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A SMALL WHOLE-BODY EXPOSURE CHAMBER FOR LABORATORY USE

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With the development of transgenic and specialized mouse strains, there is an increased need for inhalation exposure systems designed for smaller exposure groups. An inhalation exposure chamber, designed specifically for the exposure of up to 40 mice, was characterized. The chamber was fabricated from 0.32-cm-thick ($^1/_8$-in) aluminum sheets with outside dimensions of 61 cm long by 32 cm high by 34 cm deep, resulting in an internal volume of 65 L. Two stainless-steel open-mesh cages, separated by an absorbent barrier, can be stacked within the central portion of the chamber. Access is provided through a gasketed door with a safety-glass face. Tests were performed to determine the chamber leakage rate, degree of mixing, and spatial variation of two aerosols within the chamber. Results indicated that the fractional leakage rate was 0.0003 min^{-1}, well below a reported criterion for an operating chamber. Chamber operation gave similar mixing performance with, or without, use of an interior fan. For aerosols with a mass median aerodynamic diameter (MMAD) of 2.56 μm and 3.14 μm, the spatial variation of particulate matter concentration resulted in coefficients of variation (CVs) of 4.8% and 11.0%, respectively. These CV values are comparable to those obtained from similar studies involving other inhalation exposure chambers.

In recent decades, the number of animals required to perform a statistically relevant animal exposure has decreased because of the increase in the sensitivity of assays used to determine a biological response, and the use of specialized strains (Thorne, 2000). Animals used for inhalation exposures are also becoming increasingly more expensive. Therefore, the large, multilevel, whole-body inhalation chambers developed in the past are often inappropriate for current experimental designs.

The nose-only chamber is a useful alternative to the large, whole-body chamber (Cannon et al., 1983; Pauluhn, 1994). These units offer the ability to place relatively small numbers of animals in a restraining device so that an aerosol or gas will be delivered directly to the nose without the possibility of interfering obstructions. For instance, when using a whole-body chamber, these obstructions are generally caused by the animal burrowing its nose within its own fur or the fur of other nearby animals. Use of a nose-

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only chamber also precludes the possibility of secondary exposure to the animal via ingestion while preening (Stephenson et al., 1988). However, use of these devices requires an animal conditioning period prior to an exposure (Jakab & Hemenway, 1989) and are relatively labor-intensive (Stephenson et al., 1988). Other issues concerning their use involve the possibility of overheating and an elevated stress level (King-Herbert et al., 1997).

Although there are many inhalation exposure studies for which the nose-only system would be the preferred chamber for exposure, a whole-body chamber system offers a more convenient alternative. We designed, built, and tested a small whole-body exposure chamber for use with relatively low-toxicity aerosol exposures lasting 8 h or less. The chamber was specifically designed to be operated within a laboratory fume hood and be easily washed and maintained. Performance criteria included uniform mixing of an aerosol or gas throughout the chamber as well as adequate animal spacing and ventilation. The purpose of this article is to describe the physical and operational characteristics of the chamber.

CHAMBER DESIGN

The chamber was fabricated from 0.32 cm ($^1/_8$-in) aluminum sheets with outside dimensions of 61 cm long by 32 cm high by 34 cm deep, resulting in an internal volume of 65 L (Figure 1). The chamber weighs 9.3 kg and is fitted with a removable door weighing 2.5 kg. Aluminum construction was chosen to make the chamber light enough to be easily cleaned in a large laboratory sink. An aluminum chamber is also conductive and therefore prevents the buildup of a static charge leading to aerosol losses as seen with polycarbonate and glass chambers. Having the door detachable, rather than hinged, facilitated cleaning the inside of the chamber. The 40.0 cm by 24.8 cm front access area allows for the insertion of 2 complete cage assemblies, one on top of the other. A frame door fitted with a pane of 0.32-cm-thick ($^1/_8$-in) safety glass is held in place with 4 turnscrews against gasket material lining the door opening. Legs were not permanently attached to the chamber, but supports were later added to place the bottom of the chamber 13 cm from the floor of the hood (Figure 2).

