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Exposure Atmosphere Generation and Characterization

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Contents

I.	Introduction	40
II.	Scope and Limitations	41
III.	Atmosphere Generation Techniques	41
	A. Particles	41
	1. Monodisperse Aerosols	41
	2. Polydisperse Aerosols	43
	3. General Considerations	46
	B. Gases	47
	1. Pressurized Cylinders	47
	2. Permeation Tubes	48
	3. <i>In Situ</i> Generation by Physical or Chemical Reactions	49
	4. Evaporation or Sublimation	50
IV.	Characterization Techniques	50
	A. Introduction	50
	B. The Breathing Zone	51
	C. What Should Be Measured?	51
	D. Aerosol Characterization	55
	E. Gas Characterization	59

V.	Eliminating Measurement Interferences.....	60
VI.	Sampling Protocols	63
VII.	Suppliers.....	64
	A. Aerosol Generators	64
	B. Aerosol Sizers and Samplers	64
	C. Gas Generators	64
	D. Gas Analyzers and Samplers.....	65
	References	65

I. Introduction

The requirements of a modern inhalation study include having rigorous control of the exposure atmosphere (known concentrations of well defined components) and ensuring the absence of significantly confounding contaminants. To achieve these requirements, purified air with a controlled temperature and humidity is intentionally modified by added substances. Two basic considerations are (1) how to generate and control the exposure atmosphere, and (2) how to adequately monitor the exposure. This chapter discusses the selection and use of particle and gas generating systems, some of the problems which arise, and representative techniques for atmosphere generation and characterization.

A typical exposure system includes an air purification system, pollutant generators, the exposure unit, and the sampling systems. Peripheral systems include air flow controllers, air humidifiers/dryers, scrubbers, or filters to remove contaminants from air exiting the exposure system and a disposal system for water used to wash the exposure apparatus.

The generation of even simple atmospheres can pose significant problems above and beyond the selection and installation of the appropriate major equipment. For example, problems arise when exposure chamber or delivery line materials are not compatible with the atmosphere being studied. For example, the use of rubber (and some types of plastic) delivery lines leading from an ozone (O_3) generator can result in large losses of O_3 . To overcome these losses, the generator must produce quantities of O_3 far in excess of those needed for the experiment. While this in itself is not a major problem, it could result in several complications, such as the co-generation of other reactive oxygen species along with O_3 and the contamination of the exposure atmosphere with volatile degradation products. Another problem arises with the use of nonconductive materials in the construction of delivery lines and chambers used for aerosol studies. The buildup of static charges on the walls of the tubes or the chamber can produce losses of particles to these surfaces and result in unstable particle concentrations. These problems are solved by the use of inert, electrically conductive materials.

II. Scope and Limitations

Since the number of materials studied in inhalation research is quite large, selected typical examples will be discussed. This chapter will focus on single component atmospheres, and complex aerosol/gas mixtures will be briefly addressed. There are too many methods for collecting, analyzing, and characterizing exposure atmospheres to permit an encyclopedic discussion. The benefits and limitations of several techniques, however, will be discussed.

III. Atmosphere Generation Techniques

A. Particles

Generation of atmospheres containing particles of known size and concentration can be especially challenging. Particle diameters of aerosols (particles suspended in gases) can range from 0.001 to over 100 μm . Monodisperse aerosols are often used in studies of respiratory tract deposition and clearance, for examining the influence of particle size on biological processes, and for directing delivery of materials to selected regions of the respiratory tract. Monodisperse aerosols contain particles of nearly uniform size and are generally defined as aerosols that have size distributions with a known median diameter and a geometric standard deviation of less than 1.2 (aerosol sizes are usually log-normally distributed). Selected methods for generating monodisperse aerosols include condensation aerosol generators (Sinclair-LaMer type generators), spinning disk atomizers, dispersion of liquid suspensions of monodisperse particles, and particle-size segregation.

Polydisperse aerosols (those which contain a broad distribution of particle sizes) are easier to generate and can be used when the characteristics of monodispersity are not necessary. Polydisperse aerosols are usually generated by nebulization (of solutions of soluble materials or suspensions of particles), suspension of dry powders or dusts by air jets, or by mechanical grinding or abrasion.

Generation techniques for monodisperse and polydisperse aerosols are discussed in several references.¹⁻⁶ Some of the more widely used methods will be presented here.

1. Monodisperse aerosols

Particles in a narrow size range can be obtained by the controlled condensation of organic vapors onto nuclei particles. Such devices were reported by Sinclair and LaMer,⁷ and variations and improvements on this methodology have been summarized by Prodi,⁸ Mercer,¹ and Hinds.⁴ Generators of this type are commercially available. In the basic approach, a low vapor pressure liquid is heated

in a boiler at a constant temperature. Nuclei particles, generated by any of several methods, are mixed with the condensable heated vapor. The aerosol is passed into a vertical chamber where it is slowly cooled, permitting the vapor to condense on the particles in a controlled manner. If the vapor is uniformly mixed with the nuclei and condensation is slow and diffusion-controlled, then monodisperse particles will be produced. By varying the flow rate of the nuclei particles through the vapor and the vapor temperature, particles having count-median diameters ranging from about 0.2 to 2.0 μm can be produced. The geometric standard deviation of the particles generated by this type of device is generally less than 1.1.

In a paper published in 1949, Walton and Prewett⁹ presented an analysis of the conditions for production of monodisperse particles that are thrown off of the edge of a rapidly spinning surface. Since that time, a variety of spinning-disk and spinning-top liquid aerosol generators has been described. These generators operate at rotational speeds ranging from a few hundred up to about a quarter million revolutions per minute, and produce primary monodisperse droplets with diameters in the range of 6 μm to 3 mm. (They also produce small secondary satellite particles that form as the main droplets separate from the edge of the spinning surface; these particles must be removed.) Subsequent drying of primary particles can be used to produce monodisperse particles as small as 1 μm in diameter. Lippmann and Albert¹⁰ designed a spinning-disk generator for producing radioactive insoluble aerosols for human and laboratory animal deposition and clearance studies. In this generator, satellite particles are separated from the main particles and collected on a filter. Precise control of the liquid feed to the spinning disk is necessary to achieve monodispersity. Optimal conditions for using the spinning-top generator to produce monodisperse aerosols are discussed by Mori et al.¹¹

A relatively simple method for producing monodisperse aerosols in a wide variety of sizes involves the aerosolization and drying of aqueous suspensions of commercially-available latex spheres (available from Duke Scientific Corp.; Palo Alto, CA). These spheres are supplied in diameters ranging from less than 0.1 μm up to over 100 μm . The particles can be labeled with fluorescent dyes, radioactive elements, or magnetic materials. Biologically active substrates also can be attached to these particles. Suspensions of these particles can be aerosolized using a variety of generators; compressed-air nebulizers are frequently used. Although the commercial particles are quite uniform, several considerations must be applied in their use. First, the nominal diameters, as supplied by the manufacturer, are based on microscopic measurements, but the aerodynamic behavior may depend on conditions in a specific investigation; therefore, aerodynamic sizes may need to be measured. When liquid suspensions are aerosolized, some droplets will contain more than one solid particle, and some will contain no particles but will contain residual contaminants (salts, surfactants, etc.). Thus, after drying, in addition to individual monodisperse particles, some agglomerates (two or more particles stuck together) and some small residue particles will be components of the aerosol. As the aerosol is made more concentrated, the agglomerate fraction increases,

and as it is made more dilute, the residue fraction is greater. Raabe¹² has given dilution factors for suspensions that allow one to keep the singlet/agglomerate ratio above a given value. As an example, using a nebulizer with a volume median droplet diameter of 5 μm and a geometric standard deviation of 1.6 to aerosolize a suspension of 1.0- μm diameter latex spheres, the percent solids (by volume) in the nebulized suspension should be below about 0.03 (i.e., 1 part in 3000) in order to keep the ratio of singlets to agglomerates above 20:1. In this case, the ratio of small stabilizer residue particles to single latex particles will be about 50:1. Monodisperse powders can also be resuspended in air using suitable fluidized bed-type generators.¹³

