2 Nose-Only Aerosol Exposure Systems

Design, Operation, and Performance

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CONTENTS

2.1 INTRODUCTION

Several methods are available for exposing laboratory animal models to aerosols, each having its advantages and disadvantages (Table 2.1). Whole-body exposure systems (chambers) have a long history in inhalation toxicology, mainly due to their animal loading capacity and adaptability to several species. Chambers are often unsuitable for use with radioactive, infectious, and other potentially hazardous exposure materials, due to the need for large amounts of exposure material, inefficient delivery of aerosols to animals, multiple routes of exposure (e.g., oral, skin, and eyes), and the external contamination of exposed subjects. Also, chambers are costly, require considerable laboratory space, require expensive air supplies, and are difficult to decontaminate.

Nose-only systems have emerged as particularly useful for exposing laboratory animals to hazardous airborne materials for several reasons including the following: (1) they can be made compact enough to fit into secondary enclosures; (2) they are well tolerated by animal subjects when designed with animal comfort in mind (and the animals undergo preliminary training sessions); (3) respiratory rates, volumes, flows, and other physiological parameters can be acquired during exposures; (4) they provide reliable, uniform, and efficient aerosol delivery; (5) in most circumstances, they are acceptable simulations of natural aerosol exposures; (6) they are relatively easy to decontaminate; and (7) several functional designs are well documented in the literature (e.g., Cheng and Moss, 1995; Phalen, 1997; Pauluhn, 2003; Jaeger et al., 2005; Wong, 2007; Stone et al., 2012).

TABLE 2.1
Comparison of Inhalation Exposure Methods

Method	Advantages	Disadvantages	Issues to Consider
Whole-body (chambers)	Accommodate a large number of subjects	Multiple routes of exposure: skin, eyes, oral	Cleaning throughput air
	Efficient for chronic studies	Variability of doses	Construction materials
	Do not require restraint	Cannot pulse exposure	Losses of study material
	Have good environmental control	Poor investigator contact with subjects	Noise, vibration, air temperature, RH[a]
		Expensive investment	Cleaning exhausted air volume
		Animal by-product air contaminants (dander, ammonia, etc.)	
Head-only	Good for repeated exposures	Stress to subjects	Pressure fluctuations
	Limits routes of exposure	Seal around neck	Losses of study material
	More efficient use of study material than chambers	Labor in handling subjects	Air temperature, RH
		Rebreathing of exhaled air	Subject comfort
			Subject restraint
Nose-/mouth-only (including masks)	Exposure mostly to respiratory tract	Stress to some species	Exposure tube design
	Uses less exposure material	Seal about face/nose	Body temperature
	Has good containment	Labor in handling subjects	Subject comfort
		Exposure times limited to hours	Losses in system plumbing
Lung-only or partial lung-only	Precision of dose	Technically difficult	Air temperature and RH
	Uses less exposure material	Invasive	Stress to subjects
	Can use unexposed control tissue from the same animal	Artifacts in dose and response	Physiological support may be required
	Bypasses the nose	Bypasses the nose	Surgery or intubation is required

[a] RH = Relative humidity.

2.2 NOSE-ONLY EXPOSURES

Typical nose-only exposure systems, including secondary containment and an aerosol generation, are depicted in Figures 2.1 and 2.2. The exposure portion has a central plenum (or exposure manifold) into which an aerosol enters after generation and conditioning (e.g., by dilution, drying, and electrical discharging). Confinement (exposure) tubes with one end protruding into the exposure aerosol can be arranged about the plenum in various ways to permit simultaneous exposure of several animals. If the exposure tubes are well designed, animals will enter, move forward, and stick their noses out through a hole at the front of the tube. Animals may breathe from a static (i.e., no airflow) plenum or have flow past, or toward (i.e., directed flow), their noses. Mixed exhaled and throughput air is exhausted from the system, where it may be cleaned free of aerosols and exposure gases prior to discharge into the environment. For nose-only systems inside a secondary containment (e.g., a chamber, biosafety hood, or a glove box), it may be necessary to use an insulated enclosure for the aerosol-generating equipment to isolate exposed animals from heat, noise, and vibration. Such a system using several glove boxes for exposing rodents is described by Hoskins et al. (1997).

