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Chemical Warfare Agent Retained Agent Test Method and Best Practices for Hazard Mitigation Technology Performance Analysis Source Document

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

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This report provides a revised methodology for testing and data analysis in the evaluation of chemical warfare agent hazard mitigation technologies. This update includes a new shim panel that enables the characterization of agent entrained in complex features that may be significantly harder to decontaminate than flat panels. Capturing entrained agent sources in testing enables a significantly more accurate representation of the decontamination efficacy that would be observed for complex full-scale assets. A best practices calculation capability is provided in the methodology that accounts for the abundance of materials and features on an asset for relating laboratory results to asset-level performance and also treats variable agent drop volume effects and variable contamination levels. Additionally, an approach to use the scale-up results to estimate an upper bound exposure assessment, a maximum potential dose (MPD), is shown, which provides vapor and contact exposure outputs that can be compared to health-based requirements.

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Decontamination	Hazard Mitigation	Retained Agent
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Shim Panel	Scale up	Best Practices Guidance

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PREFACE

The work described in this report was authorized under project no. CB10409. The work was started in October 2021 and completed in December 2023. This report contains an overview of a test methodology. The test methodology is provided as an appendix to the report.

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Chemical Warfare Agent Retained Agent Test Method and Best Practices for Hazard Mitigation Technology Performance Analysis Source Document Concurrence Sheet

The Contamination Mitigation (CM) Capability Area Process Action Team (CAPAT) concurs with the methodology and best practice calculation guidance contained here. T&E WIPTs will determine how and where this methodology and best practice calculation guidance are implemented into their program for use. Formal implementation of the methodologies described here will follow the Test Operations Procedure process. For any non-concurrences, a dissenting paper will be attached.

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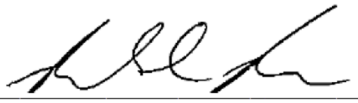
Joint Requirements Office for Chemical, Biological, Radiological and Nuclear Defense (JRO-CBRND)	 MAJ Michael C. Moore	09 APR 2024
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CHEMICAL WARFARE AGENT RETAINED AGENT TEST METHOD AND BEST PRACTICES FOR HAZARD MITIGATION TECHNOLOGY PERFORMANCE ANALYSIS SOURCE DOCUMENT

Current hazard mitigation test methods are documented in Test Operating Procedure (TOP) documents for vapor emission (VE) in TOP 08-2-060A and residual agent (RA) and contact transfer (CT) in TOP 08-2-061B. These TOPs each describe a treatment process (application of contaminant and a hazard mitigation technology) and an analysis methodology that measures post-treatment agent, in addition to multiple other decontamination tests (e.g., material compatibility).

The methodology procedure source document provided herein supplements the existing TOPs and may be considered when updating these TOPs following established TOP update procedures. In the interim the current TOPs (08-2-060A and 08-2-061B) remain in place for VE and CT testing. Users conducting tests per TOP 08-2-060A and 08-2-061B should review this methodology and best practice guidance for consideration in test planning and analysis.

The updates to the methodology include the addition of a shim panel to represent complex features and changes to treatment options and sampling, however, there are fundamental (*revolutionary rather than evolutionary*) changes in data interpretation and calculations. While the primary focus of the methodology update is the addition of a shim panel to enable the characterization of entrained agent, contamination specifications for testing have been updated to use a new per drop normalization approach rather than a surface-averaged starting challenge (i.e., contamination density). Differences in efficacy were observed for the same starting challenge when different agent drop volumes were used.[1,2] This change in definition of contamination provides significant simplification to calculations and enables an easier translation of laboratory results to operational contexts, including variable operational contamination conditions. Contamination density is now accounted for in the scale-up calculations, rather than a test condition applied to a panel. The best practice scale-up calculation and implementation of shim panels require the use of per drop normalization and how panel contamination is specified.

A best practice calculation for maximum potential dose (MPD) is introduced that assumes that all agent mass associated with the asset produces vapor or contact exposures, which enables the use of RA data to evaluate technologies to health-based decontamination requirements. This MPD output provides an upper bound (worst case) exposure estimate associated with the asset of interest. The use of RA data to produce an MPD output is estimated to be *20 times less resource intensive* (i.e., 20 times less resources and time) to characterize compared to acquiring the VE and CT data used to perform explicit exposure assessments. It is an ideal *first step* to build an understanding of how operational conditions and mitigation technology factors integrate to enable the mitigation of potential health effects in personnel using the decontaminated assets. The MPD provides the simplest approach to answer the relevant questions with acceptable accuracy using the least resources necessary.

This report provides the source methodology, as appendices A–C, to be used in generating the new TOP methods. The *data elements* produced by the current TOPs and this test methodology *cannot be directly compared to requirements or key performance parameters*. Rather, the data elements are inputs to calculations that integrate descriptions of how assets are

contaminated (i.e., contamination vignettes used in scale-up calculations) and how personnel use the asset after decontamination (i.e., exposure vignettes) to enable comparisons to requirements or key performance parameters such as health-based criteria. These calculations are provided as best practice guidance in Appendix C.

Measurement of decontamination efficacy for complex surfaces (i.e., not flat and horizontal) and as a function of small and variable size agent drop applications is not possible with the current TOPs. The test methodology provided here directly addresses these gaps with the introduction of a shim panel (two materials separated by a fixed thickness shim washer) to represent complex surfaces and a new data normalization scheme to address variable size agent drops. Most importantly, these updated procedures show how data generated by the methodology in the laboratory can be analyzed for operational relevancy (i.e., to health effect levels). This end-use perspective is required to ensure the methodology and calculations are providing the most accurate and reproducible results in the right context, using the simplest and least resource intensive approach possible. The rationale, details, and demonstration of these new concepts and capabilities are provided in a separate methodology assessment report.[2]

The source methodology covers the procedures for testing and the best practice calculation guidance for evaluating the efficacy of chemical agent decontaminants. The *treatment procedure* includes the contamination and application of hazard mitigation technologies to test materials. For each test panel, one of three laboratory *analysis methods* may be performed including retained agent (RA), vapor emission (VE), or contact transfer (CT). Vapor emission (VE) and contact transfer (CT) analysis methods, and associated exposure assessment outputs, are not addressed in this effort. The analysis methods generate the measured data elements used in *calculations* to produce various *outputs* depending on the type of data that is collected. The shim panel, RA analysis method, and updated calculations, together, comprise a significantly improved capability for evaluation of hazard mitigation technologies.

The methodology procedures are provided in Appendix A. The procedures describe the actions to contaminate, decontaminate, and analyze test panels using the RA analysis method. The RA analysis produces the *Data Element* output of the methodology, the total agent mass associated with a sample after a treatment procedure. The RA data element may be used in multiple analysis procedures according to program needs. Pre-test activities are described in Appendix B. Best practice guidance on the calculations that can utilize the RA data elements is provided in Appendix C. The calculation guidance demonstrates how the data elements may be used to generate outputs ranging from relative source reduction (Output Type A) to scaling the results represent full-scale assets (Output Type B), including a new upper bound health-based MPD.

The methodology assessment report[2] covers the evaluation of the method to meet numerous validation criteria including assessments of method reproducibility and intermediate precision standard deviation. The assessment report also provides a validation of the scale-up calculation to demonstrate the accuracy of the scale-up equation.

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2. Mantooth, B.A.; Schenning, A.M.; Nichols, D.S.; Pearl, T.P.; Hawbaker, N.A.; Davies, J.P.; Sheahy, M.L.; Eikenberg, J.H.; Myers, J.P.; Gehring, D.G.; Burns, J.R.; Chesebrough, M.J.; Ruth, J.L.; Schenning, C.S. *Methodology Assessment for Chemical Hazard Mitigation Technology Evaluations Using Multiple Panel Types*; DEVCOM CBC-TR-1873; U.S. Army Combat Capabilities Development Command Chemical Biological Center: Aberdeen Proving Ground, MD, 2024. Public Release Report (AD000).

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Appendix A: Test Methodology Procedures

HAZARD MITIGATION RETAINED AGENT PERFORMANCE ANALYSIS TEST METHOD FOR CHEMICAL WARFARE AGENTS

1. Scope

a. This Method covers the procedure for testing the efficacy (retained agent) of chemical agent decontaminants. The procedure includes the contamination and application of hazard mitigation technology(s) to test materials, this collective process is referred to as the *treatment procedures* (Figure A-1). In decontamination testing each test panel[a] may be analyzed by one of three *analysis methods* including retained agent (RA), vapor emission (VE), or contact transfer (CT). This methodology document covers only the RA analysis method, VE and CT testing methods use the existing TOP 08-2-060A (vapor) and 08-02-061B (contact) documents. This method provides the source term data to enable efficacy calculations such as log reduction, or as inputs to exposure assessment calculations for vapor or percutaneous exposure assessments using a Maximum Potential Dose (MPD) concept. These methods may be used for multiple stages of technology evaluation. They can be used to optimize a technology, to perform sensitivity analysis to identify critical materials for testing, and can be used for the evaluation of a technology to key performance parameters.

a The term panel refers to any test article, with simplified geometry, meant to represent the surface of an asset, synonymous with the term coupon. Panels can be flat, shim, horizontal, vertical, or composite, and can range in size provided they conform to available test infrastructure. This method focuses on the testing of panels, as opposed to assets (which can be full-scale or scaled down equipment items).

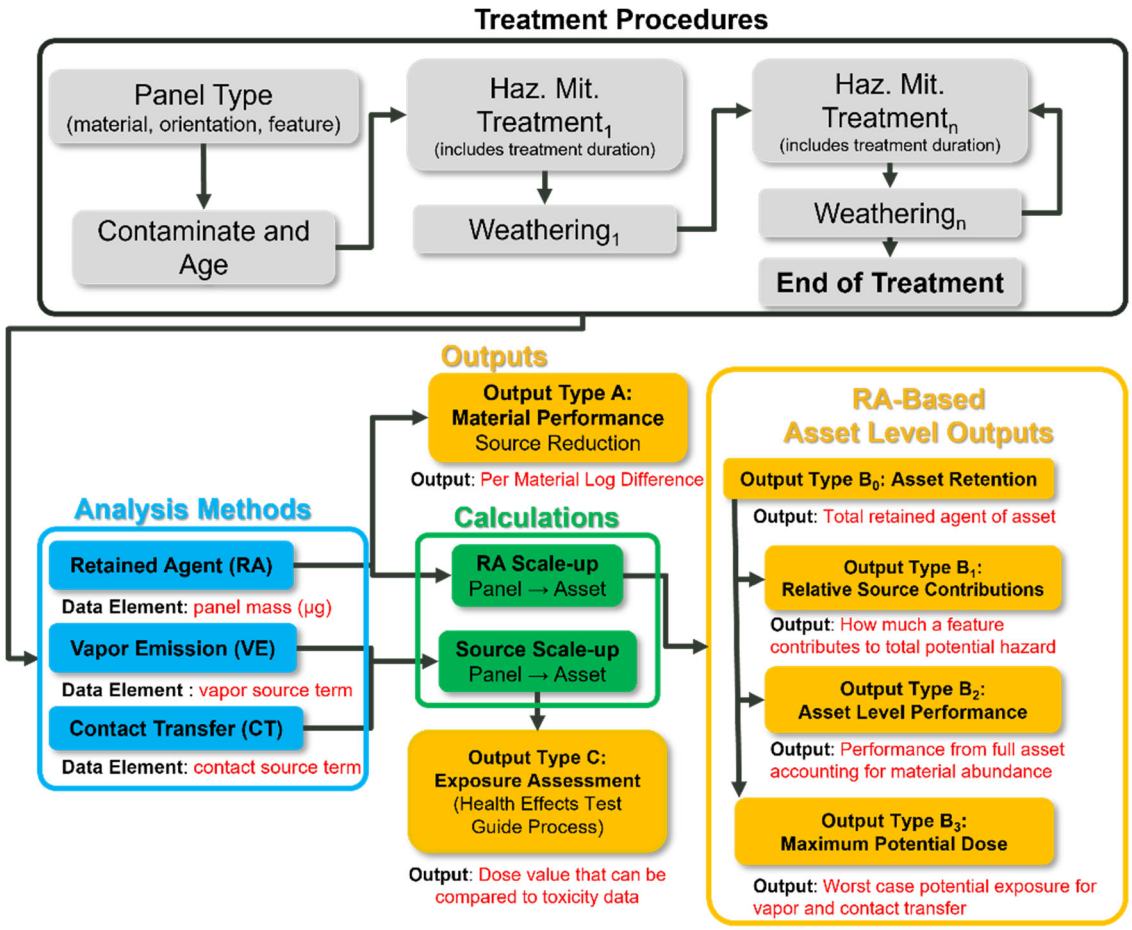

Figure A-1. Methodology components and outputs.

1.1 Purpose

a. This method contains the procedures for establishing the performance of a decontaminant (with or without an applicator) against chemical warfare agents (CWAs).

b. RA Analysis Method: The RA method extracts the panel to quantify the total agent mass associated with the panel after the treatment process. This represents total mass that may be available for exposure to personnel either by vapor emission or contact transfer.

c. Output Types: The data elements generated by this methodology are the individual RA extracted mass results, and the per drop normalized RA as a function of volume output (Table A-1). These data may be used in best practice calculations to produce measures of efficacy and to enable the estimation of health-based effects for personnel interacting with full-scale assets (Table A-2).

