

# EXPOSURE ASSESSMENT and SAMPLING PLENARY SESSION

## Part II: Exposure Assessment of the Biological Components in Metalworking Fluids

### Session Arranger / Moderator:

**TAI L. CHAN, PhD, CIH**, Mgr. Occup. Health & Safety Research, General Motors Corporation

**HOWARD J COHEN, PhD, CIH**, Professor, University of New Haven; Editor, AIHA Journal

**DANIEL LEWIS, PhD**, Chief, Immunology Branch, NIOSH

**DAVID A. SCHWARTZ, MD, MPH**, Professor, Dept. of Medicine, University of Iowa

### Technical Presenters:

**KLAUS WILLEKE, PhD, CIH and SERGEY A. GRINSHPUN PhD**, Professors in the Department of Environmental Health, University of Cincinnati

*CURRENT METHODS and ADVANCES in BIOAEROSOL SAMPLING*

**JANET M. MACHER, ScD, MPH**, Air Pollution Research Specialist, California Dept. of Health Sciences

*STATISTICAL CONSIDERATIONS in BIOAEROSOL SAMPLING*

**MIRIAM K. LONON, PhD**, Research Microbiologist, NIOSH

*BACTERIA in METALWORKING FLUIDS*

**HARIET A. BURGE, PhD**, Associate Professor, Harvard School of Public Health

*MICROBIOLOGY of METALWORKING FLUIDS*

**DONALD K. MILTON, MD, DrPH**, Asst. Professor of Occup. Medicine, Harvard School of Public Health

*ENDOTOXIN EXPOSURE ASSESSMENT in MACHINING OPERATIONS*

**PETER S. THORNE, PhD**, Assoc. Professor of Toxicology and IH, University of Iowa

*BIOAEROSOLS and AIRBORNE ENDOTOXINS in AUTOMOTIVE  
MACHINING PLANTS*

## Current Methods and Advances in Bioaerosol Sampling

Klaus Willeke and Sergey A. Grinshpun

Aerosol Research Laboratory, Department of Environmental Health,  
University of Cincinnati, P.O. Box 670056, Cincinnati, OH 45267.

### ABSTRACT

The goal of this research has been and continues to be the performance evaluation of existing bioaerosol samplers and the development of new ones. The collection efficiencies of several commercially available and newly developed impactors and liquid impingers have been measured with PSL particles ranging from 0.5 to 5  $\mu\text{m}$  in aerodynamic diameter and with different microorganisms, such as *Pseudomonas fluorescens*, *Micrococcus luteus*, and *Bacillus cereus*, aerosol-ized by a Collison nebulizer. The bioaerosol samplers were operated at flow rates ranging from 2 to 40 L/min. An aerodynamic particle size spectrometer (Aerosizer) measured the number of particles and the particle size distribution upstream and downstream of the sampler.

It was found that a high sampling flow rate may result in a high impaction/impingement velocity and thus a high collection efficiency. At the same time, it may cause microbial stress, which reduces the number of microorganisms recovered after collection and therefore decreases the collection efficiency of the viable sampler. The survival of microorganisms collected in agar surface impactors was found to be a non-linear function of the impaction velocity. For sufficiently high flow rates, the bacterial stress increased with increasing sampling flow rate. However, at the lowest flow rates (resulting in the lowest collection efficiencies), the collected bacteria survived less readily than at the slightly higher flow rates. We attribute this finding to insufficient embedding of the microorganisms in the agar when sampling was conducted at low impaction velocities. When studying the collection efficiency of impingers, we found that it may be significantly affected by microbial bounce and re-aerosolization from the collection fluid. At

higher impinger flow rates, we observed that the shallow liquid layer under the capillary is removed by the pressure created by the air jet, and particles are impacted directly onto the bottom of the vessel from which they may bounce. In the latter case, the particles may either escape with the effluent air flow or be impacted into the liquid which is pushed against the glass wall of the collection vessel. Another effect that was observed during microbial sampling into impingers - the bursting of air bubbles at the top of the liquid - may result in significant aerosolization of some of the collection fluid and its contents, which reduces the impinger collection efficiency.

### INTRODUCTION

Assessment of the hazards due to biocontaminants released by metal working fluids requires sampling techniques that, on the one hand, provide a high collection efficiency of airborne microorganisms, and, on the other hand, allow optimum recovery of the collected microbial particles with minimum death or injury to the organisms. Available bioaerosol sampling techniques include impaction, impingement and also other techniques, such as filtration, electrostatic precipitation, gravity settling and cyclone scrubbing. The differences in the physical and bio-logical sampling efficiencies of existing bioaerosol samplers (the variability in samplers' inlet and collection geometry, sampling flow rate, and microbial recovery characteristics) often cause difficulties and confusion when comparing results obtained with these devices. Therefore, reliable and accurate methods for sampling and analyzing airborne microorganisms in the metal working environment are needed.

### New Bioaerosol Samplers and Comparison

### Tests with Existing Samplers

A new concept has been developed for the sampling and evaluation of bioaerosol samplers, involving sampling from the ambient air into the inlet, collection onto solid or into liquid media, and physical and microbiological analyses. Three new samplers were designed with the same inlet and collection geometry but with different collection media - a glass slide, a moving agar-coated slide, and a liquid, as shown in Figure 1.<sup>(1)</sup>

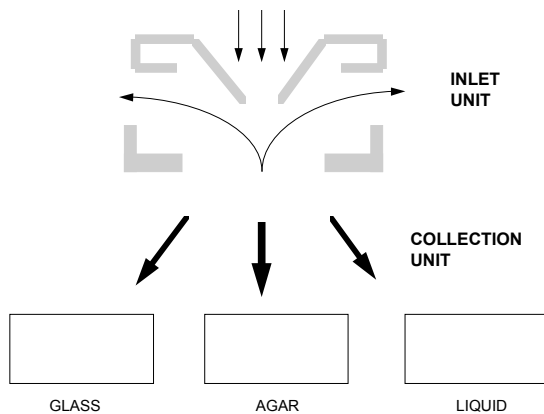


Figure 1. Three microbial collection techniques (schematic presentation).

They were evaluated as to their inlet and particle collection efficiencies, as well as their abilities in maintaining the viability of microorganisms. Our recently-developed semi-empirical models for the aspiration and transmission efficiencies of samplers,<sup>(2-4)</sup> and the counting efficiency of colonies on an agar surface<sup>(5)</sup> were used for the theoretical evaluation, and our new Bioaerosol Sampler Evaluation Facility<sup>(6)</sup> was used for the experimental work. The same performance characteristics were also determined for several conventional bioaerosol impactors (Andersen Two-Stage and Six-Stage Viable Samplers, Mattson-Garvin Air Sampler, and Casella Slit Sampler) and impingers (AGI-4 and AGI-30). The optimal sampling regimes and the appropriate collection times were determined for the new and conventional samplers.

The laboratory tests were performed with biologically inert PSL particles of typical bacterial size (from 0.3 to 2.0  $\mu\text{m}$ ) and with micro-

organisms, such as *Pseudomonas fluorescens* ATCC 13525, *Micrococcus luteus* ATCC 4698, *Bacillus cereus* ATCC 11778, and *Bacillus subtilis* ATCC 6051. A new aerodynamic particle sizer (Aerosizer, Amherst Process Instruments, Inc., Hadley, MA) was used to measure the particle concentration upstream and downstream of the sampler and to determine the collection efficiency of the sampler. This instrument, evaluated with several microorganisms, was found to be well-suited for measuring the sizes and concentrations of bacteria down to an aerodynamic particle size of 0.5  $\mu\text{m}$ . The physical characteristics of the test bacteria were determined using in parallel aerodynamic particle sizing (Aerosizer) and optical particle sizing (LAS-X, Particle Measuring System, Inc., Boulder, CO).<sup>(7)</sup> The LAS-X, measuring an optical particle diameter range of 0.1-3.0  $\mu\text{m}$ , was used to obtain information on the residue particles resulting from the nutrients, salt and cellular debris in dried droplets which did not contain bacteria. The laboratory evaluation and intercomparison of the bioaerosol samplers' performance showed that the sampling efficiencies of the new samplers are better than those of the commonly used ones. This laboratory study resulted in several design modifications of our new samplers, such as the moving slide for the new agar-slide impactor.<sup>(1)</sup> The samplers' calibration, flow rate monitoring, decontamination, and cleaning procedures were also evaluated.

Our preliminary field studies, which followed the bioaerosol samplers' laboratory tests, suggested further modifications to improve the sampling efficiencies of the new samplers. For example, it was found that the distance from the slit to the collection surface for the agar-slide impactor needed to be increased by several millimeters. This increase provided bacterial and fungal colonies room to grow after collection. The field studies focused primarily on the ability of the agar-slide impactor to recover airborne bacteria and fungi in comparison with available high-volume (Compact Surface Air Sampler, SAS) and rotating plate (Mattson-Garvin)

impactors in an office air environment under quiescent and aggressive (during carpet vacuuming) conditions. In addition, the agar-slide impactor was tested in outdoor environments where the mean fungal concentration was in the range of  $10^2$  -  $10^3$  CFU/m<sup>3</sup>. The preliminary field data indicate that the new agar-slide impactor performs well compared to the other samplers, relative to its surface area and reproducibility of results and has the potential to be either an environmental or a personal bioaerosol sampler. The new bioaerosol impactors and impingers, as well as conventional ones, were also used to study the physical and microbiological aspects of collection and survival.

### Microbial Collection on Glass Surface

The new glass-slide impactor was used to study the collection of sub- and supermicrometer PSL particles on the solid surface at sampling flow rates varying from 2 to 16 L/min. The collection efficiency of PSL particles of 1  $\mu$ m in diameter was 35-38% when sampling directly onto the glass surface. Addition of a special coating to the glass slide resulted in an increase of the collection efficiency of these particles to 92-97%, because it significantly reduced the particle bounce from the collection media and reentrainment into the air. Similar results were obtained for *P. fluorescens*, but the bounce effect was found to be less pronounced than with PSL particles.

### Microbial Collection on Agar Surface

The new moving agar-slide impactor, operated at a flow rate varying from 3.8 to 40 L/min, was employed to study the recovery of aerosolized *P. fluorescens* (relatively sensitive organism) and *M. luteus* (relatively resistant to stress) as a function of the impaction velocity. The effect of impaction on microbial stress was quantified experimentally by combining dynamic particle-sizing methods with microbial colony enumeration. The survival of collected microorganisms was found to be a non-linear function of the impaction velocity.<sup>(8)</sup> The highest relative

recoveries for *P. fluorescens* (ca. 51%) and *M. luteus* (ca. 62%) were obtained on a non-selective medium (TSA) at impaction velocities of 40 and 24 m/s, respectively. Bacterial injury data, obtained with different selective media (Minimal salts glucose agar, MacConkey agar and TSA containing 5% NaCl), showed generally greater metabolic and structural injuries for the higher impaction velocities.

The effect of impaction stress on microbial recovery on an agar surface was found to depend not only on impaction velocity, but also on the degree to which the microorganisms may be embedded in the collection medium. The act of microbial collection causes immediate "mechanical" stress which is higher at the higher impaction velocities. However, an additional "desiccation" stress may occur during the time that the microorganism is "sitting" on the collection medium. Collection of microorganisms at lower impaction velocities may result in a "soft landing," while at higher impaction velocities the particles may become embedded in the agar. The organisms, which adhere to the top of the nutrient surface after their "soft landing," experience increased drying due to the sampling air stream and have a limited ability to obtain the nutrients, moisture, and warmth during the time the sampler operates. This results in microbial stress, and therefore, a decreased chance of surviving and growing into colonies. The microorganisms embedded in the agar are expected to experience lower stress during the sampling time. The interaction of "mechanical" and "desiccation" stress on the microbes during collection on an agar surface explains the non-monotonic function of microbial recovery versus sampling flow rate found in our experiments.

The new agar-slide impactor was also used to determine the optimal sampling time for different impaction regimes (which cause different degrees of microbial stress). The moving slide in this sampler allowed us to test the masking effect and counting efficiency directly (when microbial stress is the same, and colony count is affected only by the colony surface density). We found the

microbial surface density to be directly dependent on four factors (bioaerosol concentration in the sampled air, sampler air flow rate, sample collection time, and collection surface area) and indirectly dependent on two factors (nutrient concentration and incubation conditions). Microbial colony diameter, in turn, was found to be a function of nutrient concentration, incubation time and temperature, and colony surface density. Colony size is also a function of the specific organism studied, its growth rate and pattern, and cell motility.

### Microbial Collection in Liquid

The collection efficiency of liquid impingers was measured over a wide range of sampling conditions. Our Bioaerosol Sampler Evaluation Facility has been modified by adding a needle valve to the sampling line in order to simulate the pressure drop across the impinger. A dry-air dilution system was also added to avoid the saturation effect downstream of the impinger, which may lead to significant vapor condensation on the particles and, therefore, may decrease the accuracy of the particle size distribution measurements downstream of the impinger. Different types of bioaerosol impingers were used in our studies, including the well-known AGI-30 and AGI-4 (which exemplify surface and subsurface impingement, respectively) and our newly developed slit impinger.

The impingers draw air through an inlet tube with a limiting capillary orifice through which particles in the air impinge into the liquid. This accelerates the particles to high velocity (in some devices to approximately sonic speed). Analogous to solid-surface aerosol impactors, this impaction is usually referred to as the driving mechanism for particle collection in liquid impingers. Therefore, the impinger collection efficiency is expected to be a monotonically increasing function of the particle inertia. However, we observed some irregularities in the impinger collection efficiency. For example, the new impinger collected the 1.1  $\mu\text{m}$  PSL particles with efficiencies increasing from 10 to 85% when

the flow rate was increased from 2 to 5 L/min, but further flow rate increases resulted in a collection efficiency reduction to 65% at 7 L/min followed by an increase to 90-95% at 10 L/min. Similar irregularities were found for conventional All Glass Impingers.

The collection efficiency measurements were performed, first, with the AGI-4 containing volumes of 20 mL and 1.5 mL of deionized water, representing subsurface and surface impingement, respectively. In our tests, the AGI-4 was operated at different sampling flow rates, ranging from 2 to about 13 L/min, with PSL particles and bacteria spores having approximately the same aerodynamic particle size. At a volume of 20 mL (the volume of collection fluid usually recommended for All Glass Impingers) and a relatively low sampling flow rate of 2 L/min, more than 50% of the PSL particles and approximately 70% of *B. cereus* spores were collected into the liquid. At increased sampling flow rates ranging from 6 to 10 L/min, the impinger collection efficiency was found to be above 90% for both the PSL and bacterial particles. Such values are theoretically expected based on the impaction mechanism of the particle collection. At the same time, a further increase of the flow rate to about 13 L/min did not improve the particle collection efficiency, but resulted in a slight decrease in the collection efficiency at 12.5 L/min. We believe that this phenomenon was caused by the bursting of air bubbles at the top of the liquid which resulted in the reaerosolization of some particles. The droplets resulting from burst bubbles may contain previously collected particles. This mechanism of particle escape during the subsurface impingement explains the collection efficiency reduction at higher sampling flow rates.

An unexpected impinger collection efficiency was also found for the AGI-4 when tested at a low level of deionized water (1.5 mL). At relatively low sampling flow rates ranging from 2 to 4 L/min, the collection efficiency was 40 and 50% for *B. cereus* spores and PSL particles respectively. However, a further increase of the flow rate to 4 - 8 L/min resulted in irregular

collection efficiencies for both types of airborne particles. The following explanation is suggested: with the flow rate increase, the shallow liquid layer under the capillary is removed by the pressure created by the air jet, and particles are impacted directly onto the bottom of the vessel from which they may bounce. The bouncing particles may either escape with the effluent air flow or be impacted into the liquid which is pushed against the glass wall of the collection vessel. In our experiments, at sampling flow rates between 4 and 8 L/min (the exact range depends on the bounce properties of the tested aerosol particles), the particle kinetic energy was high enough to allow particles to bounce from the vessel bottom. Thus, some of the particles escaped from the impinger with the effluent air flow, causing a decrease in the collection efficiency. At higher flow rates (6 to 10 L/min), the particles may bounce several times from the two opposite surfaces, the vessel bottom and the lower surface of the thick-walled capillary. Due to such multiple bounce, the particle kinetic energy is reduced significantly, so that the particles are finally impacted onto either the dry or wet surfaces of the collection vessel. This resulted in the higher collection efficiency values that we have observed at flow rates between 8 and 13 L/min.

When using impingers, we observed bubbles that can collect particles onto their surface rising through the liquid. When the bubbles burst, jet droplets, enriched with suspended materials, can form new aerosols. The mechanism of aerosol generation by the bursting bubbles was also studied in our laboratory using an impinger containing phosphate buffer solution. We tested the AGI-4 containing 20 mL of this solution without microorganisms and operated at 12.5 L/min in a filtered air environment. A high concentration of aerosol particles ( $10^6$  to  $10^8$   $m^{-3}$ ) was measured for the high and low air humidities. Our preliminary calculations have shown that the impinger containing phosphate buffer may generate 6 to 10  $\mu m$  liquid droplets. Droplets of this size may contain a solid residue of 1  $\mu m$  in

diameter (typical size of a single bacterium) or greater. Additional analyses of the data will be performed to describe the phenomenon of the microbial reaerosolization in impingers.

The collection efficiency of the new slit impinger was also measured at different flow rates (2 to 13 L/min). The air jet velocity in our new impinger was found to be about four times lower than that of the AGI-4 and AGI-30. This reduces the particle bounce effect and improves the microbial recovery characteristics of the sampler. Because of the bubble bursting observed, we had expected particle bounce, rather than reaerosolization, to be the predominant factor affecting the impinger collection efficiency. Similar to the results obtained with the AGI-4, the collection efficiency of our impinger was found to be a non-monotonic function of the sampling flow rate.

## CONCLUSIONS

This study compared three collection techniques widely used for bioaerosol sampling - impaction on a glass slide and on an agar surface and impingement into a liquid. The effect of impact stress on microbial recovery in agar-surface impactors was investigated and found to be dependent on impaction velocity and the degree to which the microorganisms may be embedded in the collection medium. Particle bounce and reaerosolization were observed, experimentally investigated during our study of aerosol sampling with impingers, and found to affect the collection efficiency of impingers. These findings on bioaerosol impaction and impingement contribute to the development of new methods and modification of conventional techniques for the accurate measurement of microorganisms in different air environments.

