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Performance of a Portable Whole-Body Mouse Exposure System

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A mobile whole-body exposure system was developed for exposing mice to concentrated ambient particulate matter smaller than $2.5\ \mu\text{m}$ in mass median aerodynamic diameter (MMAD). Each 20-L exposure cage was designed to hold 9 mice within individual compartments. This allowed for transport and subsequent exposure. Airflow mixing and the potential for stagnant areas within the compartments were modeled using computational fluid dynamic modeling (CFD). CFD analysis showed no stagnant areas and good mixing throughout the exposure cage. The actual performance of the exposure system was determined for 0.5 to $2.0\ \mu\text{m}$ diameter aerosols by measuring (1) uniformity of aerosol distribution and (2) particle deposition in the tracheobronchial and pulmonary regions of mice exposed in the system. A $0.6\text{-}\mu\text{m}$ MMAD (GSD = 2.0) cigarette smoke aerosol was used to experimentally measure the uniformity of aerosol distribution to the nine individual compartments. The average data from three runs showed no statistically significant difference among individual compartments. Particle deposition efficiency in adult male BALB/c mice was measured after exposure (30 min) in the system using monodisperse fluorescent polystyrene latex particles (0.5, 1, and $2\ \mu\text{m}$ aerodynamic diameter). The measured deposition efficiency in this mobile exposure system for the combined tracheobronchial and pulmonary regions of the adult male BALB/c mice was 21% for $0.5\ \mu\text{m}$, 11% for $1.0\ \mu\text{m}$, and 6.5% for $2.0\ \mu\text{m}$ particles. These deposition efficiencies are similar to those reported for mice exposed in a nose-only exposure system, which indicates that particle losses to animal fur and exposure system surfaces were acceptable.

The Southern California Particle Center and Supersite is involved in exposure of mice to concentrated ambient air particulate smaller than $2.5\ \mu\text{m}$ mass median aerodynamic diameter (MMAD) at several sites in the Los Angeles air basin. The sites provide a range of exposure intensities according to the downwind proximity to freeways. In order to conduct these exposures, rapid development of a compact, portable, whole-body exposure system for mice was required. The exposure system had to fit into a modified van, mate to the outlet of an ambient particulate air pollutant concentrator (Kim et al., 2001a, 2001b), and serve as a housing system for up to 8 h. The system developed has modular cages, each divided into individual mouse compartments.

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The compact (20-L) whole-body exposure cages (Figure 1) enable exposure of 9 mice simultaneously in individual compartments. The compartments were numbered as shown in Figure 2. The dimensions of each modular cage in this exposure

The compact (20-L) whole-body exposure cages (Figure 1) enable exposure of 9 mice simultaneously in individual compartments. The compartments were numbered as shown in Figure 2. The dimensions of each modular cage in this exposure

FIG. 1. Drawing of the mobile exposure cage showing the rectangular stainless-steel pan (50 cm length $\times$ 27 cm width $\times$ 15 cm height), perforated stainless-steel floor, partitions, copper aerosol inlet and distribution pipe, and copper aerosol outlet. The aerosol return pipe below the floor is not shown. The aerosol inlet was designed to connect to the outlet of an ambient aerosol concentrator.

system (50 cm length $\times$ 27 cm width $\times$ 15 cm height) allow several cages to be transported to various locations in the Los Angeles basin in a full size passenger van. This exposure cage size was chosen because the volume of the nine mice were less

