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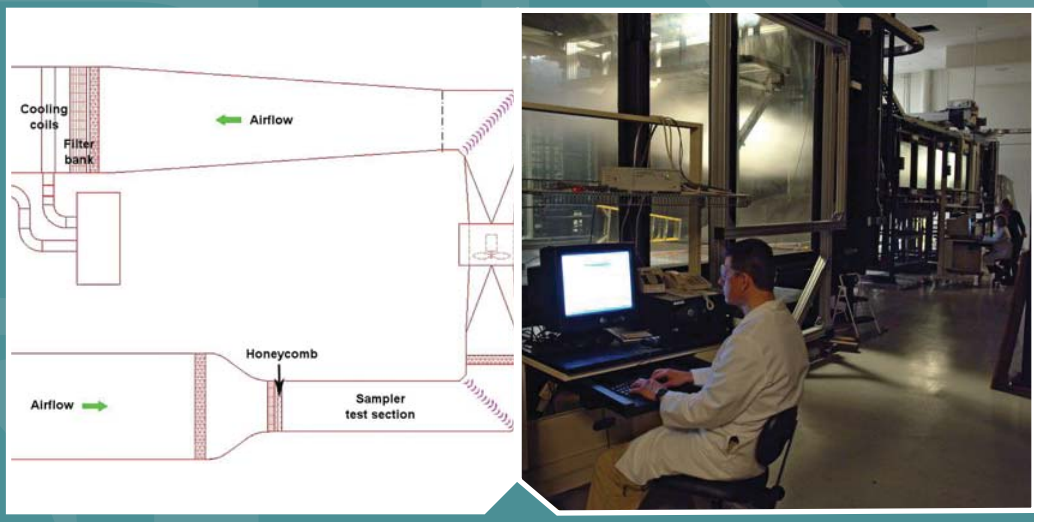
United States
Environmental Protection
Agency

Determination of the Sampling Efficiency of Biosamplers to Collect Inhalable Particles



Office of Research and Development
National Homeland Security Research Center

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U.S. Environmental Protection Agency
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Research Triangle Park, NC 27711

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Notice

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development's National Homeland Security Research Center, funded and directed the research described herein under Contracts EP-D-05-065 and EP-D-10-070 with Alion Science and Technology. It has been reviewed by the Agency but does not necessarily reflect the Agency's views. No official endorsement should be inferred. EPA does not endorse the purchase or sale of any commercial products or services.

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Foreword

Following the events of September 11, 2001, EPA's mission was expanded to address critical needs related to homeland security. Presidential Directives identify EPA as the primary federal agency responsible for the country's water supplies and for decontamination following a chemical, biological, and/or radiological attack.

As part of this expanded mission, the National Homeland Security Research Center (NHSRC) was established to conduct research and deliver products that improve the capability of EPA to carry out its homeland security responsibilities. One focus area of this research is the compilation, development, and evaluation of information on the ability to measure potential exposure to pathogens that might be used by terrorists. Such information is critical to understanding the risks associated with biological contamination and supporting the development of site-specific cleanup goals, treatment technologies, and detection limits.

NHSRC has made this publication available to assist the response community in preparing for and recovering from disasters involving microbial contamination. This information is intended to move EPA one step closer to achieving its homeland security goals and its overall mission of protecting human health and the environment while providing sustainable solutions to our environmental problems.

Jonathan Herrmann, Director
National Homeland Security Research Center

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Acronyms and Abbreviations

°C	degrees Celsius
σ_g	geometric standard deviation
µm	micrometer
AE	aspiration efficiency
APS	Aerodynamic Particle Sizer
ATF	Aerosol Test Facility
AWT	aerosol wind tunnel
CE	collection efficiency
CFR	Code of Federal Regulations
cm	centimeter
CV	coefficient of variation
dp_{ae}	particle aerodynamic diameter
EPA	U.S. Environmental Protection Agency
h	hour
hp	horsepower
HPLC	high-performance liquid chromatography
Hz	hertz
in.	inch
iso	isokinetic
K	Kelvin
km	kilometer
kPa	kilopascal
kW	kilowatt
L	liter
lb	pound
m	meter
MFC	mass flow controller
min	minute
mL	milliliter
mm	millimeter
mph	miles per hour
NaOH	sodium hydroxide
ng	nanogram
NHSRC	National Homeland Security Research Center
PSL	polystyrene latex
PSU	portable sampling unit
R^2	coefficient of determination
RH	relative humidity
rpm	revolutions per minute
SD	standard deviation
SE	sampling efficiency
SOP	standard operating procedure
Stk	Stokes number
STS	sampler test section
TE	transmission efficiency
TI	turbulence intensity
VOAG	vibrating orifice aerosol generator

Executive Summary

Three bioaerosol samplers were chosen for evaluation of sampling efficiency (SE) in the aerosol wind tunnel according to 40 CFR Part 53 Subpart D (U.S. EPA, 1998): the XMX/2L-MIL, manufactured by Dycor Technologies, Ltd.; the portable sampling unit (PSU) sampler, manufactured by Hi-Q Environmental Products; and the DryClone™ sampler, manufactured by Evogen, Inc. Wind tunnel testing of the samplers was carried out with monodisperse liquid aerosols (oleic acid and uranine) with particle diameters of 5, 10, 15, and 20 µm. The DryClone™ and PSU samplers were tested in the wind tunnel in triplicate with each of the four particle sizes at wind speeds of 2, 8, and 24 kilometers/hour (km/h). The sampling efficiency for inhalable particulate (5 to 20 µm aerodynamic diameter, dp_{ae}) is reported versus dp_{ae} and the square root of the Stokes number (a nondimensional number relating velocity and aerodynamic particle size to relaxation time). Neither of the samplers showed wind speed insensitivity, and both had a rapid decline in efficiency with rising Stokes number.

The XMX sampler was tested with 5-µm aerosol at 2 and 8 km/h, but the SE did not compare well with the manufacturer's results for solid monodisperse aerosols under the same experimental conditions. The manufacturer indicated that testing with liquid aerosol was not appropriate. For this reason, we suggest retesting the XMX sampler using solid particles prepared from fluoroscein and ammonium hydroxide.

1.0 Introduction

This research was conducted as part of the efforts of the U.S. Environmental Protection Agency (EPA) National Homeland Security Research Center (NHSRC) to determine the sampling capability of biological aerosol samplers expected to be used for homeland security field support to collect respirable and inhalable particles resuspended after an accidental or intentional release of bioagents. The specific objective of this research was to determine the sampling efficiency (SE) of several biological samplers to characterize their performance for a number of particle aerodynamic diameters and wind speeds. For this work, the PM_{10} test criteria published in Title 40 Part 53 Subpart D of the Code of Federal Regulations (40 CFR Part 53 Subpart D) formed the basis of the wind tunnel evaluation.

Direct assessment of particle concentration in the atmosphere is based on aerosol sampling by different measurement devices. Small airborne particles are well entrained in the airflow and are collected by the sampling device with high efficiency. In contrast, larger particles can deviate from fluid stream lines (aspiration loss) and can deposit inside sampler inlets and not reach the filter or measurement zone (transmission losses). The presence of the sampler and the action of sampling can cause disturbances in the surrounding air and affect particle movement, leading to a significant difference between the particle concentration measured by the sampler and the particle concentration in the free-stream air or ambient atmosphere. The SE, defined as the ratio of the measured mean particle concentration to that in the free-stream air, characterizes these discrepancies.

The SE of a sampler can be further broken down into three factors: aspiration efficiency (AE), transmission efficiency (TE), and collection efficiency (CE), as $SE = AE \times TE \times CE$ (Kesavan et al., 2003; Brockmann, 2011). AE is the efficiency of transport of particles from the free-stream air into the inlet of the sampler (ratio of concentration that enters the sampler inlet to free-stream concentration) and is influenced by the wind speed and configuration of the sampler inlet. TE is the efficiency of transport of particles from the sampler inlet to the filter or measurement zone. CE is the efficiency with which the sampler collection medium (filter, impactor, impinger, etc.) collects and retains particles. All three efficiencies are particle size dependent. In the wind tunnel testing portion of this study, the SE of each sampler was determined by comparing the test samplers to reference measurements.

2.0
Experimental Methods

2.1 Aerosol Wind Tunnel

To evaluate the samplers according to the 40 CFR Part 53 Subpart D acceptance criteria, a wind tunnel is required to provide a controlled environment with well-defined velocity profiles and monodisperse particle concentrations. An overview of the Aerosol Test Facility (ATF) wind tunnel, part of NHSRC, is shown in Figure 1. In plan view, the aerosol wind tunnel (AWT) is rectangular with outside dimensions of approximately 20 meters (m) by 14 m. Flow through the recirculating wind tunnel during all operations is counterclockwise with few flow obstructions. A number of doors with magnetic locks allow access to all sections of the wind tunnel for cleaning. At the sampler test section (STS), the wind tunnel cross-section is 1.75 m wide by 1.45 m high by 6.1 m long.

