontamination efficacies than the pH 5 modified surfactant bleach decontaminant after the 5-min dwell time.

5.3 Clustering of Fentanyl Powder

In many of the decontamination tests, agglomerated fentanyl was observed visually on surfaces following the application and dwell time of the decontaminant. This clustering or clumping of fentanyl on the surface results in a slower mass transfer rate between the decontaminant and fentanyl. Hence, higher amounts were recovered on occasion even in the presence of an otherwise effective decontaminant. Such behavior may also occur in actual remediation efforts and should be watched for.

REFERENCES

1. Oudejans L., D. See, C. Dodds, M. Corlew, and M. Magnuson, Decontamination Options for Indoor Surfaces Contaminated with Realistic Fentanyl Preparations. Journal of Environmental Management 297 (2021), 113327.
2. Doerflein, J. Case Study: A Fentanyl Incident. https://www.epa.gov/sites/production/files/2020-02/documents/doerflein_fentanyl.pdf Last accessed April 30, 2021.
3. Fisher, R. A. (1942). The Design of Experiments, 3rd Edition, Edinburgh: Oliver & Boyd.
4. Miller, R. G., Jr. (1981). Simultaneous Statistical Inference. New York: Springer-Verlag.
5. Tukey, J. W. (1953). "The Problem of Multiple Comparisons." In Multiple Comparisons, 1948–1983, edited by H. I. Braun, vol. 8 of The Collected Works of John W. Tukey (published 1994), 1–300. London: Chapman & Hall.
6. SW-846 Method 8000D, Determinative Chromatographic Separations. Revision 5, March 2018, Final Update V to the Third Edition of the Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, EPA Publication SW-846.

Attachment A – Fentanyl Certificate of Analysis

Cayman Chemical Co.
1180 E. Ellsworth Rd
Ann Arbor, MI 48108
www.caymanchem.com

Contact Us:
1-800-364-9897 or (734)971-3335
Fax: (734)971-3640
Email: crmquality@caymanchem.com

For R&D purposes only. Not intended for human or animal use.

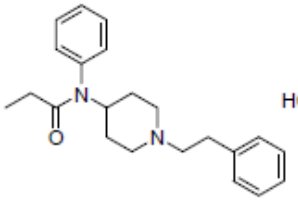
CERTIFICATE OF ANALYSIS

ACCREDITED
ISO/IEC 17025
#AT-1773
ISO Guide 34
#AR-1774

Fentanyl (hydrochloride)

N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]-propanamide, monohydrochloride

Item #: 14719
Batch #: 0530926
CAS Registry Number: 1443-54-5



Expiry Date: 01JUN2023 (valid from date of certification)
Description: neat solid
Storage and Handling: Store at -20°C. Warm to room temperature prior to opening.
Safety: Poison

ANAB ACCREDITED ISO GUIDE 34 REFERENCE MATERIAL PRODUCER

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY

Compound Information

Chemical Formula	$C_{22}H_{28}N_2O \cdot HCl$
Formula Weight	**372.90 amu**
UV λ_{max}	**205nm**

Quality Information

Qualifier	Method	Limit	Result	Meets Specification
Appearance	**Visual inspection**	**White / off-white solid**	**Off-white solid**	**Y**
Chromatographic Purity, HPLC	**Cayman Method TST SD132**	**≥98.00%**	**99.59% ± 0.18%**	**Y**
Identity, LCMS	**Cayman Method TST SD13, +ESI**	**337.2 ± 0.5 amu**	**337.4 amu**	**Y**
Identity, GCMS	**Cayman Method TST SD12**	**Conforms**	**Conforms**	**Y**
FTIR	**USP<851> (diamond ATR)**	**Conforms**	**Conforms**	**Y**
***Identity, NMR**	**^{1}H NMR**	**Conforms**	**Conforms**	**Y**
Loss on Drying	**Cayman Method TST SD24**	**≤10.00%**	**0.38% ± 0.49%**	**Y**
Residue on Ignition	**Cayman Method TST SD06**	**≤3.00%**	**<0.10% ± 0.22%**	**Y**

*NMR is provided as supplemental info but is not within scope of ISO accreditation

Property values are traceable to SI units through an unbroken chain of measurements.

Measurement Uncertainty
All measurement uncertainties are expressed as expanded uncertainties in accordance with ISO 17025 and Guide 34 at the approximate 95% confidence interval using a coverage factor of k=2.