Table A-1. RA-based Data Elements

Data Element	Name	Description
RA	Retained Agent	Mass of agent extracted from a coupon based on a specific contamination and treatment process, often reported in µg.
RA(V)	Retained agent as a function of drop volume	Per drop normalized function that indicates how the RA changes as a function of agent drop volume, specific to a treatment process, often reported in µg/drop. This function is required to enable scale-up and asset level health assessment outputs.

Table A-2. Best Practice Calculation Outputs

Output Type	Name	Description
A	Local Performance	Quantifies the relative reduction of retained agent from the material relative to a reference value (e.g., dose confirmation sample)
B_0	RA-based asset level outputs: Total Retained Agent	Quantifies the total mass of agent associated with a full-scale asset
B_1	Relative Source Contributions	Identifies the relative contribution of a given part or material of an asset compared to the total agent retained by the asset. This output can be used to identify high impact material-feature combinations.
B_2	Asset Performance	Quantifies the reduction of agent from the total asset compared to a reference value (e.g., starting challenge or applied mass). This is the net effect of decontamination at the asset level, rather than the material level (Output A).
B_3	Maximum Potential Dose	Provides the worst-case exposure that could result if personnel were exposed to all of the agent associated with the asset

d. Item Under Test: This methodology focuses on panels of materials for characterizing individual materials or for representing a full-scale asset by treating the asset as the sum of individual materials. This method addresses three test item cases: flat horizontal, entrained agent features using a shim panel, and vertical orientation panels.

1.2 Limitations

a. This method includes procedures for analyzing the decontamination of equipment and infrastructure. Skin (personnel) decontamination is not covered by this method.

b. These methods are specific to liquid contamination, other contaminant phases (e.g., vapor or solid) may require additional considerations and sampling approaches.

c. Use of this test method allows for the characterization of RA. VE and CT testing are covered under TOP 08-2-060A and 08-02-061B, respectively.

1.3 Considerations

a. A hazard mitigation treatment process is conducted for which the application process (e.g., vapor, pipette, spray application, wipe, etc.) is considered as a part of the technology being evaluated. After the treatment process is completed, the sample is analyzed. The quantity of agent present on the material after the treatment process is the focus of the analysis to enable the characterization of potential exposure risks to personel that would interact with the materials after treatment. The change in quantity of agent may be due to the combination of treatment conditions (e.g., weathering, evaporation, degradation) and the action of the hazard mitigation technology (i.e., physical removal or chemical reaction).

b. The process to represent a full-scale asset uses a bottom-up construct; the individual components used to represent an asset are identified, tested, and considered in output calculations. Test resources are limited, and it is not likely every material (to include orientation and feature type) on an asset can or should be tested. The test design should identify the most influential materials to test and include in calculations. Some materials may contribute orders of magnitude more than others. An influential material is identified by the combination of contaminated material abundance (e.g., area) and source term magnitude. Output Type B_1 is a process designed to identify significantly influential materials on an asset, which, if omitted, can result in a bias of the efficacy assessment. It is critical to have a concept of the range of applicable assets to ensure the appropriate materials are included in testing. As all possible assets cannot be realistically captured, representative assets (encompassing key functions and a cross section of potential materials) should be considered.

c. When a material is contaminated with agent, various agent–material interactions and mass transfer processes redistribute the agent in and on the material. The agent distribution is generated as a function of multiple mass transfer processes that may include liquid spreading on the material surface, liquid absorption into the material, liquid evaporation, and liquid adsorption onto the material surface. The quantity of agent that strongly interacts with a material is dependent on the physical and chemical properties of both the agent and the material. For a decontaminant to remove the agent from the material, it must access the distributed agent. Different apparent decontaminant performance may result due to different rate limiting mechanisms associated with accessing each agent distribution. The processes that alter agent distribution tend to be time dependent. For example, during contamination the agent may spread, evaporate, and absorb over time. Timing of decontamination can have a large influence because the resulting quantity of agent and its distribution change over time. As a general observation, any parameter that alters or influences agent distribution will likely influence decontamination efficacy and/or post-decontamination source terms. Therefore, it is important that the timing associated with any test be reflective of the operational use conditions associated with the applicable vignette. Testing should also consider the effect of sequential hazard mitigation steps (e.g., interdependent contamination mitigation concepts such as the sequential use of multiple technologies), to ascertain the cumulative effect of all vignette actions on agent distributions such as pre-treatments.

d. The direct measurement of panels for RA testing provides a representation of how agent interacts with a material and the ability of a hazard mitigation technology to reduce RA. This per material characterization can enable the calculation of metrics such as log reduction or percent efficacy for a given material. The translation of the results to an operational context requires

specific calculations that account for the abundance of material and contamination that may occur on an aggregate material asset.

e. The purpose of a hazard mitigation technology is to *reduce* the quantity of agent that may result in an exposure dose to unprotected personnel as reflected in a *change* in quantity of agent or source term, quantified using the output A or B_2 analyses that provide log differences. This reduction in source terms, when combined with other inputs regarding operational context (i.e., a vignette), can be used to perform an *exposure assessment* that indicates if the use of the technology was specifically responsible for preventing negative health effects (output type C). For example, to determine if personnel could reduce protective posture without experiencing negative health effects. This integrated *systems level* analysis considers the technology being evaluated with many other inputs and the resulting output is specific to the vignette used in the exposure assessment. The exposure assessment process using MPD is provided as a best practice guidance in Appendix C.

f. The context of data interpretation provided assumes reactive or removal-based treatment technologies. The test and evaluation of encapsulation technologies may be able to use the same methods, but the interpretation of RA in the material may be different.

2. Facilities and Instrumentation

2.1 Facilities

Table A-3. Facilities

Item	Requirement
Chemical agent facility	Constructed to ensure safe and secure storage, handling, analysis, and decontamination of chemical agents. Facility must be equipped and certified for work with chemical agents.
Chemical agent test chamber	Constructed to allow test item contamination and decontamination and extended residual hazard sampling of small items of equipment deliberately contaminated with chemical agent/simulant in a temperature and humidity-controlled environment. The chamber must have sufficient volume to allow free air circulation around the test item. Ability to control temperature, relative humidity (RH), and wind speed may be required.
Chemical agent test laboratory	Equipment, interior surfaces, tools, and waste must be easy to decontaminate. Must have certified fume hoods for the containment of toxic chemicals including chemical warfare agents. All exhaust air must be filtered and monitored to prevent any chemical release to the environment. The facility design should ensure safe transfer, handling, challenge, and disposal of chemical contaminants, decontaminating solutions, and solvents.

2.2 Instrumentation

These values are minimum requirements. Actual instrumentation may have greater precision, and actual values must be reported. Calibration of chromatographic instruments and permissible error of measurement shall comply with local SOPs. The accuracy for analytical chromatography instrumentation allowed by this method is typically ± 20%, or within ± 25 percent at the device Minimum Quantification Limit (MQL). The chromatography MQL should be set to enable the goals of the test.

Table A-4. Instrumentation

Parameter	Measuring Device	Permissible Error of Measurement
Temperature	Thermocouple, remote temperature device, thermometer, or equivalent.	± 1° Celsius (C).
RH	Hygrometer or equivalent.	± 5 percentage points.
Visual record (still)	Digital color camera.	Image resolution and frame capture rate adequate to document details of testing.
Visual record (motion)	Digital video camera.	Resolution adequate to document details of testing.

2.3 Test Controls.

These values are minimum requirements. Actual instrumentation may have greater precision, and actual values must be reported.

Table A-5. Test Controls

Item	Requirement
Process quality samples for GC, LC, or equivalent. These may be samples of a known mass or periodic calibration standards, known as continuous calibration verification (CCV).	Contaminant in mass/volume, ± 20 percent, or at the MQL ± 25 percent. For tests with a "pass/fail line," a standard reflecting this line should be included and checked periodically during each injection sequence, to demonstrate accuracy at the point of highest interest.
Positive control (contaminated but not decontaminated) using coupons.	Contaminant mass per sample (mass) ± 20 percent, as measured by chromatography. Demonstrates output without a treatment process.
Cross contamination control (neither contaminated nor decontaminated to determine if the test procedures introduce cross contamination).	No requirement, output documents extent of potential (vapor) transport cross contamination
Negative control (not contaminated, but decontaminated) using coupons.	No requirement, output documents process related cross contamination
Dose confirmation samples. May be taken before, during, and after test article contamination.	Contaminant per sample (mass) ± 20 percent.

3. Test Guidance

3.1 Test Planning

The test plan must be prepared, coordinated, and approved before testing begins. The test procedures described herein shall be used as the basis for the test plan. The procedures may require modification for unique items or materials to satisfy specific testing requirements in the TEMP or other program documentation. Deviations from these procedures will be coordinated and approved with all concerned organizations in advance of any testing, after giving consideration to the possible effects the changes may have upon the validity and adequacy of the data. Any deviations from this method and the rationale for the deviation will be described in the test plan. As a best practice, a test readiness review will be conducted before the test begins.

3.2 Operational Vignette Context

An operational vignette is a key element to the design of a test plan. The operational vignette describes the relevant assets (and associated materials) to be decontaminated, the timing of contamination, decontamination, and post-decontamination exposures. These data are used to refine the test parameters and provide context to enable MPD outputs.

Consideration of what assets a hazard mitigation technology will address is a key element in identifying what materials should be included in a test program. It is recommended that the higher throughput, lower cost RA testing be used in combination with Output Type B to identify the influential materials that should be the focus of further testing.

3.2.1 Contamination Considerations

Decontaminant performance is significantly influenced by the agent distribution in and on materials. The contamination conditions should produce a distribution similar to what the technology is expected to address in the operational environment. Multiple factors contribute to generating a representative distribution, the factors identified in Sections 3.3.2–5 should be addressed in a test plan.

3.2.2 Contamination Density and Agent Drop Volumes

The contamination of the material is a critical component of the test method. Starting challenge (mass of agent per unit area of material, g/m^2), also known as contamination density, is an operational context that describes the extent of contamination on an asset surface. The same starting challenge generated with different drop volumes could produce different results.[1,2] Data analyzed on a per drop basis is independent of the starting challenge. Contamination based on drop volume, rather than contamination density, is appropriate. If the operational drop volumes are different than the laboratory drop volumes, there may be a bias in the efficacy measured in the laboratory relative to what would be expected in the field. Operational drop volumes typically follow log-normal distributions spanning a wide range of volumes. The threat community should be engaged to identify an appropriate drop volume range based on the anticipated threat scenario(s). Applicators used in laboratory-scale operations can typically generate drop volumes in the range of 0.2–4 µL, and this range should be considered in the absence of more specific threat information.

Characterization of decontaminant efficacy as a function of agent drop volume, RA(V), enables a characterization of potential operational performance across variable contamination densities and can account for the difference in agent drop volumes used in the laboratory and what may be observed operationally. The ability to generate the RA(V) data element requires specific laboratory test conditions. The TOP 08-2-060A and -061B methods specified contamination as a starting challenge (grams of agent per square meter of material) with no specifications regarding the agent drop volume. The updated method applies a specific number of drops of the same drop volume to a panel, specific drop volume conditions are acquired to characterize RA(V). The number of drops applied to a panel may be varied; however, it is recommended that shim panels are contaminated with only one drop of agent. Two approaches were developed to characterize RA(V), a more accurate multivolume fit technique that requires variable agent drop volume data or a less accurate extrapolation technique where only one agent drop volume is tested. The comparison of accuracy is provided elsewhere.[1]

Starting challenge approaches typically collected five replicates per starting challenge condition for each agent-material-feature-treatment combination. The methodology assessment demonstrated that the variability of output may be different for each agent-material-feature-treatment combination.[1] The replication required to provide a specific statistical power may vary for each agent-material-feature-treatment combination. The number of replicates required for variable agent drop volume analysis is distributed across the drop volumes tested (i.e., the degrees of freedom in the fit). Variable drop volume testing could utilize multiple sampling schemes and tested drop volumes shown in Table A-6.

Table A-6. Contamination Test Designs

Test Design	Contamination	Replication	Scale-up Accuracy	Limitations
Contamination Density*	Apply a specific starting challenge (g agent / m^2 material)	A set of replicates is typically acquired for each starting challenge of interest.	N/A*	Assumes no drop volume effects on efficacy
Extrapolation	Per drop normalization, one or more drops of a specific volume is applied to the material	Similar to contamination density, however the number of drops does not have to be adjusted to achieve a specific starting challenge	less accurate, especially as the operational drop volumes diverge from the tested agent drop volume[1]	Assumes no drop volume effects on efficacy
Fit	Per drop normalization, one or more drops of a specific volume is applied to the material, and *a range of drop volumes are individually tested*	Replication is distributed across agent drop volumes, more replication provides more degrees of freedom in the analysis and more accurate estimates of mean and standard deviation outputs	more accurate	Requires more test samples

* data collected with contamination density parameters could be reinterpreted using the extrapolation technique to use in the scale-up calculations presented here, or material area based scaling could be used.

To obtain a per drop volume relationship, materials are tested with different drop volumes and drop counts. One or more drops of identical volume are applied to a panel. A linear regression is performed for each tested condition using

$$\log_{10}(RA(V)/drop) = m \log_{10}(V) + b \qquad \text{(Equation 1)}$$

Where (RA(V)/drop) refers to the residual agent per drop of agent drop volume, V.[2] If only one drop volume is tested, the extrapolation technique is used to determine the RA response for different drop volumes in equations provided in Appendix B.

All replicates acquired across the agent drop volumes contribute to the degrees of freedom in the regression. Notional agent drop volumes and replication are provided in Table A-7 for each contamination and analysis context. The exact scheme to acquire the necessary data should be selected based on the objectives of the test. Acquiring more samples using more drop volumes

will tend to provide improved accuracy in mean and standard deviation results, at the expense of additional testing.