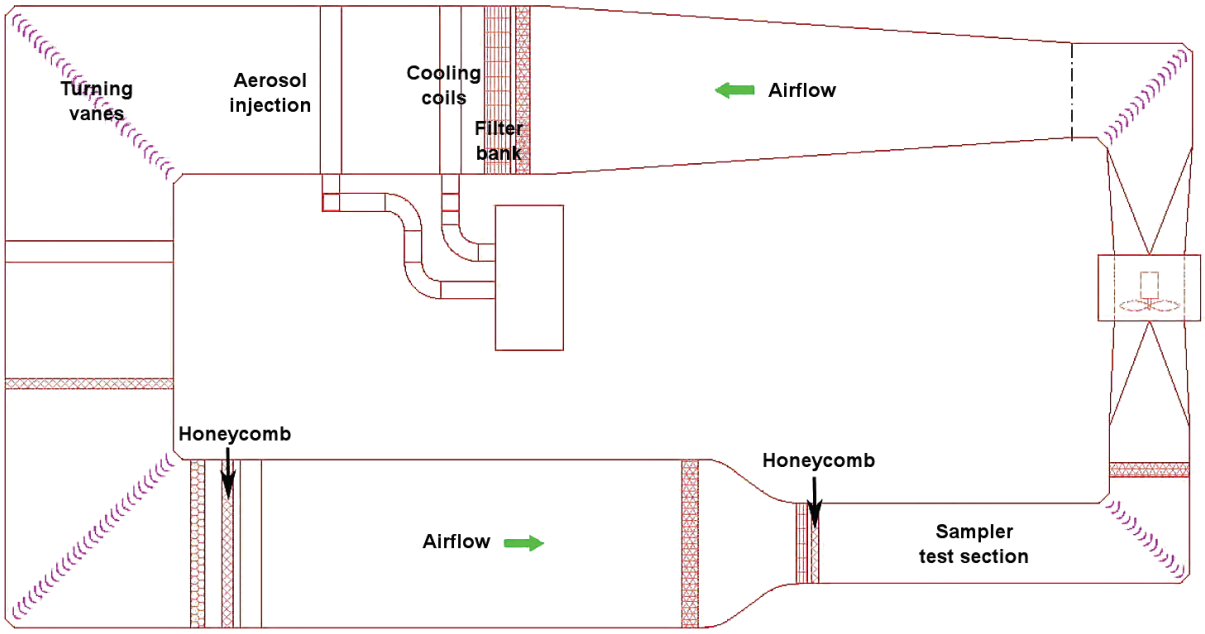

Figure 1. Plan view of the EPA NHSRC aerosol wind tunnel.

Since the wind tunnel is of fixed geometry, varying wind speeds are achieved by controlling the volumetric flow rate. Major flow through the wind tunnel is provided by a direct-drive, adjustable-blade, vane-axial fan (Twin City Fan and Blower, Minneapolis, MN) capable of providing approximately 2002 m^3/min (71,500 ft^3/min) against 0.97 kPa (3.89 inches of water) pressure drop at 1133 rpm at a power requirement of 56 kW (75 hp). This blower is capable of producing wind speeds up to 48 km/h (30 mph) in the STS. Wind speed is controlled through a variable-speed drive combined with a fan pitch system that regulates the rotational rate

of the fan. High-speed operation of the wind tunnel adds a significant amount of heat to the recirculating air stream, so a cooling coil/chilled water system is used to control the tunnel temperature. Controlled recirculation of chilled water through the cooling coil counteracts the continued heat input and allows the wind tunnel to be operated at specified temperatures. Temperature was maintained at 20 °C for the experiments presented here.

Humidity in the wind tunnel can also be controlled and is achieved by the combination of a desiccant dehumidifier and a deionized water steam humidifier. After the humidity reaches the target condition, the dehumidifier operates at a constant (low) setting and the humidifier output is automatically controlled to maintain the target set point. Relative humidity (RH) was maintained at 50% for the experiments described here.

The wind tunnel includes a bank of high-capacity, mini-pleated filters downstream of the test section to remove aerosols not collected by the samplers. This primary filter bank effectively prevents the continuous accumulation of material in the tunnel interior, dramatically reducing the background level of particles in the air stream.

Performance specifications outlined in 40 CFR Part 53 require that the velocity profile in the test section be well characterized at each of three wind speeds—2, 8, and 24 km/h—and meet specific acceptance criteria. Wind speed and turbulence intensity (TI) must be measured by hot-wire anemometry at a minimum of 12 points in a cross-sectional area of the test section. The wind tunnel meets velocity acceptance criteria if the mean wind speed in the test section is within 10% of the desired mean and the variation at any test point in the test section does not exceed 10% of the measured mean. TI must be measured and recorded, but there are no specifications for either the magnitude or the scale of the turbulence. Wind velocity measurements were made in the ATF wind tunnel during the sampler evaluation using a sonic anemometer and were found to meet the specified acceptance criteria. TI was calculated during the evaluation and was found to be 2.8% at an average wind speed of 2 km/h, 1.0% at 8 km/h, and 0.80% at 24 km/h.

2.2 Selected Bioaerosol Samplers

Three bioaerosol samplers were selected and evaluated during the project. These samplers are listed in Table 1 along with their respective operating specifications.

Table 1. Bioaerosol Samplers and Operating Specifications

Vendor	Sampler ID	Collection Media	Flow (L/min)	Particle Size Range (µm)	Cabinet Dimensions (in.)	Inlet Dimensions (in.)	Total Weight (lb)
Dycor Technologies, Ltd. (2006)	XMX	47-mm filter, 5-mL liquid extraction	650 primary* 12 secondary	1–10	11 x 17.5 x 13.5	Inlet height above cabinet, 9.125	37.5
HI-Q Environmental Products (2012)	PSU	47-mm filter	100	Not available	18 x 18 x 12	Inlet height above cabinet, 44	55
Evogen, Inc. (2012)	DryClone™	13-mL liquid extraction	400	≤100	8 x 8.5 x 16.5	Inlet height above cabinet, 33.25	17
*Reported to be 800 L/min in another evaluation (Black, 2011) and measured to be 720 L/min for this study.							

2.2.1 XMX Bioaerosol Sampler

The XMX/2L-MIL is manufactured by Dycor Technologies, Ltd. (Edmonton, Alberta, Canada; www.dycor.com) and was designed as a weatherproof, outdoor aerosol collector to sample and concentrate aerosols in the respirable particle size range. The XMX has a high sampling flow rate (measured to be 720 L/min volumetric for this study) and concentrates aerosols between 1 and 10 µm using a two-stage virtual impactor. The particles are impinged into a centrifuge tube containing a liquid (e.g., phosphate-buffered saline) that the user removes for subsequent analysis. For this study, we used the XMX dry filter unit option to replace the liquid vial. This unit collects the particles onto a filter (37-mm mixed cellulose ester), which is then removed and extracted in liquid for further analysis.

2.2.2 Portable Sampling Unit

The portable sampling unit (PSU) (U.S. DHS, 2009) is manufactured by Hi-Q Environmental Products (San Diego, CA; http://www.hi-q.net) and is designed to pull air samples through a filter for the collection of biological aerosols. The filters used for the PSU were 47-mm-diameter glass-fiber filters produced by Millipore Corporation (Billerica, MA). The PSU encloses a pump, flow controller, flowmeter, filter holder, and all electronics inside two individual lockable, weather-resistant boxes. The PSU sample inlet is adjustable between 48 and 72 inches above the ground and samples air at a flow rate of approximately 100 L/min.

2.2.3 DryClone™ Sampler

The DryClone™, developed by Evogen, Inc. (Kansas City, MO; www.evogen.com), is a prototype instrument designed to collect and concentrate airborne aerosol in a liquid medium. The specially designed system is particularly important for collecting viable samples of biologics and other low vapor pressure aerosols. The aerosol is separated by inertial impaction onto the inner (dry) surfaces of the cyclone. The aerosol is collected into the liquid medium by means of an automated rinse phase. The air velocity decreases and liquid is injected into the air stream to wash the walls of the cyclone. The particle-laden rinse solution is then removed for further analysis.

2.3 Liquid Aerosol Generation

Adhering to the guidance in 40 CFR Part 53.42, liquid aerosols were generated using a vibrating orifice aerosol generator (VOAG). The volume of the ATF wind tunnel required several VOAGs to produce sufficient quantities of monodisperse aerosols to challenge each candidate sampler adequately. The VOAGs used to generate the aerosols in the wind tunnel were equipped with a 20-µm nominal diameter orifice (see Figure 2). Liquid flow through the orifice (0.160 cm^3/min) was provided by a Scientific Systems (State College, PA) high-performance liquid chromatography (HPLC) pump that was calibrated independently at the design flow rate. Their applied AC signal was produced by a B-K Precision (Yorba Linda, CA) Model 4040A frequency generator. The VOAGs were routinely operated at a vibrational frequency of 60,000–200,000 Hz. Monodisperse aerosols of the required diameter were produced through proper selection of liquid feed rate, vibrational frequency, and solution composition. Flow rate for the VOAGs was determined by fluorometric analysis.

