Dean T. Tompkins, PhD, PE
Professional Engineer
2843 Charleston Dr.
Madison, WI 53711

Terry Kurzynski, MS
Advanced Microbiologist
5610 Sandpiper Lane
Madison, WI 53716

August 14, 2002

John Hayman
President
KES Science and Technology
3625 Kennesaw North Ind. Pkwy.
Kennesaw, GA 30144
(800) 627-4913

RE: Performance of AiroCide in Controlling Bacterial Spores
(June 2002 Test Period)

Dear President Hayman,

Please find below the results from testing the AiroCide device (KES Science and Technology; Kennesaw, GA) as a technology for controlling air-borne concentrations of bacterial spores. These tests were conducted in June of 2002. Several figures are found in the Appendix.

Experimental Methods

The Experiment. The AiroCide device was challenged by introducing a commercial preparation of *Bacillus thuringiensis* (*B. thuringiensis*) spores (Thuricide, American Brand, Thermo Trilogy) directly into the intake of the AiroCide. [NOTE: In lieu of testing a virulent spore form of *Bacillus anthracis* (the anthrax spore), tests were conducted with a non-virulent form of bacillus – *B. thuringiensis* – which is a spore-forming bacillus that is very similar to *B. anthracis*. See Figure 1.] A suspension of *B. thuringiensis* was obtained by dilution with purified (Milli-Q) water. A spore suspension was placed in the receptacle of a Collison™ nebulizer. The nebulizer was connected to a compressed air cylinder, which provided a feed stream of air at 20 pounds per square inch (psi). In this manner, the nebulizer generates a fine particle aerosol (1-5 μm). Prior to introducing spores into the AiroCide, the device (internal fan and light sources) was energized for about 45 min to achieve steady-state (SS) conditions within the unit. [NOTE: The exhaust air from the unit during SS operation measured a constant 55°C.] Once steady-state was reached, the nebulizer was activated to generate an aerosol which was fed directly into the intake of the AiroCide (Figure 2). To allow for direct feed of the aerosol into the AiroCide, the device was placed upright on its side (Figure 3). The time marked by the activation of the nebulizer was the experimental start. Also at the start of the experiment, two large (14-cm diameter) Petri™ plates containing 70 ml of Trypticase Soy Sheep's blood agar were placed side-by-side at the AiroCide exit to completely cover the device's exhaust grille (Figure 4). In so doing, spores exiting the AiroCide will

Dean T. Tompkins, PhD, PE
Professional Engineer
2843 Charleston Dr.
Madison, WI 53711

Terry Kurzynski, MS
Advanced Microbiologist
5610 Sandpiper Lane
Madison, WI 53716

impact the culture plates and be captured for subsequent growth-plate study. After 10 min of aerosolization, the nebulizer was deactivated, effectively terminating the introduction of spores into the AiroCide device. Then, over the next 60 min, twelve additional blood-agar plates (two every 10-min interval), were placed at the device's outlet as described previously to capture spores exiting the AiroCide. After this hour-long, post-nebulization sampling period, the AiroCide device was deactivated and the experiment ended.

Post Experiment Spore Analysis. Because of the experimental design and the AiroCide configuration, spore distribution within the AiroCide is limited to three locations (Figure 5): 1) spores attach to the surfaces of the fan and ante-chamber located upstream of the AiroCide *reactor zone*, 2) spores are captured, immobilized, and are either active or rendered inactive in the *reactor zone*, or 3) spores exit the AiroCide via the exhaust grille. The approach to determine the colony forming units (CFUs) surviving the experiment for these three locations are as follows, with the procedure to determine the number of spores introduced into the AiroCide discussed first:

Inoculum size) The volume of spores nebulized and introduced into the AiroCide is based on mass analysis of the pre- and post-weights of the nebulizer when containing the spore suspension.

Location #1) After the experiment, the fan and ante-chamber, located upstream of the AiroCide's reactor zone were mechanically removed from the AiroCide. All surfaces to which the spores can contact were sampled for spores by thoroughly swabbing with water-moistened, sterile gauze pads and swabs. The cotton pads and swabs were then placed in a sterile jar containing 100 ml of water and vigorously agitated for 1 min. Duplicate, 100-µl samples of the suspension were plated, incubated overnight at 35°C, and CFUs were counted the following day.