Monodisperse droplets can be made in relatively small quantities by the vibrational breakup of a liquid stream.¹⁴⁻¹⁶ In some devices, a liquid stream exiting from a small orifice (10 to 15 μm in diameter) is broken into droplets by a vibrating piezoelectric crystal. Vibration of the orifice at frequencies ranging from 50,000 to 500,000 Hz (cycles per second) leads to droplets of sizes from 18 to 40 μm that vary in volume by less than 1%. A vibrating orifice, monodisperse aerosol generator is commercially available (Model 3050, Thermo Systems Inc.; St. Paul, MN), after the design by Berglund and Liu.¹⁷ Modifications which improve the reliability of this type of generator have been reported by Mitchell et al.¹⁸

2. Polydisperse aerosols

A great variety of techniques has been used for controlled generation of polydisperse aerosols. Even a simple listing of these techniques would be cumbersome, but certain methods are especially useful because they produce aerosols in the size ranges similar to those inhaled by people in various situations. The source material for the aerosol can be a liquid (solution or suspension) or a solid. Liquids can be aerosolized using air blast or ultrasonic nebulizers. Examples of generators for solid substrates include the Wright dust feeder for dispersion of dry compressed powders, the air-blast nebulizer for aerosolization of liquids, the fluidized-bed generator for dispersion of loose powders, the exploding-wire generator for vaporizing solid metals, and the combustion generator for generating fuel-derived particles. Several references treat methods for generating polydisperse particles.¹⁻⁶ A few methods will be presented here.

Droplet aerosols are used in several ways in inhalation studies: (1) the droplet itself may be passed into an exposure system for direct inhalation; (2) the droplets may carry a suspended solid that is inhaled after evaporation of the droplet; (3) a solution may be aerosolized, leaving a residue particle upon drying; and (4) the droplets may be used as input to another stage of an aerosol generator (for example as input to the heater of a condensation aerosol generator). Air-blast and ultrasonic nebulizers produce polydisperse droplets suitable for many scientific purposes (Table 4.1). Each generator has its unique characteristics and any one may be preferable over the other for a given application. If a study requires a high number of droplets per liter of air and

TABLE 4.1
Two Polydisperse Droplet Aerosol Generators

Type	Operating principle
Air-blast nebulizer	High-velocity air directed over a liquid feed tube produces breakup of entrained liquid filament
Ultrasonic nebulizer	Vibration of a piezoelectric crystal forms a fountain of liquid that emits droplets from its tip

Source: Phalen, R. F., *Inhalation Studies: Foundations and Techniques*, CRC Press, Boca Raton, FL, 1984, 89.

large droplets can be tolerated, an ultrasonic nebulizer may be used. Compressed air nebulizers are useful for providing smaller droplets at somewhat lower output rates.

Compressed air nebulizers are commonly selected for inhalation studies due to their relative low cost, stable operation, and droplet size characteristics. Table 4.2 presents the operating characteristics of some commonly used compressed air nebulizers. In this type of generator, an air jet shatters a liquid stream which is drawn from a reservoir into the path of the air jet by the reduced pressure within the jet or as a result of positive pressure in a reservoir. The shattered liquid forms large and small droplets, and the larger droplets impact on a nearby surface (an adjacent wall, a ball, a baffle, etc.). Smaller droplets follow air streams around the impaction surfaces and exit from the nebulizer. The air that entrains the particles will be saturated with the vapor of the fluid in the reservoir, so the concentration of the dissolved or suspended material may steadily increase with time. Three methods are used to control this effect: (1) the reservoir may be cooled to reduce evaporation; (2) the reservoir may be enlarged or continuously renewed; or (3) the feed air can be pre-saturated with liquid vapor. Although the majority of the droplets impact within the nebulizer, usually about 10^6 to 10^7 particles per cubic centimeter of air exit as useful aerosol. These droplets typically have a distribution of sizes that is describable by a log-normal distribution function with a geometric standard deviation between about 1.3 and 2.2. Freshly nebulized droplets usually are highly electrically charged and are often passed through a bipolar ion field for discharging.¹⁹

When a solution is nebulized and the droplets dried to a residue aerosol, the resultant dry particles will be smaller than the original droplets. The theoretical relationship between the mass median droplet diameter (D_d) and the mass median residue particle diameter (D_p) for spherical residue particles is

$$(D_p)^3 = (D_d)^3 (C) (\rho_d/\rho_p) \quad (4.1)$$

where ρ denotes density (subscripts relate to the droplet, d, and to the residual particles, p) and C is the mass fraction of solute. The geometric standard deviation of the residue aerosol will be approximately that of the droplet aerosol.

TABLE 4.2
Operating Characteristics of Compressed Air Nebulizers

Nebulizer	Applied pressure (lb/in. ²)	Air flow (l/min)	Aerosol output conc. (g/m ³)	Vol. median drop diameter ^a (μm)
3-Jet Collison	15	6.1	8.7	—
(available from BGI, Inc.)	30	9.4	9.0	—
	50	13.6	10.4	—
De Vilbiss® No. 40	15	12.4	15.5	4.2
with closed vent	20	16.0	14.0	3.2
(available from De Vilbiss)	30	20.9	12.1	2.8
Lovelace	20	1.34	(34) ^b	6.9
(available from In-Tox)	30	1.81	(22) ^b	4.7
	40	2.28	(15) ^b	3.1
	50	2.64	(19) ^b	2.6
(Nebulizer chilled to 0°C)	20	1.34	55	—

^a G.S.D. (geometric standard deviation) for these methods ranges from 1.6 to 2.3.

^b Calculated.

Source: Phalen, R. F., *Inhalation Studies: Foundations and Techniques*, CRC Press, Boca Raton, FL, 1984, 90.

TABLE 4.3
Selected Dry Dust Aerosol Generators

Generator	Operation principle
Wright dust feed	Powder which has been compressed into a block is scraped by a rotating blade and entrained in a dry air stream.
Timbrell-type fibrous dust generator	Similar to Wright dust feed, but blades rotate at high speed over compacted asbestos or fibrous glass plug.
Fluidized bed	Upward flowing air fluidizes (suspends) a bed made of large (180 μm) "beads" into which dust to be aerosolized is mixed. The air carries particles upward into a vertical elutriator. New powder is continuously fed into the fluidizing section.
Turntable dust feed	Powder flows from a hopper (agitated) onto a turntable, which rotates toward an aerosolizing air jet.