Table A-7. Replication for each agent-material-feature-treatment combination

Agent Drop Volume	Contamination Density*	Extrapolation from V = 1 µL	Fit, replication low	Fit, replication medium	Fit, replication high
0.2	-	-	2	3	2
0.6	-	-	-	-	2
1.0	5	5	2	3	2
2.0	-	-	-	-	2
4.0	-	-	2	3	2
Total samples	5	5	6	9	10

* corresponds to the contamination density-based testing rather than per drop approaches

Panels must be sized to allow for the requisite number of drops to be applied, the hazard mitigation technology to be applied, and as such, a minimum panel size of 0.5 inches (1.25 cm) in diameter (about 0.25 in^2 or 1.5 cm^2) is recommended. The maximum size is limited by the ability to analyze the sample (e.g., extraction glassware size limitations). Panels may be any geometry (e.g., square), however round panels tend to fit through container necks and sit flat in smaller extraction containers which provide more working hood space and can significantly influence test throughput.

3.2.3 Contamination Applicator

The ability to deliver small drops of contamination may be challenging especially if thickened agents are used. Generally, air piston pipettes should not be used for volatile or viscous liquids (i.e., non-aqueous solutions). Typical contamination applicators may include: positive displacement repeater pipettes (typically limited to minimum volumes of 1 µL), syringes (with repeaters or syringe motors) that can achieve as low as ~0.1 µL drops, or other specialized dispensing systems.

3.2.4 Agent Purity

Chemical agent purity must be determined and a purity certificate must be available. Purity determination should follow the methods outlined in TOP 08-2-073. The purity of the agent must be analytically demonstrated at a frequency based on TOP 08-2-073, determined by the testing organization, or based on experience with the agent used. Purity analysis must have been conducted within 12 months of the test (except for VX, which must be purity-analyzed within 3 months). Note that mass purity of the agent is not as influential on RA(V) compared to the presence of specific types of impurities, which are often agent-specific.[3,4] Also, additives such as thickeners may have significant influence on agent distribution and efficacy resulting from differences in viscosity, solubility, rate of dissolution, and adhesion to material surfaces.[5] In the absence of more specific guidance, Chemical Agent Standard Analytical Reference Material (CASARM) agent shall be used, and purity analysis for the lot of agent used should be kept on file.

3.2.5 Contamination Aging Period and Weathering Period(s)

The contaminant-material aging period is the amount of time that the contaminant resides on the test material until an active hazard mitigation action is conducted. This period is often referred to as the *aging period*. Aging period should not be confused with *weathering period*, which refers to time intervals between decontamination steps, after decontamination but prior to analysis, or in the case where no active hazard mitigation steps are conducted, the time between contamination and analysis. Mass transport processes (e.g., sorption, evaporation, liquid spreading) during the aging period generates the agent distribution that a hazard mitigation technology must address. The duration should be selected to represent the operational time scale on which the hazard mitigation technology is to be used. Very short durations may not generate absorbed agent distributions, and exceedingly long contamination durations may result in a decrease of agent mass from evaporation, or may result in significant absorption (especially for lower volatility agents).

3.3 Panel Types and Sizing

3.3.1 Panel Features

There are two primary types of panels in the context of panel features, i.e., flat panels and shim panels. Shim panels are two materials separated by a specific thickness shim that generates a gap that may entrain agent. Details of shim panel construction are documented below. Shim panels are not intended to exactly replicate all complex geometries that may be found on an asset (material interfaces, recessions, fasteners, etc.), rather shim panels are intended to broadly represent constrained geometry complex features.

3.3.2 Panel Orientation

There are two primary types of panel orientation, horizontal and vertical. For flat panels, the horizontal orientation captures agent-material interactions and the ability of a decontaminant to access and remove the agent from a material in the absence of gravity-driven liquid flow. In contrast, a flat panel in a vertical orientation allows for flow of the agent and/or decontaminant down (and off) the surface of interest. The liquid flow may influence the agent distribution and the duration of actual interaction between the hazard mitigation technology and the material. A reusable stand is used to hold panels in a vertical orientation. Shim panels are typically only tested in the horizontal orientation, as capillary effects are expected to be much more influential than gravitational flow effects, and to keep test size reasonable.[1] Flat panel results typically exhibit minimal to no statistical difference as a function of orientation and deviations from this trend are specific to agent-material interactions (e.g., agent run off) and characteristics of the treatment process (e.g., decon run off).[1]

Application of contaminant to a vertical orientation panel is performed by placing the panel in a horizontal orientation, applying contamination, and then returning the panel to a vertical orientation as quickly as reasonable (i.e., to prevent agent from coming off the surface).[6] An example of this process is illustrated in Figure A-2.

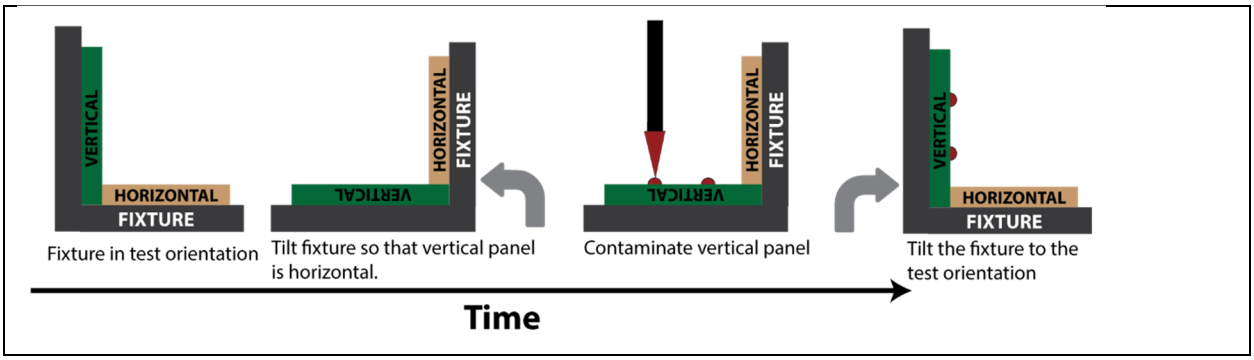

Figure A-2. Contamination process for vertical orientation panels.

Decontamination application for vertical panels should be conducted with the panel in a vertical orientation to capture any application and flow effects.

Limitations: the current vertical orientation test focuses on the interaction of agent placed on the panel. In the context of asset scale-up, a coupon represents a small area that could have contaminated regions above or below the small region represented by the coupon. The test does not consider the effects of agent runoff to material 'below' the test coupon, or agent runoff onto the test coupon from contaminated material that would be 'above' the test coupon. Other larger scale tests using frangible panels have been designed for this purpose, but are out of the scope of this methodology.

3.3.3 Flat Panels

(a) Width/Diameter

Panels can range from 0.5 inch diameter disks (or other two dimensional shapes) to larger sizes that facilitate the desired test capability and sample mounting (e.g., vertical surface mounts) or vapor chamber size limitations. Historically, a 2 inch diameter disk has been the most frequently utilized flat panel size. The use of per drop normalization does not require that a specific starting challenge (mass of agent per unit area of material) is generated in testing.

(b) Thickness

Thickness can influence test results if agent transport during any treatment process results in breakthrough, i.e., agent reaches the other side of the material. Unless the material has a defined thickness (e.g., paint coatings), the thickness of the material should be chosen to be sufficiently thick to capture transport as would be observed in the operational context, while precluding any unintended effects. This is an especially important factor for porous materials that may permit deeper agent distributions, e.g., environmental materials relevant to terrain decontamination. Procedures for ensuring that agent does not reach the opposite surface are included in Methodology Procedure Appendix A.

(c) Vertical Orientation Stand

The stand should be made from materials that do not sorb contaminant (e.g., metals), and can be decontaminated between tests to ensure no test-to-test cross contamination. Guidance is that any fixture used should not influence contamination or decontaminant flow. Example stands are

shown in Figure A-3. In this example the binder clips were above all agent drops and did not influence the flow of contaminant down the vertical surface.

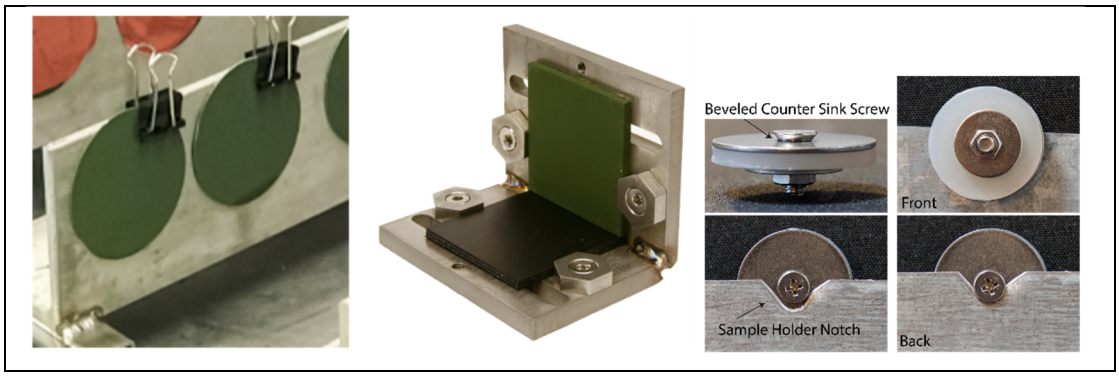

Figure A-3. Example vertical test fixtures

3.3.4 Shim Panels

The details of the standard shim panel design and construction are shown in **Figure A**-4.

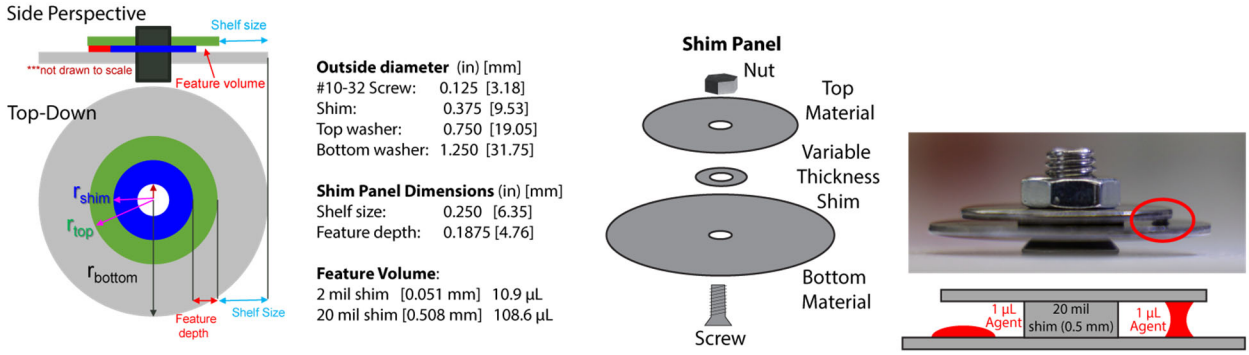

Figure A-4. Shim Panel Design

All design parameters are fixed except for the materials of the top and bottom washers, and the shim thickness (since these are the test materials of interest). Fixed parameters are given in Table 7, the pieces of the hardware listed, i.e., low profile screw, low profile hex nut, shim material, are 18-8 stainless steel.

Table A-7. Fixed Shim Panel Parameters

Parameter	Dimensions [mm (inch)]
Low Profile Screw Length	6.4 (1/4)
Low Profile Screw Thread Diameter	4.8 (#10-32*)
Narrow Hex Nut Thickness	2.8 (7/64)
Narrow Hex Nut Inner Diameter	4.8 (#10)
Narrow Hex Nut Outer Diameter	7.9 (5/16)
Shim Inner Diameter	6.4 (1/4)
Shim Outer Diameter	9.5(3/8)
Bottom and Top Washer Inner Diameter	5.1 (#10)
Bottom Washer Outer Diameter	31.8 (1.25)
Top Washer Outer Diameter	25.4 (1)
Bottom and Top Washer Thickness	0.8 to 1.2 (.033 to .047)

*Notation indicates a number 10 screw with 32 threads per inch.

(a) Shim Thickness

Generally, the smaller the gap and the more wetting the agent-material interaction, the more significant the effect of agent being drawn into the gap. As the gap becomes larger, the efficacy will approach that observed for flat panel testing. It is recommended to select shim thickness representative of the features on asset(s) of interest (if known). In the absence of additional guidance, default values of 0.002 and 0.020 in (50 and 508 μm) are recommended. These gaps represent generically 'small' and 'moderate' gaps that may be commonly found on a wide range of assets. Use of elastomers or soft materials may require a rigid spacer to prevent compression of the material.[1]

It is recommended to confirm shim gap spacing for batches of material-features ideally within ± 50% of the specified gap spacing using tools such as a feeler gague. Significant changes in efficacy may require order of magnitude changes in gap spacing, therefore the accuracy of the gap spacing is less critical[1].

(b) Contaminant Application to Shim Panels

Traditionally, flat panel testing has used a repeater pipette to enable the reproducible delivery of multiple drops to material surface. However, the repeater pipette uses an impulse to eject drops from the pipette tip, rather than a more controlled touch off. Shim panel contamination requires delivering the contaminant into the gap of the panel. Either a syringe or single action positive displacement pipette is used to control and 'touch off' the liquid agent into the feature.