Location #2) The reactor zone consists of 52 glass sleeves and catalyst-coated rings and for the purpose of this study was divided into 3 nearly equally sized zones – Zone 1, Zone 2 and Zone 3, with Zone 1 closest to the fan and ante-chamber. After testing the contents of each zone were removed from the AiroCide and kept separated. Spores attached to the sleeves, chamber walls, sides, top, bottom, and entrance, support and exit grilles were sampled with water-moistened, sterile gauze pads. The rings and sampling pads were placed in a sterile, macerating unit (Osterizer) containing 600-ml sterile saline. The mixer was energized for 5 min, pulverizing the rings and thoroughly mixing the contents. Samples of the solution were plated, incubated overnight at 35°C, and CFUs were counted the following day. All three zones were sampled independently in this manner.

Dean T. Tompkins, PhD, PE
Professional Engineer
2843 Charleston Dr.
Madison, WI 53711

Terry Kurzynski, MS
Advanced Microbiologist
5610 Sandpiper Lane
Madison, WI 53716

Location #3) After each 10-min sampling period, culture plates were covered and labeled. A total of 14 culture plates were incubated overnight at 35°C. The following day, the CFUs of *B. thuringiensis* were counted and recorded for each culture plate.

Experimental Results

A total of 71,750 *B. thuringiensis* spores were introduced into the AiroCide (Fig. 6). The fan was found to contain 10,400 CFUs, or 14.49% of the original inoculum. Likewise, the ante-chamber was found to contain 485 CFUs, or 0.676% of the original inoculum. Therefore, a total of 60,865 spores entered the reactor zone. Of this inoculum, a total of 5 CFUs exited the device and were collected on the surface of the seven sets of blood agar plates over the 70-min sampling period. Zones 1, 2 and 3 had 485, 291, and 0 CFUs, respectively, remaining after treatment.

Terry A. Kurzynski, MS
Advanced Microbiologist
Date: August 14, 2002

Dean T. Tompkins, PhD,
Professional Engineer
Date: August 14, 2002

Dean T. Tompkins, PhD, PE
Professional Engineer
2843 Charleston Dr.
Madison, WI 53711

Terry Kurzynski, MS
Advanced Microbiologist
5610 Sandpiper Lane
Madison, WI 53716

