FINAL PERFORMANCE REPORT

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"Microbiological Air Contamination from Machining Fluids"

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ABSTRACT

Workers exposed to machining fluid aerosols may have an increased risk in developing a variety of respiratory and skin diseases, such as allergies, asthma, hypersensitivity pneumonitis, and dermatitis. About 1.2 million workers in the United States are occupationally exposed to metalworking fluids (MWFs). Microbial contamination of water-based MWFs is one of the suspected causes for respiratory diseases but the exposure-response relationship is not well understood. One reason for this knowledge cap is that insufficient information has been available so far on the composition and concentration of airborne microorganisms at metalworking sites. We have studied the aerosolization of microorganisms and mist with a laboratory-scale set-up, which allows investigating one variable at a time. As a major part of this study, a laboratory-scale grinding simulator was developed and tested. It was experimentally proven that the microbial concentration in the MWF or the MWF type did not affect the aerosolization potential of microorganisms. Hydrophobic microorganisms were found to be easier to aerosolize from MWFs than hydrophilic ones and increasing microorganism size resulted in decreasing the aerosolization potential. When MWF was contaminated with bacteria the aerosolization of fine and ultrafine (0.2 -1 μm) particles was found to increase by a factor of 25 for a soluble oil and by a factor of 50 for a semisynthetic fluid. Endotoxin analysis confirmed that fine particles contained microbial cell wall fragments. Field test showed that the concentrations obtained in the laboratory simulated realistic field situations and that fine and ultrafine particles can easily migrate from production areas to other areas in the plant.

SIGNIFICANT FINDINGS

Prior to this study, the dispersion of mist and microorganisms from MWFs has not been sufficiently understood as various field studies have revealed contradictory results. Using a laboratory-scale MWF simulator, we found that the cell wall characteristics of microorganisms affect the aerosolization potential of microorganisms. Hydrophobic microorganisms are easier to be aerosolized than hydrophilic ones. Thus, the fraction of hydrophobic microorganisms (e.g., bacterial and fungal spores, and *Mycobacteria)* is likely to be higher in the air than in the bulk MWF, whereas the situation is opposite for hydrophilic ones (bacterial vegetative cells). Most significant finding of this study was that the microbial contamination of MWFs increases the aerosolization of fine particles and that these fine particles can contain microbial fragments. The presence of microbial fragments is important because studies on particle exposures in outdoor air and in some occupational environments (e.g., beryllium) have demonstrated that the fine and ultrafine particles can be more harmful to human health than larger particles of the same composition. Our field study showed that fine and ultrafine particles can efficiently migrate from production areas to other areas in the plant and thus, can cause exposure of the people in the adjacent areas.

USEFULNESS OF FINDINGS

The results indicate that the estimation of workers' exposures cannot be based solely on the analysis of bulk MWF as there are differences among microorganisms in their aerosolization potential. The microbial fragments cannot be detected by traditional microbiological methods, cultivation and microscopic counting. To fully understand the workers' exposure to microbial contaminants in MWF-environments, sampling and analysis methods have to be modified to account for microbial fragments. This will include collection of particles with methods that are efficient below the size range of intact microbial cells (for example filter sampling or low-pressure impactors) and analysis of the samples with methods that can detect microbial fragments (for example LAL assay for endotoxin and ß-glucan, or immunochemical assay for microbial allergens). For fluid maintenance, it is not enough to kill the microorganisms by using a biocide. More importantly, the total microbial biomass in the fluid has to be kept under control in order to prevent the aerosolization of microbial fragments.

LIST OF PUBLICATIONS

Peer-reviewed journal articles that have resulted from this grant with acknowledgement to the grant

1. Wang, H.X., Reponen, T., Adhikari, A., Willeke, K., Grinshpun, S.A. (2004) Effect of fluid type and microbial properties on the aerosolization of microorganisms from metalworking fluids. *Aerosol Science and Technology* 38:1139-1148.

2. Wang, H.X., Reponen, T., Li, W., Martuzevicius, D., Grinshpun, S.A., Willeke, K. (2004) Aerosolization of fine particles increases due to microbial contamination of metalworking fluids. *Journal of Aerosol Science* (in press).

