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## **Methyl Iodide Fumigation of *Bacillus anthracis* Spores**

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### **Abstract**

Fumigation techniques such as chlorine dioxide, vaporous hydrogen peroxide, and paraformaldehyde previously used to decontaminate items, rooms, and buildings following contamination with *Bacillus anthracis* spores are often incompatible with materials (e.g., porous surfaces, organics, and metals), causing damage or residue. Alternative fumigation with methyl bromide is subject to U.S. and international restrictions due to its ozone-depleting properties. Methyl iodide, however, does not pose a risk to the ozone layer and has previously been demonstrated as a fumigant for fungi, insects, and nematodes. Until now, methyl iodide has not been evaluated against *Bacillus anthracis*. Sterne strain *Bacillus anthracis* spores were subjected to methyl iodide fumigation at room temperature and at 55°C. Efficacy was measured on a log-scale with a 6-log reduction in CFUs being considered successful compared to U.S. Environmental Protection Agency biocide standard. Such efficacies were obtained after just one hour at 55°C and after 12 hours at room temperature. No detrimental effects were observed on glassware, PTFE O-rings, or stainless steel. This is the first reported efficacy of methyl iodide in the reduction of *Bacillus anthracis* spore contamination at ambient and elevated temperatures.

### **Introduction**

Following the Amerithrax attacks in 2001, a variety of federal, postal, and privately owned buildings in Washington, DC; New York; New Jersey; and Florida were contaminated with resilient *Bacillus anthracis* spores (*B. anthracis*, the causative agent of “anthrax”). Three gases were used to fumigate those facilities—chlorine dioxide gas, vaporous hydrogen peroxide, and paraformaldehyde (Canter et al., 2005). Chlorine dioxide gas penetrates into porous surfaces (U.S. Environmental Protection Agency [U.S. EPA], 2005), but reacts with a wide range of materials (National Research Council, 2005) and is known to cause corrosion of some metals including aluminum, iron, and copper found in plumbing and electrical equipment (U.S. EPA, 1999). Vaporous hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, VHP) penetrates poorly into porous surfaces, may react with organics (U.S. EPA, 2005), and is known to cause corrosion of some metals including those important to plumbing and electrical infrastructure (U.S. EPA, 1999). Paraformaldehyde (PFA, nominally OH[CH<sub>2</sub>O]<sub>n</sub>H where *n* = 8 to 100) offers high penetration into porous materials and is relatively unreactive with most materials. PFA gas reacts with oxidizers and some organics (Centers for Disease Control and Prevention [CDC], 2005); however, it is a probable human carcinogen (U.S. Department of Health and Human Services [DHHS], 2011) and may leave a residue on surfaces.

Methyl bromide (CH<sub>3</sub>Br, MeBr) has been shown to be effective in the fumigation of insect and fungal infestations and has been used experimentally for the fumigation of *B. anthracis* spores (Corsi, Walker, Liljestrand, Hubbard, & Poppendieck, 2007; Juergensmeyer, Gingras, Scheffrahn, & Weinberg, 2007; Kolb & Schneiter, 1950; U.S. EPA, 2010). MeBr penetrates into porous surfaces and does not typically react with organics, making it an attractive method for the fumigation of buildings and agricultural areas. MeBr may react with unprotected

aluminum and may react with rubber and sulfur-containing articles depending on the delivery method. It is a neurotoxin and an animal carcinogen, although not proven in humans (DHHS, 2011). Additionally, MeBr is subject to the Montreal Protocol on Substances That Deplete the Ozone Layer (Albritton & Watson, 1992) and its use has largely been phased out (with exceptions for importing and exporting of goods and certain critical uses) through the Clean Air Act (2004).

