1.0 INTRODUCTION

Medical waste treatment systems have yet to be effectively evaluated to assess their potential for biological emissions and possible associated public health risks. Environmental risks include the possibility of a release of waste components or residues to groundwater, surface water, or air. The potential for emitting infectious microbiological components of medical waste is of concern, particularly for medical waste handlers and treatment operators. A literature review was thus conducted regarding biological emissions from various medical waste treatment processes.

2.0 BACKGROUND

The Medical Waste Tracking Act of 1988 required the U.S. Environmental Protection Agency (EPA) to identify alternative (or non-regulatory) approaches to the management of medical waste. Of major concern, has been the effectiveness of medical waste treatment by alternative technologies, with little attention to the occupational and environmental impacts from potential biological, chemical, and particulate emissions.

3.0 OBJECTIVE

At the present time, there is little information available regarding bioemissions from regulatory or alternative medical waste treatment technologies. The objective of this report is to summarize available information regarding bioemission test organisms and monitoring methods for various medical waste treatment systems. Such systems include incineration, and alternative destructive and/or non-destructive processes such as steam autoclaving, mechanical/chemical, and microwave treatment. The report focuses on specific bioemissions data and sampling methodologies, and includes a summary of the various microorganisms recovered from spiked (with indicator organisms) and non-spiked medical waste processes.

4.0 INCINERATION

4.1 Indicator Microorganisms

Bacillus stearothermophilus spores [1]
Bacillus spp, staphylococci, *Staphylococcus aureus*, *Pseudomonas fluorescens* [2]
Bacillus subtilis [3]
Bacillus subtilis var. *niger* spores [4,7,8]
Salmonella [5]
Total bacteria, total coliforms, fecal coliforms [6]
Serratia marcescens [7,8]

Segall et al, developed methods to use *Bacillus stearothermophilus* spores as indicator microorganisms in incinerator air emissions and ash residue. Spores were shown to survive exposure to incinerator stack air emissions and exposure to ash residue. The spores can also be effectively recovered from residue test pipes spiked with spores and placed in the incinerator. [1] Blenkharn and Oakland showed that gram positive *Bacillus* spp., coagulase negative staphylococci and *Staphylococcus aureus*, and some gram negative *Pseudomonas fluorescens* and other pseudomonads, could be recovered from the exhaust gases of an oil-fired hospital waste incinerator treating ordinary non-spiked waste under normal operations. [2] Allen et al, burned waste spiked with *Bacillus subtilis* in a hospital incinerator and recovered other non-spiked bacteria upon sampling the exhaust gases. Their results suggested that the source of the recovered bacteria was the combustion make-up air. [3]

A semi-portable oil or gas-fired incinerator was challenged with *Bacillus subtilis* var. *niger* spores to determine the minimum operating temperatures needed to prevent the release of spores to the atmosphere when they are present in either solid or liquid refuse. [4]

Field studies at four municipal incinerators of different operational design determined that enteric pathogens can survive the incineration process when present in non-spiked solid waste. [5] Solid waste samples were taken from four different incinerators, before and after treatment, and were tested for total bacteria, total coliforms, fecal coliforms, and heat resistant spore formers. Of the four incinerators tested, only one produced residue with no fecal coliforms, with the other three producing between 2 to 2,400 colony forming units (CFU) per gram of waste. [6] Two, semi-portable, metal air incinerators were tested with aerosols of liquid and dried suspensions of *Bacillus subtilis* var. *niger* spores and dried vegetative cells of *Serratia marcescens* to determine the minimum operating conditions to sterilize contaminated air. [7]

Barbieto and Gremillion tested an industrial waste incinerator with *Bacillus subtilis* var. *niger* spores and *Serratia marcescens.* [8] The vegetative *S. marcescens* was used to verify proper incinerator operating conditions before the *B. subtilis* var. *niger* spores were introduced. Liquid spore suspensions were aerosolized into the firebox and dry powdered spores were mixed with animal bedding were dumped into the firebox. The stack gases were monitored and sampled at various temperatures inside the firebox. *B. subtilis* var. *niger* spores were recovered as the temperature of the firebox was varied. Minimum temperatures and residence times were determined for the prevention of viable spore release.