Cayman Chemical certifies that this standard meets the specifications stated in this certificate and warrants this product to meet the stated acceptance criteria through the expiration date when stored unopened as recommended.

Approval: *Title: Cayman Chemical ISO Quality Manager* *Certification Date: 01JUN2018*

Certificate # 14719-0530926-01

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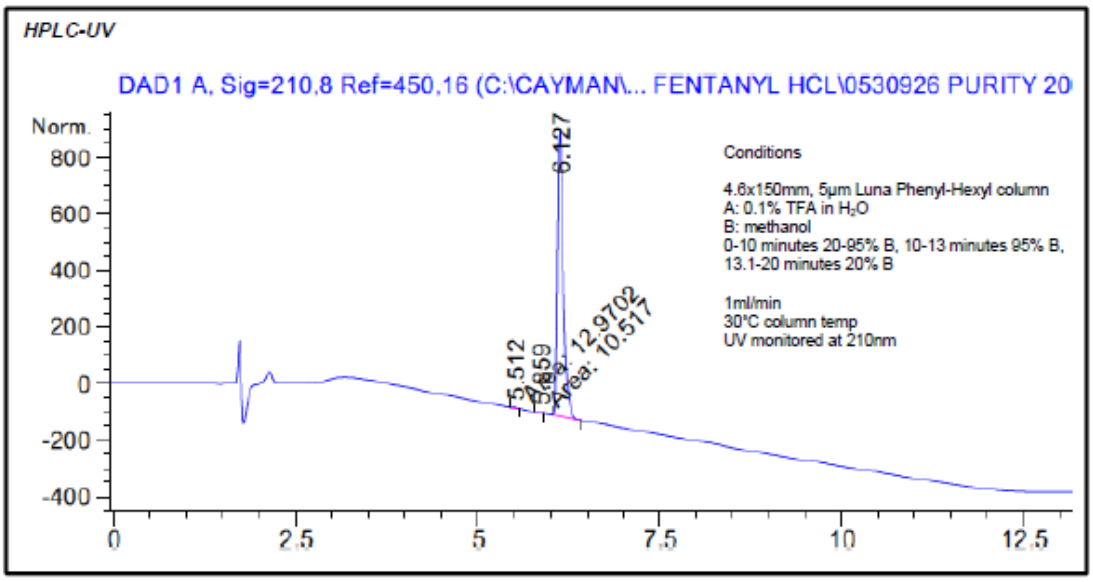
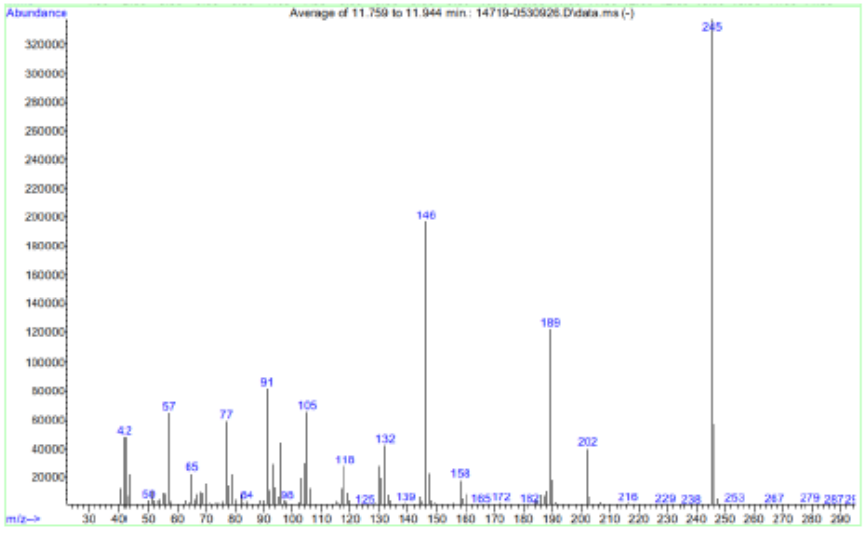
Supplemental Data