Methyl iodide (CH<sub>3</sub>I, iodomethane, MeI) is a commercially available alternative to MeBr for fumigation (Ohr, Sims, Grech, Becker, & McGiffen, 1998) and is a registered pesticide in several countries around the world with fungicide, herbicide, insecticide, nematicide, and soil disinfectant properties similar to those of MeBr. In September 2006, U.S. EPA granted the Japanese company Arysta LifeScience, Inc., an experimental use permit for MIDAS (methyl iodide) in seven U.S. states. In October 2007, U.S. EPA approved the use of MeI across the U.S. By 2010, Arysta registered MeI use in Japan, Turkey, New Zealand, Morocco, and Mexico, with planned registration in other countries including Guatemala, Australia, Israel, Chile, Egypt, and South Africa. In October 2012, following significant pressure from health professionals, environmental experts, and communities (*San Jose Mercury News*, 2011; *The New York Times*, 2012), Arysta LifeScience withdrew their product from U.S. markets and voluntarily requested the cancellation of MIDAS registration in the U.S. citing economic viability concerns in the U.S. marketplace (Arysta LifeScience, 2012; U.S. EPA, 2012).

A comparison of the inhalation exposure standards for both MeBr and MeI are shown in Table 1. The calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of mice and rats (LC50) for MeI is higher than that for MeBr. This health standard suggests that higher concentrations of MeI are needed to cause death compared to MeBr. The regulatory standard of time-weighted permissible exposure limit given by the Occupational Safety and Health Administration suggests that MeBr represents a slightly lower risk of illness compared to MeI, perhaps because of limited evidence of cancer in animals exposed to subcutaneous and intraperitoneal MeI (International Agency for Research on Cancer, 1977) compared to no evidence of cancer in animals exposed to MeBr in an inhalation study (National Toxicology Program, 1992). Advisory standards such as the American Industrial Hygiene Association's emergency response planning guidelines and the time-weighted American Conference of Governmental and Industrial Hygienists' threshold limit value suggest that workers may be exposed to higher concentrations of MeI without irreversible or adverse health effects compared to MeBr.

The boiling point of MeI is 42.4°C and the liquid density is 2.28 g/cm<sup>3</sup> at 20°C (Hayes, 2013). The compound has restrictions on both the shipment volume and method (no aircraft transport). MeI is not subject to the Montreal Protocol or the U.S. Clean Air Act. In fact, U.S. EPA found that "Once volatilized, iodomethane degrades rapidly in the lower atmosphere via direct photolysis and lasts in the atmosphere less than 12 days, as compared with two years for methyl bromide. Therefore, iodomethane is unlikely to reach upper atmosphere to have an impact upon the ozone layer. However, global uncertainty on volatilization rates, residence time in soil, photolytic degradation of iodomethane, and the removal of iodine radicals from the troposphere means that the possibility of detrimental effects of iodomethane on ozone layer and a contribution to global warming cannot be excluded entirely (U.S. EPA, 2007)."

Despite withdrawal of MeI from the U.S. agricultural market, MeI may prove to be a useful replacement for MeBr in the fumigation of *B. anthracis* spores due to the strict control and use of MeBr. A study was commissioned to evaluate the efficacy of MeI fumigation, including

whether a 6-log reduction in viable *B. anthracis* CFUs could be achieved at two different temperatures, namely room temperature and an elevated temperature that would lead to higher gas-phase concentrations of MeI. Such a reduction would meet U.S. EPA's biocide standard.

Experiments were performed using the *B. anthracis* Sterne strain (34F2) (CDC, 2009) on stainless steel at ambient room temperature and elevated temperature (55°C) corresponding to below and above the boiling point of methyl iodide. It should be noted that in a sealed vessel, a liquid cannot completely evaporate; rather, the concentration in the gas phase increases until equilibrium is reached between the gas phase and the remaining liquid.

## **Methods, Materials, and Equipment**

### ***Materials***

Methyl iodide (99% stabilized with copper/silver, 141.97 g/mol) was stored in an amber glass bottle.

Biological indicator ribbons were used (304 stainless steel ribbon) containing  $3 \times 10^6$  *B. anthracis* Sterne spores.

