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Chapter 5

Inhalation Exposure Methods

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Contents

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I. Introduction

A. Goals

When selecting an inhalation exposure system, the basic requirement is that it deliver a stable, well characterized study atmosphere to a subject or subjects for a specified duration. Inhalation exposure durations vary from a few seconds to a major fraction of the lifespans of the subjects (months or years). Secondary, but important, goals are to minimize stress to the subjects, to prevent exposure to substances other than those under study, to protect laboratory personnel from exposure to the test substances, and to prevent or limit nonrespiratory tract exposure (dermal and gastrointestinal) of the study subjects. In addition, a particular study may have additional objectives, such as permitting exercise or varying the exposure concentration on a predetermined schedule. Achieving all of the above goals simultaneously may be challenging, even for an experienced investigator and team. Several excellent papers and general reference works describing exposure systems that have been successful in meeting these goals are available.[1-23]

B. Systems Available

Over the past 50 years, a large variety of sophisticated inhalation exposure systems has been designed and used for exposing human and animal subjects to various test agents. A useful means of classifying these inhalation exposure systems is to consider how much of the subject's body is in direct contact with the atmosphere during an exposure (Table 5.1). Whole-body exposure (or immersion) systems employ chambers in which the subjects can move about, either unrestrained or in cages. Such systems permit exposure of large numbers of subjects, are relatively nonstressful, are efficient with respect to the technician labor involved, and are good for chronic exposures where daily exposure durations are 6 to 24 hours. On the other hand, such systems expose all surfaces of the subjects and often their food and water; they can require large volumes of study material and throughput air; they are expensive to build, install, and maintain; and the exposure atmosphere is often heavily contaminated by dander, excreta, microorganisms, and ammonia.

Head-only exposure systems are of two basic types: helmets and portals that permit insertion of the head into a chamber. These systems work well for brief, repeated exposures (because food and water usually are not able to be provided), prevent much of the dermal exposure, require less study material than do chambers, and allow one to quickly vary the exposure concentration

TABLE 5.1
The Major Advantages and Disadvantages Associated with Various Modes of Inhalation Exposure

Mode	Advantages	Disadvantages
Chamber	• Large number of subjects • Suitable for chronic studies • Minimal restraint • Can house animals in chambers • Labor efficient	• Dermal, eye, and oral exposure in addition to inhalation • Large amounts of test material required • Expensive • Excreta can interact with pollutants
Head-only	• Good for repeated exposure • Minimal skin contamination • Efficient dose delivery • Better control of dose	• Can be stressful • Pollutant losses can be large • Neck seal problems • Labor intensive
Nose/mouth-only	• No skin contamination • Can be used for repeated exposures • Uses much less material • Exposures can be pulsed • Personnel and facility contamination minimized	• Can be stressful • Needs good face seal • Labor intensive • Technically difficult
Lung-only	• Precision of dose • Uses less material (efficient)	• Anesthesia or tracheostomy required • Bypasses nose (could be an advantage) • Artifacts in deposition and response • Technically difficult

due to the air volume being much smaller than that needed for a whole-body chamber. Disadvantages associated with these systems include the possibility of producing stressful restraint for the subjects, the possibility of losing much of the study material to helmet surfaces, difficulty in maintaining a closed but comfortable neck seal, and the additional labor involved in positioning subjects and repeatedly checking neck seals.

Nose-only or mouth-only exposure systems include tapered tubes (for rodents and other burrowing animals), masks (for humans, dogs, rabbits, etc.), and oral-cavity apparatuses that position an exposure system tube inside the mouth (these often require full anesthesia to prevent stress or chewing on the tube). The disadvantages associated with these systems are similar to those of head-only systems, plus they are technically more demanding because they require that the system be engineered to fit the subject with more precision. Despite these problems, the advantages of nose-only exposures are considerable. They totally eliminate nonrespiratory pathways of exposure, are very efficient with respect to the volumes of study material and throughput air that are needed, eliminate contamination of the air with dander and excreta, and

the control of the study material can be very precise. Because of the success and versatility of nose-only exposure systems and the availability of tried-and-true designs, this chapter will emphasize their use.