The liquid solution for production of liquid aerosols consisted of a mixture of oleic acid and uranine dissolved in pure ethanol. Liquid flow rate, vibrational frequency, and solution

concentration were varied to produce particles of the desired aerodynamic diameter. The aerosol solution was prepared by measuring quantities of oleic acid and uranine and adding enough 200-proof ethanol to reach a total volume of 2000 mL. The mixture was homogenized for use with the VOAG. The product of each vibration was an oleic acid droplet "tagged" with uranine in a 10:1 ratio. This fluorescent tracer provided a metric for quantitative analysis.

The liquid droplets that were produced were then dispersed using a dispersion air flow rate of 1700 cm^3/min and diluted at a flow rate of 3 m^3/h. Because the liquid nebulization process produced electrically charged droplets, a Kr-85-based neutralizer (Model 3054, TSI, Inc., Shoreview, MN) was used to produce an electrically neutral aerosol. The neutralized aerosols were then introduced into the wind tunnel through heated copper pipes.

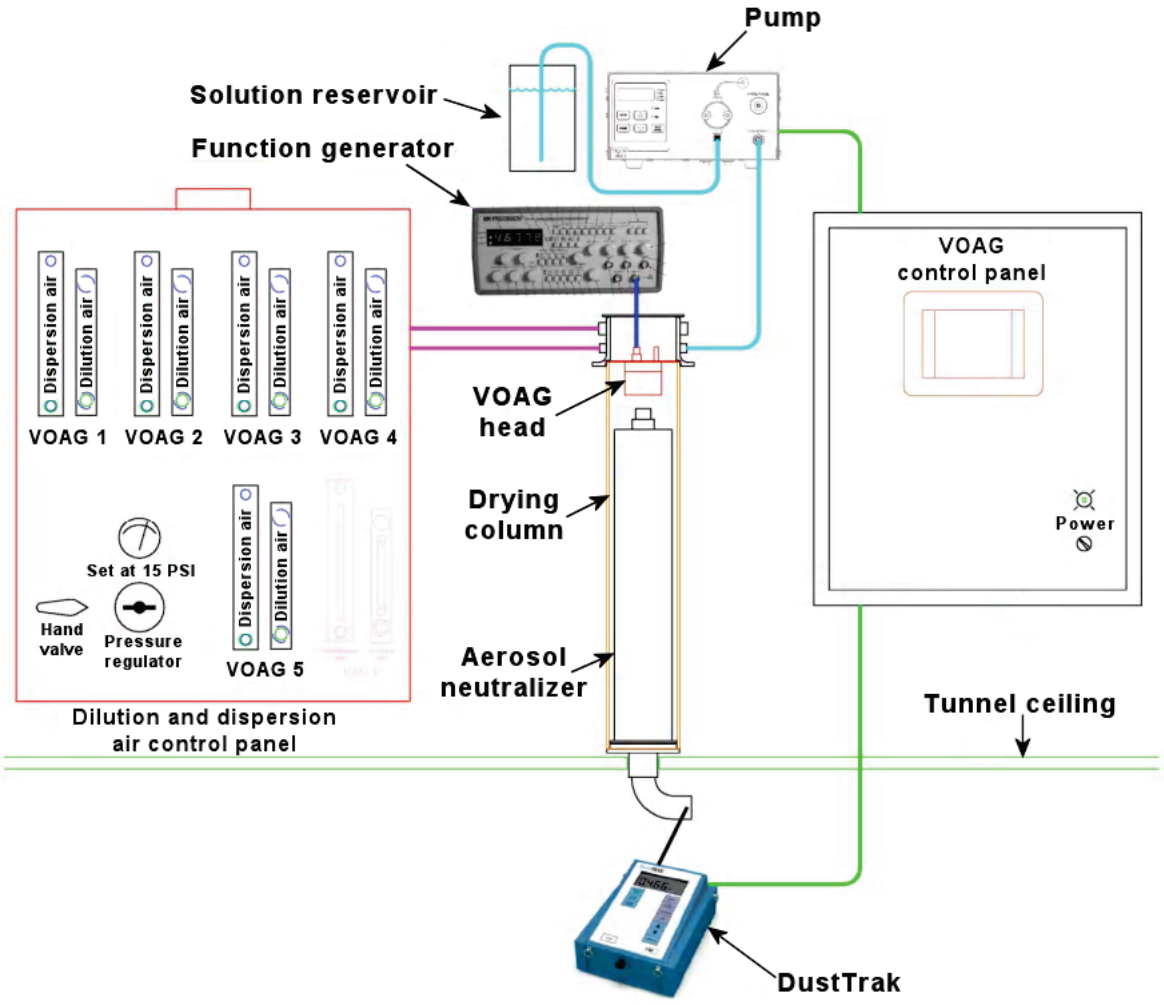

Figure 2. Aerosol generation system

2.4 Aerosol Uniformity

The 40 CFR Part 53 Subpart D requires that the test aerosol within the test section be spatially uniform. Aerosol concentrations must be measured at a minimum of five test points, and an acceptable uniformity is defined as a measured coefficient of variation (CV) not to exceed 10% at each wind speed. Title 40 CFR Part 53.42d sets the criteria for proper sample collection to confirm aerosol uniformity within a wind tunnel as follows:

> (d) The concentration of particles in the wind tunnel is not critical. However, the cross-sectional uniformity of the particle concentration in the sampling zone of the test section shall be established during the tests using isokinetic samplers. An array of not less than five evenly spaced isokinetic samplers shall be used to determine the particle concentration uniformity in the sampling zone. If the particle concentration measured by any single isokinetic sampler in the sampling zone differs by more than 10 percent from the mean concentration, the particle delivery system is unacceptable in terms of uniformity of particle concentration. The sampling zone shall be a rectangular area having a horizontal dimension not less than 1.2 times the width of the test sampler at its inlet opening and a vertical dimension not less than 25 centimeters. The sampling zone is an area in the test section of the wind tunnel that is horizontally and vertically symmetrical with respect to the test sampler inlet opening.

In the ATF wind tunnel, an array of isokinetic nozzles, called a rake, samples in a symmetrical pattern around the sampler test section to assess particle uniformity. The diagrams in Figure 3 illustrate two rake configurations used during this work. The first was designed for a previous study where similar wind speed and particle size combinations were attempted. Then 40 CFR Part 53.42d was revisited and a second configuration was designed. Each sampler in the second configuration consists of a commercial 47-mm-diameter in-line filter holder with a conical nozzle replacing the usual inlet of the filter holder. Each nozzle projects approximately 10 cm upstream of the filter holder and is made with a sharp-edged entry leading to a gradually expanding conical section. Different nozzles were used for conducting uniformity tests at 2, 8, and 24 km/h**.** The flow rate through each nozzle was controlled using mass flow controllers (MFCs) (Model 5851E, Brooks Instrument, Hatfield, PA). Each MFC was adjusted to provide isokinetic sampling at each wind speed. The filters used in the rake samplers were 47-mm glass-fiber filters (Type A/E, Pall Life Sciences, Ann Arbor, MI), which were chosen because of their collection characteristics and low background fluorometric content.

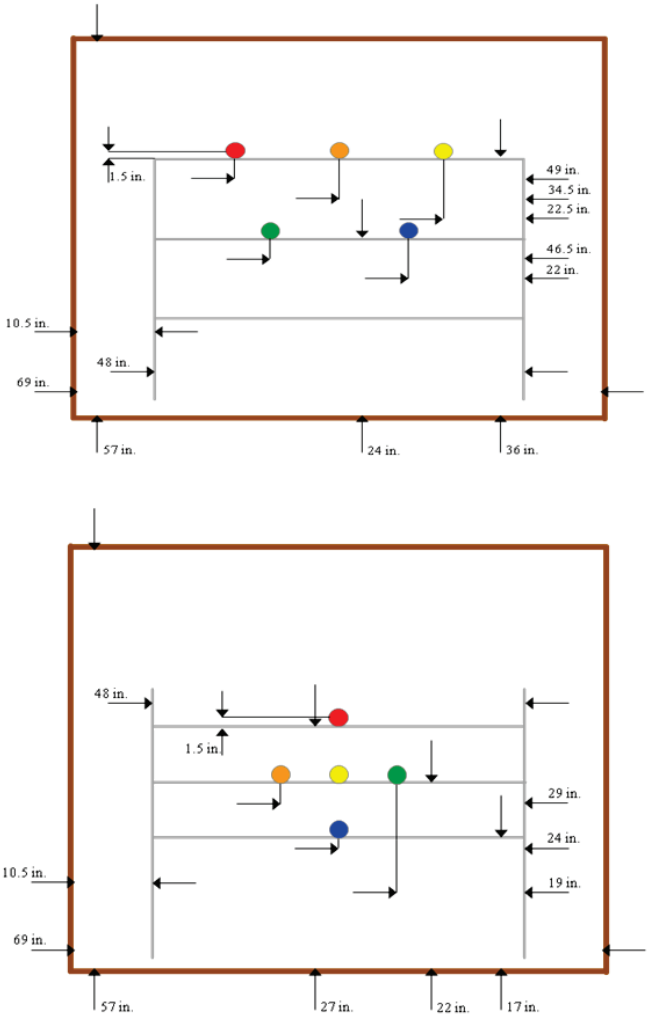

Figure 3. Critical dimensions of rake samplers showing locations of aerosol sampling points for two configuration.