3. Reponen, T., Wang, H.X., Grinshpun, S.A. (2004) Effect of microbial contamination of water-based metalworking fluids on the aerosolization of particles and microbial fragments. *Journal of ASTM International* (submitted).

Proceeding article with acknowledgement to the grant:

4. Reponen, T., Cho, S.-H., Wang, H.X., Grinshpun, S.A. (2004) Microbial fragments – a new challenge for exposure assessment of bioaerosols. In: *Proceedings of the European Aerosol Conference, Journal of Aerosol Science,* Vol. 1, pp. S399-S340.

Presentations in conferences:

5. Reponen, T., Wang. H.X., Martuzevicius, D., Adhikari, A., Grinshpun, S.A., and Willeke, K. (2002) Aerosolization of microorganisms from metalworking fluids.

Abstracts of the ACGIH symposium on Health Effects of Mineral Oil Mist & Metalworking Fluids, Cincinnati, Ohio.

6. Wang, H.X., Reponen, T., Adhikari, A., Sivasubramani, S.K., Grinshpun, S.A., Trunov, M., and Willeke, K. (2002) Evaluation of the aerosolization of microorganisms from metalworking fluids. *Student Abstracts of the American Industrial Hygiene Conference & Exposition*, San Diego, California.

7. Wang, H.X., Reponen, T., Martuzevicius, D. Li. W., Lee, S.-A., Grinshpun, S.A., and Willeke, K. (2003) Increase of fine particle aerosolization resulting from microbial contamination of metalworking fluids. *Student Abstracts of the American Industrial Hygiene Conference & Exposition*, Dallas, Texas.

8. Wang, H.X., Adhikarti, A., Li, W., Martuzevicius, D., Willeke, K., Grinshpun, S.A., Reponen, T. (2004) Aerosolization of microorganisms and microbial fragments from metalworking fluids. *Abstracts of the Annual Meeting of the American Association for Aerosol Research*, Atlanta, Georgia.

9. Reponen, T., Wang, H.X., Grinshpun, S.A. (2004) Microbial contamination of metalworking fluids increases the aerosolization of fine particles. *Abstracts of the ASTM meeting on "Recovery and Enumeration of Mycobacteria in Metalworking Fluids"*, Tampa, Florida.

10.Wang, H.X., Reponen, T., Grinshpun, S.A. (2005) Aerosolization of Fine Particles and Endotoxin from Metalworking Fluids Contaminated with Microorganisms. *Abstracts of the Annual Meeting of the American Industrial Hygiene Conference,* Anaheim, California. (accepted)

RESEARCH RESULTS CONTAINED IN THE JOURNAL ARTICLES

1. Wang, H., Reponen, T., Adhikari, A., Willeke, K., Grinshpun, S.A. (2004) Effect of fluid type and microbial properties on the aerosolization of microorganisms from metalworking fluids. ***Aerosol Science and Technology*** **38:1139-1148.**

In this study, we investigated the effects of fluid type, microorganism concentration in the liquid, and the microbial species on the aerosolization of microorganisms from MWFs. Three microorganisms were selected to represent different size and surface characteristics: *Bacillus subtilis* bacterial endospores (hydrophobic particles with aerodynamic diameter, $d_a = 0.9$ μm), *Pseudomonas fluorescens* bacterial vegetative cells (hydrophilic, $d_a = 0.8$ μm) and *Penicillium melinii* fungal spores (hydrophobic, $d_a = 3.1$ μm). Two water-based MWFs were selected: semisynthetic and soluble oil. This article describes two set-ups that were developed for the laboratory aerosolization of MWFs: the first system was based on Collison nebulizer and the other was a MWF simulator. The latter one is a laboratory-scale set-up simulating the mist generation during grinding

process. Furthermore, the cultivation and analysis methods for both fluid and air samples are described in this article.