### ***Equipment***

Pressure-rated glassware (heavy-walled borosilicate glass, 48-mL capacity) was used to contain MeI gas during fumigation. Vessels were sealed with accompanying PTFE screw caps and front-seal FETFE O-rings (#15) and vessels were wrapped with aluminum foil to prevent light from causing photodegradation of the MeI. The reaction of short-wavelength photons leads to the formation of methanol, iodide ions (I<sup>-</sup>) and protons (H<sup>+</sup>) in moist/wet environments (Gan & Yates, 1996).

A generic 5-L digital water bath with a secondary National Institute of Standards and Technology (NIST)-traceable digital thermometer (-50°C to 300°C) was used to verify the water temperature and provide an alarm against overheating.

The work was performed in a Class-II type B2 cabinet that was vented directly outside the building because of the combined hazards of *B. anthracis* and volatile MeI.

### ***Methyl Iodide Fumigation***

Undiluted methyl iodide (20 mL) was placed in the pressure-rated glassware and the biological indicator ribbon was suspended above the solution using a PTFE sample holder or nylon thread. The vessel was then sealed and (in the case of the elevated temperature experiment) lowered into a 5-L digital water bath set to 55°C such that the water was level with the bottom of the screw cap (minimizing condensation of MeI at the top of the vessel). Ambient (room temperature) experiments determined to be between 19.4°C and 21.1°C were placed in an unheated water bath. A NIST-traceable digital thermometer was used to verify the water temperature and provide an alarm against overheating. Once the desired reaction time was achieved, the vessel was removed from the water bath and the samples were removed.

The vapor pressure of MeI was calculated from a previously published relationship (Lorenz, Osborne, Collins, Manning, & Malinauskas, 1976), which states that

$$\log_{10} P(\text{mm}) = \frac{1475}{T} + 7.56$$

where  $P(\text{mm})$  is the vapor pressure in mmHg,  $T$  is the temperature in Kelvin. The corresponding concentration of MeI in the gas phase was calculated using

$$P(p)V = nRT$$

where  $P(p)$  is the vapor pressure of MeI (Pascals),  $V$  is the volume of gas above the MeI liquid in the pressure-vessel,  $n$  is the number of moles of methyl iodide in the gas phase,  $R$  is the molar gas constant ( $8.314 \text{ m}^3 \cdot \text{Pa} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$ ), and  $T$  is the temperature (Kelvin). The number of moles of MeI in the gas phase was then converted to a mass (mg) per volume (L) using the molar mass ( $141.9 \text{ g/mol}$ ) and accounting for the headspace volume above the liquid in the pressure vessel (28 mL). Partial pressures (and therefore molar ratios) were subsequently determined by comparing the number of moles of MeI to the sum of the number of moles of an ideal gas ( $0.044 \text{ mol/L}$ ). The theoretical concentration of MeI gas in the experiments can then be plotted (Figure 1).

Several control samples were evaluated during each test, including vessels containing water and others vessels containing no liquid to study the relative effect of temperature without MeI.

### ***Measurement of MeI Fumigation Efficacy***

A strict aseptic technique was used during all of the procedural steps involving sample handling and dilutions. After exposure to MeI, the ribbons containing *B. anthracis* spores were placed into individual sterile test tubes containing 10 mL of sterile distilled water. Using sterile forceps, the ribbon was first bent into a coil such that the spores were on the inside edge at the end of the coil (this had previously facilitated placement in the pressure vessel for MeI fumigation). The coiled ribbons were soaked for one hour followed by sonication for one hour. Tenfold serial dilutions were then performed using vortex-mixing (high speed for 10 seconds) prior to removing aliquots. Dilutions of  $10^{-1}$  (ribbon in 10 mL) to  $10^{-4}$  were plated as 100  $\mu\text{l}$  each onto duplicate tryptic soy agar (TSA) plates. The remaining liquid from each  $10^{-1}$  suspension (nominally 8.9 mL) was filtered through a  $0.45\text{-}\mu\text{m}$  filter and the filter was placed onto a TSA plate. Plates were incubated overnight at  $30^\circ\text{C}$ .

