UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF
PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

September 13, 2007

MEMORANDUM

SUBJECT: Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held July 17 - 18, 2007 on Guidance on Test Methods for Demonstrating the Efficacy of Antimicrobial Products for Inactivating *Bacillus anthracis* Spores on Environmental Surfaces

TO: Debbie Edwards, Director
Office of Pesticide Programs

FROM: Joseph E. Bailey, Designated Federal Official
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

THRU: Steven Knott, Executive Secretary
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

Clifford J. Gabriel, Director
Office of Science Coordination and Policy

Attached, please find the meeting minutes of the FIFRA Scientific Advisory Panel open meeting held in Arlington, Virginia on July 17 - 18, 2007. This report addresses a set of scientific issues being considered by the Environmental Protection Agency pertaining to guidance on test methods for demonstrating the efficacy of antimicrobial products for inactivating *Bacillus anthracis* spores on environmental surfaces.

Attachment

cc:

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Lynne M. Sehulster, Ph.D.

SAP Minutes No. 2007-05

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Guidance on Test Methods for Demonstrating the Efficacy of Antimicrobial Products for Inactivating *Bacillus anthracis* Spores on Environmental Surfaces

July 17 - 18, 2007
FIFRA Scientific Advisory Panel Meeting
held at the
Environmental Protection Agency Conference Center
Arlington, Virginia

Notice

These meeting minutes have been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). The meeting minutes represent the views and recommendations of the FIFRA SAP, not the United States Environmental Protection Agency (Agency). The content of the meeting minutes does not represent information approved or disseminated by the Agency. The meeting minutes have not been reviewed for approval by the Agency and, hence, the contents of these meeting minutes do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

The FIFRA SAP is a Federal advisory committee operating in accordance with the Federal Advisory Committee Act and established under the provisions of FIFRA as amended by the Food Quality Protection Act (FQPA) of 1996. The FIFRA SAP provides advice, information, and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the Environmental Protection Agency, Office of Pesticide Programs (OPP), and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. FQPA Science Review Board members serve the FIFRA SAP on an ad hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at http://www.epa.gov/scipoly/sap/ or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Joseph E. Bailey, SAP Designated Federal Official, via e-mail at bailey.joseph@epa.gov.

In preparing these meeting minutes, the Panel carefully considered all information provided and presented by EPA, as well as information presented by public commenters. This document addresses the information provided and presented by EPA within the structure of the charge.

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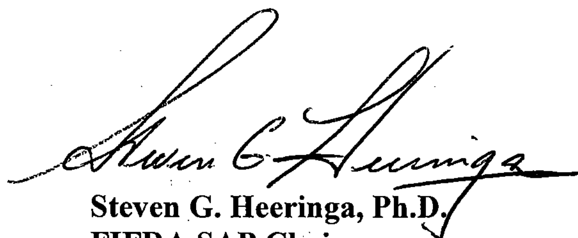

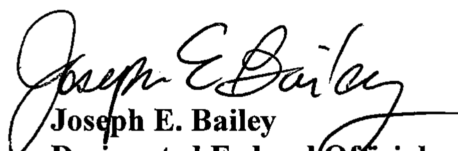

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FIFRA SAP Chair
FIFRA Scientific Advisory Panel
Date: SEP 13 2007

Joseph E. Bailey
Designated Federal Official
FIFRA Scientific Advisory Panel
Date: SEP 13 2007

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
July 17 - 18, 2007

Guidance on Test Methods for Demonstrating the Efficacy of Antimicrobial Products for Inactivating *Bacillus anthracis* Spores on Environmental Surfaces

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INTRODUCTION

The FIFRA Scientific Advisory Panel (SAP) has completed its review of Guidance on Test Methods for Demonstrating the Efficacy of Antimicrobial Products for Inactivating *Bacillus anthracis* Spores on Environmental Surfaces.

Advance notice of the SAP meeting was published in the *Federal Register* on March 14, 2007. The review was conducted in an open panel meeting July 17 – 18, 2007 held in Arlington, Virginia. Dr. Steven G. Heeringa chaired the meeting. Joseph E. Bailey served as the Designated Federal Official.

EPA regulations require that product performance (efficacy) studies be submitted to support registration of an antimicrobial product for which public health claims, such as "disinfect" or "sterilize" are made (40 CFR Part 158.640). In addition, any claim of inactivation of a specific microorganism should be supported by valid data that demonstrate the efficacy of the product against that particular microorganism. At this SAP meeting, the EPA presented draft guidance concerning efficacy testing to support a claim that an antimicrobial product inactivates *Bacillus anthracis* spores on inanimate environmental surfaces. EPA sought the assistance of the FIFRA SAP to review and comment on the scientific basis for efficacy data requirements to support registration of products that make such claims. Specific topics the FIFRA SAP considered included available qualitative and quantitative sporicidal efficacy test methods, use of various coupon materials to conduct tests, use of test surrogates for *B. anthracis*, and the adequacy of performance standards for quantitative sporicidal testing. Further, EPA requested the SAP to review and comment on the scientific basis for using a "simulated use test" for gases and vapors to support registration of sterilants and sporicides.

Debbie Edwards, Ph.D., Director of the Office of Pesticide Programs, provided opening remarks at the meeting. The agenda for this SAP meeting included an introduction of the issues under consideration provided by Jeff Kempter, Antimicrobials Division, Office of Pesticide Programs, EPA. Presentations of technical background materials were provided by Stephen Tomasino, Ph.D., Microbiology Laboratory Branch, Biological and Economic Analysis Division, EPA; Vipin Rastogi, Ph.D., Edgewood Chemical and Biological Center (ECBC), Department of Defense; Marty Hamilton, Ph.D., Montana State University; Shawn Ryan, Ph.D., National Homeland Security Research Center, EPA; and Michele Wingfield, Antimicrobials Division, Office of Pesticide Programs, EPA.

PUBLIC COMMENTERS

Oral statements were presented by:

Joan Stader, Ph.D., Clean Earth Technologies, LLC
E. Barry Skolnick, M.S.

Written statements were provided by:

E. Barry Skolnick, M.S.

SUMMARY OF PANEL DISCUSSION AND RECOMMENDATIONS

During the two days of discussions, the Panel was impressed with EPA efforts to improve the scientific basis of its regulatory process. This includes both the improvements carried out to date as well as EPA's willingness to seek input as it continues these efforts. Establishing properly validated, scientifically accurate tests of decontamination strategies is a complex and labor intensive task, and the Panel applauds EPA for meeting this arduous challenge head-on.