The testing was first performed using a Collison nebulizer to aerosolize microorganisms from three fluids: water, semi-synthetic MWF, and soluble oil. No significant difference in the aerosolization ratio (microbial concentration in the air normalized to the microbial concentration in the liquid) was observed among the three fluids. For all tested microorganisms, the concentration in the air increased proportionally with the increase of the microbial concentration in the liquid. The aerosolization ratio of *B. subtilis* endospores was greater than that of *P. fluorescens* cells and *P. melinii* spores. To explore the aerosolization of microorganisms from MWFs under the conditions that are closer to industrial settings, the tests were conducted with the MWF simulator. Simulator tests showed the same trend with respect to microbial aerosolization as those performed with the Collison nebulizer. This was further confirmed by a separate experiment, in which the Collison nebulizer and the MWF simulator were tested with liquids containing polystyrene latex (PSL) particles. In summary, this study showed that the microbial concentration in the MWF or the MWF type did not affect the aerosolization potential of microorganisms. Hydrophobic microorganisms are easier to aerosolize from MWFs than hydrophilic ones and that increasing microorganism size is likely to result in decreasing aerosolization ratio.

2. Wang, H.X., Reponen, T., Li, W., Martuzevicius, D., Grinshpun, S.A., Willeke, K. (2004) Aerosolization of fine particles increases due to microbial contamination of metalworking fluids. *Journal of Aerosol Science* (in press).

In this article, the aerosolization of microorganisms from metalworking fluids (MWFs) was studied using the laboratory-scale set-up simulating grinding operations (developed and described in article 1). An optical particle counter (OPC), a condensation nucleus counter (CNC), an electrical low pressure impactor (ELPI), and a photometric aerosol mass monitor were used to measure the airborne particles and microorganisms aerosolized from MWFs. The tests were performed using a semi-synthetic MWF with and without bacterial contamination (*Pseudomonas fluorescens*). The effect of rotation speed of the grinding wheel (800-8000 rpm) and fluid application rate (0.4-1.6 l/min) were investigated. Viability of *P. fluorescens* in MWF was also tested.

It was found that increasing the rotation speed increased both the particle number and mass concentration. Fluid application rate also increased the particle concentrations. Furthermore, it was found that microbial contamination of the MWF increased the number and mass concentrations of aerosolized particles by a factor of 2 (as measured by the OPC and the photometric aerosol mass monitor, respectively). At the same time, there was an up to 50-fold increase in the concentration of fine and ultafine particles (0.02 - 1 μm), as measured by the CNC. The data collected with the ELPI showed that the peak of the fine particle number concentration was at 0.37 μm and the aerosol contained a high number of ultrafine particles. Viability of *P. fluorescens* bacteria decreased rapidly after it was mixed with MWF indicating stress-related reactions in

bacterial cells, which may lead to release of bacterial fragments. The results indicate that MWF mist may contain high concentrations of microbial fragments, which may not be detected by traditional microbial analysis methods, such as cultivation or microscopic counting.

3. Reponen, T., Wang, H.X., Grinshpun, S.A. (2004) Effect of microbial contamination of water-based metalworking fluids on the aerosolization of particles and microbial fragments. *Journal of ASTM International* (submitted).

This article describes similar experiments as performed in article 2, except that the focus was to compare two water-soluble fluids: semisynthetic and soluble oil. This study also includes analysis of aerosolized endotoxin in different two size fractions: 0.636 – 0.99 μm representing intact bacterial cells and 0.254 - 0.392 μm representing bacterial fragments.

It was found that aerosolization of soluble oil results in higher mass concentration, but lower particle number concentrations than aerosolization of semisynthetic MWF. Thus, particles aerosolized from soluble oil are larger than those aerosolized from semisynthetic MWF, which is due to higher mineral oil content in soluble oil. Microbial contamination of soluble oil increased the aerosolization of fine and ultrafine particles 25 times compared to the non-contaminated soluble oil. The same amount of endotoxin (about 13 EU/m^3) was found in the bacterial size fraction than in the fragment size fraction indicating that the fine fraction of aerosolized particles contains microbial fragments. Thus, it was concluded that the increase in the fine particle concentration that was observed for contaminated fluids is at least partially due to the increase in the microbial cell wall components (fragments).