HPLC-UV



GC-MS



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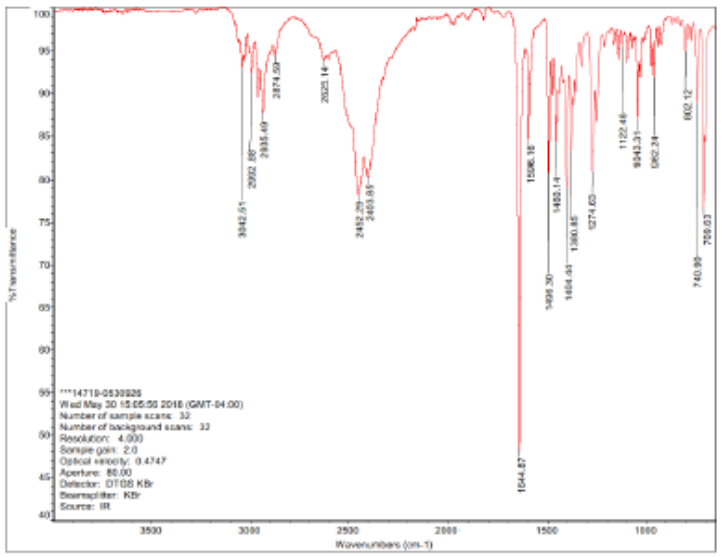
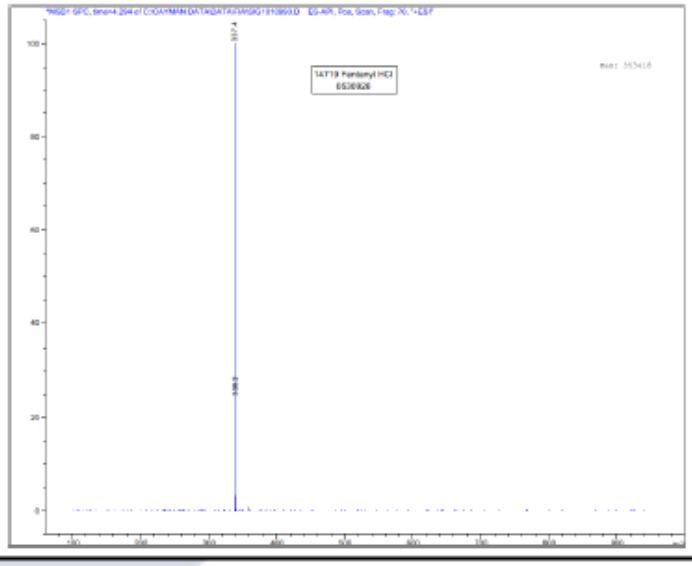
FTIR



Conditions

Thermo Nicolet iS10 FTIR /
Diamond SmartATR (single bounce)
32 scans / 32 background scans
Range: 650-4000cm^{-1}
Resolution: 4.000
ATR and background corrected

ESI-MS



Conditions

Agilent 1100 HPLC / G1956MSD
FIA-MS (no column)
Mobile phase: 50:50:0.1
MeOH/H_2O/acetic acid
Flow: 0.5mL/min
+ESI 100-1000m/z range
Nebulizer: 60psi
Desolvation Gas: 13L/min
Desolvation Temp: 350°C
Electrospray Voltage: 4kV
MS collected across peak width
at half height

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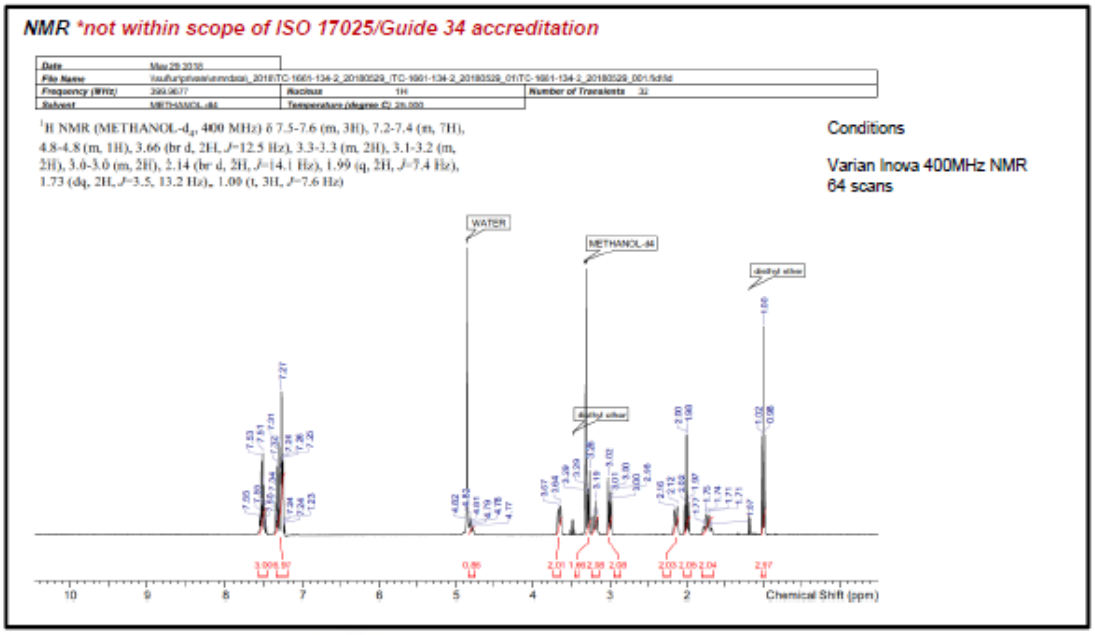
***NMR** *not within scope of ISO 17025/Guide 34 accreditation*



