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PERFORMANCE OF THE BUTTON AEROSOL SAMPLER FOR TOTAL AND VIABLE ENUMERATION OF AIRBORNE MICROORGANISMS. S. Grinshpun, V. Aizenberg, T. Reponen, Z. Wang, R. Gorny, K. Willeke, University of Cincinnati, Cincinnati, OH

The Button Aerosol Sampler was previously evaluated for stationary and personal monitoring of nonbiological particles in work environments. In this study, its performance was tested under controlled laboratory conditions with respect to the total and viable enumeration of airborne microorganisms.

The physical collection efficiency was determined by measuring the concentrations of monodispersed particles upstream and downstream of the Button Sampler using a model 1.108 Grimm dust monitor. The total microbial count was performed using microscopic techniques, and the viable count was done through culturing. For the total bioaerosol enumeration, the Button Sampler was challenged with inert PSL particles ranging from 0.44 ?m to 5.10 ?m in diameter as well as with bacterial and fungal spores (*Streptomyces albus*, *Bacillus subtilis*, *Cladosporium cladosporioides, Penicillium brevicompactum*, and *Penicillium melinii*) ranging from 0.84 ?m to 3.07 ?m in diameter.

For the viable count, the tests were conducted with spores of *P. melinii* and *B. subtilis* and vegetative cells of *Pseudomonas fluorescens* at a relative humidity (RH) of 30% and 85%. The collection efficiency of the Button Sampler was close to 100% for the entire particle size range studied.

The uniformity of the particle deposition on the Button Sampler filters was found to be very consistent. A modified CAMNEA method, involving vortexing and ultrasonic filter agitation, followed by 96%–98% efficient suspension extraction, inoculation, staining, and epifluorescent microscopic counting, was developed to analyze the viable counts. *P. melinii* demonstrated consistent culturability when sampling for 30 minutes to eight hours, while less than 20% of *B. subtilis* spores formed colonies after the four-hour sampling. *P. fluorescence* vegetative cells were unable to form colonies at RH = 30%, but were able to recover at RH = 85% if sampled for 2–10 minutes.

The Button Aerosol Sampler was found suitable for the total enumeration of airborne microorganisms. It was also shown to be suitable for the viable enumeration of resistant spores.

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SAMPLING OF AIRBORNE BACTERIA AND FUNGAL SPORES IN METALWORKING FLUID ENVIRONMENTS USING THE BIOSAMPLER®. T. Reponen, A. Freeman, G. Mainelis, K. Willeke, S. Grinshpun, University of Cincinnati, Cincinnati, OH

Workers exposed to metalworking fluid (MWF) aerosols are at increased risk of developing a variety of respiratory and skin diseases. Microbial contamination of MWFs is suspected to be one of the causative factors for these adverse health effects. Little information is available on the composition and concentration of airborne microorganisms in metalworking sites, since none of the available air sampling methods allows long-term collection of a broad-spectrum of microorganisms.

Previous laboratory and field testing of the newly developed BioSampler® have shown that the BioSampler provides better collection efficiency, more gentle collection of microorganisms, and longer sampling times than the conventional all-glass impingers. In this field study, we compared the performance of the BioSampler with the 2-stage Andersen impactor in two metalworking plants.

Plant 1 had confirmed cases of hypersensitivity pneumonitis and occupational asthma as well as workers with a variety of respiratory complaints. The workers at Plant 2 did not have any significant health complaints. The BioSampler operated for four hours with mineral oil as the collection fluid. Ten 5-minute and 10 15-minute samples were taken with the Andersen impactor during the 4-hour sampling period. The measurements were performed in winter and in spring.

Overall, the bacterial and fungal concentrations were relatively low in both plants, below 1100 CFU/m^3, while the bacterial concentrations were up to 50 times higher in Plant 1 than Plant 2. Furthermore, the composition of airborne bacteria was different in Plant 1 from that in Plant 2 and in outdoor air.

The Andersen sampler indicated lower microbial concentrations during 15-minute sampling than during 5-minute sampling. This finding can be attributed to the effect of desiccation and impaction stress. The BioSampler gave equivalent or higher concentrations than the Andersen sampler.

These results indicate that the BioSampler is a promising tool for exposure assessment of airborne microorganisms in MWF sites.