3.4 Decontaminant

3.4.1.1 Decontaminant Applicator

The application of the hazard mitigation technology to the test material should be carefully considered in laboratory testing. The introduction of vertical surfaces and capillary gaps produces restricted geometries and the potential for flow of the agent and decontaminant (liquid,

vapor, or aerosol delivery). The angle, flow rate, pressure, or drop size may be influential if the decontaminant penetrates into a feature, sticks, or flows over a vertical surface (or bounces off the material). The test plan should document the rationale for how decontaminant is applied to account for specific fluid dynamics considerations (whether liquid or gas). For instance, in 'sprayer' based technologies, shear forces may be relevant to consider while local air velocity (direction and magnitude) may be relevant for vaporous treatment processes. If a post-treatment rinse process is utilized, similar considerations should addressed and documented. In some cases the post-treatment rinse may be performed specifically to facilitate sample analysis (e.g., remove decontaminant to prevent sampling interference).

3.4.2 Decontaminant Quantity

The rationale for how much decontaminant to apply to a panel should be documented in the test plan. Types of decontaminants vary, and the appropriate metric should be considered. For example, the "quantity of decontaminant" may vary significantly between a wipe, a vaporous treatment, a dry powder, and an applied liquid (or slurry) treatment. In most cases, it is expected that the decontaminant will be in significant excess relative to the agent quantity and that the decontaminant will not act as the limiting reagent. Several options exist for selecting the quantity of decontaminant to use and are briefly summarized below.

The manufacturer recommended quantity of decontaminant, if available, should be considered first. The rationale used to establish such a recommendation should be understood, to ensure it does not conflict with the basic tenets of how the user community intends to apply the decontaminant in the field (concept of use).

Pre-studies can be conducted to characterize how the quantity of decontaminant may affect test results, and a decision should be made based on a thorough consideration of both performance and non-performance related factors.

Requirements documentation should be considered. If the requirements document relevant to the hazard mitigation technology to be tested explicitly states a coverage area requirement, this can be factored into the selection of decontaminant quantity to be applied. Coverage area may pertain to the amount of surface area per unit mass or volume of decontaminant that a hazard mitigation technology must achieve, the mass or volume of agent that must be mitigated using a hazard mitigation technology, or other factors. Coverage area may also apply to the "capacity" of a hazard mitigation technology, for instance, a wipe may become "saturated" or otherwise ineffective after decontaminating a specific amount of agent or after being applied to a specific surface area.

There are many considerations pertaining to the selection of decontaminant quantity (and application procedures), and they differ for disparate technologies. Selection of decontamination parameters should therefore be carefully considered before beginning a test program. In all cases, the nature of application for the hazard mitigation technology should reflect the anticipated application in an operational environment.

3.5 RA Analysis Method Planning

RA is characterized by extracting agent from the panel using an agent-specific solvent-based extraction process, such that the resultant data element is the mass of agent remaining in or on

the surface of the panel after hazard mitigation has been employed. Agent has a high affinity for the solvent and transfers from the contaminated panel to the liquid phase. The solution comprised of agent dissolved in solvent is analyzed for the presence of agent using chromatographic techniques. The selection of the chromatographic technique is based on not only the agent of interest, but also the anticipated concentration of agent within the extraction solvent. There are several pre-studies that may be done in advance of a test, depending on the circumstances of the test. These pre-studies ensure that data obtained from testing is valid, characterize the degree of validity, and verify that there are no gaps in understanding the factors that influence the determination of residual agent that would skew understanding of the true result. These pre-studies are summarized in Table 9, and are discussed in further detail in Appendix B. In all cases, existing analysis may be used in lieu of experimentation, where appropriate (and demonstrated) methodology already exists. It is important that the extraction solvent selected for RA testing be chosen to maximize the extraction efficiency and that the volume of solvent used be sufficient to fully submerge the panel, but not so much that the residual concentration of agent in the sample is diluted below the MQL of the analytical instrumentation.

Table A-9. Summary of Typical RA Pre-studies

Pre-study	Purpose
Agent-Material Extraction Efficiency	Characterize the degree to which agent can be extracted from the material, applicable to extraction techniques
Rinsate Extraction Efficiency (where applicable)	Characterize the degree to which agent can be extracted from the rinsate (if used)
Stability of Agent in Solvent	Ensure that the agent does not degrade over time in the extraction solvent
Sample Matrix Interference	Characterization of extraction samples to determine bias or interference in chromatographic analysis
Decontaminant Interference	Characterize and mitigate any effects (particularly reactivity) that may lead to an inability to perform chromatographic analyses, or may reduce the concentration of agent in the extraction solvent over time
Material Thickness Adequacy	Ensure that the panel is of sufficient thickness to preclude migration of agent or decontaminant to the distal surface of the panel

Accommodations for shim panel testing may be required. The shim panel has more height than typical panel samples. As such, moving to narrower diameter extraction containers enables full immersion of the panel without increasing the volume of extraction solvent.

3.6 Method Detection Limits

The method detection limits (MDL), the lowest detectable mass, should be as low as reasonable and documented within the test plan. Setting the MDL relative to any health-based requirements must recognize that laboratory data are *scaled* to generate the appropriate context results; *there is no direct 1:1 relationship* between MDL and minimum environmental concentration for exposure assessments. Laboratory testing offers the highest sensitivity with the least environmental interference to characterize hazard mitigation technologies. Data generated

by the RA analysis method is a source term describing what happens on a per drop basis. Any health-based requirement value reflects exposure to personnel interacting with a full-scale asset in the operational environment. For a given vignette, a laboratory MDL may be determined based on the scaling calculations (e.g., material abundance, contamination density), and exposure assessment parameters (e.g., transport and dispersion for vapor exposure).

4. Test Procedures

4.1 Receipt Inspection

a. The test articles (which may include coupons, panels, or small items of equipment) will be subjected to a visual receipt inspection in accordance with (IAW) TOP 08-2-500A. For equipment, user/technical manuals should also be consulted. Evidence of damage or irregularities to the test articles will be recorded in the laboratory recordkeeping system and will be documented by still photographs.

b. Each test article's model, serial number, nomenclature, identifier, manufacturer, lot number, and other pertinent information/indicators, if applicable, will be recorded in the laboratory recordkeeping system. Assignment of a test item control number (TICN) to the test article is mandatory for future identification and tracking. The TICN will be marked on small items of equipment in a location that will not interfere with test procedures. Coupons/panels may be labeled on their reverse side, or on the container or tray holding the material during testing with the TICN. The preferred method for marking the TICN is engraving. Other methods for labeling must consider the potential for analytical interference and record results of the methodology used to determine that no interference exists. The TICN and other pertinent information about the test article must be linked in the laboratory recordkeeping system.

c. If any items are determined to be unfit for testing, they will be rejected and replaced with items that are in suitable condition for testing.

4.2 Trial Preparation

a. Chemicals used for preparation of decontaminant formulations will be used as-received. Composition/purity will be established based on supplied documentation, such as Certificates of Analysis/Conformance. If no documentation is available, other means of characterizing composition, such as assays, may be used at the direction of the test sponsor. Chemicals used as solvents, as well as simulants, will be purchased in the highest purity available from the manufacturer or distributor, or as directed by the test sponsor.

b. Candidate decontaminants will be prepared IAW the manufacturer's instructions, or as directed by the test sponsor. Fielded decontaminants will be prepared IAW applicable military technical manuals. Quality checks will be performed as necessary by routine analytical methods [such as pH (hydrogen-ion-concentration) measurement, titration, etc.]. The pot life as specified by the manufacturer will not be exceeded. Pot life may also be determined through experimentation, where typically such experiments would examine efficacy as a function of time from decontaminant preparation and follow the basic tenets of this method.

c. The test setup, labeling of vials, trays, jars, etc., and other associated pretest tasks will be completed.

d. Coupons/panels may require cleaning before testing to remove cutting oils or other preparation contaminants. The marked coupons/panels or small equipment articles will be stored in a secure, environmentally-controlled location. The test articles will be protected from unrelated environmental contaminants and degradation.

e. All calibrated instrumentation will have a current calibration date.

f. Timing charts for staggering contamination, decontamination, and other test events may be required. The timing charts will assist in minimizing data scatter that may be caused by subtle differences in coupon treatment.

g. If required, a log of test parameters and adherence to said parameters should be developed to support test sponsor Data Authentication activities. The format and data contents of such a log should be established in advance of the test between the laboratory and test sponsor.

4.3 Preconditioning Procedure

a. Any required preconditioning of the test materials described in the test planning documentation will be performed.

b. Controls will be preconditioned using the same method as for the test articles.

4.4 Panel Treatment Procedures

4.4.1 Contamination Procedure

a. Chemical agent will be applied to the test article (positioned horizontally during initial contamination). Document (quantitatively or photographically) the appearance of the drop(s) at the time of application and before applying the decontaminant. For vertical panels, the test stand can be rotated so the vertical panel is in a horizontal orientation for agent application. Immediately after contamination, the stand is carefully oriented to put the panel in a vertical orientation. For shim panels, the agent is applied directly to the gap interface with a positive displacement tool (e.g., syringe or pipette), rather than ejected from a repeater pipette.

b. Test articles may be photographed to document the contamination surface coverage. If desired, a dye may added to the contaminant to allow for better visualization of contamination; however, it should be demonstrated in advance that the dye does not impact the behavior of the contaminant (e.g., spreading) nor the data results, or the behavior should be documented in pre-test activities.

c. During testing, the test article should remain uncovered when representing field conditions. The contaminated test article will be allowed to weather/age for a time specified in the test plan.

4.4.2 Decontamination Process

a. The decontamination process will be performed as described in the test plan, based upon vendor or test sponsor instructions, or IAW applicable tactics, techniques, and procedures (for fielded decontaminants). If scrubbing/brushing of the decontaminant is required to mimic operational use, a standard hard bristle toothbrush may be used to replicate this process. A new toothbrush should be used for each sample to prevent cross contamination.

b. If sequential decontamination is to be employed (i.e., to represent the application of time phased hazard mitigation processes for sequential phases of decontamination such as immediate, operational, thorough), such decontamination should be performed consistent with applicable CONOPs. Often, periods of weathering are applicable between sequential decontamination processes, and these weathering periods should be reflected in the overall hazard mitigation approach.

c. Periodic mixing of decontaminant(s) may be required, as specified by the test sponsor.

d. The surface shall be exposed to the decontaminant for the amount of time specified in the test plan. This duration is referred to as the dwell time (or in the case of constant exposure as may be seen in vaporous decontamination systems, this duration may be referred to as the duty cycle), and may be influenced by requirements, intended CONOPs, or based on performance "optimization." When attempting to optimize performance, non-performance factors such as resource consumption, physiological burden, or other factors may be considered and should be discussed with the stakeholder community. Experimentation to determine the appropriate dwell time may be conducted as a pre-study, and will typically follow the tenets of this test method.

e. If pre-wash or post-rinsing is required, the nature of the wash or rinse should be consistent with anticipated operational use. For panels, where applicable, and in the absence of further guidance, a given pre-wash or rinse will entail the application of 20 mL of water (a surfactant may be added where applicable), twice, to the front of the panel, and 20 mL of water, once, to the back of the panel. The 20 mL volume has traditionally been used for 2 in diameter disk shaped panels, the rinse volume may be adjusted as needed. The method of applying the rinse may involve the use of a pipette, pump, or other means and shall be as directed by the test sponsor. Where possible, the rinse may be applied to replicate intended rinse applicators (such as an M12 or M26 decontamination apparatus) or may use the apparatus itself for large scale laboratory operations. If a rinse is used solely to preclude analytical interference effects caused by the decontaminant, it may be necessary to capture and analyze the rinsate to ensure proper mass balance. In this case, the rinsate may be examined for the presence of agent using extraction techniques. Analytical interference effects should be considered.

f. Any visible degradation of the test article caused by contamination and/or hazard mitigation, will be recorded via photography or qualitative description, as appropriate. For example, HD is known to react with plastics under certain conditions, causing a warping/bubbling effect.

5. Analysis Methods

5.1 Retained Agent Analysis Method

a. Each panel will be placed in a container with extraction solvent. Shim panels are not disassembled for extraction. Vertical orientation panels are removed from the stand for extraction. The size of the panel is an important consideration in this procedure. The larger the test article size, the more solvent that will be required to extract the contaminant. The more extraction solvent used, the more diluted the contaminant concentration will become, making it less likely to be detected. For most materials, the contaminated side will be placed face-up; however, if the material being tested floats in the solvent, the sample will be placed face-down so that solvent contact occurs. Complete immersion of the test article is required.

b. The container will be sealed with a lid lined with Teflon® polytetrafluoroethylene [(PTFE) DuPont™, E.I. du Pont de Nemours and Company, Wilmington, Delaware], or equivalent lid liner that does not result in chromatography interference.

c. In order to facilitate contaminant extraction, the container will be agitated using a means consistent with that used during extraction efficiency pre-study work. This may include manual agitation such as swirling, automatic means such as the use of a mechanical agitator or developer, or sonication.

d. The test article will remain in the extraction solvent for a period of time consistent with extraction efficiency pre-study work. A period of 60 min or greater is typical.

e. At the end of the extraction period, the container will be swirled for 30 s before the lid is opened. After swirling, an analytical vial will be opened, and a clean pipette tip will be used to place an aliquot into the vial for analysis.

f. The sample may require dilution to be within the calibration range of the chromatography method, internal standards (if used) are also added to the sample.

g. The extraction sample is analyzed using the applicable chromatography methods providing an output of mass of agent retained, typically in units of µg of agent.

h. Samples not receiving immediate analysis may be stored until analysis can be completed. Samples should be stored in accordance with processes demonstrated in stability pre studies conducted in accordance with Appendix B.3 (e.g., stored at a reduced temperature and analyzed within a set time period).