than 5% of the exposure cage volume (Silver & Aresnal, 1948), each individual compartment complied with the area (77.4 cm²) and height (12.7 cm) recommendations of the National Research Council *Guide for the Care and Use of Laboratory Animals* (1996), and several cages fit into the allotted space in the van. The exposure cages consist of a rectangular stainless-steel pan, 1-cm-thick acrylic plastic top, perforated stainless-steel removable floor, perforated stainless-steel removable compartment partitions, and copper aerosol distribution and return line (Figures 1 and 2). The transparent top permits direct observation of the animals during the exposures and transport. The floor and partitions are manufactured from perforated stainless steel (0.4-cm-diameter round holes in a staggered pattern providing 63% open area). The floor is suspended 2.5 cm from the base of the pan for animal hygiene. The removable partition extends from the cage floor to the Plexiglas top with appropriate holes for the aerosol distribution pipes. The aerosol distribution and return pipes are manufactured from 0.3-cm (ID) copper tubing. The distribution pipe is at the top of the cage and has 0.3-cm-diameter downward facing holes spaced every 2 cm. The two ends of the distribution line were left open, forming the end outlet holes (0.3 cm diameter). The aerosol return pipe is shaped into a 10-cm square at the bottom of the cage (below the perforated floor) with 0.3-cm-diameter inward-facing holes spaced every 2 cm. Copper tubing is used instead of stainless steel because it is easier to work with, which facilitates rapid

FIG. 2. Drawing of mobile exposure cage without the perforated stainless-steel floor, showing the stainless-steel partitions, copper aerosol inlet and distribution pipe, and copper aerosol return pipe and outlet. Exposure compartments are numbered.

refinement of shape and hole spacing. Also, reactive gases such as ozone are effectively removed by the concentrator. If the exposure system was used for reactive atmospheres replacement of the copper with stainless steel would be indicated. During 2 years of use there has been no evidence of corrosion. Closed-cell polyurethane gasket material is used to seal the Plexiglas top to the rectangular pan. During transport and exposures, air-flow through each cage is controlled with calibrated rotometers connected to a vacuum pump and the cages are at a slight negative pressure (<5.0 cm H_2O). The mice received HEPA-filtered air that passed through activated charcoal and Purafil (buffered potassium permanganate impregnated on activated alumina pellets; Purafil, Inc., Norcross, GA) to remove particulate and gaseous contaminants (Mautz & Kleinman, 1997) during transport to and from the exposure sites. Access to food and water was not provided during transportation and exposure. In order to assess the performance of this exposure system, three studies were conducted: (1) computational fluid dynamic modeling (CFD) to predict mixing and airflow patterns; (2) an experimental aerosol distribution study; and (3) an experimental deposition efficiency study in mice.

METHODS

CFD Methods

A numerical simulation was performed for an exposure cage to examine the airflow patterns and perform an initial evaluation of the flow field. Approximations were necessary to keep the total number of mesh elements below 1 million, so that the computational capacity of the computer was not exceeded, while maintaining acceptable element shape and size to minimize numerical errors. The geometry included the rectangular cage with nine compartments separated by perforated walls and the aerosol distribution and return line (Figure 2). The 0.4-cm-diameter round hole staggered pattern was simulated with area-equivalent square holes in straight rows such that total mass flow through each hole was consistent with experimental conditions. The airflow patterns below the perforated floor mesh were not of concern and therefore the subfloor region was excluded from the analysis. The aerosol distribution pipe was simulated by etching out an S-shaped square channel, 0.65 cm wide and deep, with 46 area-equivalent square holes on the channel bottom so that air entered the cage vertically down, and 2 matched-size holes on each end simulating the pipe ends from which air entered the cage horizontally. The aerosol return pipe was simulated with 24 area-equivalent square holes on the bottom surface of the cage. The geometry was meshed with a total of 850,000 tetrahedral elements. The grid was examined for an acceptable amount of equiangle skewness and edge aspect ratio. An average uniform velocity was assumed at the inlet so that the total flow rate into the cage was consistent with the experimental condition of 3 L/min. Outlet boundary conditions were set to atmospheric pressure. The three-

dimensional (3D), nonlinear, coupled Navier–Stokes equations were solved using finite-element methods and the Galerkin form of weighted residuals using commercially available software packages (GAMBIT preprocessor & Fluent CFD codes, all by Fluent, Inc., Lebanon, NH). The presence of mice was not simulated because during exposures the mice are frequently moving about their individual compartments. Attempting to simulate this behavior exceeds the modeling and computational capacity of currently available commercial CFD software. Additionally, because the volume of the 9 mice is less than 5% of the exposure cage volume, it is not anticipated that their presence would significantly affect the computation results (Silver & Arsenal, 1948).