## REFERENCES

1. **Juozaitis, A; K Willeke, SA Grinshpun, and J Donnelly:** Impaction onto a glass slide or agar versus impingement into a liquid for the collection and recovery of airborne microorganisms. *Appl. Environ. Microbiol.*

- 60: 861-870 (1994).
2. **Grinshpun, SA; K Willeke, and S Kalatoor:** A general equation for aerosol aspiration by thin-walled sampling probes in calm and moving air. *Atmos. Environ.* 27:1459-1470 (1993).
  3. **Grinshpun, SA; CW Chang, A Nevalainen, and K Willeke:** Inlet characteristics of bioaerosol samplers. *J. Aerosol Sci.* 25:1503-1522 (1994).
  4. **Hangal, S; K Willeke:** Overall efficiency of tubular inlets sampling at 0 to 90 degrees from horizontal aerosol flows. *Atmos. Environ.* 24:2379-2386 (1990).
  5. **Chang, CW; YH Hwang, SA Grinshpun, JM Macher, and K Willeke:** Evaluation of counting error due to colony masking in bioaerosol sampling. *Appl. Environ. Microbiol.* 60:3732-3738 (1994).
  6. **Thompson, M; J Donnelly, SA Grinshpun, A Juozaitis, and K Willeke:** Method and test system for evaluation of bioaerosol samplers. *J. Aerosol Sci.* 25:1579-1593 (1994).
  7. **Qian, Y; K Willeke, V Ulevicius, SA Grinshpun, and J Donnelly:** Dynamic size spectrometry of airborne microorganisms: laboratory evaluation and calibration. *Atmos. Environ.* 29:1123-1129 (1995).
  8. **Stewart, SL; SA Grinshpun, K Willeke, S Terzieva, V Ulevicius, and J Donnelly:** Effect of impact stress on microbial recovery when sampling on an agar surface. *Appl. Environ. Microbiol.* 61:1232-1239 (1995).

#### ACKNOWLEDGMENT

This study was supported by the U.S. Environmental Protection Agency through Cooperative Agreement No. CR822065.

## Statistical Considerations in Bioaerosol Sampling

Janet M. Macher (A), Kai-Shen Liu (A), and Yu-Lin Chang (B)

(A) Environmental Health Laboratory Branch, California Department of Health Services,  
2151 Berkeley Way, Berkeley, California 94704-1011

(B) Biostatistics Department, Roche Labs, Palo Alto, California 94303

### ABSTRACT

Understanding the generation of bioaerosols from metal-working fluids provides the basis for evaluating potential adverse health effects and for recommending engineering controls and work practices to reduce or eliminate harmful exposures. Investigators studying bioaerosols apply statistical methods in three broad areas: (1) study design; (2) quality assurance during sample collection and analysis; and (3) data analysis and interpretation. The exact statistical methods investigators use vary from situation to situation and often involve professional judgement on the parts of the epidemiologists, medical professionals, statisticians, microbiologists, and aerosol specialists involved. This paper discusses: (1) potential sources of error in bioaerosol sampling; and (2) approaches to recognizing and minimizing such errors to obtain the best estimates of bioaerosol exposures.

### INTRODUCTION

Growth of bacteria and fungi in metalworking fluids (MWFs), as a result of storage and use, is a concern because the microorganisms can degrade the fluid and generate odors, and because biocides often are needed to control microbiological growth. Microorganisms and their metabolites can also become aerosolized along with MWF mists<sup>(1-2)</sup> and may cause respiratory problems for exposed workers. Microorganisms isolated from MWFs include aerobic and anaerobic bacteria and molds and yeasts.<sup>(1)</sup> Health effects associated with exposure to bioaerosols include infectious diseases (e.g., Legionnaires' disease and other pneumonias), hypersensitivity diseases (e.g., hypersensitivity pneumonitis and allergic asthma),

irritant effects (mucous membrane irritation), and toxic effects (e.g., endotoxemia and mycotoxicosis).<sup>(3-4)</sup>

Investigators use statistics when studying bioaerosols: (1) to design studies; (2) to ensure data quality; and (3) to analyze the data, e.g., to summarize data, to test hypotheses, and to develop predictive models. Eudey *et al.*, in a chapter on biostatistics in a book on bioaerosols, mention that investigators in problem-solving studies involving bioaerosols have often failed to consider the statistical principles that allow them to draw conclusions about the sampled populations.<sup>(5)</sup> Other chapters in recent books on bioaerosols address the statistics involved in studying particle size distribution<sup>(6)</sup> and bioaerosol dispersion.<sup>(7)</sup> This paper draws on examples of bioaerosol sampling from sources other than MWF to illustrate the application of statistics in this field.

### STATISTICS and STUDY DESIGN

The design of a study is the most important determinant of its quality, and the use of sophisticated statistical techniques cannot compensate for poor study design.<sup>(8-9)</sup> Investigators plan studies and address critical questions, such as those outlined in Table 1, keeping in mind their purpose for sampling, i.e., the hypotheses they ultimately will test and the data they need to do this.

Many decisions on study design hinge on knowledge of the underlying distributions of the measurements.<sup>(10)</sup> For example, investigators must know or estimate the distribution of bioaerosol concentrations to calculate the number of samples needed to characterize worker exposure accurately.

**Table 1. Examples of details of bioaerosol study design and execution.****What to collect**

- Specific microorganisms or general groups of microorganisms
- Bacterial endotoxin and other bacterial materials  
Fungal mycotoxin and other fungal materials

**How to collect the material**

- Consider the expected air concentration, anticipated particle size, sampler collection efficiency, and compatibility of collection medium and assay system

**Where / Who to sample**

- Area samples or personal samples
- Sample systematically (e.g., alternate sites or workers, every fifth worker)
- Randomize workers (to determine exposure range and variability)
- Randomize workers within homogeneous strata (to determine exposure range and variability within job categories or plants)
- Most highly exposed worker (to determine maximum exposure)

**When / How often to sample**

- Frequent sampling of short duration (to determine short-term peak exposures)
- Continuous sampling of long duration (to determine work-shift averages)

**Sample duration / volume**

- Consider the sampler's air flow rate, maximum and minimum anticipated air concentrations, and the assay's upper and lower detection limits

**How many samples**

- Consider the stability of exposures by worker and sampling site
- Consider the desired level of precision in the measurements

**Data analysis and reporting**

- Data collection, coding, entering, and cleaning
- Data screening, redefining, and transformation
- Descriptive summary statistics (e.g., means, medians, and standard deviations)
- Hypothesis testing using appropriate statistical procedures
- Data presentation in tables and figures

The magnitude of the potential health effect under study (e.g., the increased risk of asthma due to workplace exposure to a MWF bioaerosol) and the background rate of the condition (e.g., asthma due to non-occupational exposures) also affect the sample size needed to detect work-related associations (i.e., the required numbers of exposed and control workers or symptomatic and asymptomatic workers).

Investigators also must specify a level of statistical significance (typically 0.05) and chance of detecting a real effect (typically 0.80) when calculating sample size for a study.<sup>(11-13)</sup> For modelling purposes, statisticians need a sufficient number of observations to identify the important determinants of workplace exposures (see Statistical Analysis of Bioaerosol Data, below).

Quality assurance programs help investigators minimize measurement errors and identify potential bias, i.e., error other than random error that compromises data validity. Study design and associated budget planning should cover pilot testing of proposed measurement methods and necessary replicate and quality control sampling throughout a study.

**BIOAEROSOL DATA QUALITY**

*Quality assurance* in industrial hygiene and microbiology aims to ensure a uniform standard of quality by addressing the accuracy and precision of data, i.e., *accuracy*, the nearness of a result to a true or accepted value, expressed as absolute or relative error, and *precision*, the reproducibility of a set of repeated measurements, expressed as a range, standard deviation, or variance.<sup>(14-19)</sup> The goal of the quality assurance process is prevention of errors, but a large component is recognition and detection of errors so they can be corrected. To this end, investigators employ such monitoring tools as field and laboratory blanks, positive and negative controls, duplicate samples and analyses, and equipment calibration, as outlined in Table 2 and discussed below.

**Table 2. General measures to assess errors in bioaerosol sampling and analysis**

Sample Type	Description	Use
<b>Field blanks</b>	Sample media (e.g., culture plates and filters) handled as samples, except that no air is collected and analyzed along with samples	To detect contamination introduced during sample collection <sup>A</sup>
<b>Laboratory blanks</b>	Unexposed sample media (e.g., culture plates, filters, and sample containers) and analytical reagents processed with samples	To detect background contamination or contamination introduced during sample analysis <sup>A</sup>
<b>Positive control samples</b>	Samples known to indicate the presence of a particular biological substance at a known concentration	To confirm proper performance of an analysis and detection of positive samples <sup>A</sup>
<b>Negative control samples</b>	Samples known to indicate the absence of a particular biological substance	To confirm detection of negative samples (see also field and laboratory blanks) <sup>A</sup>
<b>Duplicate samples</b>	Samples collected simultaneously at the same location	To determine the precision of a collection method
<b>Replicate samples - split samples</b>	Analyses run repeatedly on portions of a sample	To determine the precision of an analytical method
<b>Spiked samples</b>	Samples to which a known amount of a biological substance has been added (see also positive control samples)	To confirm proper performance of an analysis, to determine recovery efficiency, and to generate calibration curves

<sup>A</sup> Question result validity when companion blanks are contaminated or control samples do not give expected results

Following are some of the criteria on which methods for bioaerosol sample collection and analysis are judged. A statistician can help a researcher decide how to measure these properties.

- (1) Yield in terms of measured *bioaerosol concentration*: Techniques that produce higher concentration estimates are considered preferable.
- (2) Yield in terms of the *number of microbial genera or species* isolated: Techniques that yield greater numbers of different organisms are considered preferable.
- (3) *Specificity* in terms of ability to select and distinguish a target bioaerosol without interference from other materials: More specific or selective techniques are considered preferable.
- (4) *Sensitivity* in terms of ability to detect small numbers or amounts of a target bioaerosol: Techniques with lower detection limits are

considered preferable.

- (5) Measurement *range* and ability to measure extremely high bioaerosol concentrations: Methods that avoid sample overload, i.e., that have high upper detection limits, are considered preferable.
- (6) Methods that provide *particle size* information and methods with known *particle collection efficiency*: Methods with sharp particle size cutpoints or that collect particles efficiently in the appropriate size range are considered preferable.
- (7) *Reproducibility* (precision) of sequential and parallel samples collected with the same or similar instruments or analyzed by the same or similar procedures: More reproducible methods are considered preferable.

(8) *Correlations* among samplers and analytical methods: Instruments and methods whose results correlate well with others and that allow sample collection or analysis by multiple techniques are considered preferable. Good correlation with traditional bioaerosol sampling methods typically is taken as a measure of a new method's accuracy because researchers can estimate accuracy only indirectly, i.e., by comparison with a reference method.<sup>(20-26)</sup>

The magnitude of error in bioaerosol measurements should be known relative to that of the other data in a study, e.g., clinical and other environmental measurements. The levels of accuracy and precision for the bioaerosol data also should be in line with the purposes for which the investigators will use the information. For example, investigators may not need to collect numerous duplicate air samples and to run many replicate analyses on each if the exposure measurements ultimately will be categorized only into high, medium, and low ranges. Likewise, investigators may not need to identify all isolated bacteria and fungi if they are interested in only one species or if they only need an estimate of the total numbers of airborne microorganisms.

### Sample Collection

Table 3 outlines some potential sources of error in bioaerosol sample collection.<sup>(20,22,25,27-29)</sup> General decisions on what bioaerosols to collect and how to collect them are made at the study-design stage. However, the field personnel control the details of sample collection, just as the laboratory personnel decide the details of how they handle and analyze samples (see below). The training, experience, and reliability of these key individuals and the completeness of the instructions they receive determine data quality to a large degree.

**Table 3. Potential sources of error in bioaerosol sample collection**

<b>Operators</b>	Lack of training or experience; Inadequate written procedures; Failure to follow directions; Human fallibility in reading and transcribing; Incorrect application of rounding rules and use of significant figures; Bias due to lack of blinding
<b>Air Sampling Equipment</b>	Failure to bring correct or sufficient supplies to test site; Inlet and internal losses; <sup>(24,28)</sup> Inappropriate particle size cutpoint; <sup>(28-29)</sup> Poor reproducibility; <sup>(20)</sup> Poor comparability; <sup>(21-23,40-41)</sup> Equipment not calibrated; Mechanical failures; Contaminant carryover between samples
<b>Collection Media</b>	Improper medium (e.g., filters, impinger solutions, or culture media); <sup>(40-42)</sup> Inappropriate, insufficient, or uneven agar in culture plates; Nonsterile media; Interfering compounds; Improper sample shipment, storage, transfer, or handling; <sup>(40-41)</sup> No duplicate or blank samples
<b>Environment</b>	Interferences (e.g., bioaerosols from sources other than MWFs or shed into personal samples, contaminants that interfere with bioaerosol assay); Temperature or humidity fluctuations; Unexpected changes in processes or work practices

### Sample Analysis

Investigators may ask qualitative or quantitative questions about biological contaminants in MWFs and MWF aerosols, e.g., What biological materials are present? or How much of a particular contaminant is present? Some of the techniques to identify and quantify biological materials include culture, microscopic identification, immunoassay, bioassay, chemical assay, and gene probes and polymerase chain reaction tests.<sup>(30-31)</sup> Table 4 lists some potential sources of error in bioaerosol sample analysis.

**Table 4. Potential sources of error in bioaerosol sample analysis**

<b>Laboratory personnel</b>	Lack of training or experience; Inadequate written procedures; Failure to follow directions; Human fallibility in reading and transcribing; Incorrect application of rounding rules and use of significant figures; Bias due to lack of blinding
<b>Sample preparation</b>	Biased selection of subsamples; Errors making dilutions; <sup>(21)</sup> Inadequate or inconsistent mixing; <sup>(40)</sup> Inaccurate sample transfers or inoculum delivery
<b>Sample analysis</b>	Incorrect choice of growth medium or incubation conditions; <sup>(16,19,23,25)</sup> Failure to follow rules for counting colonies and stained cells; <sup>(17-18,35)</sup> Failure to account for multiple-cell impaction; <sup>(34-36)</sup> Inaccurate calibration curves for colorimetric and other assays; Inaccurate microorganism identification; <sup>(16,19)</sup> Insufficient replicate analyses per sample; No positive and negative control samples; Samples beyond detection limits
<b>Media and reagents</b>	Variation among extraction methods; <sup>(26,40)</sup> Variation in batches of media and reagents, e.g., ingredients, sterility, pH, inhibitory compounds
<b>Equipment</b>	Variation in autoclaves, incubators, and refrigerators; Failure to calibrate or maintain balances, pipetting devices, micrometers, gas chromatograms; Mechanical failures; Contaminant carry-over between samples

## STATISTICAL ANALYSIS of BIOAEROSOL DATA

Following sample collection and analysis, investigators must clean their data and check for errors in data coding and entering. Investigators often assign estimated values to measurements above or below a detection limit (when they cannot repeat the samples using a more suitable collection time or air flow rate) using a scheme suitable for the data involved. Investigators sometimes can estimate *missing data* by averaging or regressing values from the available data.<sup>(32)</sup> Methods are

also available to identify possible *outliers* (i.e., observations that appear to deviate markedly from others)<sup>(33)</sup> and which may be merely extreme cases of random variability (in which case they should be retained) or be the result of methodological errors (in which case they should be discarded). Methods to correct particle or colony counts for masking, i.e., the inability to distinguish individual units that overlap, have been developed for some bioaerosol analyses.<sup>(34-36)</sup>

Investigators describe data using suitable *summary statistics*, e.g., the arithmetic mean and standard deviation for normally distributed data or (more typical for measurements of bioaerosol concentrations) the geometric mean and geometric standard deviation for lognormally distributed data. Many commonly used statistical tests require that investigators assume their measurements are *independent, normally distributed*, and have *homogeneous variance*. Lognormal data *transformed* to a normal distribution will meet the normality requirement and generally the variances become more homogeneous. Investigators can apply standard statistical procedures such as Pearson correlation, linear regression, and factor analysis to transformed data with less likelihood of violating the assumption of normality.<sup>(37)</sup>

Statistical modeling is practiced widely, e.g., researchers have developed predictive models based on bioaerosol sampling in experimental chambers<sup>(20-21,24,35,38)</sup> and in natural environments<sup>(10,21,23,25,39)</sup> as well as on theoretical sampler performance.<sup>(28,36)</sup> Models based on laboratory experiments allow investigators to explore relationships between possible *explanatory (independent) variables* and *outcomes (dependent variables)*, e.g., type of filter used and amount of endotoxin recovered.<sup>(40,42)</sup> However, the laboratory setting may not predict the real world very well where variables other than those anticipated may influence an outcome. Models based on actual workplace situations can allow investigators to assess the relative impact of several factors that affect a dependent variable. Even so, independent variables typically explain only a small portion of the variation in

occupational exposure data.

## SUMMARY and CONCLUSIONS

An investigator's ability to see an effect depends on the quality of the exposure data collected. Researchers providing measurements of bioaerosol exposures should deliver data that is precise, accurate, representative, and complete and should present the data in an easily retrieved and analyzed form. Essential to this effort is advice from statisticians familiar with the essential details of bioaerosol collection and analysis and close coordination with a project's epidemiologists. Investigators should critique their work thoroughly to identify and remedy points on which their findings could be faulted, such as small sample size, bias (e.g., non-representative samples), and methodological weaknesses (e.g., inaccurate or unsuitable sample collection and analysis or inappropriate statistical data analysis). Investigators should consider how to ensure bioaerosol data quality throughout a project, i.e., (1) in study design; (2) during sample collection and analysis; and (3) when analyzing and interpreting data.