6. Data Required

(1) RA quantitative values of analytes (e.g., mass) for all samples.

(2) Agent identification, purity, number and size of drops applied to each sample.

(3) Material, geometry, feature (e.g., shim and gap size), and orientation for each sample

(4) Instrument calibration results and MQL (upon request).

(5) Trial date, start time, and end time.

(6) Laboratory environmental conditions (such as temperature and RH/AH and windspeeds).

(7) Test infrastructure environmental conditions (for controlled environment tests).

(8) Extraction solvent used and purity.

(9) For test articles: TICN and sample location(s) and receipt inspection results.

(10) Description of key equipment, such as model designation, applicable standards.

(11) Visual observations to include photography (upon request).

(12) Decontaminant lot number, preparation, application method.

(13) Rinsate used, method of application, and volume.

(14) Surface wind speed for tests conducted within a fume hood or glovebox.

(15) Control sample and quality check standard values.

(16) Dose confirmation values.

(17) Rinsate values (as applicable).

(18) All timing associated with key aspects of the test (e.g., age time, treatment duration, weathering period, extraction duration, etc.).

(19) Any deviations from the test plan.

7. Presentation of Data

7.1 Data Review

a. Methodology assessment efforts identified that the RA data generated by these methods are heteroscedastic, present censored distributions (i.e., method detection limits), and may often produce skewed distributions. Data may be most appropriately analyzed with log transformations.[1] Outlier analysis, which will be conducted via a DAG meeting, should be conducted with extreme caution on the appropriate scale (e.g., log transformed).

b. The data may be tested for outliers during data analysis. A standardized method such as American Society for Testing and Materials (ASTM) Standard E0178,[7] will be used. Although ASTM Standard E0178 discusses multiple methods to test for outliers, the method discussed in Paragraphs 6.1 through 6.2 of ASTM Standard E0178 is recommended. Data points determined to be outliers, which will be determined by the T&E community during the PoR DAG meeting, may be excluded from statistical data analysis and should be flagged as being excluded, but must be reported along with the appropriate data set.

c. Large data variances are not always an indication of a poorly executed test and must be investigated in conjunction with the with PoR T&E WIPT and DAG meetings before data is rejected.

7.2 Data Acceptance Parameters

The following are the set point tolerance ranges in which trials should be conducted in order for the data generated to be acceptable for use.

a. Temperature: temperature should maintain the target temperature ± 3 °C. Temporary temperature excursions in excess of ± 3 °C should be reported, data should be reviewed to determine the effect the temperature.

b. RH: RH measurements should be within ± 5 percentage points of the target RH. Temporary humidity excursions in excess of ± 5 percentage points should be reported, data should be reviewed to determine the effect of humidity.

c. Dose confirmation samples (mass or volume) will be within ± 20% of the target.

d. Decontaminant acceptance parameters are specific to the technology being employed, as will be established in the test plan.

e. Timing will be within ± 10% of the target for each timing element.

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ABBREVIATIONS

AD No.	DTIC accession number
ASR	agent-simulant relationship
ASTM	American Society for Testing and Materials
ATEC	US Army Test and Evaluation Command
ATTN	Attention
C	Celsius
CBC	US Army DEVCOM Chemical and Biological Center
CT	contact transfer
Ct	concentration time (vapor dose context)
CWA	chemical warfare agent
DA	Department of the Army
DTIC	Defense Technical Information Center
FID	flame ionization detection
GC	gas chromatograph
GD	Soman
HD	distilled mustard
IAW	in accordance with
LC	liquid chromatograph
MPD	maximum potential dose
MQL	minimum quantification limit
MS	mass spectrometer
NA	not applicable
NRT	near real-time
NTA	nontraditional agent
OPSEC	operations security
OTA	Operational Test Agency
PTFE	polytetrafluoroethylene
QA	quality assurance
QC	quality control
RA	retained agent
RH	relative humidity
VE	vapor emission

Appendix B
Pre-Studies: Retained Agent

This appendix provides guidance for recommended pre-studies pertaining to RA analysis. It is important to understand that the nature of pre-studies must be tailored to the objectives and specific circumstances of each test. Which pre-studies are necessary to ensure a valid test event, and the specific nature of those pre-studies, should be carefully considered by both the performing laboratory as well as the test sponsor and the pertinent stakeholders. Prior to executing empirical pre-study work, a literature search should be performed to ensure that adequate data does not already exist from prior test events. The results of pre-study activities should be documented in the test plan, and presented at the final Test Readiness Review (TRR) to ensure the validity of the test event.

B.1 Agent-Material Extraction Efficiency

The mass of agent in a panel measured during an RA analysis is obtained by extracting the agent from the panel using an appropriate solvent. In an ideal process, the solvent would be capable of extracting all of the agent remaining in the panel (this would correspond to an efficiency of 100%); however, in practice, the extraction process is imperfect. As such, it is critical to an RA analysis to understand the degree to which remaining agent can be extracted by the extraction process. The degree to which agent can be extracted from the panel is termed the "extraction efficiency," and is calculated by dividing the mass of agent recovered in the extraction process by the total agent mass applied to the panel (dose). Extraction efficiency studies should be conducted in a horizontal orientation, even if the test event calls for vertical orientations to be used.

Agent extraction efficiency depends on the agent-material pair, and can be influenced by the distribution of agent within the material, as well as chemical and physical properties of both the agent and the material. Longer aging times can allow the agent to penetrate into permeable or porous materials, making the agent more difficult to extract. The agent may chemically bind to the material, or may otherwise have an affinity for retention, e.g., entrainment within material texture or morphology, making the agent more difficult to extract.

Additionally, agent mass losses during the extraction process, due to effects such as evaporation of agent or reactivity of agent within the material matrix, can alter the amount of agent mass available for extraction. The ability to quantitatively account for mass loss to poor extraction efficiency, evaporation, or chemical degradation is typically *not feasible*. It is important to understand these effects, but in practice they can often be extremely difficult and resource intensive to characterize. As such, there are no established acceptability criteria for extraction efficiency; however, experimentation should seek to find the process (extraction procedures and solvent selection) that maximizes extraction efficiency. If extraction efficiency is less than 80%, it may be important to properly caveat RA results to ensure those results are not misinterpreted. In general, the use of "correction factors" accounting for extraction efficiency, that is, the practice of adjusting RA results based on the observed extraction efficiency, *is not appropriate*.

It is important that the timescales associated with extraction efficiency studies are in line with the extraction timing within the test event itself. This is because extractions within the test event may occur minutes or hours after contamination, and the distribution of agent within the material (and possibly extractability) may change significantly between the point of

contamination and the point of extraction. The nature of the agent and panel material(s) will influence how this distribution changes over time. Consider the case where an agent is to be extracted from a material 2 hours after contamination in a test event. For an impermeable material such as stainless steel, it may be appropriate to conduct extraction of the panel shortly after contamination in an extraction efficiency study, as the agent will not significantly penetrate the material even over long timescales. For a porous material such as concrete, conducting extraction shortly after contamination may not be appropriate, as the distribution of agent at this time (and the ability to extract the agent from the concrete) may not reflect the distribution at 2 hours after contamination. It is often appropriate to characterize the extraction efficiency as a function of time after agent application.

The apparent efficiency of an extraction process can change based on the amount of agent remaining in the panel. It is typically easier to obtain higher extraction efficiencies for large masses of agent than for small masses of agent; the relative quantity of loss is often more significant when smaller quantities of mass are applied. As such, extraction efficiency should be examined for different applied doses. These applied doses should range from a full dose (that is, the dose expected to be applied to the panel surface during the contamination portion of the test event), to a low dose (above but within the same order of magnitude as the MQL). Extraction efficiency pre-studies should examine a minimum of 2 dose levels to ensure efficiency is fully characterized over the range of potential RA values. Extraction efficiency is not expected to be dependent on agent drop volume.

Finally, in order to establish the proper extraction period, that is, the duration of time the panel remains in the extraction solvent prior to analysis, it is important that different timescales be investigated until an optimum extraction time can be determined. This can be accomplished by withdrawing aliquots of the extraction solvent at different time-points. A minimum extraction period of 60 minutes is recommended, but longer timescale may be appropriate. Shim panels may require 24 hours of extraction. The decision of what extraction period to ultimately select will depend on not only optimizing extraction efficiency, but also may take into account laboratory throughput, possible matrix and stability effects, and other considerations. This test design factor should be agreed upon between the laboratory and the test sponsor based on results and test sponsor objectives. There are cases where longer extraction periods may start to extract interferents from the sample and degrade the sample analysis.

Many factors can affect extraction efficiency, and these factors are integrally tied not only to the agent and material but also the circumstances of the test. As such, a thorough and well-planned extraction efficiency test aimed at maximizing not only the extraction efficiency itself, but also insights gained into the nature of any inherent inefficiencies, is vital to a proper RA analysis. Extraction processes used during the record test should match the processes established during extraction efficiency pre-studies. For shim panel samples, extraction is conducted fully assembled. If the extraction recovery is low, longer extraction durations, loosening of the shim panel nut may be considered but is not recommended.

A minimum of five replicates for each combination of agent, material, aging time, and dose shall be implemented in extraction efficiency pre-studies.

A generic extraction efficiency protocol follows, and should be tailored properly for each test event.

1. The panel is contaminated with the appropriate mass of agent. For a full dose, agent should be applied in a manner matching the circumstances of contamination (total mass and drop size) anticipated for the record test. For doses lower than the full dose level, the agent may be diluted in an appropriate solvent to achieve the desired dose level.
2. Agent is allowed to age on the panel for the prescribed period of time.
3. Following aging, the panel is placed into the extraction container (i.e., jar).
4. An appropriate amount of the selected solvent is added to the container, ensuring full submersion of the panel, and the container is sealed.
5. The panel is allowed to reside in the container for a minimum of one hour. The contents of the container may be agitated during this time using manual or automated methods. Aliquots may be withdrawn and analyzed at preselected time-points. Prior to withdrawing aliquots, the container should be manually swirled for 30 seconds.
6. The container is unsealed, and duplicate aliquots are withdrawn and placed into chromatography vials.
7. The vials are analyzed for the presence of agent using the appropriate chromatographic techniques. Duplicate samples may be retained for future analysis.

B.2 Rinsate Extraction Efficiency

If the test event calls for a rinse that is performed solely to preclude chromatography interference effects from the decontaminant, the rinsate must also be captured and analyzed for the presence of agent. In this case, the RA is the sum of agent extracted from the panel and agent extracted from the rinsate.

Rinsate extraction efficiency pre-studies have the same basic objectives, and many of the same considerations, as panel extraction efficiency pre-studies. Procedures may differ when analyzing rinsate. For instance, the rinsate is typically extracted shortly after collection, and so aging timescales are not a significant factor. Also, in a rinsate extraction efficiency study, the agent is typically added directly to the rinsate solution in the container, along with an appropriate, i.e., contaminant-specific, extraction solvent.

B.3 Agent Stability in Solvent

The inherent stability of the agent of interest in the selected solvent should be demonstrated in advance of test. This ensures that the agent does not degrade in the extraction solvent over the timescales that may be realized during the test event. Degradation of agent in the solvent may lead to the erroneous conclusion that extraction efficiency was poor during an extraction efficiency pre-study. Also, if the sample is not analyzed immediately after removal of aliquots, any degradation occurring in the solvent prior to analysis may cause RA to be underreported, leading to a positive bias in efficacy results.

Agent stability in solvent can be determined by adding known dose levels of agent to the selected solvent, then analyzing the solution at different time-points, with the maximum time being how long the laboratory expects before analysis during the test event. Again, multiple dosing levels should be used in this pre-study.

B.4 Matrix Interference

During extraction, it is possible for certain materials that a constituent of the material may "leach out" into the extraction solvent and cause analytical interference, or may itself react with the agent in the solvent and degrade the agent. Whether to conduct this pre-study is dependent on the material under consideration. For example, this may not be an issue for a material such as stainless steel, but may be a concern for materials such as concrete, asphalt and coating materials. For this test, a single panel may be extracted to generate multiple samples for spiking and analysis, five replicate spike samples are recommended. An example procedure follows:

1. A *negative sample matrix* sample is generated by extracting a panel of the material in the extraction solvent. The solvent and solvent volume should match that to be used during testing. The duration of extraction should match the extraction duration to be used in testing. The solution generated from extracting the material for the specified duration is referred to as the *sample matrix*, which contains any chemicals removed from the material during the extraction process. These extracted chemicals can interfere with the chromatography analysis and could artificially enhance or suppress the analyte (i.e., agent) signal. Analysis of this sample should return a value that is below the minimum detection level (MDL).
2. A *spike reference sample* is generated by adding a low level dose of the agent of interest into a fixed volume of solvent (the solvent and solvent volume should match that to be used during testing). An internal standard should be added to the solvent as well. The solution is allowed to reside in the solvent for the prescribed period of time, the sample is swirled to ensure homogeneity, and analyzed for the presence of agent. This establishes a reference value, free of matrix effects, against which to analyze the spiked sample matrix data.
3. Lastly, a *negative control spike* sample is generated by delivering the same mass(es) of agent as in the spike reference sample to the sample matrix (generated in step 2). The negative control spike sample provides a known analyte concentration in the sample matrix. Any difference in the measured agent concentration between the spike reference and the negative control spike is attributed to matrix interference and indicates a suppression or enhancement of agent signal (positive or negative bias). The method requires the spike reference and the negative control spike to differ by no more than 15%. If outside of method specifications, the operators should modify the chromatography or extraction conditions to facilitate the evaluation.