SATISFACTION OF SPECIFIC AIMS

<u>Specific Aim A:</u> **To quantify the dispersion of mist and microorganisms under controlled laboratory conditions by using a simulator for MWF aerosol generation under different machining conditions while utilizing different types of MWFs.**

Task 1: Selection of MWFs and microorganisms for the laboratory study.
Addressed in articles 1-3.

Task 2: Modification of the cultivation, analysis, and aerosolization methods for MWF microorganisms.
Addressed in articles 1-2.

Task 3: Laboratory tests on the aerosolization of microorganisms from original (not previously used) MWFs with and without inoculated microbial contamination.
Addressed in articles 1-3.

Task 4: Laboratory tests on the aerosolization of microorganisms from used MWFs collected from the field testing sites.
See technical report below.

Specific Aim B: To verify the laboratory results on the aerosolization of microorganisms from MWFs under field conditions.

Task 5: Selection of field sites.
See technical report below.

Task 6: Field measurements on the dispersion of mist and microorganisms from MWFs.
See technical report below.

Task 7: Comparison of the laboratory and field data.
See technical report below.

TECHNICAL REPORT FOR TASKS THAT ARE NOT ADDRESSED IN PUBLISHED ARTICLES (Manuscript on these results is under preparation)

Specific Aim 1, Task 4: Laboratory tests on the aerosolization of microorganisms from used MWFs collected from the field testing sites.

Three used fluids were obtained from field sites. Two of them were soluble oils and the third one was a synthetic MWF (MWFs #2-4 in Table 1). The microbial contamination of the fluids was analyzed by cultivation (culturable bacteria and fungi) and by the LAL assay (endotoxin). For comparison, laboratory contaminated semisynthetic fluid was also tested (MWF #1). Furthermore, identical non-contaminated fluids of each type were also included in the testing (MWFs #5-8).

The used fluids were placed one at a time in the MWF simulator, which was operated at a rotation speed of 8000 rpm. The concentration of aerosolized particles was measured using the Electrostatic Low Pressure Impactor (ELPI, described in article 2). Based on data obtained reported in article 2, the ELPI data were divided into two size fractions: (i) 0.029 - 0.636 μm (representing the size range of microbial fragments) and (ii) 0.636 - 10.19 μm (representing the size range of intact bacterial cells and cell aggregates). The particles were collected onto polycarbonate filters (pore size 1 μm) on the 13 ELPI stages. Endotoxin concentration on these filters was analyzed using the LAL-assay.

Table 1. MWFs tested in Task 4.

MWF #	Sample source and type	MWF type	Total count (#/ml) or Culturable count (CFU/ml)		Endo-toxin (EU/ml)
			Bacteria	Fungi	
1	Laboratory, contaminated	Semi-synthetic MWF	*P. fluorescens*: 10^8 #/ml	N/A	Not analyzed
2	Field, used	Soluble oil 1	Gram-negative bacteria: 1.9×10^6 CFU/ml	ND	280
3	Field, used	Synthetic MWF	Gram-negative bacteria: 6.8×10^4 CFU/ml	ND	301
4	Field, used	Soluble oil 2	Gram-negative bacteria: 1.0×10^7 CFU/ml	ND	287
5	Laboratory, non-contaminated	Semi-synthetic MWF	N/A	N/A	N/A
6	Field, unused	Soluble oil 1	N/A	N/A	N/A
7	Field, unused	Synthetic MWF	N/A	N/A	N/A
8	Field, unused	Soluble oil 2	N/A	N/A	N/A

ND = not detected

The results on the concentrations of particles aerosolized from used MWF are presented in Table 2. The highest number concentrations of particles in the fragment size range were aerosolized from MWF #1. This can be attributed both to the higher level of bacterial contamination in this fluid and to the MWF type. We reported in article 3 that the highest concentration of fine and ultrafine particles was aerosolized from the contaminated semisynthetic fluid. The ratio of the number of particles in the fragment size range to that in the intact cell size range varied from 390 to 2.7. The highest ratio was found for the laboratory contaminated fluid. This ratio was generally lower for non-contaminated fluids than for the respective contaminated fluids. This supports our previous observation (reported in articles 2 and 3) that microbial contamination of MWFs increases the aerosolization of fine particles.