Charge Questions 1 and 2 focus on the use of AOAC International (AOAC) Official Method 966.04. The Panel believes that, in its overall framework, this method is suitable to support antimicrobial product claims for inactivation of *Bacillus anthracis* spores. However, there are significant concerns with many of its technical details. The Panel believes that the establishment of Method II by EPA scientists is a very important and successful step in the direction of improving AOAC Official Method 966.04. The Panel strongly encourages continuation of the process of improvement represented by the establishment of Method II. In particular, the Panel recommends production of spores using one of the conventional liquid sporulation methods. Such a method is technically easier and faster than the one described in AOAC Official Method 966.04, Method I, and will generate a preparation of spores with much more uniform physiological features and also produce an adequate number of spores for testing purposes. The Panel also recommends updating the spore purification, storage and validation procedures. In particular, phase contrast microscopy should be employed as a primary method of quality control at all stages of preparation and purification.

Charge Question 3 asks whether achieving a 6-log reduction in spore titer is a valid criterion for stating that a product inactivates *B. anthracis*. The Panel believes that a quantitative test is superior to a qualitative test and, in general, the 6-log criterion is valid. However, there are several critical caveats that must be stated. First, in real world situations, there will inevitably be environmental "hotspots", where the spore density will be such that a 6-log inactivation will be insufficient. Second, the effect of organic burden and other materials present with spores can affect the use of this criterion. Third, current sampling methods may be inadequate for environmental surfaces, thereby preventing accurate measurements of spore titers. Fourth, the level of acceptable residual risk has not been explicitly considered, as this is outside the scope of the Panel's charge. Nonetheless, this is still an important point that needs to be addressed. Fifth, positive controls of disinfection efficiency using known sterilants should be included. Overall, the Panel sees its charge as asking whether a 6-log reduction in spore activity is an adequate measure of the quantitative sporicidal efficacy, particularly *vis a vis* qualitative tests and, on this focused question, the Panel agrees that it is.

Charge Question 3.1 asks for comment on EPA criteria for selection of coupon material. The Panel believes that the EPA criteria are reasonable, but incomplete. The Panel identified many areas where additional analysis is needed in development of a process for selection of coupon material. Among the important issues identified by the Panel are the following. 1) It is unclear how many coupon material types should be specified for an adequate test. Clearly, a relatively large number (more than two) would be needed to fully represent the diversity of surfaces in a typical environment, such as an office. 2) The consequences, for any subsequent test, of cleaning and sterilizing coupon surfaces need to be characterized. 3) The interactions between the coupon and the product being tested need to be characterized. 4) The impact of coupon porosity on all aspects of testing needs to be addressed.

Charge Question 4 addresses the possible use of surrogates. The Panel feels that use of a surrogate would enhance ease of testing and should be encouraged so long as the surrogate is realistic and accurately representative of virulent *B. anthracis*. A minority view was that identification of a surrogate that would eliminate the need for testing against a virulent strain might not be realistic.

The Panel believes that the best candidate surrogate is the Sterne strain of *B. anthracis,* a non-select agent that can be used safely and very easily in Biosafety Level 2 (BSL-2) conditions, and is very closely related to virulent *B. anthracis* strains. The Sterne strain could differ to some degree from any given virulent *B. anthracis* strain, with respect to some resistance properties. Nonetheless, in general, this variation will be vastly reduced, relative to the variation with any given non-*B. anthracis* strain, since those other strains (and *B. subtilis* especially) differ much more from virulent *B. anthracis* strains than does the Sterne strain.

Charge Question 5 addresses the need for a simulated use test. The Panel believes such a test could provide useful information and should be considered. However, the Panel also believes that such a test is usually highly complex, and scale- and scenario-dependent. Therefore, it is difficult to specify a generally applicable simulated use test. Conducting these tests requires specialized technical expertise that is not necessarily available to all product developers. The overall complexity of this potential requirement likely requires EPA to provide guidelines to prospective stakeholders regarding the parameters of any scenario-specific tests they may wish to develop.

The Panel also noted that there are significant deficits in some basic science issues relating to spore properties, including spore surface characteristics and physiological variation among *B. anthracis* strains. These deficits may impede EPA efforts to develop the optimal testing regimens.

PANEL DELIBERATIONS AND RESPONSE TO CHARGE

The specific issues addressed by the Panel are keyed to the background documents, references, charge questions provided by EPA and presentations made by EPA and DOD.

Issue 1. Whether a sterilant or sporicide product may claim that it inactivates *B. anthracis* spores on inanimate surfaces.

EPA's existing efficacy testing guidance specifies that a product that passes the AOAC Sporicidal Activity of Disinfectants test (AOAC Official Method 966.04) may be registered as a sterilant/sporicide. The Agency is now proposing that a sterilant/sporicide may also bear a claim that it inactivates *B. anthracis* spores if it passes confirmatory testing using the AOAC 966.04 with virulent *B. anthracis* spores on carriers made of porcelain penicylinders and silk loops, which represent nonporous and porous surfaces, respectively.

The rationale for EPA's position is that the AOAC Method and associated EPA performance criteria (e.g., no growth from any carriers tested) have been required historically by EPA for evaluating the performance of sporicidal chemicals for regulatory purposes. The AOAC Method specifies the use of two spore-forming microbes, *B. subtilis* and *Clostridium sporogenes;* however, the Agency believes that the method is also suitable for testing spores of other spore-forming bacteria such as *B. anthracis*. The Agency has recognized deficiencies in the AOAC 966.04 Method and has improved the procedure through the official AOAC modification process – the modifications appear in AOAC 966.04 Method II. The Agency strongly prefers the use of the Method II to support the registration of sterilants/sporicides and for confirmatory testing of *B. anthracis*. In addition, the Agency has successfully revised the method editorially (see Method II), thus providing a more standardized protocol for use by stakeholders. The modifications are presented in AOAC 966.04 Method II. See Reference 1 for the entire method; see References 2 and 3 for experimental details.

<u>Charge Question 1.</u> Please comment on the scientific basis that confirmatory testing of a sporicide/sterilant using AOAC Method 966.04 with virulent *B. anthracis* spores demonstrates that a product inactivates *B. anthracis* spores on inanimate surfaces.

Panel Response

This question and its attendant discussion center on the use of AOAC methodology to provide laboratory data on disinfectant potency as required for the registration of a sporicide/sterilant, an existing category of disinfectant products. Historically, the EPA has accepted the Sporicidal Activity of Disinfectants Test (Method 966.04) as published by the AOAC for this purpose. This standardized, qualitative carrier test is both rigorous and conservative and is relevant to clinical settings. Method 966.04 (known as Method I) utilizes two spore-forming organisms (*Bacillus subtilis* and *Clostridium sporogenes*) and two types of carriers (porcelain penicylinders and silk suture loops) intended to represent non-porous and porous surfaces respectively. The text of Method I indicates that the method is applicable for use with other spore-formers.