B.5 Decontaminant Interference

For all RA analysis, confirmation should be made that once extraction begins, the decontaminant is no longer playing an active role in the experiment. If decontaminant enters the extraction solution, then reaction with the agent in the solvent can continue, leading to agent mass loss that can be misinterpreted as decontaminant efficacy. Decontaminant can also act as an interference in the chromatography analysis itself.

Before seeking to employ mitigation techniques to minimize decontaminant interference, it is important to know whether an interference effect is even occurring. A generic procedure for initially characterizing the existence of decontaminant interference effects is given below.

1. Fill an extraction container with the appropriate amount of the pre-selected solvent.

2. Add the desired dose level of agent to the solvent. Seal the container and swirl for 30 seconds. Multiple dose levels should be considered as discussed above.
3. Apply decontaminant to an uncontaminated panel in a manner consistent with that to be used during test.
4. Allow the decontaminant to dwell on the panel for a duration consistent with that to be used during test.
5. At the end of the dwell period, place the panel into the extraction solvent, and begin the extraction process (to include any agitation) for the prescribed extraction time. Aliquots may be drawn for analysis at the end of this period, or at pre-selected time-points within this period. The aliquots will be analyzed and compared to the original dose to determine if interference has occurred.

If interference is detected, it may be necessary to mitigate these effects by rinsing the panel prior to placing it into the solvent, adding a quench solution (a reagent that reacts with the decontaminant to prevent further reaction with the agent) to the decontaminant or the extraction solvent, or other methods.

B.6 Material Thickness Adequacy

It is important that neither agent nor decontaminant reach the distal surface of the panel during a hazard mitigation test (i.e., chemical breakthrough). This is important not only for RA analysis, but for CT and VE analysis as well. Distributions of agent and decontaminant within the material must be consistent with what will be seen in an operational environment for the test results to be a suitable predictor of operational results. For instance, if agent reaches the distal surface, it will evaporate, react with decontaminant, and spread in a manner inconsistent with an operational setting, reducing the correlation that can be drawn between laboratory and operational results. This is particularly important for VE analysis, where results may be quite sensitive to changes in agent behavior.

Breakthrough is not a concern for all panel materials, but may be a significant risk for very permeable or porous materials, such as those typically examined for terrain decontamination applications (i.e., concrete, asphalt, sand, etc.).

An initial qualitative screening evaluation can be used when only gross characterization of breakthrough of agent is needed, for instance when examining a requirement that allows for a large mass of residual agent to remain after decontamination. In such a screening, a dye sensitive paper such as M8 may be placed under the panel to evaluate whether agent that is applied to the panel surface breakthroughs after aging. For most tests, a higher resolution, quantitative approach is desired. A generic procedure for a quantitative approach is provided below.

1. A Polystyrene Divinylbenzene (DVB) pad (or similar) is placed under the panel. The pad area should match the panel area. This pad is used to collect any agent that permeates through to the bottom surface of the sample. The extraction efficiency of the pad material should be determined in advance.
2. An outer latex gasket is placed around the pad, with the inner surface of the gasket flush with the edge of the pad. This is used to block the collection of agent vapor from outside sources (other coupons or around the edge of the panel).

3. The panel is contaminated in a manner consistent with that to be used during test.
4. The panel is allowed to age for the specified period of time, consistent with test. Note that if breakthrough is expected, it may be useful to examine breakthrough as a function of time.
5. The panel is decontaminated in a manner consistent with that to be used during test. It is important that the decontaminant not be allowed to migrate around the edge of the panel.
6. The decontaminant is allowed to reside on the surface for the appropriate dwell time consistent with that to be used during test.

The latex gasket is removed, and the pad is analyzed for the presence of agent.

Appendix C Best Practice Calculations

C.1 Best Practice Calculation: Log Difference (Output A)

This calculation provides a log difference (assuming unequal variance) between two conditions, e.g., replicates in two data sets, and provides a 95% confidence interval (CI) on the difference. If the span of the CI does not include zero, the difference is statistically significant at the 95% confidence limit.

Relative performance-metric calculations (such as log difference) are used to determine if a hazard mitigation technology provides an improvement compared to a specified reference (e.g., positive control, reference technology, or alternate treatment condition). This analysis is used most often for Methodology Output A or B_2.

Relative performance metrics require a reference condition. The reference condition may be ageing alone, a different hazard mitigation technology, a different treatment parameter (e.g., different duty cycle), or other conditions of interest. Examples of different questions that can be answered by selecting different reference conditions are given in Table B.1.

Table C-1. Example reference and test conditions for a log difference calculation

Reference Condition	Test Condition	Evaluation
Initial Contamination	Technology A	Log reduction from starting challenge
Soapy Water Immersion	Technology A	Log reduction compared to mild surface treatment (removes bulk surface liquid); enables the determination of how well Technology A removes *absorbed* agent.
Technology A	Technology B	Relative performance of two technologies
Technology A, Duty Cycle A	Technology A, Duty Cycle B	Relative reduction in agent resulting from different duty cycles

The following calculations use data sets for agent mass collected for the two test conditions, *A* and *R*. Data set *A* is the condition being evaluated while data set *R* is the specified reference condition. For all variables in the following calculations, subscripts *A* and *R* will indicate the data set being used. The subscript *Z* indicates that both data sets, *A* and *R,* are processed using the same calculation.

The relative decontaminant performance factor (*PF*) metric is defined as the ratio of the reference condition *R* to the technology condition *A*

$$PF \equiv \frac{R}{A} \qquad \text{Eq. C-1}$$

If condition *A* is more effective than condition *R*, i.e., the measured agent mass in *A* is less than in *R*, the *PF* will be greater than 1 and condition *A* is *PF* times more effective than condition *R*. If *PF* is less than 1, condition *A* is less effective than condition *R*, by a factor of 1/*PF*. The data collected from these methods are left-censored (i.e., mass detected must be greater than zero). The data used in a performance calculation may also differ by orders of magnitude. The use of a log transform ensures the left-censored characteristic of the data is maintained and simplifies data interpretation. Eq. B.1 is log-transformed to present relative *PF* as a log difference (LD).

$$log_{10}(PF) = log_{10}\left(\frac{R}{A}\right) = log_{10}(R) - log_{10}(A) \quad \text{Eq. C-2}$$

Using this approach, an LD of zero indicates that conditions *A* and *R* are equivalent. If LD is greater than 0, condition *A* is LD orders of magnitude more effective than condition *R*. If LD is less than 0, condition *A* is LD orders of magnitude less effective than condition *R*. The LD is calculated as the difference of the arithmetic mean of the $\log_{10}$ transform of the reference and test conditions.

$$LD = \text{mean}[log_{10}(R)] - \text{mean}[log_{10}(A)] \quad \text{Eq. C-3}$$

The 95% CI for the difference in mean values, assuming unequal variance, is expressed as:

$$CI = t_{(\alpha/2,DF)}SE \quad \text{Eq. C-4}$$

$$DF = \frac{\left(\frac{{S_R}^2}{n_R} + \frac{{S_A}^2}{n_A}\right)^2}{\frac{\left({S_R}^2/n_R\right)^2}{(n_R - 1)} + \frac{\left({S_A}^2/n_A\right)^2}{(n_A - 1)}} \quad \text{Eq. C-5}$$

$$SE = \sqrt{\frac{{S_R}^2}{n_R} + \frac{{S_A}^2}{n_A}} \quad \text{Eq. C-6}$$

Where:

CI	=	confidence interval for LD (units vary)
t	=	t value (unitless)
α	=	probability level (unitless)
DF	=	degrees of freedom (unitless)
n_R	=	number of replicates for condition R (unitless)
n_A	=	number of replicates for condition A (unitless)
SE	=	standard error of the difference of the means (units vary)
S_R	=	standard deviation of the log-transformed data for condition R (units vary)
S_A	=	standard deviation of the log-transformed data for condition A (units vary)

CIs can be calculated for different probability levels, expressed as 1-α. In this case, to calculate a 95% CI, α = 0.05. The t value is a function of α and the DF in the system. The t value can be obtained from reference tables or from commercial software packages.

The calculation of LD ± CI enable the characterization of the performance between two conditions. If the CI includes zero (CI > LD) then the conditions are statistically similar and the LD does not indicate a confident difference in performance, in this case the LD can be considered as zero. If CI is less than LD (the CI does not include zero), then the two conditions

have a statistically significant difference in performance as described by LD ± CI. The result could be expressed as a PF, as $PF = 10^{LD}$.

C.2 Best Practice Calculation Guidance for RA-Based Asset Level Output Calculations (Output B)

The RA test is high throughput and much less resource intensive than VE or CT testing. The RA test quantifies the total remaining agent resulting from an agent-material-treatment process on a per drop basis, whereas the VE and CT tests measure the rate (i.e., source terms) RA leaves the material via vapor emission or contact transfer. Therefore, the RA can be used to approximate a 'maximum potential dose' (MPD) from a given condition. If a given sample has lower RA, there is less mass available for VE or CT. The use of RA will tend to provide an overestimate of VE or CT as the total mass retained may not contribute to potential exposure source terms over the time period of interest.

The following calculations use laboratory panel test data to approximate agent retention for a full-scale asset. The analysis enables multiple calculations for technology characterization. Calculations include total asset agent retention (B_0), relative source contribution (B_1), asset level performance (B_2), and maximum potential dose (B_3) as shown in Figure A-1.

A critical aspect of this calculation is describing how much agent is applied to the asset and which material-features are contaminated. The contamination of an asset is assumed to be a random distribution of drops interacting with different parts of the asset. An approach to indicate the probability of contamination for each material-feature of an asset is used to enable the randomized contamination of an asset. These calculations require documenting the materials of construction for the asset of interest and quantifying the material area, materials of construction, presence of complex features, and the area of each material. Using a contamination vignette, specific regions of the asset (e.g., the full asset or one side of the asset) could be specifically contaminated. It is assumed that the relative area of each contaminated part to the total contaminated area can be used to identify a contamination probability, if the contamination is evenly distributed on the contaminated parts. Realistic asset contamination is a random process, a variable number of drops of variable drop volume will interact with different asset parts. Random sampling (i.e., Monte Carlo) approaches enable randomized contamination to characterize the range of response that could be observed from randomized contamination of an asset.

Overall, the RA-based outputs are generated by conducting the following steps:

1. Define the asset and probability of contamination for each material-feature
2. Determine mass of agent associated with the contamination of the full-scale asset
3. Conduct many iterations of the following panel to asset scale-up process
    a. Generate a list of agent drop volumes, based on a drop volume distribution, that generates the mass of contamination
    b. Distribute drops on asset according to the contamination probability
    c. Calculate the post-treatment total asset mass (M_{Asset})
4. Use the M_{Asset} output distributions to calculate relative source contribution (B_1), asset level performance (B_2), and maximum potential dose (B_3) for vapor and/or contact exposures

C.2.1 Define the Asset of Interest and Contamination Probability

The contamination probably will be calculated as the relative area of each feature compared to the total contaminated area of the asset. Therefore, the asset of interest must be identified and the surfaces and materials of construction must be documented. The asset may be segregated into different 'parts' (e.g., sides, doors, closures, tires, screens, etc.). Document the size and materials of construction and features of each part.

Figure B.1 illustrates a simplification of a multi-tool into simplified areas that are used to generate contamination probabilities, as shown in Table .1. In this case, each face of the box is assigned a part name. Each part may comprise multiple components (flat areas, large and small gaps) and relative areas for each component were approximated. For example, for the corkscrew side 30% of the part area was estimated as flat steel areas. The component area is estimated as the part area times the component relative area (scaled 0-1). The actual material and feature of construction is documented (e.g., steel flat or steel 2 mil gap). Laboratory data for the material of construction may not be available and an alternative or surrogate laboratory-tested material will need to be used in calculations as identified in the test source column in Table C-2.

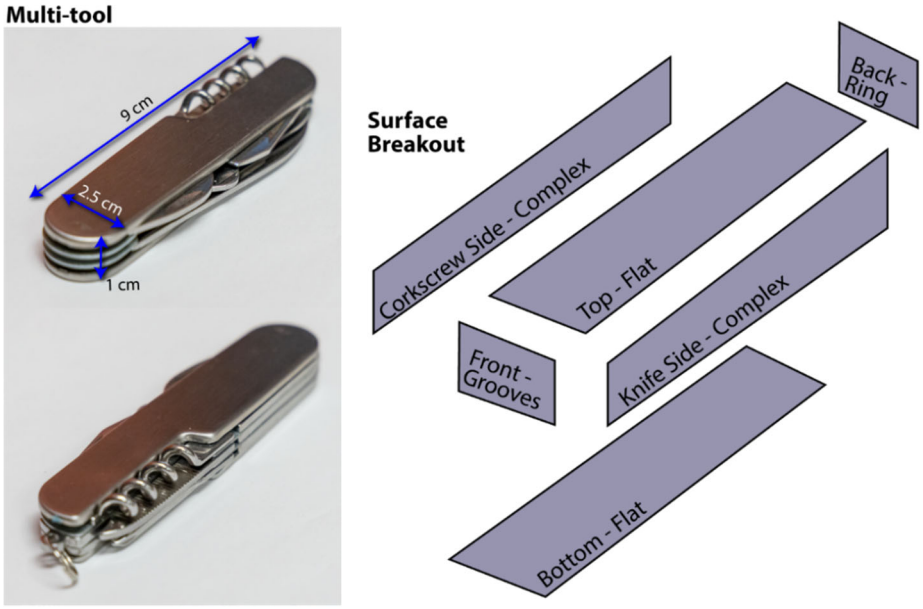

Figure C-**1.** Breakout illustration of multi-tool surfaces.