The results on endotoxin analysis are shown in Figure 1. The maximum concentrations of airborne endotoxin was found at about size of 3.97 μm for soluble oil 1 (MWF #2), at about 1.61 μm for soluble oil 2 (MWF #4), and at about 0.636 μm for synthetic MWF (MWF #3). Endotoxin was found in the fragment size range (<0.636 μm) for two of the three tested fluids (MWFs #3 and #4). These results show that particles aerosolized from synthetic MWFs are in the smaller size range than those aerosolized from soluble oil. This experiment also confirms that airborne particles in the fragment size range can contain microbial cell wall fragments.

Table 2. Number concentration of particles in fragment (<0.636 μm) and intact cell (≥0.636μm) size range. (Five-minute average ± standard deviation of three repeats)

MWF #	Number concentration (#/m^3)		Ratio of two size ranges (Fragment / Intact cell)
	Fragment size (<0.636μm)	Intact cell size (≥0.636μm)	
1	$(4.23\pm0.26)\times10^{11}$	$(1.09\pm0.05)\times10^{9}$	390
2	$(1.06\pm0.02)\times10^{10}$	$(2.00\pm0.03)\times10^{9}$	5.3
3	$(3.69\pm0.03)\times 10^{9}$	$(7.65\pm0.07)\times10^{8}$	4.8
4	$(1.75\pm0.03)\times10^{9}$	$(5.39\pm0.09)\times10^{8}$	3.2
5	$(2.91\pm0.21)\times10^{9}$	$(5.01\pm0.34)\times10^{8}$	5.8
6	$(3.77\pm0.15)\times10^{9}$	$(8.41\pm0.69)\times10^{8}$	4.5
7	$(4.50\pm0.08)\times10^{9}$	$(5.79\pm0.01)\times10^{8}$	7.8
8	$(2.71\pm0.20)\times10^{9}$	$(9.93\pm0.67)\times10^{8}$	2.7