Appendix

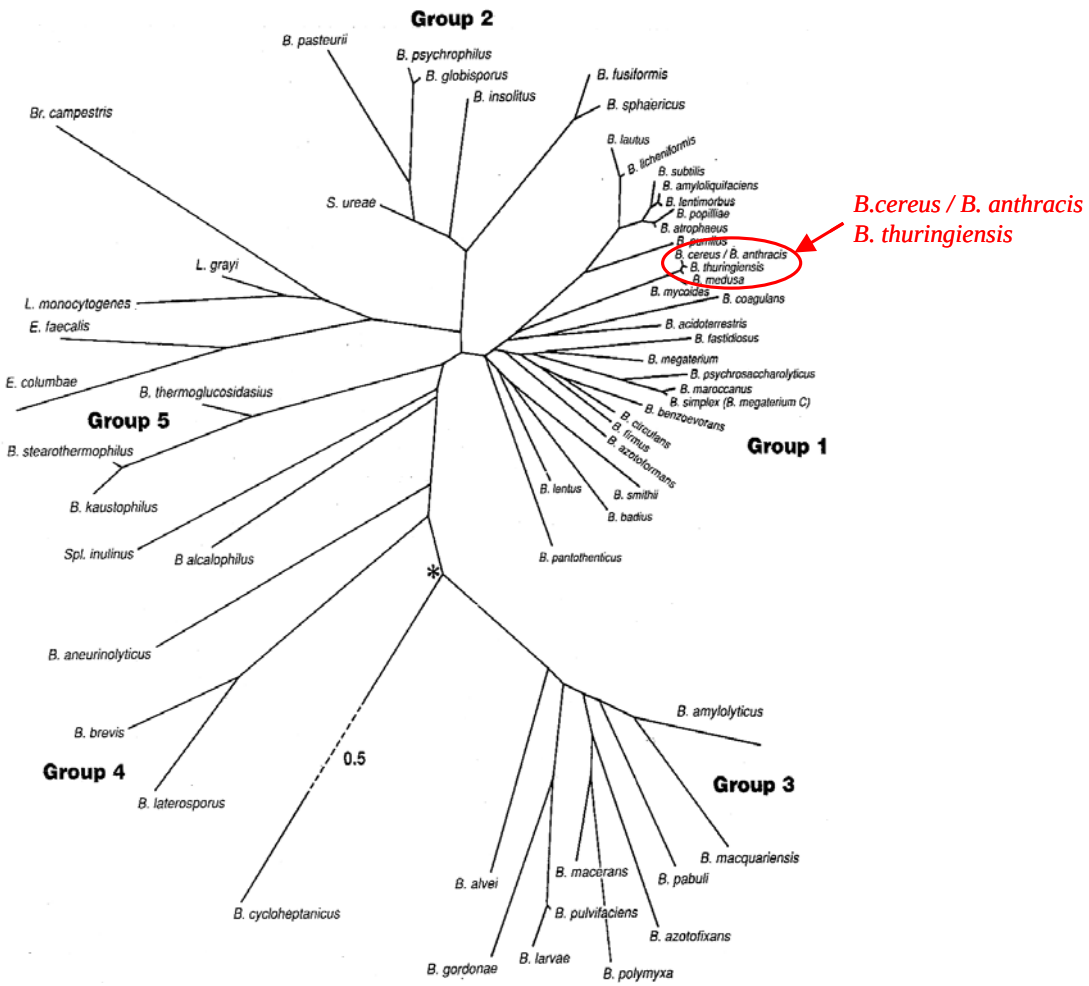

Figure 1 – Phylogenic tree of genus *Bacillus.* Note group similarity between *B. anthracis and B. thuringiensis*.

Dean T. Tompkins, PhD, PE
Professional Engineer
2843 Charleston Dr.
Madison, WI 53711

Terry Kurzynski, MS
Advanced Microbiologist
5610 Sandpiper Lane
Madison, WI 53716

Figure 2 – Suspension of *B. thuringiensis* spores in nebulizer and introduced into the AiroCide.

Figure 3 – Experimental set-up. Air from a cylinder aerosolizes suspension of *B. thuringiensis* spores.

Dean T. Tompkins, PhD, PE
Professional Engineer
2843 Charleston Dr.
Madison, WI 53711

Terry Kurzynski, MS
Advanced Microbiologist
5610 Sandpiper Lane
Madison, WI 53716

Figure 4 – Experimental set-up. Petri dishes at exit of AiroCide;
in position to capture spores in effluent from the AiroCide.

