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EXECUTIVE SUMMARY

The Medical Waste Tracking Act of 1988 required the Environmental Protection Agency to identify alternative (or non-regulatory) approaches to the management of medical waste. Until now, scientific evaluation of medical waste treatment systems potential for biological emissions had not been done. This report presents the results of investigation of potential bioemissions from three medical waste treatment technologies: steam autoclaving, microwave, and mechanical/chemical processes. The investigation consisted of a sequential approach which included: 1) a literature review; 2) a laboratory evaluation of selected indicator microorganisms; and 3) an assessment of bioemission potential for the three technologies at four sites, using indicator microorganisms and air and fluid sampling and analysis, during the treatment of regulated medical waste.

The literature review was conducted to summarize available information, knowledge gaps, and research needs regarding bioemission test organisms, monitoring methods, and various medical waste treatment technologies. It showed that the preferred indicator microorganisms were the spores of *Bacillus stearothermophilus* (BST) and *Bacillus subtilis* var. *niger* (BSN). BST and BSN spores are standard sterilization indicators for moist and dry heat inactivation respectively, and are also intrinsically resistant to chemical inactivation. BST is not commonly found in medical waste, and can be isolated selectively due to its thermophilic growth property. BSN produces a distinctive orange pigmentation which can help differentiate it from other *Bacillus* species. The review also showed that all-glass impingers, sieve impactors, and slit-to-agar samplers are recognized as commonly used bioaerosol samplers that have been used successfully to monitor airborne microorganisms from a variety of processes, to include medical waste treatment. Additionally, such samplers are required for such procedures as the ASTM *Standard Practice for Sampling Airborne Microorganisms at Municipal Solid-Waste Processing Facilities*, and the National Sanitation Foundation's Standard Number 49 on Class II Biohazard Cabinetry.

Prior to field monitoring for potential bioemissions during medical waste treatment, laboratory investigations were carried out to determine: 1) that each of the indicator microorganisms could be effectively recovered from the same sample mixture; 2) that spore viability could be preserved during sampler operations, and; 3) that the loss of collected spores could be minimized during sampling. Typical BST colonies are cream-colored and grow at 55°C, while BSN colonies are pigmented orange and will not grow at elevated temperatures, but will grow at 37°C. Pure and mixed cultures of

BST and BSN spores were plated onto five different candidate agar media and incubated five days at 37°C (BSN) and 55°C (BST). Resultant colony counts for both spore types were greatest on Trypticase Soy Agar (TSA). No interference was observed in mixed cultures when plates were incubated at selective temperatures for each organism. BSN colonies were shown to produce black pigmentation on Tyrosine agar after 72 hours, which can be used to confirm identification of BSN from samples. Survival of non-injured spores during operation of AGI-30 standard impingers was assessed for one hour periods. After 20 minutes, mean BSN survival was 26%, and declined progressively. Mean BST survival was 89% at 20 minutes, and declined progressively. Heating spores to 100°C for 20 minutes to simulate medical waste treatment by thermal inactivation destroyed BSN viability, but 81% of exposed BST spores survived for 20 minutes in operating impingers. Losses, due to spore slippage from the AGI-30 impingers, were evaluated and shown to be minimal (0.2% for BSN, and 0.3% for BST). These laboratory studies showed the suitability of *Bacillus stearothermophilus* and *Bacillus subtilis* var. *niger* spores for use in sampling for emissions from medical waste treatment systems, identified appropriate recovery and confirmation media, and showed that impingers could effectively recover the microorganisms, although operation should not exceed 20 minutes.

The primary objective of this research was to assess the bioemission potential of selected alternative medical waste treatment technologies as they processed both spiked and non-spiked regulated medical waste. Spiked waste was seeded with large numbers of intrinsically chemical and heat resistant BST and BSN endospores in suspension, or dried onto filters to mimic spilled, dried organisms on the surface of waste materials. The monitoring of each treatment system focused on demonstrating the presence or absence of the indicator spores from previously identified exhaust air and fluid emission points during spiked waste treatment, as compared with indicator spore data from ambient and non-spiked waste treatment conditions. Bioaerosol monitoring was conducted using samplers required or recommended in published standards and guidelines, primarily the American Society for Testing Materials (ASTM) *Standard Practice for Sampling Airborne Microorganisms at Municipal Solid-Waste Processing Facilities* (E884-82).

Steam autoclave treatment was evaluated for bioemissions using challenges of indicator spores dried onto multiple membrane filters contained in open petri dishes secured to the waste loads. Minimum spore challenges for the on-site hospital laboratory medical waste treatment gravity displacement autoclaves were 5.3×10^8 and 1.1×10^8 for BST and BSN respectively; while challenges for the large commercial

off-site facility's vacuum autoclave were at least 2.0 x 10^9 BST spores and 7.4 x 10^8 BSN spores. Sampling was conducted from air and fluids exhausted from the chamber before, during, and after treatment conditions, as well as from the plume exiting the chamber as the door was opened. For both steam autoclave systems during spiked medical waste treatment, no indicator organisms were recovered above background levels from all sampling/emission points. In particular, none were recovered from the initial exhausting and pressurization of the chambers, which was the phase of operation considered to provide the greatest potential for bioemissions.

Microwave treatment was evaluated for bioemissions with challenges of indicator spores in sealed glass vials used to seed four consecutive waste batches. Total spore challenges for 40 vials of each indicator organism for the four waste batches was at least 1.9 x 10^9 for the microwave resistant BST spores, and 8.8 x 10^8 for BSN spores. Sampling was done at four potential emission points: 1) the top of the grinding chamber as the door opens to receive a batch of waste; 2) the access port to the untreated waste transport auger; 3) the roof vent for exhaust of HEPA filtered air from the grinding chamber; and 4) the end of the waste exit tube. Recovery of both indicator spores, above background levels, from the untreated waste access port and the waste exit tube showed the potential for bioemissions from the treatment system.

Mechanical/chemical treatment was evaluated for bioemissions with challenges of 4.3 x 10^9 BST spores and 2.2 x 10^9 BSN spores distributed throughout 24 bags of regulated medical waste. Sampling was done at four potential emission points: 1) the waste conveyor; 2) the drum screen/conveyor interface; 3) HEPA exhaust air filter; and 4) the sewer drain at the sedimentation tank. Both types of spores were recovered from impinger samples collected at the drum screen/conveyor interface, which is not enclosed. No indicator spores were recovered from this sampling point during non-spiked waste processing. BSN spores were also recovered from air above the waste conveyor at the start of the treatment process, and BST spores were recovered from air from the HEPA filtered exhaust. Some of both spore types were recovered from fluid collected in the sedimentation tank. Which showed potential for bioemissions.

In summary, this study has provided new information relative to the assessment of potential bioemissions from alternative medical waste treatment technologies. It has identified and evaluated suitable indicator microorganisms, bioaerosol samplers, and recovery techniques; and has shown that bioemissions can be generated during access to untreated waste destruction, and from non-enclosed phases of waste treatment.