Source: Phalen, R. F., *Inhalation Studies: Foundations and Techniques*, CRC Press, Boca Raton, FL, 1984, 91.

Table 4.3 lists some useful dry dust aerosol generators. The dust loaded into the generator can have a variety of forms, including fibers, spheres, plates, etc. The dispersed phase will have a size distribution that may differ from that of the bulk dust material, due to breakup or agglomeration of primary particles.

The fluidized-bed elutriator is often used in inhalation studies because of its stability over long periods of generation and its favorable elimination of larger agglomerates and large primary particles by elutriation (settling downward). Modern fluidized-bed elutriators usually incorporate two important features: continuous feeding of material to the generator and electrical discharging of the aerosol within the elutriation column. Continuous feeding of material into the generator is often necessary because material in the generator initially loses the fine particle fraction, leaving the larger particles in the generator. Thus, unless material is continuously supplied to the generator, the emitted size distribution will change with time. Fluidized bed generators impart electrical charges to the aerosol they emit, and thus passage through an bipolar ion field is recommended.²⁰ The relationship between fluidized bed operating conditions and the aerosol produced has been reviewed by Lua.²¹ Key factors are the fluid bed height and the airflow through the bed after the minimum fluidizing velocity is exceeded.

The dust feeder described originally by Wright²² has been used to generate aerosols from a wide variety of dry materials. The material is first formed into a plug, which is then scraped by a blade and aerosolized by entrainment in a flowing air stream. An important limitation of this generator is that the material must pack into a stable plug with sufficient integrity to avoid fragmentation during the scraping process.

3. General considerations

Selection of appropriate aerosol generators for inhalation studies is made on the basis of several criteria. Some of the more important considerations in aerosol generator selection include the following:

1. Are the aerosol characteristics appropriate? These characteristics include chemical composition and physical properties such as shape, size, surface area, dispersity, and number of particles per unit volume of air.
2. Is the generator and its aerosol stable over the time period of intended use?
3. Does the generator waste input materials? Some materials that are aerosolized are expensive or rare, and economy of operation can be important.
4. Is the generator hazardous to laboratory personnel or experimental subjects?
5. Does the generator introduce unwanted foreign materials into the aerosol?
6. Does the generator require a large amount of maintenance?
7. How sensitive are the characteristics of the output aerosol to perturbations in inputs or environmental parameters such as voltage, input air pressure, barometric pressure, temperature, and relative humidity?
8. Has the aerosol generator been described and/or evaluated in publications in the available literature?

Persons not experienced in aerosol generation must be very careful when selecting generators for inhalation studies. When in doubt, one is wise to contact aerosol scientists to discuss one's requirements.

B. Gases

The generation of gases usually involves the controlled introduction of a concentrated gas into a stream of purified air. The mixture is then introduced into a chamber or other dynamic exposure system. Alternatively, the gas is injected into a static system, which may be useful for certain purposes, such as measuring respiratory system uptake. The most widely used methods of generating gases include metering from pressurized cylinders, diffusion of the gas through a permeation tube source, vaporization of volatile fluids, and creation of the gas by controlled reactions.

In some respects, the generation of gases is less difficult than the generation of particles; stabilization of particle size is not a consideration, and usually it is easy to achieve uniform mixing of a gas in an airstream. Also, the devices used to control the injection of a gas into dilution air are comparatively reliable and simple; however, the gas must be of high purity. It is essential that the materials used in the construction of the generator, metering device, and delivery lines not introduce contaminants (including particles), cause chemical degradation of the gas, or otherwise consume the gas. It is frequently necessary to pre-condition the generator and delivery and exposure systems so that stable concentrations are achieved for the exposures.

1. Pressurized cylinders

Cylinders containing pure gases (or mixtures of gases) at pressures up to a few thousand pounds per square inch (psi) are available commercially or can be prepared in the laboratory. Cylinders are prepared by first evacuating them to a sufficiently low pressure to remove contaminants, introducing a known amount of the desired gas, and then filling under pressure with a carrier gas (usually purified air or nitrogen). Mixing a filled cylinder can be achieved by rotating the cylinder or, alternatively, by gently heating and cooling an end of the cylinder. While some gases may be stable for long periods when stored, others require cylinders made with nonreactive materials (e.g., stainless steel or aluminum) or the use of inert carrier gases (e.g., nitrogen or helium) to extend shelf life.

Commercially available gases of extremely high purity are available; however, some gases will spontaneously decompose over time. For example, nitrogen dioxide (NO_2) under pressure at high concentrations (>1%) will invariably contain a small amount of nitric oxide (NO), a dissociation product. In addition, carrier gases can contain small amounts of contaminants such as hydrocarbons, carbon dioxide, and water vapor. In most inhalation studies, the pressurized gases are diluted many-fold, and the resulting amount of contamination at the breathing zone is negligible. In those cases where even a low level of contamination is a problem, scrubbers can be used to reduce contaminant concentrations. Gases for administration to humans and that conform to U.S. Pharmacopeia (USP) standards can be obtained commercially.

The cylinders from commercial suppliers generally have a valve with a threaded outlet which conforms to a substance-specific standard thread which fits only specific pressure regulators. This practice helps to minimize the possibility of mixing incompatible gases (e.g., hydrogen with oxygen), or of contaminating a system with gas residuals in a pressure regulator previously used with another gas.

Pressure regulators are used to reduce the pressure of the compressed gas to a safe working level. Two-stage regulators are recommended for most purposes. The first stage reduces the pressure from that in the tank to a pre-set intermediate level. Because the pressure fed to the second stage is fixed by the first stage, the delivery pressure is not affected by changes in the cylinder pressure, thus providing precise control of gas delivery during the course of a study. Regulators for delivery of high purity gases, or corrosive gases, are constructed of high-quality stainless steel (e.g., 316-grade stainless), with stainless steel or fluorocarbon-lined diaphragms and fluorocarbon or stainless steel seats and seals. Prices for regulators can range from about \$200 for general models to about \$1000 for delivery of ultra-high-purity, or corrosive, gases.

To control exposure concentrations precisely it is necessary to carefully meter the gas into the airstream by using a flow controller. Flow controllers can be a fixed-flow type (critical orifices, capillary tubes, or porous plugs) or variable-flow type (needle-valve-equipped rotameters, orifice meters, heated wire anemometers, or electronically controlled mass-flow meters). Flow controllers should be calibrated using devices such as a bubble meter, a spirometer (primary flow calibrators), or a previously calibrated wet-test or dry-gas meter (a "transfer" standard).

Compressed gases are often purchased for inhalation studies at very high concentrations. Small leaks from the delivery system into the laboratory can potentially result in relatively high exposures of personnel. All connections should be checked carefully for leaks, all cylinders should be properly secured, and, if possible, periodic area monitoring should be performed. Although it is a rare occurrence, regulator diaphragms can crack, releasing high pressure gas into unsealed portions of the regulator and then into the room. Therefore, two-stage regulators for toxic gases are equipped with a pre-set safety valve that protects the second stage from overloading and can be connected to a vent line to safely exhaust released gases.

