UNIVERSITY OF CINCINNATI

Date: July 19, 2005

I, HONGXIA WANG, hereby submit this work as part of the requirements for the degree of:

DOCTOR OF PHILOSOPHY

In:
ENVIRONMENTAL HEALTH

It is entitled:

AEROSOLIZATION OF FINE PARTICLES AND MICROBIAL CONTAMINANTS FROM METALWORKING FLUIDS CONTAMINATED WITH MICROORGANISMS

This work and its defense approved by:

Chair: Tiina Reponen
Sergey Grinshpun
Andrew Freeman
Eugene White
Jagjit Yadav
AEROSOLIZATION OF FINE PARTICLES AND MICROBIAL CONTAMINANTS FROM METALWORKING FLUIDS CONTAMINATED WITH MICROORGANISMS

A dissertation submitted to the Division of Research and Advanced Studies Of the University of Cincinnati In partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
In the Department of Environmental Health Of the College of Medicine

2005

By Hongxia Wang
B.E. in Food Engineering, Ocean University of Qiangdao, China, 1994
M.S. in Food Science, Shanghai Fisheries University, China, 1997
M.S. in Environmental Health, University of Washington, USA, 2001

Committee Chair: Tiina Reponen, Ph.D.
ABSTRACT

In this study, the aerosolization of microorganisms from metalworking fluids (MWFs) was studied in laboratory conditions. Furthermore, the aerosolization of particles and endotoxin from MWFs was investigated under three conditions: 1. MWFs inoculated with microorganisms were aerosolized with a laboratory-scale set-up (MWF simulator) in the laboratory, 2. MWF collected in the field were aerosolized with the MWF simulator in the laboratory, 3. MWFs were aerosolized during routine field operations.

For the aerosolization of microorganisms from MWFs, the effects of fluid type, microbial concentration in the fluid, and the characteristics of the microorganisms (size and surface characteristics) were tested. Three microorganisms were employed to represent different size and surface characteristics: Bacillus subtilis bacterial endospores (hydrophobic particles with aerodynamic diameter = 0.9 µm), Pseudomonas fluorescens bacterial vegetative cells (hydrophilic, 0.8 µm) and Penicillium melinii fungal spores (hydrophobic, 3.1 µm). The testing was first performed using a Collison nebulizer to aerosolize microorganisms from three fluids: water, semi-synthetic MWF, and soluble oil. No significant difference in the aerosolization ratio (microbial concentration in the air divided by the microbial concentration in the liquid) was observed between the three fluids. For all tested microorganisms, the concentration in the air increased proportionally with increasing the microbial concentration in the liquid. The aerosolization ratio of B. subtilis endospores was greater than that of P. fluorescens cells and P. melinii spores. To explore the aerosolization of microorganisms from MWFs under the conditions that are closer to industrial settings, the tests were conducted with a MWF simulator (a laboratory-scale set-up simulating the mist generation during grinding
process). Simulator tests showed the same trend with respect to microbial aerosolization as those performed with the Collison nebulizer. As a result, the study showed that hydrophobic microorganisms were easier to aerosolize from MWFs than hydrophilic ones and that increasing microorganism size was likely to result in decreasing aerosolization ratio.

For the aerosolization of particles from MWFs, under condition 1 (MWFs inoculated with microorganisms were aerosolized with the MWF simulator in the laboratory), an optical particle counter (OPC), a condensation nucleus counter (CNC), an electrical low pressure impactor (ELPI), and a photometric aerosol mass monitor were used to measure the concentrations of airborne particles. For a semi-synthetic MWF, microbial contamination increased the number and mass concentrations of aerosolized particles by a factor of 2 (as measured by the OPC and the photometric aerosol mass monitor, respectively). At the same time, there was an up to 50 times increase in the concentration of aerosolized fine particles (0.02-1 µm), as measured by the CNC. For a soluble oil MWF, there was an up to 15 times increase in the concentration of aerosolized fine particles due to microbial contamination. The data collected with the ELPI showed that the peak of fine particle number concentration aerosolized from contaminated MWF (semi-synthetic) was at 0.37 µm. The endotoxin analysis showed that the particle size ranges representing fragments (0.37 µm) and intact cells (0.66 µm) contain about the same amount of endotoxin. The results indicate that MWF mist may contain high concentrations of microbial fragments.

For the experiments under conditions 2 (MWF collected in the field were aerosolized with the MWF simulator in the laboratory) and 3 (MWFs were aerosolized
during routine field operations), the airborne particle and endotoxin concentrations in different size fractions were measured using the ELPI. In addition, the culturable counts of microorganisms and total endotoxin concentrations in the air and in the MWFs were measured during the field sampling. The data of the mass and number concentrations of airborne particles was also recorded in the filed. The results show high concentrations of airborne fine particles in areas that were affected by MWFs. The particle concentrations decreased linearly with increasing particle size, except in the reference area. High endotoxin concentrations were measured at the particle sizes of \( \leq 0.39 \, \mu m \), which is below the size of intact bacterial cells (0.5 \( \mu m \)). This confirmed the finding under condition 1 (laboratory study with artificially contaminated MWFs) that the aerosolized fine particles contain microbial fragments. High airborne microbial (up to \( 3.1 \times 10^5 \) CFU/m\(^3\)) and endotoxin (up to \( 83.1 \pm 12.4 \) EU/m\(^3\)) concentrations were detected in the filed sit although the total particle mass was lower than the NIOSH recommended exposure limit (REL) for MWF mist (0.5 mg/m\(^3\)).

The results of study consistently showed both in laboratory and field conditions that the particles aerosolized from MWFs contaminated with microorganisms contain high concentrations of fine particles and endotoxin in submicrometer size range. The results show the importance of size-selective measurements in exposure assessment in MWF environments.
ACKNOWLEDGEMENTS

This dissertation is the result of four years of work whereby I have been accompanied and supported by many people. It is a pleasant aspect that I have now the opportunity to express my gratitude for all of them.

I would like to express my deepest gratitude to my advisor, Dr. Tiina Reponen for her expert guidance and mentorship, for her encouragement and support at all levels. She inspired me not only with her scientific knowledge but also with her passion for the scientific research, her genuine respect to every one around her. It is such a pleasure work for her.

I would specially thank Dr. Sergey Grinshpun, for his valuable advices, help and guidance in each step of my study. His scientific knowledge always pulled me to the right direction when I dropped into a myth.

I would like to thank Dr. Klaus Willeke, for his warm welcome when I first entered this laboratory, his advice on the buildup of the experimental setup, and his Monday lunch stories.

Many thanks to my other committee members: Dr. Andrew Freeman (University of Cincinnati), Dr. Jagjit, Yadav (University of Cincinnati), and Dr. Eugene White (Cimcool, Inc.) for their constructive suggestions and critics for my research.

I would like thank Mr. Jerry Byers for providing the metalworking fluid samples and commenting on part of the dissertation.

I wish to thank all the members in the Environmental and Occupational Health program, for their help and support during my graduate study in University of Cincinnati.
I would like to express my special appreciation to all the team members in the Centers for Health Related Aerosol Studies for their constant support in my work and daily life. I thank Dr. Atin Adhikari, Dr. Shu-An Lee, Mr. Dainius Martuzevicius, and Dr. Mikhail Yermakov for their contributions towards my research. Special thanks are offered to Ms. Nancy Burton, Ms. Anne South, and Dr. Seung-Hyun Cho for their help and friendship.

Finally, I would like to thank my dear husband, Weixin Li, and daughter, Norah Li, for their unwavering love and support. I would like to dedicate this dissertation to them.
LIST OF PEER-REVIEWED PUBLICATIONS

The results of this research have been presented in the following four peer-reviewed journal articles:


The full texts of these papers are attached in Appendices A1 through A4. These four papers essentially comprise the main body of this dissertation.

Paper 1 reprinted from Journal of Aerosol Science with permission from Elsevier, 6277 Sea Harbor Drive, Orlando, FL 32887.

Paper 2 reprinted from Aerosol Science and Technology with permission from Taylor & Francis Inc., 325 Chestnut St, Ste 800, Philadelphia, PA 19016.

Paper 3 reprinted from Journal of ASTM International with permission from ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428.
TABLE OF CONTENTS

ABSTRACT ................................................................................................................. 3

ACKNOWLEDGEMENTS .......................................................................................... 6

LIST OF PEER-REVIEWED PUBLICATIONS .......................................................... 8

TABLE OF CONTENTS ............................................................................................. 9

LIST OF TABLES ....................................................................................................... 12

LIST OF FIGURES .................................................................................................... 13

BACKGROUND ....................................................................................................... 15

OBJECTIVE, HYPOTHESIS, AND SPECIFIC AIMS ................................................. 21


1.1. Materials and Method ....................................................................................... 24

1.1.1. Part I. Aerosolization of microorganisms and polystyrene latex (PSL) particles by a Collison nebulizer ................................................................. 24

1.1.1.1. Experimental set-up .................................................................................. 24

1.1.1.2. Test microorganisms and preparation of microbial suspensions ............ 24

1.1.1.3. Measurement of microbial concentration in the air ................................. 26

1.1.1.4. Measurement of microbial concentration in the fluid ............................ 27

1.1.1.5. Experiments with PSL particles ............................................................... 28

1.1.1.6. Viscosity measurement of microorganism suspensions in MWFS .......... 30

1.1.2. Part II. Aerosolization of microorganisms and PSL particles by the MWF simulator ........................................................................................................... 30

1.1.2.1. Experimental set-up ................................................................................ 30

1.1.3. Statistical analyses ...................................................................................... 33

1.2. Results and Discussion ................................................................................... 33
1.3. Conclusions for Specific Aim 1 ................................................................. 39


2.1. Materials and methods ........................................................................... 41
   2.1.1. Experimental set-up ......................................................................... 41
   2.1.2. Preparation of MWFs ...................................................................... 42
   2.1.3. Experimental procedures ................................................................. 43
   2.1.4. Particle concentration measurements ............................................ 44
   2.1.5. Endotoxin measurements ................................................................. 45
   2.1.6. Viability of *P. fluorescens* in the test fluids ..................................... 46
   2.1.7. Validation of the laboratory simulator through field sampling .......... 48
2.2. Results and Discussion ........................................................................... 48
2.3. Conclusions for Specific Aim 2 ............................................................. 55

SPECIFIC AIM 3: INVESTIGATE THE PROPERTIES OF PARTICLES AEROSOLIZED FROM USED MWFS COLLECTED IN THE FIELD USING A LABORATORY-SCALE SIMULATOR ......................................................... 57

3.1. Materials and Methods .......................................................................... 57
   3.1.1. Experimental setup ......................................................................... 57
   3.1.2. Experimental procedures ................................................................. 58
   3.1.3. Measurements of airborne particle size distribution ...................... 58
   3.1.4. Measurements of airborne endotoxin concentrations in different
           Particle sizes ...................................................................................... 58
   3.1.5. Measurements of the concentrations of airborne gram-negative
           bacteria in different size ranges ......................................................... 59
3.2. Results and Discussion .......................................................................... 60
3.3. Conclusions for Specific Aim 3 ............................................................. 61
SPECIFIC AIM 4: INVESTIGATE THE PROPERTIES OF AIRBORNE PARTICLES AEROSOLIZED FROM MWFS IN THE FIELD

4.1. Materials and Methods

4.1.1. Field sites

4.1.2. Measurement of airborne particles

4.1.3. Microbial air samples

4.1.4. Measurements of airborne endotoxin concentrations in different particle sizes

4.1.5. MWF samples

4.2. Results and Discussion

4.3. Conclusions for Specific Aim 4

OVERALL CONCLUSIONS

REFERENCES

TABLES

FIGURES
LIST OF TABLES

Table 1-1. The physical characteristics of the tested microorganisms.

Table 1-2. Viscosity of microorganism suspensions in two types of MWFs and in water as measured by a viscometer (25 °C).

Table 2-1. Comparison of the geometric mean (d_g) and geometric standard deviation (σ_g) of the particles generated by the simulator in the laboratory with those measured in the field by an optical particle counter.

Table 2-2. Endotoxin concentration of particles aerosolized from semi-synthetic MWF contaminated with *P. fluorescens*. Particle number concentration was measured using an electrical-low pressure impactor and endotoxin analysis was performed from samples collected on two ELPI stages. The results represent an average and standard deviation of three repeats.

Table 4-1. Microbial and endotoxin values reported in the literature for facilities using metalworking fluids.

Table 4-2. Mist concentrations measured in the field sites.
LIST OF FIGURES

Fig. 1-1. Experimental set-up for the aerosolization of microorganisms from metalworking fluids by the Collison nebulizer.

Fig. 1-2. Laboratory-scale MWF simulator for the aerosolization of microorganisms from metalworking fluids.

Fig. 1-3. Effect of the fluid type on the aerosolization of *P. melinii* spores (Collison nebulizer, air pressure = 15 psi).

Fig. 1-4. Aerosolization of microorganisms from water suspension of different microbial concentrations (Collison nebulizer, air pressure = 12 psi).

Fig. 1-5. Effect of microorganism type on the aerosolization from semi-synthetic MWF (MWF simulator).

Fig. 1-6. Effect of particle size on the aerosolization of polystyrene latex (PSL) particles from water suspension by the Collison nebulizer and by the MWF simulator.

Fig. 2-1. The effect of tool rotation speed (rpm) on the number concentration and size distribution of particles aerosolized from semi-synthetic MWF. The data were collected with an optical particle counter. Each data point indicates an average of five-minute measurement. Standard deviations are so small that they do not show in the figure.

Fig. 2-2. The effect of tool rotation speed (rpm) on the mass concentration and size distribution of particles aerosolized from semi-synthetic MWF. The data resulted from converting the number concentration, measured by an optical particle counter, to the mass concentration assuming that the density of the particles is 1 g cm\(^{-3}\). Each data point indicates an average of five-minute measurement. Standard deviations are so small that they do not show in the figure.