## REFERENCES

1. **Mattsby-Baltzer, I; M Sandin, B Ahlström, S Allenmark, M Edebo, E Falsen, K Pedersen, N Rodin, RA Thompson, and L Edebo:** Microbial growth and accumulation in industrial metal-working fluids. *Appl. Environ. Microbiol.* 55:2681-2689 (1989).
2. **Chan, TL; JB D'Arcy, and J Siak:** Size characteristics of machining fluid aerosols in an industrial metalworking environment. *Appl. Occup. Environ. Hyg.* 5:162-170 (1990).
3. **Lacey, J; J Dutkiewicz:** Bioaerosols and occupational lung disease. *J. Aerosol Sci.* 25:1371-1404 (1994).
4. **Wald, PH; GM Stave, ed.:** *Physical and Biological Hazards of the Workplace.* New York, NY: Van Nostrand Reinhold, (1994). pp. 237-492.
5. **Eudey, L; HJ Su, and H. Burge:** Biostatistics and bioaerosols. In *Bioaerosols.* H.A. Burge, ed., Boca Raton, FL: Lewis Pub., (1995). pp.269-307.
6. **Heber, AJ:** Bioaerosol particle statistics. In *Bioaerosols Handbook.* C.S. Cox and C.M. Wathes, eds., Boca Raton, FL: Lewis Publishers, (1995). pp. 55-75.
7. **Lighthart, B:** Dispersion Models of Microbial Bioaerosols. In *Atmospheric Microbial Aerosols.* B. Lighthart and A.J. Mohr, eds. New York, NY: Chapman and Hall, (1994). pp. 285-303.
8. **Fisher, RA:** *Statistical Methods, Experimental Design, and Scientific Inference.* Oxford: Oxford University Press, (1991).
9. **Kish, L:** *Statistical Design for Research.* New York, NY: John Wiley & Sons, (1987).
10. **Heederik, D; JSM Boleij, H Kromhout, and T Smid:** Use and analysis of exposure monitoring data in occupational epidemiology: an example of an epidemiological study in the Dutch animal food industry. *Appl. Occup. Environ. Hyg.* 6:458-464 (1991).
11. **Cohen, J:** *Statistical Power Analysis for Behavioral Sciences.* New York, NY: Academic Press, (1977).
12. **Hoel, PG:** *Introduction to Mathematical Statistics.* 5th ed. New York, NY: John Wiley & Sons, (1984).
13. **Lehman, EL:** *Testing Statistical Hypotheses.* New York, NY: John Wiley & Sons, (1959).
14. **Ratliff, TA:** *The Laboratory Quality Assurance System: A manual of quality procedures with related forms.* 2nd ed., New York, NY: Van Nostrand Reinhold, (1993).
15. **Kelley, WD; C Nenadic:** *Basic Statistics for Laboratories: A primer for laboratory workers.* New York, NY: Van Nostrand Reinhold, (1992).
16. **Bartlett, RC:** Quality Assurance in the Clinical Microbiology Laboratory. In *Manual of Clinical Microbiology.* A. Balows, ed. Washington, DC: American Soc. for Microbiology, 1991. pp. 36-43.
17. **American Public Health Association:** *Compendium of Methods for the Microbiological Examination of Foods,* 2nd ed. Washington, DC: APHA, (1984). pp. 47-123.
18. **American Public Health Association, American Water Works Association, and Water Pollution Control Federation:** *Standard Methods for the Examination of Water and Wastewater,* 17th ed. Washington, DC: APHA, (1989). pp. 9.4-9.8, 9.52-9.66.
19. **American Society for Microbiology:** Quality control, media, reagents, and stains. P. Nash, ed. *Manual of Clinical Microbiology.* 5th ed. Washington, DC: ASM, (1991). pp. 1203-1289.
20. **Buttner, MP; LD Stetzenbach:** Monitoring airborne fungal spores in an experimental indoor environment to evaluate sampling methods and the effects of human activity on air sampling. *Appl. Environ. Microbiol.* 59:219-226 (1993).
21. **Henningson, EW; MS Ahlberg:** Evaluation of microbiological aerosol samplers: A review. *J. Aerosol Sci.* 25:1459-1492 (1994).
22. **Jensen, PA; WF Todd, GN Davis, and PV Scarpino:** Evaluation of eight bioaerosol samplers challenged with aerosols of free bacteria. *A. I. H. A. J.* 53:660-667 (1992).

23. **Smid, J; E Schokkin, JSM Boleij, and D Heederik:** Enumeration of viable fungi in occupational environments: A comparison of samplers and media. *Am. Ind. Hyg. Assoc. J.* 50:235-239 (1989).
24. **Upton, SL; D Mark, EJ Douglass, DJ Hall, and WD Griffiths:** A wind tunnel evaluation of the physical sampling efficiencies of three bioaerosol samplers. *J. Aerosol Sci.* 25:1493-1501 (1994).
25. **Verhoeff, AP; JH van Wijnen, JS Boleij, B Brunekreef, ES van Reenen-Hoekstra, and RA Samson:** Enumeration and identification of airborne viable mould propagules in houses. A field comparison of selected techniques. *Allergy.* 45:275-84 (1990).
26. **Walters, M; D Milton, L Larsson, and T Ford:** Airborne environmental endotoxin: A cross-validation of sampling and analysis techniques. *Appl. Environ. Microbiol.* 60:996-1005 (1994).
27. **Willeke, K; PA Baron:** Sampling and interpretation errors in aerosol monitoring. *Am. Ind. Hyg. Assoc. J.* 51:160-168 (1990).
28. **Grinshpun, SA; CW Chang, A Nevalainen, and K Willeke:** Inlet characteristics of bioaerosol samplers. *J. Aerosol Sci.* 25:1503-1522 (1994).
29. **Nevalainen, A; J Pastuszka, F Liebhaber, and K Willeke:** Performance of bioaerosol samplers: Collection characteristics and sampler design considerations. *Atmos. Environ.* 26A:531-540 (1992).
30. **Burge, HA, ed.:** *Bioaerosols*. Boca Raton, FL: Lewis Publishers, (1995).
31. **Cox, CS; CM Wathes, eds.:** *Bioaerosols Handbook*. Boca Raton, FL: Lewis Publishers, (1995).
32. **Affifi, AA; SP Azen:** *Statistical Analysis: A Computer-Oriented Approach*. New York, NY: Acad. Press (1979)
33. **American Society for Testing and Materials:** Standard Practice for Dealing with Outlying Observations. Standard E178-80 *1994 Annual Book of ASTM Standards. General Test Methods, Nonmetal; Laboratory Apparatus; Statistical Methods; Forensic Sciences; Chromatography*. Vol. 14.02. Philadelphia, PA: ASTM, (1989). pp. 95-111.
34. **Chang, CW; YH Hwang, SA Grinshpun, JM Macher, and K Willeke:** Evaluation of counting error due to colony masking in bioaerosol sampling. *Appl. Environ. Microbiol.* 60:3732-3738 (1994).
35. **Eduard, W; O Aalen:** The effect of aggregation on the counting precision of mould spores on filters. *Ann. Occup. Hyg.* 32:471-479 (1988).
36. **Macher, JM:** Positive-hole correction of multiple-jet impactors for collecting viable microorganisms. *Am. Ind. Hyg. Assoc. J.* 50(11):561-568 (1989).
37. **Affifi, AA; V Clark:** *Computer Aided Multivariate Analysis*. Belmont, CA: Lifetime Learning Pubs. (1984)
38. **Gordon, T:** Acute respiratory effects of endotoxin-contaminated machining fluid aerosols in guinea pigs. *Fundam. Appl. Toxicol.* 19:117-123 (1992).
39. **Su, HJ; A Rotnitzky, HA Burge, and JD Spengler:** Examination of fungi in domestic interiors by using factor analysis: Correlations and associations with home factors. *Appl. Environ. Microbiol.* 58:181-186 (1992).
40. **Douwes, J; P Versloot, A Hollander, D Heederik, and G Doekes:** Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl. Environ. Microbiol.* 61:1763-1769 (1995).
41. **Thorne, PS; JL Lange, P Bloebaum, and GJ Kullman:** Bioaerosol sampling in field studies: Can samples be express mailed? *Am. Ind. Hyg. Assoc. J.* 55:1071-1079 (1994).
42. **Gordon, T; K Galdanes, and L Brosseau:** Comparison of Sampling Media for Endotoxin-Contaminated Aerosols. *Appl. Occup. Environ. Hyg.* 7:472-477 (1992).

## Bacteria in Metalworking Fluids

**Miriam Kay Lonon**

Department of Health and Human Services, United States Public Health Service  
Centers for Disease Control, National Institute for Occupational Safety and Health  
4676 Columbia Parkway, R-7, Cincinnati, Ohio, 45226

### ABSTRACT

Used metalworking fluids from a variety of sources were inoculated onto agar media for isolation and identification of bacterial species. Types of fluids examined were either synthetic or semisynthetic. Forty-five isolates were grown in pure culture. Thirty of these were identified to species. The most commonly isolated species belonged to the genus *Pseudomonas*. Other genera identified include *Acinetobacter*, *Rhodococcus*, *Variovorax*, *Bacillus*, *Serpens*, *Xanthomonas*, and *Providencia*.

### INTRODUCTION

Microbial degradation of metalworking fluids is a well known problem in the metalworking trades. Microbial enzymes may cause changes in viscosity, altering lubricity and cooling properties. Acid products of microbial metabolism may lower pH, causing corrosion. Growth of sulfate-reducing bacteria often produces foul odors, causing "souring" of the fluids. Excessive growth may clog filters and nozzles and may interfere with the metalworking operation.

Microbially contaminated metalworking fluids also may pose special hazards for workers exposed to them. Many of the organisms isolated from contaminated fluids are opportunistic pathogens that may infect susceptible individuals. In addition, the fluids may become contaminated by frank pathogens, that infect healthy as well as compromised hosts.<sup>(1,2)</sup> The aerosols generated by the machining processes are truly bioaerosols, containing many live and dead microorganisms. Inhalation of these aerosols may cause a serological or immunological response. Microbial enzymes and toxins accumulating in the fluids may act directly on the tissues of exposed workers

or may aggravate the effects of existing chemical irritants. Toxic metabolites, such as ammonia and hydrogen sulfide, may be evolved as gasses directly into the work space.

The bacteria that grow in metalworking fluids include many Gram negative species. Gram negative organisms produce endotoxin, which, in large doses, by injection or by gut absorption, is fatal, and in smaller doses, by either route, may result in fever, circulatory and blood pressure disturbances, depressed immune response, and hypersensitivity to drugs and chemicals<sup>(3)</sup>. Inhalation of endotoxin has been associated with the inflammatory disease, hypersensitivity pneumonitis.<sup>(4)</sup>

### METHODS

In this study, eighteen samples of used synthetic or semisynthetic metalworking fluids from a variety of sources were examined and inoculated onto agar media for isolation and identification of bacterial species. The fluids were streaked onto trypticase soy agar (TSA) and incubated at 28 degrees centigrade until colonies appeared. After that, the plates were kept at room temperature. Isolated colonies were picked and subcultured on TSA until only one kind of colony appeared and the Gram stain showed a homogenous population. Catalase and oxidase tests were performed on each isolate. These isolates were then analyzed, using the Biolog Microbial Identification System (Biolog, Inc., Hayward, CA). The Biolog system identifies bacteria based on a metabolic profile generated from a ninety-six well microtiter plate. The wells are filled with substrate containing ninety-five different carbon sources (the A1 well is a negative control with no carbon source) and a redox dye that undergoes a color change to indicate that respiration

(utilization of the carbon source) is taking place. The wells were filled with a suspension of the unknown organism in physiological saline and, after a suitable incubation time (4 or 24 hours), the plate was read in a microtiter plate reader. The Biolog system compares the metabolic profile generated by the unknown organism to those stored in a library of reference strains and identifies the unknown using a best fit analysis.

## RESULTS

Attempts to cultivate pure cultures resulted in the isolation of forty-five unknowns. Thirty of these strains were identified to species by Biolog (Table 1).

**Table 1.** Bacterial species identified in contaminated metalworking fluids.

---



---

<i>Pseudomonas putida</i> Type B1
<i>Pseudomonas pseudoalcaligenes</i>
<i>Pseudomonas putida</i> Type A1
<i>Pseudomonas diminuta</i>
<i>Pseudomonas stutzeri</i>
<i>Pseudomonas vesicularis</i>
<i>Pseudomonas viridilivida</i> A
<i>Acinetobacter</i>
<i>radioresistens</i> /Genospecies 8
<i>Acinetobacter lwoffii</i> /Genospecies 8
<i>Acinetobacter johnsonii</i> /Genospecies 7
<i>Rhodococcus globerulus</i>
<i>Rhodococcus erythropolis</i>
<i>Alcaligenes faecalis</i> ss <i>faecalis</i>
<i>Variovorax paradoxus</i>
<i>Bacillus pasteurii</i>
<i>Bacillus brevis</i>
<i>Serpens flexilis</i>
<i>Xanthomonas maltophilia</i>
<i>Providencia rettgeri</i>

---



---

Of these, twelve belonged to the genus *Pseudomonas*. Seven of the twelve were identified as *P. putida*. Other *Pseudomonas* species included *P. stutzeri*, *P. vesicularis*, *P. pseudoalcaligenes*, *P. diminuta*, & *P. viridilivida*.

Four of the isolates were identified as *Acinetobacter* and three are thought to be *Rhodococcus*. Other genera identified include *Variovorax*, *Bacillus*, *Serpens*, *Xanthomonas*, and *Providencia*.

## DISCUSSION

The results of this study are consistent with similar studies reported in the literature. The majority of the organisms isolated from metalworking fluids are aerobic, nonfermenting Gram negative rods with *Pseudomonas* being the genus most commonly reported.<sup>(2,5,6)</sup> The pseudomonads produce a great number of extracellular enzymes that enable them to degrade macromolecules into simpler substrates. They are able to grow on a wide variety of hydrocarbons from simple fatty acids to aromatics. Many are resistant to antimicrobial agents. They are ubiquitous in the environment and can be isolated readily from soil or water. They are important opportunistic pathogens, causing severe local and systemic infections in susceptible individuals.

*Acinetobacter*, another genus often reported from metalworking fluids,<sup>(7)</sup> is also able to use a wide variety of simple and complex hydrocarbons as an energy source. Like *Pseudomonas*, *Acinetobacter* is an opportunistic pathogen, causing septicemia, meningitis, pneumonia, and endocarditis. Clinical isolates are resistant to most antibiotics.

Three of the isolates in this study were identified as *Rhodococcus* species. The rhodococci also are able to use unusual hydrocarbon and nitrogen-containing compounds as substrates and often have been isolated from soils contaminated with petroleum.<sup>(8)</sup> Some rhodococci are pathogenic.

Some of the isolates from this study were not identified. This may have been due to the lack of a suitable reference in the library. In addition, some of the isolates grew very poorly on the isolation media, making it very difficult, in some cases, to harvest enough material for analysis. Furthermore, the Biolog system requires that the

unknown isolates be in pure culture. Although great care was taken to ensure the preparation of pure cultures, it is difficult, when working with environmental samples, to be sure that the cultures are, in fact, pure. Slow-growing contaminants may be passed from one culture to the next, unseen because they are overgrown by the predominating species. However, they may still produce metabolic products that interfere with the Biolog assay, resulting in a pattern that does not match anything in the reference library.

## CONCLUSIONS

This study is the preliminary investigation of a larger study to identify and characterize potential hazards associated with bacterial contamination of metalworking fluids. The species identified from these samples are characterized by great nutritional versatility as the result of their ability to produce a wide variety of extracellular enzymes. Many of these extracellular enzymes have been characterized as virulence factors, contributing to disease by enabling the organism to use the macromolecules found in human tissues as substrates for growth. Many of these enzymes cause damage when applied directly to tissues, especially lung tissues. This phase of the study confirmed the presence of organisms such as *Pseudomonas* and *Acinetobacter*. The next phase will investigate the possible accumulation of bacterial enzymes and toxins in the fluids and the potential hazards posed by exposure to fluid aerosols.

## REFERENCES

1. **Herwaldt, LA; GD Gorman, T McGrath, et al:** A new *Legionella* species, *Legionella feeleii* species Nova, causes Pontiac fever in an automobile plant. *Ann Intern Med* 100:333-338(1984).
2. **Tant, CO; EO Bennett:** The isolation of

pathogenic bacteria from used emulsion oils. *Appl. Microbiol.* 4:332-338(1956).

3. **Holdom, RS:** Microbial spoilage of engineering materials. Part 3: Are infected oil emulsions a health hazard to workers and to the public? *Tribology Int.* 271-280 (December 1976).
4. **Rylander, R; P Haglund:** Airborne endotoxins and humidifier disease. *Clin. Allergy* 14:109- 112(1984).
5. **Bennett, EO:** The biology of metalworking fluids. *Lubr. Eng.* 28:237-247(1972).
6. **Wort, MD; GI Lloyd, and J Schofield:** Microbiological examination of six industrial soluble oil emulsion samples. *Tribology Int.* (February 1976).
7. **Salmeen, I; JA Brown Jr, S Foxall-Van Aken, and RH Olsen:** Presence of *Acinetobacter* species among the predominant bacteria found in a contaminated metalworking fluid. *Tribology Int.* 20:218-221(1987).
8. **Nesterenko, PA; SA Kasumova, and EI Kvasnikov:** Microorganisms of the *Nocardia* genus and the '*rhodochrous*' group in soils of the Ukrainian SSR. *Mikrobiologiya* 47:866-870(1978). Cited in *Bergey's Manual of Systematic Bacteriology* by Michael Goodfellow. Baltimore, Maryland: Williams and Wilkens, (1984) p.1473.

## ACKNOWLEDGMENTS

The author acknowledges the technical assistance of visiting scholar Ladina Saluz and intern Linda Pham and thanks them for their help in deriving pure cultures and preparing them for analysis.

## Microbiology of Metalworking Fluids: Pilot Studies for a Large Scale Exposure Assessment Experience

Harriet A. Burge (A), Michael Muilenberg (A), and Thomas Robins(B)

(A) Harvard School of Public Health, Dept of Environmental Health, 665 Huntington, Boston MA, 02115

(B) University of Michigan School of Public Health, Ann Arbor MI, 48109

### ABSTRACT

Aqueous metalworking fluids always contain bacteria and fungi and exposure can result in infections, and hypersensitivity and toxic reactions. In pilot studies preceding a larger study, we used filter cassettes with polycarbonate filters for total bacterial counts. Bacteria were washed from filters and counted using acridine orange (AO) staining and UV microscopy. Bulk samples were also stained and counted, and serially diluted up to  $10^{-7}$  and spread-plated on R2A and Malt agar with and without antibiotics. Andersen N-6 and 2-stage culture plate samples were collected on the same media. Common bacterial isolates were Gram stained and Gram negative organisms identified using two commercially available ID kits. We collected air and machine and sump fluid samples twice each day for 4 consecutive days.