4.2 Bioemissions Monitoring

Most of the air sampling conducted on waste incinerators involved the use of standard stack gas sampling equipment. From the cited papers, Segall et al, used a water cooled glass probe with impingers to recover *Bacillus stearothermophilus* spores[1], while Brenniman and Rosemary used aluminum tubing fitted to Shipe impingers to recover *Bacillus subtilis* and a variety of other *Bacillus* and *Staphylococcus* bacteria. [3] Others used instruments such as Hurricane samplers (Gelman Model 16003), [4,7,8] modified exhaust blowers, [4,7] and a Staplex sampler (Model TFIA). [7] These devices basically pump measured volumes of stack gas through tared paper or glass fiber filters and can be operated either isokinetically or non-isokinetically within the stack gases. The filters were used to recover *Bacillus subtilis* var. *niger* spores, [4,7,8] and *Serratia marcescens.* [7] Blenkharn and Oakland used a somewhat novel approach to viable stack gas sampling by using a stainless steel hose connected to a Casella Slit impactor sampler which recovered *Bacillus spp.*, *Staphylococcus aureus*, other staphylococci, and *Pseudomonas fluorescens.* [2]

Solid waste was evaluated microbiologically in several studies by taking grab samples of the waste before and the ash after incineration, or solely of the ash after incineration. [1,2,5,6]

4.3 Incineration References

1. Segall, R.R., G.C. Blanchan, W.G. DeWees, K.M. Hendry, K.E. Leese, L.G. Williams, F. Curtis, R.T. Shigara, and L.J. Romesburg. 1991. Development and evaluation of a method to determine indicator microorganisms in air emissions and residue from medical waste incinerators. *Journal of Air and Waste Management Association*, 41:1454-1460.

2. Blenkharn, J.I. and D. Oakland. 1989. Emission of viable bacteria in the exhaust flue gases from a hospital incinerator. *Jour of Hospital Infection*, 14:73-78.

3. Allen, R.J., G.R. Brenniman, and R.L. Rosemary. 1989. Emission of airborne bacteria from a hospital incinerator. *Jour of Air Control Pollution Assoc*, 39:164-168.

4. Barbeito, M.S. and M. Shapiro. 1977. Microbiological safety evaluation of a solid and liquid pathological incinerator. *Jour of Medical Primatology*, 6:264-273.

5. Peterson, M.L. and A.J. Klee. 1971. Studies on the detection of salmonellae in municipal solid waste and incinerator residue. *Intern Jour of Environmental Studies*, 2:125-132.

6. Peterson, M.L. and F.J. Stutzenburger. 1969. Microbiological evaluation of incinerator operations. *Applied Microbiology*, (18)1:8-13.

7. Barbeito, M.S., L.A. Taylor, and R.W. Seiders. 1968. Microbiological evaluation of a large-volume air incinerator. *Applied Microbiology*, (16)3:490-495.

8. Barbeito, M.S. and G.G. Gremillion. 1968. Microbiological safety of an industrial refuse incinerator. *Applied Microbiology*, (16)2:291-295.

5.0 STEAM AUTOCLAVE TREATMENT

5.1 Indicator Microorganisms

Bacillus pumilus (North American Science Associates, Inc., Northwood, Ohio), *Vaccinia* virus strain WR. [3]

Bacillus stearothermophilus spores. [2,4,5,6,7,9]

Bacillus stearothermophilus spores NCA 1518. [1]

Bacillus subtilis var. *niger* spores, *Serratia marcescens*, and T1 coliphage. [8]

Bacillus stearothermophilus has been widely studied as an indicator organism for the efficacy testing of steam sterilization. Its spores are routinely used for confirming biological treatment in autoclaves. [1,2,4,6,7,9] Many types of commercially available indicators contain known numbers of *B. stearothermophilus* spores for the purpose of testing the efficacy of hospital and clinical autoclaves on a routine basis. Cole states that biological indicators (using spores of *B. stearothermophilus*) should be run with

actual loads on a daily or weekly basis depending on frequency of use. [4] In one study, Stinson et al, used *Bacillus pumilus* spore strips as a heat resistant surrogate of HIV in a sterilization study, [3] however, *Bacillus stearothermophilus* spore strips could have been used as an even more heat resistant challenge. Currently, there is little information regarding biological emissions from steam autoclaves. Biological emissions from steam treatment systems are primarily of concern when potentially contaminated medical waste is processed either in a hospital or other clinical setting or in large, offsite, medical waste autoclave treatment facilities.

5.2 Bioemissions Monitoring

Barbieto and Brookey addressed microbial emissions from the exhaust of two Ortho-Vac high vacuum sterilizers.[8] The larger of the two was first injected with aerosols of *Bacillus subtilis* var. *niger* spores, *Serratia marcescens*, *Escherichia coli* B containing T1 bacteriophage, and spores of *B. subtilis* var. *niger* dried on bulk discard materials, then evacuated before the pressurized steam was applied. Four all-glass impingers (AGI-30s) were used to sample the exhaust air for the indicator organisms prior to the pressurized steam cycle. In addition, eight sieve samplers (similar to Andersen samplers with agar plates) were positioned near the exhaust vent. Vegetative cells of *S. marcescens* were recovered when a test concentration of 1.3×10^9 organisms/ft^3 space was used but not when a concentration of $1.3 \times 10^7/ft^3$ was used. Spores of *B. subtilis* were recovered (1.3×10^1 to $1.3 \times 10^7/ft^3$) over atmospheric concentrations. T1 coliphage was recovered immediately above the atmospheric vent pipe when test concentrations of 3.6×10^7 and $3.6 \times 10^9/ft^3$ were used. Sampling at intervals with the AGI-30s indicated that test organisms were released throughout the evacuation cycle. All control samples were negative for *S. marcescens* and T1 coliphage. Low background concentrations of *B. subtilis* were found in a small percentage of the samples.