Two holes were drilled and tapped into the front face of the chamber on each side of the door. On the left side, the holes were tapped to receive a $^1/_4$-in and $^3/_8$-in standard nominal pipe thread (NPT) fitting. The larger hole was placed above the smaller one for use as an inlet for gases or aerosols. A tube with a static discharging element can be attached to this port. The smaller hole is used for introducing filtered dilution air through the chamber. On the right side are two holes tapped to receive $^1/_4$-in NPT fittings. These holes are used for sampling and chamber exhaust. In addition, two $^1/_4$-in NPT tapped holes were located at the top of the chamber for the attachment of a static pressure gauge and another sampling device as needed.

Insert cages were special-ordered from Laboratory Products, Inc. (Seaford, DE). These stainless-steel, open-wire mesh cages were modifications

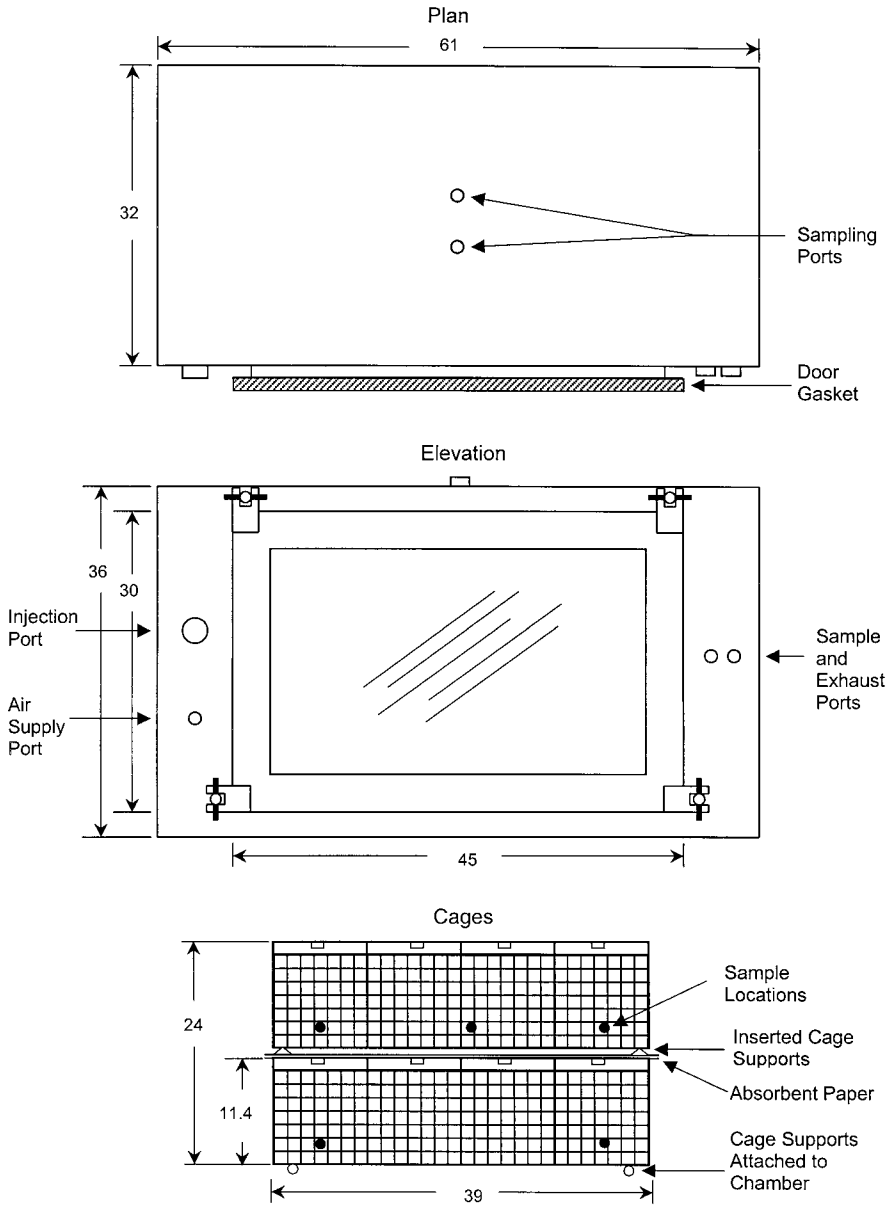

FIGURE 1. Cutaway plan view and exterior elevation view of the chamber and mouse cages with sampling locations for spatial analysis (units in cm).

of standard cages (model H-1162) sold by the company and measure 38.7 cm long by 11.4 cm high by 24.1 cm deep. Each cage system has four compartments and a removable lid unit with four individual hinged lids. The spring-loaded lid catches were bent flat so that two cages could be stacked within the chamber. We limit the number of animals to 5 per area

