DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service
Centers for Disease Control
and Prevention (CDC)

Memorandum

Date: October 28, 2003

From: Adele M. Childress, Ph.D., Program Official
Office of Extramural Programs, NIOSH, E-74

Subject: Final Report Submitted for Entry into NTIS for Grant 5 R01 OH003463-05.

To: William D. Bennett
Data Systems Team, Information Resources Branch, EID, NIOSH, P03/C18

The attached final report has been received from the principal investigator on the subject NIOSH grant. If this document is forwarded to the National Technical Information Service, please let us know when a document number is known so that we can inform anyone who inquires about this final report.

Any publications that are included with this report are highlighted on the list below.

Attachment
cc: Sherri Diana, EID, P03/C13

List of Publications

Willeke K, Lee S, Mainelis G, Adhikari A, Reponen T, Grinshpun SA, Cho SH, Wang H, Trunov M: Airborne Microorganisms Collection by a New Electrostatic Precipitator. Proceedings of 9th International Conference on Indoor Air Quality and Climate, Monterey, Calironia, pp. 396-401, June 3 - July 5, 2002

Mainelis G, Willeke K, Adhikari A, Reponen T, Grinshpun SA: Design and Collection Efficiency of A New Electrostatic Precipitator for Bioaerosol Collection. Aerosol Science and Technology 36:1073-1085, 2002

Mainelis G, Adhikari A, Willeke K, Lee S-A, Reponen T, Grinshpun SA: Collection of Airborne Microorganisms by A New Electrostatic Precipitator. J of Aerosol Science 33:1417-1432, 2002

Mainelis G, Gorny R, Willeke K, Reponen T, Grinshpun SA, Yadav J, Baron PA: Effect of Electrical Charges and Fields on Viablity and Injury of Airborne Bacteria. Biotechnology and Bioengineering 79:229-241, 2002

Mainelis G, Willeke K, Baron PA, Grinshpun SA, Reponen T: Induction Charging and Electrostatic Classification of Micrometer-Size Particles for Investigating the Electrobiological Properties of Airborne Microorganisms. Aerosol Science and Technology 36:479-491, 2002

Mainelis G, Willkeke K, Baron PA, Grinshpun SA, Reponen T: Electrical Charges on Airborne Microorganisms. J of Aersol Science 32:1087-1110, 2001

Title: Electrostatic Sampling of Airborne Microorganisms
Investigator: Klaus Willeke, Ph.D.
Affiliation: University of Cincinnati
City & State: OH
Telephone: (513) 558-0506
Award Number: 5 R01 OH003463-05
Start & End Date: 4/1/2001–3/31/2003
Total Project Cost: $344,250
Program Area: Exposure Assessment Methods
Key Words:

Final Report Abstract:
Each year millions of respiratory allergies and infections are caused by airborne microorganisms present in agricultural, industrial and indoor environments. The level of exposure indicated by bioaerosol samplers depends on the instrument used and the sensitivity of the microorganisms. In an effort to collect such microorganisms more gently, at low power and at minimal pressure drop, an electrostatic sampling technique has been developed and evaluated. As a major part of this development, an electrostatic particle size classifier and a microorganism dispersion device with optional induction charging were developed to study the electric charges on airborne microorganisms. It has experimentally been proven that laboratory dispersed indoor air bacteria, such as Pseudomonas fluorescens, have a net negative charge. Some of the bacteria were found to carry several thousand negative or positive charges. In contrast, particles of non biological origin were found to carry very few positive or negative charges. This finding suggests that a bioaerosol sampler utilizing electrostatic means may collect airborne microorganisms by its electrostatic collecting field without first charging the microorganisms in the inlet section, thus reducing the complexity and power consumption for sampling in occupational environments. Such a sampler has been developed, built and tested in the laboratory and under various field conditions. The sampler is capable of efficiently collecting airborne microorganisms without charging in the inlet section. The percentage of airborne microorganisms enumerated after collection depends on the sensitivity of the microorganism and the analysis method used.