The test materials for the smaller autoclave were contaminated with either suspended or dried *B. subtilis* spores or *S. marcescens* cells. The pre-steam vacuum exhaust was sampled for the test organisms by a modified exhaust blower equipped with a funnel-shaped 8 x 10 inch filter holder extended over the atmospheric vent pipe. Before each test cycle, AGI-30 and sieve sampler controls were taken at the sterilizer atmospheric vent to estimate background contamination. Dried *B. subtilis* spores were shown to be aerosolized by evacuation cycle, but none were recovered when loaded as spores or vegetative cells in liquid suspension or dry powder.

5.3 Steam Autoclave References

1. Block, S.S. 1991. *Disinfection, Sterilization, and Preservation*, 4th Edition, Lea and Febiger, Philadelphia, PA.

2. Rayburn, S.R. 1990. *The Foundations of Laboratory Safety*. Springer-Verlag Inc., New York, NY.

3. Stinson, M.C., M.S. Galanek, A.M. Ducatman, F.X. Masse, and D.R. Kuritzkes. 1990. Model for inactivation and disposal of infectious human immunodeficiency virus and radioactive waste in a BL3 facility. *Applied and Environmental Microbiology*, (56)1:264-268.

4. Cole, E.C. 1987. The Application of Disinfection and Sterilization to Infectious Waste Management. In: Proceedings of Strategies for Improved Chemical and Biological Waste Management for Hospitals and Clinical Laboratories. N.C. Board of Science and Technology and Duke University.

5. Gardner J.G. and M.M. Peel. 1986. *Introduction to Sterilization and Disinfection.*

6. Andrew, M.H.E. and A.D. Russel, eds. 1984. *The Revival of Injured Microbes.* Academic Press, Inc., Orlando, FL.

7. W.A. Rutala, et al. 1982. Decontamination of laboratory microbiological waste by steam sterilization. *Applied and Environmental Microbiology*, 43:1311-1316.

8. Barbieto M.S. and E.A. Brookey, Jr. 1976. Microbiological hazard from the exhaust of a high-vacuum sterilizer. *Applied and Environmental Microbiology*, (32)5:671-678.

9. Mayernik, J.J. 1972. Biological Indicators for Steam Sterilization. A U.S.P. Collaborative Study; *Bull. Parent. Drug Association*, 26:205-211.

6.0 MICROWAVE TREATMENT

6.1 Indicator Microorganisms

Bacillus subtilis ATCC 6633, *Enterobacter cloacae* ATCC 23355, *Klebsiella pneumoniae* ATCC 23357, *Serratia marcescens* ATCC 8100, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Proteus mirabilis* (clinical isolate), *Enterococcus* (Clinical isolate), and alpha streptococcus, Group D. [1]

Streptococcus faecalis, Saccharomyces cerevisiae [2]

Escherichia coli, Bacillus subtilis var. *niger* [3]

6.2 Treatment Testing Results

Latimer and Matsen in conducting treatment effectiveness testing in a 2450 MHz microwave oven showed that 5 ml broth cultures of clinical isolates of *E. coli, P. mirabilis, P. vulgaris, P. aeruginosa, S. marcescens, S. aureus, S. epidermidis,* and enterococcus were killed by a 5 minute exposure to microwave irradiation. [1] Also, *B. stearothermophilus* spore strips were sterilized after 5 minutes of microwave exposure and the spore strip kill was correlated with the sterilization of approximately 100 petri dish plates contaminated with the above listed common clinical isolates. Microwave sterilization was attributed to the heat generated by the dielectric properties of water in the test materials and was recommended for contaminated media and test tubes, but not for dry cloth, paper, glass, or newly prepared media.

Lechowich et al, showed that the continuous application of microwaves to suspensions of 10^8 to 10^9 ml *Streptococcus faecalis* or *Saccharomyces cerevisiae*, appeared to produce no lethal effects other than those produced by heat. [2] Similarly, Goldblith and Wang concluded that when suspensions of *E. coli* and *B. subtilis* were exposed to microwaves, that a 6 $\log_{10}$ cycle reduction in viability for *E. coli* and the degree of inactivation for *B. subtilis* was identical to that of conventional heating with no "other intrinsic" effects of the microwaves. [3]

6.3 Bioemissions

Latimer and Matsen indicated the need for good ventilation in the room where decontamination is to be carried out due to the odors created by the action of microwaves on bacteria. [1] However, no mention is made of the potential for microbial

aerosol emissions, or of the need for emissions monitoring. No other literature citations relevant to bioaerosol emissions from microwave treatment of medical waste were identified.