2.5 Aerosol Quality

To verify the quality and mean size of the generated aerosols, samples were collected in the test section using a specially designed slide impactor. This impactor withdrew a representative sample of the aerosol from the test section and then impacted the sampled particles on a microscope slide. The slides were pretreated using a solution of 2% NyeBar® (Type CT or Type K) oliophobic surfactant, which resisted the spread of the oleic acid droplets. The slides were then baked for 1 h at 100 °C.

Following the aerosol collection, a polarizing optical microscope (Labophot™ Pol, Nikon, Melville, NY) was used to verify that the mean particle size and aerosol uniformity met acceptance criteria outlined in the CFR. The eyepiece micrometer of the microscope was calibrated periodically using a certified stage micrometer (Model 1400, American Optics, Burlington, ON, Canada).

Two Aerodynamic Particle Sizer (APS™) spectrometers (TSI, Inc., Shoreview, MN) were installed in the wind tunnel to allow continuous observation of the aerosol quality and to check for the presence or absence of satellites. If large secondary particles (larger than one-fifth of the desired aerosol) were detected, first an attempt was made to locate the malfunctioning VOAG and then, if the problem could not be resolved expeditiously, the experiment was aborted.

A spread factor established by Olan-Figueroa et al. (1982) was used to calculate the spherical droplet size from the deposited drop and was checked against the particle diameter calculated by knowledge of the feed rate, the frequency, and the oleic acid and uranine concentration in the feed solution. The calculated value was used in plotting experimental sampling effectiveness results. The target agreement between the microscopy check and the calculated size was generally within 0.5 μm or 5% and well within the EPA specification of 0.5 μm or 10%. The geometric standard deviation (σ_g) of the generated particle size distribution was typically less than 1.05 and also within the EPA specification of 1.10.

2.6 Fluorometric Analysis

Per the CFR and the Tolocka et al. (2001a) method, the aerosol particle mass was measured by fluorometry. Following aerosol collection, the 47-mm-diameter glass-fiber filters from the isokinetic samplers were each placed in clean, labeled 50-mL centrifuge tubes equipped with resealable lids, and 30 mL of 0.01N sodium hydroxide (NaOH) was added to each container. The containers were then sealed and placed in an ultrasonic bath for 10 min. To ensure that the extract was homogeneous, the contents of each container were gently swirled with a spiraling motion. Disposable pipettes were used to transfer an aliquot of each container into separate cuvettes for fluorometric analysis. Comparison tests showed that centrifugation of the solutions was not required to obtain reliable test results.

The fluorometric content of the solutions was determined using a calibrated Quantech™ fluorometer (Barnstead-Turner, Dubuque, IA) containing an excitation filter (NB360) and an emission filter (NB460). Different calibration curves were used depending on the concentration and particle size being analyzed. Each curve consisted of at least five analytical standards. A coefficient of determination (R^2) of 1.00 was used as the indicator that the curves were linear. After the curve was set, a sample was read in the fluorometer to determine if the samples were over-range or if the working standards were sufficient. If the sample was over-range, a new curve was set or the sample was diluted. Three fluorometric measurements were recorded for each sample, the curve was checked intermediately, and blank filters (at least one per run) and spikes were analyzed for quality assurance.

The aerosol concentration in the wind tunnel was calculated from fluorometric measurements of the uranine concentrations. If the glass-fiber filter fluorescence was significant compared to the collected aerosol mass, then the calculated concentration would significantly overpredict the actual concentration. To quantify the effect of the filter's natural fluorometric content, unexposed filters were analyzed as laboratory blanks. Since the values measured from the blank filters were significantly lower than from the sample filters, blank corrections were not made to the measured filter concentrations during the course of the project.

To determine percent recovery from the filter, a solution of known uranine concentration was prepared and spiked onto a blank 47-mm glass-fiber filter (simulating the rake and sampler filters). The solution also was added to an empty centrifuge tube to serve as a blank. The filters and the blank were washed with 30 mL of 0.01N NaOH. The average percent recovery on the spikes was 91%.

2.7 Test Protocol

To evaluate the aerosol sampling performance of the biosampler monitors appropriately, most of the reference and equivalent method instrument test protocols for PM_{10} were used, as described in 40 CFR Part 53 Subpart D. The CFR provides strict test protocols and procedures for the determination of reference sampler or equivalent sampler status for PM_{10}. The test protocol required a comparison between particle concentration measured by isokinetic nozzles and the test sampler performed at fixed wind velocities and with monodisperse particles (40 CFR Part 53; Tolocka et al., 2001a, 2001b; Ranade et al., 1990). The experiments were conducted according to the CFR except that only four particle sizes were tested: 5, 10, 15, and 20 μm.

The isoknetic sampling rake was installed in the wind tunnel. Prior to a sampling run, new 47-mm filters were installed in each isoknetic (iso) sampler and clean isokinetic sampling nozzles were installed. The flow on the iso samplers was set at the isokinetic flow rate using certified MFCs.

The biosampler was installed in the wind tunnel. A clean filter was loaded in the filter assembly located within the instrument housing. For the experiments, each sampler was oriented so that its inlet was on the upwind side of the sampler. The samplers were placed in the wind tunnel so that the inlet was centered both horizontally and vertically in the test section, approximately 2 m downwind of the iso sampler rake. Because of the designs of the samplers and locations of the motors, only a portion of the PSU and DryClone™ samplers could be placed in the wind tunnel. Plywood plates were built to surround the sampler housings and fit into the floor of the tunnel. Caulking and tape were used to fill the gaps in the tunnel floor around the samplers. The XMX sampler was placed completely inside the wind tunnel. Using this configuration the blockage of the cross-sectional area of the STS was 4.8% for the XMX, 1.3% for the PSU, and 6.0% for the DryClone™. Each met the CFR requirement of no more than 15% blockage.

The typical test procedure for the biosampler evaluation was as follows. At the start of the day, ambient temperature and pressure conditions were checked. Checks were then made to verify that the wind tunnel was ready to operate and that all mixing fans were operating and in the correct orientation. The wind tunnel temperature and humidity controls were turned on. The tunnel's blower was turned on and its rotational speed adjusted to attain the desired wind speed. The wind tunnel air temperature and relative humidity were monitored throughout the tests. The monodisperse aerosol generators were brought online and allowed to stabilize for at least 30 min. Aerosol was then introduced into the tunnel and a representative sample was collected on the slide impactor during a sampling time of at least 30 min. The aerosol

quality (particle size and geometric standard deviation) was then verified using the optical microscope, and any necessary adjustments were made to the aerosol generation and dispersion system. Aerosol samples were monitored continuously using an APS spectrometer (TSI, Inc.), and the aerosol quality was verified based on the geometric standard deviation. All routine wind tunnel operations were carried out following established ATF procedures. The aerosol concentration in the wind tunnel was then allowed to stabilize for at least 15 min before sampling began.

Once the system was verified as ready for sampling, the test biosampler and iso rake samplers were turned on and operated for 1 h. At the end of the sampling period, the iso samplers and the test sampler were turned off and the stop time and the final volume sampled were recorded for each. Once the experimental runs were completed, the iso filters, iso nozzles, biosampler filter, and biosampler inlet unit were collected from the wind tunnel and prepared for analysis. Samples were collected from the iso rake from right to left as standard practice to avoid mislabeling of samples. Figure 4 shows an example of the uranine-tagged particles collected on the iso sampler filter.

Filters were placed in prelabeled centrifuge tubes with 30 mL of 0.01N NaOH and sonicated for 10 min. The internal walls of the iso nozzles were washed into prelabeled centrifuge tubes, each with 30 mL of 0.01N NaOH. The collected aerosol deposits were extracted and quantified fluorometrically using procedures described in Section 2.6.

Figure 4. Isokinetic sampler filter showing collected uranine-tagged particles.