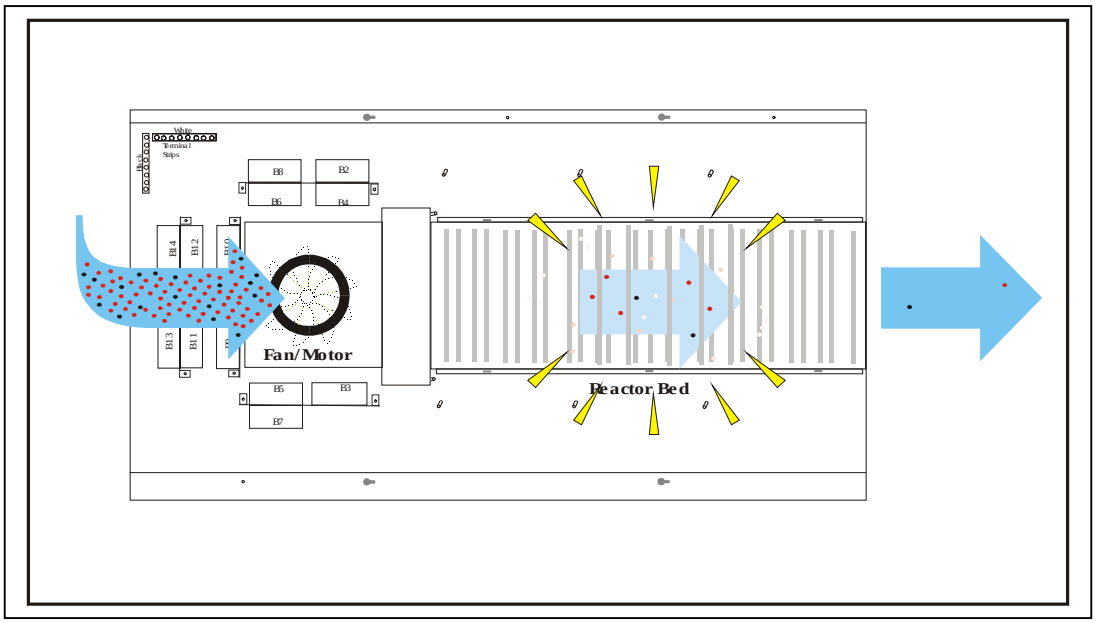

Figure 5 – AiroCide Operation: Spore-filled air enters through the fan and is pressurized into the reactor bed. Spores are immobilized onto the photocatalyst surfaces (and other interior surfaces), where they are exposed to surface-bound radicals (e.g., hydroxyl radical – OH·) and ultraviolet germicidal irradiation (UVGI).

.

Dean T. Tompkins, PhD, PE
Professional Engineer
2843 Charleston Dr.
Madison, WI 53711

Terry Kurzynski, MS
Advanced Microbiologist
5610 Sandpiper Lane
Madison, WI 53716

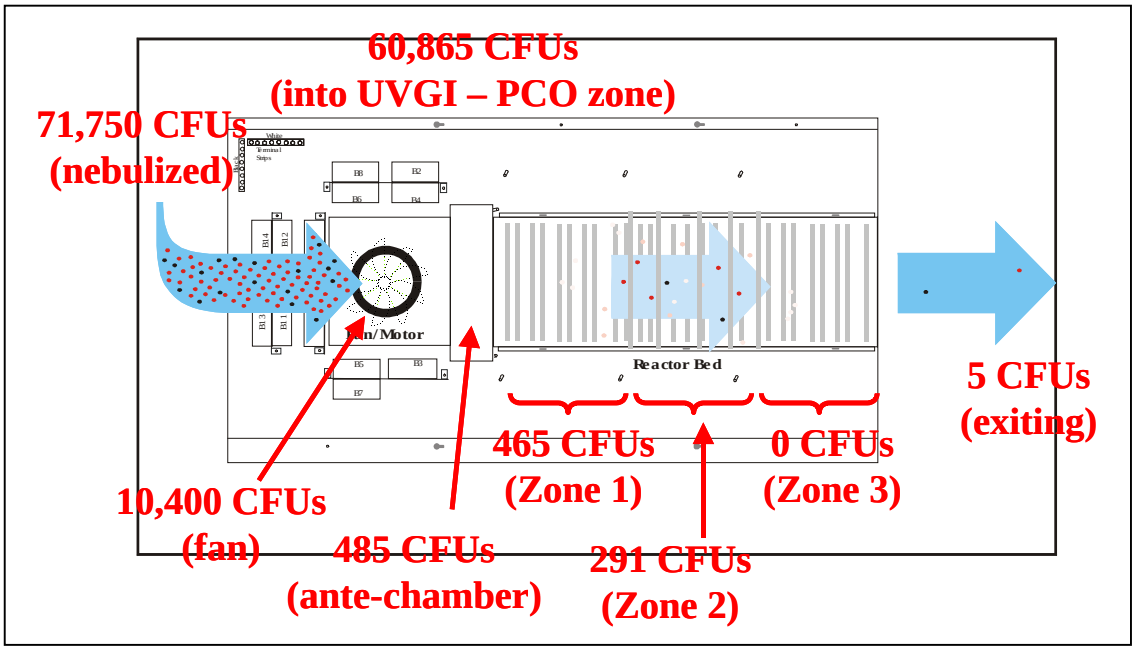

Figure 6 – Summary of testing results overlaid on the AiroCide Zones 1, 2, and 3 had 465, 291, and 0 CFUs, respectively, during 70 min of operation.