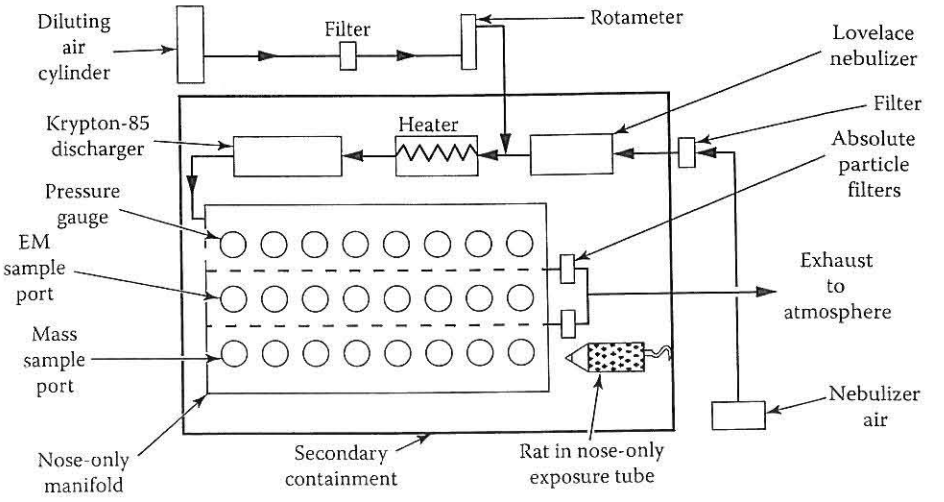

FIGURE 2.1 A nose-only exposure system with secondary containment (stainless steel chamber) suitable for radioactive aerosol exposures. (Courtesy of the Air Pollution Health Effects Laboratory, University of California, Irvine, CA.)