Homogeneity
Homogeneity was assessed by visual inspection and replicate purity analyses. The recommended minimum quantity for use is 2.0µg. Quantities below this have not been evaluated.

Short Term Stability Summary
No decrease in purity was observed at ambient conditions or 60˚C after two weeks. This data supports shipping of this product at ambient temperature.

Long Term Stability
Long term stability data predicts 5 years stability at the -20˚C storage temperature.

Certificate # 14719-0530926-01

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For R&D purposes only. Not intended for human or animal use.

Revision History

Revision #	Date	Reason for Revision
01	01JUN2018	Initial version

Disclaimers

Material Safety Data
This material should be considered hazardous until information to the contrary becomes available. Do not ingest, swallow, or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. This information contains some but not all of the information required for the safe and proper use of this material. Before use, review the complete Material Safety Data Sheet, which has been sent via email to your institution.

Warranty and Limitation of Remedy
Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material will meet our specifications at the time of delivery.
Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence.
This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.
Buyer's exclusive remedy and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specification.
Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver of Buyer of all claims hereunder with respect to said material.
For further details, please refer to our Warranty and Limitations of Remedy located on our website and in our catalog.

Attachment B – Environmental Data

Table B1. Environmental Conditions in Experimental Chamber with Spray Setup

Test Description	Temperature Range (°C)	Relative Humidity (%)
Quench Method development (no spray)	19.8 – 21.1	22 - 27
Meth Remover® decontamination (60 min)	19.9 – 22.0	56 - 94
ZEP® decontamination (60 min)	19.7 – 22.2	60 - 94
Dahlgren Decon™ decontamination (60 + 60 min)	20.1 – 22.4	57 - 76
pH 5 modified surfactant bleach decontamination (60 + 60 min)	19.6 – 22.1	60 - 91
Diluted Dahlgren Decon™ decontamination (various time points up to 15 min)	19.4 – 21.6	63 - 92
Diluted Dahlgren Decon™ decontamination (5 min)	20.9 – 22.7	56 - 99
pH 5 modified surfactant bleach decontamination (5 min)	19.3 – 21.5	64 - 97

Attachment C – Spray Characterization Data

Table C1. Decontaminant Spray Delivery Mass per Position, Meth Remover®

Position	Sprayed Decontaminant Weight (g)						
	Rep 1	Rep 2	Rep 3	Average	St Dev	% RSD	Percent of Target
1	1.30	1.31	1.22	1.28	0.049	3.9%	110%
2	1.23	1.31	1.11	1.22	0.101	8.3%	105%
3	1.23	1.35	1.07	1.22	0.140	11.5%	105%
4	1.30	1.35	1.01 [A]	1.22	0.184	15.0%	105%
5	1.37	1.35	1.27	1.33	0.053	4.0%	114%
6	1.38	1.28	1.31	1.32	0.051	3.9%	114%
7	1.21	1.36	1.28	1.28	0.075	5.8%	110%
8	1.38	1.32	1.24	1.31	0.070	5.3%	113%
9	1.22	1.37	1.31	1.30	0.075	5.8%	112%
10	1.24	1.38	1.33	1.32	0.071	5.4%	113%
11	1.26	1.24	1.14	1.21	0.064	5.3%	104%
12	1.23	1.26	1.32	1.27	0.046	3.6%	109%
13	1.32	1.36	1.39	1.36	0.035	2.6%	117%
14	1.28	1.39	1.35	1.34	0.056	4.2%	115%
15	1.19	1.35	1.35	1.30	0.092	7.1%	111%
16	1.24	1.32	1.36	1.31	0.061	4.7%	112%