*B. anthracis* colonies on the TSA plates were counted manually by visual inspection after 16 hours of incubation at  $30^\circ\text{C}$ . Colonies were confirmed to have morphology consistent with *B. anthracis*, namely flat or slightly convex colonies with irregular borders and ground-glass appearance. The colony counts were multiplied by the dilution factor to give the total number of CFUs for each biological indicator.

The efficacy of MeI fumigation was then calculated and expressed as a “log-reduction” value by subtracting the average log CFU measured on samples after fumigation ( $\text{CFU}_f$ ) from the average log CFU on unexposed positive control samples ( $\text{CFU}_c$ ), as follows:

$$\text{Efficacy} = \log_{10}\text{CFU}_c - \log_{10}\text{CFU}_f$$

### **Results and Discussion**

The effects of exposure times (and therefore of gas-phase MeI dosage) from one hour (11.7 parts per million per hour [ppm-hr] MeI) to 72 (842.4 ppm-hr MeI) hours at ambient room temperature are shown in Table 2 and Figure 2. The number of CFU was reduced by almost 50% after one hour (12 ppm-hr). A further reduction in the number of CFU was observed after four hours (50 ppm-hr) and the U.S. EPA biocide standard 6-log reduction was achieved (leaving no viable spores to measure) after 12 hours of MeI exposure at room temperature (141 ppm-hr). As expected, no significant reduction in CFU was observed in experiments containing water, and no

dry experiments were performed at room temperature because no additional heat was applied. In some cases (noted by a standard deviation of N/D), duplicate samples were not determined.

Table 3 and Figure 2 show that at 55°C, a 5.6-log reduction in CFUs was observed after only 30 minutes (18 ppm-hr MeI). Efficacies greater than U.S. EPA's biocide standard 6-log reduction were achieved after just one hour, corresponding to a 36 parts per million by volume per hour (ppmv-hr) exposure, leaving no viable spores to measure. Analysis of the dry air controls at elevated temperature show that the increased efficacy at higher temperature is not due to heat-inactivation of the spores, and as such the increase in efficacy gained by using elevated temperature is due to the increased concentration of methyl iodide in the gas phase. Despite some level of humidity inside the vessel, the humidity was certainly less than 100% (experiments were performed in a laboratory with no forced humidity control, not a saturated environment). A reduction of less than one order of magnitude was observed in some of the samples suspended above water in the method control samples and subjected to 55°C. These results are consistent with those at 90% RH and 60°C for 24 hours (Buhr et al., 2012) causing an average log reduction in *Sterne* spores from 0.2 to 2.7 on a variety of materials. Comparing the results from both room temperature and elevated temperature exposure experiments (Figure 2) shows how temperature affects the required time to achieve reduction in CFU. Not surprisingly, at higher temperatures (and therefore higher concentrations of MeI in the gas phase), shorter exposure times are required. Increasing the temperature to 55°C over room temperature increased the concentration in the gas phase threefold.

Elevated temperatures such as 55°C (131°F) would be difficult to achieve for large buildings and may only be practical for small items that can be placed inside heated chambers. It is important to note, however, that military equipment such as planes and vehicles are likely to withstand more extreme elevated temperatures, suggesting that rapid fumigation could be achieved.

In the first documentation of MeBr fumigation of *B. anthracis* (Juergensmeyer et al., 2007) a 7-log reduction in spores was achieved using 80 mg/L for 48 hours (~984,600 ppm-hr). Room temperature MeBr fumigation tests performed by U.S. EPA (2010) at 25°C for nine hours at 211 mg/L (~14,769,200 ppm-hr) did not meet a 6-log efficacy standard for *B. anthracis* spores. Instead, an 18-hour exposure was required at 37°C to achieve a 6-log reduction in their experiments. The work presented here shows that MeI achieved a 6-log reduction in spores, meeting U.S. EPA's biocide standard at a lower temperature and with a significantly lower concentration compared to MeBr. It should be noted, however, that the demonstrations by both previous studies of MeBr fumigation of *B. anthracis* involved significantly larger volumes than the 28 mL headspace in which the biological indicator strips were suspended in this work. Additionally, the effect (and control) of relative humidity was not evaluated in our study.