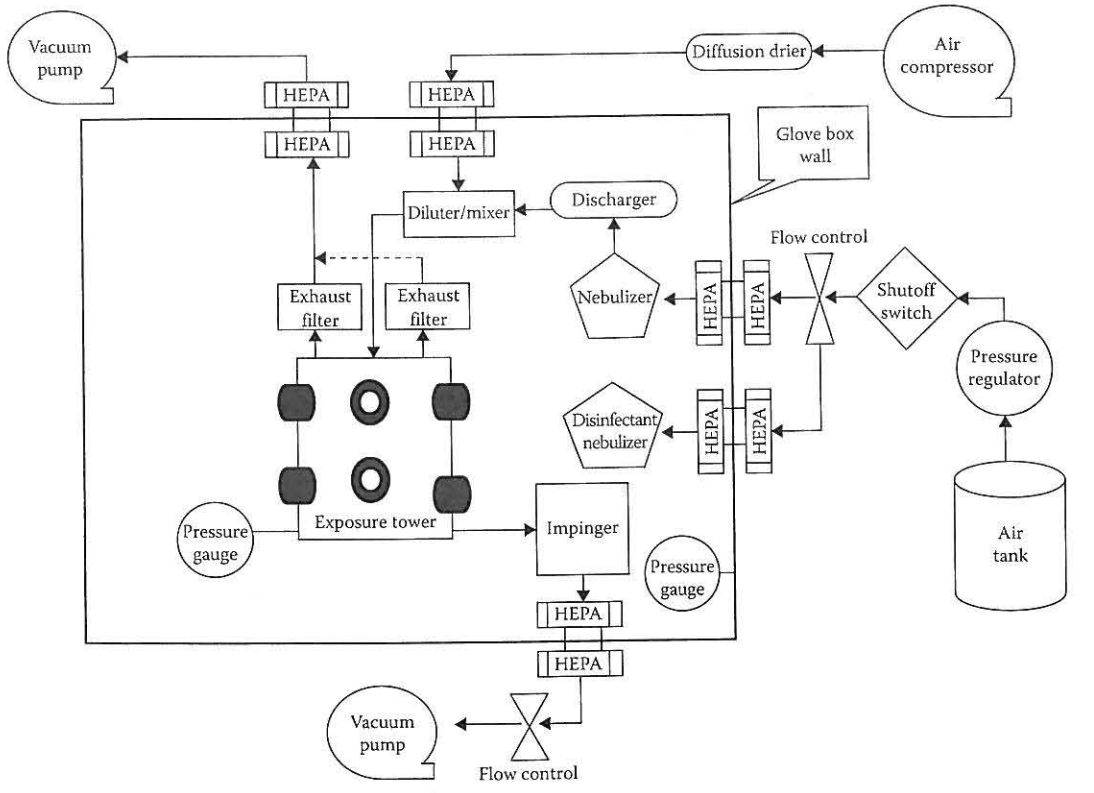

FIGURE 2.2 A high-containment nose-only exposure system with internal disinfectant capability suitable for infectious aerosol exposures. Double HEPA filters prevent contamination of the laboratory. (Courtesy of the Air Pollution Health Effects Laboratory, University of California, Irvine, CA.)

In order to prevent contamination of the laboratory during exposure, the nose-only system can be maintained at a pressure negative with respect to the secondary containment, and the secondary containment held at a pressure negative with respect to the exposure room. If environmental contamination is a concern, the entire laboratory building can have a negative pressure relative to the outside environment.

Nose-only exposures can be labor intensive and stressful to the animal subjects if the system is not well designed. Training animals to accept restraint in nose-only tubes is recommended to reduce stress and produce normal physiological states. Also, thermoregulation of the subjects must be considered both in tube design and by cooling and/or isolation from heat sources (e.g., pumps and heaters used for drying aerosols).

Rodents are particularly adaptable to close confinement in nose-only exposure tubes. Rabbits, ferrets, and most other small species will also accept such exposures, but they may require additional training. Some species, for example, sheep, pigs, rabbits, dogs, and equines, can be trained to accept nose-only exposure muzzle masks, but they may require sedation prior to attaching them to exposure systems and may require restraint during exposures. Nonhuman primates are usually exposed in head-only systems (using a full helmet-like enclosure of the head), but sedation may be required (Dabisch et al., 2010).

2.3 HISTORY

Nose-only exposure systems have a long history of use. Early exposure studies and systems include those reported by Barnes (1947), Henderson (1952), Bair et al. (1961), Casarett (1964), Thomas (1969), Johnson and Ziemer (1971), and Raabe et al. (1973), to name a few. When the first nose-only exposure was used in an inhalation study is unknown, and perhaps unknowable, as it seems reasonable that the method would be used by any investigator who wanted to minimize the amount of exposure material required and/or to avoid contamination of the fur (as by infectious, toxic, or radioactive materials) during an inhalation study. MacFarland (1983), Cheng and Moss (1995), Phalen (1997), Pauluhn (2003), Jaeger et al. (2005), and Wong (2007) have reviewed nose-only exposure systems and included some historical information. Several systems are in current use based on designs by Raabe et al. (1973) and Cannon et al. (1983). New designs have emerged, often for specific applications (e.g., Yeh et al., 1987; Pauluhn, 1994; Jaeger et al., 2005; Oldham et al., 2009; Werley et al., 2009; Stone et al., 2012). Several of these systems are commercially available.

2.4 MINIMIZING ANIMAL STRESS

Unless careful attention is paid to the entire nose-only system design, animals may experience stressful conditions during aerosol exposures. Foremost is the requirement for close fitting yet comfortable exposure tubes that invite the animals to enter and move forward where the nose enters the exposure atmosphere. The body enclosure may be snug, but it must be comfortable, especially for nonsedated animals. The portion of the tube enclosing the head should be designed to painlessly fit the head of the species used. If the tubes are poorly designed, awake animals may not enter freely, may struggle or turn around, and even suffocate in their attempts to escape. Poorly designed exposure tubes can produce abnormal physiological states and thereby distort exposure-related data.