2. Permeation tubes

Permeation devices are polymeric tubes that are permeable to vapors of the liquids which are sealed within them. Hundreds of organic and inorganic chemicals are commercially available in permeation tubes. The permeation rate of the gas out of the tube is a function of the vapor diffusion coefficient, vapor pressure, temperature, atmospheric gas pressure, solubility coefficient of the vapor in the tube polymer, surface area of the tube, and the thickness of the tube wall. The vapor permeation rate is determined by weighing the

tube at timed intervals. In use, the permeation tube is inserted into a special chamber which is flushed with purified air at a known, constant rate. The chamber is maintained at a fixed temperature by immersion in a constant temperature bath or by use of a thermostated heater. The vapor-laden air which exits the permeation tube system can be diluted with a known flow of purified air to produce a stable delivery rate. Permeation tubes can be purchased or constructed, in various sizes and with various permeation rates. For heat-stable materials, the temperature of the permeation tube chamber can be increased to increase the permeation rate. Multiple permeation tubes can be used in parallel to increase output concentrations, if necessary.

Some precautions are necessary to ensure good results when using permeation tubes. Careful handling of the tube is necessary because damage to the tube or the presence of finger oils or other contamination can alter the permeation rate. The tube should never be handled without appropriate gloves and should only be handled by the sealed ends to avoid damaging the permeable surface of the tube. A temperature-controlled chamber and an accurate balance are needed to maintain and to check the permeation rate. Note that even at room temperature, permeation tubes continue to emit vapors; hence, tubes should be stored between uses at a low temperature in a sealed container. The container should only be opened in a well ventilated area, preferably an exhaust hood, to avoid exposure of personnel.

3. *In situ* generation by physical or chemical reactions

Several physical and chemical processes lead to the production of gases. Such processes can be exploited when the gas to be generated is not commercially available or if the gas is unstable. Physical processes that lead to the generation of gases include thermal transformations, photochemical reactions, and electrolysis from molten salts.

Chemical reactions that produce gases can be used to produce compounds that otherwise would be unavailable. The rates of production depend upon a number of factors that must be rigorously controlled in order to maintain a stable output with respect to purity and concentration. Reaction rates depend upon thermodynamic factors such as temperature, pressure, and humidity, as well as kinetic factors such as the concentrations of the reactants, the rate of reactant mixing, and the rate of removal of products. Impurities in the reactants or in the reaction vessel may lead to contamination of the product or modification of the reaction rates and pathways. In some situations the evolved product, which might be obtained in a liquid or vapor form, can be collected, purified, and metered into the exposure system using metering pumps or motor driven syringes.

As a specific example of a gas generated by physical processes, consider O_3 . To produce O_3 , diatomic oxygen (O_2) should be used as a starting material, because the use of air could result in production of unwanted contaminants (such as nitrogen oxides). A laboratory O_3 generation system might consist of a cylinder of compressed pure O_2 , a two-stage pressure regulator, a needle

valve-equipped rotameter to meter O_2 flow to the reactor, and a voltage-controlled electrical discharge or ultraviolet reactor. The $O_2 + O_3$ mixture should exit the reactor through preconditioned inert tubing prior to being mixed with purified air and injected into the exposure system. Concentrations of O_3 in the exposure system should be monitored with a continuous monitor and adjusted as needed by controlling the flow rates or the voltage applied to the reactor. Depending upon the metering rate of O_2 into the generator and the exposure system air throughput rate, the chamber O_2 concentration could be higher than ambient, which might be important if metabolic measurements are being made as part of the study. Nitrogen gas can be metered into the chamber to adjust the concentration of O_2 to normal ambient levels.

4. Evaporation or sublimation

Controlled generation of vapor by evaporation or sublimation can be useful for generating some gases (such as formaldehyde or nitric acid vapor). The rate of gas evolution is temperature dependent, so precise control of the generator temperature is required. Immersion of the reactor vessel containing the liquid or solid into a thermostatically controlled bath will often suffice. Carrier gas for the generator can be heated to the required temperature by passage through a metal coil immersed in the same bath. Many vapors at high concentration are reactive, explosive, or flammable. In such cases, an inert carrier gas, such as nitrogen, should be used. If it is necessary to use air, the vapor should be rapidly diluted to a concentration below the lower explosive limit (LEL); for safety, a maximum concentration of 10% of the LEL should be considered.

IV. Characterization Techniques

A. Introduction

Careful characterization of the exposure is a necessary part of experimental inhalation toxicology studies and thus deserves both appropriate planning and attention to detail. The usual objective in an aerosol inhalation study is to establish a quantitative relationship between an observed biological response and the measurable physical and chemical properties of the test aerosol. The relevant physical and chemical properties that produce the expected biological effects must be known. Certainly the composition, number and mass concentrations, median size, and size distributions come immediately to mind. Other particle properties, including the surface area, state of electrical charge, surface character, hygroscopicity, particle shape characteristics, dissolution rates in lung fluids, and amounts and types of adsorbed materials often will be relevant to the study. Selection of the exact parameters for measurement is not trivial. Consider the size parameter. Which sizes are important — geometric, aerodynamic, or

both? Should size distributions be based on particle number, surface area, volume, or mass? For a size distribution, is the mode, mean, or median most relevant?

Although gases are usually easier to characterize than particles, some issues must be considered. How short should the sampling intervals and instrument response times be? Should concentration data be summarized by the mean and standard deviation or by the median and geometric standard deviation? Should the peak value be recorded? The answers depend upon the study objectives.

Assuming that one has identified those relevant physical and chemical properties and has selected an appropriate battery of equipment, one is faced with additional questions. Where should one sample? How many samples must one acquire? A good inhalation study may involve expending as much effort in atmospheric characterization as is spent in measuring the biological response. In the following sections, some counsel will be offered on atmospheric characterization, but no simple or certain rules can be given that can be applied in all cases.

B. The Breathing Zone

Usually, one wishes to characterize the material in an inhalation study as it exists just before it is inhaled by a subject. Because it is often impractical to place a sampler inlet beside the subject's nose, the concept of the breathing zone will be introduced. The breathing zone is that volume or space from which the subject breathes. It extends from above the lowest to below the highest elevation commonly reached by the subject's nose during the exposure, and laterally it includes the full range of travel of the head. For a caged, unrestrained rat this may be the entire volume of the cage. For the masked animal, it will be the small volume in front of the nostrils. The major point to be made is that the breathing zone is not above or below the cage, it is not 2 ft into a pipe leading to a mask, nor is it at the outlet of the aerosol generator.

Implicit in this discussion of the breathing zone is the assumption that this zone has a uniform composition. If it does not, one should consider either additional confinement of the subject, better mixing, or sampling at sufficient sites so that an average composition can be described. It should be noted that subjects may preferentially breathe from areas in the breathing zone that contain less toxicant.

C. What Should Be Measured?

All of the particle properties that significantly contribute to biologic effects in the study should be measured. Table 4.4 lists 21 candidate properties that should be considered for measurement. Properties 1 through 5 nearly always

must be considered. Property 6 (electrical charge) is often conveniently controlled by use of bipolar ion sources for reducing the state of electrical charge. In specific cases, the other listed properties may be important. For example, sodium chloride particles, upon entering the elevated relative humidity of the respiratory airways, become liquid and exhibit hygroscopic growth (the 7th property), which can alter the amount and distribution of material deposited. A well known example of the importance of property 9 (particle shape) is asbestos. Such particles, by virtue of their extreme length, can be retained in the deep lung for long periods. Practical considerations will limit the number of particle properties that can be measured in any given investigation, so good judgment on the part of the investigators is essential to the success of the sampling strategy.