The mode of action for MeI on *B. anthracis* spores is assumed to be the same as that for MeBr, namely DNA alkylation. The alpha/beta-type small, acid-soluble spore proteins do not protect the spore DNA against alkylating agents (Setlow, Tautvydas, & Setlow, 1998).

Further tests should evaluate fumigation of spore control strips in larger enclosures with controlled temperature and relative humidity as well as material compatibility studies. Since MeI is a liquid at room temperature, mitigating condensation during or after fumigation will be an important factor in implementing future tests and scaled up field testing. Field tests should be directed at small rooms, enclosures, or military vehicles to demonstrate efficacy and practicality on scales larger than bench-top experiments. Potential users of MeI for *B. anthracis* fumigation include U.S. EPA and military organizations, or their international equivalents.

## **Conclusion**

The scoping study described in this article clearly shows that MeI is an efficient sporicide in the neutralization of *B. anthracis* Sterne spores. Efficacy was measured on a log scale and 6-log spore reduction in spores was observed after one hour at 55°C and after 12 hours at room temperature, making MeI a viable alternative to current fumigation techniques such as chlorine dioxide, vaporous hydrogen peroxide, paraformaldehyde, and MeBr. Recommended follow-on studies include examination of efficacy in the presence of organic materials and evaluation of the effect of methyl iodide on simple electronic equipment and on valuable materials such as paper documents. The effect of relative humidity on MeI efficacy should also be subject to investigation.

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**Table 1**  
**Published Inhalation Exposure Health, Regulatory, and Advisory Standards for Methyl Bromide (MeBr) and Methyl Iodide (MeI)**

Inhalation Exposure Limits	MeBr, mg/m <sup>3</sup>	MeI, mg/m <sup>3</sup>	Reference
LC50 mice <sup>a</sup>	1,540	5,000	U.S. Department of Health and Human Services, 1993
LC50 rats <sup>a</sup>	1,173	1,300	U.S. Department of Health and Human Services, 1993
OSHA PEL <sup>b</sup>	80	28	Occupational Safety and Health Standards, Toxic and Hazardous Substances, 1998
AIHA ERPG-2 <sup>c</sup>	195	290	American Industrial Hygiene Association, 1998
ACGIH TLV <sup>d</sup>	4	10	American Conference of Governmental Industrial Hygienists, 1999

<sup>a</sup>The calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of mice and rats.

<sup>b</sup>Occupational Safety and Health Administration's permissible exposure limit.

<sup>c</sup>American Industrial Hygiene Association's emergency response planning guidelines.

<sup>d</sup>American Conference of Governmental and Industrial Hygienists' threshold limit value.

**Table 2**  
**Average Number of *B. anthracis* CFU Surviving After Exposure to 12 Parts per Million by Volume Methyl Iodide (MeI) at Room Temperature (~20°C)**

Time (hrs)	Average Positive Control, CFU (SD)	Average Water Control, CFU (SD)	Average Post-MeI Exposure, CFU (SD)	Average Log Reduction From MeI
1	2.28 x 10 <sup>6</sup> (±8.31 x 10 <sup>4</sup> )	2.23 x 10 <sup>6</sup> (±2.83 x 10 <sup>4</sup> )	1.28 x 10 <sup>6</sup> (±3.82 x 10 <sup>5</sup> )	0.25
2	2.27 x 10 <sup>6</sup> (±3.25 x 10 <sup>5</sup> )	2.07 x 10 <sup>6</sup> (±2.12 x 10 <sup>4</sup> )	1.06 x 10 <sup>6</sup> (±4.23 x 10 <sup>5</sup> )	0.33
4	2.27 x 10 <sup>6</sup> (±3.25 x 10 <sup>5</sup> )	2.14 x 10 <sup>6</sup> (±7.42 x 10 <sup>4</sup> )	2.48 x 10 <sup>4</sup> (±3.35 x 10 <sup>4</sup> )	1.96
12	2.38 x 10 <sup>6</sup> (±N/D <sup>a</sup> )	2.31 x 10 <sup>6</sup> (±N/D)	0 (±0)	6.38
24	2.09 x 10 <sup>6</sup> (±N/D)	2.74 x 10 <sup>6</sup> (±7.78 x 10 <sup>4</sup> )	0 (±0)	6.32
72	2.27 x 10 <sup>6</sup> (±N/D)	2.14 x 10 <sup>6</sup> (±N/D)	0 (±0)	6.36