Table C-2. Asset Source Description and Contamination Probability Demonstration

Describe Asset							Determine Contamination Probability		
Part	Part Area (cm^2)	Component Description	Relative Component Area	Component Area (cm^2)	Actual Material-Feature of Construction	Test Source Material-Feature	Contaminated (1 or 0)	Contaminated Area (cm^2)	Contamination Probability*
Top	22.5	Flat side	1	22.5	Steel-flat	Steel-flat	1	22.5	0.6618
Bottom	22.5	Flat side	1	22.5	Steel-flat	Steel-flat	0	0	0.0000
Front	2.5	Grooved surface	1	2.5	Steel-20 mil	Steel-20 mil	1	2.5	0.0735
Back	2.5	Rounded surface	0.75	1.875	Steel-flat	Steel-flat	0	0	0.0000
		Key ring	0.125	0.3125	Steel-2 mil	Steel-2 mil	0	0	0.0000
		Large groove	0.125	0.3125	Steel-20 mil	Steel-20 mil	0	0	0.0000
Knife Side	9	Small gaps	0.5	4.5	Steel-2 mil	Steel-2 mil	1	4.5	0.1324
		Large gaps	0.5	4.5	Steel-20 mil	Steel-20 mil	1	4.5	0.1324
Corkscrew Side	9	Flat region	0.3	2.7	Steel-flat	Steel-flat	0	0	0.0000
		Large gaps	0.5	4.5	Steel-20 mil	Steel-20 mil	0	0	0.0000
		Small gaps	0.2	1.8	Steel-2 mil	Steel-2 mil	0	0	0.0000
Total	68	-	-	68	-	-	-	34	1.0

*This is a demonstration contamination probability were the specified parts were selectively contaminated (as indicated by a 1 in the contaminated column).

The next step is to identify the parts that will be contaminated based on the contamination vignette, for example if the full asset is uniformly contaminated or if only parts of the asset may be contaminated. In this example contamination vignette the top, front, and knife side of the tool were contaminated (indicated with a 1 or 0 in the contaminated column). The component area is multiplied by the contaminated column to produce the contaminated area. The total contaminated area (34 cm^2) is the sum of the contaminated areas of each contaminated component. Many variations of contamination could be considered in an evaluation.

Contamination probability, the probability that any given agent drop would interact with a component, is assumed to be proportional to the relative contaminated area (i.e., the contamination is evenly distributed across the contaminated components). The contamination probability is calculated as the *contaminated* area for each component divided by the *total contaminated area*. For this particular asset and contaminated parts, the top-flat side has a contamination probably of (22.5 / 34) = 66.18%.

In later steps, random sampling methods assign drops to a specific part-component according to the contamination probabilities. Repeating this random drop distribution many times will produce the range of responses that may be observed from different numbers of drops and volumes interacting with each asset feature.

It is recognized that the process of defining the parts and components of assets to determine contamination probability may become significantly more complicated as the asset becomes larger or more detailed, such as vehicles. Future efforts may need to identify the level of detail

required to provide acceptable accuracy since the output is a combination of the ability to accurately describe the contamination vignette and the parts, components, and contamination probability of the asset, and the laboratory source term data. It is anticipated that there is diminishing returns for specifying higher levels of detail as the accuracy of the contamination probabilities will be challenging to confirm and because of the uncertainty in other contamination parameters (e.g., actual mass applied to the asset, regions of the asset that would be contaminated).

C.2.2 Contamination Context

Decontamination requirements often specify the quantity of agent to be decontaminated as a starting challenge, expressed as grams per square meter (g/m^2). This value has historically been interpreted as grams of agent per square meter of *material* and consequently, contamination conditions of panels in tests have been specified this way.[2] However, the contamination density value is more likely associated with grams of agent per square meter of terrain, the distribution of agent in the environment. This interpretation has a significant influence on the quantity of agent that may be associated with an asset for a given contamination event. The following descriptions provide a new perspective on how to determine the contamination of an asset to enable scale-up calculations. The first step is to determine the total mass of agent that would be applied to the asset. The proposed approach defines an approximate area term that can be used with contamination density to determine the mass of liquid agent that contaminates the asset. Subsequent calculations will subdivide the total mass into drop distributions and apply drops to various regions of the asset to enable the per drop scale-up calculations.

The starting challenge is the environmental contamination density which describes the grams of agent per square meter of terrain. If an asset were present in the environment, some portion of its area will receive the agent that would otherwise have impacted the terrain. The total material area of an asset may be significantly different than the area that was contaminated depending on the contamination source and the exposed areas of the asset materials, thus there is a significant difference between grams of agent per *material* area and grams of agent per *terrain* area. If the contamination source was imagined as a light source, the resulting *shadow* of the asset would describe the area of terrain that would have been contaminated if the asset was not present (Figure B2). The mass of agent contaminating the asset would be the shadow area times the contamination density. The material area of the asset is constant, but the material area that could be contaminated may change significantly depending on the configuration of the assets (e.g., open or closed, for the multi-tool or a vehicle) and orientation of the asset (long face versus edge on).

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2 A 2 inch diameter flat panel presents a material area of 20.2 cm^2, when contaminated with 2 × 1 µL drops of VX (1.852 mg of VX) the resulting contamination density is calculated as 0.91 g/m^2.

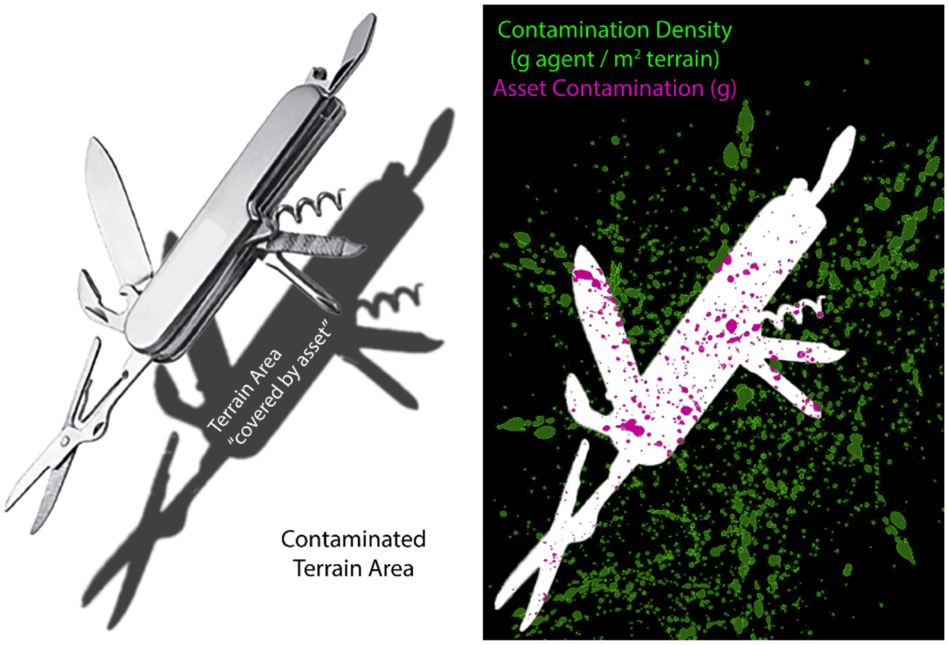

Figure C-2. Conceptual image of an item and the terrain area cast by its shadow (left). The asset would be contaminated by the (pink) drops that would have landed on the terrain had they not impacted the item.

This provides a more versatile approach for describing contamination of assets in many different situations and quantifying outputs from technology evaluations for relevancy in health-based assessments. Here, starting challenge is used to identify the mass of agent contaminating the asset, the calculations translate this mass into drop distributions to enable the scale-up calculations.

C.2.3 Calculate the Mass Applied to an Asset

When liquid droplets are dispersed in an environment resulting in a contamination density for exposed surfaces, the agent mass associated with an object or asset in that environment can be expressed as

$$M_c = AC \qquad \text{Eq. B-7}$$

Where

M_c	is the total mass of agent that contaminated the asset (g)
A	is the projected area of asset that would receive contamination (m^2)
C	is the contamination density (g agent / m^2 terrain)

Determining the projected area for the asset may be non-trivial and is not addressed in detail here. Rather, initial efforts could make simplifications such as using the area for 1–3 sides of an

asset (e.g., top, front edge, side edge) as an order of magnitude estimate for the area. The operational environment would likely include many permutations of how assets may be contaminated, detailed precision for the area may not be required to capture the range of possible areas to consider. Note that factors such as the distance of an asset from the contamination source can have significant influence on the 'local' contamination density that an asset would experience.

C.2.4 Panel to Asset Scale-up

This section covers how to calculate the total asset retained agent, M_{Asset}, used in subsequent output B calculations. RA data elements are provided as inputs to the scale-up equation as the log-transformed mean (μ) and standard deviation (σ) for each agent-material-feature-treatment process used. For each contamination distribution considered, a list of *K* drops of randomly sampled drop volume is identified (i.e., generating the agent drop distribution). These drops are assigned to the asset components according to the contamination probability. Then the laboratory source terms are randomly sampled and aggregated to calculate M_{Asset}. Multiple iterations are conducted for each contamination distribution, and multiple iterations of different contaminations may be conducted.

C.2.4.1 Generate the Agent Drop Distribution

This process will convert the M_c mass into a list of drops and drop volumes.

Contamination sources that disperse liquid drops generate a range of drop diameters, which tend to follow a log-normal distribution. The drop volumes may generally range from about 0.05 μL to 10 μL depending on many factors including agent properties and the nature of how the agent was dispersed. Drop volume distributions are often specified using log-normal statistical distributions of the drop diameter in mm, such as a mean, μ_d, of 0.9 $\log_{10}$(mm) and a standard deviation, σ_d, of 0.25 $\log_{10}$(mm). Note that drops of a fixed volume could be generated if the standard deviation is set to zero. The diameter and volume of randomly sampled drop volumes can be expressed as Equations Eq. B-8and Eq. B-9. The mass of the drop is a function of the drop volume, the liquid density (ρ), and the agent mass purity (ψ), as shown in Eq. B-10.

$$D_{drop} = antilog(randn(\quad)\sigma_d + \mu_d) \qquad \text{Eq. B-8}$$

$$V_{drop} = \frac{4}{3}\pi\left(\frac{D_{drop}}{2}\right)^3 \qquad \text{Eq. B-9}$$

$$m_{drop} = \rho\psi V_{drop} \qquad \text{Eq. B-10}$$

To generate the contamination of an asset, drop volumes are randomly sampled and sequentially aggregated until the total mass of drops adds to M_c. For the following calculations the total number of drops is indicated by capital *K*, and each specific drop is denoted by lower case *k*.

C.2.4.2 Assign Drops to Asset Features

Using the contamination probabilities of the asset, randomly distribute the K drops.

Most statistical programs provide a random sampling function (e.g., randsample() in MATLAB), that will take the agent drop list and assign each drop to a given component according to the contamination probability. This is a random (stochastic) process, the same drop distribution will not be generated each time this process is performed. An example output is provided in Table C-3.

Table C-3. Example table showing the random contamination of the multi-tool asset

k (Drop ID)	Part-Component	Material-Feature Source for drop k	V_k (µL)
1	Top-Flat side	Steel-flat	0.391
2	Top-Flat side	Steel-flat	0.411
3	Top-Flat side	Steel-flat	0.764
4	Front-Grooved surface	Steel-20 mil	0.133
5	Front-Grooved surface	Steel-20 mil	0.346
6	Knife Side-Small gaps	Steel-2 mil	2.816

C.2.4.3 Obtain Laboratory Source Terms

The RA data elements measured in laboratory testing (i.e., the source terms) provide the mass of agent retained by the material-feature per drop after the treatment process based on the agent drop volume. It is assumed that the experimental responses are log-normal, and all data is analyzed with a $\log_{10}$ transform. There are two methods to provide the RA source term inputs of mean as a function of agent drop volume, µ(V), and standard deviation, σ. The preferred and more accurate method, the multivolume fit regression, requires variable agent drop volume data. The less accurate extrapolation method can be conducted when only one agent drop volume data are available.[1]

Multivolume Fit Regression

Perform a least squares regression according to

$$log_{10}\left(\frac{RA}{n_{tested}}\right) = m\, log_{10}(V) + b \qquad \text{Eq. C-11}$$

Where:

m is the slope for $\log_{10}$(RA/drop) versus $\log_{10}$(V) analysis

b is the intercept for $\log_{10}$(RA/drop) versus $\log_{10}$(V) analysis

RA	is the RA mass for agent-material-treatment-panel (µg)
V	is the agent drop volume (µL)
n_{tested}	is the number of drops applied to a panel in testing (unitless).