Publications:
Mainelis G, Grinshpun SA, Willeke K, Reponen T, Ulevicius V, Hintz PJ: Collection of Airborne Microorganisms by Electrostatic Precipitation. Aerosol Science and Technology 30:127-144, 1999

Mainelis G, Willkeke K, Baron PA, Grinshpun SA, Reponen T: Electrical Charges on Airborne Microorganisms. J of Aersol Science 32:1087-1110, 2001

Mainelis G, Willeke K, Baron PA, Grinshpun SA, Reponen T: Induction Charging and Electrostatic Classification of Micrometer-Size Particles for Investigating the Electrobiological Properties of Airborne Microorganisms. Aerosol Science and Technology 36:479-491, 2002

Mainelis G, Gorny R, Willeke K, Reponen T, Grinshpun SA, Yadav J, Baron PA: Effect of Electrical Charges and Fields on Viablity and Injury of Airborne Bacteria. Biotechnology and Bioengineering 79:229-241, 2002

Mainelis G, Adhikari A, Willeke K, Lee S-A, Reponen T, Grinshpun SA: Collection of Airborne Microorganisms by A New Electrostatic Precipitator. J of Aerosol Science 33:1417-1432, 2002

Mainelis G, Willeke K, Adhikari A, Reponen T, Grinshpun SA: Design and Collection Efficiency of A New Electrostatic Precipitator for Bioaerosol Collection. Aerosol Science and Technology 36:1073-1085, 2002

Willeke K, Lee S, Mainelis G, Adhikari A, Reponen T, Grinshpun SA, Cho SH, Wang H, Trunov M: Airborne Microorganisms Collection by a New Electrostatic Precipitator. Proceedings of 9th International Conference on Indoor Air Quality and Climate, Monterey, Calironia, pp. 396-401, June 3 - July 5, 2002

Final Invention Statement

NIOSH Grant 5 R01 OH003463, Years 1 through 5
"Electrostatic Sampling of Airborne Microorganisms"

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Grant Period: 9/30/1997 – 3/31/2003
(The first 3-year funding cycle ended 9/29/00 and was extended to 3/31/01; the 2-year continuation grant started 4/1/01 and ended 3/31/03)

April 2, 2003

No inventions related to this grant were conceived.

Klaus Willeke

Final Performance Report

NIOSH Grant 5 R01 OH003463, Years 1 through 5
"Electrostatic Sampling of Airborne Microorganisms"

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Grant Period: 9/30/1997 – 3/31/2003
(The first 3-year funding cycle ended 9/29/00 and was extended to 3/31/01; the 2-year continuation grant started 4/1/01 and ended 3/31/03)

April 2, 2003

Abstract

Each year millions of respiratory allergies and infections are caused by airborne microörganisms present in agricultural, industrial and indoor environments. The level of exposure indicated by bioaerosol samplers depends on the instrument used and the sensitivity of the microorganisms. In an effort to collect such microorganisms more gently, at low power and at minimal pressure drop, an electrostatic sampling technique has been developed and evaluated. As a major part of this development, an electrostatic particle-size classifier and a microorganism dispersion device with optional induction charging were developed to study the electric charges on airborne microorganisms. It has experimentally been proven that laboratory-dispersed indoor air bacteria, such as *Pseudomonas fluorescens*, have a net negative charge. Some of the bacteria were found to carry several thousand negative or positive charges. In contrast, particles of non-biological origin were found to carry very few positive or negative charges. This finding suggests that a bioaerosol sampler utilizing electrostatic means may collect airborne microorganisms by its electrostatic collecting field without first charging the microorganisms in the inlet section, thus reducing the complexity and power consumption for sampling in occupational environments. Such a sampler has been developed, built and tested in the laboratory and under various field conditions. The sampler is capable of efficiently collecting airborne microorganisms without charging in the inlet section. The percentage of airborne microorganisms enumerated after collection depends on the sensitivity of the microorganism and the analysis method used.

Significant Findings

The overall objective of this research was to use electrostatic mechanisms for the efficient collection of airborne microorganisms in a nutrient medium, and to develop a prototype electrostatic bioaerosol sampler. These objectives have been met: Several studies were undertaken on the behavior of airborne microorganisms in an electrostatic filed and a prototype electrostatic bioaerosol sampler has been developed and tested.