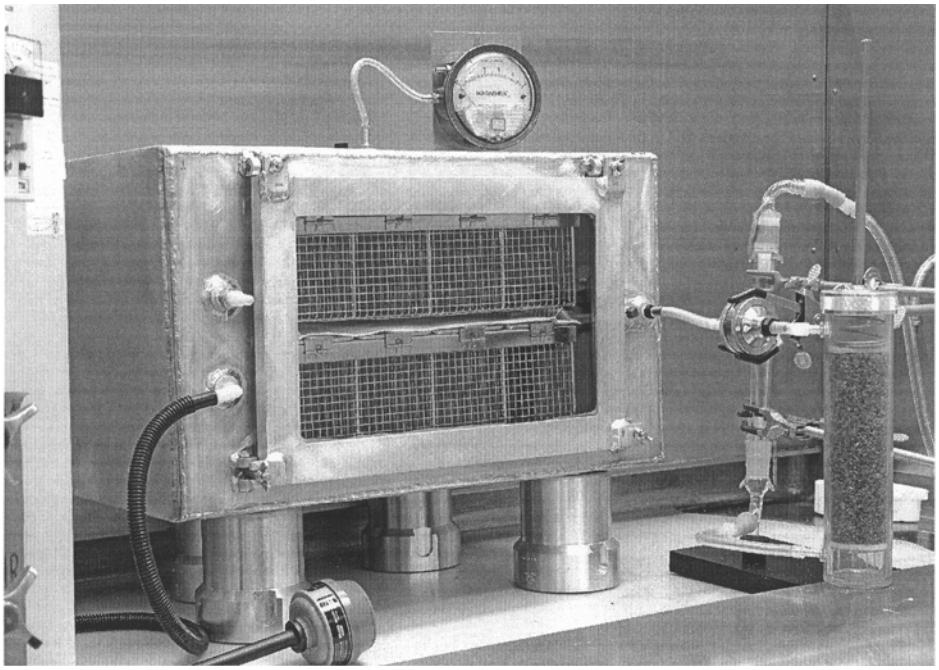

FIGURE 2. Photograph of chamber supported on aluminum cylinders with cages, inlet filter, pressure gauge, and exhaust flow apparatus.

for a maximum of 40 animals per exposure trial. The lower cage is suspended above the bottom of the chamber by resting on two rods in line with the bottom of the front opening and 5 cm from the chamber floor. The floor area is covered with absorbent paper to catch excreta during an exposure. Likewise, absorbent paper with impermeable polyethylene backing (e.g., Versi-Dry Surface Protectors, Fisher Scientific, Pittsburgh, PA) is cut to fit the top of the lower cage, and aluminum angle iron pieces are placed on either side to support, and allow an air space below, the upper cage. Simply applying an absorbent barrier between the cages avoided the unnecessary cost of custom fabricating a pan to hold the paper, although such a pan could be applied for this purpose.

Airflow through the chamber is controlled by adjusting the total exhaust flow rate drawn through the chamber with the use of a $^1/_3$ HP, rotary vane, vacuum pump. This pump is capable of pulling up to 33 L/min of air through the chamber, equivalent to an air exchange every 2 min, or 0.5 air exchanges per minute (ACM). This is comparable to the volume-to-flow ratio typically employed when using commercially available chambers (Cheng & Moss, 1995). The pump was placed in the cabinet space below the exhaust hood to reduce noise, and pump exhaust was directed back into the hood with polyvinyl chloride tubing. Air exhausted from the chamber typically goes through a particulate filter, a column containing desiccant, a

calibrated rotameter, and a valve before exiting through the vacuum pump (Figure 3).

Inlet airflow, above that supplied by the aerosol generator, was initially supplied via a 0.45-μm "mini-capsule" filter (Gelman Sciences, Inc., Ann Arbor, MI) attached to the inlet port. However, this filter produced a highly negative static pressure within the chamber, especially at high flow rates, because of the substantial pressure drop across this filter (–7.25 inches water gauge pressure (in w.g. at 32.5 L/min). An alternative was found that offered a much lower resistance to flow. This apparatus consisted of a sampling wand for an infrared analyzer (Thermo Environmental, Inc., Franklin, MA) that contained an in-line holder for a respirator filter. One end of this unit has threads to attach a respirator filter and the other end affixes to the chamber (Figure 2). As they are designed for human breathing, the pressure drop across these filters is minimal while still maintaining a high removal efficiency (–0.5 in w.g. at 32.5 L/min). A combination chemical/particulate cartridge can also be used to remove volatile organic compounds as well as particulates if necessary. Similarly, the entire sampling wand can be used on the exhaust side to place a particulate filter in-line between the chamber exhaust port and the vacuum pump (Figure 2 shows an in-line 47-mm filter holder instead).