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BIOCIDAL EFFECTIVENESS OF HIGH INTENSITY ULTRAVIOLET IRRADIATION IN METALWORKING FLUIDS. D. Johnson, T. Pearce, M. Phillips, University of Oklahoma Health Sciences Center, Oklahoma City, OK; B. Hollander, UV Water Systems, Inc., Minden, NV

Metalworking fluid (MWF) aerosols have been associated with adverse health effects such as hypersensitivity pneumonitis in exposed workers. These effects are believed to be associated with microbial infestation of in-use fluids, and control measures have focused on use of chemical biocides.

UV irradiation is an attractive alternative to chemical biocides because it leaves no chemical residual or byproducts (such as those associated with chemical oxidation), requires no hazardous materials storage or handling, and poses no fire hazard or risk of accidental chemical exposure to workers. It has been believed to be impractical for opaque fluids, however.

In this pilot work, we evaluated the biocidal effectiveness of new technology corrosion-resistant, high-output, long-duration submergible nonglass UV lamps in both synthetic and soluble oil MWF under laboratory conditions. Three transparent synthetic MWFs, three opaque soluble oil MWFs, and water were evaluated. Each fluid was innoculated with *Pseudomonas fluorescens* bacteria and exposed to UV radiation in a circulating system for up to one hour. Aliquots were withdrawn at intervals, diluted with sterile distilled dionized water, and surface-plated on tryptic soy agar.

The number of colony-forming units (CFUs) were counted after incubating the plates overnight at 35°C. Control experiments were run under identical conditions except that the UV lamp was not turned on. The lamps were shown to be extremely effective in all fluids tested, with a classic exponential decrease providing at least 3-log reductions in culturable concentrations.

Culturable concentrations remained essentially steady in the absence of UV irradiation. These results were somewhat unexpected for the opaque MWFs, suggesting that UV microbial control in such fluids may be more feasible than previously believed.

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CLASSIFICATION OF BACTERIA USING FTIR SPECTROSCOPY. S. Chunduri, Advanced Micro Devices, Austin, TX; J. Rock, Texas A&M University, College Station, TX

Bacterial FTIR spectra are fingerprint-like patterns resulting from superimposed absorption bands of all constituents of the cell. These spectra can be used for rapid identification of bacteria.

Standard practice of classification and identification of bacteria start with a streaking of bacteria on a plate for obtaining a pure culture. Two or three generations are usually sufficient. After obtaining a pure strain, series of tests are performed for classification and identification. Some of the tests are Gram-stain, heat resistance tests, acid production from sugars, polymeraise chain reaction, phase contrast microscopy, light microscopy, morphological property such as, endospore production, sheaths, holdfasts, acid fastness, cysts, stalks, type of motility and selective media growth properties.

The spectrum of each bacteria is a function of chemical composition, concentration, sampling point on the growth curve, and growth conditions (e.g., temperature, pH, type of growth media). Simultaneous control of these conditions poses enormous challenges for collecting reproducible spectra. Different sampling methods, such as attenuated total reflectance, diffused reflectance, transmission, etc., produce different quality of spectra. Selecting a proper method has a impact on classification. A new method using polyethylene film was developed for bacteria spectra collection. This method yields reproducible spectra.

Nine species of bacteria were selected to evaluate these methods. Bacteria used in this study are *B. subtilis 6633, B. cerues, E. coli K12, E. coli DH5A, E. coli T10F, E. coli BL21, E. coli BL21 GFP, S. typhimurium TA100* and *S. typhimurium TA98*. The cluster analysis technique was used to classify bacteria. Holding out one spectrum from the group of spectra cluster analysis methods was evaluated for classification of unknown spectra. The results indicate a fair amount of success ranging from 70% to 30%. Classification of bacteria by FTIR spectra is an emerging technology showing great potential.

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ELECTROSTATIC CHARGE DISTRIBUTIONS ON AIRBORNE MICROORGANISMS. G. Mainelis, K. Willeke, S. Grinshpun, T. Reponen, S. Trakumas, University of Cincinnati, Cincinnati, OH; P. Baron, NIOSH, Cincinnati, OH

Several studies showed that bacteria suspended in liquid may carry as many as 10,000 elementary charges. This study was undertaken to determine the electrical charge distributions on bacteria in the airborne state. This information is important, since high electric charges on airborne microorganisms may result in high transport losses in flow systems. High electric charges also suggest that the sampling of airborne microorganisms by an electrostatic collection technique, a potentially "gentle" bioaerosol collection method, may be possible without the need for prior charging.