AO counts were compared after 1, 2 and 3 days of filter storage. Slight increases stimulated culture comparison of UV and propylene oxide (PO) for killing. PO prevented growth; UV suppressed fluorescence (or staining). Cells were efficiently washed from filters ( $96.5 \pm 2.9\%$ ) using 0.02% Tween 20. R2A recovered slightly more bacteria than other media and antibiotics did not improve recoveries. Duplicate Andersen samples varied by less than 10% and average in machining areas exceeded 5000 CFU/m<sup>3</sup>. More than 50% of colonies were recovered on stage 2 (<1 $\mu$ m) of 2-stage Andersen. Culturable bacteria in bulk samples ranged from  $10^5$  -  $>10^9$ /ml. Overall, levels were not different between sump and machine or for AM and PM, but individual paired samples were not correlated. Levels varied significantly between areas and between the first and all subsequent days of sampling. AO bacterial counts and particle mass on air samples were correlated

and strength of correlation depended on area ( $r^2=0.5 - 0.75$ ). Nearly all bacteria were Gram negative rods. Of 40 isolates, 12% were identified as the same and 50% different for the two ID kits, and 28% were not identified by either kit. Overall, 97% of the bacteria recovered were not identified. Identifiable taxa in bulk and air varied between but not within the three major sites. *Fusarium* was the dominant fungus in both air and bulk samples.

These studies make clear that exposure to bacterial and fungal aerosols can be intense and that exposure assessment presents a significant challenge due both to the intensity of the aerosols and the variability by site and time. AO counts of air and sump samples are useful alternatives to culture. Identification of the Gram negative bacteria common in machining fluids is difficult and the expense and effort may not be justified until differential health effects between the different types can be documented.

### INTRODUCTION

Microbial contamination of aqueous metalworking fluids is inevitable (e.g., Lee & Chandler 1940, Foxall-VanAken *et al* 1986). Organisms present in machining fluids become part of the aerosol produced by the machining processes (Chan *et al.*, 1989). Both bacteria and fungi can become abundant, and exposure to these organisms can result in infectious disease (e.g., Legionnaires' disease), hypersensitivity diseases involving the development of specific antibodies or sensitization of the cellular immune system to microbial components and metabolites, and toxic reactions caused by exposure to structural or metabolic toxins produced by bacteria and fungi. Rossmore (1981) reports *Candida*, *Fusarium*, and *Cephalosporium* as common contaminants of

cutting fluids. Some species of *Fusarium* and *Cephalosporium* produce trichothecene toxins under some circumstances (Shank 1981). Bacteria and fungi also produce volatile organic compounds that are odoriferous and often irritating. These objectionable odors often prompt addition of biocides to machining fluids on Monday mornings following a weekend shutdown. Health effects resulting from exposure to these compounds have not been studied.

Field collections have determined that bacterial levels of  $10^5$  -  $10^7$  are commonly identified in water-based cutting fluids, and, in fact, this range has been proposed as a range of acceptable contamination (Rossmore 1981). *Pseudomonas* species are most commonly recovered from contaminated machining fluid (Lee & Chandler 1940, Rossmore 1981, Rycroft 1980). However, Foxall-VanAken *et al* (1986) report that 7 of 12 bacterial strains isolated from contaminated fluid were *Acinetobacter* species. Their studies also indicate that these 7 strains were able to multiply in noncontaminated fluid, and to utilize the very low levels of the fatty acids that are present in the fluids (oleic and linoleic acids). The *Pseudomonas* strains that were recovered were unable to multiply in clean oil, or to utilize these carbon sources. *Acinetobacter* species may be pioneer organisms, and pseudomonads secondary invaders that utilize metabolic products from the initial colonizers.

This report presents data from pilot studies designed to develop an exposure assessment protocol for a large epidemiologic study of respiratory effects of exposure to machining fluid aerosols. Our primary goals were to choose and test appropriate sampling and analytic methods, and to develop a sampling plan that would accurately represent acute exposures occurring during the larger studies.

## METHODS

### Sample collection methods

#### Filter samples and acridine orange counts.

Disposable endotoxin free filter cassettes were loaded with preweighed 0.8 $\mu$ m polycarbonate

filters and samples were collected with DuPont personal sampling pumps operating at 2 L/min. Replicates were counted at 1, 2, and 3 days following collection. Because some increases in counts occurred during storage, replicate filters were treated with either ultraviolet light or propylene oxide (PO) (45 minutes). Subsequently all filters were treated with PO, dried, and weighed to estimate total mass collected. Each filter was then placed in a test tube and the collected particles, including microorganisms, washed from the filter using three 2 ml washes of filter-sterilized 0.02% Tween 20 in distilled water (diluent) with 1 minute vortex agitation between each wash. All 6 ml of wash suspension were combined and microorganisms were fixed by adding formaldehyde to a final concentration of 1%. Fluid samples taken directly from machines or sumps were diluted 1:4 with the Tween solution and treated with formaldehyde as above. These wash suspensions and dilute machining fluid samples were stored at 4°C until stained.

For staining, 1 ml of filter wash suspension, or 5  $\mu$ l of 1:4 fluid in 1 ml dilution fluid, was filtered through a black-stained 0.2 $\mu$ m pore 25mm diameter polycarbonate filter (Poretics Corp, Livermore CA) in a 25 ml filter tower attached to a vacuum source. One ml of filter-sterilized 0.01% acridine orange (AO) crystals in distilled water was added and after 5 minutes pulled through the filter followed by two 5 ml volumes of diluent to remove excess stain. The filter was removed from the tower, placed on a glass microslide, allowed to air dry, and mounted in Cargille immersion oil (1.586 refractive index) under a cover glass. Bacteria were counted using 1000x magnification on an Olympus epifluorescence microscope with a 100W mercury burner, a 490 nm exciter filter, 500 nm dichroic mirror, and 515 nm barrier filter. All fluorescent particles of "bacterial size and shape" (that is, spherical and rod-shaped particles between about 0.5 and 2  $\mu$ m diameter) were counted. All bacteria in every third field across the diameter of the filter were counted. Using known flow rates, exposure times, and dilutions, bacterial cells/m<sup>3</sup> of air or per ml of machining fluid were calculated.

**Cultural sampling.** Duplicate N6 Andersen samples (1 minute samples at 28 liters/min, and single Andersen 2-stage samples (Andersen Inc., Atlanta GA) (2 minute samples at 28 L/min) were used with four culture media: R2A, R2A with cycloheximide (R2A/C), Malt extract agar (MEA) and MEA with streptomycin sulfate (MEA/S). Andersen samplers were cleaned between samples using alcohol swabs followed by air drying and examination to verify that holes were clear. R2A and R2A/C plates were incubated at 30°C for 48 hours. MEA and MEA/S plates were incubated at room temperature under near UV illumination (GE Gro lites) for at least 5 days, with daily monitoring for overgrowth by fast-growing fungi. All colonies were counted, converted for multiple impactions, and results reported as colony forming units (CFU) per m<sup>3</sup> of air sampled.

Bulk fluid samples were collected using sterile 100cc syringes, and placed in clean dry french square bottles and transported to the laboratory (unrefrigerated) on the same day. Samples were refrigerated on arrival in the lab. Each sample was serially diluted 1:10 - 1:10<sup>8</sup> with sterile saline and 0.1 ml of each dilution plated on each of the media described above, plus tryptone soy agar with and without cycloheximide (TSA, TSA/C). Colonies were counted and converted to CFU/ml of fluid. Colony counts were recorded for all dilutions where discrete colonies were visible. Dilutions with about 10 - 50 fungal colonies and 30-100 bacterial colonies were selected for further analysis. For all culture plates, fungi were identified microscopically and common bacteria were subcultured and incubated for 24 hours. Subcultured bacteria were Gram-stained and examined microscopically. Gram negative organisms were identified to genus or species where possible using either the API NFT method or the Biolog method for identification of Gram negative bacteria

**Burkard samples.** Burkard recording spore trap was loaded with Lubriseal-greased Melenex tape, and operated at 10 liters/min for 4 days. Burkard tapes were visually examined and determined to be unacceptable due to buildup of fluid. The Burkard

samplers were not used further for this project.

### Sampling plan

Air and bulk samples were collected in three machining areas (case, valveB, and valveC) in the morning and afternoon of 4 successive days (Monday - Thursday). Bulk samples were collected from the main fluid sumps for each area and from a machine in each area. In addition, air samples only were collected from a control area (final assembly) and outdoors on each day.

## RESULTS

### Acridine orange counting

The presence of machining fluid did not prevent AO staining and counting of bacteria. AO counts on filters increased slightly after 3 days of storage (Figure 1). Propylene oxide effectively killed cells on filters and did not prevent staining. UV did not kill cells, and interfered with staining (Figure 2). Cells were efficiently washed from air sample filters for counting ( $96.5 \pm 2.9\%$ ) using 0.02% Tween 20.

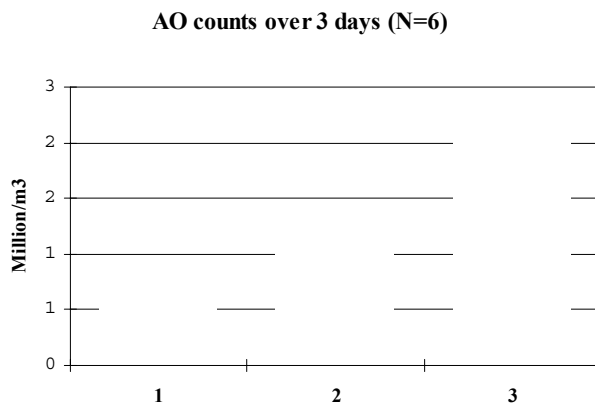
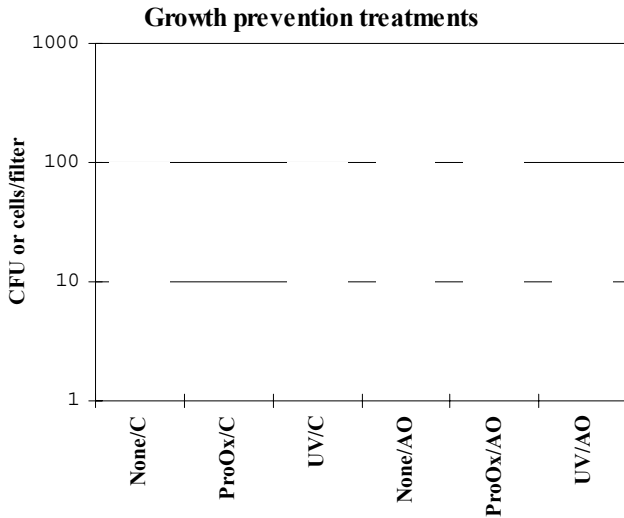


Figure 1. Comparison of AO counts on filters after 1, 2, and 3 days of storage.

### Culture media comparisons

There were no significant differences between levels of recovery on the different culture media used. For some samples R2A and for others TSA produced higher counts (Table 1).



Error! Switch argument not specified.  
 Figure 2. Comparison of different treatments for prevention of bacterial multiplication on filters.

**Andersen samples**

There was less than a 10% geometric mean (g $\times$ ) difference between paired N6 Andersen samples. The overall g $\times$  (range based on the geometric standard deviation) for all samples from two sites was 5671.9 (4211.7-7638.4). The g $\times$  of the absolute differences between duplicate samples

was 507.2 (251.8-1021.6). Individual pairs of replicates were significantly correlated ( $r^2 = 0.846$ ) and were not different by paired ttest. Many samples from one area were overloaded and are not included in this analysis.

Table 1. Andersen N6 counts for bacteria on 4 culture media. P values were derived from Student T-tests on log transformed data.

	Assembly AM	ValveB AM
R2A	5687 $\pm$ 124	7359 $\pm$ 149
R2A/Cyclo	4454 $\pm$ 324	10352 $\pm$ 398
TSA	4595 $\pm$ 374	4930 $\pm$ 498
TSA/Cyclo	5053 $\pm$ 373	4754 $\pm$ 747
R2A/TSA P <	0.1341	0.9369
R2A/R2AC P <	0.4675	0.4097
TSA/TSAC P <	0.8056	0.7487

**Bacterial identification comparisons**

The API method uses 20 biochemical tests to evaluate the probable identification of each isolate. The Biolog method uses 96 tests. Table 2 presents comparisons on 40 isolates identified using both methods.

Table 2. Comparison of two bacterial identification methods

11 strains No ID, either test		
5 strains Same ID, both tests	(2) <i>Ps. vesicularis</i> <i>Fl. breve</i> (2) <i>Sp. paucimobilis</i>	
	<b>APT-NFT</b>	<b>Biolog</b>
9 strains Close match	(4) <i>C. testosteroni/Ps. alcaligenes</i> (2) <i>C. testosteroni/Ps. alcaligenes</i> <i>Ae. salmonicolor./ Ps. vesicularis</i> <i>Flavobacterium sp.</i> <i>Ps. vesicularis</i>	<i>Sp. pseudoalcaligenes</i> <i>C. terrigena</i> <i>Ps. vesicularis</i> <i>Fl. balustinum</i> <i>CDC gr II H*</i>
5 strains No API NFT ID		(2) <i>Delaya aesta</i> (.574, .623) <i>Brucella abortus</i> (.505) <i>Moraxella bovis</i> (.610) <i>Altermonas halophilus</i> (.581)
4 strains No Biolog ID	<i>Ps. vesicularis</i> <i>P. vesicularis</i> <i>Sph. pauci A</i> <i>Fl. Indologenes</i>	<i>CDC gr II H</i> (.485) <i>Capnocytophaga canim.</i> (.450) NO REACTIONS <i>CDC gr II H</i> (.416)
6 Different ID	<i>CDC gr IV C2</i> (99.2%) <i>Ps. stutzeri</i> (2) <i>C. testosteroni/ Ps. alcaligenes</i> <i>C. testosteroni/ Ps. alcaligenes</i> <i>Ps. vesicularis</i>	<i>Gilardi pink neg rod</i> (.777) <i>Ps. diminuta</i> (.900) <i>Ps. diminuta</i> (.894, .763) <i>Al. faecalis II</i> (.688) <i>Delaya aesta</i> (.623)

C = Comamonas, Ps=Pseudomonas, Ae=Aeromonas, Sp=Sphingomonas, Fl=Flavobacterium, Al=Alcaligenes. Numbers in parentheses preceding name indicate number of strains; numbers following names indicate probability of correct ID (% for API-NFT, decimal for Biolog).

**Levels, spatial and temporal variation in bulk samples**

Complete sets of bulk sample data were retrieved over the 4 day study for two sites. The third sump was treated with biocides during the week of sampling and culturable levels fell to zero, making these comparisons impossible. There were no significant differences between sumps and machines within sites, but both were significantly different between sites (Table 3).

Morning and afternoon bulk fluid samples did not differ overall, and were correlated for each day ( $r^2=0.882, 0.898, 0.877, 0.973$ ). However, there were significant differences across days for all three sites (Table 4).

machining areas served by different sumps.

	CASE	VALVE B	CASE/VALVE SUMP
<b>SUMP</b>	5.6E+08 (5.06E+08- 6.17E+08)	3.05E+08 (2.62E+08- 3.55E+08)	
<b>MACHINE</b>	5.49E+08 (4.85E+08- 6.22E+08)	2.63E+08 (2.15E+08- 3.21E+08)	
<b>PAIRED T TEST</b>	p=0.76	p=0.09	p=5.15E-06

Table 3. Comparison of bulk recoveries from two

Table 4. Day by day comparisons of overall recoveries of bacteria from fluids (p values based on paired t-test on log-transformed data).

	30 MARCH	1 APRIL	2 APRIL
29 MARCH	0.001384	0.021288	0.010743
30 MARCH		0.666957	0.232654
1 APRIL			0.284901

If the site at which biocide was added is eliminated, then significant day to day differences are apparent only for 29-30 March (p=0.009).

Air samples collected on 2-stage Andersen samplers produced the following data from two sites, indicating that a significant fraction of viable bacteria are carried on respirable-size particles (Table 5).

Table 5. Air-borne bacterial counts (Andersen 2-stage).

Stage	Assembly AM	Assembly PM	ValveB AM	ValveB PM
1	1021 ± 149	1564 ± 334	5563 ± 996	5317 ± 1046
2	3239 ± 199	6267 ± 697	5910 ± 548	5106 ± 647

## DISCUSSION

### Sample handling

Because of the time required for sample collection and for dilution plating, and personnel limitations, it was necessary to refrigerate samples overnight before analysis. Limited tests comparing recoveries before and after this refrigeration step indicated a variable effect on total colony counts that was usually less than a factor of 10. Tests were not conducted to examine effect on species composition. However, this is a factor that should be considered in future studies, assuming taxon is considered an important variable. Bottles for bulk samples were not sterilized because, in our experience, fewer than 1 fungal colony/ml and fewer than 10 bacterial colonies/ml can be recovered from sterile water exposed to bottle surfaces as used ("clean" and dry).

### Sample analysis

AO counting. While the use of acridine orange epifluorescence is an accepted method for monitoring total (viable and non-viable) bacteria in fluids (i.e., water, milk), only recently has it been successfully used to estimate concentrations of airborne microbes (Palmgren *et al.*, 1986). The technique has not been tried (or at least reported) in oil mist situations. There is a slight possibility that certain machining fluids or additives could reduce staining efficiency or interfere with our ability to observe the fluorescence (e.g., high background levels). However, in our study, staining and counting were relatively straightforward.

We are aware that our counting method for the filters did not account for non-random distribution of particles on the filters. A mathematical adjustment could be made to account for this source of error. However, given the inherent variation in these kinds of samples, we decided that such adjustment was not necessary to achieve the goals of these studies (i.e., examining relationships between changing levels and health outcomes).

Bacterial identification. Many bacteria remained unidentifiable, at least in part due to poor growth in culture on any of the media used. In some cases (especially in Valve B) the dominant organisms grew very poorly in culture. We attempted to recover some of these on media containing machining fluid but were not successful. We assume that these organisms were adapted to the machining fluid environment, and contained only enough residual nutrient material to enable minute colony formation. Subsequent transfers failed to produce growth because some essential nutrient was probably lacking.