Fig. 2-3. The effect of fluid application rate on the number concentration and size distribution of particles aerosolized from semi-synthetic MWF, as measured by an optical particle counter. Each data point indicates an average of five-minute measurement and the error bars indicate one standard deviation.

Fig. 2-4. The effect of tool rotation speed (rpm) on the total number concentration of particles aerosolized from *Pseudomonas fluorescens* suspension in semi-synthetic MWF as measured by an optical particle counter. Each data point indicates an average of five-minute measurement and the error bars indicate one standard deviation.

Fig. 2-5. The effect of tool rotation speed (rpm) on the mass concentration of particles aerosolized from *Pseudomonas fluorescens* suspension in semi-synthetic MWF, as measured by a photometric aerosol mass monitor.
Fig. 2-6. The effect of tool rotation speed (rpm) on the number concentration of particles aerosolized from *Pseudomonas fluorescens* suspension in semi-synthetic MWF as measured by a condensation nucleus counter. Each data point indicates an average of five-minute measurement and the error bars indicate one standard deviation.

Fig. 2-7. The effect of tool rotation speed (rpm) on the number concentration of particles aerosolized from *Pseudomonas fluorescens* suspension in soluble oil MWF as measured by a condensation nucleus counter. Each data point indicates an average of five-minute measurement and the error bars indicate one standard deviation.

Fig. 2-8. The normalized number size distribution of particles aerosolized from different fluids at a tool speed of 8000 rpm, as measured by an electrical low pressure impactor. Each size distribution was normalized by its highest concentration. Each data point indicates an average of five-minute measurement and the error bars indicate one standard deviation.

Fig. 2-9. Viability of *P. fluorescens* cells after mixing with water and semi-synthetic MWF. Each data point indicates an average of three repeats and the error bars indicate one standard deviation.

Fig. 3-1. Number size distribution of particles aerosolized in the laboratory simulator as measured by an electrical low-pressure impactor.

Fig. 3-2. Airborne endotoxin concentration and gram-negative bacterial concentration as a function of particle size. The experiment was performed in the laboratory-scale MWF simulator using an electrical low-pressure impactor for the collection of endotoxin and a six-stage viable impactor for the collection of gram-negative bacteria.

Fig. 4-1. Number size distributions of particles in the field sites as measured by an electrical low-pressure impactor (P#1= Plant #1, P#2= Plant #2).

Fig. 4-2. Airborne endotoxin concentration as a function of particle size in the field sites as collected by an electrical low-pressure impactor (P#1= Plant #1, P#2= Plant #2).

Fig. 4-3. Concentrations of bacteria and gram-negative bacteria in the MWFs (A) and in the air (B) in the field sites (BD- below detection limit, N/A- non-applicable since no MWF fluid was used in the reference areas).

Fig. 4-4. Endotoxin concentrations in the MWFs (A) and in the air (B) in the field sites (N/A- non-applicable since no MWF fluid was used in the reference area).
BACKGROUND

Metalworking fluids (MWFs) are used in machining and grinding processes to lubricate contact surfaces, to dissipate heat, and to remove fines and swarfs. MWFs can be divided into four general categories: straight oils (mineral oils), soluble oils, semi-synthetic MWFs, and synthetic MWFs (NIOSH, 1998). Straight oil MWFs are primarily composed of mineral oils with other organic additives. They are water-insoluble, and are used undiluted. The last three categories are water-based fluids and are used as suspensions containing up to 95% water by volume. The main difference between the three water-based MWFs is the amount of mineral oil they contain in their concentrates. Soluble oil MWF concentrates contain up to 80 percent mineral oil with emulsifying agents added to form emulsions when mixed with water. Semi-synthetic MWFs concentrates contain 5-30 percent mineral oil. Synthetic MWFs contain no mineral oil and are completely water-soluble (NIOSH, 1998).

Health effects associated with human exposure to MWFs include dermatitis, respiratory symptoms, and asthma. (O’Brien, 2003; Popendorf et al., 1996; Taibjee and Foulds, 2003). The association between MWF exposures and hypersensitivity pneumonitis is still controversial. About 1.2 million workers in the United States are occupationally exposed to MWFs (NIOSH, 1998). It has been reported that MWF exposure was the second most common cause of work-related asthma in the state of Michigan (Rosenman et al., 1997).

Outbreaks of hypersensitivity pneumonitis, bronchitis, and asthma in MWF environments have prompted numerous epidemiological and microbiological studies on the contributions of microbial contamination of water-based fluids (Bukowski, 2003; Fox
et al., 1999; NIOSH, 1998). Since water-based MWFs are often reused in the work place, microbial contamination often occurs and is addressed by fluid management practices. Antibodies to a common MWF bacterium, *Pseudomonas*, have been found in the serum of MWF-exposed workers, suggesting an association between microbial exposure and allergy-based health effects (Bernstein et al., 1995). Microorganisms contain a variety of substances that can also stimulate inflammatory and toxic reactions when inhaled. Lewis et al. (2001) detected endotoxin (a component of the outer membrane of gram-negative bacteria) from bulk MWF and from MWF aerosols.

Gram-negative bacteria, especially *Pseudomonas* spp. have been found by many investigators to represent the major bacterial contaminants in MWFs (Bernstein et al., 1995; Lonon et al., 1999; Sondossi and Rossmore, 1985). Other microorganisms have also been identified in used industrial MWFs, such as *Mycobacteria* (Kreiss and Cox-Ganser, 1997; Moore et al., 2000), *Aspergillus niger*, *Staphylococcus capitas*, and *Bacillus pumilus* (Bernstein et al., 1995). Mattsby-Baltzer et al. (1989) showed that bacteria could be present in industrial MWFs at high concentration (> $10^8$ cells/ml) even after large quantities of biocides had been added. Veillette et al. (2004) studied the progression of microbial contamination for 6 months after dumping, cleaning and recharging (DCR) of a large semi-synthetic MWF system managed with several biocides. They found a rapid progression in the total bacterial counts as determined by fluorescence microscopy: $5.7 \times 10^7$ cells/ml in the pre-DCR used fluid, no measurable bacteria in the fresh fluid, $6.9 \times 10^6$ cells/ml after 12h and $2.2 \times 10^6$ cells/ml, $3.6 \times 10^8$ cells/ml, and $6.1 \times 10^8$ cells/ml after 1, 3 and 6 months, respectively.
Scientists are exploring new methods to determine the microorganisms in the MWFs to yield the results more rapidly and accurately. A filtration-based rapid adenosine triphosphate bioluminescence assay, which takes less than 10 min to perform, was evaluated by Webster et al (2005) as a test method for estimating total bacteria population in MWFs. The results indicate the rapid adenosine triphosphate assay is strongly correlated to the standard plate count method for soluble and semi-synthetic fluids.

Yadav and his colleagues (Izhar and Yadav, 2004; Selvaraju et al., 2005; Yadav et al., 2003) developed methods using PCR to identify and quantify Mycobacteria and Pseudomonas in MWFs.

A few field studies have attempted to link the concentrations of microorganisms and endotoxin in the fluids with those in the air (Thorne et al., 1996; Virji et al., 2000). Thorne et al. (1996) monitored microorganisms and endotoxin in the air and in the bulk MWFs in an engine plant for four seasons. The concentration of airborne total bacteria (culturable + nonculturable) varied widely from 5,560 cells/m$^3$ to 468,000 cells/m$^3$. Endotoxin concentration ranged from 39 to 166,000 endotoxin units (EU)/ml in the bulk MWF and from below detection (<4 EU/m$^3$) to 790 EU/m$^3$. The study revealed that airborne endotoxin concentrations demonstrated significant associations with bulk endotoxin and with bulk total microorganisms. Virji et al. (2000) used a multivariate model to determine the major factors associated with the microbial levels. These investigators found that fluid-related factors (pH level of the fluid and presence of tramp oil) were the most important characteristics related to the microbial levels in the bulk MWF, while process-related factors (worker’s distance from the machine, bulk microbial levels, machine enclosures) were the major characteristics associated with the microbial
levels in the air. Due to the large variation of microbial concentrations in the above field studies, it remains unclear how much the aerosolization of microorganisms from MWFs depends on the MWF type and the characteristics of the microorganisms (size and surface characteristics).

Several investigators have studied the factors that affect the aerosolization of mist from pure MWFs using machining centers. Heitbrink et al. (2000) investigated the particle size and concentration of mist aerosolized from soluble oil as a function of tool rotation speed, fluid application rate, and tool cutting rate in an enclosed machining center. The mist concentration was found to increase with increasing tool speed and fluid application rate. The shape of the size distribution was unaffected by the experimental variables. Dasch et al. (2002) investigated the effect of the following variables on the mass concentration of mist generated in a machining center: fluid itself (concentration, type, volatility, age, temperature, and the presence of tramp oil), fluid application (fluid velocity and through-tool application), and machining parameters (tool speed, tool diameter, tool feed, depth-of-cut, and tool wear). It was shown that tool speed had the largest impact on the mist mass concentration. Turchin and Byers (2000) studied the mass concentration of aerosolized mist in a laboratory-scale mist generator, and demonstrated that the presence of fluid contaminants (e.g., tramp oil) had a major effect on the mist level.

The importance of knowledge about particle size for assessing potentially associated health hazards has long been recognized. NIOSH recommends that occupational exposures to MWF aerosols be limited to 0.4 mg/m$^3$ for thoracic particulate mass (which corresponds to approximately 0.5 mg/m$^3$ for total particulate mass) (NIOSH, 1998). The
The relationship between inhalable and total aerosol concentrations in MWF sites was reevaluated by Donovan et al. (2005) and Wilsey et al. (1996). The effect of different variables on the size distributions of the particles were investigated on pure MWFs using machining centers as described above (Dasch et al., 2002; Heitbrink et al., 2000).

Only a few studies (Gorny et al., 2004; Woskie et al., 1994) have measured the particle concentrations in specific size ranges in MWF sites in the field that had possible microbial contamination. Gόrny et al. (2004) measured the particle concentrations in four size fractions in a steelwork: particles with aerodynamic sizes up to 2.5 µm (PM 2.5), up to 5 µm (PM 5), up to 10 µm (PM 10), and total suspended particles (TSP). They reported the following particle concentrations: 143 ± 14 for PM 2.5, 232 ± 55 for PM 5, 351 ± 66 for PM 10, and 446 ± 82 for TSP µg/m³. Woskie et al. (1994) measured workers’ personal exposures to particles in different size fractions in MWF environments. Both of these studies revealed considerable concentration levels for small particles (< 2.5 µm).

As described above, previous particle measurements in MWF sites have mostly focused on mass-based sampling. Very few studies have reported particles and endotoxin in the fine particle size range (< 1µm). Due to their small size and large surface area, the fine particles may be even more damaging to human health than the larger ones (Ferin et al., 1990; Oberdorster, 1996). There are several reasons for that. Firstly, because of their small size, fine particles can penetrate to the gas-exchange area of the lung when inhaled. Secondly, for a given mass of particles, the particle number and surface area dramatically increase as particle size becomes smaller. The large number of particles deposited in the lung may exceed the ability of macrophages to phagocytize them, and prolongs the
interaction between the particles and epithelial cells. Oberdorster’s study (1996) showed that for “non-toxic” materials such as carbon and titanium dioxide, the effective dose (the dose that mediates toxicity) appears to be a function of surface area of particles. Thirdly, because of their small size, fine particles can even across the lung membrane and enter the blood circulation system (Nemmar, 2003). Therefore they can reach the secondary target organs such as the heart and liver. Fine particles depositing in the nasal region may also be able to translocate to the brain via the olfactory nerve (Oberdorster et al., 2004).
OBJECTIVE, HYPOTHESIS, AND SPECIFIC AIMS

Objective: The objective of this study was to investigate the aerosolization of particles and microorganisms from MWFs contaminated with microorganisms. The effects of the following factors were investigated: the machining processes (the tool rotation speed, and the fluid application rate), the characteristics of the microorganisms (size and surface characteristics), as well as the MWF types. The results of this study will be useful in the selection of MWFs and machining parameters to reduce workers’ exposure to microorganisms and particles. The results will also give more insights for the exposure assessment in MWF environments.

Hypothesis: The aerosolization of particles and microorganisms from MWFs depends on the machining parameters (the tool rotation speed, and the fluid application rate), the characteristics of the microorganisms (size and surface characteristics), and the MWF types.

In order to achieve the objective of this study and test the hypothesis, the following specific aims were accomplished:

Specific Aim 1: Investigate the effect of fluid type, the microbial concentration in the fluid, and the characteristics of the microorganisms (size and surface characteristics) on the aerosolization of microorganisms from MWFs.

Specific Aim 2: Under laboratory conditions, determine the relationship between the concentrations and characteristics of airborne particles with the MWF type, and the machining processes (the tool rotation speed and the fluid application rate).
**Specific Aim 3:** Investigate the properties of particles aerosolized from used MWFs collected in the field using a laboratory-scale simulator.