Through this research, it was found that airborne microorganisms may naturally carry significantly higher levels of electrical charge than non-biological particles, particularly when dispersed from a liquid. This implies that one may be able to collect such microorganisms without first charging them.

In order to perform laboratory tests on airborne microorganisms, a bioaerosol generator was developed that gently imposes electric charges on the microorganisms by electric charge induction. Conventional electrical charging by corona discharge is known to kill sensitive microorganisms.

In order to study the viability of airborne microorganisms carrying a specific positive or negative electrical charge, an electrical charge classifier was developed. It was found that even sensitive microorganisms can maintain a high negative charge without loosing their viability. However, high positive charges may kill them. We attribute this to naturally present negative charges in the cell walls of the microorganisms.

A sampler utilizing electrostatic means has been developed, built and tested in the laboratory and under various field conditions. The sampler is capable of efficiently collecting airborne microorganisms without charging in the inlet section. The percentage of airborne microorganisms enumerated after collection depends on the sensitivity of the microorganism and the analysis method used.

The new sampler differentiates between positively and negatively charged microorganisms. In most of the tested environments, the net charge on the airborne microorganisms was found to be negative. The charge distribution on the microorganisms adds a signature that may assist in their identification or differentiation.

Laboratory and field tests with the new sampler suggest that future design modifications should consider: 1. Placing agar plates on the positive and negative electrodes, so that positively and negatively charged microorganisms can be collected at the same time. 2. Placing an ionizer for charge enhancement not in the inlet, but between the precipitation electrodes, so that the loss of highly charged microorganisms to surfaces other than the agar plates is minimized. 3. Shaping the inlet into a narrow slit with a modest pressure drop to reduce the ambient turbulence level and to assure uniform distribution of the microorganisms between the precipitation electrodes.

Usefulness of Findings

Each year millions of respiratory allergies and infections are caused by airborne microorganisms present in agricultural, industrial and indoor environments. A new method utilizing electrostatic means has been developed for monitoring the presence and concentration of airborne microorganisms. This method collects airborne microorganisms efficiently and consumes very little power. It differentiates positively from negatively charged microorganisms, which adds a signature to their identification or differentiation. The new method also gives us a tool for assessing the electrical charge distribution on airborne microorganisms. For each species, the electrical charge distribution depends on the time since dispersal of the microorganisms into the air.

Peer-reviewed Journal Articles that Have Resulted from this Grant (with Acknowledgement to the Grant. Copies of the Articles are Attached to this Report)

1. Mainelis G, Grinshpun SA, Willeke K, Reponen T, Ulevicius V, Hintz PJ: Collection of Airborne Microorganisms by Electrostatic Precipitation. Aerosol Science and Technology 30:127-144, 1999

2. Mainelis G, Willeke K, Baron PA, Grinshpun SA, Reponen T: Electrical Charges on Airborne Microorganisms. J. of Aerosol Science 32:1087-1110, 2001

3. Mainelis G, Willeke K, Baron PA, Grinshpun SA, Reponen T: Induction Charging and Electrostatic Classification of Micrometer-Size Particles for Investigating the Electrobiological Properties of Airborne Microorganisms. Aerosol Science and Technology, 36:479-491, 2002

4. Mainelis G, Gorny R, Willeke K, Reponen T, Grinshpun SA, Yadav J, Baron PA: Effect of Electrical Charges and Fields on Viability and Injury of Airborne Bacteria. Biotechnology and Bioengineering 79:229-241, 2002

5. Mainelis G, Adhikari A, Willeke K, Lee S-A, Reponen T, Grinshpun SA: Collection of Airborne Microorganisms by a New Electrostatic Precipitator. J. of Aerosol Science 33:1417-1432, 2002

6. Mainelis G, Willeke K, Adhikari A, Reponen T, Grinshpun SA: Design and Collection Efficiency of a New Electrostatic Precipitator for Bioaerosol Collection. Aerosol Science and Technology 36:1073-1085, 2002

7. Mainelis G, Gorny RL, Willeke K, Reponen T: Rapid Counting of Liquid-borne Microorganisms by Light Scattering Spectrometry. J. of Microbiological Methods, submitted, 2003.