Exposure systems that expose only portions of the respiratory tract have also been used when precise delivery of a material to a limited area of tissue is required. Full anesthesia is required, frequently along with surgical implanting techniques. These systems tend to be nonphysiological in their delivery of test agents and are used only in specialized circumstances. The use of techniques other than inhalation, such as instillation of liquids, is frequently criticized on the basis of the trauma produced locally in airways; however, any method may be very useful for specific research purposes.

C. Ethical Considerations

Ethical considerations and good scientific practice require that the investigator have an intimate knowledge of stresses placed on the animals. The ethical challenges involved in conducting inhalation studies are not trivial. In an inhalation experiment, the subject is critically dependent on the exposure system for delivery of a breathable atmosphere and a comfortable environment. Without adequate monitoring systems, animals can be placed in situations with high air concentrations of ammonia and carbon dioxide, extremes in humidity and temperature, and uncomfortable confinement. Inexperienced investigators may have no awareness of these stressors. Poorly fitted masks, collars, slings, and other restraint devices can produce great stress. Padding, sedation, or anaesthesia are necessary in many cases. Horses, donkeys, pigs, and some other animals require freedom of movement of the head when they are restrained in an unanesthetized state for more than brief periods. Animals can overheat in close-fitting enclosures unless provision is made for cooling. Rats cool via their tails, so they are more stable in systems that do not confine their tails. Training the animals by putting them through trial sham exposures ("dry runs") can help them to relax and thus improve their performance during actual exposures. A good rule is to only use an exposure system in which you would be willing to be exposed. Also, laboratory personnel should be trained to respect and properly handle animals, as well as to recognize distress and to quickly alleviate it.

D. Safety

Inhalation studies pose some relatively serious safety problems. In addition to the safety considerations that are associated with the use of any major laboratory equipment, exposure systems have special requirements. The study material may be hazardous, so seals should be checked, the atmosphere should be under slight negative pressure in relation to the surroundings, and some

means of detecting the contamination of laboratory air or personnel should be considered in the study design. Other safety considerations relate to the study subjects themselves. Rodents are notorious for generating airborne allergens that can sensitize laboratory personnel, making their tasks unpleasant or, in some cases, nearly impossible to perform. In addition, many species of laboratory animals will bite when they are tired, uncomfortable, disoriented, agitated, handled roughly, or, for a few species and strains, whenever the opportunity presents. Bites are prevented by gentle, proper handling; eliminating stress; acclimating the animals to the procedures; and using soft gloves. Only if absolutely necessary should one use chain-mail gloves with small animals (mice and hamsters), since such gloves are sometimes associated with producing tissue damage in small animals. Anticipating safety hazards and applying preventative measures, as opposed to responding to preventable emergencies, are signs of an experienced inhalation toxicologist.

II. Problems Encountered

A. Materials

Exposure systems are usually constructed of relatively inert materials such as metals, glass, and plastics. Problems arise when the study material is chemically reactive. For example, acidic aerosols and several nonacidic salts can corrode many metals or even react to produce hazardous byproducts. Various grades of stainless steel are available and often are used, but they can greatly increase the expense of the system. Nonconductive materials (glass, rubber, and plastic) can easily acquire electrostatic charges that collect study aerosols. This problem can be severe enough to nearly totally deplete the atmosphere of particulate prior to it being inhaled. Antistatic coatings can be applied, and some investigators dampen the surfaces of their systems with aqueous detergent, followed by air-drying (no touching or rubbing with cloth or paper), in order to destroy islands of electrical charge. The aerosol particles can also be discharged by passage through an ion field prior to use.[9]

B. Pollutant Mixing

A poorly designed exposure system can have poor mixing characteristics. That is, the study material is not uniformly dispersed in the throughput air, producing a uneven exposure of the subjects. Careful attention must be paid to how the study material is initially dispersed into the air. Use of venturi mixers (in which the pollutant/air mixture is contracted and then expanded), turbulent mixers, and multiple injection ports (often in a radial design) have been used; however, the uniformity of exposure must be verified in each case.