6.4 Microwave References

1. Latimer J.M. and J.M Matsen. 1977. Microwave oven irradiation as a method for bacterial decontamination in a clinical microbiology laboratory. *Journal of Clinical Microbiology*, (6)4:340-342.

2. Lechowich R.V., L.R. Beauchat, K.I. Fox, and F.H. Webster. 1969. Procedure for evaluating the effects of 2,450-megahertz microwaves upon *Streptococcus faecalis* and *Saccharomyces cerevisiae*. Applied Microbiology, (17)1:106-110.

3. Goldblith, S.A., and D.I.C. Wang. 1967. Effect of microwaves on *Escherichia coli* and *Bacillus subtilis*. Applied Microbiology, (15)6:1371-1375.

7.0 MECHANICAL/CHEMICAL TREATMENT

7.1 Indicator Microorganisms

Bacillus subtilis var. *niger* (ATCC 6633), *Candida albicans* (ATCC 10231), *Enterococcus faecalis* (ATCC 29212), *Mycobacterium fortuitum* (ATCC 6841), *Serratia marcescens* LSPQ 3028 (pigmented strain), and bacteriophages ΦX174 and f2. [1]

Bacillus subtilis (clinical isolate), *Enterococcus faecalis* (ATCC 29212), *Mycobacterium fortuitum* (ATCC 3571), *Serratia marcescens* (pigmented clinical isolate), *Acinetobacter anitratus* (ATCC 33498), *Aspergillus sp.* (clinical isolate), *Candida albicans* (clinical isolate), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella sp.* (clinical isolate), and *Staphylococcus aureus* (ATCC 29213). [2]

7.2 Monitoring Results

Medical SafeTEC Inc. (Indianapolis, Indiana) has developed a medical waste treatment system in which waste is ground and shredded in a hammermill while mixed with high concentration sodium hypochlorite. Liquid effluent is disposed of in the sanitary sewer. The company makes three models: the Z-5000, the Z-5000HC, and

the Z-12500. Denys tested the Z-12500 model for treatment efficacy with *Serratia marcescens*, *Enterococcus faecalis*, *Bacillus subtilis*, and *Mycobacterium fortuitum*. [2] In the same study, it was evaluated for microbial aerosol emissions with a clinical isolate of *Serratia marcescens*. For bioemissions monitoring, AGI-30 impingers were placed 1-2 ft around the periphery of the unit, with one placed directly in contact with the exhaust air. In addition, two Mattson-Garvin volumetric slit-to-agar (1 CFM flow rate) samplers were placed on either side of the hammermill. A series of gravity settle plates were also used during system operation. Surfaces were monitored with sterile swabs moistened with 1% sodium thiosulfate. No aerosols of *S. marcescens* were identified during the disposal process in the absence of sodium hypochlorite. An average of 6-8 colonies of *S. marcescens* were recovered on settle plates inside the HEPA filter cabinet. All microorganisms recovered by the AGI-30s and Mattson-Garvins were either gram positive bacteria, or fungi ubiquitous to air. *S. marcescens* was recovered from surfaces that were saturated with water: hopper autoremover/ separator door gaskets, feed belt and feed drape, in the absence of sodium hypochlorite. Repeat cultures after hypochlorite treatment yielded rare gram positive spore forming bacilli or cocci (possible contaminants).

In another study by Jetté and Lapierre, the Medical SafeTEC Z-5000 was evaluated for treatment effectiveness with *B. subtilis*, *Enterococcus faecalis*, *Candida albicans*, *Serratia marcescens*, and for most tests, with *Mycobacterium fortuitum* and bacteriophages ΦX174 and f2. Bioaerosol air sampling was conducted around the unit with one Andersen air sampler located 1m in front of the conveyor belt, and with exposed gravity settle plates located in 43 places around the unit. Air sampling was conducted three separate times for each run, 60 minutes before testing, during testing, and 60 minutes after testing. The primary indicator organism for the bioaerosol sampling was a pigmented strain of *S. marcescens*. Additional surface monitoring utilized contact plates taken in 44 places around the unit before and after test runs. No colonies of *S. marcescens* were detected by aerosol sampling when the unit was run with hypochlorite. When the unit was run three times with water only, *S. marcescens* was recovered from aerosol sampling from all three tests. The Andersen samplers detected no *S. marcescens* before or after testing, but all plates during testing were positive. Of 123 settle plates, 2 were positive for *S. marcescens* before testing, 117 were positive during testing, and 1 was positive after testing. Of 127 contact plates, none were positive before testing, and 62 were positive after testing. [1]