8. Lee, S-A, Willeke K, Mainelis G, Adhikari A, Wang H, Reponen T, Grinshpun SA: Assessment of Electrical Charge on Airborne Microorganisms by a New Bioaerosol Sampling Method. American Industrial Hygiene Association J, submitted, 2003

Proceeding Article (with Acknowledgement to the Grant)

9. Willeke K Mainelis G, Grinshpun SA, Reponen T, Baron P: Measurement of Electrical Charges on Airborne Microorganisms, Journal of Aerosol Science 31: S957-958, 2000

10. Willeke K, Lee, S, Mainelis, G, Adhikari, A, Reponen T, Grinshpun SA, Cho SH, Wang H, Trunov M: Airborne Microorganisms Collection by a New Electrostatic Precipitator, Proc of 9th International Conference on Indoor Air Quality and Climate, Monterey, California, 396-401, June 30-July 5, 2002

11. Lee, SA, Willeke K, Mainelis G, Adhikari A, Reponen T, Grinshpun SA, Sivasubramani SK, Gorny RL, Shukla R: New Method for Collecting Airborne Microorganisms by Electrostatic Precipitation. Proc 6th International Aerosol Conference, Taipei, Taiwan, 193-194, September 9-13, 2002

The research results have also been presented at the annual American Industrial Hygiene Conferences in 1998 (Atlanta), in 1999 (Toronto), in 2000 (Orlando), in 2001 (New Orleans), and in 2002 (San Diego); at the annual American Association for Aerosol Research Conferences in 1998 (Cincinnati), in 1999 (Tacoma), and in 2000 (Saint Louis);

in 2000 at the European Aerosol Conference (Dublin, Ireland); and in 2002 at the 7th International Congress on Aerobiology (Montebello, Canada).

Research Results Contained in the Journal Articles

<u>First Article</u>: **Mainelis G, Grinshpun SA, Willeke K, Reponen T, Ulevicius V, Hintz PJ: Collection of Airborne Microorganisms by Electrostatic Precipitation, Aerosol Science and Technology 30:127-144, 1999.**

Before designing a new electrostatic sampler for bioaerosol collection, we decided to first gain experience with the electrostatic precipitation of bacteria by modifying and using a commercially available electrostatic aerosol sampler that was developed for the collection of non-biological particles. We acquired an Electrostatic Aerosol Sampler (EAS) (model 3100, TSI Inc., St. Paul, MN), consisting of an inlet, a flow straightener (honeycomb), a corona charging section, an electrostatic precipitation section and an outlet. In order to use this device for the collection of bacteria, a 0.8 cm deep, 4.8 cm wide and 18 cm long well was milled into the collection area so that a rectangular trough could be placed inside. The trough was filled with agar or water for collecting and subsequently incubating bacteria. We also used a filter as the collection medium; in this case, a conducting support was placed into the trough underneath the filter to ensure that the collection surface was at the same height as when sampling with agar or water. The electric field in the precipitation section was 4.2 kV/cm; the corona discharge in the charging section was produced by a 0.05 mm diameter tungsten wire maintained at a voltage of 3.5 kV.

The major findings of relevance to the development of an electrostatic bioaerosol sampler were as follows. Tests conducted with three bacterial species and with agar, water, and filter as collection media have shown that the electrostatic precipitation method is suitable for collecting viable airborne microorganisms. When collecting *Bacillus subtilis* var *niger* (BG) spores on agar, about 50 to 60 % of the collected culturable microorganisms formed colonies in the EAS. However, a much lower recovery rate was obtained for *Pseudomonas fluorescens* and *Mycobacterium bovis* BCG vegetative cells, which suggests that these bacteria may have sustained injuries in the charging section through the corona discharge (which produces ozone and nitric oxides). Additional embedding of the collected bacteria into agar did not result in an increase of the bacterial recovery rate. When the EAS pump was turned off and tests were performed with the three bacterial species inoculated on agar or in water in the collection section (i.e., no prior exposure to corona discharge) while exposing them to 4.2 kV/cm, the culturability was not reduced during test periods of up to two hours. We concluded from these data that even sensitive bacteria, such as *Pseudomonas fluorescens*, were not inactivated by the electric field after their collection on agar or in water. This finding encouraged us to find ways to avoid the use of corona discharge in the charging section and to find ways of measuring the electric charge distribution on airborne microorganisms.