[A] Position 4, rep 3 small amount of decontaminant spilled before weighing

Table C2. Decontaminant Spray Delivery Mass per Position, ZEP®

Position	Sprayed Decontaminant Weight (g)						
	Rep 1	Rep 2	Rep 3	Average	St Dev	% RSD	Percent of Target
1	1.18	1.07	1.13	1.13	0.055	4.9%	97%
2	1.19	1.13	1.11	1.14	0.042	3.6%	98%
3	1.28	1.19	1.07	1.18	0.105	8.9%	102%
4	1.30	1.21	1.17	1.23	0.067	5.4%	106%
5	1.26	1.20	1.24	1.23	0.031	2.5%	106%
6	1.36	1.24	1.29	1.30	0.060	4.6%	112%
7	1.35	1.26	1.24	1.28	0.059	4.6%	111%
8	1.28	1.13	1.09	1.17	0.100	8.6%	100%
9	1.00	0.96	0.99	0.98	0.021	2.1%	85%
10	0.98	0.97	0.98	0.98	0.006	0.6%	84%
11	0.95	0.97	0.98	0.97	0.015	1.6%	83%
12	0.99	1.00	0.99	0.99	0.006	0.6%	86%
13	1.01	0.98	0.99	0.99	0.015	1.5%	86%
14	1.02	1.00	1.02	1.01	0.012	1.1%	87%
15	1.05	1.01	0.97	1.01	0.040	4.0%	87%
16	1.03	0.99	0.94 [A]	0.99	0.045	4.6%	85%

[A] Position 16, rep 3 small amount of decontaminant spilled before weighing.

Table C3. Decontaminant Spray Delivery Mass per Position, pH 5 Modified Surfactant Bleach

Position	Sprayed Decontaminant Weight (g)						
	Rep 1	Rep 2	Rep 3	Average	St Dev	% RSD	Percent of Target
1	1.08	1.19	1.08	1.12	0.064	5.7%	99%
2	1.12	1.13	1.20	1.15	0.044	3.8%	102%
3	1.16	1.16	1.12	1.15	0.023	2.0%	101%
4	1.18	1.16	1.11	1.15	0.036	3.1%	102%
5	1.19	Lost [A]	1.14	1.17	0.035	3.0%	103%
6	1.17	1.13	1.10	1.13	0.035	3.1%	100%
7	1.15	1.16	1.10	1.14	0.032	2.8%	100%
8	1.11	1.09	1.11	1.10	0.012	1.0%	97%
9	1.05	1.05	1.07	1.06	0.012	1.1%	93%
10	1.12	1.08	1.06	1.09	0.031	2.8%	96%
11	1.10	1.09	1.09	1.09	0.006	0.5%	97%
12	1.14	1.07	1.07	1.09	0.040	3.7%	97%
13	1.09	1.09	1.11	1.10	0.012	1.1%	97%
14	1.13	1.21	1.19	1.18	0.042	3.5%	104%
15	1.10	1.17	1.14	1.14	0.035	3.1%	100%
16	1.11	1.15	1.18	1.15	0.035	3.1%	101%

[A] Position 5, rep 2 sample lost (spilled).

Table C4. Decontaminant Spray Delivery Mass per Position, Dahlgren Decon™

Position	Sprayed Decontaminant Weight (g)						
	Rep 1	Rep 2	Rep 3	Average	St Dev	% RSD	Percent of Target
1	1.34	1.11	1.54	1.33	0.215	16.2%	104%
2	1.22	1.15	1.39	1.25	0.123	9.8%	98%
3	1.20	1.08	1.24	1.17	0.083	7.1%	92%
4	1.19	1.30	1.20	1.23	0.061	4.9%	96%
5	1.28	1.18	1.30	1.25	0.064	5.1%	98%
6	1.42	1.33	1.13	1.29	0.148	11.5%	101%
7	1.36	1.38	1.23	1.32	0.081	6.2%	103%
8	1.25	1.53	1.28	1.35	0.154	11.4%	106%
9	1.50	1.25	1.34	1.36	0.127	9.3%	107%
10	1.47	1.28	1.66	1.47	0.190	12.9%	115%
11	1.45	1.27	1.61	1.44	0.170	11.8%	113%
12	1.32	1.15	1.51	1.33	0.180	13.6%	104%
13	1.38	1.24	1.43	1.35	0.098	7.3%	106%
14	1.36	1.20	1.20	1.25	0.092	7.4%	98%
15	1.40	1.14	1.28	1.27	0.130	10.2%	100%
16	1.31	1.31	1.30	1.31	0.006	0.4%	102%