C. Contaminants

Even if the air supply systems are highly filtered and scrubbed of gaseous pollutants, unwanted contaminants can be present at the breathing zones of the subjects. Such contaminants arise from the air-moving and air-conditioning equipment and from the subjects themselves. Animal dander can add a substantial quantity of unwanted contaminant particles to the breathing zone of the exposure system. A severe problem arises when bacteria in the excreta generate ammonia.[24] State-of-the-art exposure systems are meticulously maintained and cleaned so that excreta-generated ammonia does not build up. In some cases, the use of antibiotics, disinfectants, or other substances where excreta accumulate can be used to suppresses ammonia. Rock salt can be used below wire cage bottoms to dry feces and prevent ammonia formation. Control of animal-generated contaminants is also discussed in Chapter 2.

D. Sampling

Samplers must be used to measure the quantities of test agents in the study atmosphere in order to characterize and control the exposure. Samplers should be properly calibrated and extract representative air samples from the breathing zones of the subjects. Otherwise, the samples may not accurately represent what is inhaled. Also, significant losses of the study material in sampling lines must be prevented. This is accomplished by using large-bore, short length, chemically inert, and electrostatically uncharged lines for sampling aerosols. Metals or conductive plastic lines will not acquire local charges that collect the study aerosol before it can be analyzed. If nonconductive materials are used, one should consider using a static-elimination procedure to treat lines and surfaces prior to the study. In cases in which reactive gases (such as ozone, nitrogen dioxide, etc.) or vapors (such as formaldehyde, etc.) are sampled, it is wise to use inert fluorocarbon tubing for sampling lines.

E. Animal Handling

Each species of laboratory animal must be handled in a unique manner to prevent stress and to promote cooperation. Gentle handling and taking care to provide secure support (to prevent dropping the animal) are essential. As a precaution, the fingers of those handling animals should not be placed in front of an animal's muzzle, as fingers can be mistaken for food, resulting in a nip or even a severe bite. Experienced animal handlers should be used to train laboratory personnel in the proper techniques. Also, gentleness in off-study daily care is essential to helping animals relax and willingly accept handling during a study.

III. A Custom-Made, Nose-Only Exposure System

Some excellent commercially available nose-only exposure systems are available (see suppliers of equipment), but it is feasible to convert a whole-body chamber into a nose-only exposure system that has excellent performance. Smith et al.[25] gave a description of such a system that was used at the Los Alamos National Laboratory for exposing rodents to mineral fibers. The authors replaced their chamber doors with two panels, each with 32 or 45 ports, to receive tapered plastic tubes with one end open for nose-only exposure of rats or hamsters. As a result of the success of this conversion, the Air Pollution Health Effects Laboratory (University of California, Irvine) converted four University of Rochester-type (1-m^3 stainless steel) chambers to permit nose-only exposures of rats.[26] In this system (Figure 5.1), which is still used, all six walls of each hexagonal chamber were modified to expose 20 rats nose-only, giving a total capacity for all four chambers of 480 rats. Inside the chambers, each nose-only port is independently supplied with exposure atmosphere by a rectangular cross-section stainless steel channel. The air flow through these channels is adjusted to be greatly in excess of each rat's ventilation requirement to prevent rebreathing of the flowing atmosphere. On the outside of each wall, short cylindrical stainless steel tubes were welded such that they lead to each internal channel. These tubes accept plastic body tubes with O-ring-fitted aluminum nose-pieces that were designed to accept the rat's heads, permitting about 1 cm of the snout to protrude into the flowing exposure atmosphere. A plastic tailgate at the rear of the body tube is dropped into one of three slots at different distances from the front of the tube, thus preventing the rats from backing out of the exposure tubes. The tailgate covers about 3/4 of the rear opening, so that the rat's tail can hang outside of the tube (rats cool themselves via blood-flow to the tail).