The problem of characterizing gaseous atmospheres is relatively straightforward in comparison to particles. In general, one must know either the concentration in terms of volume (e.g., ppm), or mass per unit volume of air (e.g., mg m^{-3}), and the total atmospheric pressure, or the partial pressure (torr, mmHg, etc.). As discussed for aerosol particles, gas concentrations must be measured in the breathing zone. Although gases tend to distribute more uniformly than particles in an air volume, local inhomogeneities in concentration may exist. Causes for this include the presence of sinks, such as reactive materials in the exposure system and leaks in the containment system. A factor which is often overlooked in characterizing a gaseous atmosphere is the determination of contaminant particles that may be present. In general, despite the precautions taken, some particles will be present which may influence the biologic effects of the gas that is being studied. For example, the results of a study that indicated a synergistic toxic effect between ozone and sulfur dioxide are now thought to be due to contaminant particles formed by gas-phase reactions between the added gases and the unpurified air used for the primary chamber throughput air.

Transient concentration spikes (or peaks) may be important in interpreting studies of gas toxicity. For example, peak ambient concentrations may be very important in observed health effects. In view of this, it is wise to record concentration maxima in inhalation studies. This type of monitoring implies the use of instruments which continuously sample, have a short cycle time, and can provide frequent concentration data (often by using an automatic data recorder). A sufficient number of samples should be taken so that the standard deviation of the gas concentration during the exposure can be calculated and reported along with the experimental results.

The toxicity of essentially all inhaled materials is influenced by the prevailing environmental conditions during exposure (Table 4.5). Any environmental parameter that produces a change in the subjects' breathing or ventilation rates will influence the dose received. Environmental exposure conditions which stress the subject can alter the susceptibility to injury, even with a given dose. Table 4.6 lists some of the most important environmental contaminants found in exposure chambers. Such contaminants should be controlled

TABLE 4.4
Aerosol Particle Properties that May Relate
to Their Biological Effects

Property	Comment
1. Chemical composition	Will influence response in many ways
2. Mass concentration (mass of particles per unit air volume)	Will influence dose deposited and, hence, biologic response
3. Aerodynamic size	Will influence inhalability and deposition pattern
4. Size distribution	Will influence dose and dose distribution
5. Geometrical size	Will influence deposition pattern, clearance rate, dissolution rate, and sensory irritancy
6. Electrical charge	Will influence coagulation rates and deposition on chamber walls, animal fur, nares, and nasal hairs
7. Hygroscopicity	Will determine growth rates in respiratory tract and, hence, deposition pattern
8. Surface area	Will determine amount of adsorbed gases carried on particles Will influence rate of dissolution in lung fluids; may influence interaction with macrophages and other lung cells
9. Particle shape	Will influence deposition via interception mechanism and may influence phagocytic efficiencies
10. Dissolution rate	Will determine persistence in lung fluids and tissues
11. Water solubility	Will influence particle stability in respiratory tract
12. Deliquescence	Will influence particle size in respiratory tract and, hence, deposition pattern
13. Irritancy	May influence breathing patterns and thus modify dose and dose distribution
14. Specific gravity	Will influence deposition pattern and may influence clearance rates
15. Number concentration (no. of particles/volume of air)	May influence deposition pattern via "cloud effect" and will influence irritancy
16. Antigenicity	May influence irritancy, induce tissue responses, and secondarily influence clearance rates
17. Odor	May induce avoidance behavior
18. Taste	May induce avoidance behavior
19. Radioactivity	Can influence electrical charge and dissolution rate characteristics, as well as toxicity to lung cells
20. Surface characteristics	May influence the interactions with macrophages and other lung cells
21. Temperature	Particle temperature will be similar to that of surrounding gas and will influence deposition pattern

Note: Many of these properties are interdependent; for example, for spherical particles, the aerodynamic diameter is a function of geometric diameter and density.

Source: Phalen, R. F., *Inhalation Studies: Foundations and Techniques*, CRC Press, Boca Raton, FL, 1984, 104.

TABLE 4.5
Environmental Parameters Which Influence Subject Responses
in Inhalation Studies

Parameter	Comments
Temperature	Will influence physical activity, ventilation rates, and several other biologic characteristics
Relative humidity	Influences thermal regulation and hence ventilation; extreme values can produce abnormalities in respiratory tract mucus
Atmospheric pressure	Influences ventilation as well as cardiac and hematopoietic functions; is directly related to driving pressure for uptake of gases by tissues
Illumination	Influences state of activity and can cause stress due to altered diurnal patterns
Air contaminants	Can act as co-toxins or lead to adapted or sensitized states; may influence ventilation and other physiologic functions
Noise	A known co-stress for mammals; often present in exposure chambers due to human activity, equipment vibrations, or flow of air through small orifices or over sharp edges
Vibration	A known co-stress that may be produced by motors or other moving apparatus

Source: Phalen, R. F., *Inhalation Studies: Foundations and Techniques*, CRC Press, Boca Raton, FL, 1984, 106.

TABLE 4.6
Common Contaminants Found in Exposure Chambers
During Inhalation Exposures

Contaminant	Comments
Ammonia	Produced by bacterial action on urine; levels can rapidly increase over the duration of an exposure
Carbon dioxide	Crowding of animals or low airflows can cause a buildup of this exhaled metabolic byproduct
Water vapor	Crowding of animals or low air flows can lead to rapid buildup
Oil mist	Present in unfiltered output air from oil-lubricated air pumps
Animal dander and fur	May be present in excessive amounts due to poor housekeeping, overcrowding, or unhealthy animals
Organic vapors	May be residual from cleaning agents or previous exposures
Viable aerosols	May be present due to inadequate chamber cleaning

Source: Phalen, R. F., *Inhalation Studies: Foundations and Techniques*, CRC Press, Boca Raton, FL, 1984, 106.

and/or measured. The importance of proper control of environmental parameters cannot be overemphasized. Unless the investigator is aware of the exposure environment under the conditions of an actual exposure, he or she is not in control of the study.

D. Aerosol Characterization

The selection and use of an appropriate array of aerosol samplers and sizing instruments are not trivial tasks. Instrument limitations are not always obvious, and untrained or inexperienced persons can be at the mercy of the claims made in advertisements and manufacturer's literature. Aerosol characterizations represent some of the most difficult of all physical measurements, and even persons with advanced degrees in physics or engineering may not have had sufficient training to undertake them with confidence. It can be safely stated that no instrument, in the hands of a novice, will consistently provide reliable aerosol data.