<u>Second Article</u>: **Mainelis G, Willeke K, Baron PA, Grinshpun SA, Reponen T: Electrical Charges on Airborne Microorganisms, J. of Aerosol Science 32:1087-1110, 2001.**

In this article, we focus on the electric charge distributions of different particles of biological and non-biological origin. We show the wide electric charge distribution on *Pseudomonas fluorescens* bacteria when no induction voltage is applied during their dispersal. When NaCl particles of the same size are dispersed in the same way, only very low particle charges are measured. We attribute this striking difference to the presence of electric charges in the cell walls of the bacteria. When NaCl particles are dispersed in a highly negative induction field, they become more highly charged than the bacteria dispersed in the same way. This indicates that the cell walls of the microorganisms have their own mechanisms of regulating their electric charges. *Pseudomonas fluorescens* bacteria are gram-negative with a rather thin cell wall. Experiments with spores of gram-positive bacteria, such as *Bacillus subtilis* var *niger*, also showed a wide electric charge distribution when dispersed without induction charging.

<u>Third Article</u>: Mainelis G, Willeke K, Baron PA, Grinshpun SA, Reponen T: Induction Charging and Electrostatic Classification of Micrometer-Size Particles for Investigating the Electrobiological Properties of Airborne Microorganisms, Aerosol Science and Technology 36:479-491, 2002.

This article deals with the development of an aerosol generator with electric charge induction and an aerosol electric charge classifier. The development of the aerosol generator with charge induction was necessary so that we would have a means for aerosolizing particles of biological and non-biological origin with or without specific charge levels imposed on them. We could have dispersed the microorganisms by more conventional means and then charged them by corona discharge. However, that would have rendered too many of the microorganisms non-viable, as documented in our first article. Therefore, we developed a more gentle method by charging the microorganisms through induction. While induction charging has been successfully performed in a modified vibrating orifice aerosol generator for the generation of particles from a liquid solution, we are not aware of any such device for the dispersion of microorganisms from a suspension and their simultaneous induction charging.

In the new aerosol generator, the microorganisms are dispersed through the center stem of a Collison nebulizer (BGI Inc., Waltham, MA), i.e., the housing of the Collison nebulizer acting as the impaction surface is removed. After dispersion by the Collison's sonic orifice air jet, the liquid-borne microorganisms pass through a second, larger orifice, surrounded by air, which separates the particles from each other due to the pressure drop across the second orifice. Addition of dry air evaporates the water so that only airborne microorganisms and much smaller droplet residues leave the aerosol generator. Large droplets settle to the bottom of the generator and are drained. When a positive or negative voltage of up to 3,000 volt is applied to the stainless steel Collison stem, while the second orifice is grounded, electric charges are induced onto the droplets as they are being formed from the liquid exiting the liquid feed tube in the Collison stem.

The charged bioaerosol particles then enter our newly developed aerosol electric charge classifier. The bioaerosol particles enter through a channel, which is parallel to clean sheath air. By applying a voltage potential across this parallel plate device, the bioaerosol particles carrying a desired electric charge range are extracted.

During the first few months of working with these new devices in our test facility we found that many of the highly charged microorganisms were lost in the tubing and other inner surfaces of the system. After reducing or eliminating every conceivable loss surface, the device worked efficiently.

The article shows the electric charge distributions measured on *Pseudomonas fluorescens* bacteria over a wide range of electric charges. The *Pseudomonas fluorescens* bacteria carry up to 13,000 positive or negative charges, even when there is no induction charging during their dispersal. The net electric charge on these bacteria is negative when there is no induction charging.

When the bacteria are dispersed in a highly negative induction field, the net charge on the bacteria becomes more negative and the number of highly negatively charged bacteria increases significantly. However, the percentage of bacteria with low charges remains about the same. When the bacteria are dispersed in a positive induction field, the net charge on the bacteria becomes positive. To our knowledge, this is the first time that electric charge distributions have been measured on airborne microorganisms.