**Specific Aim 4:** Investigate the properties of airborne particles aerosolized from MWFs in the field.
SPECIFIC AIM 1


In Specific Aim 1, the effects of fluid type (distilled water, semi-synthetic MWF, and soluble oil), microorganism concentration in the fluid, and the characteristics of the microorganisms (size and surface characteristics) on the aerosolization of microorganisms from MWFs were investigated. Three microorganisms were employed to represent different size and surface characteristics: *Bacillus subtilis* bacterial endospores (hydrophobic particles with aerodynamic diameter=0.9 µm), *Pseudomonas fluorescens* bacterial vegetative cells (hydrophilic, 0.8 µm) and *Penicillium melinii* fungal spores (hydrophobic, 3.1 µm). The testing was first performed using a Collison nebulizer to aerosolize microorganisms from three fluids: water, semi-synthetic MWF, and soluble oil. To explore the aerosolization of microorganisms from MWFs under the conditions that are closer to industrial settings, a laboratory-scale set-up (MWF simulator) was developed to simulate the mist generation during grinding process, and the tests were conducted with the MWF simulator.
1.1. MATERIALS AND METHODS

1.1.1. Part I. Aerosolization of microorganisms and polystyrene latex (PSL) particles by a Collison nebulizer

1.1.1.1. Experimental set-up

The experimental set-up utilized in the first part of this Specific Aim is schematically shown in Figure 1-1. A six-nozzle Collison nebulizer (BGI Inc., Waltham, MA) generated aerosols either from microorganism suspensions or PSL suspensions at a flow rate of 6 l/min. The aerosol flow was diluted with HEPA-filtered clean air at a flow rate of 30 l/min and entered a test chamber with a Button Inhalable Aerosol Sampler (SKC Inc., Eighty Four, PA) placed in the center. In addition, an optical particle counter (OPC) (Grimm Technologies Inc., Douglasville, GA) was placed beside the Button Sampler to monitor the stability of the aerosol concentration in the chamber. The entire set-up was located in a biological safety cabinet (Sterilchem-Gard Class II, Baker Co., Sanford, ME).

1.1.1.2. Test microorganisms and preparation of microbial suspensions

The testing was performed with three species of microorganisms: Bacillus subtilis bacterial endospores, Pseudomonas fluorescens bacterial vegetative cells, and Penicillium melinii fungal spores. These microorganisms have been commonly found in used MWFs (Lonon et al., 1999; Lummus et al., 1998; Mattsby-Baltzer et al., 1989; Thorne et al., 1996). They were selected to represent different surface characteristics of microorganisms (hydrophilic or hydrophobic) and different sizes, see Table 1-1. B. subtilis endospores were received from the US Army Edgewood Laboratories (courtesy
of Agnes Akiyemi and Dr. Edward Stuebing, Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, MD). The spores were activated at 60°C for 25 min and washed twice with sterile deionized water by centrifugation at 7000 rpm for 7 min. *P. fluorescens* (ATCC 13525) culture was obtained from the American Type Culture Collection (Rockville, MD). It was subcultured by incubating in a trypticase soy broth at 28°C for 18 h. The cells were then washed twice, similar to the procedure used for *B. subtilis* spores. *P. melinii* strain had previously been isolated from a moldy building. Prior to their use in the experiments, they were cultured on malt extract agar and subsequently incubated at 25°C for 14 days. Spores were collected from matured sporulating cultures by applying 1 g of dry, autoclaved glass beads (0.45-0.5 mm in diameter, B. Braun Biotech International, Melsungen, Germany) per Petri plate as described by Schmechel et al. (2003). The lid was put back into place and the plates were gently shaken back and forth. This allowed the spores to attach to the beads. The beads were transferred into a 50-ml tube containing sterile deionized water and spores were suspended and separated from the beads by briefly shaking the tube and decanting the spore suspension.

The freshly prepared suspensions of *B. subtilis*, *P. fluorescens* and *P. melinii* were diluted with test MWFs or with sterilized deionized water, depending on the experiment, until desired concentrations were achieved. Two types of MWFs tested in this study, a semi-synthetic MWF and a soluble oil, are commercially available. They were used in this study as 5% water solutions.
1.1.1.3. Measurement of microbial concentration in the air

*Button Sampler*

The Button Personal Inhalable Aerosol Sampler operating at a flow rate of 4 l/min collects particles on a 25-mm membrane filter. The filter is located behind the porous metal inlet screen, which has a spherical surface with a subtended angle of 160° and a porosity of 21%. Orifices of 381 µm diameter are evenly spaced throughout the inlet surface resulting in uniform particle deposition on the entire filter area. The Button Sampler was found to be significantly wind-insensitive under laboratory conditions (Aizenberg et al., 1999) as well as outdoor field conditions (Adhikari et al., 2003). Black polycarbonate filters with a pore size of 0.2 µm (Osmonics Inc. Westborough, MA) were employed for the collection of *P. fluorescens* and *B. subtilis* at a sampling time of 10 minutes. Black polycarbonate filter with a pore size of 3.0 µm was employed for the collection of *P. melinii* to allow a longer sampling time (30 min) without blockage of the filter. Although *P. melinii* spores were extracted directly from culture plates, we found it challenging to obtain a large amount of spores for the liquid suspension. As a result, the number of *P. melinii* spores aerosolized from the liquid into the air was also relatively low. In order to collect a sufficient number of fungal spores that could be adequately quantified by microscopic counting, an extended sampling time was required for *P. melinii*. Lee et al. (2004) reported that the collection efficiency of 3.0 µm particles on a polycarbonate filter with 3.0 µm pores was 95% at a flow rate of 7.2 l/min.
Total microbial concentration in the air

For determining the total microbial concentration in the air, the black polycarbonate filter was removed from the Button Sampler and directly stained by the acridine orange method, as described by Wang et al. (2000). An epifluorescence microscope was used to count the microorganisms on the filter at a magnification of 1000× (E. Leitz, Inc., New York). Forty randomly chosen microscopic fields were counted.

The total microbial concentration in the air, $C_{\text{total-air}}$ (org/m³), was determined as follows:

$$C_{\text{total-air}} = \frac{N_1 A_1}{(A_2 Qt)} \quad (1-1)$$

where $N_1$ is the average microbial count on each microscope field in the air sample, $A_1$ is the effective collection area of the Button Sampler filter (360 mm², i.e., the area on which particles are deposited, which is 22-mm in diameter on the entire 25-mm filter), $A_2$ is the area of the microscopic field (0.02404 mm²) and $Q$ is the air flow rate of the Button Sampler (m³/min), $t$ is the sampling time (min).

1.1.1.4. Measurement of microbial concentration in the fluid

For determining the total microbial concentration in the fluid, 1 ml of the fluid sample was stained by the acridine orange method and filtered through a 25-mm diameter black polycarbonate filter, as described by Wang et al. (2000). The epifluorescence microscope was used to count the microorganisms on the filter at a magnification of 1000×. Forty randomly chosen microscopic fields were counted.

The total microbial count in the fluid, $C_{\text{total-fluid}}$ (org/ml), was determined as follows:
\[ C_{\text{total\_fluid}} = N_2 \left( \frac{A_s}{A_A V} \right) \]  

(1-2)

where \( N_2 \) is the average microbial count on each microscope field in the fluid sample, \( A_s \) is the effective filtration area of the 25-mm filter (210 mm\(^2\)), \( V \) is the volume of the suspension that was stained and filtered (1 ml).

The aerosolization ratio [(org/m\(^3\) in the air)/ (org/ml in the liquid)] of each microbial species is defined as the total concentration in the air (\( C_{\text{total\_air}} \), org/m\(^3\)) divided by the total concentration in the fluid (\( C_{\text{total\_fluid}} \), org/ml):

\[
\text{Aerosolization ratio} = \frac{C_{\text{total\_air}}}{C_{\text{total\_fluid}}} 
\]  

(1-3)

1.1.1.5. Experiments with PSL particles

PSL particles and preparation of the suspension

PSL particles (Bangs Laboratories Inc., Fishers, IN) of two aerodynamic sizes, 1.0 µm and 3.4 µm, were used in this study to investigate the effect of aerodynamic size on the aerosolization of particles with the same surface characteristics (hydrophobic). These two sizes were selected to simulate the spores of two of our three test microorganisms, \( B.\ subtilis \) and \( P.\ melinii \), which are both hydrophobic and have the aerodynamic diameters of 0.9 µm and 3.1 µm, respectively. Thirty microliters of PSL suspension were diluted with deionized water until a desired concentration was reached.
**PSL particle concentrations in the air**

The OPC was used to measure the PSL particle concentration in the air. During our preliminary experiments, the aerosolization of 1 µm PSL particle solution resulted in a concentration peak between 0.5 and 1.0 µm, as measured by the OPC. This is different from the actual aerodynamic size of the PSL particles (1 µm) since the OPC determines the optical diameter. Thus, the OPC count of particles ranging from 0.5 to 1.0 µm was used for determining the aerosol concentration of 1 µm PSL particles. Similarly, the particle count recorded by the OPC within the size range of 2.0 to 5.0 µm represented the 3.0 µm PSL particles as their concentration peak was observed in this size range.

**Measurement of PSL particle concentration in the fluid**

PSL particle concentrations in the liquid were measured by a hemacytometer (Hausser Scientific, Horsham, PA). The test liquid was placed on the hemacytometer chamber and mounted using a cover glass. A light microscope was used to count the PSL particles on the hemacytometer at a magnification of 400× (Model Laborlux S; E. Leitz, Inc., New York). Eighteen squares were counted on both upper and lower chambers of the hemacytometer. The total concentration of PSL particles in the fluid, $P_{total\_fluid}$ (particles/ml), was determined as follows:

$$P_{total\_fluid} = f \times N_3$$

(1-4)

where $N_3$ is the average PSL count on the squares, and $f$ is the hemacytometer constant ($2.5\times10^5$).

The aerosolization ratio of PSL particles, defined as the ratio of particles/m³ in the air to particles/ml in the liquid, was determined as:
Aerosolization ratio = \( \frac{P_{\text{total, air}}}{P_{\text{total, fluid}}} \)  

1.1.1.6. Viscosity measurement of microorganism suspensions in MWFs

The viscosities of our test fluids were measured using a Cannon-Fenske Routine viscometer (Cannon Instrument Company, State College, PA). Ten milliliters of the test fluid were pipetted into the viscometer. Then the viscometer was vertically aligned in a water bath. The sample was kept in the water bath for 10 minutes, which allowed it to stabilize to the bath temperature (25°C). Suction was applied to draw the liquid above a specified mark of the viscometer and then the liquid was let to flow down. The time for the liquid to flow a specified distance (approximate 4 cm) was recorded as the efflux time. The kinematic viscosity (mm²/s) of the sample was calculated by multiplying the efflux time (in seconds) by the viscometer constant (0.1569 mm²/s²).

1.1.2. Part II. Aerosolization of microorganisms and PSL particles by the MWF simulator

1.1.2.1. Experimental set-up

To study the aerosolization of microorganisms under more realistic conditions that occur in industrial settings, a laboratory-scale set-up that simulates the mist generation during grinding process (MWF simulator) was built. Similar type of approach has previously been used for studying mist generation by Turchin and Byers (2000), Thornburg and Leith (2000), and White and Lucke (2003). As our simulator was developed primarily for the investigation of aerosolization of microorganisms from MWFs, one of the main goals in the design was to avoid contamination of laboratory air
and cross-contamination of the set-up between experiments with different microorganisms. The MWF simulator is schematically shown in Figure 1-2. It was made of two enclosed chambers, an inner chamber and an outer chamber. The aerosol was generated in the inner chamber (5 liters) when a liquid pump ejected MWF through a nozzle against a rotating aluminum rod (3.8 cm diameter). The actual metal grinding was intentionally omitted in this experimental design. Earlier study (Turchin and Byers, 2000) showed that mist levels were higher when the machine was idling with the fluid flowing, but no metal was being ground. Apparently, the metal part tended to block the fluid spray and minimize misting. Thus, in a laboratory-scale test of mist aerosolization, the grinding wheel appears to be the most critical part, particularly as the focus was the aerosolization of microorganisms, not metallic particles. The fluid flow rate was 1 l/min which resulted in an ejection velocity of the fluid of 85 cm/s. This value was close to the lowest fluid velocity used in the study of Dasch et al. (2002). The rotation speed of the rod was 8000 rpm, resulting in a surface speed of 4064 cm/s, which is a typical speed for grinding processes. The rod rotation speed was measured by a tachometer (Monarch Instrument, Amherst, NH). The largest fluid droplets hit the walls of the inner chamber, drained downward and were collected at the bottom of the chamber, from where the fluid was recirculated by a variable flow chemical transfer pump (Control Company, Friendswood, TX). A motor (Milwaukee Electric Tool Ltd., Milwaukee, WI) driving the rod was placed in the outer chamber (120 liters) to prevent its contamination by MWF mists. A small opening was drilled into the wall of the inner chamber to allow the motor’s shaft to enter the chamber.
HEPA-filtered air entered the inner chamber either directly ($Q_{\text{Air}1}$) or by passing air ($Q_{\text{Air}2}$) into the outer chamber and then through the hole surrounding the rotating shaft into the inner chamber. The flow rates ($Q_{\text{Air}1} = 70 \text{ l/min}$ and $Q_{\text{Air}2} = 30 \text{ l/min}$) were constant throughout the experiment. The airflow rate into and out of the test environment of the MWF simulator was therefore 100 l/min. The flow of $Q_{\text{Air}1}$ passing from the outer chamber through the opening for the motor’s shaft to the inner chamber created a positive pressure difference between the outer and inner chambers. This prevented the mists from passing into the outer chamber through the small opening. Before applying this positive pressure difference, we had frequently noticed mists in the outer chamber when the rod rotated at a high speed. The air flow rates of each airway were monitored by calibrated rotameters (Dwyer Instrument, Inc., Michigan City, IN). The air pressures in the inner and outer chambers were monitored by two pressure meters, $P_1$ and $P_2$, respectively (Dwyer Instruments Inc, Michigan City, IN).

The aerosol flow from the inner chamber was drawn past a baffle for removal of the larger droplets before entering the sampling chamber. One Button Sampler at a time was placed in the sampling chamber facing the air flow. The sampling probe for the OPC also intruded into the sampling chamber parallel to the air flow. The remaining aerosol flow was exhausted from the sampling chamber through a HEPA-filter into the surrounding biological safety cabinet. The OPC body was also placed inside the biosafety cabinet to avoid biocontamination.