AOAC methods are extensively vetted through a process of collaborative laboratory studies coordinated by the EPA. EPA recognized several aspects of Method I that could be

further standardized and modernized. The proposed modifications were evaluated by four laboratories, and data from this collaborative study indicated that the modified method provides a similar outcome as the existing AOAC 966.04 Method I. The proposed modifications have been reviewed and adopted by AOAC as 966.04 Method II (Tomasino and Hamilton, 2006). The major modifications incorporated into Method II include: 1) replacement of soil extract nutrient broth with nutrient agar supplemented with 5 µg/mL manganese sulfate; 2) establishment of a minimum spore titer per carrier; 3) confirmation of disinfectant neutralization activity; and 4) evaluation of an alternative non-porous carrier (i.e., stainless steel). Although Method I indicates it can be used to evaluate liquids and gases, Method II has only been validated for liquids; additional work is needed to validate Method II for gases.

A candidate sporicide/sterilant would be challenged first with *B. subtilis* and *C. sporogenes*. EPA prefers the use of Method II whenever it is appropriate to do so. A confirmatory test using a specific spore-former (*B. anthracis* in this instance) is done in support of specific agent label claims. Although there are some differences in biophysical and biochemical properties between *B. subtilis* and *B. anthracis*, their similarities are such that the use of Method II (as developed using *B. subtilis*) is appropriate for use with *B. anthracis* spores for the purpose of empirically demonstrating disinfectant potency against *B. anthracis*.

The discussants applauded the work done by the EPA and its collaborators in efforts to make the AOAC 966.04 a more consistent test, and they took the opportunity to offer additional aspects of the test process that could be improved. Designating a single provider of bacterial spores could be a significant step towards consistency and enhancement of quality assurance; however, caution must be exercised in selecting a provider as some commercial sources do not have good quality control in spore production, as noted in response to Charge Question 2. It is relatively easy and probably best for quality control to produce spores in-house. Spore quality was viewed as an important factor that can influence the outcome of the experiment. When asked about procedures to evaluate gases and vapors, EPA indicated that currently this is handled on what amounts to a case-by-case basis. Efforts are underway to validate modern methods for gases and vapors; a full collaborative study is necessary to accomplish this validation. EPA also indicated that at present there are no plans to include other carriers, but research is currently underway on a variety of carrier materials. If a company's product successfully passes this AOAC method, the product can be used on porous and/or non-porous surfaces. EPA indicated that the Agency would look at intended uses. When asked if the disinfectant product label should reflect a "process" rather than a "product," EPA indicated that manufacturers will be required to include a remediation action plan that incorporates precleaning, sterilant/sporocide use and environmental testing. At present the primary focus of EPA is whether or not the product works.

In conclusion, the Panel believes that confirmatory tests using *B. anthracis* spores and AOAC 966.04 (Methods I and II as appropriate) are feasible. The confirmatory test is conducted as a rigorous qualitative procedure that can be used in support of specific agent label claims primarily for liquids. Method II will result in greater consistency and more quantitative results. It is anticipated that this method will be sufficiently tested shortly and will soon replace Method I. It is in many respects simpler and more accurate and will be available to laboratory companies of all sizes. Its use at this time is encouraged.

Issue 2. Whether a sporicidal decontaminant product may claim that it inactivates *B. anthracis* spores on inanimate surfaces, when tested solely with AOAC Method 966.04 using virulent *B. anthracis* spores.

`The Agency is proposing that an antimicrobial product may be registered as a "sporicidal decontaminant" if it is tested with the AOAC Method 966.04 using virulent *Bacillus anthracis* spores (instead of *B. subtilis* or *C. sporogenes*), or using a surrogate acceptable to EPA, on porcelain penicylinders and/or silk loops, which represent nonporous and porous surfaces, respectively. The Agency's rationale is consistent with the use of the AOAC 966.04 method for sterilant/sporicidal agents; however, the Agency will not require registrants to conduct the entire AOAC Method 966.04 to support a sporicidal decontaminant claim. The Agency believes that product efficacy against spores of *C. sporogenes*, an anaerobic spore-forming species relevant to clinical environments, has limited applicability to decontamination scenarios involving spores of *B. anthracis,* and thus testing against *C. sporogenes* will not be required. Furthermore, the registrant will be allowed to test against porcelain and/or silk loops (which represent nonporous and porous surfaces, respectively) depending on the proposed claims. Continued use of EPA product performance criterion (e.g., no growth from any carriers tested) is appropriate for testing the efficacy of sporicidal decontaminants against spores of *Bacillus anthracis.*

<u>Charge Question 2.</u> Please comment on the scientific basis that use of the AOAC Method 966.04 with virulent *B. anthracis* spores demonstrates that a sporicidal decontaminant product inactivates *B. anthracis* spores on inanimate surfaces.

Panel Response

AOAC 966.04 Method II has been assessed in four independent laboratories and shown to be effective in reducing the population of *Bacillus anthracis* spores with tests both on the pathogenic Ames strain and the Sterne strain cured of the plasmid pXO1, i.e., plasmid free. It is promising that an appropriate surrogate can be substituted and there is strong consensus among the Panel that a *B. anthracis* strain (probably Sterne) should be used. *Clostridium* spores are clearly not suitable and *B. subtilis* spores are sufficiently different, especially in surface properties, that they would not be a suitable surrogate. This is discussed in detail in response to Charge Question 4. In addition, as discussed in response to Charge Question 3, testing for a 6-log reduction by plating for survivors will provide more reliable quantitative data that can be subjected to critical statistical analysis. The tests on both a stand-in porous and/or non-porous surface are promising, but not conclusive, as these types of surfaces can vary substantially in porosity, ability to absorb spores, etc.

Option #2 (use of AOAC Method 966.04 with *B. anthracis* spores alone) does provide a more up to date and simpler procedure than the established AOAC Method 966.04 using *B. subtilis* and *C. sporogenes* with confirmatory testing using *B. anthracis* spores. The Panel suggests that some consideration be given to the following modifications of Method II:

1. Preparation of spores in a liquid rather than solid sporulation medium with incubation at 37C for 24-30 hours should be sufficient. Longer incubations can be problematical and can lead to preparations of uneven consistency. If there is a poor yield of spores after 24 hours, it is better

to start the experiment again than to allow the culture to incubate for extended periods of time. This protocol has been shown to result in a very high frequency (greater than 95%) of "phase-bright" heat resistant spores and at titers greater than 1 x 10^9. Some key spore properties such as exosporium structure do differ between liquid and solid medium spores. These differences may be reflected in the hydrophobicity of the spores and, thus, in their ability to adhere to surfaces and to clump, properties that are relevant to sporicide analysis. It should be noted that the susceptibility of spores produced in liquid and solid medium may differ. Therefore, this issue would be worth addressing experimentally before establishing a spore preparation procedure.