3.0
Results and Discussion

Sampling efficiency (SE) was calculated for the biosamplers from the wind tunnel experiments. SE is defined as the ratio of the measured mean particle concentration (C_m) to that in the free-stream air (C_0):

$$\mathrm{SE} = \frac{C_m}{C_0}.$$

All concentrations were calculated as mass concentration of uranine in units of nanograms per liter (ng/L). C_0 was calculated from the iso sampler results as

$$C_0 = \frac{M_{f,iso} + M_{w,iso}}{Q_{iso} \cdot t},$$

where $M_{f,iso}$ was the mass collected on the iso filter (ng), $M_{w,iso}$ was the mass collected from the iso nozzle wash (ng), Q_{iso} was the iso calibrated flow rate (L/min), and t was the sampling time (min). The measured concentration for the biosampler was calculated as

$$C_m = \frac{M_f}{Q \cdot t},$$

where M_f was the mass collected on the biosampler filter (ng), Q was the biosampler calibrated flow rate (L/min), and t was the sampling time (min). The inlet concentration for the biosampler was calculated as

$$C_i = \frac{M_f + M_w}{Q \cdot t}$$

where M_w was the mass collected from the biosampler inlet wash (ng).

The SE data were plotted as a function of the aerodynamic particle size. To determine the significance of the data, data were plotted as a function of the square root of Stokes number (Stk). The Stokes number is a dimensionless number that describes the ability of particles to follow the flow of the carrier fluid. Stk is defined as the ratio of the stopping distance of a particle to a characteristic dimension of the obstacle, or

$$\mathrm{Stk} = \frac{{d_p}^2 \rho_p U}{18 \mu D},$$

where d_p is the calculated particle diameter (μm), ρ_p is the density of the particle (kg/m^3), U is the free-stream velocity (m/s), μ is the viscosity of air (Pa·s), and D is the inlet diameter of the sampler (m). The larger the Stk, the less the particles will follow the air stream lines, making them less likely to be captured by the sampler. Since the sampler inlets are not simple circular openings, D is defined for each sampler as the diameter of a circle with the same area as the opening in the sampler inlet. For this purpose, the d_p was calculated using the VOAG operating conditions, as it is considered more accurate than measuring the particles microscopically (Liu et al., 1982). The Stokes numbers were calculated using AEROCALC, an Excel spreadsheet calculator for aerosols written by Dr. Paul Baron (Baron, 2001).

3.1 Aerosol Uniformity Results

Acceptable uniformity was achieved for wind speeds of 2, 8 and 24 km/h for particle sizes of 5, 10, 15, and 20 μm with all samplers tested. Stairmand disks and axial fans were employed to homogenize the aerosol within the air stream to properly challenge the candidate samplers. The rake, which provides a measure of the aerosol homogeneity, was reconfigured after the addition of the mixing instruments. The aerosol uniformity data are presented in Table 2.

Table 2. Aerosol Uniformity Results

Date	d_p (μm)	U (km/h)	Mass Concentration (ng/mL) Nozzle 1	Nozzle 2	Nozzle 3	Nozzle 4	Nozzle 5	Average	SD	CV
2/10/2011	5	2	11.79	11.79	11.88	12.35	12.16	11.99	0.25	2.1%
2/10/2011	5	2	14.32	14.54	14.03	15.13	15.04	14.61	0.47	3.2%
2/10/2011	5	2	12.87	12.65	12.49	13.34	13.50	12.97	0.44	3.4%
2/11/2011	5	8	8.99	9.19	8.90	8.78	8.71	8.91	0.19	2.1%
2/14/2011	5	8	9.27	8.84	8.57	8.38	8.08	8.63	0.45	5.3%
3/8/2011	5	8	14.89	13.60	14.80	13.09	13.25	13.92	0.86	6.2%
8/24/2011	5	24	5.78	5.33	5.81	6.13	6.09	5.83	0.32	5.5%
8/24/2011	5	24	6.19	6.05	6.43	6.82	6.43	6.38	0.29	4.6%
8/24/2011	5	24	3.70	3.73	3.96	3.89	3.75	3.81	0.11	2.9%
6/7/2011	10	2	6.17	5.66	5.30	6.03	5.72	5.78	0.34	5.9%
6/9/2011	10	2	4.85	4.56	4.75	5.03	4.56	4.75	0.20	4.2%
6/10/2011	10	2	5.14	4.86	4.81	5.33	4.61	4.95	0.28	5.7%
6/8/2011	10	8	11.15	10.09	10.91	12.28	10.44	10.97	0.84	7.6%
6/9/2011	10	8	8.13	7.81	8.83	9.52	8.69	8.60	0.66	7.7%
6/13/2011	10	8	26.31	26.57	26.22	27.36	28.19	26.93	0.84	3.1%
8/9/2011	10	24	25.18	26.98	27.27	30.15	27.15	27.35	1.78	6.5%
8/9/2011	10	24	24.22	24.47	25.85	27.51	25.46	25.50	1.31	5.1%
8/10/2011	10	24	14.54	14.99	15.64	17.43	15.75	15.67	1.10	7.0%
8/19/2011	15	2	14.94	16.46	16.53	14.67	17.56	16.03	1.21	7.5%
8/22/2011	15	2	16.62	19.49	18.98	17.57	21.25	18.78	1.79	9.5%
8/22/2011	15	2	14.16	16.69	15.22	15.25	18.25	15.91	1.59	10.0%
8/18/2011	15	8	56.67	56.23	57.22	57.88	58.75	57.35	1.00	1.7%
8/19/2011	15	8	56.14	56.37	56.88	56.72	58.20	56.86	0.80	1.4%
8/19/2011	15	8	63.47	61.68	63.36	63.26	65.52	63.46	1.37	2.2%
8/18/2011	15	24	21.94	23.94	22.89	25.10	23.71	23.51	1.18	5.0%
8/19/2011	15	24	21.81	24.05	24.19	25.20	23.11	23.67	1.28	5.4%
7/27/2011	15	24	33.79	37.98	38.22	39.90	37.33	37.44	2.25	6.0%
8/5/2011	20	2	5.00	5.43	5.24	5.37	5.96	5.40	0.35	6.5%
8/29/2011	20	2	1.50	1.39	1.51	1.56	1.66	1.52	0.10	6.5%
8/15/2011	20	8	27.04	26.86	27.31	26.92	29.51	27.53	1.12	4.1%
8/15/2011	20	8	22.76	24.39	24.94	25.28	26.82	24.84	1.47	5.9%
8/17/2011	20	8	26.01	28.89	30.17	29.95	31.27	29.26	2.00	6.8%
8/15/2011	20	24	12.29	15.09	13.50	14.48	12.98	13.67	1.13	8.2%
8/16/2011	20	24	14.36	17.45	16.01	17.91	16.01	16.35	1.40	8.6%
8/16/2011	20	24	14.27	17.54	15.67	17.97	15.16	16.12	1.58	9.8%

3.2 XMX Sampler Results

The total flow rate for the XMX sampler during the tests was 720 L/min. Five test runs were performed with 5-µm liquid particles (oleic acid) at wind speeds of 2, 8, and 24 km/h using the filter collection option on the sampler. Data collected for two of the tests with this sampler were not acceptable because a uniform distribution of the particles across the STS could not be achieved. During the runs with acceptable aerosol distribution, the SE was less than 30%. The manufacturer, Dycor, stated that the sampler was not designed for liquid particles and that the standard EPA tests as defined in this report would likely not yield representative SEs. In tests performed under the direction of Dycor, the SE for 1.9-µm polystyrene latex (PSL) spheres was reported to be approximately 54% at both 2 and 8 km/h. For 5-µm PSL spheres at 2 and 8 km/h, the results were reported to be 88% and 83.6%, respectively. We suggest retesting the sampler using solid monodisperse particles prepared from fluoroscein and ammonium hydroxide to determine SE.

3.3 PSU Sampler Results

The Hi-Q PSU bioaerosol sampler was configured to collect samples on 47-mm glass-fiber filters. The flow rate was set to 100 L/min. Test runs using 5-, 10-, 15-, and 20-µm particles at wind speeds of 2, 8, and 24 km/h were completed in triplicate. Some runs were repeated when the aerosol uniformity or particle size did not meet the method criteria. Data for the failed runs are not included here. SE was calculated from the analytical data, and the averages are plotted in Figure 5 and presented in Table 3 along with the square root of Stk.

3.4 DryClone™ Sampler Results

Test runs with the DryClone™ sampler using 5-, 10-, 15-, and 20-µm particles at wind speeds of 2, 8, and 24 km/h were completed in triplicate. Additional test runs were performed if the data did not meet QA requirements. These runs were marked as outliers and the data were not included in the averages. SE was calculated from the analytical data, and the averages are plotted in Figure 6 and presented in Table 4 along with the square root of Stk. In tests performed under the direction of Evogen, the SE of the DryClone™ for 5-µm solid particles was reported to be 93–103%. The SE from our tests at 2 km/h compares well with the reported Evogen data.

3.5 Sampling Efficiency

The SE data for each sampler were fitted to a Gompertz function for regression analysis (Ratkowsky, 1983). The Gompertz curves are given by

$$\mathrm{SE} = a\mathrm{e}^{b\mathrm{e}^{c\cdot \mathrm{Stk}^{0.5}}},$$

where Stk is based on the VOAG particle size estimate, e is the base of the natural logarithm, and a, b, and c were fit to the data by regression with the limits $0 < a \leq 1$, $-1 < b < 0$, and $c > 0$. The values for a, b, and c from the regressions are given in Table 5 for the PSU sampler and Table 6 for the DryClone™ sampler.