Table C5. Decontaminant Spray Delivery Mass per Position, Diluted Dahlgren Decon™

Position	Sprayed Decontaminant Weight (g)						
	Rep 1	Rep 2	Rep 3	Average	St Dev	% RSD	Percent of Target
1	1.31	1.18	1.23	1.24	0.066	5.3%	107%
2	1.26	1.18	1.21	1.22	0.040	3.3%	105%
3	1.20	1.17	1.22	1.20	0.025	2.1%	103%
4	1.18	1.24	1.15	1.19	0.046	3.9%	102%
5	1.25	1.18	1.17	1.20	0.044	3.6%	103%
6	1.25	1.17	1.15	1.19	0.053	4.4%	102%
7	1.17	1.17	1.01	1.12	0.092	8.3%	96%
8	1.19	1.14	1.14	1.16	0.029	2.5%	100%
9	1.13	1.07	1.11	1.10	0.031	2.8%	95%
10	1.20	1.12	1.12	1.15	0.046	4.0%	99%
11	1.12	1.07	1.01	1.07	0.055	5.2%	92%
12	1.02	0.99	0.98	1.00	0.021	2.1%	86%
13	1.06	1.07	1.09	1.07	0.015	1.4%	92%
14	1.16	1.02	1.10	1.09	0.070	6.4%	94%
15	1.15	1.02	1.06	1.08	0.067	6.2%	93%
16	1.15	1.11	1.10	1.12	0.026	2.4%	96%

Attachment D – Average Mass Recovery and Decontamination Efficacy Data

Table D1. Average Mass Recovery, Meth Remover®

Decontaminant	Material	Sample Description	Average Recovery											
			Coupon				Runoff				Total Mass (Coupon + Runoff)			
			Mass (µg)	St. Dev. (µg)	RSD (%)	% of Total Mass	Mass (µg)	St. Dev. (µg)	RSD (%)	% of Total Mass	Mass (µg)	St. Dev. (µg)	RSD (%)	% Recovery vs Pos Avg
Meth Remover®	Painted drywall	Positive Controls	1077	189	18%	-	-	-	-	-	-	-	-	-
		Test Coupons	677 [A]	169	25%	81%	149	29	19%	19%	827	143	17%	77%
	Coated steel	Positive Controls	1379	254	18%	-	-	-	-	-	-	-	-	-
		Test Coupons	346 [A]	124	36%	58%	235	31	13%	42%	582	139	24%	42%
	Laminate	Positive Controls	1180	144	12%	-	-	-	-	-	-	-	-	-
		Test Coupons	438 [A]	102	23%	54%	381	105	28%	46%	818	105	13%	69%
	Wood	Positive Controls	1251	252	20%	-	-	-	-	-	-	-	-	-
		Test Coupons	337	54	16%	61%	211	44	21%	39%	548	31	5.6%	44%

[A] Solid material observed on replicate coupon surfaces at time of extraction.

Table D2. Average Mass Recovery, ZEP®

Decontaminant	Material	Sample Description	Average Recovery											
			Coupon				Runoff				Total Mass (Coupon + Runoff)			
			Mass (µg)	St. Dev. (µg)	RSD (%)	% of Total Mass	Mass (µg)	St. Dev. (µg)	RSD (%)	% of Total Mass	Mass (µg)	St. Dev. (µg)	RSD (%)	% Recovery vs Pos Avg
ZEP®	Painted drywall	Positive Controls	1235	5.4	0.44%	-	-	-	-	-	-	-	-	-
		Test Coupons	529 [A]	241	46%	50%	513	150	29%	50%	1042	183	18%	84%
	Coated steel	Positive Controls	1375	143	10%	-	-	-	-	-	-	-	-	-
		Test Coupons	306 [A]	166	54%	30%	694	198	29%	70%	999	196	20%	73%
	Laminate	Positive Controls	1351	195	14%	-	-	-	-	-	-	-	-	-
		Test Coupons	424 [A]	54	13%	36%	744	120	16%	64%	1168	172	15%	86%
	Wood	Positive Controls	1266	59	4.7%	-	-	-	-	-	-	-	-	-
		Test Coupons	545 [A]	139	25%	79%	138	67	49%	21%	683	79	12%	54%

[A] Solid material observed on replicate coupon surfaces at time of extraction.