2. There are issues in regard to spore purification. Water washing may be adequate to remove some contaminating cells and debris but will not remove some mother cell components that adhere tightly to the exosporial surface. The best documented case is for the vegetative cell S-layer protein, but there are likely to be others such as one or more of the toxins. These could alter the surface properties and thus the ability to adhere to surfaces.

On the other hand, further purification involves centrifugation through a high density gradient composed of Renografin (or sucrose) to separate the very dense dormant spores from cells and other material. These spores may be "purer" but may have lost some legitimate spore coat or exosporial components. Whether they will differ in resistance/adherence properties is not known. Here again, it should be noted that the spore preparation method can have an effect on spore susceptibility. Similarly, it would be worthwhile to test the susceptibility of spores purified in various ways before a spore preparation procedure is established.

3. Whatever the protocol, the preparations should be monitored in the phase microscope at the harvest and purification stages to be sure there is a very high percentage of "phase bright" endospores (greater than 90% with greater than 10^9/ml). In order to ensure uniformity and high quality dormant resistant spores, it would also be beneficial to check their heat resistance and perhaps some other resistance property (to a chemical other than HCl) or even the completeness of germination in response to optimal germinants (inosine plus L-alanine would be a good choice). HCl causes spores to "pop" but is not a quantitative procedure and thus not a reliable method for determining the uniformity of a spore population. Purified spores should not be stored longer than 2-4 weeks at 4°C, although storage at -20°C in lots to be thawed only once would be preferable. One should be aware of the fact that there may be discrepancies between microscopic and plate counts (Turnbull, et al., 2007), but hopefully these values will not vary among spore batches. These steps would ensure that high quality, uniform preparations would be tested every time.

4. Purified spores are best kept frozen to avoid any autogermination and probably should be stored no longer than 2-4 weeks. It would also be beneficial to keep many stocks of the species to be tested frozen at -70°C in glycerol rather than on plates. This would help to ensure uniformity in the spore preparations and avoid the very likely genetic changes that can occur during repeated culturing.

The Panel believes that these suggested changes in the protocol, precautions regarding procedures, as well as awareness of variability in spore properties will improve the reliability and reproducibility of the spore preparations (and thus of the tests), and bring them more into line with current generally used laboratory practices.

Several additional concerns were noted by various panel members:

1) Spores should not be purchased from commercial sources unless they are very thoroughly vetted, since at least some commercial vendors have been known to have very poor quality control.

2) It would be useful to know the degree to which a given preparation method causes spore clumping. Certain treatments (such as fumigants) might have difficulty reaching the interior of a clump. An important question is the degree to which clumping is relevant to the application being tested. Clumping can be analyzed very easily by phase contrast microscopy.

3) Whatever method is chosen should address the question of how many of the spores added are washed off prior to the actual action of the decontamination agent. Washed off material should not be confused with killed material, when final spore titers are measured.

Issue 3. Whether a six (6)-log ($\log_{10}$) reduction is an adequate measure of success when employing a well developed, quantitative sporicidal efficacy test.

The Agency is proposing that an antimicrobial product may be registered as a "sporicidal decontaminant" if it is tested using a well developed, quantitative sporicidal test method acceptable to EPA using virulent *Bacillus anthracis* spores (or a surrogate acceptable to EPA) on nonporous and/or porous inanimate surfaces and the testing of the product achieves at least a six (6)-log reduction (or a minimum 1×10^6 spores per carrier) of virulent *B. anthracis* spores (or a surrogate acceptable to EPA). The use and adoption of standardized quantitative methods for testing the performance of sporicidal decontaminants for regulatory purposes is supported by EPA. The AOAC Method 966.04 is a qualitative procedure (i.e., provides only positive/negative or pass/fail results). Quantitative procedures provide an estimate of actual spore kill, usually based on the $\log_{10}$ scale, and can be adapted for multiple product formulations and carrier materials. Several well-developed quantitative procedures are available for use. The Agency believes that a performance standard for quantitative laboratory-based assays is essential to establishing consistent product efficacy under actual decontamination scenarios in the field. The proposed 6 log reduction performance standard is a scientifically valid and rigorous standard. The standard will give the Agency reassurance that sporicidal decontaminants when applied per the product's label claims are effective against spores of *B. anthracis.*

The technical basis of the Agency's selection of a minimum 6-log performance standard includes the following:

(1) The target spore titer currently allowed in AOAC method 966.04 (Method II) is a minimum of 1×10^5 to approximately 1×10^6 spores per carrier);

(2) The quantitative methods currently available and published can reliably generate control carrier spore titers necessary to measure a 6-log reduction;

(3) A minimum 6-log reduction in viable spores has been measured for commercially available sporicidal agents and technologies designed for treating sites contaminated with *B. anthracis*; and

(4) Environmental sampling pre- and post-application of the sporicidal decontaminant will determine the need for re-treatment.

Charge Question 3. Please comment on the scientific basis that achieving a six (6)-log reduction using a well developed, quantitative sporicidal test method demonstrates that a product inactivates *B. anthracis* spores on inanimate surfaces.

Panel Response

The EPA product performance criteria for AOAC method 966.04 (Method II) is equivalent to a 5- to 6-log reduction (from 1 x $10^{5\text{-}6}$ spores/carrier to no growth from any carriers tested). Published quantitative methods, such as ASTM E 2111-05, ASTM E 2414-05 and ASTM E 2197-02, can reliably generate control carrier spore titers necessary to measure a 6-log reduction. The proposed 6-log reduction is comparable to the current performance criteria. Commercially available sporicidal agents and technologies designed for decontaminating *B. anthracis* have been shown to achieve a 6-log reduction.

While a 6-log reduction is appropriate as a starting point in a lab setting for product development, it is understood that for so called "hot spots", a much higher degree of kill would be needed and also, in an actual life setting, organic burden will play a major role in decontamination efforts. A survey for "hot spots" for special treatments, a pre-clean up procedure, etc., may be required for actual environment decontamination. Also current surface sampling methods may not be adequate for actually enumerating environmental samples. Furthermore, the level of destruction required in a field application needs to be based on the acceptable residual level of risk, which is outside the scope of the Panel's consideration. The charge to the Panel is whether a 6-log reduction is an adequate measure of the quantitative sporicidal efficacy, particularly *vis a vis* qualitative tests and, on this focused question, the Panel agrees that it is.

A quantitative test method is more appropriate and useful for the types of applications that EPA is considering; that is, testing products and processes to remediate from *B. anthracis* contamination. EPA is to be commended for the development of this type of assay. Quantitative testing is a far better approach than the qualitative tests under the AOAC protocols.