Gram negative organisms were always dominant in fluid samples. The occasional Gram positive organisms recovered were those commonly associated with human skin, and were assumed to be accidental contaminants and not part of the fluid ecosystem. The Biolog system uses many more tests for each identification, and contains a larger data base of species than the API NFT system. In

addition, more species were identified with a greater confidence with Biolog than with NFT. However, even the Biolog system failed in many cases, demonstrating the primarily clinical focus of the system. In order to accurately identify many of the isolates in the fluid, which are not commonly associated with clinical specimens, standard (old-fashioned) microbiological techniques would have to have been used. These were too time-consuming and expensive to be used in these studies.

It is not clear that species identification of Gram negative organisms is important for the health outcomes usually related to exposure to Gram negative bacteria unless one hypothesizes that endotoxin from different organisms has more or less of an effect, or that other toxins might be involved. One report exists of hypersensitivity pneumonitis associated with exposure to *Pseudomonas* aerosols (Gordon *et al* 1973). However, in view of the massive exposures to Gram negative organisms that does not result in HP, one must question whether or not the *Pseudomonas* was actually at fault in the humidifier case. Other organisms could have been present that actually caused the disease, possibly in conjunction with endotoxin. However, cultural analysis to verify that a "standard" Gram-negative population is dominant is essential. Hypersensitivity pneumonitis has been reported in association with exposure to Gram positive, acid-fast organisms in machining fluid (Muilenberg *et al* 1993).

## REFERENCES

1. **Chan, TL; JB D'Arcy, and J Siak:** Size characteristics of machining fluid aerosols in an industrial metalworking environment. *Appl Occup Environ Hyg* 5:162-170, 1990.
2. **Foxall-VanAken, S; JA Brown, et al:** Common components of industrial metalworking fluids as sources of carbon for bacterial growth. *Appl Environ Microbiol* 51:1165-1169, 1986.
3. **Gordon, DS; RG Hunter, RJ O'Reilly, and BP Conway:** *Pseudomonas aeruginosa* allergy and humoral antibody-mediated hypersensitivity pneumonia. *Am Rev Respir Dis* 108(1):127-31, 1979.
4. **Lee, M; AC Chandler:** A study of the nature, growth and control of bacteria in cutting compounds. *J Bacteriol* 373-386, 1940.
5. **Muilenberg, ML; HA Burge, and T Sweet:** Hypersensitivity pneumonitis and exposure to acid-fast bacilli in coolant aerosols. *J Allergy Clin Immunol* 91(1)pt 2:311 1993.
6. **Palmgren, U; G Strom, and G Blomquist, et al:** Collection of airborne microorganisms on Nuclepore filters, estimation and analysis - CAMNEA method. *J Appl Bacteriol* 61(5):401-406, 1986.
7. **Rossmore, HW:** Antimicrobial agents for water-based metalworking fluids. *J Occup Medicine* 23:247-253, 1981.
8. **Rycroft RJG:** Bacteria and soluble oil dermatitis. *Contact Dermatitis* 6:7-9, 1980.
9. **Shank, RC:** Mycotoxins and N-nitroso Compounds: Environmental Risks. CRC Press, Boca Raton FL, 1981.

## Endotoxin Exposure Assessment in Machining Operations

Donald K. Milton and Daryl K. Johnson

Occupational Health Program, Harvard School of Public Health, Boston, MA

### INTRODUCTION

Endotoxin, a substance derived from Gram-negative bacteria, is associated with acute and chronic changes in lung function among workers in a range of industries from agriculture and cotton textiles to fiberglass manufacturing. Endotoxin is everywhere in our environment, but can be found in high levels where Gram-negative bacteria flourish, such as in metal working fluids (MWF). In the environment, endotoxin consists of whole cells and cell fragments. The substance with the specific toxic activity known as endotoxin can be purified from Gram-negative bacteria by isolating the lipopolysaccharide (LPS) that makes up the outer membrane of Gram-negative bacteria.

Because a range of LPS chemical structures all have the property of being endotoxins, and because the Limulus in-vitro bioassay is more sensitive and specific for endotoxin than are chemical assays for LPS, endotoxin exposure assessment presents unique challenges. We performed several experiments to validate techniques for endotoxin sampling and measurement in MWF. The methods validated in these experiments were then applied in two recent epidemiological studies.

### METHOD

Initial experiments determined that LPS added to MWF could be detected in the Limulus bioassay, and that the recovery of endotoxin activity was 100%. Because at least brief storage of samples was logistically necessary, experiments were conducted to determine optimal techniques for preservation of endotoxin in the fluids. Samples were collected for us by the epidemiologic study teams in endotoxin free glass vials containing either no preservative, formaldehyde (to make 1.5% final concentration) or antibiotics (cycloheximide 50 mg/ml and

chloramphenicol 100 mg/ml final concentrations). All samples were kept refrigerated except during transport. Only minor differences were observed in endotoxin concentration obtained by the various methods. There were no significant differences between endotoxin concentrations in the formaldehyde and antibiotic preserved samples when assayed over the first 4 to 8 weeks of storage at 20°C. However, after four months there was significant loss of endotoxin activity in the fluids regardless of preservation method. Subsequently, all fluid samples were preserved by addition of formaldehyde and refrigeration. Freezing caused a large reduction in endotoxin potency of used fluids and was therefore avoided.

In preparation for air sampling, we tested filter media to determine whether endotoxin could be recovered from them after drying onto the filter. As reported previously, a wide range of filter media avidly bound LPS during an incubation typical of conditions used to extract endotoxin from aerosol samples. However, capillary-pore polycarbonate membranes did not significantly adsorb LPS. In an examination of the feasibility of measuring endotoxin in samples from the Kennedy study, LPS was added to MWF and known amounts of the MWF-LPS mixture were dried onto woven Teflon filters used in that study. Only a small fraction of the endotoxin activity thus applied could be recovered by extraction in aqueous media. A larger fraction of the endotoxin could be recovered by extraction in an organic solvent. However, the dose-response slope of endotoxin in the Limulus assay was significantly altered so that it could not be accurately quantified, after evaporation of the organic phase into water.

In contrast to the above results, we found that when known amounts of MWF containing endotoxin were dried onto capillary-pore polycarbonate membranes, the majority of the

endotoxin activity was recovered by extraction in a phosphate-triethylamine buffer with bath sonication. Therefore, in subsequent work, all samples were collected on 0.4-mm polycarbonate membrane filters. Sampling was performed in closed-face, polystyrene cassettes. Samples were desiccated with individual silica gel containers attached to each cassette, and refrigerated except during transport.

## RESULTS

Endotoxin was measured in a total of 810 fluid and air samples provided by two epidemiologic study teams working at GM plants. The studies are reported at this symposium. Personal breathing zone and area samples were assayed for selected subjects among the machinists and assemblers, in Phase 1 of the Indianapolis study. Bulk samples of MWF from the small sumps supplying the exposed workers were also sampled for certain subjects. All subjects in Indianapolis-Phase 2 were sampled for breathing zone endotoxin levels on 4 days. Area samples and paired bulk samples of sump and point of use fluids were assayed for endotoxin in Rounds 1 and 2 in Warren, MI. In Round 3, personal breathing zone samples as well as area and sump samples were assayed for endotoxin.

All samples were assayed for endotoxin with a kinetic-chromogenic Limulus lysate from BioWhittaker (Kinetic-QCL), and all endotoxin results are reported in endotoxin units (EU) relative to reference standard EC5. Analyses were internally controlled for validity using the KLARE method. Approximately 2% of the data were rejected because of assay interference detected by the KLARE method. All blanks were approximately 100 fold lower than the lowest field samples.

The personal exposure levels measured in the Warren plant (Table 1) were higher than those in the Indianapolis plant. Thus, the range of exposures between studies was significantly greater than that within studies.

**Table 1.** Personal Exposure to Airborne Endotoxin in two Plants (EU/m<sup>3</sup>)\*

Area	Mean	Geometric Mean	GSD	N
WARREN				
Assembly	16.9	12.6	2.8	44
Valve	49.3	31.6	2.5	32
Case	444	254	3.1	52
INDIANAPOLIS				
Classroom	2.24	0.63	4.8	9
Unexposed	8.55	3.39	5.3	24
Exposed	14.5	8.55	3.4	70

\* Data are from Warren (Round 3), Indianapolis Phase 1

Of particular note is that the low exposure group in Warren had higher mean endotoxin measurements than any of the groups in Indianapolis. It is unlikely that the high levels in the Warren assembly area were spurious because they were consistent over repeated rounds of sampling. Also, a small number of area samples from the assembly area were submitted for GC-MS analysis of 3-hydroxy fatty acids (3-OHFA), a chemical marker of LPS. This assay, while insensitive at the low levels present in these samples, found evidence suggesting that LPS was present at detectable levels in some of these samples. At normal background levels 3-OHFA from LPS are expected to be below the limit of detection except for high-volume samples.

Endotoxin in the MWF bulk samples from Warren and Indianapolis were similar (Table 2). The Warren samples came from two areas - valve with two sumps, and case with one sump. The samples from Indianapolis came from a large number of small sumps attached to individual machines and are reported as an average over all the sump samples. Within the Warren plant, the area with highest airborne endotoxin had the higher average sump endotoxin level. The mean sump endotoxin at Indianapolis was intermediate between the levels seen in Warren, and more variable.

**Table 2.** MWF Sump Endotoxin Concentrations (EU/mL)\*\*

Area	Mean	Geometric Mean	GSD	N
WARREN				
Valve	29,300	21,700	2.3	45
Grind	16,900	13,100	2.1	21
Body	40,100	33,900	1.9	24
Case	144,000	124,000	1.9	27
INDIANAPOLIS	76,500	41,900	3.3	58

\*\* Data are from Warren (Rounds 2 & 3), and Indianapolis Phase 1

Based on these data one would expect Indianapolis to have levels of airborne endotoxin similar to those in Warren. Thus, fluid endotoxin levels while potentially useful within plant to identify areas with higher airborne endotoxin levels, did not appear to explain differences in airborne endotoxin levels between the plants. Further examination of the characteristics of the exposure and aerosol sources at each plant may be useful in defining the causes and in finding potential controls for endotoxin exposure from MWF.

## CONCLUSIONS

In conclusion, it is appropriate to put the endotoxin levels measured in the two automotive plants in perspective by comparison with other environments. Endotoxin has been associated with acute effects on lung function in experimental studies of cotton dust with thresholds at 90 to 330 EU/m<sup>3</sup>. A recent epidemiologic study reported that exposure above 42.5 EU/m<sup>3</sup> was associated with acute effects on lung function among workers in a fiberglass wool manufacturing plant. A recent study of grain and postal workers found that grain workers exposed to endotoxin above 100 EU/m<sup>3</sup> had significantly more respiratory symptoms and lower baseline lung function, even though acute effects on lung function were not found even above 1000 EU/m<sup>3</sup> in that study. However, other reports of endotoxin exposure in agriculture found acute effects above 1000 EU/m<sup>3</sup>. Thus, it seems likely that only one of the plants studied had endotoxin levels in a range that would be of concern. Furthermore, a comparison of the two plants may yield more information about the potential effects of endotoxin than can be obtained internally from either study.

## Bioaerosols and Airborne Endotoxins in Automotive Machining Plants

Peter S. Thorne, Jeannine A. DeKoster, and Periyasamy Subramanian  
The University of Iowa, Department of Preventive Medicine  
& Environmental Health, Iowa City, IA 52242-5000

### ABSTRACT

Although airborne microorganisms and endotoxins are recognized hazards in a variety of occupational environments, there has been relatively little study of these contaminants in machining plant aerosols. In this investigation bioaerosol monitoring was undertaken in a 185,000 m<sup>2</sup> engine plant across 4 seasons. Sampling was performed in 9 locations: 4 sites were on an older (1970s) engine line (4 9), 4 sites were located on a new engine line (NS) with improved engineering controls, and one site was in assembly. The machining sites represented areas supplied with 3 different metal working fluid (MWF) formulations from 8 separate sump systems. Bulk in-use MWF samples were collected at the machining sites and compared with air measurements at the same sites. Gravimetric concentration of aerosol averaged 1.24 mg/m<sup>3</sup> on the older engine line and 0.74 mg/m<sup>3</sup> on the new line. The inhalable fraction was 87% of the aerosol and 57% was respirable. Formaldehyde yielded from biocides averaged 0.22 mg/m<sup>3</sup> and was as high as 0.62 mg/m<sup>3</sup>. Endotoxin concentration ranged from 39 to 166,000 EU/ml in the bulk MWF and from below detection (<4 EU/m<sup>3</sup>) to 790 EU/m<sup>3</sup> in air. Airborne viable fungi was generally low (<470 colony forming units (CFU)/m<sup>3</sup>) while viable mesophilic bacteria ranged from 40 to 4,000 CFU/m<sup>3</sup>. Airborne total bacteria (culturable + non-culturable) ranged from 5,560 org/m<sup>3</sup> to 468,000 org/m<sup>3</sup>. Concentrations of endotoxin in the MWF were significantly correlated with bulk viable mesophilic bacteria (p<0.0001, r=0.62) and Gm- bacteria (p=0.014, r=0.54). Airborne endotoxin concentration demonstrated significant associations with bulk endotoxin (p=0.022, r=0.44) and bulk total organisms (p=0.016, r=0.80), but correlations with airborne organisms were weak. Airborne endotoxin was

strongly correlated with the gravimetric aerosol concentration (p<0.0001, r=0.83) suggesting that a standard based upon gravimetric aerosol concentration is a reasonable surrogate for endotoxin in this environment. Perhaps most striking about the measured bioaerosol concentrations was the tremendous temporal and spatial variability and the dependence upon adjustments of MWF constituents. The dynamics of the microorganisms in the MWF and the biocides added to control them suggested that close monitoring of MWF and early intervention when *microbiota* increase can help prevent excessive concentrations of airborne endotoxin.

### INTRODUCTION

Aerosols of metal working fluids (MWF) are generated in the process of cooling, flushing and lubricating machine tools and metal parts during machining operations such as drilling, grinding, honing, hobbing, and milling. While mineral oils are most commonly used for honing, these other machining processes use water-based MWF, typically containing 2 to 20 % emulsified oil. While in small job shops each machine may have its own sump for MWF, large machining facilities such as automotive engine and transmission plants use central systems with up to 750,000 L (200,000 gal) capacity and an array of piping and flumes to deliver the MWF to the line. Whether large or small, these MWF systems become complex mixtures of water, emulsified oils, antifoam agents, rust inhibitors, biocides, microorganisms, and microbial products. The MWF have half-lives in the sump systems typically of 3 to 6 months. When these MWF are sprayed onto machine tools bioaerosols are generated. Both bacteria and fungi can effectively colonize MWF and serve as sources of microbial toxins. Bioaerosols are known hazards

in a variety of occupational environments<sup>(1)</sup> and epidemiologic studies have shown that inhalation of mists from in-use MWF can result in acute respiratory symptoms<sup>(2)</sup> and cross-shift declines in lung function.<sup>(3)</sup> Thus, the purpose of this research was to characterize airborne contaminants in machining plants with a focus on bioaerosols and endotoxin.

## METHODS

### Aerosols

Concentrations of non-volatile aerosols were determined gravimetrically using DM-800 filters (0.8  $\mu\text{m}$  pore size, Gelman, Ann Arbor, MI) connected to personal sampling pumps (GilAir-5, Gilian, Caldwell, NJ) calibrated at 4.0 L/min in an open-face configuration. Particle size distributions were determined using 8-stage personal cascade impactors (Marple Series 290, Graesby-Andersen, Atlanta, GA) connected to personal sampling pumps calibrated at 2.0 L/min. Sampling media were desiccated and weighed before and after sampling using a Mettler MT-5 microbalance. Real-time monitoring of MWF aerosols was performed using Mini-RAMs (GCA Corporation, Bedford, MA) with PDL-10 data loggers (Mie, Inc., Bedford, MA). Formaldehyde was assayed as its hydrazone derivative by HPLC following sampling onto 2,4-dinitrophenyl hydrazine-coated glass fiber filters at a flow rate of 0.5 L/min.

### Bioaerosols

Sampling for airborne viable microorganisms used 2-stage jet-to-agar cascade impactors (Graesby-Andersen). Malt extract agar (MEA, Difco, Detroit, MI) was used for sampling fungi, R2A agar with 1% cycloheximide was used for sampling mesophilic bacteria, and eosin methylene blue (EMB) agar was used for sampling Gm- bacteria. All cultures were incubated at  $23 \pm 2^\circ\text{C}$ . Field blanks and control plates were included for each set of samples. Colonies on the plates were corrected for coincidence using the positive hole correction method.<sup>(4)</sup> Fungi that could not be identified by colony characteristics alone were analyzed microscopically with lacto-phenol blue

stain<sup>(5)</sup> and identified using standard slides and atlases of fungal spores.<sup>(6)</sup> Predominant bacteria and fungi were subcultured and speciated using standard microbiology methods. Bulk samples were collected from MWF spray nozzles at machining operations at the air monitoring sites. They were cooled to  $4^\circ\text{C}$  and transported under refrigeration in sterile 1L polypropylene bottles and cultured as described above within 4 to 20 hr of collection.

Total airborne culturable and non-culturable bacteria and fungi were quantified using the fluorescence microscopy Nuclepore filtration/elution method (FM-NFE).<sup>(7-10)</sup> Samples were collected onto 25 mm black polycarbonate filters connected to personal sampling pumps calibrated at 1.5 L/min. The adhered organisms on these filters were stained directly with 0.1 mg/ml acridine orange solution, pH 7.2 in phosphate buffer (EM Science, Cherry Hill, NJ). Viable and non-viable microorganisms were then enumerated under epifluorescence microscopy and the total airborne concentrations were determined as described previously.<sup>(9)</sup> Microorganisms in the bulk MWF were serially diluted, filtered onto black polycarbonate media and analyzed in a fashion similar to the air samples described above.

### Endotoxins

Endotoxin analysis was performed on DM-800 filters following gravimetric analysis using the endpoint Quantitative Chromogenic *Limulus* Amebocyte Lysate Assay (BioWhittaker, QCL-1000, Walkersville, MD). Filters were extracted into 30 ml of pyrogen-free water with heating to  $68^\circ\text{C}$  for 30 min and periodic shaking. Serial dilutions of filter extract and endotoxin standards were prepared using sterile pyrogen-free water in glass tubes which had been heat treated for at least 4 hours at  $200^\circ\text{C}$ . Absorbance measurements were performed at 405 nm using a BT2000 Micro-Kinetics microplate reader (Bio-Tek Instruments, Palo Alto, CA). Change in absorbance relative to the assay reagent blank was calculated and a standard curve of absorbance versus endotoxin concentration was generated. The standard curve

ranged from 0.1 to 1.0 Endotoxin Units (EU) of National Institute of Standards and Technology traceable EC-5 standard endotoxin (10 EU= 1 ng endotoxin). Bulk MWF endotoxin concentrations were measured with the QCL-1000 method as described above. Chemical and dye inhibition assays were run to assess augmentation or interference with the assay and were negative at the dilutions used for the endotoxin assays. Selected bulk fluids were repeatedly analyzed over a period of 4 to 6 weeks and were stable in storage at 4°C.