Maintaining physiologically tolerable environmental conditions during nose-only exposures is another essential design requirement. Thermal stress in exposure tubes can lead to animal fatalities, so thermally conductive or vented construction materials are sometimes used. Heat sources, such as pumps and heated aerosol drying devices, should be isolated from the animals, and environmental temperatures monitored and controlled within tolerable limits. Rats in particular regulate body temperatures by adjusting blood flow through their hairless tails, so close tail confinement without provision for cooling should be avoided if possible, unless exposure durations are short. Provision of

cool air flowing over the exposure tubes may be required for any species. Rodents require frequent access to drinking water, which limits exposure durations to a few (e.g., up to 6) hours. Inhumane exposure durations are species dependent and must be avoided.

Stress may also be reduced by progressive subject training that involves an initial brief sham exposure followed by confinement periods that will actually be used in the study. Such training for rats and mice may require up to 14 days for respiratory and cardiac functions to completely normalize (Narciso et al., 2003). Properly trained animals will usually enter the tubes freely and relax during exposures without attempting to escape. When animals struggle during confinement, the reason should be determined and the problem remedied. Sedation is seldom required to obtain low-stress exposures, but it can be used if necessary. Exposures that produce significant acute respiratory tract irritation should be avoided unless sedation or pain relief is provided.

Vibration, noise, and unfamiliar odors also stress rodents and other species. As many rodents are active at night, daytime exposures may be preferable. If elevated respiratory rates are required, CO_2 can be added to the exposure atmosphere. In any case, it is wise to measure the physiological status of the animals under exposure conditions. Control groups simultaneously exposed to a sham atmosphere are strongly recommended, as cage controls will not experience the handling, confinement, and environmental conditions associated with the exposure.

2.5 ACQUIRING BREATHING DATA

Breathing data, especially frequencies and tidal volumes, are needed to estimate inhalation doses, and they are also useful measures of the effects of exposures. Mauderly (1990) reviewed several methods for acquiring such data for laboratory animals during inhalation exposures. One method, plethysmography, is readily adaptable to nose-only exposures. Plethysmography is any of several techniques for measuring and recording volume changes in a subject or a portion of the subject. Whole-body, head-out, and limb- or digit-only are descriptions of plethysmographic techniques. When the enclosed portion of the subject changes size (e.g., due to respiration or changes in blood flow), the pressure in the plethysmograph enclosure varies. Measurement of the pressure change or airflow in and out of the enclosure is recorded. If the device is calibrated (e.g., using a syringe or ventilator pump), the recording can be converted to provide accurate volume changes. Such a technique has been described for unsedated guinea pigs by Murphy and Ulrich (1964) and for unsedated rats by Mautz and Buffalino (1989). The apparatus used by Mautz (1997) is shown in Figure 2.3. Similar methods have been described by Mauderly (1986, 1990). For masked animals, flow meters or spirometers (in series or parallel with the animal) can be used to acquire breathing data. Boecker et al. (1964) described such a system for dogs, which could be adapted to other species.

2.6 INHALABILITY

Inhalability has had several definitions in inhalation toxicology, including exposure efficiency of the deep lung and that portion of the exposure material that enters the trachea. The modern concept of *aerosol particle inhalability* evolved from sampler efficiency studies (e.g., Ogden and Birkett, 1978). Standards for inhalable particle sampling have been developed for application to adult workers (Soderholm, 1989). The aerosol inhalability (I, AI, or IF) is defined as the fraction of aerosol in the immediate breathing zone that is actually inhaled into the nose and/or mouth. Inhalability is also referred to as the aspiration efficiency or the sampling efficiency of the exposed subject. It is analogous to the sampling efficiency of an aerosol monitor or collector. For workers, the sampling efficiency depends on the external wind speed, ventilation rate, orientation of the face to the wind, and most importantly the aerosol particle aerodynamic diameter. Table 2.2 shows the American Conference of Governmental Industrial Hygienists (ACGIH®, 2012) inhalability values for particles up to 100 μm aerodynamic diameter for low wind speeds averaged over all orientations