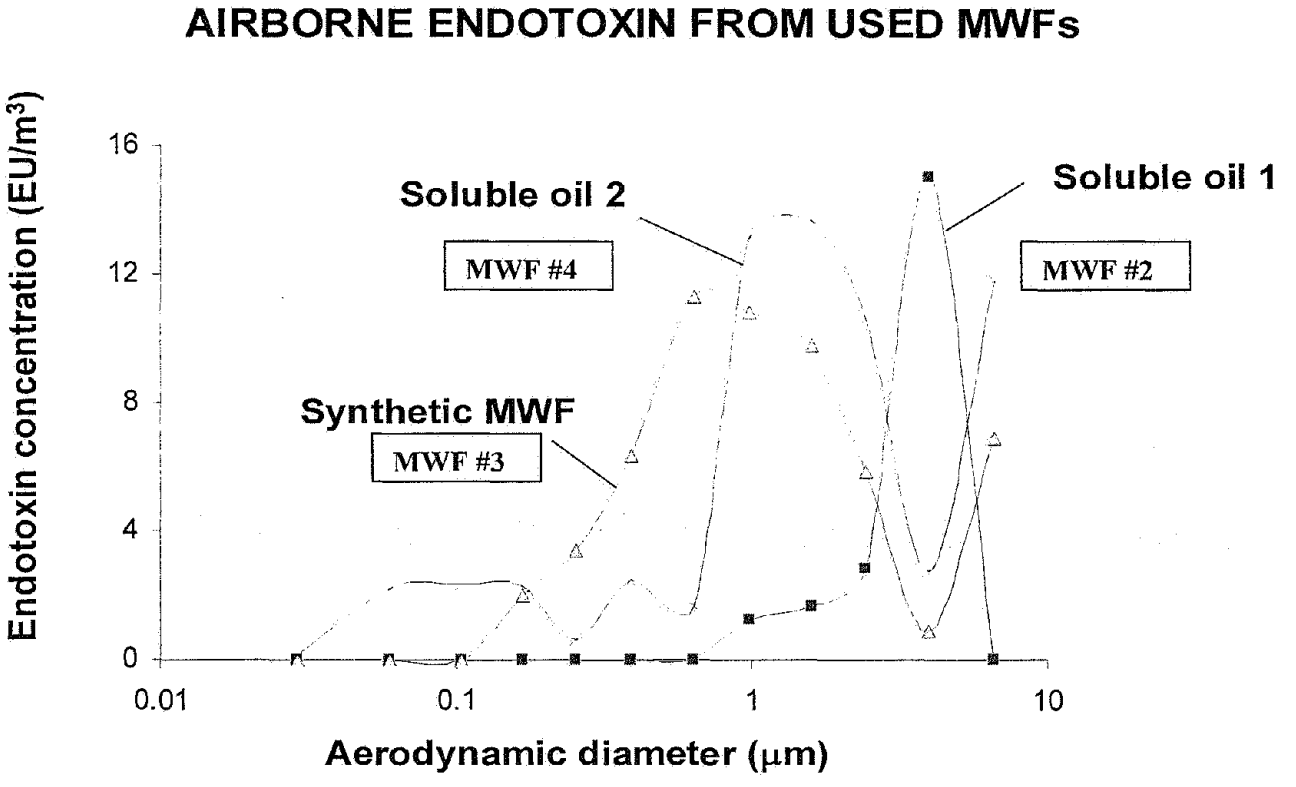

Figure 1. The size distribution of airborne endotoxin aerosolized from used fluids and collected onto 13 stages in the ELPI.

Specific Aim B: To verify the laboratory results on the aerosolization of microorganisms from MWFs under field conditions.

Task 5: Selection of field sites.

In order to find and get access to field sites, several different parties were contacted, for example major automanufacturing companies (e.g., Daimler Chrysler, Ford, and General Motors), United Autoworkers (UAW), local industrial hygiene consultants working with MWF companies, fluid manufacturers (e.g., Milacron, Castrol), owners of local small metal shops as well as colleagues in other univeristies and at NIOSH. Despite tremendous effort put in this task only one company agreed to let us in for field measurements. This turned out to be well-maintained and without any misting or microbial problems at the time of the measurments as shown below.

Task 6: Field measurements on the dispersion of mist and microorganisms from MWFs.

Three areas were selected for test locations on the field site: crank area, headline area, and reference area. In the crank area, metal pieces were cut at a speed of 0.6 mm/min. The machining was enclosed and had local exhaust ventilation. The machine had its own separate MWF tank. The door to the enclosure opened automatically between the cuts. The MWF used in this site was synthetic MWF. It had not been changed in two years as no microbial problems had been reported. The headline area had 26 enclosed grinding machines, which were served with one central MWF tank. This area had had misting problems and microbial problems in the fluid in the past. The fluid used in these grinding machines was semi-synthetic MWF. The reference area was a conference room about 20 meters away from the production area and separated by the front lobby. The door to the production areas was closed, although it was opened frequently by people entering and exiting the production area.

Fluid samples were collected from the tanks serving the crank and headline machines and analyzed for culturable total count of bacteria and fungi, and culturable count of gram-negative bacteria. Total culturable count of bacteria was determined by cultivating the sample on tryptic soy agar, total culturable count of fungi by cultivating on malt extract agar, and the culturable count of gram-negative bacteria by cultivating on eosine methylene blue agar. All plates were incubated at 25°C for two days. Endotoxin concentration was determined by LAL assay. Air samples were collected on the three sites using methods listed in Table 3. Culturable counts of microorganisms from air samples collected into the liquid by the BioSampler were determined similarly as from the MWF samples.

Table 4 shows the results on cultivation analysis. As seen, the fluids did not have microbial contamination at the time when our sampling occurred. Also endotoxin concentration in the fluids was low.

Table 3. Methods used for the air sampling in the field sites.