The quantitative test approach also places this field on a comparable basis to other environmental disinfection applications, such as disinfection in water, wastewater and biosolids. The evidence that quantitative sporicidal tests produce equivalent measures of spore inactivation is persuasive (i.e., a 6-log reduction demonstrated via the quantitative test appears equivalent to a "pass" on the qualitative tests.)

It would be appropriate for both this quantitative test, as well as the qualitative tests, to incorporate positive controls of disinfection efficiency with known sterilants in order to check the consistency of the bioassay organism.

It must be recognized that in the field some chance of poorer performance will arise due to mixing imperfections and other physical heterogeneity. Great caution should be given to extrapolating results beyond the bounds of the bioassay -- either to lesser or greater levels of

inactivation than actually observed due to nonlinearity in survival curves.

Issue 3.1. What criteria should be used when selecting coupon materials for quantitative sporicidal tests.

The Agency is proposing to allow only certain nonporous and porous materials to be used in the quantitative sporicidal tests based on specific criteria. The rationale for EPA's position is that in order to achieve reproducible results across laboratories, and to ensure that test materials are suitable to support a particular claim (i.e., the material type to be treated), basic criteria should be established. The EPA also recognizes that the nature of the test material may impact product performance. The criteria that EPA intends to establish for the selection of carriers and carrier materials include the use of standardized materials (e.g., quality, grade and consistency), relevancy of materials to the use site, material availability, data on spore recovery, ability to clean and sterilize prior to inoculation, and potential for interaction with the product's active ingredients.

Charge Question 3.1. Please comment on the EPA's criteria for selecting coupon materials to represent nonporous and porous surfaces in quantitative sporicidal efficacy tests.

Panel Response

Overall, the Panel believes that the criteria for coupon material selection put forth by the EPA represent desirable targets. However, it would be very difficult to identify a single coupon material, or even a small number of such materials, that meet all the stated requirements. Nonetheless, the Panel recognizes that EPA needs to designate appropriate materials in the short term to support regulation of appropriate sporicidal agents for use in contaminated areas. The need to use standardized materials that are readily available should somewhat limit material choice and, as a result, simplify the criteria for selection of materials. However, identification of coupon materials will be challenging if a single set of materials (whether porous or nonporous) will be used to test both liquid and gas disinfectants.

The problem of identification of coupon materials still remains incompletely analyzed. To clarify the relevant issues, several areas need to be further discussed.

1. Choice of materials and number of material types needed to adequately model a contaminated site. How many coupon materials are needed to represent the diversity of surfaces found in a typical environment, such as a contaminated office building (as was the case in September/October 2001)? Are porcelain and silk relevant to any given contaminated area? In the case of an office environment, these two materials may well serve as representatives of cement or carpet. However, they are not representative of many other surfaces in this environment. How will the number of representative materials to be used be determined?

At least one critical material that should be represented is paper. Would this require an additional coupon or is it adequately represented by silk suture loops? A *Yersinia* survival study on environmental surfaces was previously performed by the Centers for Disease Control and Prevention (CDC) which examined paper material (Rose et al., 2003). This study, although not a decontamination study, examined strictly the adherence and survival of *Yersinia pestis* on paper product. However, it seems to be relevant to consider seriously the use of paper as a coupon

material. The Panel recognizes that while surface decontamination of paper material might not be practical, gas sterilization may be effective.

Additional materials that would seem to be relevant are sensitive electronic components (representing computer components and other electronic devices) and plastics. Gas decontamination, rather than surface decontamination would probably be appropriate for most of these classes of materials.

2. Consequences of cleaning and sterilizing coupon materials prior to inoculation. Protocols for appropriate cleaning and sterilization of coupon materials should be available. Surface decontamination or steam sterilization will likely be effective at removing organisms from most nonporous materials. However, chemicals used for surface decontamination may leave a residue that could interfere with testing. Where no other decontamination method can be used, this difficulty will need to be addressed.

The need for pre-sterilization protocols poses a serious challenge for most porous material (such as silk loops or paper), due to the likelihood that pre-sterilization will likely alter the material. The use of irradiation may be a solution in some cases. However, because few investigators will have access to an irradiator, this is not a general solution. One research group autoclaved brown manila envelope coupons (Rose et al., 2003). It would be useful to know how autoclaving in general affects material integrity.

In general, because sterilization or other cleaning processes can change a material such that it differs from what is found in the field, sterilization protocols can affect the reliability of the testing regimens. For example, surface changes may be induced in a carrier during an autoclave process. Conceivably, this could lead to test results different than what might be encountered at a contaminated field site (where surfaces will not have been similarly treated). This issue should be considered in the selection of carrier substrates, and the development of test materials preparation and testing protocols. Test material (coupon) preparation issues will need to be addressed during the validation phase of methods employing carriers intended to represent materials encountered in buildings, etc.

3. Potential for interactions between the product being tested and the coupon material. Ideally, a decontamination product will not react with the coupon material. Coupon materials will need to be examined for their stability when treated with a given product. The potential for interaction is likely to be less for nonporous materials as these should be able to withstand testing of liquid disinfectants. However, porous material (silk loops, paper, carpet, lumber etc.) would likely react with liquids. Gaseous sterilization agents are probably much more suited for decontamination of porous materials.

4. Other criteria to be considered for coupon materials.

a) Standards of quality for porous material, such as a painted surface and untreated pine wood, should include demonstration of the degree of porosity and demonstration of relative ability to recover spores. For measuring the relative porosity of a surface, the ability to absorb water might be used. For this measurement, a set surface area of the material is exposed to water for a set time, and the amount of water weight gained is determined. It should also be recognized that the physical and chemical properties (e.g., permeability) of wood vary widely by species.

Wood is anisotropic with permeability varying dramatically with respect to grain orientation, moisture content and between earlywood and latewood.

b) The interactions between the coupon and spores in a powder form are not well understood. The criteria already described would be sufficient only to test for contamination by a liquid or slurry. The differences between spore-coupon interactions, when spores are dry or wet, could significantly affect the outcome of the sporicidal assay.

c) Coupon materials need to reflect material surfaces as they are found in the field, not only as those materials appear just after manufacture. Specifically, after use or weathering, a material may be quite different than just after production. Weathering due to natural processes can include oxidation, exposure to carbon dioxide or other atmospheric constituents, or long term reaction with water. Specific effects of weathering can include alteration of the surface energetics (such as the surface tension) that affect the degree to which spores disperse or clump.

As alluded to, a major difficulty is the application of liquid sporicidal agents to porous coupons. Most likely in a contaminated building, surface decontamination by a liquid would not be practical for paper, carpet and ceiling tiles. These areas would need to be sterilized with vapor/gas sterilants. Therefore, it may be more relevant to establish criteria that would separate decontamination with liquid and vapor/gas sporicidal efficacy tests.