The concentrations of microorganisms and PSL particles in the air and in the liquid were determined as described in Part I.
1.1.3. Statistical analyses

Three replicates were obtained for each experiment. The Kruskal-Wallis test was used to compare the aerosolization ratios between the three test fluids or between the three microorganisms. The Wilcoxon test was used to compare the aerosolization ratio of PSL particles between the Collison nebulizer and the MWF simulator.

1.2. RESULTS AND DISCUSSION

Figure 1-3 shows the effect of fluid type on the aerosolization of microorganisms by the Collison nebulizer. Three fluids were tested, *P. melinii* in water, *P. melinii* in semi-synthetic MWF, and *P. melinii* in soluble oil. *P. melinii* was selected as the test microorganism because it allowed reliable testing of all the three fluids with the same microorganism. Soluble oil is very viscous and has a milk-white color, which made it difficult to enumerate small *B. subtilis* spores and *P. fluorescens* cells under the microscope. For all three fluids, the *P. melinii* concentrations in the liquid were about $10^7$ org/ml. For the *P. melinii* in water suspension, the aerosolization ratio was 0.47±0.08 $[(\text{org/m}^3 \text{ in air})/ (\text{org/ml in liquid})]$. The respective value was 0.62±0.14 for the *P. melinii* in semi-synthetic MWF and 0.55±0.1 for the *P. melinii* in soluble oil. There was no statistically significant difference (Kruskal-Wallis, p=0.23) in the aerosolization ratio between these three fluids. We speculate that this is due to the characteristics of MWFs, which were close to water suspensions since MWFs contain 95% water and 5% MWF concentrate. Table 1-2 shows the viscosity of microorganism suspensions in two types of MWFs and in water as measured by a viscometer. The efflux time and kinematic viscosity were measured for three type of fluids: microorganisms in water,
microorganisms in semi-synthetic MWF, and microorganisms in soluble oil. The result shows that the differences of the viscosities between these fluids were less than 20%.

Since the fluid type had no significant effect on the aerosolization ratio of the microorganisms, the effects of microorganism species and microbial concentration in the liquid on aerosolization were tested using water suspensions in the Collison nebulizer. The results are shown in Figure 1-4. The microorganism concentrations in the liquid varied from $10^7$ to $10^9$ org/ml and the microorganism concentrations in the air varied from $10^6$ to $10^8$ org/m$^3$. A linear regression line was drawn to fit the data points for each microorganism species. When the microorganism concentration in the liquid is zero, the microorganism concentration in the air should also be zero. Moreover, the microorganism concentration in the air should always be positive. Therefore, the regression lines were forced to go through the origin according to a method described by Armitage (1971). For all test species, a significant linear relationship was found between the microorganism concentration in the air and the microorganism concentration in the liquid ($p<0.001$ for \textit{B. subtilis}, $p<0.001$ for \textit{P. fluorescens}, and $p=0.017$ for \textit{P. melinii}). It demonstrates that the microbial concentration in the liquid does not affect the aerosolization ratio of microorganisms.

The aerosolization ratio was $0.44\pm0.14 \ [\text{(org/m}^3 \ \text{in the air)}/ \text{(org/ml in the liquid)}]$ for \textit{B. subtilis}, $0.23\pm0.04$ for \textit{P. fluorescens}, and $0.20\pm0.04$ for \textit{P. melinii}. Wilcoxon tests showed that the aerosolization ratio of \textit{B. subtilis} was significantly different from that of \textit{P. fluorescens} and of \textit{P. melinii} ($p<0.001$ for both). The aerosolization ratios obtained for \textit{P. fluorescens} and \textit{P. melinii} were not significantly different (Wilcoxon, $p=0.556$). For the same concentration in the liquid, the \textit{B. subtilis} concentration in the air was much
higher than that of *P. fluorescens* or *P. melinii*. *B. subtilis* spores and *P. fluorescens* cells have approximately the same aerodynamic sizes (0.9 \( \mu \text{m} \) for *B. subtilis* and 0.8 \( \mu \text{m} \) for *P. fluorescens*), but different physical surface characteristics (*B. subtilis* spores are hydrophobic and *P. fluorescens* cells are hydrophilic). Thus, our results indicate that hydrophobic microorganisms are easier to aerosolize with the Collison nebulizer than hydrophilic ones. *B. subtilis* spores and *P. melinii* cells have the same surface characteristics (both are hydrophilic) but different sizes (0.9 \( \mu \text{m} \) for *B. subtilis* and 3.4 \( \mu \text{m} \) for *P. melinii*). The particle size seems to play a role as well since the larger microorganisms were more difficult to aerosolize than the smaller ones. The data show that *P. fluorescens* and *P. melinii* had similar aerosolization ratios although they have different sizes and different surface characteristics. It appears that the effect of size and surface characteristics compensated each other for these two species. All these experiments were conducted with the air pressure = 12 psi (82.7 kPa) for the Collison nebulizer. This resulted in a lower aerosolization ratio for *P. melinii* compared to the one presented in Figure 1-3 for which the air pressure = 15 psi (103.4 kPa). At the same concentration of particles in the liquid, the concentration of aerosolized particles increases with increasing air pressure in the Collison nebulizer (Sterk, 1983).

To confirm the results under conditions that would be closer to field settings, tests were also conducted with our MWF simulator that simulates the mist generation in grinding process in manufacturing. The relationship to industrial operations was validated by comparing the particle size distributions generated by the MWF simulator and measured with the OPC to similar data obtained in working environments as described in Specific Aim 2. The three microorganisms in semi-synthetic MWF were
tested and the results are shown in Figure 1-5. The microorganism concentrations in the
liquid were about $10^7$ org/ml for all three test fluids. The aerosolization ratio of *B. subtilis*
in MWF was significantly higher than that of *P. melinii* (Kruskal-Wallis $p<0.001$). *P. melinii* and *P. fluorescens* had similar aerosolization ratio (Wilcoxon, $p=0.227$). The MWF simulator tests showed the same trend as the Collison nebulizer tests (Figure 1-4): *B. subtilis* was easier to aerosolize than *P. fluorescens* and *P. melinii*. The results demonstrate that aerosolization of microorganisms in an industrial setting showed the same trend as in a laboratory setting: hydrophobic microorganisms tend to be easier to aerosolize than hydrophilic microorganisms and small microorganisms are easier to aerosolize than large microorganisms. It should be pointed out that the aerosolization of microorganisms by our MWF simulator in the laboratory is not fully identical to the aerosolization during grinding operation in the field. For example, in the MWF simulator, a baffle was installed in front of the sampling chamber to remove large particles. It could cause the loss of the relatively large *P. melinii* spores. However, no changes were observed in the trend of the microorganism aerosolization as the calculated loss of 3.1 µm particle by the baffle in the MWF simulator was found to be below 1% (Stk = 0.0006).

To further examine the effect of particle size on the aerosolization ratio, 1 µm and 3.4 µm hydrophobic monodisperse PSL particles (surrogates for *B. subtilis* and *P. melinii* spores, respectively) were aerosolized from water suspensions by the Collison nebulizer and the MWF simulator. The results are shown in Figure 1-6. For the Collison nebulizer, the aerosolization ratio of 1 µm PSL particles was approximately 7 times higher than that of 3 µm PSL particles and for the MWF simulator, it was 20 times higher. Thus, similar trend was found with PSL particles than with microorganisms (Figures 1-4 and 1-5):
smaller particles are easier to aerosolize than larger particles. There might be two reasons for the more pronounced effect of the particle size on the aerosolization of PSL particles than microorganisms. First, PSL particles have more uniform size than microorganisms (we observed their size variability under the microscope). As a result, PSL particles of different sizes demonstrated a more distinct difference in their aerosolization ratio. Second, the concentrations of PSL particles and microorganisms were measured with different methods. For the microorganisms, the particle concentrations in the air and in the fluid were measured by acridine orange staining and epifluorescence microscope counting. For the PSL particles, the concentration in the air was measured by the OPC whereas the concentration in the liquid was measured by the hemacytometer method.

Both MWFs and PSL suspensions contain small amount of surfactants. The surfactants in MWFs work as corrosion inhibitor and emulsifier. Surfactants in the PSL suspension serve as a stabilizer to prevent PSL particle flocculation. The surface active ability of the surfactants could decrease the surface tension of the liquid. Surfactants could also interact with particles in the fluids and consequently change their surface characteristics. In this study, surfactants did not seem to play an important role at affecting the aerosolization of particles from suspension. This may due to the fact that both MWFs and the PSL suspensions contained high concentration of particles ($10^7$ #/ml), and the amount of surfactants in the fluids was not sufficient to change the trend of the particle aerosolization.

Aerosol generation from pure liquid (without any microorganisms or solid particles) by the Collison nebulizer has been explored quite extensively (Gussman, 1984; May, 1973). In the Collison nebulizer, a gas is used to aspirate the liquid into a sonic velocity
gas jet. The jet then impacts against the inside of the jar to remove the larger droplets. However, the aerosolization of microorganisms or solid particles from the Collison nebulizer suspension has not been quantitatively characterized. We assume that, in the Collison nebulizer jar, hydrophobic particles are easier to get into the liquid/gas jet since the force between the particles and the liquid in the jar is lower. The large droplets will be removed by impaction onto the inner wall of the jar and the smaller droplets will enter the sampling chamber (these droplets may or may not contain microorganisms). As a result, hydrophobic particles have a higher aerosolization ratio than hydrophilic particles. Larger particles are easier to be removed by impaction onto the wall of the jar. Therefore, the larger particles have a lower aerosolization ratio than the smaller ones.

In field situations, MWFs mists can be generated by centrifugal forces on the liquid coating the turning rod, by impaction of the liquid on walls, or by condensation of the vapors produced by the high temperature in the metal being cut (Thornburg, 2000). Our MWF simulator included the first two mechanisms of aerosol generation, the centrifugal force being the predominant one. The evaporation/condensation mechanism is not relevant to the aerosolization of intact microbial cells or spores. Hence, this mechanism was not considered when designing the MWF simulator. When the fluid is continuously fed onto the rotating rod, a liquid film develops on the body of the rod. With time, this film grows in thickness until the centrifugal forces overcome the surface tension of the liquid. Part of the film then spins off in long threads that look like tadpoles. The heads of the “tadpoles” subsequently break off as primary droplets while the long tails break into several smaller satellite droplets. Each droplet may contain one or more microorganisms or solid particles, depending on the droplet and microorganism size.
Many or most of the droplets may not contain any microorganisms. Since MWF is water-based, the water evaporates quickly from the droplet. The presence of microorganisms may change the aerosol characteristics of the mists. Hydrophobic particles have lower surface tension than hydrophilic particles. Consecutively, hydrophobic particles are easier to be detached by centrifugal forces and easy to be spun off in the MWF simulator compared to hydrophilic ones. A similar role was played by particle size on the aerosolization of particles as the one described for the Collison nebulizer.

1.3. CONCLUSIONS FOR SPECIFIC AIM 1

The effects of fluid type, microorganism concentration in the liquid and the characteristics of the microorganisms (size and surface characteristics) on the aerosolization of microorganisms from MWFs were examined. Three microorganisms were employed to represent different particle sizes and surface characteristics: *B. subtilis* bacterial endospores (hydrophobic with an aerodynamic diameter of 0.9 µm), *P. fluorescens* bacterial vegetative cells (hydrophilic, 0.8 µm), and *P. melinii* fungal spores (hydrophobic, 3.1 µm). The testing was first performed using a Collison nebulizer. It was observed that the fluid type and the microorganism concentration in the liquid did not affect the aerosolization of microorganisms. In contrast, the characteristics of the microorganisms showed an effect. *B. subtilis* was easier to aerosolize than *P. fluorescens* and *P. melinii*. To confirm the results under conditions that are closer to an industrial setting, the aerosolization of three microorganisms was also tested with the MWF simulator. The results showed the same trend as the results from the Collison nebulizer.
testing...

Experiments with PSL particles confirmed that aerosolization mechanisms employed both by the Collison nebulizer and by the MWF simulator resulted in a higher aerosolization of small particles (representing the bacterial size range) than larger ones (representing the fungal spore size range). In summary the findings in both the laboratory environment and in an industrial setting showed that hydrophobic microorganisms were easier to aerosolize from MWFs than hydrophilic microorganisms and that increasing microorganism size was likely to result in a decrease of the aerosolization ratio.
SPECIFIC AIM 2


In Specific Aim 2, aerosolization of particles from metalworking fluids (MWFs) was studied using a laboratory-scale set-up (MWF simulator) as described in Specific Aim 1. An optical particle counter (OPC), a photometric aerosol mass monitor, a condensation nucleus counter (CNC), and an electrical low pressure impactor (ELPI) were used to measure the particle concentrations. A semi-synthetic MWF and a soluble oil were contaminated with *Pseudomonas fluorescens*. The fluid application rate varied from 0.4 to 1.6 l/min and the rotation speed of the rod varied from 800 to 8000 rpm. The endotoxin concentrations of particles in two size fractions - the bacterial fragment fraction and in the intact bacterial fraction - aerosolized from contaminated semi-synthetic MWF were tested.