CHAMBER VALIDATION

A series of trials were performed to validate chamber performance under various flow conditions. These trials included a chamber leakage test, chamber mixing tests, and aerosol spatial variation tests.

Chamber Leakage Test

A chamber leakage test was conducted by measuring the change in chamber pressure relative to atmospheric pressure as described by Mokler and White (1983). After applying a negative static pressure to the chamber, shut-off valves were used to close the supply and exhaust ports to seal the chamber. A pressure transducer, connected to a port in the top of the cham-

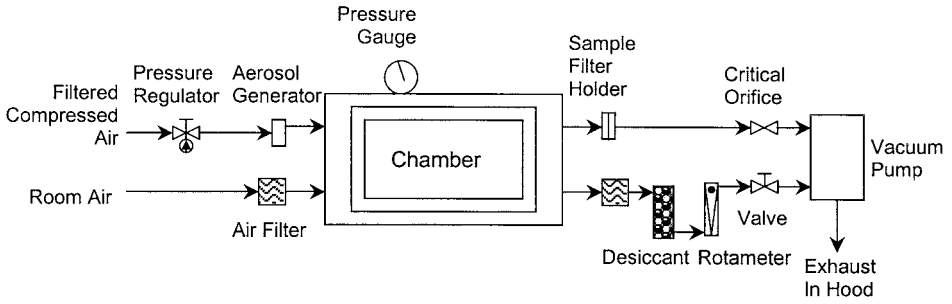

FIGURE 3. Typical chamber operating schematic when exposing mice to a dry aerosol (static discharging device not shown).

ber, was used to record static pressure every 0.2 min. Although the chamber static pressure is negative relative to atmospheric pressure, determining a pressure differential, $\Delta_P = P_{chamber} - P_{atmos.}$, results in a positive value that will decay exponentially after the chamber is sealed. An indication of the leakage rate of the chamber can therefore be determined by calculating the first-order rate constant, k, associated with the exponential decay in Δ_P between a starting pressure differential, $\Delta_P(0)$, and a subsequent pressure differential, $\Delta_P(t)$, as given in Eq. (1).

$$\Delta_P(t) = \Delta_P(0)e^{-kt} \tag{1}$$

Furthermore, Eq. (1) can be linearized by taking the natural logarithm of $\Delta_P(t)$:

$$\ln \Delta_P(t) = \ln \Delta_P(0) - kt \tag{2}$$

Therefore, k can be computed from the negative of the slope resulting from a linear regression of the log-transformed pressure differential and time.

Mokler and White (1983) suggest a leakage criterion based on determining a fractional leakage rate, Q_L/V, which is related to the chamber volume, V, and the flow rate of air through all leaks, Q_L. Because this leakage rate will necessarily diminish as Δ_P decreases, Mokler and White recommend computing Q_L/V when the chamber static pressure has increased to –1 in w.g. from the lower pressure obtained at the start of the test, $[Q_L/V]_{1\ in}$. Because Q_L cannot be measured directly, the leakage rate can be determined after computing k with the use of Eq. (3):

$$\frac{Q_L}{V} = \frac{k}{P_a}\Delta_P e^{-kt} \tag{3}$$

where P_a is the atmospheric pressure at the time of the test in the same units as Δ_P (for example: P_a is near 407 in w.g. for Δ in inches of water). Based on their personal observations, Mokler and White suggest that $[Q_L/V]_{1\ in}$ should be less than 0.001 min^{-1} as a performance criterion for new or refurbished chambers.

Chamber Mixing Test

A well-mixed chamber is desired as this condition will ensure a uniform distribution of gas or aerosol throughout the chamber, and therefore the concentration breathed by test animals will be uniform regardless of the position of the animal within the chamber. Typically, chambers have been designed to provide a high degree of mixing by producing turbulent airflow through the chamber rather than with the addition of fans or other mechanical agitators. Because higher air flows result in more turbulent conditions, it is generally assumed that greater mixing will occur at the higher flows.