Thus, to investigate the charge on airborne bacteria we built a new experimental system, in which bacteria are aerosolized and then channeled into a parallel plate mobility analyzer. By adjusting the electric field inside the analyzer we selected bacteria

carrying known charge ranges. By comparing their concentrations with those entering the analyzer, we obtained the electrical charge distributions on the bacteria.

Our tests with *Pseudomonas fluorescens* bacteria, commonly found in air environments, have shown that airborne bacteria have a net negative charge, but the individual bacteria can be charged either negatively or positively. When the bacterial suspension was aerosolized with compressed air, the bacteria acquired up to ?14,000 elementary charges per bacterium; 50% of these bacteria carried between -1000 and +400 elementary charges.

When the same bacterial suspension was aerosolized using bursting bubbles, the bacteria acquired maximum charges of ?1500 and 50% of the bacteria had between -200 and +150 electrical charges. Thus, aerosolized bacteria have sufficiently high electric charges to be collected by an electrostatic field without prior charging.

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EXPOSURE ASSESSMENT OF EMPLOYEES LAND-APPLYING BIOSOLIDS. N. Burton, D. Trout, NIOSH, Cincinnati, OH

In response to a management request for assistance, NIOSH investigators conducted an evaluation of worker exposure during the land application of biosolids (treated sewage sludge). At the wastewater plant, biosolids were loaded into large dump trucks with a front-end loader, transported to a farm field, and dumped. The biosolids were then loaded into a side discharge sludge spreader and sprayed on the field. A tractor was used to disk the sludge into the soil.

All five employees reported at least one episode of gastrointestinal illness occurring soon after performing this work. Environmental monitoring was conducted, which included the collection of area and/or personal breathing zone air samples for culturable bacteria, endotoxins, volatile organic compounds (VOCs), and metals. Bulk samples of sewage sludge were analyzed for coliform bacteria. The geometric mean bacterial area air concentrations ranged from 412 to 2356 colony forming units per cubic meter of air (CFU/m³).

All bacterial genera identified in these samples are associated with outdoor environments or mammals; some are considered opportunistic human pathogens. Airborne endotoxin levels ranged from 20 to 39 endotoxin units per cubic meter (EU/m³), which are similar or below levels found in wastewater treatment plants. The geometric mean concentrations of coliform bacteria and *Escherichia coli* in the bulk sewage sludge samples were 2.7×10^4 CFU and 2.2×10^4 CFU per gram of sample, respectively. The concentrations of metals and VOCs, including toluene, were low and well below current occupational exposure limits.

The nature and timing of the employees' reported symptoms suggest occupational exposure by direct contact with the biosolids as a probable cause of these symptoms. Recommendations were made for minimizing the growth of microorganisms during the storage process, increasing hand washing, adding filtration to the air-conditioning units on the tractor and front-end loaders, and improving the use of personal protective equipment.

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AN INVESTIGATION OF FUNGAL CONTAMINATION UNDERNEATH CARPET TILES IN AN OFFICE FACILITY. L. Hung, U.S. Public Health Service, Federal Occupational Health, Philadelphia, PA

Fungal proliferation was detected on the rubber backing of carpet tiles in a facility that had resulted in loosing of carpet tiles and employee health complaints. Elevated airborne fungal levels (10^3 CFU/m³) — with *Aspergillus* species as the predominant fungi — were detected at the floor level of fungi-proliferated areas.

The facility went through a decontamination and carpet replacement project. This included relocation of employees, removal of all furniture, full containment of the facility with negative air pressure, removal of carpets and vinyl tiles, decontamination of the concrete floor, sealing of the concrete floor, and carpet/vinyl tile replacement.

Microbiological samplings were conducted at different periods: 1) Before the initiation of the project; 2) after decontamination (clearance sampling); and 3) after reoccupancy of the facility. Andersen air samples, contact plate samples on wall and ceiling tile surfaces, swab and contact plate samples on concrete floor surfaces, and vacuum carpet dust samples were collected for fungal analysis.

Results showed a significant reduction of fungal levels on concrete floor surfaces underneath carpeting (from a mean of 9.5×10^5 CFU/in² to below the detection limits of 40 CFU/in²). The decontamination and carpet replacement did not significantly increase fungal loading on wall and ceiling tile surfaces. Before decontamination, the average indoor fungal level was higher than that of outdoors. Moreover, *Aspergillus* species (*Aspergillus versicolor* included) were the predominant fungi detected indoors while *Basidiomycetes* dominated outdoor fungal flora. After decontamination and facility reoccupancy, the mean indoor airborne fungal level was lower than that of outdoors (1726 CFU/m³ vs. 160 CFU/m³) and fungi detected indoors were those of outdoors.