2.1. MATERIALS AND METHODS

2.1.1. Experimental set-up

The laboratory-scale metalworking simulation facility has been described in detail in Specific Aim 1.
2.1.2. Preparation of MWFs

Two commercially available water-based MWFS were selected for this study: a semi-synthetic and a soluble oil. They were commonly used in factories as a 5% water solution. *Pseudomonas fluorescens* was used as the test bacterium. Numerous studies have found that gram-negative bacteria, especially *Pseudomonas* spp. represent the major bacterial contaminants in MWFs (Sondossi and Rossmoore 1985; Bernstein et al. 1995; Lonon et al. 1999). *P. fluorescens* (ATCC 13525) culture was obtained from the American Type Culture Collection (Rockville, MD). It was subcultured by incubating in a trypticase soy broth at 26±2°C for 18 h. The cells were then washed twice with sterile deionized water by centrifugation at 7000 rpm for 7 min. Then the *P. fluorescens* suspension was diluted with the test MWF or with sterilized deionized water, depending on the experiment, until the desired concentration was achieved (10^8 cells/ml). A fresh bacterial suspension was prepared daily for the tests.

The following three fluids were tested in this study:

- Semi-synthetic MWF (consisting of 95% water and 5% commercial semi-synthetic MWF concentrate),
- Soluble oil MWF (consisting of 95% water and 5% commercial soluble oil MWF concentrate),
- *P. fluorescens* suspension in water (10^8 cells/ml),
- *P. fluorescens* suspension in semi-synthetic MWF (10^8 cells/ml), and
- *P. fluorescens* suspension in soluble oil (10^8 cells/ml).

No biocide was added to any of the test fluids.
2.1.3. Experimental procedures

In addition to the fluid type, the tool rotation speed and the fluid application rate were selected as the major variables in this study. Previous studies have shown that these three variables have the greatest effect on mist generation (Dasch et al., 2002; Heitbrink et al., 2000; Thornburg, 2000).

Test fluids were prepared on the same day prior to the experiment. One liter of the test fluid was transferred into the inner chamber at the beginning of the experiment. Each experimental run lasted 13 minutes. The experiment started within 5 minutes after the bacteria were introduced into the MWF. At first, the rotation rod was adjusted to the assigned speed using a Variac (Staco Energy Products Co., Dayton, OH) that was connected to the motor. Five rotation speeds were used in the tests: 800, 2000, 4000, 6000, and 8000 rpm. As the diameter of the rod is 3.8 cm, the tool rotation speed of 8000 rpm corresponds to a surface speed of 3180 cm/s, which represents the typical surface speed for grinding process.

A time period of 5 minutes was sufficient to stabilize the rotation speed. Then a chemical pump was turned on to apply the test fluid onto the rotating rod. Five fluid application rates were tested: 0.4, 0.7, 1.0, 1.3, and 1.6 l/min. Preliminary measurements made with an optical particle counter (OPC) indicated that the mist concentrations required 3 minutes to reach steady state. During our experiments, therefore, we waited 3 minutes and then continuously recorded the particle concentrations for 5 minutes, as described below.
2.1.4. Particle concentration measurements

The aerosol concentration in the sampling chamber was continuously monitored using an optical particle counter (OPC), a photometric aerosol mass monitor, a condensation nucleus counter (CNC), and an electrical low pressure impactor (ELPI). A one-minute averaging time was used for all the instruments.

The OPC (Portable Dust Monitor, Grimm Technologies Inc., Douglasville, GA) continuously monitored the particle number concentration and size distribution. The Grimm counter utilized an 8 cm long, 3 mm diameter probe to sample at a flow rate of 1.2 l/min. The particles were size-classified into 15 channels: > 0.3, >0.4, >0.5, >0.65, >0.8, >1.0, >1.2, >2.0, >3.0, > 4.0, >5.0, >7.5, >10.0, >15.0 and >20.0 µm. The particle number concentration was converted to mass concentration using the following equation:

\[ C_m = \sum_{i=1}^{n} \left( \pi d_i^3 \rho_p / 6 \right) \times C_i \quad (2-1) \]

where \( C_i \) is the particle number concentration in each channel, \( d_i \) is the average particle diameter of each channel, and \( \rho_p \) is particle density (assumed \( \rho_p = 1 \text{ g/ml} \)).

The photometric aerosol mass monitor (DustTrak aerosol monitor, TSI Inc., St. Paul, MN) continuously monitored the total particle mass concentration within the particle size range of 0.1 to 10 µm. It sampled through an 8 cm long, 6 mm diameter probe at a flow rate of 2 l/min.

The CNC (P-trak fine particle counter, TSI Inc., St Paul, MN) was used to continuously monitor the total number concentration of fine particle. It sampled through an 8 cm long, 3 mm diameter probe at a flow rate of 0.7 l/min. The P-trak can measure particles ranging from 0.02 µm to those exceeding 1 µm.
The ELPI (Dekati Ltd., Tampere, Finland) was used to continuously monitor the particle concentration and aerodynamic size distribution in a size range that included the fine and coarse aerosol particle fractions. It has an inlet of 10 cm length, 13 mm in diameter, and operates at a flow rate of 30 l/min. The 50% cut diameters for the 13 impactor stages are 0.029, 0.059, 0.103, 0.165, 0.254, 0.392, 0.636, 0.99, 1.61, 2.45, 3.97, 6.58 and 10.18 µm. Unlike the Grimm OPC, it allows the measurement of particles below 0.3 µm. The benefit of using the ELPI over the CNC is that the ELPI records particle size-selective data. The inlet efficiency (including the aspiration and transmission) in the sampling chamber for all the instruments was calculated according to the methods described by Baron and Willeke (2001). For particles <1 µm, the inlet efficiency ranged from 0.981 to 0.999. The lowest inlet efficiency was calculated for the combination of OPC and 5-µm particles (0.883). As the focus of this study was on fine particles, we concluded that the sampling in the sampling chamber was representative. The representativeness of the sampling was also assured by positioning the four inlets at a distance of at least 5 inlet diameters from each other.

2.1.5. Endotoxin measurements

The airborne endotoxin was collected onto polycarbonate filters (Millipore Inc. Billerica, MA) placed on the impactor stages of the ELPI. The filters of certain stages were selected for endotoxin analysis that represented the microbial fragments size and the intact bacterial size. The sampling time was 120 min. After the collection, the selected filters were transferred into pyrogen-free tubes. They were sent to DataChem
Laboratories, Inc. for endotoxin analysis that used the quantitative chromogenic *Limulus* amoebocyte lysate (LAL) assay.

The endotoxin concentration was reported as endotoxin units (EU)/filter. The endotoxin concentration in the air in each size range, $C_{\text{endotoxin-size } (i)}$, EU/m$^3$, was calculated as follows:

$$C_{\text{endotoxin-size } (i)} = \frac{E_{\text{filter } (i)}}{Qt}$$

(2-2)

where $E_{\text{filter } (i)}$ is endotoxin concentration on filter $i$ of the 13 ELPI filters (EU/filter), Q is the sampling flow rate of the sampler (0.03 m$^3$/min), and $t$ is the sampling time (120 min).

### 2.1.6. Viability of *P. fluorescens* in the test fluids

To investigate possible changes in the characteristics of *P. fluorescens* during the test, the viability of *P. fluorescens* was examined by measuring the culturable and total bacterial count at the following time points after the preparation of the test fluids: 0, 0.5, 1, 2, and 3 hours.

**Culturable microbial count in the fluid**

Immediately after taking the fluid sample, 1 ml of the fluid was diluted and cultivated on trypticase soy agar (DIFCO Laboratories, Detroit, MI) in triplicate. The culture plates with *P. fluorescens* were incubated at 26±2° C for 48 hours. The colony-forming units on plates were counted and an average of the three repeat cultures was used for the data analysis.

The culturable microbial count in the suspension was calculated as follows:
\[ C_{\text{cfu}} = (\text{cfu} / 10^{-n}) \times (V_1 / V_2) \quad (2-3) \]

where \( \text{cfu} \) is the average number of total colony-forming units on three culture plates, \( n \) is the dilution factor, \( V_1 \) is volume of the suspension analyzed (1 ml), and \( V_2 \) is the volume of diluted suspension spread on each plate (0.1 ml).

**Total microbial count in the fluid**

For determining the total count, 1 ml of the fluid sample was stained by the acridine orange method and filtered through a black polycarbonate filter, as described by Wang et al. (2000). An epifluorescence microscope was used to count the microorganisms on the filter at a magnification of 1000× (Model Laborlux S; E. Leitz, Inc., available from W. Nuhsbaum Inc., McHenry, IL). Forty randomly chosen microscopic fields were counted.

The total microbial count in the fluid, \( C_{\text{total}} \), was determined as follows:

\[ C_{\text{total}} = N \left( \pi R^2 / AV_3 \right) \quad (2-4) \]

where \( N \) is the average microbial count on each microscope field, \( R \) is the effective radius of the filter (8.5 mm), \( A \) is the area of the microscopic field (0.02404 mm\(^2\)), and \( V_3 \) is the volume of the suspension that was stained and filtered (1 ml).

The viability of \( P. \text{fluorescens} \) in the fluid is defined as the culturable count (\( C_{\text{cfu}} \)) divided by the total count (\( C_{\text{total}} \)):

\[ \text{Viability} = \frac{C_{\text{cfu}}}{C_{\text{total}}} \quad (2-5) \]
2.1.7. Validation of the laboratory simulator through field sampling

Prior to the laboratory experiments, the simulator was validated by comparing the particle size distributions generated by the device and measured with the OPC under laboratory conditions to the data obtained in the working environment. Field sampling was conducted at a grinding site in a plant, where semi-synthetic MWF was applied during the machining operation. The samples were collected at a stationary site as close to the workers’ breathing zone as possible without disturbing the normal working activities. The particle size distributions and number concentrations of the aerosol generated in the simulator were compared with those measured with the OPC in the field.

2.2. RESULTS AND DISCUSSION

Table 2-1 shows the size distribution characteristics of geometric mean diameter ($d_g$) and geometric standard deviation ($\sigma_g$) of the particles aerosolized in the simulator and measured in the field. The two size distributions were compared by comparing their geometric mean diameters using a student’s $t$ test as described by Baron and Willeke (Baron, 2001). The result showed that there is no significant difference between them ($p=0.25$) indicating that particles generated in the simulator represent well those in the field.

After the initial validation, the simulator was used for a detailed study on the properties of airborne particles aerosolized from MWF with and without microbial contamination. The results on the aerosolization from pure semi-synthetic MWF are presented in Figures 2-1 to 2-3. Figure 2-1 shows the effect of the tool rotation speed on the number concentration and size distribution of particles aerosolized from the pure
MWF. The particle number concentration, measured by the OPC is presented as a function of the particle optical diameter. As seen from Figure 2-1, the increase of the tool rotation speed increased the particle number concentration. For a specific rotation speed, the aerosol particle concentration increased with decreasing particle size in the measured particle size range of 0.3 to 20 µm. The trend was more pronounced with higher rotation speeds. The effect of the tool rotation speed on the mass concentration of particles aerosolized from semi-synthetic MWF was evaluated by converting the particle number concentration measured by the OPC into particle mass concentration \( C_m \) through the use of equation (2-1). Figure 2-2 shows the effect of tool rotation speed on the particle mass concentration and size distribution. Similar to the number concentration, the particle mass concentration increased with increasing rotation speed. Dasch et al. (2002) has shown a similar trend with soluble oil. As seen in Figure 2-2, the shape of the mass size distribution is essentially unaffected by the tool rotation speed, as was also reported by Heitbrink et al. (2000). The mode of the mass size distributions shown in Figure 2-2 was approximately 4.5 µm, which is lower than the 8-10 µm mode obtained in Heitbrink’s study. This may be caused by the different fluid type and different measurement instruments (Heitbrink et al. used soluble oil and performed their measurements with the aerodynamic particle sizer and MOUDI). The difference may also be associated with machining parameters, such as the fluid application rate and the tool diameter. Figure 2-3 shows that the number concentration of the aerosolized particles increased with increasing fluid application rate. At the same time, the particle number size distribution was not affected by the fluid application rate. This is consistent with the results reported by Heitbrink et al. (2000).
Our experiments conducted with the pure semi-synthetic MWF showed the same trends as what was reported in previous studies regarding the effect of tool rotation speed and fluid application rate on the mist concentration and size distribution (Dasch et al., 2002; Heitbrink et al., 2000; Thornburg, 2000). Thus, we concluded that our simulator was suitable to represent the aerosolization of mist during machining operations. Following this conclusion, experiments with microbiologically contaminated MWFs were initiated.

The properties of particles that were aerosolized from *P. fluorescens* suspension in semi-synthetic MWF and in water, respectively, were examined and compared with those obtained with pure semi-synthetic MWF not containing microbial contamination. The results are shown in Figures 2-4 to 2-6. Figure 2-4 presents the effect of the tool rotation speed (rpm) on the total particle number concentration for different fluids as measured by the OPC. The test results were compared for three fluids: pure semi-synthetic MWF, *P. fluorescens* suspension in water, and *P. fluorescens* suspension in semi-synthetic MWF. For a specific fluid type, the total number concentration of the aerosolized particles increased with increasing rotation speed. Among the different fluids, the total particle number concentrations were very close to each other and close to zero at speeds below 2000 rpm. The difference among the fluids increased with increasing the tool rotation speed. The lowest total particle number concentration was observed with pure semi-synthetic MWF and the highest with *P. fluorescens* suspension in semi-synthetic MWF. At 8000 rpm the total number concentration aerosolized from *P. fluorescens* suspension in semi-synthetic MWF was about 2 times of that from pure semi-synthetic MWF.
Figure 2-5 shows the effect of the tool rotation speed on the total mass concentration of particles aerosolized from the three different fluids as measured by the photometric aerosol mass monitor. They were pure semi-synthetic MWF, *P. fluorescens* suspension in water, and *P. fluorescens* suspension in semi-synthetic MWF. For a specific fluid type, the particle mass concentration increased with increasing tool rotation speed. The highest total particle mass concentration was measured with *P. fluorescens* suspension in semi-synthetic MWF and the lowest with *P. fluorescens* suspension in water. At 8000 rpm, the total mass concentration aerosolized from *P. fluorescens* suspension in semi-synthetic MWF was about 2 times of that from pure semi-synthetic MWF. As the diameter of the rod is 3.8 cm, the tool rotation speed of 8000 rpm corresponds to a surface speed of 3180 cm/s. The total mass concentration of particles generated in our simulator at this velocity and a fluid application rate of 1 l/min was 0.65 mg/m³. This is close to the value that has been obtained by Dasch et al. (2002) under similar laboratory conditions. In that study, the total mass concentration was 0.36 mg/m³ at a fluid application rate of 4.9 l/min and tool rotation speed of 2320 cm/s. Our mass concentration data are also consistent with those reported by Ball (1997) based on the measurements conducted in eight manufacturing plants: the mean value of the MWF mist concentration obtained in her study was 0.85 mg/m³.