A test to determine the mixing characteristics of a chamber relies on a determination of the average residence time, τ, of a tracer gas in the chamber. If the chamber is perfectly mixed, the measured residence time, τ_m, of the gas will be equivalent to the theoretical residence time, τ_t, computed as chamber volume (V) divided by the flow rate through the chamber (Q).

There are two simple methods for determining τ_m. The first method involves injecting a bolus of gas into the chamber and measuring the sudden rise and fall of concentration at both the inlet and outlet of the chamber on a real-time basis (Hemenway et al., 1982). The resulting response curves can be analyzed to determine the mean times of passage (Levenspiel, 1972), and these means subtracted to determine a chamber residence time. This method is especially appropriate when attempting to determine the residence time of long, narrow volumes such as the horizontal chamber shelves described by Hemenway et al. (1982) where the contaminant is expected to diffuse longitudinally but not mix well laterally.

When the chamber volume is presumed to be "well mixed," a second method for determining τ_m involves injecting a gas to obtain a steady level of the gas concentration, C_O, and then abruptly switching off the gas source. The resulting gas concentrations, C_t, will decay exponentially and the residence time can be calculated from the rate constant, K, associated with the equation:

$$C_t = C_O e^{-Kt} \tag{4}$$

where $K = 1/\tau_m$. As described earlier, K, and hence τ_m, can be calculated by determining the slope of the natural logarithm of concentration regressed on time.

The second method just described was utilized for this study. However, when implementing this method, the computation of τ_t relies on an exact knowledge of the chamber volume, which may include the additional volume of inlet and outlet tubing as well as the reduction in volume when cages are inserted, and an exact knowledge of flow rate, which may be difficult to precisely determine. Furthermore, the averaging time of the real-time instrument used to measure the gas concentration can influence the resulting value of τ_m. Therefore, the τ_m values were compared to residence times computed after performing an identical test with the addition of a 10-cm box fan in the chamber to ensure complete mixing by the mechanical action of the fan. Comparisons were then made between the chamber residence time determined by regression analysis with the fan turned on relative to the residence time determined when the fan was absent and cages were either present or absent. Since this comparison did not involve an assessment of τ_t, an accurate knowledge of the chamber flow rate and volume was not required.

Three sets of trials were performed that included various combinations of placing a fan or cages in the chamber: (1) fan on, cages absent; (2) fan

absent, cages absent; and (3) fan absent, cages present. When performing each mixing trial, an infrared analyzer (MIRAN, Thermo Environmental, Inc., Franklin, MA) was used to read the concentrations of sulfur hexafluoride (SF_6) injected into the chamber. The sampling wand of this instrument was connected to the sampling port on the right side of the chamber. Likewise, during each trial set, the exhaust valve was set to create total chamber flow rates (instrument plus vacuum pump) of 8.0, 16.5, and 32.5 L/min, approximately equivalent to air exchange rates of 0.125, 0.25, and 0.5 ACM, respectively. Because it was found that the sampling rate of the instrument was highly dependent on the static pressure in the chamber (unable to pull effectively against a negative chamber pressure), the inlet filter was removed to maintain nearly atmospheric pressure within the chamber regardless of flow rate.

Spatial Variation Study

The study described earlier utilized a gas to indicate the overall degree of mixing within the chamber. A separate study was also performed to measure the spatial variability of an aerosol within the chamber under different flow conditions. As explained by Cheng et al. (1989), the spatial variability of an aerosol within an exposure chamber can be measured by simultaneously sampling in various locations within the chamber. Spatial variation can then be defined as the coefficient of variation (CV = std. dev./mean) among all samples taken after correcting for the temporal variation in concentration measured at a single sampling point. Such an analysis is performed under the assumption that the spatial and temporal variations are independent of each other.

To conduct this study, an aerosol consisting of ISO fine test dust (Powder Technology, Inc., Burnsville, MN) was generated with the use of a Wright dust feed (BGI, Inc., Waltham, MA). A second, somewhat finer, aerosol was also generated from TiO_2 powder. In both cases, trials were performed with total flow rates to achieve 0.125, 0.25, and 0.5 ACM. These flow rates resulted from a combination of air pulled by the vacuum pump and the addition of a cascade impactor (InTox Products, Albuquerque, NM) to the right sampling port operating at 2 L/min. The impactor allowed for determination of the aerosol mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD).