		Number of samplers in each area		
Measured parameter	Method	Crank area	Headline area	Reference area
Number concentration of fine and ultrafine particles (0.1 -1 μm)	Condensation nucleus counter, CNC (P-Trak)	1	1	1
Number concentration of fine and large particles (0.3 -10 μm)	Optical particle counter, OPC (Grimm 1.108)	1	1	1
Mass concentration (0.1-10 μm)	Photometer (Dust-Trak)	1	1	0
Culturable count of microorganisms	BioSampler	3	3	3
Endotoxin	Button Inhalable Aerosol Sampler + white polycarbonate filter	3	3	3

Table 4. Concentrations of fluid and airborne microorganisms and endotoxin.

Sampling area	**MWF type**	**Culturable count in the fluid (CFU/ml)**			**Endotoxin conc. in the MWF (EU/ml)**
		Bacteria	**Fungi**	**Gram-negative bacteria**	
Crank area	Synthetic	ND	32	ND	1.0
Headline	Semi-synthetic	46	ND	ND	19
Reference	N/A	ND	ND	ND	N/A
Sampling area		**Culturable count in the air (CFU/m^3)**			**Endotoxin conc. in the air (EU/m^3)**
		Bacteria	**Fungi**	**Gram-negative bacteria**	
Crank area		ND	$12*10^3$	ND	ND
Headline		ND	ND	ND	ND
Reference		ND	ND	ND	7.6

ND = not detected

Figure 2 shows the results on airborne particle concentrations measured with the three devices. The concentration of fine and ultrafine particles (size range of 0.2 -1 μm) measured by the CNC was twice higher in the production area compared to the one in the reference area. This difference was lower than expected. This may be due to the proximity of the reference area to the production area and frequent opening of the door leading to the production area. The concentration of larger particles (0.3 -10 μm) as measured by the OPC was higher in the crank and headline area than in the reference area by a factor of 22 and 31, respectively. The finding that there was a more clear difference in the data obtained by the OPC than with the CNC between the production and reference areas can be attributed to the particle size. Larger particles (measured by the OPC) do not migrate as efficiently to the adjacent areas than smaller particles (measured by the CNC). The mass concentration was measured only in the two production areas (due to the lack

of multiple instruments) and was found to be higher in the headline area than in the crank area. When comparing the results in Figures 2 a-c it can be concluded that the mist aerosolized in the crank area was in the smaller particle size range than the mist aerosolized from the headline area. As there was minimal microbial contamination in the fluids, no correlation could be made between the microbial contamination of the MWF and concentration or size distribution of mist.