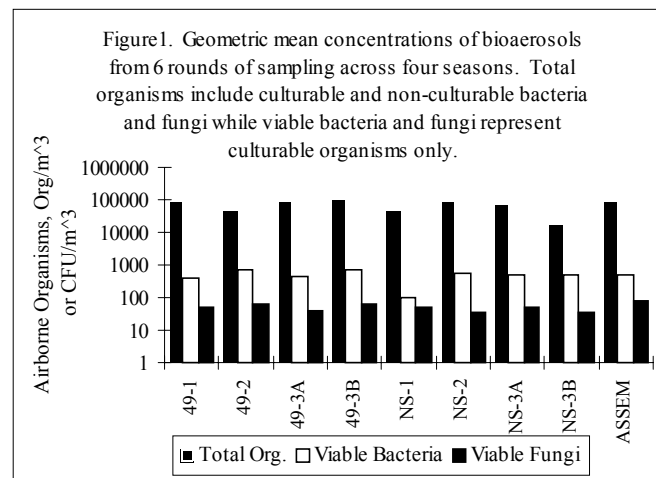
## RESULTS

Gravimetric concentrations of MWF aerosol in the air were all less than 5 mg/m<sup>3</sup> and were significantly higher in the 49 areas than in the NS areas. Samples collected in assembly were lower by 10-fold or more in aerosol concentrations. Real time monitoring of MWF aerosol by light scattering demonstrated that there were large temporal and spatial variations in aerosol concentration within the plant depending on proximity to wet machining operations and whether or not mist collection systems serving a particular area were functioning. Average temperature and relative humidity at the 8 machining sampling locations ranged from 23.6°C and 61.2% in Winter to 26.1°C and 79.3% in Summer. CO<sub>2</sub> concentration, an indicator of general exhaust ventilation was reduced from 850 ppm to 450 ppm as ventilation was increased.

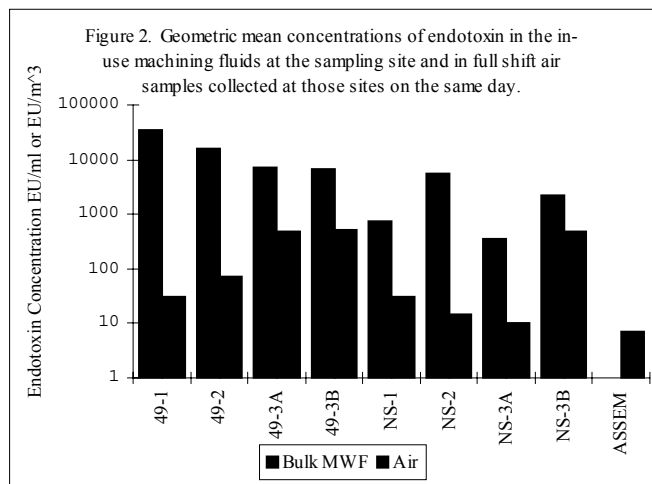
Bulk MWF samples had low concentrations of fungi due to continuous aeration of the MWF, competition from bacteria, and the effective use of fungicides. Most MWF samples had fewer than 50 viable fungi/ml although 2 of 50 samples had higher concentrations (2,890 and 10,100 CFU/ml). The concentrations of bacteria in the bulk fluids were much higher. Viable bacteria in the MWF ranged from 7 CFU/ml to 7.6 x 10<sup>6</sup> CFU/ml and total bacteria ranged from 1.5 x 10<sup>7</sup> to 1.7 x 10<sup>8</sup> org/ml. On average, Gram negative bacteria accounted for 65% of the bulk viable bacteria. The predominant bacteria isolated from the MWF samples were *Pseudomonas pseudoalcaligenes*, *Pseudomonas alcaligenes*, several unidentified *Pseudomonas* sp., and

*Micrococcus luteus*.

Geometric mean concentrations of bioaerosols over six rounds of sampling spanning all 4 seasons are illustrated in Figure 1.



Overall, airborne viable fungi ranged from below detection to 3,800 CFU/m<sup>3</sup> and mean concentrations at each site were all below 60 CFU/m<sup>3</sup>. Outdoor viable fungi averaged 244 CFU/m<sup>3</sup>. Airborne viable bacteria demonstrated site-specific mean values from 81 to 550 CFU/m<sup>3</sup>. As a point of reference, these concentrations were not markedly different from indoor air in homes in the U.S. midwest.<sup>(11)</sup> Total airborne organisms were 2 orders-of-magnitude higher than viable organisms. Figure 2 illustrates the bulk and airborne concentrations of endotoxins by sampling site for Spring and Summer samples. Individual bulk MWF samples yielded endotoxin concentrations that ranged from 39 EU/ml up to 166,000 EU/ml. The highest values were observed during episodes of rapid growth of Gram negative bacteria in which these organisms exceeded 10<sup>6</sup> CFU/ml. Airborne concentrations of endotoxin ranged from below detection (<4 EU/m<sup>3</sup>) to 787 EU/m<sup>3</sup>. This is above the response threshold reported by Castellan *et al.*<sup>(12)</sup> for cotton textile workers of 90 EU/m<sup>3</sup>, but below exposures typically encountered in agricultural poultry and swine houses (1,000 to 10,000 EU/m<sup>3</sup>). Forty percent of the air samples from the older 4.9 L engine line exceeded 90 EU/m<sup>3</sup> while 17% from the new NS engine line were over this threshold.



## CONCLUSIONS

This study demonstrated that the airborne levels of endotoxin in automotive machining plants may exceed the thresholds identified for acute respiratory health effects. Airborne endotoxin concentrations were strongly correlated with the bulk MWF endotoxin concentration and were particularly high when microbial growth in MWF was excessive. Thus, a program of careful monitoring and control of *microbiota* and endotoxin in machining fluids is of vital importance to minimizing inhalation exposure to endotoxin among machinists.

## ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Ms. Sylvia McGuire with in-plant sampling.

“This project was funded in whole by joint funds from the UAW-GM National Joint Committee on Health and Safety which does not necessarily support or endorse the findings and opinions herein. The findings and opinions contained within this publication are solely the responsibility of and attributable to the authors.”<sup>(13)</sup>

## REFERENCES

1. **Lacey, J; J Dutkiewicz:** Bioaerosols and occupational lung disease. *J. Aerosol Sci.* 25:1371-1404 (1994).
2. **Sprince, NL; PS Thorne, W Pependorf, MI Selim, C Zwerling, and ER Miller:** Respiratory

symptoms and lung function abnormalities among machinists in automobile production. (submitted).

3. **Kennedy, SM; IA Greaves, D Kriebel, EA Eisen, TJ Smith, and SR Woskie:** Acute pulmonary responses among automobile workers exposed to aerosols of machining fluids. *Am. J. Ind. Med.* 15:627-641 (1989).
4. **Leopold, SS:** “Positive Hole” statistical adjustment for a two-stage Anderson air sampler. *A.I.H.A.J.* 49:A88-89 (1988).
5. **Kozak, P Jr.; P Gallup, LH Cummins, and SA Gillman:** Currently available methods for home mold surveys. I. Description of techniques. *Ann. Allergy* 45:85-89 (1980).
6. **Smith, EG:** *Sampling and Identifying Allergenic Pollens and Molds.* San Antonio: Blewstone Press, 1984.
7. **Hobbie, JE; RJ Daley, and S Jasper:** Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33:1225-1228 (1977).
8. **Palmgren, U; G Strom, G Blomquist, and P Malmberg:** Collection of airborne microorganisms on Nuclepore filters, estimate and analysis-CAMNEA method. *J. Appl. Bact.* 61:401-406 (1986)
9. **Thorne, PS; MS Kiekhaefer, P Whitten, and K Donham:** Comparison of bioaerosol sampling methods in barns housing swine. *Appl. Environ. Microbiol.* 58:2543-2551 (1992).
10. **Thorne, PS; JL Lange, PD Bloebaum, and GJ Kullman:** Bioaerosol sampling in field studies: Can samples be express mailed? *Am. Ind. Hyg. Assoc. J.* 55:1072-1079 (1994).
11. **DeKoster, JA; PS Thorne:** Bioaerosol concentrations in non-complaint, complaint and intervention homes in the midwest. *Am. Ind. Hyg. Assoc. J.* 56:573-580, 1995.
12. **Castellan, RM; SA Olenchock, KB Kinsley, and JL Hankinson:** Inhaled endotoxin and decreased spirometric values. *New Eng. J. Med.* 317:605-610 (1987).
13. **UAW-GM National Joint Committee on Health and Safety:** Research agreement between UAW-GM and The University of Iowa. pg. 5, 1991.

## DISCUSSANT'S COMMENTS and OPEN DISCUSSION

**Dr. TAI CHAN, General Motors:** The fundamental basis of bioaerosol sampling and the various issues are critical, and we know now that the metalworking fluid aerosol should be treated as a metalworking fluid bioaerosol. I think now we have an opportunity to hear the Discussants and field your questions, and I'm sure there must be quite a few questions. Our first Discussant is Howard Cohen and since I have introduced him already, I don't think he needs any further introduction and Howard is going to focus his comments on the first two papers. Of course, he's free to comment on the other papers as well, so let's start with Howard.

**Dr. HOWARD COHEN, University of New Haven:** I'll keep my remarks fairly short so that you can have time for questions and answers. First of all, I would like to set the stage which is maybe why Tai has me up here.

First of all, I'm an Industrial Hygienist and I am Editor of the AIHA Journal and that's really why I'm up here now. That the whole issue of metalworking fluids is a critical issue in occupational health as I see it. There are lots of workers who are exposed to metalworking fluid, not just from the automotive companies, but from other manufacturing as well. It's one of those exposures that you can find where workers still complain, and where relative to other control methods that I think we have out there, are still fairly primitive.

One of the things that really impressed me in doing a study that I described earlier this morning was that in going to eight facilities, I saw a variety of workplaces, a variety of union/management interactions and a variety of people. I found workers who love their jobs, workers who hated their jobs, workers who liked to see me, workers who wished I was dead, a whole variety of different types of personalities and people, which is exactly what you would expect.

But what I found was that everybody either complained about metalworking fluids or had concerns about irritation to metalworking fluids. When the whole study was done, which for me was a very short study, the issue that gave me the most lasting impression was the people who were happiest, who loved their jobs, had problems working with metalworking fluids. They could have been in management, provided that they were out in the workshop. If they were in their office, they didn't have so many problems. They could be employees. No matter who it was, they complained about these fluids.

It became obvious that it's a problem which is really not well controlled. When I first came into the field of industrial hygiene, one of my first jobs was monitoring metalworking fluid operations as we made rifles for the Winchester Rifle Company. It was one of the most awful jobs we had in the whole facility. People walked away covered with metalworking fluid and breathing it. We had a variety of controls which certainly didn't make it better and perhaps at times made it worse. We never exceeded five milligrams per cubic meter, although there was fluid on my skin, clothes, face and everything else every day, but we never could exceed 5 milligrams per cubic meter using traditional monitoring methods.

So it's interesting to come back here and listen to this and I will say that there's a lot more knowledge than probably was around 20 years ago and some of the levels are dropping, but nevertheless, it's still an important industrial hygiene issue. It's an important occupational health issue and I'm very impressed, quite truthfully, to see how much knowledge has been forwarded by groups like the UAW-GM. It has made a big difference. I would call these nontraditional funding sources because these are, in fact, going to be some of the funding sources in the future in this country for looking at occupational health.

Given these points, let me add one more

thing to my collection here. The other reason this is a very interesting area is that the issues are very complex, as you have heard and they're complex from the things that concern me. Air sampling, analysis, the biological issues, control technologies. All of those make this a fascinating area.

Before moving on to microbiology, which is what the speakers talked about, let me mention that the whole area of biological hazards, bioaerosols, in our field is becoming very important. We see far more papers being submitted to our journal from research, primarily outside the U.S., quite truthfully, but even within the U.S. Our Journal started in 1940 and through about 1990, we never seemed to have a need to have either a microbiologist or someone who had expertise in biohazards on our Editorial Board. I'm pleased to say our first person to join us was Peter Thorne, one of our speakers behind me. We now have, in the short period of time we have added Peter to our Board, two others and they reflect simply the volume of papers that we're seeing in this area, so it's a very important area.

When I go listening to indoor air quality problems, what I hear are problems related to biological hazards, the growth of microorganisms which is contributing to the variety of problems we see in indoor workplaces. And what you hear is all the efforts being done to remove the substrates that microorganisms and biological agents are living on, primarily water, but in other cases dirt and other materials. When you come to the metalworking fluid area, what do you find? When you go through the plants, you find rivers, rivers flowing of things that microorganisms are going to grow on and live in a luxurious lifestyle.

So from an indoor air quality point of view, it's going to be very difficult to say, okay, we know the source, let's just get rid of the metalworking fluids. It's not a reasonable control for this industry. I think it is very useful, looking at all the problems we see in indoor air pollution and looking at some of the complaints we see regarding irritation in the workplace to look very carefully at bioaerosols. It shouldn't be too

surprising that that's where our focus is. And I think that's what makes the papers we heard today very important.

Some of the key issues we heard, if you look at Klaus' [Willeke] and Sergey's [Grinshpun] first paper on monitoring, let me back up by saying I've known Klaus for a number of years and our Journal has been very fortunate to have a number of fine papers from him and his co-workers and his laboratory, and one of the things that always impresses you with Klaus is that he and his workers and his collaborators are usually on the cutting edge of developing new technologies for us. And that's what always gets me rather excited to get some of his papers to review. And that's what you saw here.

New methods, how do we collect bioaerosols, what are the methods to doing it. His paper here had some very basic information for the audience, but nevertheless, one comes away with it that these are the sampling tools of the future if we want to go into workplaces and do bioaerosol sampling. If, in fact, we are going to do this routinely, it's not going to be the microbiologist, maybe it is going to be if we have that many microbiologists available. I have a feeling it's going to be engineers, chemists, industrial hygienists and others, who are going to somehow have to find a way to use these tools to take samples that are appropriate.

We do, obviously, have a lot of microbiologists involved in metalworking fluids and I think it may have been Janet Macher that pointed out, it's pretty rare to get people who understand how to sample for microorganisms together at the same time, where we have people who understand how to do air sampling.

The analysis that we have heard and all the complexities, and my background isn't chemistry, the complexities are not that dissimilar to what we find out in chemistry. And that is that there's lots of interferences. It's very hard to understand what a true value is, we do things different ways so we come up with different results.

Perhaps the more difficult thing in the whole area of bioaerosols are that we're looking at

a whole host of microorganisms, so a much more complex analysis than say for formaldehyde. More like if you would do a pixie analysis where you're looking at all elements together.

One of the things that's clearly very important is comparison to standards and I think Peter [Thorne] talked about this. When we go collect samples, we have microorganisms, we have bacteria on our body, we have it outside, we have it everywhere. When we get these levels, what are the important values. What are the important values to look at. Let me, as I have just a few minutes left, seconds left, talk about Dr. Macher's work for a minute.

I thought Janet focused in on key issues on study design, how you have to do a study. One of the things it reminded me of is when I go buy a new car at a dealer. I can buy the car, or I can buy every option that goes with the car. Usually I can't afford to buy every option, so I have to start making some decisions as what's valuable to me, and in our sampling and control strategies, we need the same thing. We usually can't take all the samples we'd like to take. It's impossible. We don't have the money, the time and whatnot and really people like Janet are going to have to help us make some of these decisions and say what is the cost-effectiveness of not doing this or not doing that. That's what's going to be critical for us so that we spend our money, develop studies that we can use in the future and know that we can't do everything that we'd like to do in a single study.

Thank you very much.

**Dr. TAI CHAN, General Motors:**

Thank you, Howard. As he pointed out, the bioaerosol area is a growth area, but we don't just want that to generate research papers. We need to at some point implement this research and help the folks on the plant floor. We need to tell them the buck stops here and we can help them do that.

The next two discussions have to do with endotoxin aspects and also the bioaerosol. Dr. Dan Lewis is Section Chief of the Immunology Branch at NIOSH Morgantown and I'll let Dan

make his comments.

**Dr DANIEL LEWIS, NIOSH:** Thank you, Tai. I want to thank you and the organizers for inviting me to this Symposium and I really must complement the audience. You are really hearty souls to still be here after three days of technical talks. I think it is wonderful that so many people take such an interest in this important and complex subject.

When Tai first asked me to come to this Symposium, I was not sure exactly why, and when I finish giving my comments, you may be asking the same question. My background is really in organic dusts and pulmonary reactions resulting from the inhalation of organic dusts rather than the bioaerosols from metalworking fluids. Organic dust exposures are related more to agricultural work environments, but many of the questions related to MWF bioaerosols turn out to be very similar to those found in the agricultural workplace. The health problems are very analogous, and after three days of listening to these MWF presentations, I find that my research has touched on many of the same concerns and problems.

One thing that struck me while listening to these talks was a comment that I heard earlier this Summer at the International Congress of Toxicologists out in Seattle, Washington. The keynote speaker at the conference was Dr. Leroy Hood, whom some of you may or may not know, but he's a very preeminent molecular biologist who is really one of the pioneers at automating and computerizing the biology laboratory. His research has changed how biologists think about things, and how they collect and analyze data. The essence of his talk dealt with the need for toxicology to become involved in interdisciplinary research in the broadest sense of the word. The engineers working with the biologists, the chemists, the statisticians as a team to understand and solve a complex problem. But they all must be able to communicate and understand each other. It is often more difficult for two professionals from different fields to understand each other than it is for two professionals from the

same field but different countries to understand each other. For example, a Russian and an American biologist may be able to understand each other's research better than an American biologist and an American engineer can understand each other, even when they are working on the same problem. The educational background and intellectual paradigms are so different for different professions that interdisciplinary research can be very difficult. Learning a common language to ensure that all members of the team can communicate with each other is one of the first and most difficult parts of interdisciplinary research.