All trials were conducted without a fan in the chamber and with both chambers separated by absorbent barrier in place. To measure the aerosol concentration at various locations within the chamber, four 4-mm polyvinyl chloride tubes of equal length were placed within the cages so that the ends were located in the bottom center of the first and fourth cage partition of both the bottom and top cage (Figure 1). The end of a fifth tube was placed beside the center partition of the top cage. These locations were only 28 cm apart horizontally and 11 cm vertically but represent the extent of variation that may be encountered by animals within the 2 cages.

To determine spatial variation, an optical particle counter (1.100 Series, GRIMM Technologies, Inc., Atlanta, GA) was used to measure the counts at each of the four sampling positions for 5 min followed by a switch to the next location in a random order. To indicate the temporal variation of the aerosol concentration, a second particle counter continuously sampled at the fifth central position throughout the sample period. Counts read at the other four locations were then normalized relative to the changes in concentration indicated at the center position. An average count concentration for all particles greater than 1.0 μm was determined for the 5-min sample period at each location and a spatial CV calculated from the resulting averages.

RESULTS

Leakage Test

A static pressure of –3 in w.g. was applied to the chamber and the resulting change in static pressure recorded for 8 min. A leakage rate constant, k, of 0.30 min^{-1} was obtained from a linear regression of the resulting decay in Δ_P over time (Figure 4, inset). The fractional leakage rate at 1 in water gauge, $[Q_L/V]_{1\ in}$, was determined with the use of Eq. (3) supplied with the time when the pressure sensor recorded a static pressure nearest to –1 in w.g. (Figure 4). The resulting value of 0.0003 min^{-1} was less than the value of 0.001 suggested by Mokler and White (1983) as indicating a chamber in need of repairs to minimize leaks.

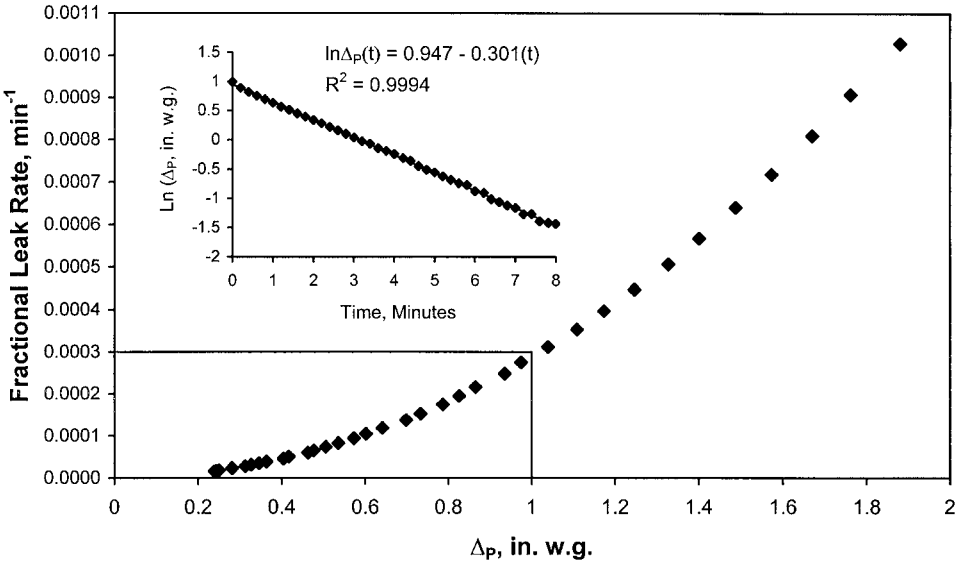

FIGURE 4. Chamber fractional leakage rate relative to Δ_P and (insert) linear regression determination of the rate constant, k.

Mixing Test

A typical gas-concentration decay curve is given in Figure 5. This curve was generated when operating the chamber at 0.5 ACM with no cages and the fan off. Curves were likewise obtained after recording the gas concentration with different chamber flow rates; with the fan on or off; and with the cages present or not. A comparison of the measured residence time, τ_m, when cages were absent or present relative to that obtained when only the fan was present and on (and complete mixing is assumed) is given in Figure 6. Without cages present, the small volume of the chamber was well mixed regardless of the flow rate, as the τ_m values obtained with both cages and fan absent were coincidental with those obtained when the fan was on. However, the two cages, and the associated absorbent barrier between them, developed τ_m values somewhat lower than obtained when the fan was on, especially at the higher flow rates analyzed.