Before discussing aerosol sizing instruments, it is necessary to consider devices that collect aerosol samples for subsequent analysis. Several sample collection devices are listed in Table 4.7. A basic sampling method, filtration, will be discussed briefly. An air filter is a device that allows the passage of air but traps particles. Collection efficiency varies with filter type, particle size, and the velocity of air passing through the filter. To collect particles, air is drawn through a filter using a pump or other method for producing a reduced pressure at the collector outlet, thus producing an air flow. Mercer¹ has extensively reviewed air filtration and has tabulated examples of filter collection efficiencies. Filters are usually of two types: pads of compacted fibers or membranes having many holes (pores) penetrating through them. Fiber filters designed for particle collection usually have fiber diameters in the micrometer size range. The fibers can be composed of a variety of materials including glass, viscose, cellulose acetate, asbestos, cotton, or other mineral and organic substances. Fibers can be coated with other materials, such as fluorocarbons, to modify chemical reactivity and hygroscopicity. Membrane filters can be made of cellulose or polycarbonate esters having pores of controlled diameter between about 0.01 and 10 μm . Membrane filters typically offer considerably more resistance to air flow than do fiber filters, but microscopic examination is easier for membrane filters, and they often have lower background concentrations of potential contaminants such trace metals or sulfates.

Filter samples taken for gravimetric analysis of airborne particle mass concentrations must be obtained with great care to avoid artifacts. Such artifacts include: changes in filter collection efficiency or throughput air flow rate due to clogging by the captured particles, leaks around the filter or through small tears, gain or loss of deposited mass due to chemical reactions, errors in determining filter mass due to loss of pieces of filter during handling, errors due to loss of sample in handling, and nonrepresentative sampling due to improper flowrate. From this list it is apparent that even this most basic method of obtaining a particle sample requires a knowledge of the physics of air flow and particle collection, the possible chemical reactions that can occur on the filter, and other relevant factors that may be specific to the application at hand. When care is taken, filter samples can provide accurate estimates of aerosol mass concentrations and the chemical species present.

TABLE 4.7
Aerosol Sample Collection Devices for Subsequent Analysis
of Aerosol Shape, Size, or Composition

Sampler	Principle	Artifacts and limitations
Filters	Air passes through holes, channels, or around fibers, but particles are trapped by interception, impaction diffusion, sedimentation, or electrostatic interaction	Flow rate may change as filter loads, air flow may be nonisokinetic, filter media may be damaged, filter holders may leak, and reactions may occur on filter
Electrostatic precipitators	Particles are charged and attracted to a collection surface	Very tiny particles may lose charge
Thermal precipitators	Particles are driven onto a relatively cool collection surface by thermal gradient forces	Aerosol particles may be altered by high temperatures; condensation may alter particle size
Elutriators	Particles settle under the action of gravity onto a collection surface	Particles smaller than about 1 μm in diameter do not readily settle because of Brownian motion
Impingers	Aerosol is passed through a tube and impacted on a plate submerged in a liquid	Collection efficiency diminishes for particles smaller than about 1 μm in diameter; agglomerated or fragile particles may break up; soluble components may be aerosolized
Cyclones	Particle-laden air is given a rotational motion, leading to inertial collection of particles	Collection efficiency diminishes for particles smaller than about 5 μm in diameter; plastic cyclones develop charges and alter size distributions

Source: Phalen, R. F., *Inhalation Studies: Foundations and Techniques*, CRC Press, Boca Raton, FL, 1984, 108.

Aerosol measuring instruments are based on exploiting size-dependent properties of aerosols (Table 4.8). Examples of size-dependent aerosol properties include terminal settling velocities, light-scattering patterns, mobilities, and abilities to serve as centers (nuclei) for condensation of supersaturated vapors. It is a well known fact in physics that detection or measurement is always associated with some perturbation of the system under measurement. This perturbation may be negligible for a macroscopic body but may be appreciable for a tiny aerosol particle. Energy from an intense light beam, for example, can be absorbed and converted to heat, which can produce wild gyrations in the motion of tiny particles and may rapidly evaporate liquid droplets.

Two very important aerosol size distribution parameters, the mass median aerodynamic diameter and the geometric standard deviation, are usually reported. A calibrated cascade impactor with a backup filter is probably the most widely used instrument for obtaining these parameters. The use of cascade impactors and step-by-step instructions on data reduction have been treated extensively by Lodge and Chan.²³ Assuming one has properly taken an impactor sample and avoided the many possible artifacts listed in Table 4.8,

TABLE 4.8
Aerosol Measuring Instrumentation for Inhalation Studies

Instrument	Principle	Artifacts and limitations
Optical microscope	Image formation using visible light and glass optics	Lower limit of resolution = 0.5 μm can lead to grossly distorted size distributions
Electron microscopes	Projected image formation using electron beam and magnetic "optics"	Evaporation of sample due to vacuum and local heating; shallow depths of focus and small field of view can bias size distributions; coating of small particles with column contaminants can lead to overestimate of size
Scanning electron microscope	Image reconstruction using scanning electron beam scattering and electronic signal processing	Evaporation of sample due to vacuum and local heating
Cascade impactor	Impaction stages in series collect successively smaller particles on basis of aerodynamic size	Cutoffs for stages can be imprecise. Particle bounce and re-entrainment produce biases; wall losses can be appreciable, and low pressures can cause evaporation of liquids; lower cutoff at about 0.3 μm aerodynamic diameter, except in low pressure models; amount of sample collected is usually small before overloading occurs; particle charges can produce artifacts
Condensation nuclei counters	Supersaturated vapor causes particle growth; particle number determined by intensity reduction of a light beam of air.	Lower limit of detection near 0.002 μm ; coincidence errors occur at high concentrations, about 10^6 or $10^7/\text{cc}$; calibration is difficult
Centrifuge spectrometers	Rotation produces increased sedimentation velocities across a clean air field where particles settle onto a tape	Inlet losses can be large for particles above a few micrometers
Diffusion batteries	Aerosol is drawn through fine screens or small channels in parallel at various air flow rates; measurements of penetrating fractions lead to size distribution	Inappropriate for particles greater than a few tenths of a micrometer in diameter; analysis is increasingly difficult as geometric standard deviation increases
Charge spectrometers	Aerosol is drawn between oppositely charged plates and deposits are analyzed to yield number of charges on particles	Operational difficulties include maintaining stable laminar air flow and prevention of arcing; analysis of polydisperse aerosols is difficult; usually custom designed
Mobility analyzers	Particles are given known charge and selectively deposited with respect to size by change in intensity of a collecting electrical field	Instruments have large internal losses that are particle-size dependent; calibration is sensitive to many environmental factors; lower size limit is undefined; upper size limit is about 1 μm

TABLE 4.8 (continued)
Aerosol Measuring Instrumentation for Inhalation Studies

Instrument	Principle	Artifacts and limitations
Surface area measurement devices	Particles are degassed and then allowed to adsorb gas on their surfaces; amount of adsorbed gas is determined gravimetrically or by other means	Requires large quantities of collected aerosol; method is sensitive to surface character, presence of cracks, and voids in particles; low pressures and high temperatures used may alter particle surface area during measurement
Elutriators	Particles travel usually horizontally and settle through clean air onto a surface; position on the surface is related to aerodynamic diameter	Impractical for submicrometer particles due to their large diffusion coefficients; charged particles may not deposit at proper locations

Source: Phalen, R. F., *Inhalation Studies: Foundations and Techniques*, CRC Press, Boca Raton, FL, 1984, 112–113.

one must accurately determine the collected mass on each of the impactor collection stages, as well as on the backup filter. In some cases, a gravimetric determination is adequate, but often a chemical analysis provides much more specific information. Obviously, one must be sure that the entire mass collected on each stage is recovered and quantitatively assayed. Proper use of impactors requires skill and understanding. If the aerosol is liquid and the impaction surface hard, for example, one may have problems with droplets breaking up and fractionating during collection. On the other hand, if the impactor collection surface is a filter or has been coated with oil or grease to prevent particle bounce, elaborate means may be necessary to remove all of the collected deposit for subsequent analysis. Although the median aerodynamic size can be obtained using a cascade impactor, effects such as particle bounce and electrostatic repulsion will tend to artificially broaden the estimated size distribution.