Figure 2-6 shows the effect of the tool rotation speed on the concentration of fine particles aerosolized from the three different fluids as measured by the CNC. They were pure semi-synthetic MWF, *P. fluorescens* suspension in water, and *P. fluorescens* suspension in semi-synthetic MWF. Similar to the findings presented in Figures 2-4 and 2-5, within the fluid type, the fine particle concentration increased with increasing the
rotation speed. However, there was a more pronounced difference among the fluid types than observed with the OPC and the photometric aerosol mass monitor. A considerable increase occurred in the number of fine particles aerosolized from semi-synthetic MWF after the fluid was inoculated with *P. fluorescens*. For example, at 8000 rpm the fine particle number concentration from *P. fluorescens* suspension in semi-synthetic MWF was 50 times higher than for the pure semi-synthetic MWF.

The effect of microbial contamination on the aerosolization of fine particles was tested also with soluble oil. Figure 2-7 shows the effect of the tool rotation speed on the number concentration of fine particles aerosolized from fresh (non-contaminated) and contaminated soluble oil as measured by the CNC. Similar to semi-synthetic MWF, microbial contamination of soluble oil increased the aerosolization of fine particles. For example, at 8000 rpm the number concentration of fine particles aerosolized from *P. fluorescens* suspension in soluble oil was 15 times higher than that from fresh soluble oil MWF.

To further explore the properties of the fine particles aerosolized from contaminated MWFs, the ELPI was used to measure the size distribution of particles aerosolized from semi-synthetic MWF contaminated with *P. fluorescens* in the size range of 0.029 to 10.18 μm. The results are presented in Figure 2-8. The horizontal axis presents the aerodynamic particle diameter as measured by the ELPI, in contrast to previous figures, which referred to the optical diameter measured by optical instruments. To facilitate the comparison of the size distributions of particles generated from two suspensions (*P. fluorescens* in semi-synthetic MWF and *P. fluorescens* in water), each size distribution was normalized by its highest concentration. In this experiment, the tool rotation speed was 8000 rpm and the
fluid application rate was 1 l/min. The mode of the size distribution of particles aerosolized from the *P. fluorescens* suspension in water was 0.66 µm. This is consistent with the result of Qian et al. (1994), who reported that the aerodynamic diameter of *P. fluorescens* was 0.7 ± 0.1 µm. The mode of the other size distribution (representative *P. fluorescens* suspension in MWF) was about 0.37 µm. The two size distributions were compared using a chi-square test as described by Baron and Willeke (2001). The test showed a significant difference between them (p<0.01). The results of Figures 2-8 thus indicate that the microbial contamination of MWFs resulted in a shift in the size distribution of aerosolized particles towards a smaller particle size.

We suspect that these fine particles aerosolized from MWFs contaminated with microorganisms contain microbial fragments. Therefore, the endotoxin concentrations of particles at the fragments size range (0.37 µm) and at the intact bacterial cell size range (0.66 µm) aerosolized from *P. fluorescens* suspension in semi-synthetic MWF were measured. The results presented in Table 2-2 showed that the two size fractions contain the same amount of endotoxin. This suggests that the size fractions below the size of intact bacterial cells contain cell wall components of bacteria.

We had anticipated that these bacterial fragments presented in contaminated MWFs were a result of cell fragmentation and other processes associated with cell death after *P. fluorescens* was mixed with semi-synthetic MWF. The viability of *P. fluorescens* was therefore tested at t = 0, 0.5, 1, 2 and 3 hours after MWF was inoculated with the bacteria. The results are shown in Figure 2-9. The percentage of viable *P. fluorescens* cells in the water suspension remained around 50-55 % during the entire testing period. When mixed with MWF, the viability of *P. fluorescens* was around 42% at t = 0. A half hour after
inoculation the viability had dropped to 10%. After 3 hours, the viability had decreased to 0.3%. These data show that the viability of *P. fluorescens* in the suspension decreased very quickly after the cells were mixed with MWF (in contrast to water). This shows that MWF could kill bacteria in a very short time. However, the total microbial count of *P. fluorescens* in the MWF remained constant during the three-hour testing period. It was $3.08 \times 10^8$ cells/ml at $t = 0$ and $3.70 \times 10^8$ cells/ml at $t = 3$ hrs. The slight increase in the total bacterial count may be due to bacterial deagglomeration during mixing. However, the increase is within the measurement error range. As the total count did not significantly change, cell rupture in the suspension was not likely the source of fine aerosol particles.

A study by Terzieva et al. (1996) also showed an increased amount of cell fragments detected in the air due to continuous bacterial nebulization. The increase of fine particles may be an indicator of bacterial death or injury that is likely to occur in the grinding process causing bacterial slime, capsular material, cell wall, or cell membrane to be broken from the cells and subsequent leakage of intracellular components, such as DNA, RNA, Mg$^{2+}$, polysaccharides, proteins, and other nutrients (Ray, 1989; Terzieva, 1996). Another reason might be that MWFs contain small amount of surfactants. Surfactants can release material from bacteria by making the cell wall highly porous. After adding bacteria into the MWF, its surface tension may be changed. This might contribute to the increasing aerosolization of fine particles.
2.3. CONCLUSIONS FOR SPECIFIC AIM 2

A laboratory-scale simulator was used to investigate the properties of particles aerosolized from microbiologically contaminated MWFs. The simulator was validated by comparing the size distributions of particles generated with the simulator in the laboratory to those obtained in a machining plant where MWF was applied during a grinding procedure. After this, the simulator was used to examine the effects of tool rotation speed (ranging from 800 to 8000 rpm) and fluid application rate (ranging from 0.4 to 1.6 l/min) on the concentration of particles aerosolized from a pure semi-synthetic MWF (without microbial contamination). As expected, the concentration of aerosolized particles increased with increasing tool rotation speed and fluid application rate. The shape of the particle size distributions was not affected by these variables. After the testing with pure MWF was completed, the aerosolization from the semi-synthetic MWF contaminated by *Pseudomonas fluorescens* bacterial cells was studied. While there was a factor of 2 increase in the total particle number concentration and mass concentration above 0.3 µm, there was a very pronounced increase in the fine particle concentration (0.02-1 µm). This phenomenon was observed when the source (semi-synthetic or soluble oil MWF suspension) was contaminated with *P. fluorescens*. There was a 50 times increase in the fine particle aerosolization after the semi-synthetic MWF was contaminated with *P. fluorescens*. For the soluble oil, the increase was 15 times. The experiments with the ELPI showed that the mode of the size distribution of the fine particles was around 0.37 µm for the contaminated semi-synthetic MWF whereas it was 0.66 µm for the fresh semi-synthetic MWF. The endotoxin analysis showed that for the contaminated semi-synthetic MWF the particle size range representing fragments and
intact cells contain about the same amount of endotoxin. This suggests that the size
fractions below the size of intact bacterial cells contain bacterial fragments. The
significant increase in the concentration of fine particles might be caused by cell death or
injury. The latter was likely to result from the interaction of *P. fluorescens* with MWFs,
which might have caused bacterial slime, capsular material, as well as cell wall, or
membranes to be broken from the cells. This in turn led to the leakage of intracellular
components and thus contributed to the fine fraction after aerosolization from the fluid.
This hypothesis is supported by the experimental evidence that cell viability decreased
quickly after *P. fluorescens* was mixed with semi-synthetic MWF. The result of this
study indicates that the fine particle size fraction of the MWF mist may contain microbial
fragments that can not be detected by traditional microbiological methods, such as
microscopic counting and cultivation.

When newly prepared microorganisms are mixed with MWF in the laboratory they
might be more fragile than those that grow in the field and get adapted to MWFs. The
fluids in the field also contain more components such as machine oil, biocides. The
biocide in the fluid collected in the field might be killing the microorganisms in the
MWFs more effectively. Therefore the aerosolization process in the field might be more
complex. For this reason, the aerosolization of particles and microbial contaminants from
MWFs was also tested under two other conditions: 1. MWF collected in the field were
aerosolized with the MWF simulator (Specific Aim 3), 2. MWFs were aerosolized during
routine field operations (Specific Aim 4).
SPECIFIC AIM 3

INVESTIGATE THE PROPERTIES OF PARTICLES AEROSOLIZED FROM USED MWFS COLLECTED IN THE FIELD USING A LABORATORY-SCALE SIMULATOR

In Specific Aim 3, MWF collected in the field were aerosolized with a laboratory-scale simulator (MWF simulator) in the laboratory. The airborne particle concentrations and airborne endotoxin concentrations in different size fractions were monitored by an electrical low pressure impactor from 0.029 to 10.18 µm. A six-stage viable impactor was used to collect the airborne gram-negative bacteria in different size from 0.65 to 7 µm.

3.1. MATERIALS AND METHODS

3.1.1. Experimental setup

The experimental setup utilized in the first part of the study was a MWF simulator. MWF fluid samples were collected from plants using MWFS in their operations. Endotoxin concentration in the fluids was measured as described below and the fluid that had the highest endotoxin concentration was selected for the laboratory test. This fluid was a synthetic MWF, and contained 360 EU/ml of endotoxin. One endotoxin unit equals 0.1 milligrams. The total grease and oil concentration in the MWF was 3390 mg/l (0.34%). The following gram-negative bacteria were identified from the fluid: Comamonas testosterone/Pseudomonas alcaligenes, Alcaligenes xylosoxidans, Burkholderia cepacia, and Methylobacterium mesophilicum, among which Comamonas testosterone/Pseudomonas alcaligenes was the dominant one.
3.1.2. Experimental procedures

One liter of the test MWF was transferred into the simulator in the beginning of the experiment. The rotation rod was adjusted to 8000 rpm. The fluid application rate was 1 l/min. The airborne particle concentrations were continuously monitored by an ELPI for 120 minutes. At the same time, the airborne endotoxin in different sizes were continuously collected onto the 13 impactor stages of the ELPI for 120 minutes to determine the size distribution of endotoxin containing particles. At the end of the ELPI measurement, a six-stage viable impactor (Thermo-Anderson, Smyrna, GA) was connected with the MWF simulator to measure the size distribution of airborne gram-negative bacteria under the same experimental conditions. The sampling time of the viable impactor was 10 min. The entire test (ELPI + viable impactor) was repeated three times.

3.1.3. Measurements of airborne particle size distribution

The ELPI was used to continuously monitor the particle size distribution in a size range that included the fine and coarse aerosol particle fractions.

3.1.4. Measurements of airborne endotoxin concentrations in different particle sizes

The airborne endotoxin was collected onto polycarbonate filters (Millipore Inc. Billerica, MA) that were placed on all 13 impactor stages of the ELPI. After the collection, the filters were transferred into pyrogen-free tubes. They were sent to
DataChem Laboratories, Inc. for endotoxin analysis that used quantitative chromogenic *Limulus* amoebocyte lysate (LAL) assay.

The endotoxin concentration was reported as endotoxin units (EU)/filter. The endotoxin concentration in the air in each of the 13 size ranges, $C_{\text{endotoxin-size (i)}}$, EU/m$^3$, was calculated using equation 2-2.

### 3.1.5. Measurements of the concentrations of airborne gram-negative bacteria in different size ranges

The six-stage viable impactor was used to collect the airborne gram-negative bacteria in different size ranges. The 50% cut diameter for the 6 impactor stages are 0.65, 1.1, 2.1, 3.3, 4.7, and 7 $\mu$m. The impactor was operated at a flow rate of 28.3 l/min. Petri dishes containing eosine methylene blue agar supplemented with cycloheximide (0.5 g/l) were used for the collection. After the measurements, the Petri dishes were incubated at 25±2 ºC for 2-5 days.

Bacterial colonies were counted and the concentration of airborne gram-negative bacteria in each size fraction, $C_{\text{gram-neg-size(i)}}$, CFU/m$^3$, was calculated as follows:

$$C_{\text{gram-neg-size}} = \frac{N_{\text{stage(j)}}}{(Qt)}$$

where $N_{\text{stage(j)}}$ is the number of colony-forming units on stage j of the 6 stages after the count was adjusted to account for multiple impaction through holes on each stage (Macher, 1989), Q is the sampling flow rate of the sampler (0.0283 m$^3$/min), and t is the sampling time (10 min).
3.2. RESULTS AND DISCUSSION

Fig 3-1 shows the number size distribution of particles aerosolized from the used MWF as measured by the ELPI. As seen, the airborne particle concentration decreased with increasing particle size. At the size of 0.042 µm, the normalized particle concentration was $2.16 \times 10^9 \#/\text{m}^3$, and dropped almost linearly to $3.99 \times 10^7 \#/\text{m}^3$ at the size of 8.18 µm.