Spatial Analysis

When aerosolizing the fine test dust, an analysis of information collected with the cascade impactor resulted in the determination of a mass median aerodynamic diameter (MMAD) of 3.14 μm and geometric standard deviation (GSD) of 2.10. The CV values determined when using this dust for each flow rate, and a pooled value for all flow rates, are given in Table 1. There was a significant difference at the 95% confidence level (p = .037)

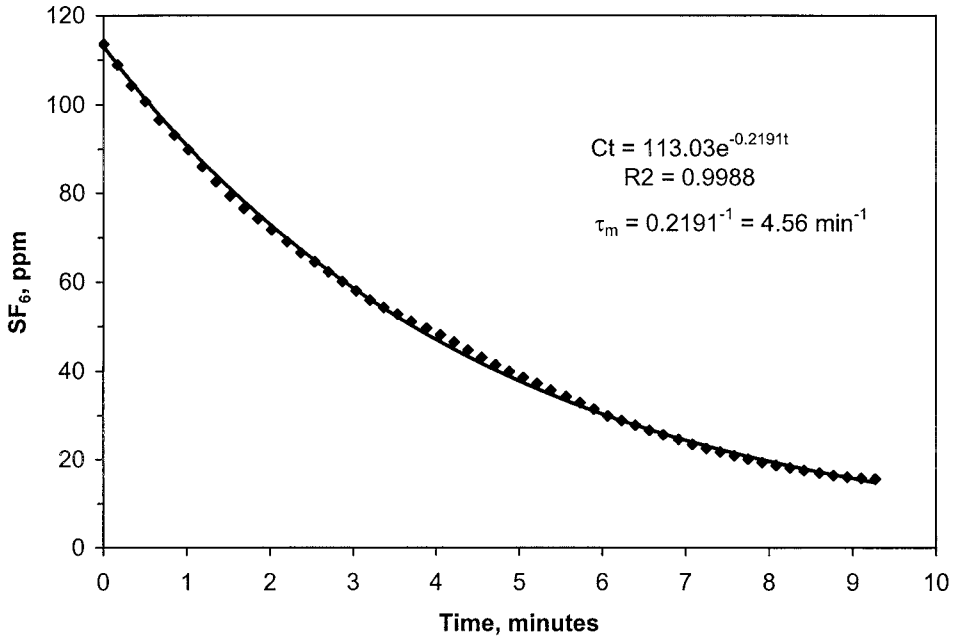

FIGURE 5. Decay in SF_6 concentrations for a flow rate of 16.5 L/min.