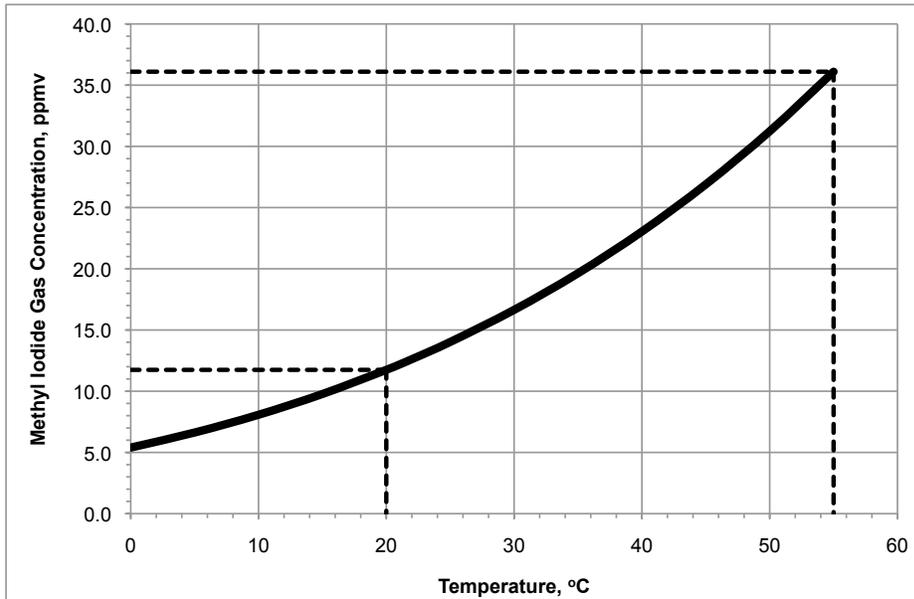
<sup>a</sup>N/D = not determined.

**Table 3****Average Number of *B. anthracis* CFU Surviving After Exposure to 36 Parts per Million by Volume Methyl Iodide (MeI) at 55°C**