Many sizing techniques, such as electron microscopy and cascade impaction, have a major shortcoming: they do not usually give a final answer rapidly. Often, many hours or even days will pass before one has a reliable result. Thus, these techniques generally are not useful for real-time adjustments of aerosol generators. To solve this problem, instruments that give real-time responses may also be required. As purchased, such instruments are calibrated against relatively ideal particles (for example, monodisperse polystyrene latex microspheres with densities near 1 g cm^{-3}). Several instruments are available that provide real-time aerosol size distribution data (Table 4.8). In most cases it is necessary to periodically check their accuracy under the specific conditions of an experiment, since instrument calibrations made using more ideal particles may not apply well to other aerosols.

Examples of commonly used real-time monitors include optical particle spectrometers, optical particle counters, condensation nucleus counters, low-pressure piezoelectric impactors, and mobility analyzers. With adequate calibration, real-time monitors can be very useful for both controlling and characterizing atmospheres in inhalation studies.

In some special studies, additional aerosol instrumentation will be required. For example, one may need information on the surface areas or electrical charge states of particles. Thus, specialized instrumentation will be necessary. In such cases, experienced scientific personnel and a thorough knowledge of the aerosol literature are essential for success.

The air-flow patterns entering sampling devices can be complex and lead to collection of a nonrepresentative sample. Mercer¹ has described conditions under which particles of various sizes may be sampled isokinetically. It is very difficult to isokinetically sample particles with diameters of 20 μm or greater, especially when sampler inlets are tubular and sampling flow rates are less than about 1000 $l \text{ sec}^{-1}$. Mercer,¹ Hinds,⁴ or Vincent²⁴ should be consulted if large particles are to be sampled.

E. Gas Characterization

Three primary methods are used for obtaining samples of gaseous materials for analysis: grab sampling, absorption sampling, and continuous sampling. In each method, the objective is the same — to acquire a representative sample, that is not altered by the sampling process, for subsequent analysis. The collection efficiency of a gas sampler or sampling procedure must be known in order to quantitatively determine an airborne concentration. The collection efficiency, C , is

$$C = \frac{\text{amount collected or detected}}{\text{amount present in sampled volume of air}} \quad (4.2)$$

The collection efficiency can be less than 1 due to factors such as absorption or adsorption in sampling lines, losses due to chemical reactions, and condensation of gases onto the surfaces of airborne particles. Estimation of the collection efficiency is typically accomplished by measuring concentrations in standardized calibration mixtures. Note that the entire sampling system should be tested in the configuration to be used in the actual study. Great care must be taken in selecting appropriate sampling line materials and in removing particles from the sampled air flow.

Grab sampling refers to the rapid acquisition of an air sample that is then transported to analytical equipment. Evacuated containers, calibrated syringes, plastic or aluminized bags, and a variety of other containers are used to hold grab samples. Problems encountered include: porosity of the container, sample

contamination by container materials or contaminant air residing in the container, and degradation of the sample within the container. Adsorption sampling refers to the capture of either a sample on a high surface substrate such as activated charcoal or in a liquid solvent such as water. The collected sample is then driven from the capture medium for analysis or is analyzed *in situ* on the medium. A related type of sampler, the cold trap, collects a gas by low-temperature liquefaction. Several types of adsorption and absorption media are in use, including glass beads (wet, dry, chilled, etc.), activated charcoal, silica gel, ion-exchange resins, impregnated filters, and a variety of other specialized materials.

The analysis of a sample of gas may be performed by traditional quantitative wet-chemistry followed by gravimetric, photometric, or some other analytical method. Other phenomena or analytical techniques that have been exploited for analysis of gases include chemiluminescence (chemically stimulated light emission), electrical conductivity of a solution, heat of combustion, thermal conductivity, coulometry (measurement of charge generated by chemical reactions), flame ionization, gas chromatography, spectrophotometry, polarography (electrolysis), radioactivity, and mass spectrometry. Table 4.9 lists some gases and representative analytical techniques applied to them. Methods for many of the commonly encountered contaminants of ambient air and workplace atmospheres have been published.²⁵⁻²⁶ The current trend in developing real time instrumentation for gases includes improvements in specificity, portability, and sensitivity. Reading the current scientific literature is essential to the proper selection of gas-monitoring instrumentation. Useful journals in this regard include: *Journal of the Air & Waste Management Association*, *Environmental Health Perspectives*, *Analytical Chemistry*, *Atmospheric Environment*, *Environmental Science & Technology*, *American Industrial Hygiene Association Journal*, and *Applied Occupational & Environmental Hygiene*.

V. Eliminating Measurement Interferences

Interference occurs when the analysis for one material is significantly altered by the presence of another. In an air sample taken during inhalation exposure, several unwanted materials are often present. Three basic techniques can be applied to the problem of interferences: selection of detection methods that do not suffer from significant interferences, removal of interfering species from the air stream, and correction of analyzer readings to remove that portion of the reading due to interfering species.

Selection of detectors that do not suffer from significant interferences has become easier in recent years. In general, various absorption and emission spectra are unique to each given material, and monitoring instruments based on such spectra tend to be relatively free of interferences. One problem that

TABLE 4.9
Representative Methods for Collection and Analysis of Gases and Vapors

Gas or vapor	Chemical techniques			Interferences
	Sorption medium	Analysis		
Ammonia	25 ml 0.1 N Sulfuric acid	Nessler reagent		—
Benzene	Activated charcoal	Gas chromatography	Other similar retention compounds	
Formaldehyde	1% 10 ml Sodium bisulfate	Color of product	Phenols, some other aldehydes	
Nitrogen dioxide	20–30 ml Saltzman reagent	Reacts with adsorbing solution	Ozone in fivefold excess; peroxyacyl nitrate	
Ozone	1% Potassium iodide in 1 N potassium hydroxide	Measure color of iodine liberated	Other oxidizing agents	
Styrene	15 ml Spectrograde isooctane	UV analysis	Other aromatic hydrocarbons	
Direct reading instruments				
Operating principle	Applications and remarks	Range	Sensitivity	
Chemiluminescence	Measurement of NO in ambient air selectively and NO ₂ after conversion to NO by hot catalyst; specific measurement of O ₃ ; no atmospheric interferences	0 to 10,000 ppm	Varies: 0.1 ppb to 0.1 ppm	
Colorimetry	Measurement and separate recording of NO _x -NO _x , SO ₂ total oxidants, H ₂ S, HF, NH ₃ , Cl ₂ , and aldehydes in ambient air	ppb and ppm	0.01 ppm (NO ₂ , SO ₂)	
Coulometry	Continuous monitoring of NO, NO ₂ , O ₃ , and SO ₂ in ambient air; provided with strip chart recorders; some require attention only once a month	Selective: 0 to 1.0 ppm overall or to 100 ppm (optional)	Varies: 4 to 100 ppb dependent on instrument range setting	
Infrared analyzer	Continuous determination of a given component in a gaseous or liquid stream by measuring amount of infrared energy absorbed by component of interest using pressure sensor technique; wide variety of applications including CO, CO ₂ , freons, hydrocarbons, nitrous oxide, NH ₃ , SO ₂ , and water vapor	From ppm to 100% depending on application	0.5% of full scale	