Early in the use of this system, exposure technicians started to experience a moderately severe inflammatory response to the airborne dander and dried excreta from the animals. To solve this problem, external box-like enclosures were installed around the exposure tubes and a negative pressure was applied to scavenge the animal-generated dust and to ventilate (cool) the enclosures. In the intervening years, this system has been used very successfully to expose thousands of animals to a variety of particles, gases, and particle-gas mixtures. Technical personnel like the system, and rats accept the exposure system well for 4 hours/day, 5 days/week, for up to 9 months. This system is a good alternative to the commercial nose-only exposure modules.

IV. Commercial Nose-Only Exposure Systems

A. Historical Development

Turning now to commercially available, nose-only exposure systems, it is useful to examine each component of the systems in some detail. A system in

Figure 5.1
Nose-only exposure system made by modifying a Rochester-type, whole-body inhalation exposure chamber.

use at the Air Pollution Health Effects Laboratory (University of California, Irvine) can be traced to an original unit designed for use at the Inhalation Toxicology Research Institute (ITRI; Albuquerque, NM) by Raabe et al.[27] The original unit had a tapered aerosol entry section on top to present a mixed, uniform concentration of particles to the main chamber below. Two walls of the rectangular main chamber each had 44 (for rats, 88 for hamsters) brass ports into which plastic nose-only exposure tubes were inserted. Like the system described in the previous section, rat noses protrude into the chamber through holes in tapered ends of the tubes (the rear of each tube is closed with a tightly-fitting rubber stopper). Inside the main chamber, a suction system draws exposure atmosphere through individual holes in front of each rat's nose and then into an exhaust manifold. This system has been used successfully for several decades. Improvements in design have been made over the years to decrease the size and cost, while still preserving the excellent containment and uniformity of exposure.

B. The Manifold

Two basic types of nose-only exposure units are commercially available; one has a tower having a circular cross-section (after a design by Cannon et al.),[28] and the other is a narrow rectangular box with exposure ports on two sides (Figure 5.2). Inside the rectangular system, air is drawn through holes to contact each animal's nose and is exhausted through radial holes surrounding each animal's nose. This ensures that each animal gets a fresh, continually renewed supply of the study material with minimal losses of material to the system surfaces. In the tower system, air flows downward past a stack of noses; side ports expose each animal separately. In both types of systems, an airflow is maintained that exceeds the ventilation requirements of the animals. Also, in each system, at least one port is used for obtaining air samples.

V. Exposure Tubes

Various designs of nose-only exposure tubes have been used for exposures. Two successful types (one all plastic and the other a plastic body tube with a machined metal headpiece) both have a tapered internal conical cavity for the animal's head. This taper is important, not only for animal comfort, but it also produces a partial seal around the nose when the animal moves forward to the front of the tube. Animals placed in such tubes usually quickly move to the front as far as possible and stick their noses out of the front hole. If the tubes are too loose around the body, animals may turn around to face the rear of the tube. If the tubes are too tight, animals will not enter them, or will be very restless when they are exposed in them. It is wise to have a collection of various-size tubes available to accommodate unusually small or large animals.