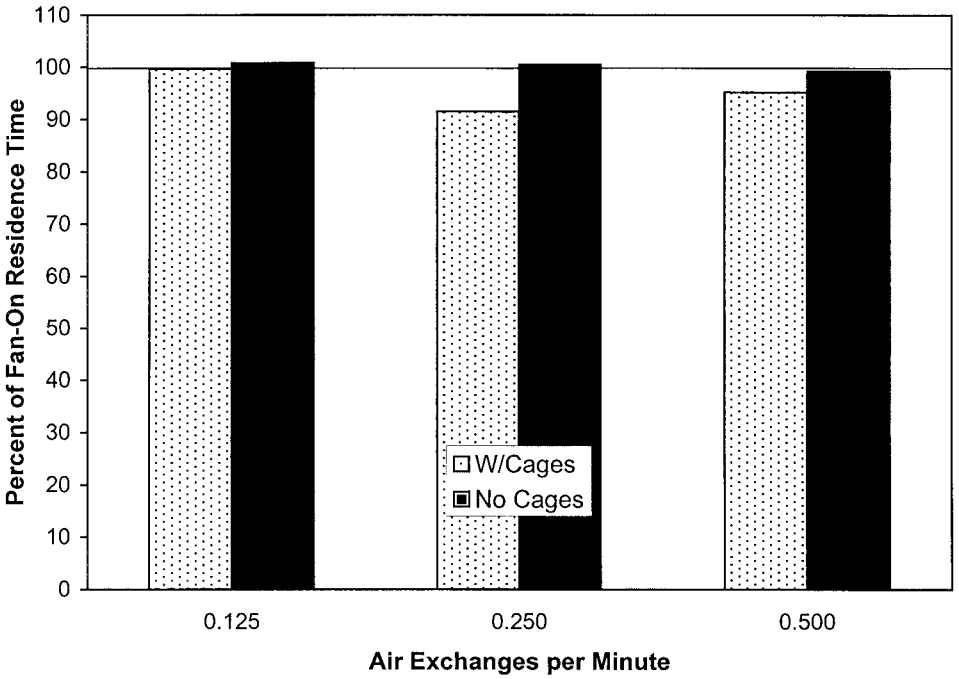

FIGURE 6. Percent change in measured residence time relative to that obtained when the fan was on.

across the time-normalized counts obtained at the various locations and there was a significant difference between counts taken in the lower left and upper right positions (p = .039). Averaged across all flow rates, the counts taken at the lower left sampling point were highest followed by those taken in the lower right, upper left, and upper right.

An MMAD of 2.56 μm and GSD of 2.76 were determined for the TiO_2 powder. As shown in Table 1, the CV values obtained for each flow rate were approximately half those obtained for the fine test dust. When aerosolizing this dust, normalized counts in each location were not significantly different (p = .892). However, the average of counts taken in the lower sampling locations were higher than those taken in the upper locations.

TABLE 1. Coefficient of variation (CV, %) values obtained when comparing the time-normalized counts taken at four locations within the upper and lower cages for the two dusts analyzed

	Chamber flow rates			
	0.125 ACM 8.0 L/min	0.25 ACM 16.5 L/min	0.5 ACM 32.5 L/min	Pooled CV
Fine test dust, 3.14 μm (2.10)	8.3	14.9	9.8	11.0
TiO_2, 2.56 μm (2.76)	5.8	2.8	5.8	4.8

DISCUSSION

The chamber leakage test indicated good performance by the criteria established by Mokler and White (1983). The soldered construction of the chamber prevents any leakage between the aluminum chamber panels; therefore, the primary opportunity for leaks exists between the door and gasket material around the opening on the face of the chamber. A pressure gauge (see Figure 2), sensitive to chamber static pressure in inches of water pressure, can be used to visually indicate that a good seal has been formed between the face and the door by showing a negative static pressure typical for the flow rate established (e.g., –0.5 in w.g. at 32.5 L/min).

Results from the chamber mixing test demonstrated a reduction in the measured residence time, τ_m, when cages were present relative to when the fan was on. This result indicates that the cages may have developed small pockets of "dead space" within the chamber as the air flowed around them. These pockets of still air effectively caused a short-circuiting of the air through the chamber, which, in essence, reduced the chamber volume occupied by the flowing air, V_{eff}, and resulted in a decrease in $\tau_m = V_{eff}/Q$. Interestingly, a larger reduction in residence time occurred for the higher flow rates, where it would be expected that mixing would be enhanced due to the turbulence created at these higher flows.

The CVs measured during the spatial-variability test are relatively low for an inhalation chamber (Cheng & Moss, 1995) and therefore indicates little difference in aerosol concentration between the cage areas. As expected, an aerosol with a larger median diameter resulted in higher CVs as has often been reported (Cheng & Moss, 1995; Cheng et al., 1989). The low CVs in general are a consequence of good mixing and the small chamber volume. The small volume also necessarily keeps animals in a localized area where the concentration is relatively uniform. The results from this test did, however, indicate that a significant difference in concentration can be obtained between cage locations when aerosolizing larger particles. This difference was most noticeable between the lower left cage area and the upper right area. Since the aerosol is injected directly into the left portion of the chamber, some may settle prior to reaching the upper right area. Rotation of animals within the various cage areas during a subchronic or chronic exposure will minimize the effect of these spatial differences on the overall dose received by each animal tested (Hemenway et al., 1983).

CONCLUSION

An inhalation exposure chamber, designed for mouse exposures and small enough to fit within a laboratory fume hood, was characterized with a variety of tests. These tests demonstrated that the chamber has an acceptable leakage rate and provides good mixing at the flow rates tested. Two of these chambers have been successfully used for over 2 yr in our laboratory. The greatest cost associated with the chamber was the purchase of the cages, fol-

lowed by the chamber itself. The chamber, with cages, pump, and filter attachments, cost approximately $4000. Large savings are achieved, however, because there is no need to have a dedicated ventilation system for the chambers.

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