In the talks that we heard this afternoon there were several important points that deserved some additional comments. From Miriam Lonon's and Harriet Burge's presentations, the first thing that struck me was that several microbiologists could make a career out of studying the microbial ecology of these fluids. There may be organisms there that have not been previously identified. There is a lot to be learned about the microbiology of these fluids. But the central question comes down to this; is it necessary? The answer to me is that both sides of the question are important, but depend somewhat on the health outcome of concern. The number of bacteria, particularly the number of Gram negative bacteria which relates to the amount of endotoxin, in both the fluid and aerosol can relate to the health effect observed. The inhalation of endotoxin is associated with certain patterns of pulmonary responses in a dose dependent manner. In my field of research this is usually referred to as an ODS reaction, or organic toxic dust syndrome. And one of the things we have learned is the severity of this pulmonary reaction is more dependent upon the amount of endotoxin or number of bacteria inhaled, and not which type of organism. At the other end of the spectrum is hypersensitivity pneumonitis which appears to be more dependent upon the type of organism inhaled. Over the years a lot of data has been collected which suggests that certain microorganisms, particularly actinomycetes and

some fungi, are more frequently associated with this disease than other microorganisms. So there is no clear answer as to whether it is more important to know how much is there or what is there. It really depends on the end point you are concerned with; that is, the health effect of concern.

There is one other point about microbial ecology that deserves mentioning. Miriam Lonon gave us a cute, easy to remember five word paradigm that will probably be one of the best remembered phrases of the Symposium. I think there is a more important message here. By understanding the microbial flora of metalworking fluids, and fully characterizing that flora, it may be possible to gain some idea of where the sources of contamination are. Where do these organisms that are growing in the fluid come from? Can we do anything to control the sources of contamination? In reality there are probably numerous sources of contamination, but possibly by controlling these sources and keeping the fluid as clean as possible, then the quantity of biocides added could be reduced and the health effects due to microbial exposure lessened. I think it is something worth considering.

One final point that I would like to make. Our laboratory does a lot of endotoxin analyses, and with some investment in equipment and planning, it is something that can be done routinely without a lot of difficulty. Dr. Thorne mentioned the idea that gravimetric determination may be a good surrogate for endotoxin since the endotoxin in the fluid paralleled what he was finding in the air. I would suggest that maybe the opposite might also be true. Because endotoxin can be measured when present in vanishingly small quantities, like picogram amounts, it may be possible to use endotoxin as the surrogate for the gravimetric determinations, allowing for increased sensitivity for air sampling techniques. Such increased sensitivity may be helpful in determining the time dependent concentration of a substance in air. Thus the exposures during different phases of an operation could be monitored to determine when and where controls

need to be put into place.

Thank you for your attention, and for the opportunity to participate in your Symposium

**Dr. TAI CHAN, General Motors:**

Thank you, Dr. Lewis. Our last Discussant is Dr. David Schwartz from the University of Iowa. He is a Professor of Medicine and he's going to give his clinical observation about some of the things discussed this afternoon. David.

**Dr. DAVID SCHWARTZ, University of Iowa:** Thank you, Tai and I want to thank you on organizing such a well attended and well organized Symposium that touches on many of the details that are important, both in terms of exposure, as well as disease.

Although this afternoon's presentations have focused on microbial contamination and sampling techniques, I want to bring to you the perspective of a pulmonologist who has a very strong interest in occupational and environmental airway disease.

The focus of my comments are going to be in three different parts. First I want to comment on the spectrum of lung disease that appears to be associated with exposure to cutting fluids. Second, I want to make some brief comments on the relative importance of bioaerosol sampling methods and I'm going to try to keep them brief because the scientists that have talked before me are much more expert about this than I am. Finally, I want to make some recommendations about some areas of research that I think would be particularly important to pursue in this area, given what's been presented at the Symposium thus far.

So listening to yesterday's presentations, I should start by saying that I came to this meeting as someone who didn't particularly believe in cutting fluids as an important health problem, but sitting through yesterday's presentations, I became convinced that workers using cutting fluids are at increased risk of developing lung disease. I was not particularly convinced by any one study, but I was persuaded by the consistency from one study

to the next in terms of the respiratory symptoms, as well as the findings that were presented by the investigators.

However, as a pulmonologist and also as a clinician, it really remains entirely unclear to me what the location of the lung disease is and also what the type of lung disease is in these individuals. I think it's important when you think about lung disease, to realize that there are basically three different areas of the lung; the airway above the vocal chords, the conducting airways below the vocal chords, and the lung parenchyma or the alveoli, the breathing sacs in the lung that transfer oxygen and carbon dioxide. And there are really three distinct disease processes that all could explain the findings presented yesterday.

One is upper airway irritation, which could be responsible for a lot of the symptomatology that was presented, as well as some of the spirometric findings.

The second is airway irritation with or without asthma. There may be an asthma-like phenomenon or a general irritation of the airway that's occurring in these individuals that's not true asthma.

Then thirdly, Dr. Burge brought up the possibility today of hypersensitivity pneumonitis and one of the points that I want to make sure that no one leaves this meeting thinking is that hypersensitivity pneumonitis is similar to asthma. These diseases are completely different processes occurring in different areas of the lung. Asthma occurs in the conducting airways, it's inflammation, irritation, narrowing of the conducting airways. Whereas hypersensitivity pneumonitis is an inflammatory response that occurs in the alveolus, the breathing sacs in the lungs. We think of hypersensitivity pneumonitis as a lung parenchymal disease, as opposed to an airway disease. These diseases are very different pathologic phenomenon and they're very different diseases in terms of location.

Although I suspect cutting fluids are capable of causing all of these diseases, putting a label on the symptoms is not nearly as important

as determining the extent and the severity of the disease, and I think that this was clearly pointed out yesterday. I think it's important to recognize that we don't yet know what percentage of workers are affected by these lung problems. And we also don't know what the impact of the lung function impairment is on their work or recreational activities and I think this too is a very, very important point in terms of understanding how impairing this disease process is.

As an investigator, I'm interested in trying to understand the mechanisms that underlie the disease process, and this is not a trivial point. This point relates very much to what interventions are appropriate in this population. And I think that there are a lot of pathologic mechanisms that could potentially be playing into this process. This is a mixture of a lot of different agents and you've heard that over and over again from many different speakers.

First of all, these agents could directly irritate the mucosa and damage the mucosa. Secondly, these agents could act to trigger a primary allergic response and we really don't yet have any information whether it's acting as a primary allergen. The fact that Susan Kennedy showed yesterday that a lot of very sensitive people leave the workplace early on in their career, suggests that potentially an allergic mechanism might be playing a role in identifying a susceptible population that end up having symptoms and end up leaving the workplace.

Thirdly, this might be a primary biologic response to either microorganisms or the toxic products of microorganisms and there was a host of data presented this afternoon that suggests that microorganisms may play a very important role in this disease process. And fourthly, this could be a nonspecific inflammatory response occurring not only in the airways, but also in the lung parenchyma.

Given the complex mixture of agents in the aerosol, I suspect that cutting fluids under different circumstances may be capable of causing different mechanisms or different pathologic processes in various components of the lung and

potentially that's why we are not seeing a clear picture in terms of the symptoms or in terms of the physiologic abnormalities that are presented in these different studies. Thus, multiple mechanisms under different exposure circumstances are probably involved in this process.

So briefly turning my attention to the exposure side of the equation, it's clear that these cutting fluids are highly contaminated with microorganisms and their products, and you've heard about that today. Although microorganisms are capable of causing lung disease, we really don't know whether these microorganisms are responsible for the development of cutting fluid induced lung disease. That's still not proven, it's just an associated phenomenon at this point.

Several other toxic agents are present in this mixture and acute changes in lung function have not been shown particularly to be strongly related to the degree of microbial contamination in the cutting fluids. This healthy degree of skepticism raises several specific concerns regarding the exposure assessment studies, much of which were discussed by Dr. Macher. And I just want to touch on a few of these elements regarding study design as they relate to the exposure assessment.

First, Dr. Macher mentioned the issue of sampling error. I refer to that as reliability or repeatability. How repeatable is that measure from one moment to the next or using different sampling mechanisms? And Dr. Cohen this morning talked about a 50 to 60 percent variability in terms of measurement error. That's a high degree of variability and you have to ask is that acceptable, and what is that telling us?

Another issue in terms of reliability is how stable are these measures over time? This is particularly important when you're looking at chronic disease outcomes. If you take one measurement and that measurement isn't stable over time, as some of Dr. Thorne's data suggests, then it's hard to relate that acute exposure, that one-time measurement to the development of chronic disease. We are going to have to come up

with better ways of assessing what the estimated level of chronic exposure is over a period of time.

The issue of association versus causation is another very important point. Is endotoxin causing the disease or is it a measure of the degree of contamination in the plant in general? Is it a measure of other contaminants that might be present that might be more strongly related to disease in terms of causation?

And finally, how do these external measures of exposure relate to what gets into the lung because after all, that's what we're interested in, in terms of causing disease. We still haven't developed the techniques or the technology to assess what the measure of deposited aerosol is in the lung.

So with this as background, I just want to suggest a couple of paths for future research projects. First, I'd like to suggest that epidemiologically there's a lot to be done. I believe that population based longitudinal studies really need to be conducted in this area. The long-term goal should be to identify the extent, as well as severity of disease in individuals who work in large plants, as well as those who work in small plants because there is probably a very different pattern of exposure and disease in these individuals, depending on the specific type of factory.

As was suggested by Dr. Milton today, I think it's very important that we build consistency within these epidemiologic studies so that we can directly compare the findings from one epidemiologic study to another epidemiologic study, and I think in terms of the planning of these studies, you really want to keep in mind the issue of consistency.

I think another very large area that I'm particularly interested in is moving beyond these epidemiologic observations. I think it's important to use these epidemiologic observations, to understand the specific exposures that have been highlighted today and perform human exposure response studies. I think the human exposure response studies are critical to understanding the components of the bioaerosol that are playing a role in the development of symptoms, as well as

physiologic changes. I think the exposure response studies will help better define the acute symptomatology, the physical response, as well as the underlying biologic mechanisms that are responsible for the development of disease. It will take us a long way toward understanding the disease pathogenesis, the mechanisms that underlie the development of disease in individuals and importantly from a clinician's perspective, they'll also help us develop intervention strategies that are relevant to the underlying biology within these disease processes.

Thanks for listening to me and I look forward to participating in the ongoing discussion.

---

**Dr. TAI CHAN, General Motors:** This session is open for questions now.

---

**Dr. Frederick Passman:** Fred Passman, Biodeterioration Control Associates. I have got a couple of comments, particularly to Miriam's [Lonon] talk in terms of succession of populations, just kind of a point of order.

Typically the aerobes colonize first, scavenge the oxygen from the environment and then create micro-environments in which the obligate anaerobes can then become established, so very rarely would you see an SRP a sulfate reducer population be established before the mucous forming what I call pioneer microbes that fix themselves to pristine surfaces and create micro-niches in which other microbes can then colonize and evolve into mature biofilms.

And in most of our metalworking systems, what we have are massive fixed film biological reactors which in many cases I find curious. It's a very growing industry in the United States, taking use of that technology and then producing products. On the other hand, within many industries these many square meters of surface area virtually get ignored when we sample.

The second comment is on the various identification kits the API and the Biolab. These are based on what we call phenotypic tests. The

ability to metabolize particular substrates, show whether or not they have particular enzymes. These tests are not surprisingly designed for the diagnostic industry, which is a very large industry compared to when a guy like me goes to an ASM meeting and tries to find another environmental microbiologist, I'm often talking to the wall. There are very few microbial ecologists, particularly dealing with industrial process fluids.

So it's not surprising that the databases don't provide good correlations between what we see in these environments. I would recommend you to some of the genotypic databases, which take a look at the genetic material databases being developed at places like University of Montana, Bozeman, I believe Ron Atlas' group at the University of Louisville and the folks at University of Maryland are doing a lot of work in microbial ecology.

**Dr. CHAN:** Excuse me, Fred, do you have a specific question?

**Dr. Passman:** No, I have two questions, yes. One, I have a question for Dr. Burge. Monday to Tuesday titer increases, were they reproducible over several weeks, or was this a phenomenon that you observed on a particular week and then I also wanted clarification, once you knocked down the bacterial titers within 24 or 48 hours you saw substantial increases in your fungal titers?

**Dr. HARRIET BURGE:** The plants that we were in very rarely used biocides and that Monday to Tuesday wasn't necessarily a knockdown. It was just Delta. It was just a difference, and our plants were not using biocides hardly ever.

**Dr. Passman:** I thought I heard you mention that in one case you had high bacterial titers, they treated it with a biocide and within about 24 hours?

**Dr. BURGE:** That wasn't me.

**Dr. Passman:** Okay. Whoever made that observation?

**Dr. TAI CHAN:** Okay, John has been very patient. John?

**Dr. John Howell:** First is a comment and then a question for Dr. Burge.

One of the factors which is going to be important it looks like in the future is looking further at the area of endotoxins, whether in the bulk fluid or in the air sampling and I had spoken during break time to Dr. Milton and Dr. Thorne and let them know that the ASTM Committee on Health and Safety of Metalworking Fluids, E34.50 had in fact started a study group on standardizing methodology for endotoxin sampling of metalworking fluids. That group's next meeting is Monday, December 4th and that is a topic which will be addressed then, so anyone who is interested in pursuing that, I'd certainly invite them to talk to me on the side and we'll get you some information. But that is in an area that I think is going to need some follow-up work.

The specific question for Dr. Burge had to do with the hypersensitivity pneumonitis outbreak or episode that you spoke of. Based upon your experience in the area of this, how frequent, is that very rare, have you heard of other episodes like that, or is it more common than perhaps others of us might think?

**Dr. HARRIET BURGE:** I have absolutely no idea of how common it is. I have heard of three outbreaks of hypersensitivity pneumonitis now related to mycobacterium colonization of coolants. How many there are out there, I have absolutely no clue. I mean everybody doesn't call me about them, so I just don't know. I have heard of three, though, just me, I alone have heard of three, so I think it's probably something that we're, that it happens more often than we imagine. I still think that it's not common. I think it's a rare disease. I think

that our physicians would agree with that.

**Dr. TAI CHAN:** I think Dr. Schwartz would like to comment on that one.

**Dr. DAVID SCHWARTZ:** Actually I just wanted to ask Dr. Burge to clarify this issue of hypersensitivity pneumonitis. It is an unusual problem, and I was wondering to what extent it was documented at this plant and how was the diagnosis confirmed?

**Dr. BURGE:** It was very well documented. It was biopsied, x-rayed, very well documented and these were very well documented cases, all of them. Not by serology, but by the usual method. I'm not a physician, but in this case, I was very convinced, I was completely satisfied that all these people actually had that disease, not asthma.

**Dr. SCHWARTZ:** This point is very important because many cases of hypersensitivity pneumonitis are simply documented by antibodies, but if it was documented by chest x-ray and lung biopsy, then it is very likely hypersensitivity pneumonitis. I am very impressed that 75 percent of the workers in that setting developed hypersensitivity pneumonitis - that percentage is quite astounding.

**Dr. TAI CHAN:** Okay. We can move over here.

---

**Mr. Lew Schultz:** My name is Lew Schultz. I'm with the UAW, I'm an international rep and I work out of the Center for Health and Safety. One of my responsibilities is health and safety audits. As a result of that, I'm somewhat pessimistic, however, I will make my responses rather short and I have two questions.

The first question goes to and I look at a lot of charts and graphs, I start wondering about the authenticity and whether they are actually supported with fact. I notice that there was no

difficulty in challenging some of the UAW-GM charts that were put up and some of the data, yet I noticed some of the other industries, in particular the oil industry, when they were challenged indicated they got it from the World Health Organization and that they got some internal study and they were presenting those as facts.

As a result of that and being somewhat pessimistic as I have indicated earlier, I'm wondering if the oil industry and other industries would be willing or prepared to provide the criteria and method by which their conclusions were arrived at, so they can be checked for accuracy. In addition to that, there was another request made on the initial day by an investigator [Susan Woskie], requesting some information from the oil industry as pertains to some of their products. I would like to know whether the oil industry is going to respond to that and whether they are going to provide that necessary data.

And then I would like to make one final comment. I go in to the plants, a lot of machining plants and the first speaker [William Lucke] said it best: When you go into these facilities, the first thing that you notice is that there is a heavy mist. After a little while you have it on your skin and your clothing and your hair. I'm talking about somebody just coming in. By about the second or third day of an audit, you become aware of other problems, a little bit of breathing difficulty.

The people that are in the plant do this every day. When you go out and look at their equipment, they've got it on the floor, they've got it on the equipment, the cleaning people are involved in it, the maintenance people are involved in it, they have got it all over them. And this is a continuous problem.

I would hope that what we come up with out of here is going to help those people. One of the facilities I was in they had in excess of 50 people being followed because they introduced something into the system and they can't identify it. And they are moving them from area to area. And a lot of times, like I said, they come in on Monday, they feel okay, by Wednesday they have a sore throat and it's just a continuous situation

until something major develops.

I would request, though, that the oil industry and the other people here who made presentations, show how they arrived at some of those conclusions. I would appreciate that. Thank you.

**Dr. TAI CHAN:** Thank you for the question. I think as far as the first question, I'm sure the oil industry would be happy to respond to you directly and it's beyond the scope of this specific session.

On the second part, however, I think Howard might want to comment on that in terms of dealing with issues on the plant floor. How difficult it is to communicate. This is a difficult problem.

**Dr. HOWARD COHEN:** He said it better than I did. He spends time in audits doing it. I'm an industrial hygienist, but it's clearly an area that needs control, needs activity, but you said it better than I do and you know it better than I do.

**Mr. Schultz:** Thank you. I know one thing. That if we as a total group don't get together and start working on these problems, we have a major, major situation going on out there. I don't have the answers for the people on the floor. We're talking to them daily and that's dealing with people. I'm talking human beings.

I can relate a little bit to what you taught me, a little microbiology, a little statistics, a little industrial hygiene, but I got to tell you, when you are talking to the people on the floor and you're talking to the families, that's much, much more difficult. It's easier to talk to you. Thank you.