Time (hrs)	Average Positive Control, CFU (SD)	Average Water Control, CFU (SD)	Average Dry Control, CFU (SD)	Average Post-MeI Exposure, CFU (SD)	Average Log Reduction From MeI
0.5	$2.28 \times 10^6$ ( $\pm 8.13 \times 10^4$ )	$2.11 \times 10^6$ ( $\pm 2.40 \times 10^5$ )	$2.30 \times 10^6$ ( $\pm 1.06 \times 10^5$ )	$7.75 \times 10^0$ ( $\pm 5.91 \times 10^0$ )	5.59
1	$2.28 \times 10^6$ ( $\pm 8.13 \times 10^4$ )	$2.25 \times 10^6$ ( $\pm 3.89 \times 10^4$ )	$2.28 \times 10^6$ ( $\pm 1.98 \times 10^5$ )	$2.50 \times 10^{-1}$ ( $\pm 5.00 \times 10^{-1}$ )	6.66
2	$2.74 \times 10^6$ ( $\pm 0$ )	$1.85 \times 10^6$ ( $\pm 2.65 \times 10^5$ )	$2.43 \times 10^6$ ( $\pm 3.18 \times 10^4$ )	$2.50 \times 10^{-1}$ ( $\pm 5.00 \times 10^{-1}$ )	6.74
4	$2.89 \times 10^6$ ( $\pm 8.49 \times 10^4$ )	$2.85 \times 10^5$ ( $\pm 1.11 \times 10^5$ )	$1.28 \times 10^6$ ( $\pm 2.09 \times 10^5$ )	0 ( $\pm 0$ )	6.46
8	$2.38 \times 10^6$ ( $\pm 2.79 \times 10^5$ )	$1.39 \times 10^6$ ( $\pm 1.41 \times 10^4$ )	$1.40 \times 10^6$ ( $\pm 2.47 \times 10^5$ )	0 ( $\pm 0$ )	6.38
16	$2.08 \times 10^6$ ( $\pm 1.17 \times 10^5$ )	$6.88 \times 10^5$ ( $\pm 1.17 \times 10^5$ )	$2.47 \times 10^6$ ( $\pm 7.42 \times 10^4$ )	0 ( $\pm 0$ )	6.32
24	$1.99 \times 10^6$ ( $\pm 1.06 \times 10^4$ )	$3.56 \times 10^5$ ( $\pm 1.05 \times 10^5$ )	N/D <sup>a</sup>	0 ( $\pm 0$ )	6.30

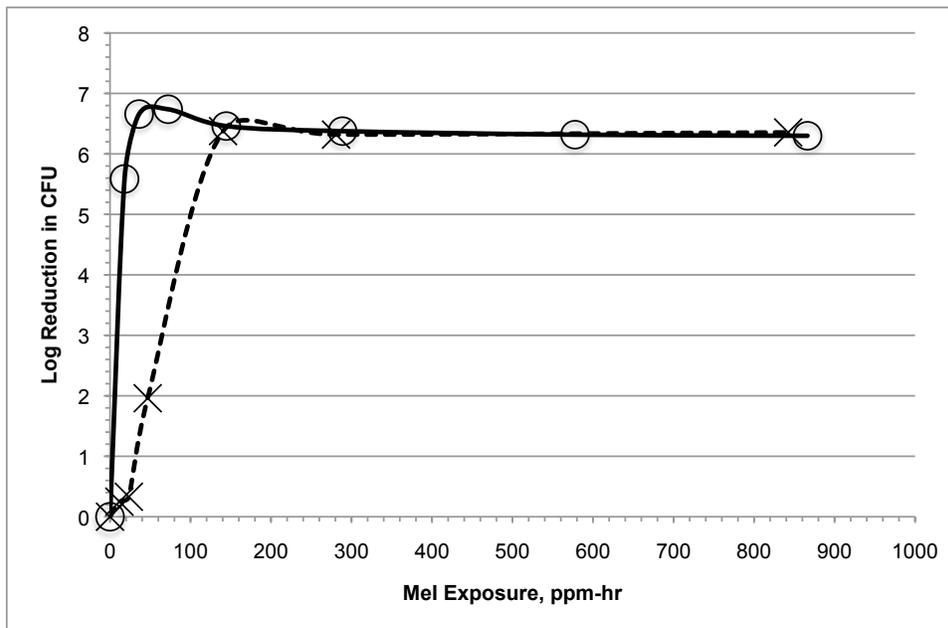
<sup>a</sup>N/D = not determined

**Figure 1: Theoretical Gas-Phase Concentration of Methyl Iodide in a 48-mL Tube Containing 20 mL Liquid at Temperatures From 0°C to 55°C**



Footnote: ppmv = parts per million by volume.

**Figure 2: Efficacy of Methyl Iodide (MeI) Gas-Phase Exposure on *B. anthracis* Sterne Strain Spores ( $3 \times 10^6$ ) at Room Temperature ( $\sim 20^\circ\text{C}$ , -X-) and Elevated Temperature ( $55^\circ\text{C}$ , -O-)**



Footnote: ppm-hr = parts per million per hour.