Source: Phalen, R. F., *Inhalation Studies: Foundations and Techniques*, CRC Press, Boca Raton, FL, 1984, 116.

arises when airborne gases are analyzed spectroscopically is that the presence of particles can lead to unwanted scattering and absorption of light and other electromagnetic radiation. Such interfering particles may be present in the breathing zone even when they are not intentionally generated. Also, particles may be formed within a sampling line or an analytical instrument due to changes in pressure, temperature, humidity, or other factors.

Removal of interfering species from a sample air stream may be necessary in order to obtain valid readings with analytical instruments. Particulate filters can be placed in gas sampling lines. Several problems are associated with such in-line particle filters, including: reaction or capture of the sampled gas on the filter material or particles trapped in and on the filter, reduction of the sampler air-flow rate due to the resistance of the filter, dilution of the sample by air leaks that occur in the filter holder, and introduction of new materials into the sample due to chemical reactions or physical desorption of materials trapped on the filter. Intentional removal of gases from the sampled air can be achieved by coating the walls of a sampling line with specific absorbers or by passage of the sample through a bed or filter containing an absorber. One must be careful in designing such scrubbers to ensure that they do not retain the sampled species.

If it is necessary to correct instrument readings for interfering materials, two general methods can be considered. One may monitor the concentration of the interfering material separately and correct the output of the primary instrument using data on the relationship of the concentration of the interfering substance and the size of the interfering signal. Alternately, one may experimentally recalibrate the primary instrument under actual exposure conditions with interfering species present. The first technique is usually superior when the interfering material has a relatively constant concentration. In order to have confidence in one's ability to correct for or eliminate interferences, it is wise to intentionally generate known amounts of interfering species and thus test the correction technique before using it in an actual inhalation study.

Stevens et al.²⁷ and Kleinman et al.²⁸ used an ammonia gas denuder to remove ammonia from sampled air to prevent neutralization of acid particles on a sampling filter. The device described by Stevens consisted of a set of 16 parallel glass tubes (30-cm length, 0.5-cm i.d.) coated with phosphorous acid. A gas diffusion denuder for removal of sulfur dioxide and/or hydrogen sulfide was described by Coburn et al.²⁹ that had a gas capture efficiency of nearly 100% and permitted 98% or more penetration of particles. Recent denuder designs can be used in tandem to separate and analyze acid gases, basic gases, and particulate species in a single sample.³⁰⁻³¹ Annular denuders have been used very successfully for inhalation studies and environmental studies.³²⁻³⁶

VI. Sampling Protocols

Ideally, all of the relevant atmospheric characteristics would be continuously monitored throughout an inhalation exposure. In practice, this is likely to be an impossibility for several reasons. Many samplers do not provide continuous data, and samples must be collected over finite periods in order to accumulate sufficient material for analysis. The total air-flow rate through a large battery of samplers can exceed the total exposure system throughput air-flow rate. Also, the instrument costs and manpower requirements for an ideal, complete monitoring scheme can be prohibitive. Therefore, practicality, ingenuity, and scientific insight are all elements in designing valid sampling protocols that are cost effective.

At a minimum, the primary experimental materials being investigated, as well as any other materials or parameters that might reasonably be expected to modify the measured responses, must be measured. The mean concentration is usually the most important datum relating to the potential biological effect of a material, but the standard deviation, range, and peak values may also be important to interpreting the observed responses. In some studies, the rate of increase or decrease in concentration of a material may be measured, as such phenomena can also influence biologic responses. When a continuous data record is made, each of the foregoing exposure parameters can, in principle, be obtained from the record. When discrete samples are obtained in order to determine a desired mean concentration value, the minimum number of samples (N) that must be taken can be calculated given two additional input parameters: the required precision with which one must know the mean (i.e., the standard error of the mean, SEM) and the expected standard deviation (SD) of the measured concentrations. Then:

$$N = (SD/SEM)^2 \quad (4.3)$$

This equation is based on the assumptions that the concentration measurement data are randomly distributed about the mean (normally distributed) and that each measurement is independent of the others.

When discrete samples are acquired, one cannot determine the maximum and minimum concentrations, as they may have occurred between sample periods or may have been masked by the finite duration of sampling. When discrete samples are acquired, one should thus report not only the number of samples taken, but also the duration of each sampling cycle.

When the time required to acquire a sample for analysis is large with respect to the total duration of the inhalation exposure, one should consider beginning the sample at the start of the exposure and continuing the sampling throughout the exposure period. In this way, the sample will represent an average which is representative of the conditions that existed throughout the exposure.

VII. Suppliers

A. Aerosol Generators

In-Tox Products
115 Quincy NE
Albuquerque, NM 87108

BGI, Inc.
58 Guinan Street
Waltham, MA 02154

TSI, Inc.
500 Cardigan Road
Shoreview, MN 55126

B. Aerosol Sizers and Samplers

In-Tox Products
115 Quincy NE
Albuquerque, NM 87108

Malvern Instruments, Inc.
10 Southville Road
Southborough, MA 01772

SKC, Inc.
863 Valley View Road
Eighty Four, PA 15330

Graseby (Andersen Samplers)
500 Technology Court
Smyrna, GA 30082

MIE, Inc.
1 Federal Street, #2
Billerica, MA 01821-3500

Climet Instruments Co.
1320 Colton Avenue
Redlands, CA 92374

TSI, Inc.
500 Cardigan Road
Shoreview, MN 55126

Particle Measuring Systems, Inc.
5475 Airport Blvd.
Boulder, CO 80301-2239

Met One, Inc.
481 California Avenue
Grants Pass, OR 97526

Quantichrome Corp.
(Surface Area Analysis)
1900 Corporate Drive
Boynton Beach, FL 33426

C. Gas Generators

Liquid Carbonic
Cylinder Gas Products
810 Jorie Blvd.
Oak Brook, IL 60521-2216

Altech Associates, Inc.
2051 Waukegan Road
Deerfield, IL 60015

Matheson Gas Products
30 Seaview Drive
Secaucus, NJ 07094

Scott Specialty Gases
6141 Easton Road
Plumsteadville, PA 18949-0310

D. Gas Analyzers and Samplers

Dasibi Environmental Corp.
506 Paula Avenue
Glendale, CA 91201

SKC, Inc.
863 Valley View Road
Eighty Four, PA 15330

Monitor Labs, Inc.
74 Inverness Drive E.
Eaglewood, CO 80112

Columbia Scientific Industries Corp.
11950 Jollyville Road
Austin, TX 78759

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