---

**Ms. Joan Tucker:** My name is Joan Tucker and I work for General Motors at a UAW represented plant. I'm one of those gophers, one of those persons that go out on the floor and does air sampling and I want to applaud the arrangers for this wonderful Symposium. It has provided a

great opportunity for me to learn many things, and makes me realize how much there is to learn outside, which I'm sure I never will, but I look at the resources, I look at the years of study, the world of academia in this plant and in this room, I look at how much energy has been put forth and how much you have given of yourselves in putting this together, the amount of knowledge that is here in this room.

I also think it's very nice in many ways that we put in the human factor of a medical doctor that deals with people at the end of this session. Just a short comment. Thank you.

**Dr. TAI CHAN:** Thank you very much. In fact, I would like to compliment AAMA for putting this Symposium together. As Lew pointed out, we need to work together to fix the problem. We're here for you. Thank you.

---

**Mr. Patrick Dugal:** My name is Pat Dugal. I'm from the Canadian Auto Workers in Windsor, Ontario, Canada. There was a comment made by Janet [Macher] this afternoon dealing with an exposure, a metalworking fluid dealing with legionella. I would just like to expound on that just a bit.

It was the plant that I worked in where that occurred at, and just to give some history to the participants here of what happened on that August, 1981 day. There's close to 700 people in that plant and out of the 700, there was over 200 affected by the outbreak. We had over 140 compensation cases that came from that. The insidious bacteria, the way it moves around inside of the plant after people from the Disease Control Center from Atlanta came in and also we were very fortunate at the time to have Dr. Mike Silverstein who was with the UAW at the time, and he took control of the situation, but just to give you some day-to-day events dealing with that outbreak, like I had people inside of my area which I was a Union Steward at the time that we're talking about this thing being a communicable disease, was it or was it not, were

they going to take it home to their families?

The amount of anxiety and fear that it caused was just incredible. I'm standing up here today just to let the participants know of what that thing was really like.

And I have a question for Janet after I'm finished here. I'm glad to say today that facilities that I work for and I go into have a control measure in place now where we do have daily testing of our coolant system and there doesn't seem to be those kind of problems today. We might have a system go bad, but nothing like that. So what I just want to say to everybody inside of this room is that the end user is the workers inside of that plant to the products that are being produced by the suppliers.

And I think it's very important, not just the suppliers, but also the manufacturers and also the buyers and also the people that are using it in the plant, understand, and I agree with the previous speaker, that we all have to work together on this thing. We really do. I don't want to have to go into a plant today then to revisit something that happened 14 years ago and I think it's important that you people hear that. I really do.

But the question that I would like to ask Janet deals with between 1981 and 1995 where we are at today, do you know of any other strains of legionella that have appeared in metalworking fluids?

**Dr. JANET MACHER:** Well, if I was going to make a comment, what I was going to say is that legionella would not grow on TSA, R2A, the agars that you heard mentioned today for the bacterial cultures. So I don't think we know if legionella is out there in any of these other materials or if anybody has data, I think we should share it.

It is a recent concern in California. We have a possible case of Legionnaire's disease in a machinist and we know that there's legionella in the coolant and I'm not at liberty now to say more than that, but I think it is an area that we probably should pay more attention to so that we don't get surprised by other outbreaks.

Does anybody have anything else to contribute?

**Dr. PETER THORNE:** I would just say we had planned to do the proper media for legionella in our study, but to do what we did, needed five different media for a few thousand runs and each of these two-stage samplers, you have two plates for each run and if you do them in duplicate, it quickly adds up, so often times the resources aren't there and you have to pick and choose and the legionella seemed a long shot compared to the basic information we were seeking about the fluids themselves, so we had to choose not to do that as part of our study and that was a choice we made with some reluctance for the reasons that we just heard.

---

**Dr. John Howell:** Again, John Howell, Castrol Industrial North America representing at this moment the Independent Lubricant Manufacturers Association.

I did want to just respond to Lew's question and say that this very afternoon that representatives from the ILMA Health and Safety Task Force did meet with Professor Susan Woskie and we talked about providing the information that she had requested on Monday afternoon and I'm satisfied that we have got a mechanism in place to get her that information and so I just want to say that ILMA is working hard to pro-actively be involved in the health and safety of the products we supply.

**Dr. TAI CHAN:** In fact Lew, my opening statement today was we are here to serve the American worker and that's the bottom line.

---

**Dr. James D'Arcy:** Jim D'Arcy from General Motors. I just wanted to comment on the legionella. In the '86-'87 work that we did, we did try to culture legionella specifically onto the proper media for that, and we were able to culture it, you know, the control cultures, but culturing

from machining fluid, we did not find any in the plant.

We did take some plant machining fluid, inoculate it with legionella and we were able to plate it back out from the legionella, but in a series of probably about 20 different samples over a period of about three or four months, we did not culture any from the fluids in the plant, even though it appeared that it would survive at least for a short period of time in the fluid.

---

**Mr. Otto Peter:** Otto Peter from General Motors of Canada. I guess we're a lot of GM people up here. I have a question for Dr. Burge. When you were talking about near the end of your presentation about systems and you were talking about some gram positive systems and maybe I misheard you, you made a comment about you want to keep your systems gram negative and I just wanted to ask that question because as I was listening to a lot of other people, I thought maybe a way we can keep our systems from being so toxic to our employees is maybe inoculate them with gram positive and that way we could keep the endotoxins out of them, you see? Maybe even add food. That was just a thought. I'm a hygienist, not a microbiologist. So I just have that question. Is that what you meant to say?

**Dr. HARRIET BURGE:** That is exactly what I meant to say. Well, I have talked about this with another industrial hygienist at this Symposium, as a matter of fact, the possibility of finding the perfect microorganism that could take over a machining coolant system and have absolutely no health effects and as a microbiologist and a long term medical school employee, I think not.

My concern with the gram positive organisms is that they are very closely associated with hypersensitivity disease, both with asthma and with hypersensitivity pneumonitis and I really do think that the severity of the disease that results from exposure to mycobacterium is much larger and much greater. It's a much more severe

disease, hypersensitivity pneumonitis, than the things that I have at least heard about related to the endotoxin exposures. Now I could be wrong, I'm not a physician, but at any rate, no, I don't think you should switch to a gram positive fluid and I did mean to say that.

**Dr. DONALD MILTON:** If I may add my two cents to that. I found it really interesting that in the Warren plant and the Indianapolis plant, the endotoxin levels in the sumps were very similar and yet the airborne endotoxin levels were much lower in one of the plants than in the other, which suggests to me that there are ways to contain these aerosols that don't have to do with trying to manipulate the flora and the things growing in the fluid and that you don't want to breath a lot of endotoxin, but you don't want to get mycobacteria or other organisms that are associated with hypersensitivity pneumonitis going in there either, so you might be better off just looking for those control technologies that can really bring down the fluid levels in the air and the associated endotoxins in the air. I think one of the things that might be very productive would be to go back and compare across those two studies, what are the factors across those plants and perhaps including Peter's [Thorne] study as well, what are the factors across multiple plants where we have a lot of data that really contributed to control of mist and control of the endotoxin and other exposures.

**Dr. TAI CHAN:** I have a question for Don. Is the difference between Warren and Indianapolis simply a difference because of the machining activity?

**Dr. MILTON:** I really don't know what caused that difference and that's what I think we need to look at. Certainly in the walk-through investigations that were made in Indianapolis, people looked at these guys putting their faces right up to the machines and assumed that they were going to have exposure because certainly in many of the small shops that I go into and Sue

Woskie and I have investigated one for example in Wooster that looks a lot like that plant, where we're seeing in excess of a milligram per cubic meter and lots more endotoxin than we saw in either of those plants, so it was actually quite surprising that the levels were so low there.

---

**Mr. Walter Diachuk:** Walt Diachuk, Helical Dynamics. I have a question in general to the panel. If you were to use a low vapor pressure fluid to machine and then totally enclose the machine so it would be totally negative to the building and the employee and then have a removal system that could remove to say a tenth of a micron or better in the particulate aerosol and if this discharge is very low on the vapor and we were to add to it ozone free ultraviolet to kill the bacteria and so forth, are we helping the environment? Are we getting close? Finally if we construct the hypothetical system from all these three days of talking, what would it do to the environment then?

**Dr. PETER THORNE:** I have one word to say. Nirvana.

**Dr. TAI CHAN:** Does anyone else want to comment?

---

**Dr. William Watt:** Bill Watt from Chrysler. Suppose I go to the office Friday and the phone rings off the hook and people want some analysis of bacteria and endotoxin levels in the plant and suppose I'm able to get someone to take a sample, what would the levels mean? Should I look for types of bacteria? Should I look for levels of bacteria? Should I look for endotoxins and how much should I worry about and how much should I not worry about? Even if I know what's there, what does it mean?

**Dr. THORNE:** I think that that raises a very good point.

**Dr. Watt:** Sorry to do that.

**Dr. THORNE:** No, and it should have come out before and it will now, in my opinion, anyway. There's many factors and the engineering controls that are in place are a big determinant of what you're going to have in the air obviously, and that relates to our last questioner who outlined a program that might reduce airborne quantities to the point where it doesn't matter what's in them, the fluid itself or what the composition of the fluid might be that if you removed all the aerosol, you enclose the operations completely, if you're filtering it so you're getting 99.3 percent of everything less than point three microns, or if there's some kind of HEPA filtration system, if there's no exposure, you can have the most toxic substance in the world and it isn't going to cause any risk.

But in the practical world where we don't have that level of engineering control in place, then I think there is some meaning to the concentration of these materials in the fluids, but that by itself is not sufficient. You need to know something about the engineering controls in place and the airborne levels that arise from these machining operations and something about the duration of exposure, who is exposed, where they are and this sort of thing as well and perhaps others want to address that.

**Dr. DONALD MILTON:** Well, I was impressed with what Peter [Thorne] stated within his plant that the correlation with total particulate was so high, between endotoxin and total particulate and that countable bacteria was another approach, so there are various approaches you can take, but what you need is something that's quick and easy and you really want to know what's the aerosol level of metalworking fluid there and if that's up over a milligram, it needs to be brought down. If following in Harriet's [Burge] footsteps here, if they have a gram positive system, maybe they should look at dumping that and just trying to start over.

**Dr. DAVID SCHWARTZ:** I'd just like to follow up that question. Are we sure that that's what's causing the disease process in these individuals and if so, well, I guess if not, what kinds of experiments need to get done or studies need to be done to identify the pathogenic origin of this process?

**Dr. HARRIET BURGE:** I'm not going to say what studies, I mean, obviously, we need to do more studies. I think that there's enough evidence that mycobacterium exposure in machine coolants have led, I think there's enough evidence that it could lead to hypersensitivity pneumonitis, that if you see mycobacterium as the dominant organism in machine coolants, it's worthwhile to dump the fluid and try to get rid of it. It's a dangerous disease, as I'm sure you will agree, that may become irreversible and I just don't think it's worth taking, I don't think we should wait until we have more research before we start being pro-active about that.

On the other hand, I'd love to see more people monitoring for mycobacterium so that we know what the incidence of it is in machine coolants. I don't think most of you all do that and then I'm sure that we could design studies that might allow detection, early detection of this kind of disease as well.

**Dr. DONALD MILTON:** I think we should move towards things like looking at endotoxin and looking at some of these other factors that are in the air, but at this stage where there is still going to be disagreement about methods, there's going to be problems with laboratories comparing with each other, that you don't want to get into doing that as a routine method and that's why I think your first line of defense, bringing down the total aerosol and that's the first step.

---

**Mr. W. Jay Scott:** I'm Jay Scott from Ford Motor Company. The question is for Dr. Burge. You said earlier that your results for your

fungi report were not out yet. You hadn't studied them and whatnot. I was wondering how we can get those results when you do and if you want to make any comments now about those. Do we have to worry about them? Is it something that, I mean mushrooms aren't going to hurt you, yeah, but is it something that maybe it's a spore or is it going to be something else that we are going to have to worry about later?

**Dr. HARRIET BURGE:** Mushrooms do hurt you, as a matter of fact, they are one of the major causes of asthma in outdoor air.

**Mr. Scott:** Only if you eat them.

**Dr. BURGE:** Well, breathing the spores does too, but they don't grow in machining coolants. That I can pretty well guarantee.

The levels of fungi that we saw in the sumps, that we looked at were very low. The levels were quite low in comparison to the levels of bacteria that we saw. Levels were in the range of less than 1,000 per milliliter of fluid, where we were seeing ten to the eighth bacteria per milliliter of fluid. The problem is, we don't know what levels of fungi are important with respect to disease, so it may be that in the long run exposure to fusarium at levels of 1,000 or 500 or even 50 per milliliter of fluid could be a risk for allergic sensitization. For example in the population in a plant that has the tendency to develop allergic asthma. I don't know that and neither does anybody else and it's going to be a long time before we know that. The kinds of research that are necessary to learn that piece of information are not there.

The best I'm going to be able to tell you is how much we recovered, what the variability is, what conditions were involved in actually seeing fungi in the fluids. The only fungus we saw with any regularity at all was fusarium and that is a toxin producing fungus, so it's the best I can offer you.

**Mr. Scott:** Okay. When are your results going to be published, or are you going to write a

paper?

**Dr. BURGE:** The person behind you would love to know that as well [referring to Dr. Tom Robins standing behind Mr. Scott]. During 1996. Actually we're starting, Laurie is maybe still listening and she is the doctoral student who is helping me work on this and during 1996, yes.

**Mr. Scott:** Okay. From what I see and what I hear, there's maybe something else out there that's causing all these problems. The facts are, maybe it is the fungi. We don't know, we don't even know what the toxic levels of that are. That's what everybody is saying, so maybe that's something we need to hit on.

**Dr. PETER THORNE:** I'm not sure we would agree with that statement.

**Mr. Scott:** You may not, but you haven't proven to me that you're not.

**Dr. THORNE:** Well, I would just offer one thing from my perspective in the plant that I studied here. The levels of airborne fungi were much lower than we find in peoples homes in Iowa. They were orders of magnitude lower than what we measure in grain handling facilities, dairy barns, a variety of other work environments in agriculture and so I don't think that that is the primary place to put our effort.

The other fact that Dr. Burge and I both find that the fusarium organisms are the primary genus that we're seeing and this is a ubiquitous fungi. It's not an odd pathogenic organism and so I think that it is something to continue looking at, but I don't think that it's the magic bullet that we're just missing the boat on. I wouldn't want you to be left with that perspective.

**Mr. Scott:** I wouldn't. No, I would have to agree there. I don't think it is either, but I don't think we should drop the ball on it. Thanks.

**Ms. Leslie Wireman:** My name is Leslie Wireman from Quaker Chemical Corporation and my question goes to Dr. Thorne, I believe, but anyone else who wants to comment.

In your one study where you compared an older grinding system to a newer grinding system in your last talk, you were talking about newer engineering controls decreasing the level of bioaerosols, endotoxin and misting, but unless my notes were wrong here and they may be, I saw an increase in the formaldehyde concentration in the newer system, and I guess we have to give thought to will these newer engineering controls, although they may help us in a lot of other areas, actually concentrate, assuming we are going to use triazine biocides or formaldehyde donors, will that concentrate that formaldehyde aerosol and will that dissipate over time or is that a concern that we should really take a look at?

**Dr. PETER THORNE:** I believe you are referring to one particular sampling site on the new engine line and it's fair to say that I was sampling this engine line as they were bringing it up. When we started our sampling, they were still going through testing. I think they were at a level of about 200 to 300 engines per day on that line and were moving up toward a present value that I'm told as of yesterday something like a thousand engines per day.

So they were still troubleshooting the system and there were a couple locations within that new engine line that had some problems early on as determined by perception, as determined by slick floor and condensate and mist problems, and they were working to correct those. So it may be that that particular site does not now have that problem, I don't know, but that was odd to see that in a line that was designed to have better controls, so I think it was one site of the four on that line and it may not be the case anymore. I haven't been back to test that so I couldn't really answer if that's a continuing thing and it was not a repeated sample, so it wouldn't give us that kind of answer.

**Dr. Tom Robins:** Tom Robins here from U of M. If I may, I just wanted to come back to this issue that was raised I guess by the speaker before the last one about what should be the level of concern about the fungal growth in machining fluids.

I guess one thing just to point out, although in terms of the data from the Warren plant that Dr. Burge was presenting, I think that plant was relatively unusual in the fact that they were using virtually no biocides at all, so I think at least there's the potential, depending on what the strategy of biocide use was, that you might be suppressing bacterial growth in other situations and allowing more fungal growth.

The other thing I wanted to mention for those of you that heard me yesterday in answer to one question, I mentioned that I was aware of several clinical cases of hypersensitivity pneumonitis or 'hypersensitivity pneumonitis *like*' disease in individuals I have seen and though I don't have this data personally, so I hesitate to say this, but I just wanted to mention that I was told in a couple of these cases that there was suspicion in fact about fungal species, I think specifically aspergillus as opposed to something like fusarium in one case possibly being related, but again, take that with a grain of salt because I don't have specific data, but I was just wondering, is there anything else that anyone on the panel would like to say any further comment about this question about whether fungi are a concern?

**Dr. HARRIET BURGE:** We actually didn't see no fungi in the Warren plant. We saw it consistently off and on all the way through at very low levels. My suspicion would be if the fungi were part of this whole syndrome, that we would have seen more asthma than we did and we really didn't see very much. Is that your impression?

It's not impossible and actually I wrote down a little note about how we might analyze the data that we have to look at that very question. I think it's a very interesting question that needs to be looked at and also needs to be evaluated maybe in some of the other studies where there were

biocides added often and fungi were higher, in higher levels in the sumps.

It doesn't worry me that the fungi weren't abundant in the air because fungal toxins and allergens could very well have been there without culturable spores. The spores are fragile and very, very large and so you would be more likely to get allergens and fungal toxins and we weren't measuring those. I don't think any of the studies measured those.

**Dr. TAI CHAN:** Actually Peter claimed that he saw some fungi in Livonia.

**Dr. PETER THORNE:** I just wanted to remind everybody that [the term] biocides include bacteriocides and fungicides, and that many compounds have activity against both types of organisms.

---

**Dr. TAI CHAN:** Well, this is unusual. We're on time. Are there any questions? How about Frank?

**Dr. Franklin Mirer:** Not now, but of course I'll reserve my right to speak the next time.

**Dr. TAI CHAN:** I want to thank the speakers and the discussants and I want to thank you for being here. Tonight's session, the poster session goes from 7:00 to 10:00 pm and it is a good transition into the control technology. Hope we see you back at the Great Lakes Center and tomorrow morning we start at 7:40. Continental breakfast at 7:00.