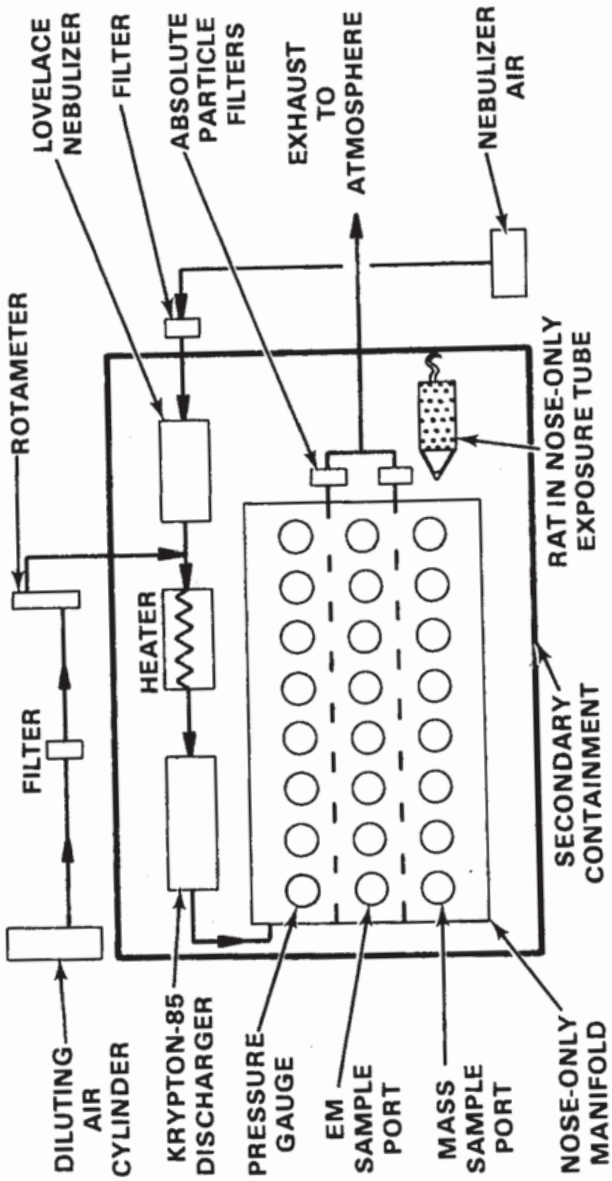

Figure 5.2
Nose-only exposure unit of the type designed by Raabe et al.[27]

Thermal stress can occur due to heat buildup inside nose-only exposure tubes. This heat can produce fatalities if the nose-only system is in a hot surrounding environment. Precautions must be taken to prevent heat stress during confinement. Several methods have proven to be successful, including perforated metal tubes,[29] allowing rat's tails to extend outside of the tubes,[15,26] and keeping the surrounding laboratory air cool.

VI. Air Supply

The air supply system should provide sufficient air to substantially exceed the ventilation requirements of the animals in order to prevent rebreathing of exhaled air. A useful empirical formula for calculating the ventilation per minute (V_m) of a resting mammal of body mass, M (in grams), was published by Guyton:[30]

$$V_m(cm^3/min.) = 2.18\ M^{3/4} \quad (5.1)$$

For a 70-kg human, this gives a minute ventilation of 9.4 *l*, and for a 200-g rat it gives a result of 116 cm^3/minute; both values are quite reasonable. To be certain that rebreathing of expired air does not occur, even if the animals become active, it is wise to multiply the value obtained using Guyton's equation by a factor of 10, and then to deliver this volume of air to each subject each minute. As was discussed in Chapter 3, the air supply should be within comfortable limits of temperature and humidity, and it should be free of all significant non-study-related contaminants.

VII. Exhaust Scrubbing

The unfiltered exhaust air from an inhalation exposure system will contain the study material and possibly substances that are added by the subjects (dander, dried particles from feces, saliva, etc.); therefore, scrubbing should be considered. For particulate material, a battery of filters — a coarse filter followed by a high-efficiency filter — is a good solution. For gaseous pollutants, adsorption, absorption, or chemical degradation may be used. Activated charcoal is a good adsorption material, as are several other high-surface-area granular materials. Absorption in a liquid via a bubbler or by passage through scrubber tubes coated with absorbing materials should be considered. Chemical degradation reactions can occur in beds of potassium permanganate impregnated on activated alumina pellets (Purafil, Inc.; Doraville, GA) and in bubblers filled with aqueous acids or bases, for example. Thermal degradation, as by passage of the exhausted air through a heated metal, glass, or ceramic tube, also can be effective. In any case, the investigators must make sure that hazardous or nuisance levels of contaminants are not emitted into the environment.

VIII. Monitoring

In order to specify and control an inhalation exposure, several parameters should be monitored. In relation to the exposure, the study material concentration at the breathing zone and air-flow rate should be known throughout the exposure. If aerosol particles are being studied, the size distribution (preferably including the mass median aerodynamic diameter and geometric standard deviation) should be measured at least once during each daily exposure, preferably more often. (See the general references listed at the beginning of this chapter and in Chapter 4 for acceptable methods.) In high-quality studies, the air temperature and humidity, both inside and surrounding the inhalation exposure system, will be monitored and corrected, if necessary.