In Figures 7 and 8, the Gompertz regressions are plotted against the square root of the calculated Stk along with the SE data for each particle size and wind speed. These plots demonstrate the quality of the overall data by providing a nondimensional estimate of the performance of the sampler. The square root of Stk provides a nondimensional relationship between the particle relaxation time and the SE. The particle relaxation time is important because it indicates how a particle will change its velocity in a changing flow field. In the case of these samplers, the particle is accelerating from its initial velocity (the wind speed) to its collection velocity in the sampler.

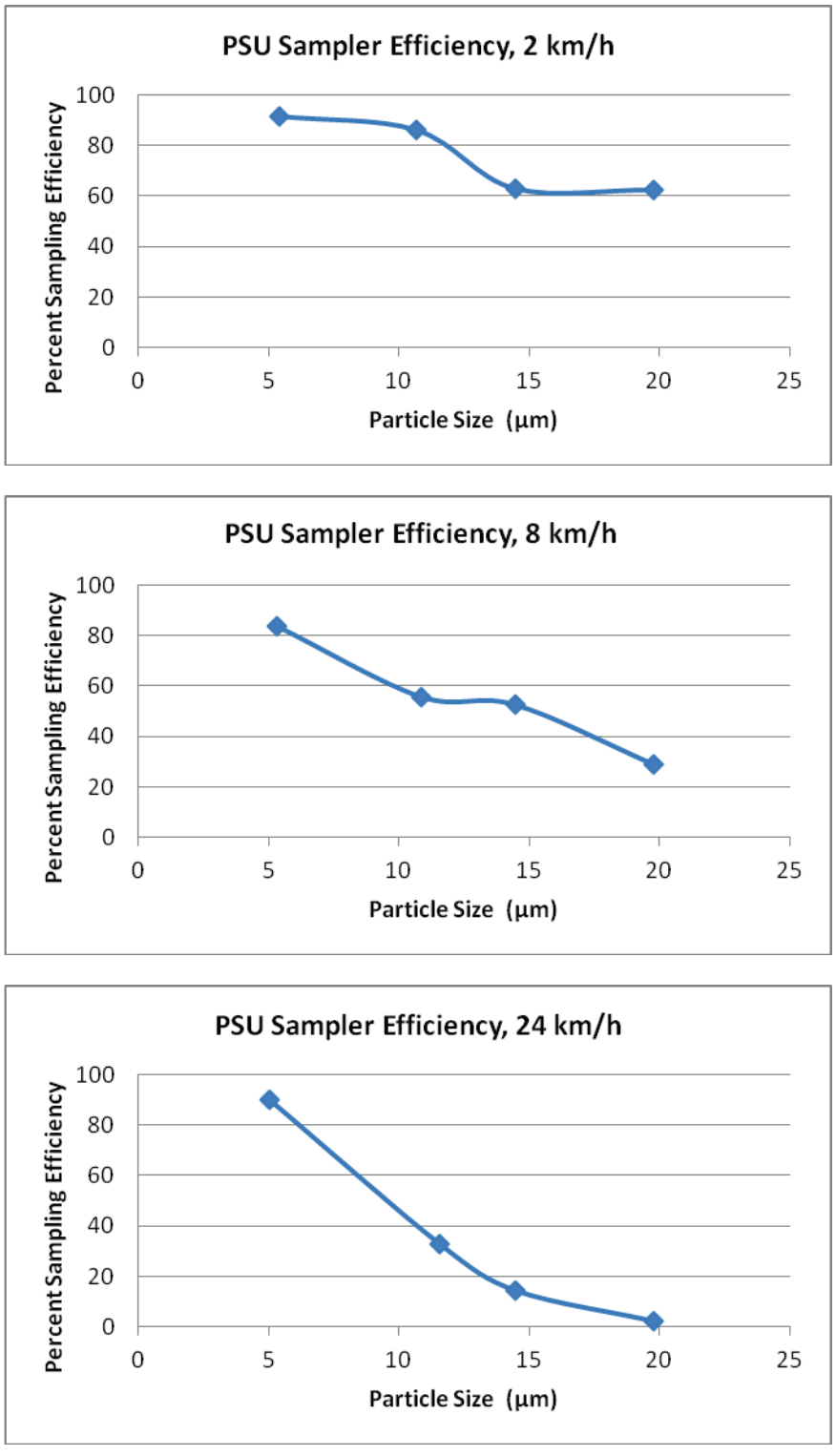

Figure 5. PSU sampler efficiency data plotted against particle size for all three test wind speeds.

Table 3. Sampling Efficiencies of the PSU Sampler

Replicate No.	Date	d_p (µm)	Rake (ng/L)	Spatial Uniformity CV (%)	Sampler (ng/L)	Sampler Efficiency (%)	Square Root of Stk*
5 µm_2 km/h							
1	2/10/2011	5.42	0.2654	2.1%	0.2428	91.5	0.0257
2	2/10/2011	5.42	0.3233	3.2%	0.3020	93.4	0.0257
3	2/10/2011	5.42	0.2869	3.4%	0.2548	88.8	0.0257
Average						**91.2**	
SD						**2.3**	
CV						**0.03**	
5 µm_8 km/h							
2	2/11/2011	5.42	0.1230	2.1%	0.0663	53.9	0.0516
3	2/14/2011	5.42	0.1191	5.3%	0.1118	93.9	0.0516
4	3/8/2011	5.18	0.1922	6.2%	0.1988	103.5	0.0493
Average						**83.7**	
SD						**26.3**	
CV						**0.31**	
5 µm_24 km/h							
1	8/24/2011	5.01	0.0624	5.5%	0.0587	94.1	0.0827
2	8/24/2011	5.01	0.0684	4.6%	0.0630	92.2	0.0827
3	8/24/2011	5.01	0.0407	2.9%	0.0339	83.1	0.0827
Average						**89.8**	
SD						**5.9**	
CV						**0.07**	
10 µm_2 km/h							
1	2/15/2011	10.36	0.3721	10.8%	0.3359	90.3	0.0487
3	2/17/2011	10.83	0.5938	7.5%	0.4988	84.0	0.0487
4	2/22/2011	10.83	0.4168	7.1%	0.3502	84.0	0.0487
Average						**86.1**	
SD						**3.6**	
CV						**0.04**	
10 µm_8 km/h							
1	2/17/2011	10.83	0.6794	2.5%	0.3319	48.8	0.1023
2	2/21/2011	10.83	0.4839	2.3%	0.3012	62.2	0.1023
4	2/23/2011	10.83	0.6158	4.4%	0.3460	56.2	0.1023
Average						**55.8**	
SD						**6.7**	
CV						**0.12**	
10 µm_24 km/h							
1	8/23/2011	11.64	0.2193	5.9%	0.0773	35.2	0.1904
2	8/23/2011	11.49	0.1961	5.0%	0.0566	28.8	0.1880
3	8/23/2011	11.49	0.1924	5.9%	0.0662	34.4	0.1880
Average						**32.8**	
SD						**3.5**	
CV						**0.11**	
15 µm_2 km/h							
1	8/19/2011	14.45	0.3547	7.5%	0.1953	55.1	0.0678
2	8/22/2011	14.45	0.4155	9.5%	0.2891	69.6	0.0678
3	8/22/2011	14.45	0.3521	10.0%	0.2243	63.7	0.0678
Average						**62.8**	
SD						**7.3**	
CV						**0.12**	

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Replicate No.	Date	d_p (µm)	Rake (ng/L)	Spatial Uniformity CV (%)	Sampler (ng/L)	Sampler Efficiency (%)	Square Root of Stk*
15 µm_8 km/h							
1	8/18/2011	14.45	0.7915	1.7%	0.4326	54.7	0.1362
2	8/19/2011	14.45	0.7848	1.4%	0.4266	54.4	0.1362
3	8/19/2011	14.45	0.8758	2.2%	0.4262	48.7	0.1362
Average						**52.6**	
SD						**3.4**	
CV						**0.06**	
15 µm_24 km/h							
1	8/18/2011	14.45	0.3173	15.0%	0.0464	14.6	0.2361
2	8/18/2011	14.45	0.2518	5.0%	0.0427	16.9	0.2361
3	8/19/2011	14.45	0.2534	5.4%	0.0290	11.4	0.2361
Average						**14.3**	
SD						**2.8**	
CV						**0.19**	
20 µm_2 km/h							
1	8/29/2011	19.78	0.0169	6.5%	0.0105	62.3	0.0926
20 µm_8 km/h							
1	8/15/2011	19.78	0.3799	4.1%	0.1223	32.2	0.1861
2	8/15/2011	19.78	0.3428	5.9%	0.1100	32.1	0.1861
3	8/17/2011	19.78	0.4038	6.8%	0.0902	22.3	0.1861
Average						**28.9**	
SD						**5.7**	
CV						**0.20**	
20 µm_24 km/h							
1	8/15/2011	19.78	0.1463	8.2%	0.0041	2.8	0.3227
2	8/16/2011	19.78	0.1750	8.6%	0.0043	2.5	0.3227
3	8/16/2011	19.78	0.1726	9.8%	0.0021	1.2	0.3227
Average						**2.1**	
SD						**0.8**	
CV						**0.39**	

*Stk was calculated using the following values: viscosity of air (μ) at temperature = 293.15 K and pressure = 101.3 kPa, D = 0.145 m, and ρ_p = 933 kg/m^3.