Table D3. Decontamination Efficacy Testing, Average Percent Efficacy

Decontaminant	Material	Avg % Efficacy ± SD [physical removal and chemical decontamination] (%) [A]	Avg % Efficacy ± SD [chemical decontamination only] (%)[B]
Meth Remover®	Painted drywall	37 ± 19	23 ± 19
	Laminate	75 ± 10	58 ± 13
	Coated steel	63 ± 10	31 ± 12
	Wood	73 ± 7	56 ± 9
ZEP®	Painted drywall	57 ± 20	16 ± 15
	Laminate	78 ± 12	27 ± 16
	Coated steel	69 ± 6	14 ± 18
	Wood	57 ± 11	46 ± 7

[A] Avg test coupon recovery vs avg pos control recovery. Combined efficacy of physical removal and chemical degradation.

[B] Avg test coupon recovery plus avg runoff recovery vs avg pos control recovery. Efficacy of chemical degradation only.

Table D4. Average Mass Recovery, Dahlgren Decon™ – Double Application

Decontaminant	Material	Sample Description	Average Recovery											
			Coupon				Runoff				Total Mass (Coupon + Runoff)			
			Mass (µg)	St. Dev. (µg)	RSD (%)	% of Total Mass	Mass (µg)	St. Dev. (µg)	RSD (%)	% of Total Mass	Mass (µg)	St. Dev. (µg)	RSD (%)	% Recovery vs Pos Avg
Dahlgren Decon™	Painted drywall	Positive Controls	1371	108	7.3%	-	-	-	-	-	-	-	-	-
		Test Coupons	0.10	0.09	83%	9.2%	1.5	0.7	46%	91%	1.6	0.62	38%	0.12%
	Coated steel	Positive Controls	1688	329 [A]	19%	-	-	-	-	-	-	-	-	-
		Test Coupons	0.04	0.02	53%	1.7%	1.9	0.49	25%	98%	2.0	0.5	26%	0.12%
	Wood	Positive Controls	1268	65	5.1%	-	-	-	-	-	-	-	-	-
		Test Coupons	1.2	0.83	72%	44%	1.2	0.40	34%	56%	2.3	1.1	47%	0.18%
	Wood with ascorbic acid	Positive Controls	1406	53 [A,B]	3.7%	-	-	-	-	-	-	-	-	-
		Test Coupons	42	40	95%	89%	2.4	1.1	46%	11%	44	39	89%	3.1%

[A] Solid material observed on replicate coupon surfaces following 1st 60-min application.

[B] Solid material observed on replicate coupon surfaces at time of extraction.

Table D5. Average Mass Recovery, pH 5 Modified Surfactant Bleach – Double Application

Decontaminant	Material	Sample Description	Average Recovery											
			Coupon				Runoff				Total Mass (Coupon + Runoff)			
			Mass (µg)	St. Dev. (µg)	RSD (%)	% of Total Mass	Mass (µg)	St. Dev. (µg)	RSD (%)	% of Total Mass	Mass (µg)	St. Dev. (µg)	RSD (%)	% Recovery vs Pos Avg
pH 5 modified surfactant bleach	Painted drywall	Positive Controls	1208	8	0.66%	-	-	-	-	-	-	-	-	-
		Test Coupons	6.1 [A,B]	3.1	50%	15%	37	15	41%	85%	43	16	39%	3.5%
	Coated steel	Positive Controls	1208	13	1.1%	-	-	-	-	-	-	-	-	-
		Test Coupons	42 [A,B]	60	145%	24%	67	49	73%	76%	109	110	101%	9.0%
	Wood	Positive Controls	1204	81	6.7%	-	-	-	-	-	-	-	-	-
		Test Coupons	139 [A,B]	183	132%	63%	52	45	85%	37%	191	227	119%	16%
	Wood with ascorbic acid	Positive Controls	1274	59	4.7%	-	-	-	-	-	-	-	-	-
		Test Coupons	148 [A,B]	117	12%	55%	106	40	37%	45%	254	132	52%	20%

[A] Solid material observed on replicate coupon surfaces following 1st 60-min application.
[B] Solid material observed on replicate coupon surfaces at time of extraction.

Table D6. Decontamination Efficacy Testing, Average Percent Efficacy

Decontaminant	Material	Avg % Efficacy ± SD [physical removal and chemical decontamination] (%) [A]	Avg % Efficacy ± SD [chemical decontamination only] (%) [B]
Dahlgren Decon™	Painted drywall	99.992 ± 0.006	99.88 ± 0.05
	Coated steel	99.998 ± 0.001	99.88 ± 0.04
	Wood	99.91 ± 0.07	99.82 ± 0.09
	Wood with ascorbic acid	97 ± 3	97 ± 3
pH 5 modified surfactant bleach	Painted drywall	99.5 ± 0.3	96 ± 1
	Coated steel	97 ± 5	91 ± 9
	Wood	88 ± 15	84 ± 19
	Wood with ascorbic acid	88 ± 9	80 ± 10

[A] Avg test coupon recovery vs avg pos control recovery. Combined efficacy of physical removal and chemical degradation.