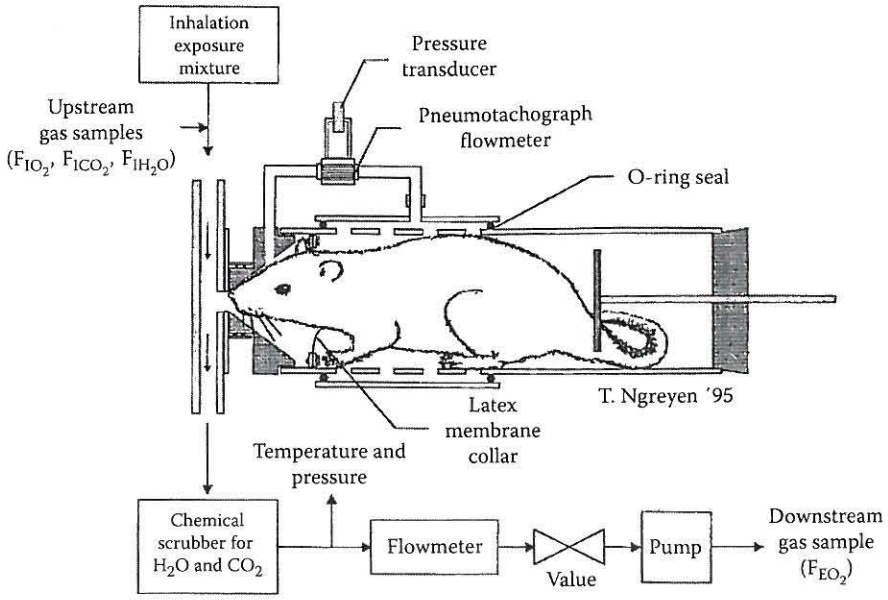

FIGURE 2.3 A plethysmograph/nose-only aerosol exposure system for rats. (From Mautz, W.J., Animal monitoring, in *Methods in Inhalation Toxicology*, Phalen, R.F., ed., CRC Press, Boca Raton, FL, 1997, Chapter 6, pp. 85–99; Courtesy of the Air Pollution Health Effects Laboratory, University of California, Irvine, CA.)

to the wind. These widely used values apply only to the *average* adult worker. Body size, level of exertion, high or zero wind speeds, and other factors modify aerosol inhalability.

Inhalability concepts have also been applied to the efficiency of aerosol delivery to animals, for example, while exposed in nose-only systems (Menache et al., 1995). In a well-designed nose-only exposure system, an unbiased sample from the breathing zone (e.g., from an unused exposure port) can be used to determine the sampling efficiency of the subject. Total deposition in the subject

TABLE 2.2
Inhalability versus Particle Aerodynamic Diameter for Workers

% Sampling Efficiency (Inhalability)	D_{ae}[a] (μm)
100	0
97	1
94	2
87	5
77	10
65	20
58	30
54.5	40
52.5	50
50	100

Source: Data from ACGIH®, *American Conference of Governmental Hygienists*, Cincinnati, OH, 2012.

[a] D_{ae} = Aerosol aerodynamic diameter.

TABLE 2.3
Percent Inhalability (I) for Rats versus Particle Aerodynamic Diameter for Miller (2000) Using Menache et al. (1995) Calculations, and Asgharian et al. (2003) as Measured during Nose-Only Exposures[a]

I, Miller (2000)	I, Asgharian et al. (2003)	Aerodynamic Diameter of Particle (μm)
97	97	0.5
95	85	0.8
93	82	1
85	68	2
77	48	3
71	37	4
65	30	5
55	21	7
44	15	10
Not done	7	20

[a] Data are averages estimated from graphs for three breathing rates; slow, normal, and fast.

divided by an accurate breathing-zone sample yields the inhalability. As shown in Table 2.3, Miller (2000), using the equations of Menache et al. (1995), published inhalability values for rats. Also shown in Table 2.3 are the lower values for rats measured in a nose-only system that were reported by Asgharian et al. (2003). Animal exposures are more complex than those of workers who typically experience orientation-averaged low wind speeds. Air velocities directed at the noses of animals in exposure tubes can be very high, which not only alters the aerosol inhalability but may also produce avoidance behavior, a sign of stress to the exposed subjects.