<u>Fourth Article:</u> Mainelis G, Gorny R, Willeke K, Reponen T, Grinshpun SA, Yadav J, Baron PA: Effect of Electrical Charges and Fields on Viability and Injury of Airborne Bacteria, Biotechnology and Bioengineering79:229-241, 2002.

In this article, we present data on the viability, metabolic injury and structural injury of charged and uncharged airborne microorganisms. In several sets of experiments, each lasting about four hours, we dispersed the microorganisms in the manner already described, passed them through the aerosol electric charge classifier and then sampled the bacteria carrying a pre-selected charge range with a BioSampler. The figures show the viability of bacteria at the beginning (t = 0) and at the end of the experiments (t = 4 hours) when there was no electric field applied across the plates of the classifier. When the induction voltage was set to zero volts during aerosol dispersion, the viability for the bacteria was found to be on the order of 50 to 60%. When a high negative charge was imposed during microbial dispersion in a highly negative induction field, the viability levels were of the same order of magnitude. However, when a high positive induction voltage was applied during dispersion, imparting a net positive charge to the bacteria, the viability was much reduced. The metabolic and structural injury levels were about the same, irrespective of the charging levels.

After the experiment at time = 0, different voltages were applied to the aerosol charge classifier and microorganisms of specific charge levels were extracted from the classifier. The negatively charged bacteria had viability levels that are normal for these bacteria. The viability of the positively charged bacteria, however, was much lower. We interpret this as follows: The bacteria naturally contain negative charges in their cell walls. When these bacteria carry positive charges, the bacterial balance is upset and the viability is reduced.

The new electrostatic sampler essentially follows the design detailed in the original grant proposal. The microorganisms are collected into a liquid which gravitationally flows down a collection surface at ground potential relative to a 4kV plate above it. The flowrate at which the bioaerosols are sampled into the device is 12.5 Lpm. The pressure drop between the inlet and the outlet of the sampler is minimal, on the order of 1 inch w.g.

<u>Fifth Article</u>: Mainelis G, Adhikari A, Willeke K, Lee S-A, Reponen T, Grinshpun SA: Collection of Airborne Microorganisms by a New Electrostatic Precipitator. J. of Aerosol Science 33:1417-1432, 2002

In this article, experiments are described that were conducted with *Pseudomonas fluorescens* vegetative cells, *Bacillus subtilis* var. *niger* (BG) endospores (used to simulate the spores of anthrax-causing *Bacillus anthracis*) and *Penicillium brevicompactum* fungal spores. It was found that 80-90% of initially "charge-neutralized" biological particles were removed from the air, when a small amount of ionization was generated in the electrostatic precipitator inlet and a precipitation voltage of ± 4000V was applied across the agar plates. The bioefficiency of the new sampler was about the same as that of the BioSampler, which was tested in parallel and requires 0.5 atm pressure drop by its pump. In experiments with sensitive Pseudomonas fluorescens vegetative cells, the electrostatic precipitator enumerated twice as many cells as the BioSampler.

<u>Sixth Article</u>: Mainelis G, Willeke K, Adhikari A, Reponen T, Grinshpun SA: Design and Collection Efficiency of a New Electrostatic Precipitator for Bioaerosol Collection. Aerosol Science and Technology 36:1073-1085, 2002

In this article, tests with non-biological NaCl particles versus *B. subtilis* var. *niger* (BG) spores and vegetative cells have shown that airborne microorganisms are collected more efficiently than non-biological particles, even when the microorganisms have first passed through an electric charge neutralizer with no additional charging applied. The difference was attributed to the natural charges contained in cell membranes or spore coats of the microorganisms. Charge-neutralized BG spores and vegetative cells were collected at 4 L/min with efficiencies close to 80%, depending on the precipitation voltage, versus 50-60 % for NaCl test particles. When incoming BG spores were charged with positive ions and then collected by a precipitating voltage of +1,300 V, about 80 % of the incoming spores were collected and more than 70 % of incoming spores formed colonies. These experiments with BG spores have also indicated that there were no significant particle losses inside the sampler. The collection efficiency of biological and non-biological particles increased to 90-100% when the particles were externally charged and the precipitating voltage was increased to more than ± 4,000 V. It has also been shown that the aerosolized BG spores (used as anthrax simulants for bioaerosol sensors) carry a net negative electric charge. Thus, the collection efficiency depends on the polarity of the electric field applied across the agar plates. These findings indicate that the collection of airborne microorganisms is possible by electrostatic precipitation without prior electric charging, if the microorganisms already carry electric charges. These are usually high immediately after their release into the air.