[B] Avg test coupon recovery plus avg runoff recovery vs avg pos control recovery. Efficacy of chemical degradation only.

Table D7. Average Mass Recovery, Dahlgren Decon™

Decontaminant	Material	Sample Description	Average Recovery											
			Coupon				Runoff				Total Mass (Coupon + Runoff)			
			Mass (µg)	St. Dev. (µg)	RSD (%)	% of Total Mass	Mass (µg)	St. Dev. (µg)	RSD (%)	% of Total Mass	Mass (µg)	St. Dev. (µg)	RSD (%)	% Recovery vs Pos Avg
pH 5 modified surfactant bleach	Saranex®	Positive Controls	1021	52	5.1%	-	-	-	-	-	-	-	-	-
		Test Coupons	224 [A]	261	116%	41%	239	111	46%	59%	463	171	37%	45%
	HazMat suit	Positive Controls	1069	57	5.3%	-	-	-	-	-	-	-	-	-
		Test Coupons	151 [A]	56	37%	35%	272	47	17%	65%	423	47	11%	40%
	Bunker gear	Positive Controls	1135	136	12%	-	-	-	-	-	-	-	-	-
		Test Coupons	118 [A]	100	85%	29%	273	57	21%	24%	391	44	11%	34%
	Neoprene	Positive Controls	1145	159	14%	-	-	-	-	-	-	-	-	-
		Test Coupons	119 [A]	151	128%	26%	320	131	41%	74%	438	35	7.9%	38%

[A] Solid material observed on replicate coupon surfaces at time of extraction.

Table D8. Average Mass Recovery, pH 5 Modified Surfactant Bleach

Decontaminant	Material	Sample Description	Average Recovery											
			Coupon				Runoff				Total Mass (Coupon + Runoff)			
			Mass (µg)	St. Dev. (µg)	RSD (%)	% of Total Mass	Mass (µg)	St. Dev. (µg)	RSD (%)	% of Total Mass	Mass (µg)	St. Dev. (µg)	RSD (%)	% Recovery vs Pos Avg
Diluted Dahlgren Decon™	Saranex®	Positive Controls	1182	322	27%	-	-	-	-	-	-	-	-	-
		Test Coupons	2.3	2.5	109%	18%	15	15	99%	82%	18	18	99%	1.5%
	HazMat suit	Positive Controls	992	52	5.2%	-	-	-	-	-	-	-	-	-
		Test Coupons	103 [A]	72	69%	90%	8.0	10	126%	9.6%	111	74	66%	11%
	Bunker gear	Positive Controls	917	36	3.9%	-	-	-	-	-	-	-	-	-
		Test Coupons	28 [A]	8.9	32%	87%	4.9	4.4	91%	13%	33	12	38%	3.6%
	Neoprene	Positive Controls	822	105	13%	-	-	-	-	-	-	-	-	-
		Test Coupons	13 [A]	6.8	52%	81%	3.4	5.0	149%	19%	16	6.4	39%	2.0%

[A] Solid material observed on replicate coupon surfaces at time of extraction.

Table D9. Decontamination Efficacy Testing, Average Percent Efficacy

Decontaminant	Material	Avg % Efficacy ± SD [physical removal and chemical decontamination] (%)[A]	Avg % Efficacy ± SD [chemical decontamination only] (%) [B]
pH 5 modified surfactant bleach	Saranex®	78 ± 25	55 ± 17
	HazMat suit	86 ± 5	60 ± 5
	Bunker gear	90 ± 9	66 ± 6
	Neoprene	90 ± 13	62 ± 6
Diluted Dahlgren Decon™	Saranex®	99.8 ± 0.2	98 ± 2
	HazMat suit	90 ± 7	89 ± 7
	Bunker gear	97 ± 1	96 ± 1
	Neoprene	98 ± 1	98 ± 1

[A] Avg test coupon recovery vs avg pos control recovery. Combined efficacy of physical removal and chemical degradation.

[B] Avg test coupon recovery plus avg runoff recovery vs avg pos control recovery. Efficacy of chemical degradation only.

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