The respiration rate and tidal volume may be abnormal in confined subjects. These parameters should be measured under actual exposure conditions. An important design requirement is to supply breathing air in excess of the animals' requirement (e.g., 2–10 times the minute ventilation). Lower supply rates can produce rebreathing of exhaled air, which complicates determinations of the dose. Rates that are too high can produce avoidance behaviors and unknown inhaled doses and even harm the animals. As breathing and inhalability parameters depend on the animal characteristics and the specific system used for exposure, literature values are seldom adequate, so actual characterization is recommended.

2.7 AEROSOL DEPOSITION

2.7.1 Measuring Aerosol Deposition

An important aspect of an inhalation study is to determine the dose delivered to the animals. Inhaled aerosol dose determinations are more complex than those associated with other common dosing techniques (e.g., oral, dermal, or injection). Although essentially all nose-only inhalation systems allow for the measurement of the aerosol concentration during the exposure, the actual aerosol deposition within the animals' respiratory tracts should be measured or at least closely estimated. Otherwise, the delivered dose will be unknown. Measuring *total* (anywhere in the respiratory tract) aerosol deposition is usually easier than measuring *regional* (e.g., nose, tracheobronchial tree, and alveoli) deposition. Counting particle concentrations in inhaled and exhaled air can be used to measure total deposition and using radioactive aerosols or sacrifice techniques can be used to measure regional deposition. Clearance curves for radioactive particles that can be acquired with external detectors have been used to measure the regional deposition of inhaled

particles by performing initial counts (for total) and analyzing the clearance at 20–24 h to separate out regional deposition. A common assumption is that poorly soluble material retained in the animal more than 24 h after exposure was deposited distal to the ciliated airways, which may not always be true.

2.7.2 Experimental Aerosol Deposition

The most complete data to date on measuring aerosol deposition after delivery with a nose-only system were published in a series of studies by Raabe et al. (1977, 1988), in which total and regional deposition of monodisperse radioactive aerosols in several species (i.e., mice rats, hamsters, guinea pigs, and rabbits) of unsedated small animals was reported. In the 1988 paper, ventilation values based on formulae published by Guyton (1947) in mice, rats, hamsters, guinea pigs, and rabbits were used. The near monodisperse particles ranged from about 0.2 to 10 μm in diameter. Tables 2.4 and 2.5 show deposition data for mice and rats. Because nose-only exposures to tracer particles lasted up to 45 min, early clearance would have occurred. Material detected in the gastrointestinal tract was assigned to the *Head and Larynx* region in Tables 2.4 and 2.5. In addition to Raabe and colleagues, other groups have reported deposition fractions for rodents using additional (both smaller and larger) particle sizes (Alessandrini et al., 2008; Thomas et al., 2008; Oldham et al., 2009) to characterize their nose-only inhalation systems and the aerosol delivery in their studies. The data imply that even for small animal models, large diameter particles have some, albeit small pulmonary deposition (as is shown in Tables 2.4 and 2.5).

TABLE 2.4
Percent of Presented Aerosols Depositing in Airways of CF_1 Mice

D_{ae}[a] (μm)	Head and Larynx	Trachea and Bronchi	Pulmonary	Total Respiratory Tract
0.27	10.5	14	45	70
1.09	43	6.1	9.7	59
3.45	90	2.7	0.9	94
4.49	99	1.1	0.23	100
5.98	98	1.4	0.4	100
9.65	99	0.66	0.04	100

Source: Data adapted from Raabe, O.G. et al., *Ann. Occup. Hyg.*, 32(S1), 53, 1988.
[a] D_{ae} = Aerosol aerodynamic diameter.

TABLE 2.5
Percent of Presented Aerosols Depositing in Airways of Fischer 344 Rats

D_{ae}[a] (μm)	Head and Larynx	Trachea and Bronchi	Pulmonary	Total Respiratory Tract
0.29	4.9	8.4	13	27
1.02	7.4	4.9	6.5	19
1.03	10	8.3	11	29
3.11	88	4.4	6.6	99
4.26	80	15	4.8	100

Source: Data adapted from Raabe, O.G. et al., *Ann. Occup. Hyg.*, 32(S1), 53, 1988.
[a] D_{ae} = Aerosol aerodynamic diameter.