Other points were also noted by panel members:

1) Characterization of coupon surface topography and other characteristics (e.g., electrostatic) could result in more reliable testing. This testing is not trivial, but can be done. For a number of common materials, this analysis is already in the literature. Possibly, these already characterized materials could be candidate coupon materials.

2) An additional approach to the choice of coupons would be to choose them on the basis of the degree of challenge they offer to the product. Coupons could be graded according to the degree of challenge they present to the decontamination technology.

3) The use of plastics such as polycarbonate, that are especially common in widely manufactured commercial products, should be carefully considered.

4) It may be worth considering adding a caveat to a label indicating that the product has not been tested against powders.

5) While weaponization may impede the effectiveness of a decontamination agent, the organic load of a spore preparation may be as much of a challenge, and is very hard to model or predict. One panel member suggested that perhaps as a "worst case" of organic loading, tests be conducted against spores embedded in dried fermentation medium.

<u>Issue 4.</u> Whether a surrogate *Bacillus* species of spores may be used in place of *Bacillus anthracis* spores in sporicidal efficacy tests.

The Agency is proposing to allow certain surrogate, avirulent *Bacillus* species to be used in place of virulent *Bacillus anthracis* spores for either qualitative or quantitative sporicidal efficacy tests based on specific <u>criteria</u>. Surrogate spores should have certain desirable attributes and be acceptable to EPA. The use of safe-to-handle surrogates of virulent *B. anthracis* spores is supported by the EPA. Surrogates are frequently used as models or representatives for virulent strains of pathogens such as *B. anthracis* Ames. Federal restrictions and biosafety issues limit the number of labs capable of testing select agents. Cost, time and resources required for managing studies on virulent *B. anthracis* spores are also limiting factors. Certain criteria should be met in order for a surrogate to be utilized in the efficacy testing of sporicidal decontaminants. To be an acceptable surrogate, a *Bacillus* spore species should generally be as resistant or more resistant to inactivation by a particular chemical on a particular surface than *B. anthracis* spores.

To demonstrate equivalent resistance of the surrogate spore type to the virulent agent, a comparative efficacy study should be performed using a well-developed/validated quantitative methodology appropriate for the test chemical and microbe to measure resistance. Testing should be conducted in accordance with the potential product claim. Replicated studies with adequate controls and with side-by-side, parallel test designs are desirable. It is also desirable to compare carriers with comparable spore populations. The same sporulation media should be utilized for all test microbes. Percent recovery of spores from carriers should be determined in advance. The strain of *B. anthracis* used in the study should be verified as a pathogenic strain. Examples of acceptable and relevant surrogate studies are provided in References 7 and 8. Pre-existing data may be appropriate to support the use of a surrogate as well.

<u>Charge Question 4.</u> Please comment on the desirable attributes for selecting surrogate *Bacillus* species for *Bacillus anthracis* in either qualitative or quantitative sporicidal efficacy tests.

Panel Response

The Panel's discussion of this issue can be broken down into three general areas: the need for use of surrogates for virulent *B. anthracis*, the extent to which surrogates should be used for product efficacy tests, and the desirable attributes for selecting a surrogate.

The need for use of surrogates for virulent *B. anthracis*.

Testing of virulent *B. anthracis* spores requires Biosafey Level 3 (BSL-3) conditions, which are not widely available and can prove quite expensive, especially for relatively small companies. Given the wide availability of avirulent, non-select agent bacterial strains that are similar to virulent *B. anthracis* in many phenotypic properties, and that can be utilized under BSL-2 conditions, there seems little reason not to allow the use of an appropriate surrogate. The Panel expressed broad support for the general use of surrogate organisms. It was pointed out that other US government agencies such as laboratories at Dugway Proving Grounds have used surrogates quite successfully.

The extent to which surrogates should be used for product efficacy tests.

Two scenarios for the use of surrogates can be envisioned. In the first, the surrogate might be used during all phases of product development, but final product efficacy demonstration would require the use of a virulent *B. anthracis* strain. This is probably the current process followed by product developers and, as far as product efficacy demonstration is concerned, would not require any change from current policy.

In the second scenario, the presentation of either previously published or newly generated data indicating that a surrogate had equivalent or greater resistance than a virulent *B. anthracis* strain to a particular chemical on a particular surface would be sufficient justification for complete product efficacy testing using the surrogate. The challenge in this situation will be determining how broadly to define the "particular chemical" and "particular surface." If the term "particular chemical" refers to a general class of sporicidal agents in a variety of formulations, then the use of a surrogate in efficacy demonstration will save time and money. If the term "particular chemical" is more narrowly defined and equivalent resistance must be demonstrated for each product formulation, then each product will end up being tested against the virulent agent as in the first scenario described above. Broad versus narrow definition of the term "particular surface" will have similar effects. This will be directly related to the number and classification of surfaces tested, as discussed in response to Charge Question 3.1, and the requirements set for labeling a product as effective on porous and non-porous surfaces.

One panel member's impression was that use of a surrogate is, in part, to reduce the burden on technology developers and potential registrants. For a given test protocol and for a particular surface, call it surface #1, surrogate A may best meet the requirement of being "as resistant or more resistant to inactivation by a particular chemical...than *B. anthracis.*" However, as some of the test data presented at the meeting implies, surrogate B may prove more resistant on some other surface #2 using the very same test protocol. A similar observation may be anticipated for surface #3 (where perhaps some other surrogate C is more resistant). Thus, in practice, a number of surrogates may be required, each requiring a side-by-side demonstration of equivalence test with virulent *B. anthracis*. So, it should be kept in mind that the savings (i.e., burden reduction) relative to tests with virulent strains may ultimately not be as great as initially hoped. This is a strong argument for identifying a single surrogate that can be used in a broad variety of applications.

In general, the Panel expressed broad, but not unanimous, support for the use of surrogates in efficacy testing, given reasonably strong evidence that the surrogate has equivalent resistance to a virulent strain under the conditions tested.

Desirable attributes for selecting a surrogate.

A) Resistance properties similar to those of a virulent strain.

Several panel members pointed out the problem with trying to define a surrogate with resistance properties "equivalent to" those of a virulent *B. anthracis* strain because of the inherent problem in defining the resistance of a generic virulent strain. Spore properties, including both resistance and surface interaction properties, can vary with any of the following

parameters: strain (Ames vs. Vollum or other virulent strains); spore preparation methods (medium, temperature, plate vs. liquid culture, washing methods, weaponization efforts); and specific conditions of spore introduction to the site.