Aerosol Distribution

Sidestream tobacco smoke generated from 1R3 research cigarettes (University of Kentucky) was used to measure the uniformity of aerosol distribution (spatial and temporal; Cheng et al., 1989) within an exposure cage without mice present. Cigarettes were placed in the center of a temperature- and humidity-controlled 4-m³ volume clean room (Mannix et al., 1996), lit, and allowed to smolder to a butt length of 2.3 cm. The resulting sidestream smoke was stirred with a small fan and maintained (target $\pm$ range) at $20 \pm 1^\circ\text{C}$ and $60 \pm 3\%$ relative humidity throughout the $2\frac{1}{2}$ -h experiment. To measure the uniformity of aerosol exposure, open-face filter cassettes (2.5 cm diameter) were placed in each of the nine compartments in the portable exposure cage. Flow rates through each filter cassette were set to approximate the minute ventilation of a BALB/c mouse (25 cm³/min) and matched to within 5%. Total airflow through the exposure cage was matched to animal exposure conditions, 3 L/min. After the exposure, filters were extracted with isopropyl alcohol and the absorbance read at 350 nm (Mannix et al., 1996). Three runs were conducted. At the midpoint of the run an impactor sample (Mercer type cascade RJR impactor: Intox, Inc., Albuquerque, NM) was obtained to determine the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the sidestream tobacco smoke.

Deposition Efficiency in Mice

Eight young adult male BALB/c mice (mean body mass = 24.7 g) were placed in the exposure cage and exposed to nebulized aqueous 0.5-, 1-, and 2- μm -diameter fluorescent polystyrene latex (PSL) particles (Duke Scientific, Palo Alto, CA). Suspensions of the particles were diluted so that no more than 5% of the aerosolized liquid droplets would contain PSL doublets (Raabe, 1968). To estimate the exposure concentration, a 2.5-cm-diameter stainless-steel open-face filter cassette with Nucleopore (Whatman International Ltd., Maidstone, England) filter (0.1 μm pore diameter) sampling at 50 cm³/min was placed in the ninth compartment (Figure 2). The mass median aerodynamic diameters of the PSL particles were calculated using size and density data provided by the supplier; the

geometric size was verified using light microscopy in our laboratory. Aerosols were generated using an Acorn II (Marquest Medical Products, Englewood, CO) compressed air nebulizer operated at $7 \times 10^{-3} \text{ kg m}^{-2}$ (10 PSI) and were diluted (10:1) with filtered dry ($\sim 5\%$ RH) compressed tank air. After 30 min of aerosol exposure the animals were sacrificed, the trachea was tied off to stop continued particle clearance, and the lungs were removed. All eight pairs of lungs were pooled into one sample to improve particle counting statistics. The sample was homogenized in $\sim 8 \text{ M NaOH}$ in order to dissolve the tissue and render the PSL visible in an ultraviolet (UV)-equipped microscope (Fluorphot, Nikon, Japan). To quantitate the number of PSL particles in the lung homogenate and open-face filter sample, a cell-counting hemacytometer (Bhalla, 1997) was used with the fluorescence microscope. The homogenate sample and open-face filter sample were sonicated for 1 h prior to counting the PSL. Complete removal of fluorescent PSL from the filter was verified using fluorescence microscopy. Deposition efficiency was calculated by dividing the average number of particles depositing in a mouse lung by the estimated number of particles inhaled by the mouse, which was estimated using the filter data.

RESULTS

CFD Predictions

The flow field was examined on horizontal and vertical planes at 1-cm intervals. The airflow velocity in the cage ranged from 0.3 to 3 cm/s. Flow path lines after 5 s of airflow are shown in Figure 3. Velocities were maximum in the center compart-

ment, minimum in compartments 3 and 7, and average in the remaining compartments. The circulation patterns indicated that no areas of stagnation were present, and that the chamber design created sufficient mixing for adequate aerosol distribution.