2.7.3 Predicted Aerosol Deposition

Traditional mechanistic respiratory tract aerosol deposition models (ICRP, 1994; NCRP, 1997; MPPDep, 2002) as well as computational fluid dynamic (CFD) techniques (Jeon et al., 2012) have been used to design and interpret results from experiments conducted in nose-only inhalation systems. Traditional mechanistic models apply the three main deposition mechanisms (i.e., inertial impaction, gravitational sedimentation, and Brownian diffusion) to particles flowing through simplified airways based on morphometric measurements. Inhaled particle deposition calculations may be regional (extrathoracic, tracheobronchial, and pulmonary), or tracheobronchial airway generation number by airway generation number. By utilizing species-specific and strain-specific anatomy in these mechanistic models, predictions of aerosol deposition can be used to guide the target exposure concentrations and/or duration to get a desired deposition dose for a study as well as to extrapolate and/or interpret results.

CFD techniques have also been used to predict regional deposition such as in the nasal cavities of rodents (Fischer 344 and Sprague Dawley rat), New Zealand white rabbit, and Rhesus monkey (Morgan et al., 1991; Kimbell et al., 1993, 1997a; Godo et al., 1995; Corley et al., 2009, 2012; Jiang and Zhao, 2010). Although these predictions mainly included gaseous toxicants (formaldehyde, acrylic acid, acetone, etc.) (Cohen Hubal et al., 1997; Kimbell et al., 1997b; Bush et al., 1998; Kimbell and Subramaniam, 2001), CFD techniques have also been used to predict aerosol deposition in the nasal cavities of rats (Garcia and Kimbell, 2009). Because of the computational requirements of CFD techniques and the complexity of tracheobronchial airways, there are few predictions for aerosol deposition in these airways (Corley et al., 2012). Currently, only a few species and strains have appropriate anatomical respiratory data suitable for use in aerosol deposition computational models.

2.7.4 Experimental versus Predicted Aerosol Deposition

A few studies have attempted to validate or verify aerosol deposition predictions with empirical data (Nadithe et al., 2003; Wichers et al., 2006; Oldham et al., 2009). In these studies, experimental aerosol deposition measurements appear to agree with predicted values of aerosol deposition. CFD techniques have also been used to design nose-only inhalation apparatus specific for nano-aerosols (Jeon et al., 2012). Dosimetry prediction differences from the measured deposition ranged from 10% up to about 40% depending on the particle diameter (Wichers et al., 2006; Oldham et al., 2009; Jeon et al., 2012). These differences in deposition efficiencies can be due to strain-specific respiratory tract anatomy, ventilation parameters (e.g., measured versus estimated), as well as the design of the nose-only inhalation systems (Oldham et al., 2009).

2.8 OTHER APPLICATIONS FOR NOSE-ONLY INHALATION SYSTEMS

A recent review (Cathcart et al., 2011) highlighted the use of exhaled breath and exhaled breath condensate in toxicology studies of large animals. Much less work has been done with rats and mice, most likely due to the challenge of collection of exhaled breath from enough animals for the reliable detection of potential markers. A nose-only inhalation system is well suited to this challenge. Nose-only exposure systems with a low internal volume provide good detection sensitivity. de Broucker et al. (2012) collected exhaled breath from a nose-only exposure system and determined that statistically significantly elevated levels of NO_x and H_2O_2 resulted from lipopolysaccharide-induced acute lung injury in rats. Among the many applications of animal nose-only exposure systems, Stone et al. (2012) designed a system for testing the effectiveness of various filter media for preventing airborne transmission of infections.