Pathogenic *B. anthracis* strains, even though very closely related genetically, can vary phenotypically. This is well illustrated by a paper showing very significant differences in LC_{50} values in mice for the Ames strain versus a Sterne strain transformed with plasmid pXO2 (Drysdale *et al.*, 2005). This result points out the complexity of defining the properties of *B. anthracis* by comparing to a particular pathogenic strain.

Thus, even if one virulent strain is chosen and a product's sporicidal activity against this virulent strain is demonstrated under a variety of conditions, this will be no guarantee of effectiveness in a real world situation because information on all the above parameters will be unknown. Post-application confirmatory testing will be required in every real world application. Therefore, the relevant question becomes, "Does the product inactivate spores that are representative of the broad range of virulent *B. anthracis* spores that might be encountered in real world applications?"

The choice of a single surrogate with "similar" resistance properties over a broad range of test conditions might be better than using varied surrogates for different products simply because the different surrogates have the most closely matched resistance properties. The surrogate's other desirable traits may be equally important. For example, a surrogate might have equivalent resistance properties under five different test conditions, but under a sixth condition show a 7-log reduction while the virulent strain shows a 6-log reduction. This may not be a reason to reject the surrogate, if it is a good match to other desirable criteria. The 7-log reduction may be within the range of what is expected for the broad class of virulent spores to be encountered in real-world situations.

The fact that a surrogate and the virulent strain have the same mechanism of resistance may be more important when choosing a surrogate for a broad class of sporicidal agents. A surrogate that is chosen simply because it has greater resistance properties than the virulent strain may fail when the particular agent is put into a new formulation. The mechanism by which such a surrogate achieves resistance may be different than that of the virulent strain and this greater resistance may then vary more greatly with changes in the chemical formulation. This argues for the use of more closely genetically related surrogates.

The virulent strain(s) chosen for equivalent resistance testing should be verified as such. Given that the virulent strains are handled in a relatively small number of locations, anyone desiring to do resistance equivalence testing will either be at such a location, will collaborate with such a location, or will obtain the virulent strain from such a location. The known origin of the strain and PCR-based genetic testing to show the presence of the pXO1 and pXO2 virulence plasmids should be sufficient to demonstrate a putative virulent strain.

B) The surrogate should have similar spore surface properties.

The exosporium of *B. anthracis* spores can have major effects on interaction of spores with surfaces. The relative interaction of a spore on a given surface, changing factors such as distance and the presence of different chemical groups, can potentially alter the chemistry of a

decontamination reagent on a surface and spore. The presence of an exosporium of similar composition to that of virulent strains is probably a good idea in order to obtain the closest simulation of spore-surface interactions in the presence of the test reagent. Changes in spore preparation conditions as noted above can potentially significantly change spore surface properties, such as hydrophobicity (Bailey-Smith et al., 2005). Ideally, a surrogate should be demonstrated to exhibit changes in spore surface properties similar to those of a virulent strain, indicating similar regulation of this phenomenon.

C) Genetic relatedness should be a major guiding factor in choosing a surrogate.

There is very little genetic diversity among *B. anthracis* strains. There is no good reason why avirulent *B. anthracis* strains cannot be used as surrogates for virulent *B. anthracis*. The Sterne strain is highly attenuated, does not have select agent status, can be utilized under BSL-2 conditions and can be expected to have spore phenotypic traits similar to those of the virulent strains. Data presented during the FIFRA SAP meeting indicated that resistance differences between the virulent Ames strain and the avirulent Sterne strain were not statistically significant under a variety of conditions. Some data indicated potentially statistically significant differences but this data is unpublished and may be incomplete. Based on the genetic similarities between the Ames and Sterne strains, the probabilities that they have similar surface properties, regulation of surface properties, and resistance mechanisms are high. The spores of these strains are more likely to behave similarly when placed on various surfaces and in the presence of varied chemical and physical conditions.

There was significant support for the idea that a single surrogate should be developed and used as widely as possible. This would more rapidly demonstrate the utility or failure of this surrogate with a wide variety of surfaces and sporicidal agents. The Panel expressed broad support for the use of *B. anthracis* Sterne as a good surrogate. It should be emphasized that the Sterne strain is very easy to work with and is safe. The following caveats concerning the Sterne strain were expressed and should be examined closely as further studies become available.

There is a possibility that plasmid pXO2, which is not present in the Sterne strain, encodes factors affecting spore properties or even regulatory factors that control spore properties. There are a number of uncharacterized open reading frames on this plasmid. As further studies of this plasmid are published, effects on spore resistance properties should be considered with regard to sporicide efficacy studies. If, in the future, a *B. anthracis* strain that carries pXO2 but lacks the relevant virulence genes from this plasmid is named as a non-select agent, then it may be a better surrogate.

There was some significant concern expressed about the use of *B. subtilis* as a surrogate for virulent *B. anthracis*, even considering the data indicating that *B. subtilis* is more resistant than *B. anthracis* to certain sporicides under some conditions. *B. subtilis* clearly differs in adherence properties, as evidenced by data presented at the meeting showing the need to use a mild non-ionic detergent to remove *B. anthracis,* but not *B. subtilis* spores, from solid, porous surfaces.

One panel member expressed the opinion that taxonomic similarity is not necessarily the most important criterion in selecting a surrogate. The key criteria should be the similarity in sensitivity, and surrogates of dissimilar taxonomy should not be overlooked. This panel

member asked, "Were there any data taken on the microbiology of the organisms remaining after the 2001 decontamination that could be used to inform potential choices of such taxonomically dissimilar organisms?" There is a strong and lengthy experience with indicators of disinfection efficiency in water and wastewater treatment, and the best indicators have very often been found not to be of similar taxa. For example, naturally occurring aerobic spore forming organisms are becoming strong candidate indicator organisms for the disinfection of *Cryptosporidium parvum* oocysts by various chemical disinfectants (Mazoua and Chauveheid, 2005).

A dissenting view was expressed by a panel member, stating that the amount of work needed to ensure that a surrogate is equivalent to the real thing may not be worth the cost.

Issue 5. Whether gas or vapor products should be subjected to a "simulated use test."

The Agency is proposing that gas or vapor sterilants, sporicides and sporicidal decontaminants be subjected to a "simulated use test" for gas or vapor products intended for use in large, enclosed spaces. The rationale for EPA's position is that efficacy testing performed in the laboratory does not necessarily demonstrate that a product will perform satisfactorily when applied in a large, enclosed space. Many factors can reduce the effectiveness of a gas or vapor product, such as inadequate distribution, breakdown by light, and absorption/breakdown by porous or reactive surfaces. Accordingly, EPA believes that a simulated use test is needed to demonstrate that a gas or vapor product will perform successfully in a large volume of space (e.g., a typical office). In addition, such a test should include monitoring to assure that key parameters (e.g., temperature, relative humidity, concentration) for an effective fumigation will be met.