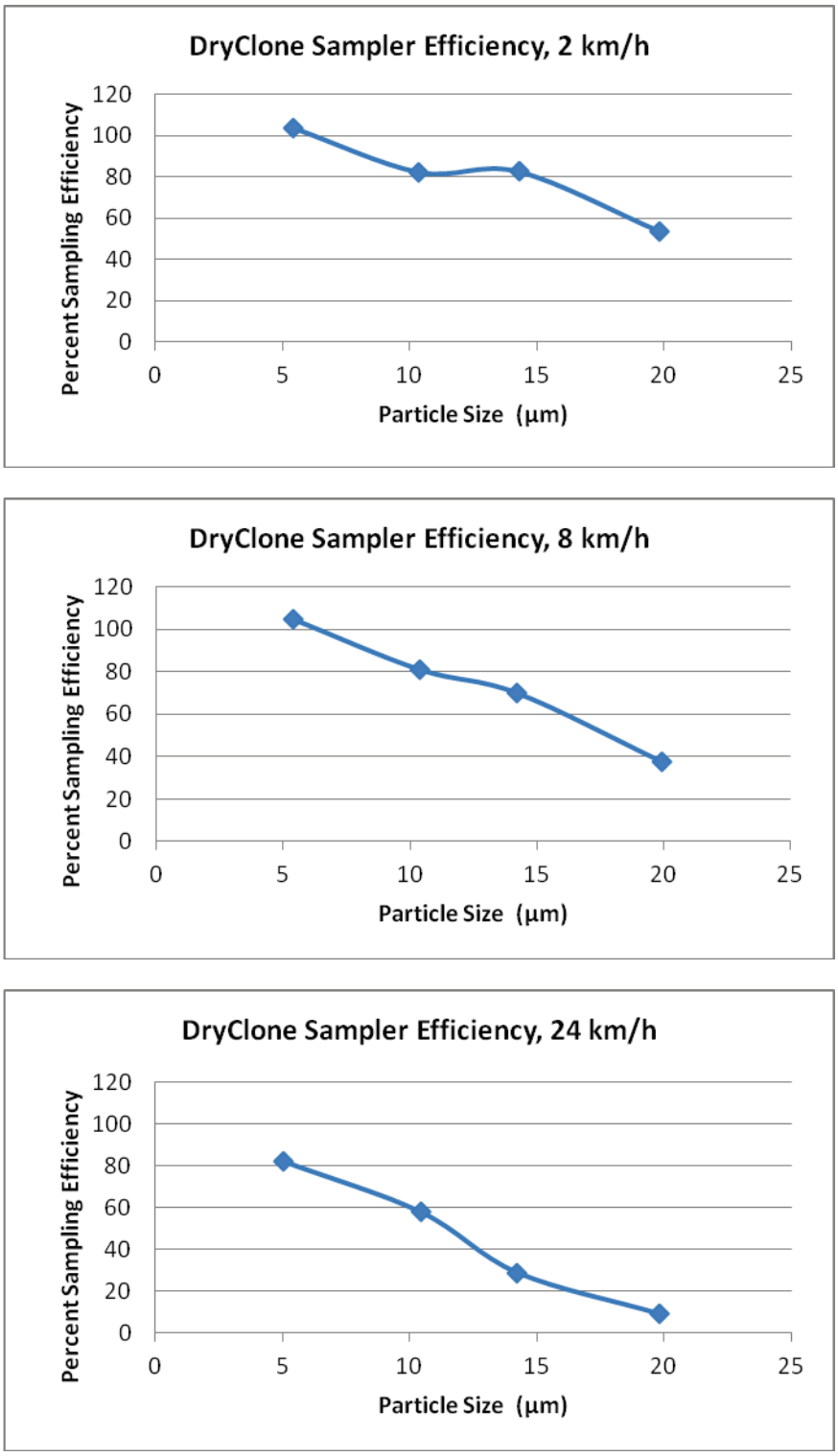

Figure 6. DryClone™ sampler efficiency data plotted against particle size for all three test wind speeds.

Table 4. Sampling Efficiencies of the DryClone™ Sampler

Replicate No.	Date	d_p (µm)	Rake (ng/L)	Spatial Uniformity CV (%)	Sampler (ng/L)	Sampler Efficiency (%)	Square Root of Stk*
5 µm_2 km/h							
1	6/1/2011	5.42	0.1544	5.9%	0.1664	107.8	0.0219
3	6/3/2011	5.42	0.1524	7.9%	0.1569	102.9	0.0219
4	6/3/2011	5.42	0.1494	5.9%	0.1497	100.2	0.0219
Average						**103.6**	
SD						**3.8**	
CV						**0.04**	
5 µm_8 km/h							
1	6/1/2011	5.42	0.1315	7.7%	0.1412	107.3	0.0439
2	6/1/2011	5.42	0.1384	4.7%	0.1507	108.9	0.0439
3	6/2/2011	5.42	0.1572	6.5%	0.1542	98.1	0.0439
Average						**104.8**	
SD						**5.8**	
CV						**0.06**	
5 µm_24 km/h							
1	8/8/2011	5.02	0.0601	11.9%	0.0465	77.3	0.0706
2	8/8/2011	5.01	0.0594	2.0%	0.0477	80.3	0.0704
3	8/9/2011	5.01	0.0627	3.4%	0.0562	89.6	0.0704
Average						**82.4**	
SD						**6.4**	
CV						**0.08**	
10 µm_2 km/h							
1	6/7/2011	10.36	0.2556	5.9%	0.2165	84.7	0.0415
2	6/9/2011	10.36	0.2102	4.2%	0.1697	80.8	0.0415
4	6/10/2011	10.36	0.2190	5.7%	0.1782	81.4	0.0415
Average						**82.3**	
SD						**2.1**	
CV						**0.03**	
10 µm_8 km/h							
2	6/8/2011	10.36	0.3029	7.6%	0.2506	82.7	0.0833
3	6/9/2011	10.36	0.2373	7.7%	0.1723	72.6	0.0833
5	6/13/2011	10.36	0.7433	3.1%	0.6540	88.0	0.0833
Average						**81.1**	
SD						**7.8**	
CV						**0.05**	
10 µm_24 km/h							
1	8/9/2011	9.84	0.2928	6.5%	0.1817	62.0	0.1372
2	8/9/2011	9.84	0.2731	5.1%	0.1802	66.0	0.1372
3	8/10/2011	11.60	0.1678	7.0%	0.0773	46.1	0.1616
Average						**58.0**	
SD						**10.6**	
CV						**0.18**	
15 µm_2 km/h							
4	19-Jul	14.67	0.123	7.2	0.100	81.8	0.0575
5	21-Jul	14.67	0.200	12.9	0.169	84.3	0.0568
6	25-Jul	14.37	0.226	13.7	0.185	81.8	0.0568
Average						**82.1**	
SD						**1.5**	
CV						**0.02**	

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Replicate No.	Date	d_p (µm)	Rake (ng/L)	Spatial Uniformity CV (%)	Sampler (ng/L)	Sampler Efficiency (%)	Square Root of Stk*
15 µm_8 km/h							
2	7/18/2011	14.23	0.6124	8.2%	0.4239	69.2	0.1142
3	7/19/2011	14.23	0.4462	5.9%	0.3102	69.5	0.1142
4	7/21/2011	14.23	1.7550	9.5%	1.2411	70.7	0.1142
Average						**69.8**	
SD						**0.8**	
CV						**0.01**	
15 µm_24 km/h							
1	7/26/2011	14.23	0.1816	11.2%	0.0532	29.3	0.1980
2	7/27/2011	14.23	0.2673	6.0%	0.0757	28.3	0.1980
3	7/27/2011	14.23	0.2549	9.2%	0.0736	28.9	0.1980
Average						**28.8**	
SD						**0.5**	
CV						**0.02**	
20 µm_2 km/h							
1	8/3/2011	19.78	0.0354	10.4%	0.0165	46.6	0.0789
2	8/4/2011	19.97	0.0183	18.6%	0.0095	51.7	0.0796
4	8/11/2011	19.78	0.0565	15.3%	0.0355	62.9	0.0789
Average						**53.7**	
SD						**8.3**	
CV						**0.15**	
20 µm_8 km/h							
1	8/2/2011	19.78	0.3689	3.7%	0.1413	38.3	0.1583
2	8/3/2011	19.97	0.4102	8.8%	0.1610	39.2	0.1600
3	8/4/2011	19.97	0.2860	6.2%	0.0992	34.7	0.1600
Average						**37.4**	
SD						**2.4**	
CV						**0.06**	
20 µm_24 km/h							
1	8/2/2011	19.78	0.0570	10.1%	0.0045	7.9	0.2747
2	8/2/2011	19.78	0.1007	12.1%	0.0086	8.5	0.2747
3	8/4/2011	19.97	0.0945	9.2%	0.0100	10.6	0.2774
Average						**9.0**	
SD						**1.4**	
CV						**0.16**	

*Stk was calculated using the following values: viscosity of air (μ) at temperature = 293.15 K and pressure = 101.3 kPa, D = 0.2 m, and ρ_p = 933 kg/m^3.