The subscript k, indicates the material-feature of that drop k interacts with. The $\mu_k(V)$ input for calculations for the agent-material-feature source term is determined using the parameters from the fit

$$\mu_k(V) = m_k \, log_{10}(V) + b_k \qquad \text{Eq. C-12}$$

where

μ_k	is the mean value output from the fit for agent-material-feature-treatment associated with drop k as a function of drop volume ($\log_{10}$(µg))
m_k	is the slope parameter from Eq. C-11 for agent-material-feature-treatment associated with drop k
b_k	is the intercept parameter from Eq. for agent-material-feature-treatment associated with drop k
V	is the agent drop volume for drop k (µL)

The root mean square error (RMSE) of the fit quantifies both the σ (random error) and the fitting error. If a regression fit is reasonable, then RMSE is an acceptable estimate of σ (random error). As sample size becomes sufficiently large the RMSE will approach the true σ value. When sample sizes are very small the regression fitting error becomes larger and can inflate the RMSE. The RMSE can be considered as the standard deviation of the fit, the σ_k input for MC calculations is the RMSE of the fit

$$\sigma_k = RMSE_k \qquad \text{Eq. C-13}$$

where

σ_k	is the standard deviation of the fit for agent-material-feature-treatment associated with drop k ($\log_{10}$(µg))

Drop Volume Extrapolation

The drop volume extrapolation source term uses the laboratory test data for one drop volume to extrapolate the results to all other drop volumes. The extrapolation approach uses the per drop regression (Eq. C-11), but assumes that the slope (m) is 1.0. This assumption implies that the efficacy (e.g., log reduction relative to the applied mass) is independent of the agent drop volume. The approach solves for the intercept B in Eq. C-11 using the single tested drop volume, then substitutes the value back into Eq. C-11, applies logarithmic identities to produce

$$\mu_k(V) = \overline{log_{10}\left(\frac{RA}{drop}\right)_{V_{tested}}} + log_{10}\left(\frac{V}{V_{tested}}\right) \qquad \text{Eq. C-14}$$

where:

V_{tested}	is the drop volume used in testing (µL)
$\left(\frac{RA}{drop}\right)_{V_{tested}}$	is the measured RA/drop response for the tested drop volume (µg/drop)

The overbar notation indicates the arithmetic mean of the $\log_{10}$ transformed responses. The standard deviation MC input for this approach, σ_k, is calculated as the standard deviation of the $\log_{10}$ transformed RA/drop results, and is assumed to be constant for all V.

C.2.5 Determine the Total Asset Retention (Output B_0)

The scale-up calculation assumes that the mass retained by an asset (M_{Asset}, *Method output* B_0) is the sum of RA due to each drop that contaminates the various materials and features of the asset. Consider an asset that was contaminated with K drops of agent. Each agent drop (k) may have a different volume (V_k) and interacts with a specific material feature that generates a specific RA per drop volume ($RA_k(V_k)$). In this case, the RA_k indicates the material-feature associated with drop k. The M_{Asset} calculation estimates the distribution of responses that may be observed for an based on the treatment process variability for each meateiral-feature. Random sampling Monte Carlo techniques use µ and σ as inputs. The random sampling for each drop will be performed using the log transformed statistics, and then inverse transformed back to the original RA mass units. The $RA_k(V_k)$ of Eq. is randomly sampled as

$$M_{Asset} = \sum_{k=1}^{K} RA_k(V_k) = \sum_{k=1}^{K} antilog_{10}\left(randn(\,) * \sigma_k + \mu_k(V_k)\right) \qquad \text{Eq. C-15}$$

Where:

$RA_k(V_k)$	is the laboratory data RA source term as a function of agent drop volume V for the material-feature associated with drop k (µg)
M_{Asset}	total retained agent mass of the asset (µg)
V_k	is the volume of drop k (µL)
randn()	is a function that provides a random draw from a standard normal distribution (unitless)

Applying this calculation to the example drop distribution produces the example output in Table 4. This corresponds to one agent drop distribution on the asset. Calculations should be run for multiple agent distributions on the asset.

Table 4. Example table showing source terms and one iteration of randomly sampled output

k (Drop ID)	Part-Component	Material-Feature Source for drop k	V_k (µL)	$\mu_k(V)$ $\log_{10}(\mu g)$	σ_k $\log_{10}(\mu g)$	$RA_k(\mu g)$
1	Top-Flat side	Steel-flat	0.391	-1.183	0.509	0.08
2	Top-Flat side	Steel-flat	0.411	-1.150	0.509	0.31
3	Top-Flat side	Steel-flat	0.764	-0.721	0.509	0.04
4	Front-Grooved surface	Steel-20 mil	0.133	0.397	0.689	152.45
5	Front-Grooved surface	Steel-20 mil	0.346	0.856	0.689	82.88
6	Knife Side-Small gaps	Steel-2 mil	2.816	3.598	0.552	3167.76
-	-	-	-	-	M_{Asset} =	**3403.5**

The M_{Asset} calculation is repeated for many iterations to generate a statistical sampling of the distribution of responses associated with the treatment process. This combined with performing many iterations of contamination distributions provides an indication of the range of responses that may be observed for a given asset for the specified contamination parameters. An upper tolerance interval of M_{asset} could be used to describe the upper bound of the MPD output. For example, it could be reported with 95% confidence that 95% of the population is less than the tolerance interval.[1,2]

C.2.6 Calculate the Relative Contribution of Each Material to the Total Asset Retained Agent (output B_1)

The assessment of relative contributions of each source are described as output B_1 of the methodology. Relative contributions of each feature type are calculated to identify what features contribute to the calculated M_{Asset} values. The relative contributions of each feature were tracked as

$$C_i = \frac{M_i}{M_{Asset,calculated}} \qquad \text{Eq. C-16}$$

Where:

C_i	is the relative contribution of agent-material feature *i*
M_i	is the total mass of RA associated with agent-material feature *i* from per drop RA panel data
$M_{Asset,calculated}$	is the calculated total mass of RA associated with the entire asset based on per drop RA panel data

The C_i results are calculated for each iteration of the MC simulation because each simulation is the result of the sum of randomly sampled drops. The relative contributions can vary significantly based on the random sampling. C_i could be conducted for the panel-level material-feature combinations (e.g., steel-flat, steel-2 mil), or at the asset-part level (e.g., a tire, a door, knife side of multi-tool).

C.2.7 Asset Level Performance (Output B_2)

The relative performance of a technology or treatment condition can be compared to another condition to indicate a realtive change in the RA_{Asset}. For example, if a technology condition were compared to the total agent mass applied to the asset, a reduction from starting challenge could be evaluated. It is recommended to use the Log Difference calculation to calculate the asset level performance.

C.2.8 Maximum Potential Dose (Output B_3)

Background: The asset level calculations enable an estimation of the total quantity of agent associated with an asset after the treatment process. Maximum potential dose (MPD) assumes that all of the agent retained results in contact or vapor exposure. Vapor exposure is assumed to be for inhalation or ocular exposure. This analysis minimizes the complexity of an exposure assessment and provides a worst-case evaluation of the potential for negative health effects. For example, if a total potential dose is below the toxicity value of interest, no exposure assessment of the same asset could exceed the total potential dose.

C.2.8.1 Maximum Potential Dose for Contact Transfer

CT MPD applies to assets where personnel have long duration close contact with the asset, such as weapons, radios, helmets (i.e., items that are worn or carried). It assumes that the personnel will interact with all of the originally contaminated materials and all of the agent would result in exposure. Cases for 'large' assets such as vehicles may need special consideration for the MPD calculation. For example, in the case of a vehicle, personnel will not interact with all surfaces of the vehicle, and may only interact with specific parts of a vehicle (e.g., door handle). In the case of large assets the MPD could focus on specific regions of the asset.

MPD estimates that include the use of shim panel data may be significant overestimates if the agent is retained deep within a feature. The entrained agent may not be accessible to transfer to skin during a touch. This agent may not present immediate exposure risks to personnel in the standard use of an asset. However, if maintenance is performed such that the asset is disassembled, this entrained agent may produce a potential exposure.

For systemic agents: the CT MPD is the total asset retained agent (output B_0), this total mass is compared to the relevant percutaneous toxicity value typically expressed as mg agent.

For localized agents: the exposure that produces an injury depends on local skin surface area, the CT MPD is the total asset retained agent divided by the relevant body region contact area typically expressed as µg agent / cm^2 of *contaminated skin* surface area. Note *this is not µg agent / cm^2 of material or total body area*.

For localized agents, the anatomical body region contacted must be identified. The skin surface area for various anatomical body regions is available from other resources.[3,4] For the average American male, the palmar surface area of the hand is between 76–107 cm^2, the smaller value of 76 cm^2 could be used to produce an upper bound skin surface concentration. The localized skin dosage is calculated as

$$D_{local} = \frac{M_{Asset}}{A_{skin}} \qquad \text{Eq. C-17}$$

Where

D_{local} is the localized skin dosage of agent (μg cm^{-2} skin)

M_{Asset} is the mass of agent associated with asset (μg)

A_{skin} is the area of skin interacting with the asset (cm^2 skin)

The resulting CT MPD, which is equal to D_{local}, is then compared to the toxicity value of interest. If the MPD is less than the toxicity value, further exposure assessment may not be necessary as this is a worst case value.

C.2.8.2 Maximum Potential Dose for Vapor Emission

For MPD vapor exposure, the B_0 output of total asset mass must be converted to a vapor source term (i.e., emission rate). The effects of the environment (e.g., room volume, air change rate, wind speed, distance from source, etc.) will influence the local vapor concentration and MPD for vapor exposure. Calculations must be conducted to translate the vapor source to an environmental vapor concentration to calculate a MPD for vapor exposure. MPD estimates for volatile agents may be more accurate where significant evaporation may occur during a relevant mission time span. However, MPD estimates may be a significant overestimate for lower volatility agents that may not completely evaporate on relevant time scales.

VE MPD assumes that all RA associated with an asset is emitted over the exposure vignette time duration at a *constant emission rate*. Experimental results show that vapor emission tends to rapidly decay over time, however, the rate of decay is not known without testing. The constant emission rate based on B_0 should provide a safe sided emission rate; typically less than 100% of the RA is emitted over 24 h time periods. For emission rates that have non-linear decay, the majority of emission tends to occur shortly after treatment, however assets may not be reissued to personnel for some time before the exposure may actually occur (**Figure C**1). The constant emission rate will overestimate the actual emission rate at longer time durations. Metrics such as the total potential VEM will be greater than or equal to the experimentally determined values, similar trends should be observed for exposure doses as well as the MPD assumes all RA mass results in exposure.

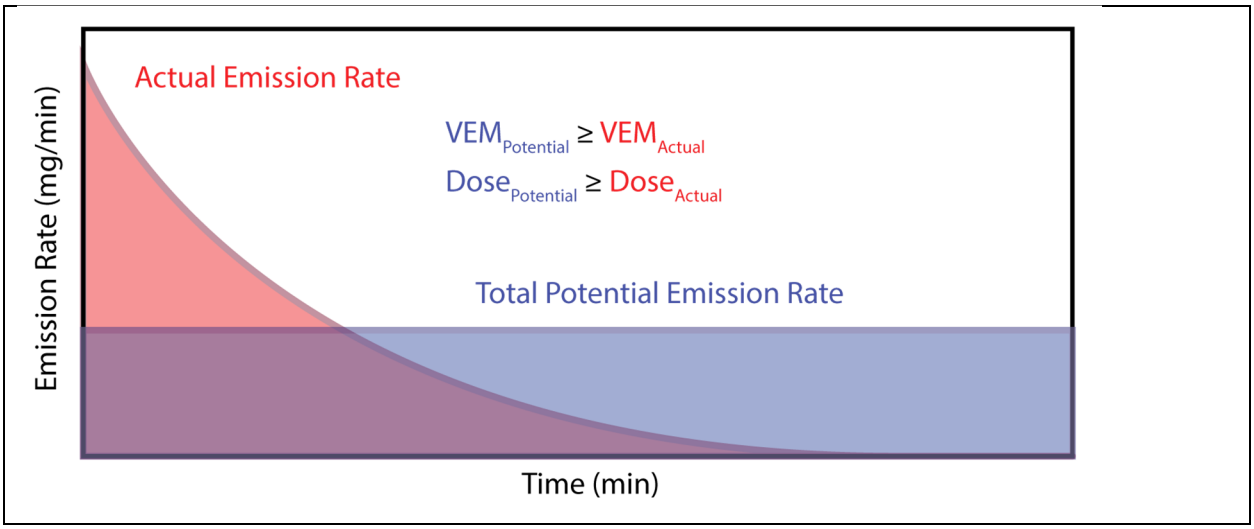

Figure C-3. Comparison of actual vapor emission rate to total potential emission rate assumption.

The constant vapor emission rate of an asset is

$$E_{Asset} = \frac{M_{Asset}}{t_{Vignette}} \qquad \text{Eq. C-18}$$

Where

E_{Asset}	constant Asset emission rate (mg min^{-1})
$t_{Vignette}$	relevant duration the asset is in the enclosed environment (min)

The $t_{vignette}$ is the duration that the asset is in the enclosed environment. The constant emission rate can be supplied to vapor vignette calculations to determine a vapor exposure dose. As a first approximation, when enclosed spaces are considered with a constant emission rate, the mass balance differential equation describing the vapor concentration in a well-mixed enclosed volume is simplified to an algebraic function that is only dependent on the ventilation rate, Q, [5]

$$C_{env} = \frac{E_{Asset(s)}}{Q} \qquad \text{Eq. C-19}$$

$$D_{MPD} = C_{env}\, t_{exposure} \qquad \text{Eq. C-20}$$

Where

$E_{Asset(s)}$	sum total of all constant emission rate assets in the vignette (mg min^{-1})
Q	fresh air flow (ventilation) rate of enclosed space (m^3 air min^{-1})
$t_{exposure}$	Duration personnel are in the enclosed environment (min)
C_{env}	Environmental vapor concentration (mg m^{-3})

D_{MPD} Maximum Potential Vapor Dose (mg min m^{-3})

The resulting vapor dosage, D_{MPD}, can be calculated for a different time duration than the asset was in the environment if desired. For example, assets could be placed in a vehicle for many hours, but personnel were only in the vehicle for a duration of 1 h where they were exposed to the enclosed environment vapor concentration.

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