Charge Question 5. Please comment on the scientific basis for conducting a "simulated use test" for a gas or vapor product intended for use in large, enclosed spaces.

Panel Response

In general, the Panel agreed with the position stated by the Agency that "efficacy testing performed in the laboratory does not necessarily demonstrate that a product will perform satisfactorily when applied in a large, enclosed space." In theory, the proposed simulated use test does provide an opportunity to assess factors that could interfere with product effectiveness in the field. However, one panel member noted that most factors likely to impact effectiveness are, generally speaking, either scale-dependent or scenario-dependent (e.g., this includes side reactions with materials within the large space, photo-degradation, restricted flow patterns, diffusion bottlenecks, etc.).

As an example, it was noted that while a technology developer may conduct a successful demonstration at the approximately 3,000 cu. ft. scale using, for example, a combination of strategically placed fans, heaters, and/or humidifiers, it does not follow directly that the same strategy will prove effective in a 30,000 cu. ft. facility of different geometry. The underlying issue is that mixing dynamics depend on the characteristic dimensions (and shape) of the enclosed space.

Another illustrative example was offered. This example supposes that an approximately 3,000 cu. ft. enclosed space with a particular arrangement of office furnishings was to be

successfully decontaminated. However, it was noted that an alternative spatial arrangement of the same furnishings may result in more aerodynamically 'dead' or stagnant zones and hence a less successful demonstration might be observed under the same test conditions. It was suggested that diffusion resistances (bottlenecks) may vary significantly with the particular scenario.

The point was made that accurate performance projections to large-scale enclosed spaces, as might be encountered in the field, may prove difficult based on a single, modest-scale simulated use test due to the fact that the scaling will likely be non-linear. One panel member noted that each of the various physical parameters scale with a different exponent, further complicating scale-up projections. Another panel member suggested that, in addition to the Agency stance, the simulation must be representative of the actual test (i.e., preserve the key physics) and perhaps a scale ratio, or percentage (maybe 10%), of the actual test should be clearly established.

It was also noted that while a simulated use test can provide some useful (albeit partial) data related to performance upon scale-up, a number of experimental "design" issues should also be addressed prior to initiation of any such tests since these may directly affect the outcome. A few things to consider are:

- Are the surfaces within the space to be pre-cleaned? If so, how? If not, how do we measure and standardize the dirt/organic load?
- Is the room to be pre-conditioned? For example, water condensed in small pores may be present and affect spore binding. Do we draw vacuum prior to the test?
- Are the same carrier materials (as used in lab scale testing) to be placed strategically in the room, or are the contents to be contaminated directly? If it is the latter, then guidance on acceptable inoculation and recovery protocols would be useful.
- For most products, it is anticipated that *some* combination of inlet concentration and time can be found that ultimately brings every surface up to the desired concentration, but at what price to the contents and building infrastructure? Is collateral damage to be quantified? How? Or is the time required the primary basis of distinguishing between techniques?

It was reiterated that a moderate size demonstration does provide insight into practical issues such as the relative rates of fumigant degradation to transport/diffusion and sensitivity to environmental factors. However, these rates should be quantified to be of general utility. It was suggested that some up-front modeling may provide useful guidance on accuracy of performance projections as a function of change in scale. It was suggested that approaches ranging from classical dimensional analysis to high fidelity computational fluid dynamics be considered as guides to designing such tests. One panel member pointed out that some of the data obtained from simulated use tests might also be useful in model validation.

Another panel member thought the rationale for a simulated use test needed to be more clearly thought through. If this simulated use test is aimed at demonstrating the maximum capacity for a given unit of equipment to achieve homogenous and steady dosing over time, then there are limitations in what can be done in small scale testing to project the capacity in large scale. Various physical and chemical processes scale differently and thus small scale tests might not fully represent the challenges that occur at large scale. For example, the surface area/volume ratio (which would be important in heat exchange with the surroundings) decreases with scale. If

the flow rate of decontaminant-containing gas is kept proportional to the system scale, then the entrance Reynold's number of the gas (which would impact mixing) would scale roughly with the $2/3^{rd}$ power of system scale. In addition, it was also suggested by this panel member that the agency should consider the use of computational models for furthering the important goal of rating capacity of different units for different size facilities. For example, various zonal flow models have been developed by National Institute of Standards and Technology (CONTAM-W) and other organizations, and for complex scenarios, the use of computational fluid dynamic models or discrete Markov chain models represent possible paths forward. This panel member also voiced concern over what would be used for controls if coupons are placed within the space during the simulated use test?

The capacity of a unit to remediate a given space will depend upon a complex series of factors that extend beyond just the total size of the space. The complexity of the floor plan, the ventilation plan, the interior building envelope materials, and the external heating or cooling loads will all play a role, as will other variables. A considerable amount of basic engineering research and development is needed to fully understand these complexities. Without this additional research and development, the field deployment of remediation will remain as much of a full scale experiment as it was in 2001.

An additional issue that was raised was that these types of tests can be highly complex and it will be important for technology developers to have good guidelines so they know what they are getting into and do not waste a lot of time on unproductive testing regimes. EPA may want to have developers submit model designs before they are used in a test, to ensure the designs are sound.

Additional Considerations

A general question was raised regarding whether any of the methods being developed by EPA for product testing would be suitable for analysis of weaponized spores. The Panel indicated that the methodologies currently being developed would not be suitable for analysis of weaponized spores. However, it was noted that the AOAC is developing a sampling method for powders, which could potentially be a pre-cursor to decontamination efficacy testing.

Suggestions from the Panel for Future Research.

It was noted during the Panel's discussion that significant research gaps exist in several basic science areas related to spore biology. The lack of research data in these particular areas prevent EPA from having all the information it needs for the best quality decisions. Three particular areas in which future research could bolster the scientific community's understanding of basic spore biology, and thereby enhance EPA's ability to develop optimum spore decontaminating testing regimes, are provided below.

1) Spore Adherence to Surfaces. Little is known about the mechanisms of adherence of spores to surfaces. This information could be obtained using available methodologies. However, federal funding is not available for this type of research. Unless this information gap is closed, it will be very difficult to accurately predict the behavior of spores with coupon materials and, therefore, to choose optimal coupon materials.

2) Variations in Virulent Strains. Little is known about variation in properties of virulent *B. anthracis* strains. This lack of information significantly retards efforts to identify suitable surrogates.

3) Properties of Coupon Materials. A firm theoretical basis on which to choose coupon materials is lacking. This is a complex and multidimensional problem, but one that would benefit from basic science of several types, including a better understanding of the materials science of coupons, so that the choice can be made more rationally.

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