Table 5. Gompertz Parameter Values from Regression Fitting for the PSU Sampler

Parameter	Estimate	Lower 95th Bound	Upper 95th Bound
a	1.0	1.0	1.0
b	-0.1010	-0.1405	-0.0615
c	13.1459	10.5746	15.7172

Table 6. Gompertz Parameter Values from Regression Fitting for the DryClone™ Sampler

Parameter	Estimate	Lower 95th Bound	Upper 95th Bound
a	1.0	1.0	1.0
b	-0.0500	-0.0741	-0.0258
c	17.0515	13.7347	20.3682

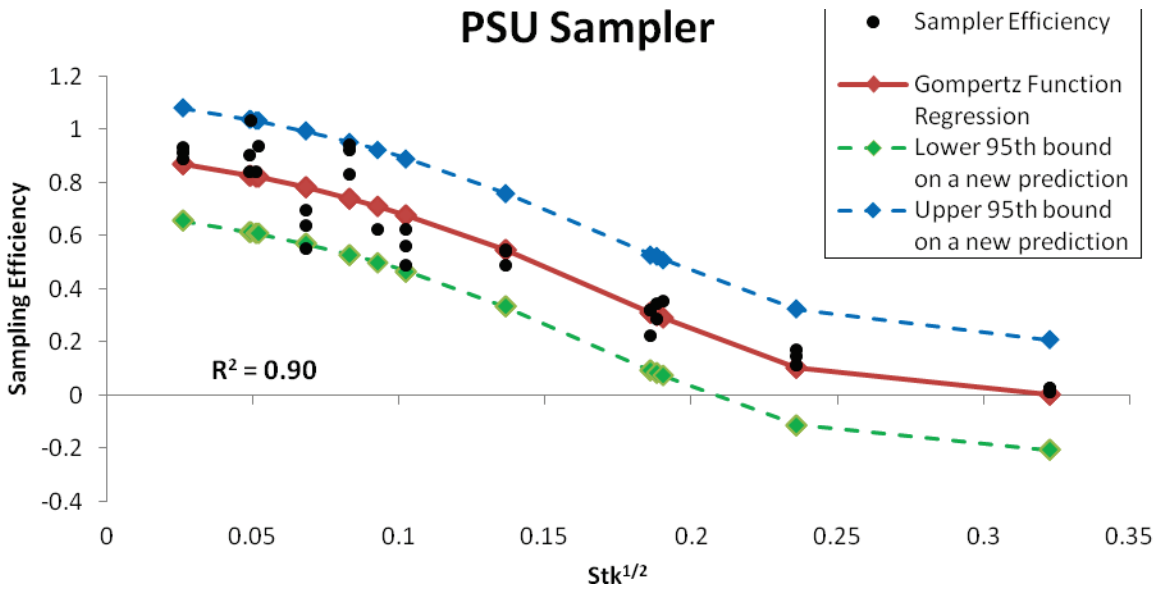

Figure 7. PSU sampling efficiency plotted as a function of the square root of Stk. Regression lines are the Gompertz functions with values of *a*, *b*, and *c* from Table 5.

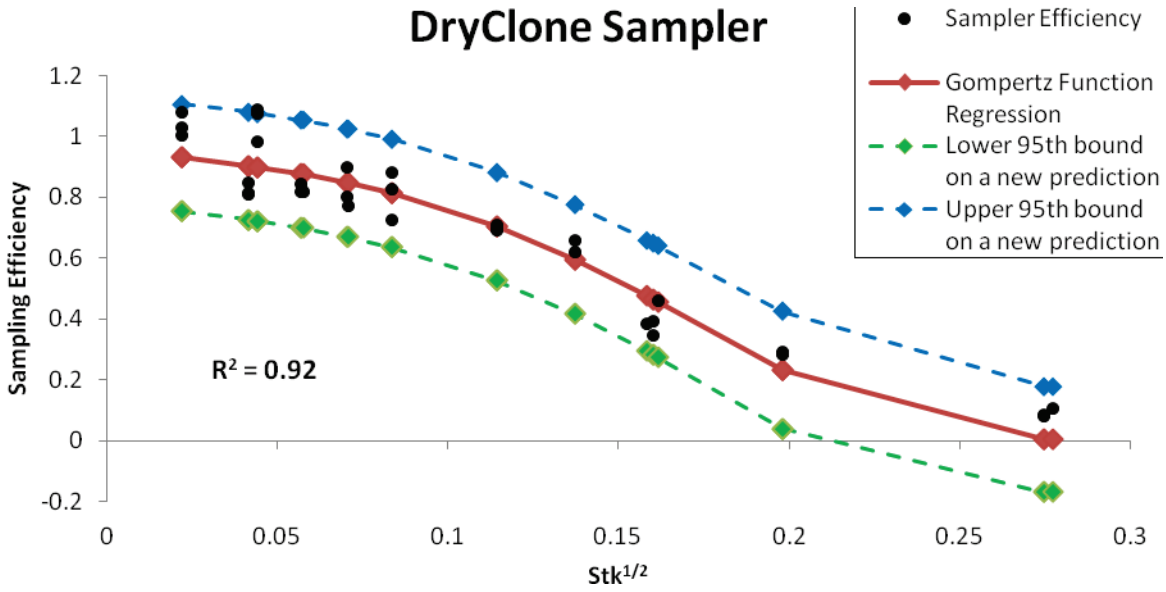

Figure 8. DryClone™ sampling efficiency plotted as a function of the square root of Stk. Regression lines are the Gompertz functions with values of *a*, *b*, and *c* from Table 6.

The data quality is indicated by the smoothness of the curves. The noise indicated by the distance of the particles off the curve is due to methodological error. For comparison, the isokinetic precision tests in Table 2 show CVs ranging from 1.4% to 9.8%, which represents the methodological error.

A few comments are in order regarding the regression fits. First, parameter *a* is estimated for both samplers to be 1.0 with a 95% confidence interval of (1,1), which reflects that this parameter provides the upper bound on the efficiency. Note that the efficiency at an Stk of 0 (i.e., the vertical axis intercept) is given by

$$a*e^{b}.$$

Although many of the sampling efficiencies at low $Stk^{0.5}$ values were quite close to 1, with some exceeding 100%, parameter *a* was constrained to be less than or equal to 1 (and greater than 0) in estimating the curves. The reason is the interplay seen between parameters *a* and *b* in this expression for the intercept at Stk = 0. (See also the description of the further role of *b* below.) Without the restriction of *a* being no greater than 1, the interplay between *a* and *b* led to unreliable estimates of both the parameters themselves and their confidence intervals for both sampler types. The estimates obtained for *a* and *b* yielded intercepts of 95% and 90% for the DryClone™ and PSU samplers, respectively.

Another point worth noting is that residual analyses indicated that some of the original data points were suspiciously aberrant. Upon review, these outlying data points had been collected during the very earliest phase of sampling and were more likely to be subject to error than data collected later after more experience had been gained with the sampling. Because of the aberrant data, the Gompertz curves were estimated after eliminating three data points from the DryClone™ sampler and one data point from the PSU sampler from the analyses. The following data points were deleted:

DryClone™: 20 μm at 2 km/h, replicates 1, 2, and 4

PSU: 5 μm at 8 km/h, replicate 2

The accompanying figures show that the Gompertz curves fit the data reasonably well. The estimated curves reflect the sigmoidal behavior of the data, and the R^2 values were quite high at 90% and 92% for the PSU and DryClone™ samplers, respectively.

The Gompertz curve has an inflection point at

$$\frac{-\ln(-b)}{c}.$$

The interpretation of the coefficients in this case is as follows. The *b* parameter reflects an exponential decay portion of the behavior, and the *c* parameter mitigates the exponential decay at lower Stk. At lower Stk (i.e., to the left of the inflection point) the sampler efficiency might be dominated by properties of the sampler itself, whereas at higher Stk (i.e., to the right of the inflection point) the exponential decay, representing particle impaction, takes over.

4.0
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