2.9 SUMMARY AND DISCUSSION

Nose-only aerosol exposure systems have a long and successful history. As not all investigators have had success with this exposure modality, it is important to consider how some of the problems can be corrected. Early nose-only systems used glass (e.g., soda bottles) or plastic (e.g., centrifuge tubes) for holding animals during exposures. Although inexpensive and convenient, such tubes seldom provide good fits to the animal's bodies or heads. Poor fit can not only cause animals to resist placement in the tubes but also cause significant abrasion damage to rodent's eyes (which may be in direct contact with the tapered end of the tube). Attempts to confine animals in the exposure tubes have included plungers that are advanced against the rear of the animal with significant force. Such force may crush the animal or at least distort its posture enough to produce significant stress. Also, the use of rubber stoppers at the rear of glass or plastic tubes can lead to overheating and, in extended confinements, even deaths. The use of well-designed animal exposure tubes that are neither too snug nor too loose and that are shaped to accommodate the animals' heads is essential for successful exposures. Animals will generally enter properly sized and shaped tubes and move forward without being forced. Even same-aged inbred rodents will vary in body size, so a selection of tube sizes should be available.

In some unsuccessful designs, thermal stress has led to fatalities. Aerosol-generating and conditioning equipment can generate significant heat, so it is imperative that such heat sources should not be placed near the exposure tubes if exposures last for more than a few minutes. Even when there are no nearby heat sources, chilled air may be needed to cool the exposure tubes in order to overcome metabolic heat production.

Rodents and other small animal species use their extensive olfactory apparatus to detect predators so unfamiliar odors can produce agitation and escape behaviors. Therefore, odor control is important. Exposure tubes should be thoroughly washed (e.g., in a high-temperature dishwasher) before each use. Technicians that handle animals should wear fresh, clean lab coats and not use perfumes or other scented personal care products on exposure days. Odor control also applies to carts used to transport animals to the exposure area and to the components of the exposure system. Lack of attention to such details can cause a nose-only exposure experiment to be stressful to subjects (and experimenters) and even fail.

Gentle handling of animals is also essential to successful exposures. Technicians should be trained in proper handling techniques. The goal is to obtain the confidence and thus cooperation of the animal subjects. It should be clear that good system design, maintenance, and operation are all required for conducting nose-only exposures.

Nose-only exposure systems just are one of the several useful techniques for exposing laboratory animals to aerosols. This exposure modality is particularly effective for use with potentially hazardous or expensive exposure materials that require control of doses and unwanted contamination. Although many designs are available, investigators should select or build systems that are appropriate for their species and study design. Animal comfort is an important consideration. When properly designed and operated, nose-only exposure systems can be considered as the gold standard for measuring particle-size-dependent deposition efficiencies in small animals.

QUESTIONS

A. Multiple choice questions: Select the best answer.

1. What type of exposure system would be preferred for a chronic study of a relatively nontoxic aerosol using rodents? Exposures are 24 h per day for 9 months.
   a. Nose-only
   b. Partial-lung-only
   c. Whole-body
   d. All of the above are suitable

   Answer: c

2. What type of exposure system would be recommended for a single 30 min ferret exposure to anthrax spores? The exposure concentrations are 1 and 10 spores per cubic centimeter of air, and the group size is 10 animals.
   a. Nose-only
   b. Partial-lung-only
   c. Whole-body
   d. All of the above are suitable

   Answer: a
3. You are designing a rat study to examine the toxicity to deep-lung macrophage cells of plutonium particles produced by an explosion. The particles are 20 μm in aerodynamic diameter, and the exposure is very brief (e.g., a few seconds). What exposure method would you select?
   a. Nose-only to prevent contamination of the fur
   b. Whole-body as it is most realistic
   c. Lung-only in order to bypass filtration in the rats' noses
   d. Substitute external gamma ray exposure to mimic the radiation effect

   Answer: c

B. Write a brief essay to answer each question.

1. You are designing a nose-only exposure system for use with mice, rats, and rabbits using aerosols that will be nebulized from a water suspension and then heat-treated to dry and fuse tiny aerosol particles together. How would you prevent thermal stresses to the animals?

   Answer for full credit: Provide isolation of the heating source from the exposure location; and use exposure tubes that conduct body heat away from the animals, and are comfortable.
2. What factors in a nose-only exposure might influence the inhalability of the exposure aerosol?

   Answer for full credit includes three of the following: the aerosol characteristics, the air velocities in the breathing zone of the animals, the exposure tube designs, and the ventilation characteristics of the animals.

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