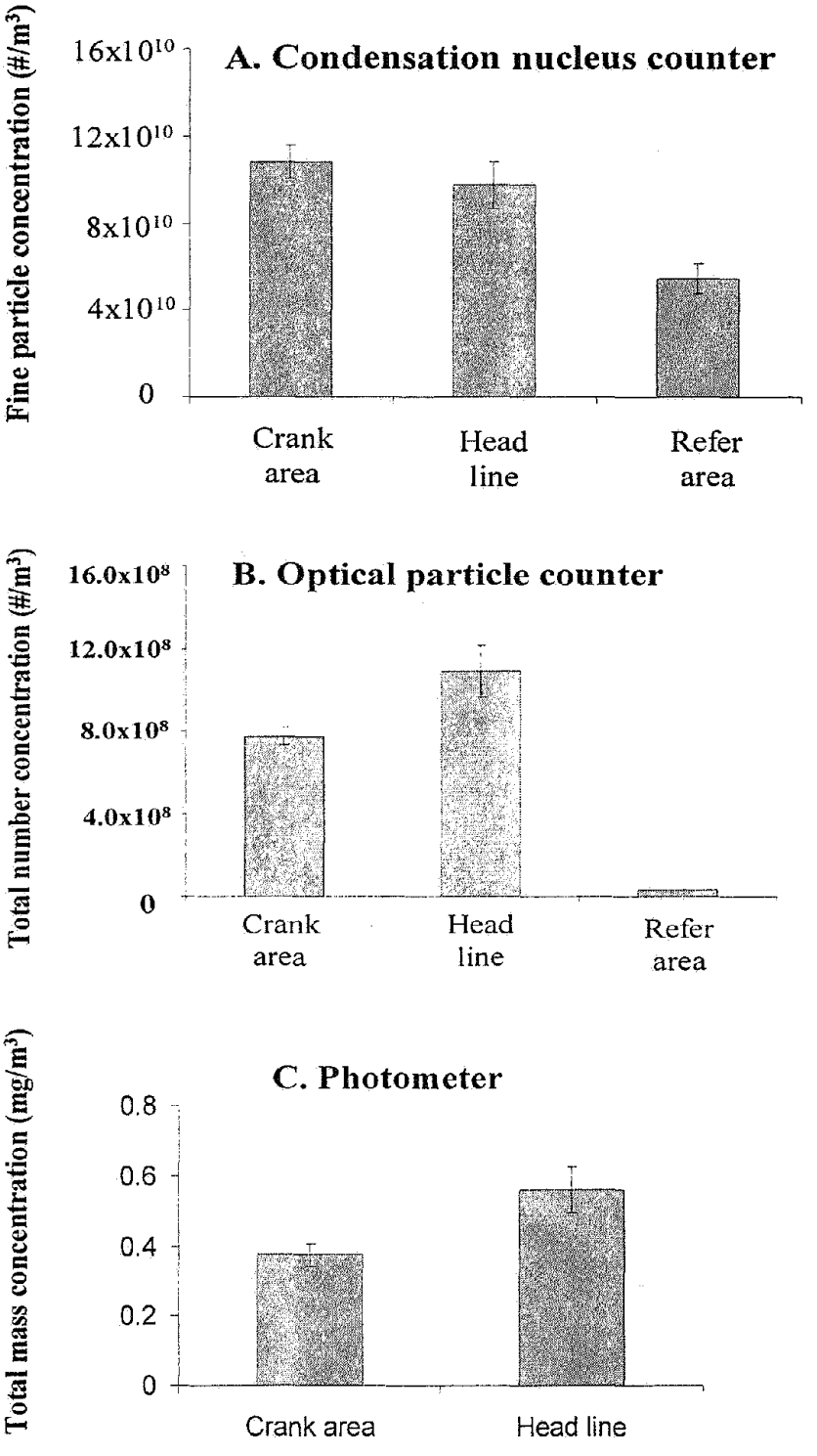

Figure 2. Concentrations of airborne particles in the three test sites. A. The number concentration particles measured by the condensation nucleus counter (0.02-1 μm); B. The number concentration particles measured by the optical particle counter (0.3-10μm); and C. The mass concentration of particles measured by the photometer (0.1- 10 μm). The bars show the average of 2-hour measurements and the error bars show the standard deviation.

Task 7: Comparison of the laboratory and field data.

Semisynthetic fluid was used in the field in the headline area. The same fluid type was tested in the laboratory experiments. This allowed us to compare these two data sets. As seen from Table 5, the concentrations measured by the OPC and the photometer in the laboratory tests and in the field site are in the same range. The concentration of fine and ultrafine particles measured in the field by the CNC is one to two orders of magnitude higher than that measured in the laboratory tests. This comparison shows that the particle concentrations obtained in our laboratory tests are realistic.

Table 5. Comparison of laboratory and field data obtained for semi-synthetic fluid.

	RANGE IN THE CONCENTRATION OF AIRBORNE PARTICLES		
Measurement location	**Condensation nucleus counter (#/m^3)**	**Optical particle counter (#/m^3)**	**Photometer (mg/m^3)**
Laboratory simulator			
non-contaminated[1]	(2.01 -2.25)×10^8	(1.12 –1.25)×10^9	0.64 -0.65
contaminated[2]	(10.24 -10.43) ×10^9	(2.36 -2.41) ×10^9	1.13 -1.25
Field measurement			
Headline area	(8.54 –11.77)×10^{10}	(9.14 -14.42))×10^8	0.427 -0.708

1. 8000 rpm
2. 8000 rpm, 10^8#/ml *P. fluorescens*