Fig 3-2 shows the concentrations of airborne endotoxin (y-axis on the left side) and airborne gram-negative bacteria (y-axis on the right side) as a function of particle aerodynamic size. The size distribution of endotoxin containing particles had two modes. One was at 2.45 µm coinciding with the mode of the size distribution of gram-negative bacteria. Another mode was in the smaller size range, at 0.39 µm. Most of the intact bacteria are larger than 0.5 µm. This indicates that the endotoxin in these small particle sizes could only come from bacterial fragments. Figs 3-1 and 3-2 confirmed our findings in Specific Aim 2 that microbial contamination of MWFs increases the aerosolization of fine particles and that these fine particles contain microbial fragments. The peak of endotoxin concentration at the particle size of 2.45 µm may be caused by bacterial agglomeration. An interesting correlation was shown between the endotoxin concentration curve and the gram-negative bacterial concentration curve in the size range of 0.84 to 5.74 µm. This indicates that at the particle size larger than the intact bacterial cells, the endotoxin were mainly from intact gram-negative bacteria.
3.3. CONCLUSIONS FOR SPECIFIC AIM 3

The laboratory test with MWF collected showed that the airborne particles contain high concentrations of fine particles and these fine particles were shown to contain microbial fragments.
SPECIFIC AIM 4

INVESTIGATE THE PROPERTIES OF AIRBORNE PARTICLES AEROSOLIZED FROM MWFS IN THE FIELD

The experiments were conducted when MWFs were aerosolized during routine field operations. The number size distribution of airborne particles and the airborne endotoxin concentrations in different sizes were measured using an electrical low pressure impactor. In addition, the microbial concentrations and total endotoxin concentrations in the air and in the MWFs as well as mass and number concentrations of airborne particles were measured during the field sampling.

4.1. MATERIALS AND METHODS

4.1.1. Field sites

Two facilities that utilize MWFs in their operations were chosen as the field sites for this study. Some workers complained of respiratory symptoms in Plant #1. Plant #2 had a history of outbreaks of hypersensitivity pneumonitis. In each plant, two machining areas and one reference area were chosen. The following criteria were applied for the selection of machining areas: possible microbial contamination, different machine operations, and different type of MWFs being used.

In Plant #1, sampling was performed in a turning lathe machining center, a milling machining center, and in reference area. There was no central ventilation system. Each machine had its own air filtration system and MWF tank. The turning lathe machining center was mainly used for cutting. Different rotation speeds and tools with different
diameters were used for the machining of different parts during the sampling. A semi-synthetic MWF used for this turning lathe center had not been changed for sometime. The milling center was used for drilling most of the time and occasionally for milling. The same semi-synthetic MWF was used in this center. The fluid in this machining center was changed 5 days prior to our sampling because an operator complained about the odor released from the fluid. The reference area was a conference room located in the office area about 20 meters away from the main machine area.

In Plant #2, the three sampling sites were: a large parts cell machining center, a small parts machining center, and a reference area. The machining processes in the large parts cell machining center involved milling the four edges of a square shaped large machine part and drilling 5 holes in the middle. The synthetic MWF used at this center was supplied by a central fluid system. The small parts machining center was primarily used for the milling and drilling of small parts. The semi-synthetic MWF used in this machine was from its attached sump. The reference area was a cafeteria located in the plant. This area was separated from the main machine site by two swinging doors.

In each machine site, our sampling station was placed at the location where workers spent most of their work time.

4.1.2. Measurement of airborne particles

The airborne particle concentrations were continuously monitored using a photometric aerosol mass monitor, an optical particle counter (OPC), a condensation nucleus counter (CNC), and an electrical low pressure impactor (ELPI). These instruments were described in Specific Aim 2. A one-minute averaging time was used for
all the instruments. The microbial concentration and total endotoxin concentration were collected with BioSamplers (SKC Inc., Eighty Four, PA).

The OPC was used to monitor the particle number concentration in 15 size fractions between 0.3 and 20 µm. The number concentrations of particles larger than 1 µm were reported as the total number concentration.

4.1.3. Microbial air samples

The total airborne endotoxin and microorganisms were collected with BioSamplers. The collection efficiency of the BioSampler filled with 20 ml of water is 79% for 0.3 µm particles, 89% for 0.5 µm particles, 96% for 1 µm particles, and 100% for 2 µm particles (Willeke et al., 1998). Filtered, autoclaved 0.01 M phosphate-buffer saline (PBS) containing 0.01% Tween 80 (SIGMA, St. Louis, MO) was used as the collection medium in the BioSamplers. During extended operations, the reservoir liquid in the BioSamplers evaporates, which can lead to collection efficiency reductions from reaerosolization and particle bouncing (Lin et al., 1999; Willeke et al., 1998). To keep collection efficiency constant, 4 ml of sterile phosphate buffer solution was added every half an hour into the collection vessel in the BioSampler to maintain the manufacturer’s recommendation level of 20 ml (Fabian, 2005). Immediately after the collection, samples were stored on ice to minimize the microbial growth. The samples were brought back to the laboratory at the end of the sampling period and stored in the refrigerator until they were processed the next day.
**Microbial cultivation of the BioSampler liquid**

In the laboratory, each liquid sample collected with the BioSampler was mixed thoroughly. The samples were diluted by serial dilutions. A 0.1 ml aliquot of each dilution was plated on triplicate onto different culture plates for different microorganisms. Total culturable fungal counts were determined by cultivating the samples on malt extract agar. Malt extract agar was supplemented with streptomycin sulphate (40 mg/l) to prevent the growth of bacteria. Total culturable count of bacteria was determined by cultivating the sample on tryptic soy agar and the total culturable count of gram-negative bacteria by cultivating on eosine methylene blue agar. Both tryptic soy agar and eosine methylene blue agar were supplemented with cycloheximide (0.5 g/l) to prevent the growth of fungi. All plates were incubated at 25±2 ºC for 2-5 days. Three field blanks and control plates were included for each set of samples.

**Endotoxin analysis from the BioSampler liquid**

Part of the BioSampler suspension (2ml) was transferred to 2 ml pyrogen-free tubes. The samples were kept on ice and sent to DataChem laboratory for endotoxin analysis by quantitative chromogenic *Limulus* amoebocyte lysate (LAL) assay. The endotoxin concentration was reported as endotoxin units (EU)/ml. The endotoxin concentration in the air, \( C_{\text{endotoxin-air}} \), EU/m³, was calculated as follows:

\[
C_{\text{endotoxin-air}} = \frac{E_{\text{fluid}} V}{Q t}
\]  

(4-1)

where \( E_{\text{fluid}} \) is endotoxin concentration (EU/ml), \( V \) is the volume of the sampling media of the BioSampler (20 ml), \( Q \) is the sampling flow rate of the sampler (0.0125m³/min), and \( t \) is the sampling time (120 min).
4.1.4. Measurements of airborne endotoxin concentrations in different particle sizes

The airborne endotoxin was collected onto polycarbonate filters (Millipore Inc. Billerica, MA) that were placed on all 13 impactor stages of the ELPI. The sampling time was 120 min. After the collection, the filters were transferred into pyrogen-free tubes. They were sent to DataChem Laboratories, Inc. for endotoxin analysis. The endotoxin concentration was reported as endotoxin units (EU)/filter. The endotoxin concentration in the air in each size range, $C_{\text{endotoxin-size (i)}}$, EU/m$^3$, was calculated using equation 2-2.

4.1.5. MWF samples

Bulk MWF samples were collected from each machining center on the testing day into 5-liter sterile, plastic containers and kept on ice. At the end of the field sampling day, they were brought back to the laboratory and kept in the refrigerator until processed the next day.

Total culturable count of fungi, bacteria and gram-negative bacteria of the fluid samples were analyzed similarly as the BioSampler samples described above. Endotoxin concentration in the fluid was analyzed similarly as described for the BioSamplers samples.
4.2. RESULTS AND DISCUSSION

Figures 4-1 to 4-4 show the results of field sampling in the facilities using MWFs in their operations. Figure 4-1 shows the number size distribution of airborne particles measured in Plant #1 and Plant #2. The data of the four machine sites in Plant #1 and Plant #2 and one reference area in Plant #2 were presented. There were two operations in the large parts site in Plant #2. They generated quite different levels of particles. As a result, the airborne particle concentration in that site was shown as operation 1 and operation 2. The number size distributions of airborne particles in the four machine sites in the field showed the same trend: the airborne particle concentrations were quite high in the small particle size range and the particle concentration decreased with increasing particle size. The airborne particle concentration in the reference area in plant #2 had the lowest concentration level in all the sizes. In addition, the particle concentration remained the same in the size range of 0.041 to 0.205 µm.

Figure 4-2 shows the airborne endotoxin concentration as a function of particle size measured in the three machine sites during the field testing. The data of the turning lathe center and the milling center in Plant #1 and the large parts site in Plant #2 are presented. These three machining sites were chosen to represent the working situation in these plants. For all the three machining sites the endotoxin concentrations were high in the submicrometer size range. For example, at the turning lathe center in Plant #1, the airborne endotoxin concentration was 4.72 EU/m³ at the particle size of 0.39 µm, and very close to the concentration at the size of 0.64 µm (6.94 EU/m³). At the large parts cell site in Plant #2, the airborne endotoxin concentration was 0.92 EU/m³ at the size of 0.39 µm, 2 times of that at the size of 0.64 µm (0.44 EU/m³). Most bacteria are larger
than 0.5 µm. It indicates that at this small particle size, this endotoxin could only come from gram-negative bacterial fragments. Figures 4-1 and 4-2 verified that the findings in Specific Aims 2 and 3 are true also in real field environments. There was a peak of the endotoxin concentration at the particle size of 2.45 µm in Figures 4-2. It may be caused by bacterial cell agglomeration.

Figure 4-3 shows the concentrations of bacteria and gram-negative bacteria in the MWFs (A) and in the air (B) measured in the two plants. As seen in Figures 4-3 (A), the bacterial concentrations in the MWFs varied in the four machining sites from the level below detection limit (<10 CFU/ml) to 3.2x10^5 CFU/ml. Most of the bacteria in the MWFs were found to be gram-negative. No fluid samples were collected in the two reference areas. As seen in Figure 4-3B, the bacterial concentrations in the air measured at the machine areas at the two plants varied from (5.2±1.1)x10^3 to (3.3±2.3)x10^5 CFU/m^3. There are no current standards or threshold limit values for the concentrations of microorganisms or endotoxin in the MWF exposure environments. Therefore, the microbial results have to be assessed against results from other studies. Table 4-1 shows the microbial and endotoxin values reported in the literature in the facilities using metalworking fluids. The airborne bacterial concentrations at the four machine areas in the two plants were in the same range as the data previously reported in the literature (Laitinen et al., 1999; Lewis et al., 2001; Thorne et al., 1996; Virji et al., 2000) but towards the higher ends. This indicates that the microbial contamination of the air in the machine areas in the studied plants is of concern. Although we did not find any bacteria in the fluid in the small parts site in Plant #2, the concentrations of bacteria and gram-negative bacteria in the air at this site were (7.4±2.1)x10^3 and (6.5±1.7)x10^3 CFU/m^3,
respectively. In this case, bacteria may come from the nearby operations. Neither bacteria nor gram-negative bacteria were detected in the air at the reference area in Plant #2. Fungi were not detected in any of the fluid or air samples.

Figure 4-4 shows the endotoxin concentrations measured in the MWFs (A) and in the air (B) in the two plants. The endotoxin concentrations in the fluids in the turning lathe center and in the milling center in Plant #1 were $3.1 \times 10^5$ EU/ml and $5.1 \times 10^4$ EU/ml. They were in the same range as the values reported in the literature (Laitinen et al., 1999; Lewis et al., 2001; Thorne et al., 1996; Virji et al., 2000) but towards the higher ends. This indicates that the fluids in the two machining sites of Plant #1 were heavily contaminated. The endotoxin concentrations in the fluids in the two machining centers of Plant #2 were low (360 and 180 EU/ml) They were in the same range as the levels reported in the literature (Laitinen et al., 1999; Thorne et al., 1996; Virji et al., 2000), but towards the lower end. This indicates that the fluids in the two machining sites of Plant #2 were only moderately contaminated.

The airborne endotoxin concentrations at the turning lathe center, milling center and reference area in Plant #1 were $83.1 \pm 12.4$, $17.3 \pm 2.7$, and $5.2 \pm 3.5$ EU/m$^3$, respectively. Thus, the endotoxin concentrations in the air at the turning lathe center, milling center, and the reference area were on average 16 and 3 times higher than in the reference room. According to the recommendations of the American Conference of Governmental Industrial Hygienists (ACGIH, 1999), control measurements should be taken when the endotoxin concentration in the air is 10 times the background level in the presence of respiratory symptoms among workers. In the absence of symptoms, the maximum action level is 30 times the background. As the operators working in that area complained about
respiratory symptoms, this shows that the air at the turning lathe center of Plant #1 was contaminated to the level of concern.

The endotoxin concentration measured by the BioSampler at the large parts site was extremely high (33417 ± 6826 EU/m³) compared to the values reported in the literature. However, the endotoxin concentration measured by the ELPI for particles larger than >1 µm was the lowest at the large parts site among the three sites included in the ELPI measurement, see Figure 4-2. At the same time, the size distribution had a local maximum at large particle size: 10.18 µm, which may contribute to the high total endotoxin concentration. As most of the total endotoxin mass was in the non-respirable size range, this set of measurements gives a good example of the value of size-selective endotoxin measurement for exposure assessment purposes.

Table 4-2 shows the mass concentration for the particle size range of 0.1 to 10 µm, total number concentration of large particles for the range of 1.0 to 20 µm, and the total fine particle concentration for the range of 0.02-1.0 µm for the aerosols measured in the two plants. At the four machining centers, the total fine particle concentrations (0.02 -1.0 µm) were 10³ to 10⁵ times higher than the total number concentration of larger particles (1.0-20 µm) in the same area. However, the total fine particle concentrations in the reference areas were almost the same as the total number concentrations of large particles measured in the same area.