It is also sometimes useful to monitor the breathing patterns of animals during exposure. This is very difficult to do if a large number of animals are exposed at once, so monitoring the breathing pattern of one, two, or three randomly selected animals is desirable. See Chapter 6 for a description of the techniques and equipment used for this purpose.

IX. Suppliers of Equipment

A. Chambers

Hazelton Systems
P.O. Box 700
Aberdeen, MD 21001

B. Nose-Only Exposure Systems

CH Technologies (U.S.A.)
263 Center Avenue
Westwood, NJ 07675

In-Tox Products
115 Quincy NE
Albuquerque, NM 87108

Lab Products, Inc.
255 W. Spring Valley Avenue
Maywood, NJ 07607

Unifab Corp.
5260 Lovers Lane
Kalamazoo, MI 49002

Machine Shop (D. Baugh & Son)
(custom-made exposure tubes for small animals)
3413 Fern Meadow Road
Palomar Mountain, CA 92060-0130

X. Sample Data

The major data that are acquired during an inhalation exposure will relate to the characteristics of the study material. Achieving the target concentrations and the target particle-size distributions (if aerosols are involved) are of primary importance. Samples of data from actual inhalation exposures are shown in Table 5.2. This study involved two gases (ozone and nitric acid vapor) that are both often difficult to stabilize. Ozone is very reactive, so system surfaces had to be clean and inert, and the generator was stabilized by use of a voltage regulator. Nitric acid adsorbs onto system surfaces, so preconditioning for several hours was necessary before any animals were placed into the exposure system.

TABLE 5.2
Summary of Atmosphere Characteristics in a Nose-Only Exposure

Exposure atmosphere	Parameter/ pollutant	Target	Measured (mean ± SD)
	4 hours/day × 1 day		
Purified air	Relative humidity	60%	60.7 ± 1.0
	Temperature	22–24°C	23.7 ± 0.8
0.6 ppm O_3	Relative humidity	60%	60.7 ± 1.0
	Temperature	22–24°C	23.7 ± 0.8
	Ozone	0.6 ppm	0.61 ± 0.02
1 mg/m³ HNO_3	Relative humidity	60%	60.9 ± 0.9
	Temperature	22–24°C	23.3 ± 0.5
	Nitric acid	1.0 mg/m³	1.08 ± 0.13
0.6 ppm O_3 + 1 mg/m³ HNO_3	Relative humidity	60%	60.5 ± 1.0
	Temperature	22–24°C	24.1 ± 0.8
	Ozone	0.6 ppm	0.60 ± 0.01
	Nitric acid	1.0 mg/m³	1.01 ± 0.12
	4 hours/day × 4 days		
Purified air	Relative humidity	60%	60.2 ± 0.9
	Temperature	22–24°C	22.5 ± 0.8
0.15 ppm O_3	Relative humidity	60%	60.2 ± 0.9
	Temperature	22–24°C	22.5 ± 0.8
	Ozone	0.15 ppm	0.15 ± 0.01
0.25 mg/m³ HNO_3	Relative humidity	60%	60.4 ± 0.9
	Temperature	22–24°C	22.3 ± 0.6
	Nitric acid	0.25 mg/m³	0.27 ± 0.08

TABLE 5.2 (continued)
Summary of Atmosphere Characteristics in a Nose-Only Exposure

Exposure atmosphere	Parameter/ pollutant	Target	Measured (mean ± SD)
0.15 ppm O_3 +	Relative humidity	60%	60.0 ± 0.8
0.25 mg/m^3 HNO_3	Temperature	22–24°C	22.8 ± 0.8
	Ozone	0.15 ppm	0.15 ± 0.01
	Nitric acid	0.25 mg/m^3	0.26 ± 0.09

Note: No aerosol-phase HNO_3 was observed; all of the HNO_3 was in the vapor phase.

Source: Adapted from Nadziejko, C. E. et al., *Inhal. Toxicol.*, 4, 343, 1992.

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