Aerosol Distribution

The mass median aerodynamic diameter of the sidestream tobacco smoke used to measure the aerosol distribution was $0.6 \mu\text{m}$ with a geometric standard deviation of 2.0. The normalized spectrophotometer absorbance readings (converted to percentage of mean for all compartments) are shown for each compartment, for each of three repetitions, in Figure 4.

A two-way analysis of variance (ANOVA) showed that after accounting for the run-to-run differences in smoke concentration ($p = .032$), there was no significant difference between individual compartments ($p = .89$).

Deposition Efficiency in Mice

The deposition efficiency in the combined tracheobronchial and pulmonary regions of the respiratory system of BALB/c mice decreased from 21% to 6.5% as particle diameter increased from 0.5 to $2.0 \mu\text{m}$ (Figure 5).

DISCUSSION AND CONCLUSIONS

Cheng et al. (1989) and O'Shaughnessy et al. (2003) assumed that spatial and temporal aerosol uniformity within their whole-body exposure chambers are independent of each other. Based upon the CFD results and the aerosol uniformity study for

FIG. 3. Flow path lines after 5 s of airflow at 3 L/min. Perforated stainless-steel floor not shown so path lines are visible. Compartments are numbered left to right such that number 1 is in the upper left corner and number 9 is in the lower right corner.

FIG. 4. Results of three runs to compare exposure compartments. Shown is percent of mean value for each compartment in an exposure cage for three test exposures. The test aerosol was sidestream tobacco smoke.

our comparatively small cages, we did not make this assumption. The aerosol uniformity obtained in our study was not as good as that reported for nose-only exposure systems (Yeh et al., 1990; Cheng & Moss, 1995) or some large stationary whole-body exposure chambers (Moss et al., 1982; MacFarland, 1983; Cheng et al., 1989; Yeh et al., 1990); however, it is consistent with the values that have been reported for other large stationary whole-body exposure chambers (Schreck et al., 1981;

MacFarland, 1983; Yeh et al., 1986; Cheng & Moss, 1995; O'Shaughnessy et al., 2003). The aerosol uniformity of the mobile exposure system mean $\pm 15\%$ was judged acceptable since exposures occur 5 days/wk for 2 wk with mice randomly placed in individual compartments. The variations in repeated measurements in compartments 2, 6, 8, and 9 may be caused by variations in filter sampling flow rates, by small inward air leaks, or other unknown factors.

FIG. 5. Comparison of deposition efficiency from current study (■) and the data of Raabe et al. (1988) (●). Error bars for the deposition efficiency of 1.09- and 3.45- μm -diameter particles of Raabe et al. (1988) are too small to be visible, but are 1.1% and 0.7%, respectively, for the trachea, tracheobronchial, and pulmonary regions.

The aerosol deposition efficiencies in BALB/c mice observed in our compact portable whole-body exposure system are similar to deposition efficiencies published by Raabe et al. (1988) for a nose-only exposure system using a different strain of mice (CF1) (Figure 5). A comparison shows that as particle size decreases the differences in deposition efficiency appear to increase. The agreement is remarkable given the significant differences in the two exposure systems and differences in the experimental methods used to measure deposition efficiency. The deposition efficiency measured in our whole-body exposure system takes into account aerosol losses on the interior cage surfaces and on the fur of the mice. In addition, exposure avoidance behavior (mice curling up on the chamber floor) did not significantly prevent exposure. Raabe et al. (1988) accounted for particle clearance by measuring radiolabeled particles in the gastrointestinal tract, while we did not account for particle clearance in our measurement of particle deposition efficiency. One concludes that our whole-body exposure system provides exposures to the respiratory tract that are not significantly different from those in nose-only exposure systems.

The results from both CFD and experimental studies demonstrate the utility of our portable exposure system. The measurement of deposition efficiency over a selected particle size range in BALB/c mice using this exposure system enables investigators to predict doses to the respiratory tract using measured particle sizes during actual exposures. Thus, calculated doses can be correlated with toxicology endpoints, instead of relying on only the exposure concentration and duration.

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