<u>Seventh Article</u>: Mainelis G, Gorny RL, Willeke K, Reponen T: Rapid Counting of Liquid-borne Microorganisms by Light Scattering Spectrometry. J. of Microbiological Methods, submitted, 2003.

In this article, the feasibility of using light scattering photometry for enumerating biological particles in liquid samples was investigated. A particle size spectrometer was used to count six species of microorganisms commonly found in air. All species were counted with and without microbial stains applied. The counts obtained were compared with those obtained with a phase-contrast microscope. It was found that the particle size spectrometer counted the microorganisms with an efficiency that was species and particle size dependent. It was also found that the difference in count between stained and non-stained microorganisms may serve as a basis for differentiating biological from non-biological particles in a liquid sample. This method is encouraging for the rapid enumeration of microorganisms collected by bioaerosol samplers.

<u>Eighth Article</u>: Lee, S-A, Willeke K, Mainelis G, Adhikari A, Wang H, Reponen T, Grinshpun SA: Assessment of Electrical Charge on Airborne Microorganisms by a New Bioaerosol Sampling Method. American Industrial Hygiene Association J, submitted, 2003

In this article, the polarity of electrical charges, the overall physical collection efficiency and the biological collection efficiency were determined in several laboratory and field environments: bacteria, fungal spores and dust dispersed from soiled carpets were sampled in a walk-in test chamber; a simulant of anthrax-causing *Bacillus anthracis* spores were dispersed and sampled in the same chamber; human bacteria were sampled in a small office with four adults engaged in lively discussions; bacteria and fungal spores released from hay and horse manure were sampled in a horse barn during cleanup operations; and bacteria in metal working fluid droplets were sampled in a metal working simulator. It was found that the new sampler differentiates between positively and negatively charged microorganisms, and that in most of the tested environments the airborne microorganisms had a net negative charge. This adds a signature to the sampled microorganisms that may assist in their identification or differentiation. Since the ESP is essentially an open channel, low power is required for the sampling flow through the ESP, making this method attractive for low power monitoring of airborne microorganisms, e.g., in an anti-bioterrorism network.

Satisfaction of Specific Aims of Original Grant

All of the Specific Aims of the five-year grant have been met:

Year 1: Development of a laboratory test system. This is described in the first and third articles that are listed and discussed above.

Experiments with electrically charged microorganisms. Papers 1-4 satisfy this aim.

Experiments on the charging of microorganisms and inert test particles. The first three papers satisfy this aim.

Years 2 & 3: Full-scale laboratory study of electrostatic sampling. The second, third and fourth paper satisfy this aim.

Year 3: Development of an electrostatic bioaerosol sampler and field testing. The fourth article satisfies this aim.

Y4 (Aim 1): Study of the physical and biological collection efficiencies of the new microbial sampler when exposed to laboratory-generated microorganisms. Papers 5, 6, 9 and 10 satisfy this aim.

Y4 (Aim 2): Development of a procedure for the dynamic counting of the particulate concentrations in a liquid, using a liquid optical particle counter. Paper 7 satisfies this aim.

Y5&6 (Aim 3): Measurements of the physical and biological collection efficiencies of the new method when sampling dry airborne microorganisms in an indoor environment. Papers 8 and 11 satisfy this aim.

Y5 (Aim 4): Collection efficiency determinations with the new method sampling liquid-borne microorganisms dispersed from metalworking fluids. Papers 8 and 11 satisfy this aim.

Y5 (Aim 5): Method evaluation in the presence of high concentrations of airborne microorganisms in an agricultural environment. Papers 8 and 11 satisfy this aim.

Note: No human subjects were tested during the grant period.