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SEVERE FUNGAL CONTAMINATION OF A HOTEL RESULTING FROM AN EFIS SYSTEM FAILURE: ENVIRONMENTAL CHARACTERIZATION AND CLINICAL OUTCOMES. K. Martinez, K. Wallingford, D. Trout, NIOSH, Cincinnati, OH

In the fall of 1998, NIOSH responded to a request for technical assistance from a municipal health department to evaluate a hotel following identification of significant fungal contamination on interior surfaces of outdoor-facing walls of the structure. The fungi were suspected to have proliferated following extensive water incursion at the seams of the external building insulating and protective facing panels.

Specifically, NIOSH was asked to assess the fungal contamination, including testing hypotheses for the existence of fungal reservoirs, consultation regarding the ongoing remediation work, and the evaluation of the potential for occupational exposures to hotel employees and to the remediation workers, including environmental and medical components.

Surveys were conducted to collect microbiological bulk and air samples (using culturable and nonculturable methods) and to collect bulk samples from contaminated wallboard to be analyzed for mycotoxin content. The results of the NIOSH investigation clearly documented the presence of active fungal reservoirs behind vinyl wall-covering of exterior hotel walls; bulk sample results ranged from 8.8 $\times 10^4$ to 5.2×10^7 colony forming units per gram of material (CFU/gm).

Mycotoxins specific to *Stachybotrys chartarum* (i.e., complex trichothecenes, satratoxin and roridin, and atranones) and *Memmoniella echinata* (i.e., griseofulvins) were identified on 8 of 18 bulk wallboard samples. In addition, air sampling indicated the dissemination of fungal spores and hyphae into the rooms of the hotel, with geometric mean concentrations indoors ranging from 294 CFU/m³ to 2690 CFU/m³. The identified fungal genera included *Acremonium, Alternaria, Aspergillus, Cladosporium, Penicillium, Phoma, Stachybotrys*, and *Ulocladium*.

The results indicate that inhalation exposures to various fungal structures, and consequently their mycotoxins, were possible at the time of the NIOSH investigation. It is not clear what impact these exposures may have had on the workers in the hotel. Two of the hotel employees who might have been the most highly exposed are being clinically evaluated for health conditions potentially related to their exposures.

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MICROANATOMICAL CHANGES IN ALVEOLAR TYPE II CELLS IN JUVENILE MICE INOCULATED INTRACHEALLY WITH *STACHYBOTRYS CHARTARUM* SPORES. T. Rand, M. Mahoney, K. White, Saint Mary's University, Halifax, Nova Scotia, Canada; M. Oulton, Dalhousie University, Halifax, Nova Scotia, Canada

We recently showed that alveolar Type II cells in young mice are sensitive to exposure to *Stachybotrys chartarum* spores and to the *trichothecene, isosatratoxin F*. This sensitivity is manifested by alterations in the normal metabolic processing of pulmonary surfactant. It is unclear from our previous studies, however, whether there are concurrent morphological and dimensional changes in the alveolar Type II cells that reflect these metabolic alterations.

The objectives of this study were to document ultrastructural and morphometric changes developing in alveolar Type II cells of juvenile mice exposed to a single intratracheal instillation of *S. chartarum* spores for 24 and 48 hours. Within these times, marked ultrastructural changes were associated with the alveolar Type II cells in *S. chartarum*-inoculated mice compared with those in saline-treated and untreated control mice. These changes included swollen mitochondria, cisternal dilation of endoplasmic reticulum, and membranal figures in lamellar bodies and alveolar spaces.

Point-count stereological analysis revealed a significant decrease in alveolar Type II cell volume compared with the control animals. In addition, lamellar body volume density and volume were also significantly decreased in the *S. chartarum*-treated animals. These results further support our position that alveolar Type II cells show high sensitivity toward *S. chartarum* spores, which is manifested by metabolic and concurrent microanatomical changes.

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CHANGES OVER TIME IN AUDIOMETRIC THRESHOLDS IN A GROUP OF AUTOMOBILE STAMPING AND ASSEMBLY WORKERSWITHIN A HEARING CONSERVATION PROGRAM. L. Gibson, Concurrent Technologies Corporation, Largo, FL; E. Talbott, J. Burks, University of Pittsburgh, Pittsburgh, PA

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