Although microbial data of the air at the turning lathe center in Plant#1 indicated that this site was severely contaminated with microorganisms and endotoxin, the mass concentration of airborne particles at the site measured by a photometric mass monitor was below the NIOSH recommended exposure limit for MWF mist (0.5 mg/m³). The
mass concentration would be even lower if the gravimetric method was used for the measurements instead of the photometer (Simpson, 2003). This indicates that severe microbial contamination can exist even when the mist mass concentration is relatively low.

**4.3. CONCLUSIONS FOR SPECIFIC AIM 4**

MWF sites were found to be contaminated with high concentrations of fine particles and these fine particles were shown to contain microbial fragments. Severe microbial contamination was found even when the mist mass concentration was low. The results call for the size-selective measurement of particles and endotoxin for more comprehensive exposure assessment in MWF sites.
OVERALL CONCLUSIONS

This study showed that hydrophobic microorganisms were easier to aerosolize from MWFs than hydrophilic microorganisms and that increasing microorganism size was likely to result in decreasing aerosolization ratio. The fluid type and the microbial concentration in the fluid did not affect on the aerosolization ratio of microorganisms. The study consistently showed both in laboratory and field conditions that the mist aerosolized from MWFs contaminated with microorganisms contain high concentrations of fine particles and endotoxin in submicrometer size range. Field measurements indicated that severe microbial contamination can exist even when the airborne mist mass concentration is low. The results of this study are useful in the selection of MWFs and in the estimation of the relative microbial levels in the air based on their microbial properties and fluidborne concentrations. The results also show the importance of size-selective measurements in the exposure assessment in MWF environments. The results of this study calls for the size-selective measurement of particles and endotoxin for more comprehensive exposure assessment in MWF sites.
REFERENCES:


Wosten HAB, Vocht ML. Hydrophobins, the fungal coat unraveled. Biochimica et Biophysica Acta 2000; 1469: 79-86.
Table 1-1. The physical characteristics of the tested microorganisms.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Type</th>
<th>Aerodynamic size (Reference)</th>
<th>Cell surface hydrophobicity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. melinii</em></td>
<td>fungal spore</td>
<td>3.1 µm (Aizenberg et al., 2000)</td>
<td>hydrophobic (Wosten and Vocht, 2000)</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>bacterial vegetative cell</td>
<td>0.8 µm (Willeke et al., 1996)</td>
<td>hydrophilic (Madigan et al., 1996)</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>bacterial endospore</td>
<td>0.9 µm (Aizenberg et al., 2000)</td>
<td>hydrophobic (Doyle and Rosenberg, 1990)</td>
</tr>
</tbody>
</table>
Table 1-2. Viscosity of microorganism suspensions in two types of MWFs and in water as measured by a viscometer (25 °C).

<table>
<thead>
<tr>
<th>Fluid Type</th>
<th>Efflux Time (s)</th>
<th>Kinematic viscosity (mm$^2$/s$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microorganism in water</td>
<td>62±1</td>
<td>0.9728±0.0157</td>
</tr>
<tr>
<td>Microorganism in semi-synthetic MWF</td>
<td>66±1</td>
<td>1.0355±0.0157</td>
</tr>
<tr>
<td>Microorganism in soluble oil</td>
<td>73±1</td>
<td>1.1454±0.0157</td>
</tr>
<tr>
<td>Distilled H$_2$O</td>
<td>61±1</td>
<td>0.9571±0.0157</td>
</tr>
</tbody>
</table>
Table 2-1. Comparison of the geometric mean ($d_g$) and geometric standard deviation ($\sigma_g$) of the particles generated by the simulator in the laboratory with those measured in the field by an optical particle counter.

<table>
<thead>
<tr>
<th>Site</th>
<th>$d_g$ (µm)</th>
<th>$\sigma_g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory simulator*</td>
<td>0.54</td>
<td>1.80</td>
</tr>
<tr>
<td>Field (grinding area)</td>
<td>0.62</td>
<td>1.96</td>
</tr>
</tbody>
</table>

*rotation speed of 8000 rpm, semi-synthetic MWF.
Table 2-2. Endotoxin concentration of particles aerosolized from semi-synthetic MWF contaminated with bacteria. Particles number concentration was measured using an electrical low-pressure impactor (ELPI) and endotoxin analysis was performed from samples collected on two ELPI stages. The results represent an average and standard deviation of three repeats.

<table>
<thead>
<tr>
<th>Particle size fraction</th>
<th>Concentration (da= 0.36 µm)</th>
<th>Intact bacteria (da= 0.66 µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle number concentration (#/m³)</td>
<td>1.7 x10⁹</td>
<td>9.3x 10⁸</td>
</tr>
<tr>
<td>Endotoxin concentration (EU/m³)</td>
<td>13.1± 6.0</td>
<td>13.9 ± 4.4</td>
</tr>
</tbody>
</table>
Table 4-1. Microbial and endotoxin values reported in the literature for facilities using metalworking fluids.

<table>
<thead>
<tr>
<th>Fungi concentration in the bulk MWFs</th>
<th>Bacteria concentration</th>
<th>Gram-negative bacteria</th>
<th>Endotoxin concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50 CFU/ml</td>
<td>7- 7.6x10⁶ CFU/ml</td>
<td>--</td>
<td>39 – 1.7x10⁵ EU/ml</td>
<td>Throne, et al., 1996</td>
</tr>
<tr>
<td>--</td>
<td>5.0x10⁵-5.0x10¹⁰ CFU/ml</td>
<td>--</td>
<td>--</td>
<td>Virji et al., 2000</td>
</tr>
<tr>
<td>--</td>
<td>--</td>
<td>--</td>
<td>BD- 1.1x10⁶ EU/ml</td>
<td>Lewis et al., 2001</td>
</tr>
<tr>
<td>--</td>
<td>BD- 6.5x10⁸ CFU/ml</td>
<td>BD – 4.8x10⁶ CFU/ml</td>
<td>0.3 – 2.5x10⁵ EU/ml</td>
<td>Laitinen et al., 2000</td>
</tr>
</tbody>
</table>

Microbial concentration in the air

| BD- 3.8x10³ CFU/m³ (mean <60 CFU/m³) | 40- 4.0x10³ CFU/m³ | --                     | 4 - 790 EU/m³           | Throne et al., 1996 |
|--|1- 8.3x10³ CFU/m³|--|--|Virji et al., 2000|
|--|--|--|1.5 ± 1.0 EU/m³|Lewis, D., et al., 2001|
|--|30- 6.0x10⁴ CFU/m³|1- 4.1x10⁴ CFU/m³|0.4 – 600 EU/m³|Laitinen et al., 2000|

BD: Below detection
-- : Not reported
<table>
<thead>
<tr>
<th>Sampling area:</th>
<th>Mass (0.1-10 µm) (mg/m³)</th>
<th>Total number (1.0-20 µm) (#/m³)</th>
<th>Total fine particle (0.02-1.0 µm ) (#/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plant #1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turning lathe center</td>
<td>0.45 ± 0.11</td>
<td>(1.1 ± 0.2) x10⁷</td>
<td>(1.73 ± 0.3) x10¹¹</td>
</tr>
<tr>
<td>Milling center</td>
<td>0.48 ± 0.11</td>
<td>(1.4 ± 1.2) x10⁷</td>
<td>(9.5 ± 3.0) x10¹⁰</td>
</tr>
<tr>
<td>Reference</td>
<td>0.11 ± 0.03</td>
<td>(5.7 ± 0.8) x10⁵</td>
<td>(6.6 ± 0.6) x10¹⁰</td>
</tr>
<tr>
<td><strong>Plant #2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large parts site (operation1)</td>
<td>4.33± 4.10</td>
<td>(3.1 ± 0.7) x10⁴</td>
<td>(2.9 ± 1.1) x10¹¹</td>
</tr>
<tr>
<td>Large parts site (operation2)</td>
<td>0.56 ± 0.37</td>
<td>(2.6 ±0.6) x10⁷</td>
<td>(9.8 ± 5.3) x10¹⁰</td>
</tr>
<tr>
<td>Small parts site</td>
<td>0.14 ± 0.07</td>
<td>(5.8 ± 0.3) x10⁶</td>
<td>(1.1 ± 0.3) x10¹¹</td>
</tr>
<tr>
<td>Reference</td>
<td>0.09 ± 0.04</td>
<td>(5.5± 2.9) x10⁵</td>
<td>(1.7 ± 0.2) x10¹⁰</td>
</tr>
</tbody>
</table>
Fig. 1-1. Experimental set-up for the aerosolization of microorganisms from Metalworking Fluids (MWFs) by a Collison nebulizer.
Fig 1-2. Laboratory-scale MWF simulator for the aerosolization of microorganisms from metalworking fluids
P. melinii

Fig. 1-3. Effect of the fluid type on the aerosolization of P. melinii spores (Collison nebulizer, air pressure=15 psi).
B. subtilis  
(y=0.4404x  \( r^2=0.951 \)  p-value<0.001)

P. fluorescens  
(y=0.2206x  \( r^2=0.950 \)  p-value<0.001)

P. melinii  
(y=0.2069x  \( r^2=0.884 \)  p-value=0.017)

Fig. 1-4. Aerosolization of microorganisms from water suspension of different microbial concentration (Collison nebulizer, air pressure= 12 psi).
Aerosolization ratio of microorganisms

\[
\text{org/m}^3 \text{ in air}/\text{org/ml in liquid}
\]

0.0
0.2
0.4
0.6
0.8
1.0

P. fluorescens
P. melinii

Fig. 1-5. Effect of microorganism type on the aerosolization from semi-synthetic MWF (MWF simulator).
Fig. 1-6. Effect of particle size on the aerosolization of polystyrene latex (PSL) particles from water suspension by the Collison nebulizer and by the MWF simulator.
Fig. 2-1. The effect of tool rotation speed (rpm) on the number concentration and size distribution of particles aerosolized from semi-synthetic MWF. The data were collected with an optical particle counter. Each data point indicates an average of five-minute measurement. Standard deviations are so small that they do not show in the figure.
Fig. 2-2. The effect of tool rotation speed (rpm) on the mass size distribution of particles aerosolized from semi-synthetic MWF. The number concentration was measured by an optical particle counter and converted to mass concentration assuming that the density of particles was 1 g/cm³. Each data point indicates an average of five-minute measurement. Standard deviations are so small that they do not show in the figure.
Fig. 2-3. The effect of fluid application rate on the number concentration and size distribution of particles aerosolized from semi-synthetic MWF, as measured by an optical particle counter. Each data point indicate an average of five-minute measurement and the error bars indicate one standard deviation.
Fig. 2-4. The effect of tool rotation speed (rpm) on the total number concentration of particles aerosolized from *Pseudomonas fluorescens* suspension in semi-synthetic MWF as measured by an optical particle counter. Each data point indicates an average of five-minute measurement and the error bars indicate one standard deviation.
Fig. 2-5. The effect of rotation speed (rpm) on the mass concentration of particles aerosolized from *Pseudomonas fluorescens* suspension in semi-synthetic MWF, as measured by an aerosol photometer. Each data point indicates an average of five-minute measurement and the error bars indicate one standard deviation.
Fig. 2-6. The effect of tool rotation speed (rpm) on the number concentration of particles aerosolized from *Pseudomonas fluorescens* suspension in semi-synthetic MWF as measured by a condensation nucleus counter. Each data point indicates an average of five-minute measurement and the error bars indicate one standard deviation.
Fig. 2-7. The effect of tool rotation speed (rpm) on the number concentration of fine particles aerosolized from *Pseudomonas fluorescens* suspension insoluble oil as measured by a condensation nucleus counter. Each data point indicates an average of five-minute measurements and the error bars indicate one standard deviation.
Fig. 2-8. The normalized number size distributions of particles aerosolized from different fluids at a tool speed of 8000 rpm, as measured by an electrical low pressure impactor. Each size distribution was normalized by its highest concentration. Each data point indicates an average of five-minute measurement and the error bars indicate one standard deviation.
Fig. 2-9. Viability of *P. fluorescens* cells after mixing with water and semi-synthetic MWF. Each data point indicates an average of three repeats and the error bars indicate one standard deviation.
Laboratory Simulator

![Graph showing the number size distribution of particles aerosolized in the laboratory simulator as measured by an electrical low-pressure impactor.](image)

**Fig. 3-1.** Number size distribution of particles aerosolized in the laboratory simulator as measured by an electrical low-pressure impactor.
Fig. 3-2. Airborne endotoxin concentration and gram-negative bacterial concentration as a function of particle size. The experiment was performed in the laboratory simulator using an electrical low-pressure impactor for the collection of endotoxin and a six-stage viable impactor for the collection of gram-negative bacteria.
Fig. 4-1. Number size distribution of particles in the field sites as measured by an electrical low-pressure impactor (P#1=Plant #1, P#2=Plant #2).
Fig. 4-2. Airborne endotoxin concentration as a function of particle size in the field sites as collected by an electrical low-pressure impactor (P#1 = Plant #1, P#2 = Plant #2).
Field

**A. MWF**

- **Bacteria**
- **Gram-negative bacteria**

**B. Air**

- **Bacteria**
- **Gram-negative bacteria**

![Graph showing microbial concentration in MWF and air](image)

**Detection limit**

Fig. 4-3. Concentrations of bacteria and gram-negative bacteria in the MWFs (A) and in the air (B) in the field sites (BD-below detection limit, N/A non-applicable as no MWF fluid was used in the reference areas).
Field

Fig. 4-4. Endotoxin concentrations in the MWFs (A) and in the air (B) in the field sites (N/A-non applicable as no MWF fluid was used in the reference areas).